Flavobacteria
across Time and Scale

Designing for Human-Microbe Engagement

Clarice Risseeuw

Flavobacteria
across Time and Scale

Designing for Human-Microbe Engagement

Dissertation

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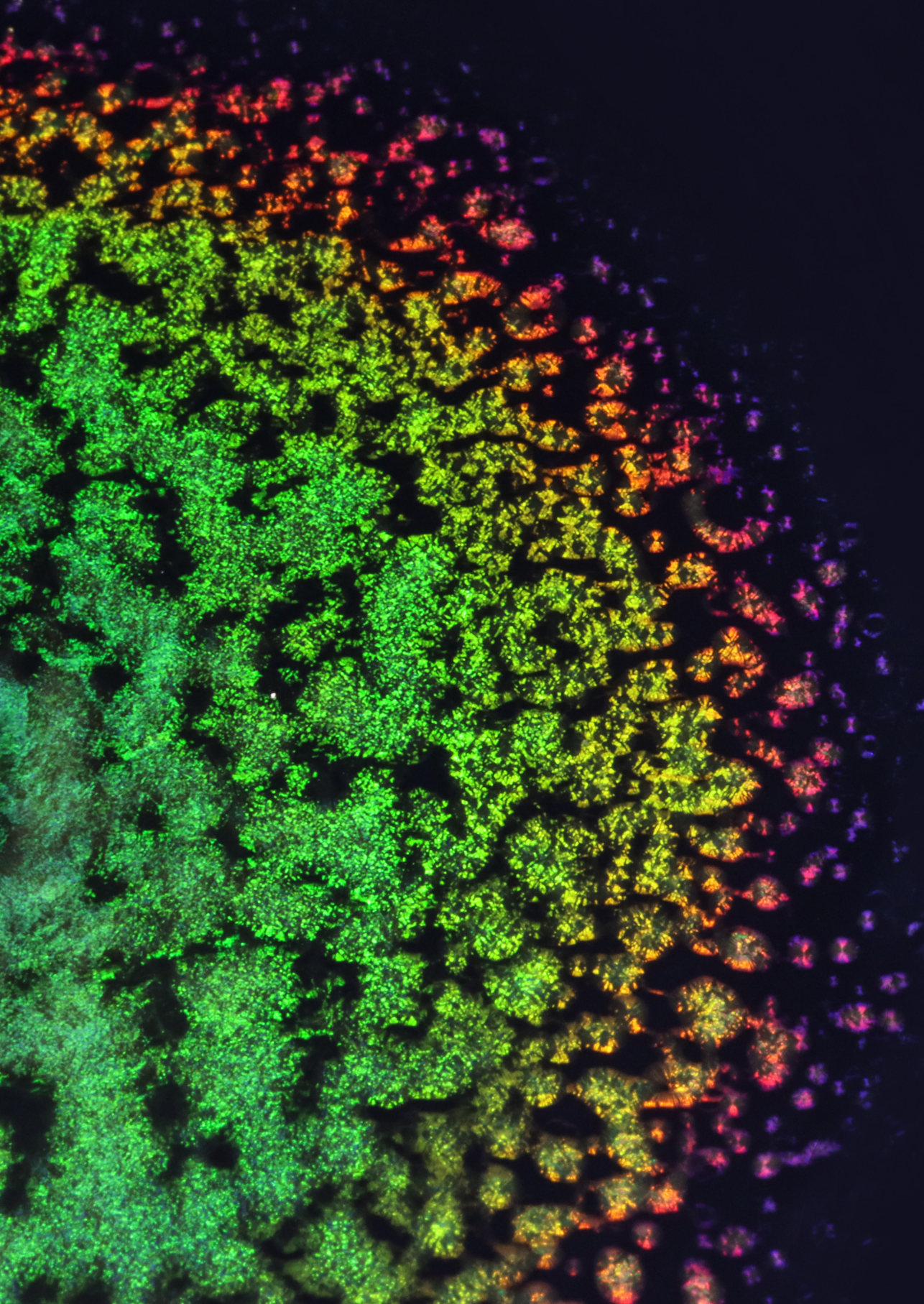
Voor papa,
die altijd in mij gelooft



<< Cellulophaga lytica PLY-A2

5-day culture, grown at room temperature

Table of Contents

Preface

This dissertation is shaped by a long-standing fascination with the natural world. From early on, I found myself drawn to gardens, forests, and beaches. I could spend hours simply watching the garden—the movement of birds near the pond, the shifting light on leaves, the subtle details of plants and flowers. I have always loved walking through forests, feeling the textures of mosses and ferns, or exploring on the beach, observing shells and watching the tides shape the coastline. These moments consistently renew my energy and sharpen my attention.

During my design education, this fascination initially translated into projects that supported people in outdoor activities and addressed sustainability questions. I was increasingly concerned with how design might respond to ecological crises and how it could support people in getting more ecologically attuned. During my MSc graduation project, I was introduced to biodesign. Here, I worked with iridescent Flavobacteria for the first time, and I was immediately captivated. Biodesign felt like the perfect way to combine my passions for design and nature, while opening up possibilities to engage people with living systems in ways that cultivate greater attentiveness and appreciation for other forms of life.

Alongside my academic work, I have maintained an ongoing engagement with nonhuman life through gardening and the everyday care of more than one hundred indoor plants. Tending to them over the years has cultivated a sensitivity to subtle temporal changes, such as the slow emergence of new growth, early signs of stress, and small adaptations after relocation. During my PhD, a similar form of attunement gradually emerged in my work with Flavobacteria. What began as curiosity and aesthetic fascination developed into a deeper familiarity. I learned to recognise their rhythms and subtle shifts in their expressions as signs of thriving or struggling. Over time, I found myself feeling connected to these microorganisms in ways I had not anticipated.

This evolving relationship shaped both the questions asked in this dissertation and the manner in which the research was conducted. My orientation toward the bacteria was not fixed: at times I approached them from a technical and analytical distance; at other times I related to them with attentiveness and care. The work unfolded within this shifting space as I moved between technical analysis and attentive forms of engagement, gradually finding my own way of working with living microorganisms.

Chapter 1

Introduction

1.1 Research Context

1.1.1 The potential of Flavobacteria's sustainable living colour

The accelerating environmental crisis—driven by industrialisation, urbanisation, and unsustainable resource use—is causing widespread climate change, pollution, and biodiversity loss [1]. These interconnected issues threaten human health, ecosystem resilience, and planetary stability, demanding urgent reconsideration of how materials and technologies are produced and used [2, 3]. Within this broader crisis, the colouring industry presents severe sustainability challenges [4–6], as most used pigments and dyes are petroleum-based or rely on inorganic compounds. Despite their aesthetic and functional importance, colourants generate pollution, hazardous waste, and recyclability barriers.

In parallel, electronic sensors used to detect and communicate environmental conditions raise sustainability concerns of their own [7–9]. They rely on complex material assemblies, scarce or hazardous resources, and energy-intensive production processes, and are rarely designed for recyclability—contributing to growing e-waste streams and resource depletion. Colour-changing materials offer a more intuitive and visual alternative to electronic sensing of environmental conditions; however, many remain embedded in synthetic, non-degradable matrices and can incorporate toxic components, such as many thermochromic materials [10].

Together, these challenges highlight the need for sustainable alternatives to dynamic and sensing materials within HCI and interaction design. This aligns with wider calls in sustainable and ecological HCI [11–15], regenerative design [16, 17], and biodesign [18, 19] for material paradigms that reduce environmental impact and rethink relationships between technology, living systems, and the environment. In response, this dissertation proposes Flavobacteria[1] as a compelling direction for exploring sustainable, living alternatives, motivated by their dynamic iridescent colourations. Specifically, I aimed to unpack the design potential of Flavobacteria's living colour, considering its temporality, responsiveness, and implications for interaction with living systems.

Flavobacteriia, a class of bacteria within the phylum Bacteroidetes, represents a diverse group of microorganisms found across a wide range of environments. Many of its strains produce dynamic colourations when growing on a surface, as their cells self-organise into so-called photonic crystals [20–22]. This collective cell organisation has been linked to their predatory behaviour, suggesting that the colourations may be a secondary effect of mechanisms used for preying on other bacteria [23]. The in-depth exploration presented in this dissertation focuses on the non-pathogenic flavobacterium *Cellulophaga lytica* PLY-A2 (Fig. 1.1), isolated from marine environments and known for its stability and

[1] The class *Flavobacteriia* contains hundreds of rod-shaped bacteria species. In this dissertation, I use the term Flavobacteria specifically to refer to flavobacterium species that produce iridescence, primarily *Cellulophaga lytica*, with which all empirical studies were conducted.

vivid colourations [24]. When illuminated, the optical structures created by this strain produce iridescent effects—hues that shift with viewing and illumination angle, akin to those seen on butterfly wings. As the bacteria grow, reproduce, and respond to their environment, the colony's structural colour continuously transforms, revealing its dynamic and temporal character. Importantly, these bacteria are widely distributed [24] and can thrive under relatively modest conditions, requiring little more than appropriate humidity, salinity, and trace nutrients. Combined with their dynamic and responsive colourations, these characteristics highlight their potential as a sustainable, living alternative to conventional colour-changing materials.

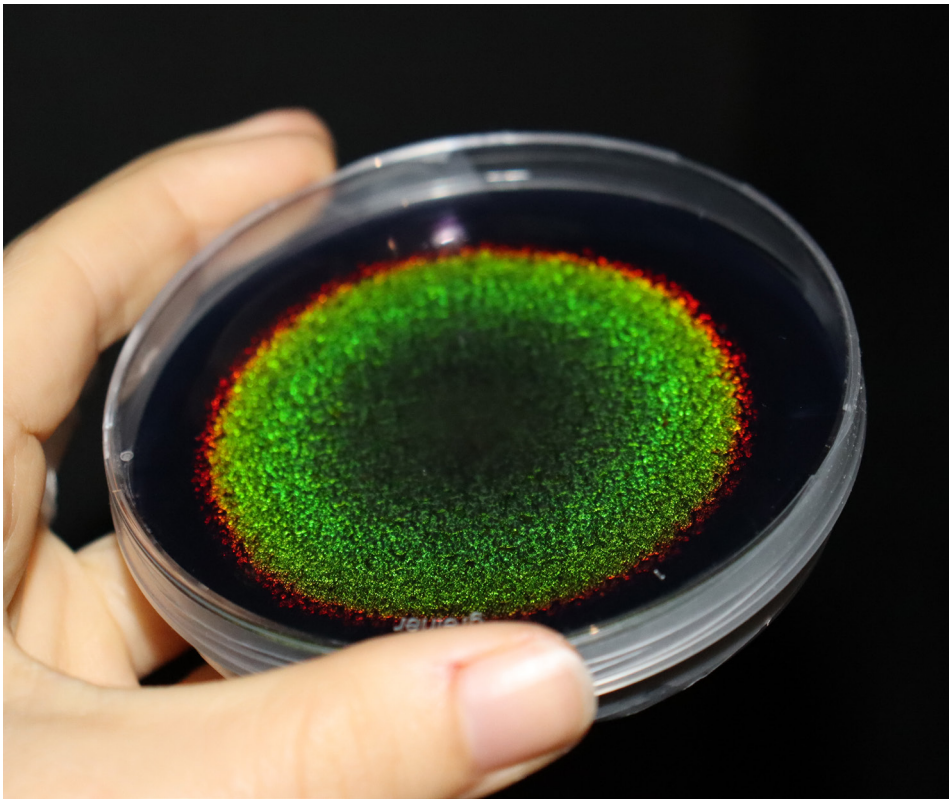


Fig. 1.1 *Cellulophaga lytica PLY-A2.*

At the start of this PhD trajectory, studies on iridescent Flavobacteria were limited, mostly focusing on the mechanisms of self-organisation and on how environmental factors such as nutrition, salinity and temperature affect this [21–28]. While some scholars noted opportunities for engineering living optical materials [25], the design potential of these iridescent bacteria had not yet been explored. In recent years, however, their striking optical properties and responsive behaviour have begun to attract wider attention—for instance, in material sciences [29] and artistic practices engaging with living colour [30, 31].

Building on these works and my initial explorations with Flavobacteria, two key challenges became central to the inquiry. These challenges, discussed in the following sections, emerged from working with this specific microorganism but are not unique to it; rather, they reflect broader challenges encountered in biodesign and in designing with living microorganisms specifically.

1.1.2 Understanding microorganisms and their design potential

Over the past decades, biodesign [32] has gained significant interest across art, product design, fashion and textile design, architecture, and human–computer interaction (HCI) [33–37]. Bridging biology and design, this interdisciplinary field challenges designers to understand the behaviours, capacities, and constraints of the living microorganisms they work with. Designing with living microorganisms demands insight into how microbes grow, interact, and respond to their environment—knowledge that extends well beyond traditional material understanding.

Much biodesign practice implements microorganisms as biological resources within production processes, highlighting both ecological benefits and the emergence of novel expressions [35]. Early pioneering work includes Suzanne Lee's cultivation of kombucha-based cellulose garments [38] and Maurizio Montalti's development of mycelium-based materials [39]. Addressing sustainability concerns within the colouring industry, biodesign practitioners have also explored pigment-producing microorganisms as alternatives to synthetic dyes [40]. Such practices require designers to understand microorganisms' activity and what they require to thrive.

Other works within biodesign extend microbial livingness into the "use time" of artefacts, as seen in the *Living Coffin*, which leverages mycelium's ability to decompose organic matter, transforming end-of-life into a regenerative act [41]. Living artefacts, as conceptualised by Karana et al. [42], offer novel responsive behaviours and interaction possibilities. For instance, *Ambio* illustrates how microbial responsiveness can be incorporated into designed interactions, as bioluminescent bacteria establish a dynamic light source that responds to touch [43]. Such interactive living artefacts highlight both the possibilities and challenges of working with living microorganisms, including the need to design suitable habitats that support microbial viability while enabling interaction. In these practices, challenges become increasingly layered and complex, requiring designers to understand microbial metabolism, sensitivities, responsive behaviour, and maintenance conditions.

Parallel explorations within bio-HCI [44] further investigate how biological systems can be integrated into computational or interactive frameworks. Concepts such as *Living Bits* [37] rethink the boundaries between biological cells and computational components, while *Living Media Interfaces* explore how organisms can function as interactive and sensing media [45]. Together, these works emphasise the difficulty of working with temporal, responsive, and

environmentally sensitive behaviours—qualities that fundamentally shape how microorganisms can be incorporated as living interactive elements into design.

Commonly, these works emphasise that designers must thoroughly understand microorganisms in order to bring their sustainable design potentials forward beyond speculative applications. Designing with Flavobacteria required me to understand the organism's needs, colour-producing mechanism, sensitivities, temporal qualities, and responsive behaviours. Such understanding was essential not only for evaluating which interactions and applications were biologically plausible but also for determining the habitat requirements of Flavobacteria living artefacts. Therefore, the first research challenge was to closely characterise the temporal and responsive dynamics of Flavobacteria's living colour.

1.1.3 Understanding and designing for human-microbe engagement

While biodesign often foregrounds functional and ecological potentials of microorganisms, one can argue that these approaches alone may not be sufficient to support truly regenerative futures. Focusing primarily on microbial capabilities risks overlooking the relational and affective dimensions of interacting with microbial life, which may be equally important for establishing more sustainable ways of living. Together, these considerations point to the need for more relational and reflective forms of engagement.

In line with this, the second line of inquiry in this dissertation aligns with wider discussions in design and HCI concerning how humans experience and relate to living microorganisms. Although living artefacts and interfaces are receiving increasing attention, it remains poorly understood how people actually experience and make sense of these living systems, particularly in everyday contexts.

To conceptualise how livingness is experienced, Karana et al. [42] introduced *Living Aesthetics*, a principle describing how the growth, reproduction, transformation, and decay of embedded organisms come to expression in artefacts and how these expressions are experienced by humans. In particular, living aesthetics concern how people experience the type, intensity, and duration of change—qualities central to how the presence and liveliness of organisms become noticeable, engaging, and meaningful. The dynamic colourations produced by Flavobacteria offer an opportunity to study how people notice, interpret, and respond to microbial expressions, as they encompass distinct temporal rhythms, intensities, and modes of change. Living aesthetics are therefore a key focus in this dissertation.

I take this exploration further and argue that engaging with living aesthetics not only helps us design living artefacts that are functional, legible, and inviting to interact with, but also plays a crucial role in shaping how humans relate to living organisms over time. More specifically, I show that Flavobacteria, with their vivid colourations and expressive responsiveness, hold potential for meaningful

human-microbe engagement that can evoke reflection on interconnectedness and open pathways for cultivating awareness and appreciation for microbial life.

In short, realising the potential of living microorganisms in design remains challenging, as it requires understanding microorganisms in detail—including their needs, temporality and responsiveness—as well as understanding how humans interact and respond to these organisms in everyday life, and how these interactions can foster a greater sense of engagement with living artefacts and beyond. This dissertation addresses this gap through an in-depth exploration of iridescent Flavobacteria, situated at the intersection of living aesthetics, interactive living artefacts, and meaningful human-microbe engagement.

1.2 Approach

Building on the research context, I present the research questions, methodological approach, and an overview of the studies and chapters that structure this dissertation.

1.2.1 Research objectives and questions

The main objective of this dissertation is to support biodesigners in designing for human-microbe engagement through knowledge, tools, and techniques. Accordingly, this work addresses the overarching research question:

How can biodesign facilitate human-microbe engagement in everyday life?

This question is explored through an in-depth exploration of iridescent Flavobacteria, as detailed in the methodology subsection. To unpack the main research question and structure the investigation, the work is guided by three interrelated sub-research questions:

1. How do Flavobacteria's temporal expressions unfold over time and in response to external conditions?
2. How can direct human-Flavobacteria interactions be designed within living artefacts to reveal responsive microbial expressions?
3. How do humans experience and engage with these microbial expressions in everyday life?

1.2.2 Methodology

This dissertation presents a mixed-methods, design-led inquiry that uses Flavobacteria's living colour as the research medium to explore human–microbe engagement. Focusing on a single group of microorganisms enabled a deep, iterative investigation across laboratory, design, and field settings.

Flavobacteria provide an especially suitable case because they display expressive qualities that are perceptible to humans and offer a broad spectrum of living aesthetics, characterised by distinct temporal rhythms, intensities,

and modes of change. In addition, Flavobacteria's cultivability and my prior 1.5 years of experience working with this microorganism ensured the feasibility of iterative, hands-on experimentation. Motivated by curiosity and fascination for this microorganism, I followed an exploratory approach that began with a close examination of the microorganism's colourations and responsiveness, which subsequently informed the development of artefacts and interaction modalities.

Figure 1.2 illustrates the overall structure of the research, highlighting the different research strands with their key activities and outcomes through which they contribute to the research questions.

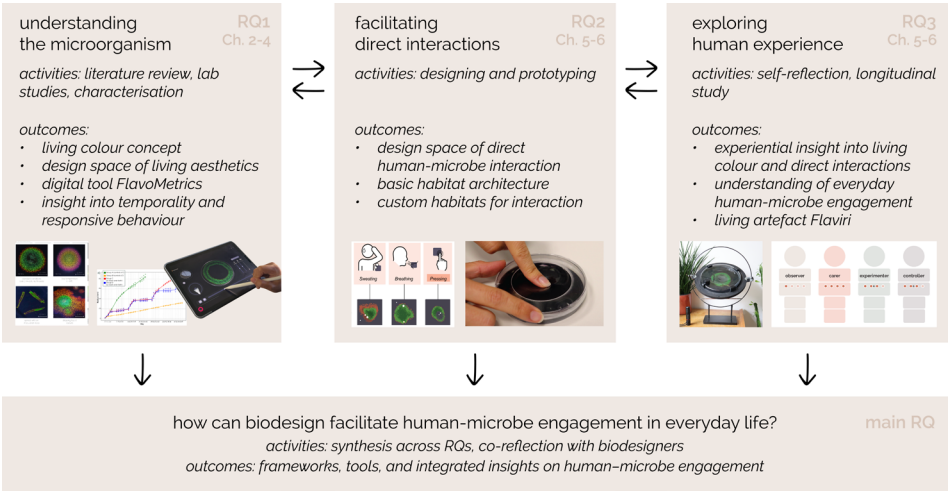


Fig. 1.2 *Overview of the three research strands and their key activities and outcomes, illustrating how each strand contributes to addressing the research questions.*

To address RQ1, I conducted an in-depth investigation into Flavobacteria's iridescent colourations, their temporality and responsive behaviour. Activities included a literature review and a series of laboratory studies, ranging from exploratory experiments involving tinkering and temporal characterisation [46] (Ch. 2, 3 and 5) to technical, in-depth investigations with microscopy and statistical analysis (Ch. 4). These methods were chosen to develop a thorough understanding of Flavobacteria's colour-producing behaviours and temporal expressions, forming the basis for subsequent design explorations.

Building on this understanding of the microorganism, I conducted design activities to address RQ2. These activities enabled direct human–Flavobacteria interactions, both in the laboratory (Ch. 5) and in domestic settings (Ch. 6), through the design of custom habitats and interaction artefacts. These designs further enriched the understanding of Flavobacteria by revealing how the microorganisms respond to diverse inputs within designed contexts.

The designed artefacts also enabled addressing RQ3 by creating opportunities to study how humans experience Flavobacteria's expressions. To explore

human experience, I first documented and reflected on my own interactions with Flavobacteria in the biolab (Ch. 5). Next, I conducted a longitudinal study situated in domestic settings with participants who had no prior familiarity with the organism (Ch. 6). The living research artefact for this study was informed by earlier investigations into Flavobacteria's colourations and their responses to the direct interactions in the laboratory. In studying human experience, I drew on the Experiential Characterisation Toolkit [47] to capture sensorial, affective, interpretive, and performative dimensions of engagement.

The main research question was addressed through the integration of all studies presented in this dissertation. This included co-reflection with biodesigners to explore how digital tools can support the understanding and exploration of living aesthetics (Ch. 3), as well as the development of design spaces and conceptual frameworks (Ch. 2 and 5) that distilled findings into structured resources for biodesign practice. Together with the outcomes of the sub-questions, these reflective and integrative activities collectively contributed to answering the overarching question of how design can facilitate human–microbe engagement.

Throughout these research activities, this dissertation foregrounds a diverse set of artefacts that were central to knowledge generation. These artefacts structured inquiry, inspired design directions, enabled direct interaction, and supported reflection on human–microbe engagement. The artefacts developed in this dissertation include:

- **Design spaces and frameworks**, which organised insights from biolab explorations, structured inquiry, and guided making. These artefacts embodied design reasoning and served as conceptual scaffolds for future biodesign practice.
- **A digital interactive tool**, which enabled dialogue and co-reflection with biodesigners on how digital tools might support the understanding and exploration of living aesthetics.
- **Custom-made habitats**, designed to support long-term microbial growth and enable direct interactions. These artefacts served as both an instantiation and an exploration of a design space, generating technical insights into microbial behaviour within designed contexts—revealing, for example, which conditions hindered or fostered growth.
- **Artefact concepts**, which made ideas and assumptions about possible interactions and human-microbe engagement tangible. These artefacts enabled critical reflection within discussions with my supervisory team and beyond, and thereby supported an evolution of my research focus—expanding from primarily exploring the design potential of Flavobacteria's living colour toward also considering how such interfaces might facilitate meaningful human–microbe engagement.
- **A living artefact**, specifically designed for a longitudinal study to enable an in-depth investigation into human-microbe engagement in everyday contexts. This artefact embodied inquiry, generated both empirical and experiential knowledge, and invited reflection among participants as well as in collaborative discussions with my supervisory team.

The supervisory team brought expertise in interaction design research, sustainable design, biodesign, and microbiology, supporting the conceptualisation of studies and framing of research questions. In addition, I had the opportunity to collaborate with Flavobacteria-specific expert scientists, in particular Dr Colin Ingham, whose expertise has been essential to this project. While the studies were collaboratively designed within the supervisory team, I conducted the empirical work in both laboratory and everyday-life settings, and created the artefacts presented in this dissertation, except for the digital interactive tool, which was developed in a close collaboration with experts in computer graphics and computational design.

1.2.3 Dissertation overview

This dissertation consists of seven chapters in total, which are visually outlined in Figure 1.3 to illustrate their connections. Chapters 1 and 7 provide the introduction and conclusion to the overall research, respectively. Chapters 2 to 6 are paper-based, presenting work published or under review in conference proceedings and peer-reviewed journals. Structured into two parts, this dissertation first focuses on the microorganism itself, exploring its temporal expressions and responsive behaviour, and then transitions to investigations of human-Flavobacteria engagement.

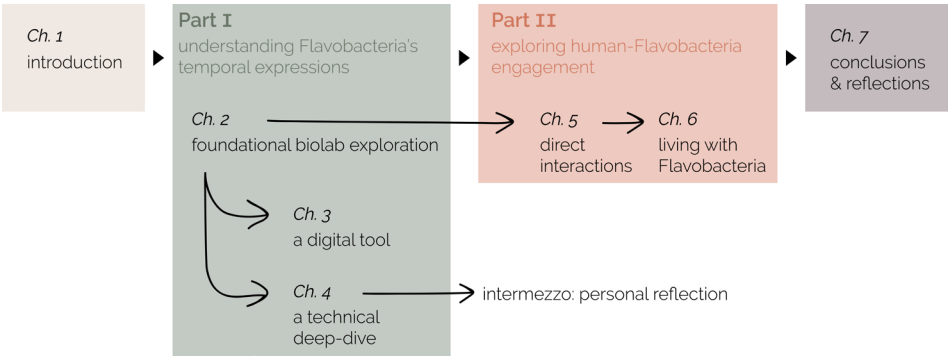


Fig. 1.3 *Overview of the dissertation chapters and their connections, including the two-part organisation of Chapters 2-6.*

Part I, comprising Chapters 2 to 4, focuses on understanding Flavobacteria's temporal expressions. Chapter 2 lays the groundwork for this dissertation by introducing iridescent Flavobacteria to HCI and design communities and providing an in-depth exploration into their living aesthetics. Building on a capture tool and empirical insights of this chapter, Chapter 3 explores how digital tools can assist biodesigners in understanding and working with the living aesthetics of microorganisms. Chapter 4 further expands upon the empirical foundation of Chapter 2 by examining Flavobacteria's responsive behaviour under varying growth conditions. While Chapters 3 and 4 follow distinct investigative paths, they both demonstrate how interdisciplinary collaborations

can support biodesigners in deepening their understanding of microorganisms' temporal expressions—a crucial aspect of working with these organisms and their unique complexities. The following intermezzo provides a personal reflection, focusing primarily on the interdisciplinary research presented in Chapter 4 and on technical approaches to working with living microorganisms.

Part II, comprising Chapters 5 and 6, presents explorations into human-Flavobacteria engagement. Chapter 5 initiates this shift by expanding the design space introduced in Chapter 2, unpacking possibilities for direct interaction and reflecting on their implications for fostering attentiveness toward microbial worlds and beyond. Building on these empirical and experiential insights, Chapter 6 explores how human-Flavobacteria engagement evolves over time in domestic settings, highlighting the role of living aesthetics in shaping these dynamics through a longitudinal study with a living artefact.

Finally, in Chapter 7, I conclude the dissertation by reflecting on the implications of the findings for both research and design communities, addressing limitations and opportunities of future work, and offering a critical reflection on my evolving position within the field.

To support coherence across the dissertation, some chapters were lightly edited for consistency in spelling, formatting, and biological terminology. The content of Chapter 3 was additionally adapted to align with the layout of this dissertation, as it was originally formatted as a pictorial publication.

1.3 Contributions

This dissertation aims to support biodesigners in designing for human-microbe engagement through an in-depth exploration of iridescent Flavobacteria. It contributes to the field of biodesign and to relevant sub-communities within HCI, including sustainable, ecological, and biological HCI. It also advances research on engineered living materials (ELMs) within the field of biotechnology. Central to this work is the bridging of two perspectives: the microorganisms—their expressions and environmental responsiveness—and the human experiences—how people perceive and respond to these microbial expressions. This dual perspective reinforces the central theme of human-microbe engagement and informs contributions across theoretical, empirical, methodological, and design dimensions. Overall, this dissertation makes the following key contributions:

- Theoretical and conceptual contributions - *new ways of thinking*

It introduces iridescent Flavobacteria into the domains of HCI and design, conceptualising living colour as communicative matter. It develops a vocabulary and design space for Flavobacteria's living aesthetics and offers a definition and understanding of direct interactions with living microorganisms.

- Empirical and experiential insights - *new knowledge from the lab and beyond*

It provides technical insight into Flavobacteria's living colour, including its temporality and responsiveness, alongside experiential insights into how

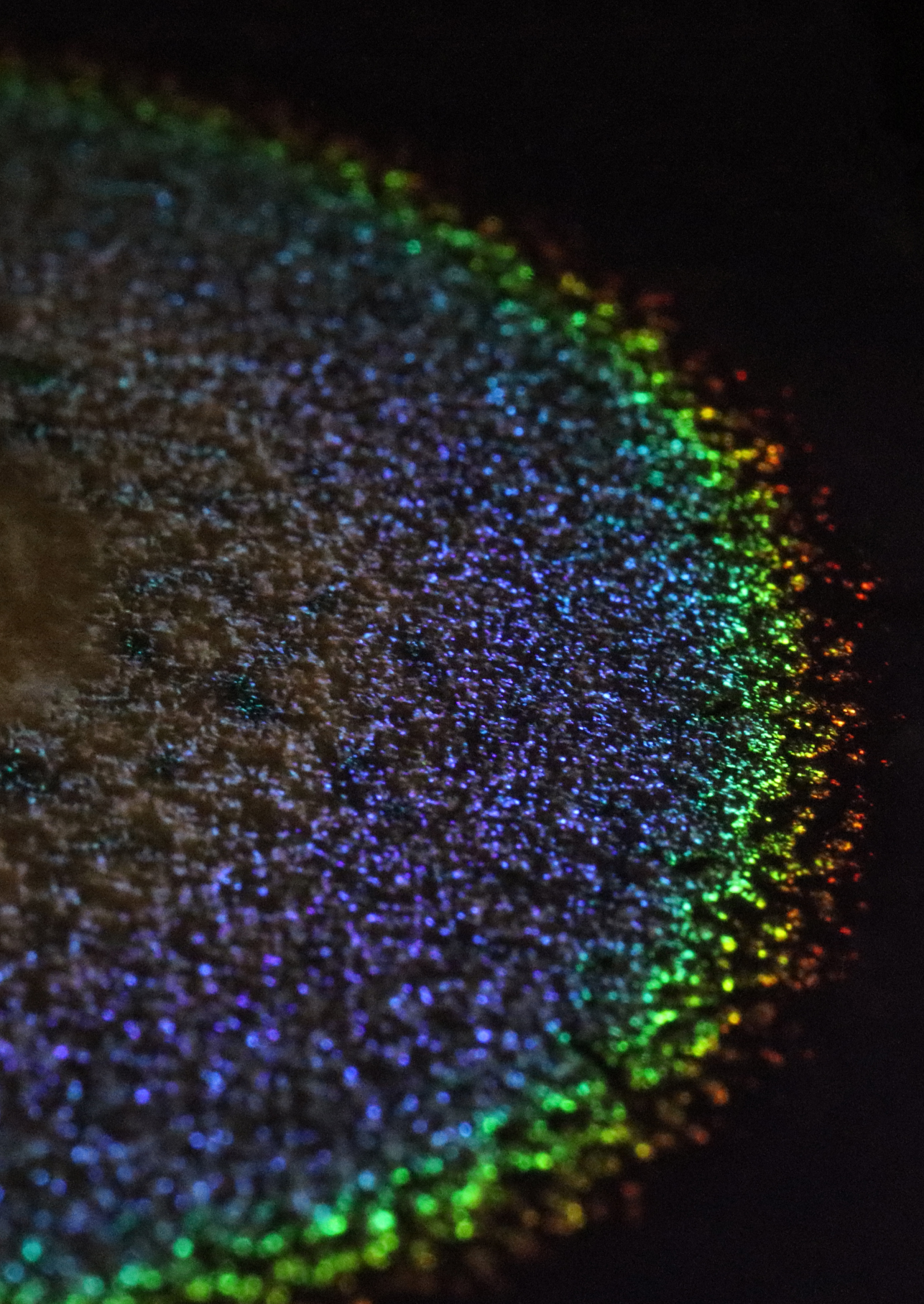
humans perceive and respond to these microbial expressions. Furthermore, it offers an empirical understanding of how living aesthetics shape human-microbe engagement within everyday life.

- Methodological contributions - *new ways of researching/designing*

It demonstrates an in-depth, microorganism-specific design research approach that integrates laboratory characterisation, interaction design, and field-based studies. It also provides a detailed methodology to investigate human-microbe engagement in everyday life.

- Design instances - *novel research artefacts and techniques*

It provides custom-made tools and techniques, including laboratory protocols, to document and characterise temporal expressions and enable direct interaction with living colour within and beyond lab environments. It also presents the digital tool *FlavoMetrics* to inspire and unpack the potential of digital tools for understanding living aesthetics in biodesign, as well as Flavobacteria living artefacts, including a longitudinal research artefact and several application concepts.



<< Cellulophaga lytica PLY-A2

side illumination revealing purple colouration

Part I

Understanding Flavobacteria's Temporal Expressions

Chapter 2

An Exploration into Flavobacteria's Living Aesthetics

This chapter marks the starting point of my dissertation. Drawing from bio-HCI, microbiology, and emerging biodesign practices, it introduces iridescent Flavobacteria to the HCI and design communities and explores their potential for living interfaces. In this chapter, I provide an in-depth exploration of Flavobacteria's living aesthetics, illustrating how their colourations shift over time and in response to their environment. The resulting vocabulary and empirical insights inform the subsequent studies in this dissertation.

Furthermore, the chapter presents a custom-made tool for capturing Flavobacteria's iridescent and temporal colourations, which I used throughout the entire PhD trajectory, and played a critical role in the development of the digital tool introduced in Chapter 3. It also presents a custom habitat for long-term growth of Flavobacteria, revealing that plate orientation significantly affects growth patterns—an unexpected insight that later inspired direct interactions through tilting in Part II.

Finally, while this initial study frames the bacteria primarily as a living medium for interaction design—an instrumental focus—it also laid the empirical and conceptual groundwork for a later broadening of perspective toward explorations of human–Flavobacteria engagement, emphasising relational dynamics, as elaborated in the second part of this dissertation.

This chapter was previously published as a conference paper:
Groutars, E. G.*, Risseeuw, C. C.*, Ingham, C., Hamidjaja, R., Elkhuizen, W. S., Pont, S. C., & Karana, E. (2022). Flavorium: An Exploration of Flavobacteria's Living Aesthetics for Living Color Interfaces. In Proceedings of the ACM Conference on Human Factors in Computing Systems. https://doi.org/10.1145/3491102.3517713
* shared first authorship

This work was presented at the CHI conference 2022 in New Orleans. The supplementary material for this chapter is available online at: https://dl.acm.org/doi/10.1145/3491102.3517713

Abstract

Flavobacteria, which can be found in marine environments, are able to grow in highly organised colonies producing vivid iridescent colourations. While much is known about the biology of these organisms, their design potential as responsive media in user interfaces has not been explored. Our paper aims at bridging this gap by providing insights into the type, degree, and duration of change in Flavobacteria's expression, i.e., their living aesthetics. We present a tool to capture and characterise these changes concerning form, texture and iridescent colour. To support the long-term study of their living aesthetics, we designed Flavorium. This bio-digital artefact provides the necessary habitat conditions for Flavobacteria to thrive for a month. Granting insights into the responsive behaviour of this organism, this work presents a design space, vocabulary, and application concepts to inspire HCI and design scholars to investigate the complex temporal qualities of living media for future user interfaces.

2.1 Introduction

The integration of living organisms into interactive systems is a growing area of interest for HCI and design researchers [32, 35, 37, 44, 45, 48–52]. Organisms have, for example, been embedded in interactive installations [53–55], hybrid computer games [56–58], wearables [59, 60] and interface designs [61–66], in which novel functionalities and interaction possibilities are achieved through substitution of computer input and output with living media. Within this body of work, some have proposed conceptual frameworks informing the HCI community on the challenges and opportunities that arise when working with living organisms [37, 45].

In parallel, a body of research in the HCI community brings to attention the social dimension of living media [62, 67–69]. For example, researchers discussed the roles living organisms could play in new ways of living and working at home [49] while raising critical questions about temporality, care, symbiosis, and cohabitation [42, 61, 70]. In line with this body of work, foregrounding livingness as a biological, ecological, and experiential phenomenon, Karana et al. [42] proposed the concept of living aesthetics, i.e., the way humans experience the type, degree, and duration of change in a living artefact that occurs due to the growth, reproduction and death of an organism. In particular, we are inspired by the unique living aesthetics of Flavobacteria, which infers them as a potential medium for future Living Colour Interfaces (LCIs).

The ability of living organisms to dynamically change the colour of an artefact has been harnessed in diverse ways in HCI and design, for example, through pigment-producing bacteria [71, 72], fluorescent bacteria [59, 63] or bioluminescent algae [61, 70]. Flavobacteria can add to this repertoire by changing the colour of an artefact through their structural colour. These bacteria, which can be found in marine environments, are able to grow as part of a densely organised colony. Through this multicellular organisation, they form photonic crystals which reflect light in specific ways, creating striking visual effects [25]. Whilst the term 'photonic crystal' may be unfamiliar to many, the effects of this form of structural colour are not. The underlying optical mechanism is similar to that of the peacock's bright feathers, which produce colour through naturally formed nanostructures rather than pigments. Microbiologists have researched Flavobacteria's ability to self-organise in relation to various abiotic factors (i.e., non-living chemical and physical parts of the environment that affect living organisms) [26], the presence of other microbes [23] and the underlying genetic pathways [25]. However, despite their vivid colourations and highly responsive behaviour, Flavobacteria are yet to be explored as a living medium for human-computer interaction design.

This research contributes to the ongoing work in Biological HCI [37, 44, 45, 49, 51], expanding the scope and design opportunities with the introduction of Flavobacteria as a medium for Living Colour Interfaces (Fig. 2.1). Specifically,

our goal is to study Flavobacteria's aesthetics over time, and how these can be tuned with different input mechanisms. Capturing and characterising Flavobacteria's living aesthetics is intriguing but not straightforward due to their angle-dependent and temporal colour that varies across the growing colony. This dynamic complexity requires a unique research approach that combines knowledge, tools and techniques from microbiology, vision and imaging sciences, and design. Informed by the iterations between these knowledge domains, combining systematic lab experiments and design explorations, this work presents the following:

- Design space introducing a variety of input mechanisms that influence Flavobacteria's living colour output (i.e., their living aesthetics),
- Vocabulary to analyse and communicate Flavobacteria's living aesthetics as well as a tool to capture and characterise these,
- First insights on how living aesthetics of Flavobacteria are tuned with a specific input mechanism (i.e., humidity), presented with rich data illustrating changes in form, texture and iridescent colour over seven days,
- Flavorium, a bio-digital artefact, which provides the necessary habitat for Flavobacteria to thrive for a longer time (at least a month), opening up new possibilities for long-term studying of Flavobacteria's behaviour for Living Colour Interfaces (LCIs),
- Application concepts proposing diverse potentials of Flavo-bacteria-based LCIs for HCI.

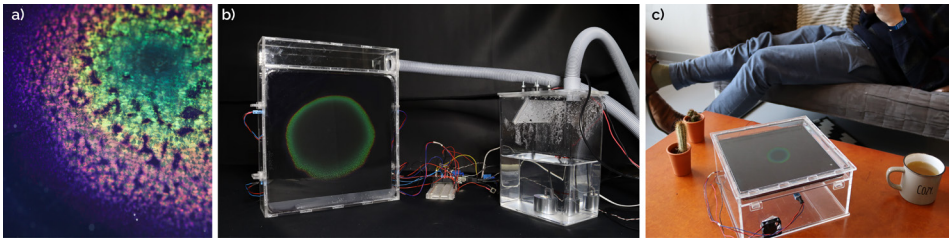


Fig. 2.1 *Flavobacteria as a medium for Living Colour Interfaces (LCIs): a) vivid colourations produced by Flavobacteria; b) Flavorium, a bio-digital artefact for Flavobacteria to thrive for a month; c) a LCI with Flavobacteria embodying users' physical activity measured by a smartwatch.*

2.2 Related Work

2.2.1 Colour-changing interfaces in HCI

Over the last few decades, we have seen growing interest among HCI and design researchers in the application of physical materials as an alternative to digital screens for interacting with computational systems [73–75]. Such physical user interfaces can potentially provide for rich multisensory experiences in everyday interactions that go beyond the capabilities of traditional graphical user interfaces. Within this field of physical user interfaces [76–79], materials

with the ability to change their colour are especially of interest for the scope of this paper. In HCI, researchers proposed thermochromic materials that change their colour in relation to temperature as a basis for interactive fabrics [80] and make-up [81], enabling wearers to seamlessly alter their appearance in more abstract, ambient and ambiguous manners than screen-based displays [82]. Also explored for novel user interfaces are electro-luminescent materials that change their colour in relation to an electrical current [83, 84] and photochromic materials, which allow for the colouring and recolouring of objects using certain wavelengths of light [85] and the intentional degradation of stains on textiles [86]. Nilsson et al. [87] experimented with the patterns of colour change in the textile upholstery on an interactive piece of furniture. Given the dynamic nature of such colour-changing interfaces, Tsuji and Wakita [88] have shown interest in understanding the aesthetics of their temporal appearance, for example, for the colours inside Japanese calligraphy. We aim to develop such an understanding for a colour-changing interface using the emergent properties of living bacteria.

2.2.2 Biological HCI

Recent years have seen multiple HCI projects that focus on integrating living organisms as design elements, bringing forth novel interaction possibilities between humans, computers and biological systems [37, 44, 45, 51, 52]. In these projects, bacteria [53, 63], fungi [62, 65], algae [61, 70] and even quasi-living viruses [89] have been proposed as design elements. Hamidi and Baljko [65] developed a fungus-based interface, where data about the usage of a digital app is visualised through the growth of the fruiting bodies of fungi. In a similar manner, bioluminescent bacteria have been used to visualise social network activity [63]. These examples highlight how living organisms can act as the actuator in interactive systems, displaying information in an ambient manner. Living organisms can also fulfil the role of a sensor, as proposed in the *Living Tattoo* project, where Liu et al. [59] developed a wearable containing transgenic bacteria, able to respond to chemical stimuli by producing fluorescent proteins.

In consonance with such developments, researchers have proposed frameworks and practical guidelines for the integration of living organisms in HCI [37, 42, 44, 45, 52]. This is an effort to consolidate the different fields of expertise involved and to highlight various opportunities, challenges and ethical dilemmas that come into play. Interviewing bio artists, community lab organisers, and DIYbio researchers, Asgarali-Hoffman and Hamidi [90] grant insights on such opportunities and challenges at the intersection of bio-art and HCI. Kuznetsov et al. [91] discuss how a design studio can be transformed into a biosafety level 1 laboratory, engaging HCI researchers in exploring biomaterials. Inspired by Tangible Bits [74] and challenging traditional boundaries between biological cells and computers, Pataranutaporn et al. [37] proposed the concept of Living Bits, a framework to support characterisation of human-microbe interactions across contexts and scales. Merritt et al. [45] proposed Living Media Interfaces (LMIs) as interfaces that "incorporate living organisms and biological materials, taking advantage of their qualities to enable different forms of interaction between

humans and digital systems". Grounded in an overview of the current design space, their research brings to the attention the Biological, Ethical, Artistic, and HCI perspectives to be considered in the design of LMIs.

Our work contributes to this line of work in HCI by proposing Living Colour Interfaces (LCIs) as a form of Living Media Interfaces [45], emphasising the ability of organisms to produce colour which changes over time and in response to the environment and user. Bringing attention to changes that occur in living artefacts during their use time (e.g., colour changes) due to the growth, reproduction and death of a living organism, Karana et al. [42] proposed *living aesthetics* as one of the fundamental principles of designing for livingness in biodesign.

2.2.3 Living aesthetics

The dynamic and temporal qualities of materials have been reflected in diverse literature crossing HCI and interaction design [83, 92–97]. Parkes and Ishii [78] brought to attention the need for a vocabulary to express behavioural transformability in shape-changing materials. Concepts such as becoming materials [92] and temporal form [97] have been put forward, referring to the temporal capacity of computational materials to assume multiple aesthetic expressions that unfold only over time and in context. In line with this body of work, Döring et al. [73] introduced the concept of Ephemeral User Interfaces, intentionally created to last for a limited time and typically incorporate materials that evoke a rich and multisensory experiences like water [98], ice [99] and soap bubbles [100]. Karana et al. [42] suggest a similar understanding of living materials as dynamic and temporal, open to change at both design and use time. These studies on computational and biological materials alike suggest that in designing with new materials, we need to re-evaluate the ways in which we understand the reaction times and manners in responsive media, as well as the modes of interaction [97, 101].

The notion of living aesthetics provides a theoretical understanding of temporality in living artefacts [42]. It brings the focus to the social dimension of living artefacts, calling for a purposeful design of change from the initial state of a living material to the end of its life, indicating how aspects of livingness (i.e., growth, reproduction and death) come to expression in the artefact, and can, therefore, be experienced. Through diverse practical examples, the researchers illustrate how designers navigate between the different dimensions of living aesthetics (e.g., immediate or gradual changes in colour, form, or function). Synthesising the concepts Ephemeral User Interfaces [73] and Living Media interfaces [45], Barati et al. [61] introduced Living Light Interfaces in exploring the living and short-lived expressions of bioluminescent algae, i.e., their living aesthetics [42]. In this specific example, the researchers illustrated how the flash characteristics of dinoflagellates in a liquid culture change under a range of kinetic stimuli, including orbital rotation, pulsation and vibration. The living light

aesthetics are presented as the intensity variations over time, textural qualities and spatial distribution [61].

Inspired by this body of work in HCI, we aim, in this paper, to provide an initial understanding of the living aesthetics of Flavobacteria, concerning the relation between diverse stimuli (e.g., humidity) and the quality of their living colour. Colour is an important element in biodesign to communicate through living media [32, 48]. For example, Smith et al. [72] embedded pigment-producing bacteria in additively manufactured masks named *Vespers*, to enable a predefined and gradual colour change over time. While the colour change in Vespers is achieved through chemically induced behaviour of genetically engineered bacteria, we can also identify projects in which such a level of control is not favoured. For example, in the creation of a living billboard, advertising the *Contagion* movie in 2011, designers cultivated multiple organisms in a giant Petri dish, providing a dramatic change of colour and texture over time and emphasising the agency of organisms themselves [102].

These works showcase the capacity of biological systems to change the colour of an artefact, whether this is controlled by humans, left to the agency of the organisms, or through a collaborative effort between humans and other living organisms. This foregrounds the need for a more extensive elaboration on how the livingness of living media comes to expression in LCIs, hence emphasising the importance of capturing and communicating their living aesthetics. The notion of living aesthetics provides us with a lens to navigate through our design explorations and experiments towards unveiling the potential of Flavobacteria for the design of novel user interfaces.

2.2.4 Flavobacteria's structural colour

Flavobacteriia, a taxonomic term for a class of bacteria from the phylum Bacteroidetes, includes many strains of bacteria that can grow as dense, highly organised colonies on surfaces, creating optical structures that interact with light. These bacteria originate from diverse environments and vary in their nutritional dependency and colour [24, 25]. In our project, we worked with a wild-type strain of Flavobacteria called *Cellulophaga lytica*. This non-pathogenic bacterium originates from marine environments and is known for its stability and brilliant colourations [24]. Flavobacteria's cells, which are 3-4 microns long, move by gliding through which they organise themselves into 2D photonic crystals (2DPC) resulting in structural colour [21, 22]. A minimum of 5 to 10 layers of cells is required to form a barely visible response [22], meaning approximately 100 to 1000 cells are required for a minimally detectable structural colour. When illuminated, these 2DPCs produce iridescent colours by the interference of scattered light of highly specific wavelengths, at highly specific angles. The periodicity and orientation of the structure of cells are critical in determining the interfering wavelength and thereby the reflected colour. In Figure 2.2, we illustrate the principle behind Flavobacteria's structural colour.

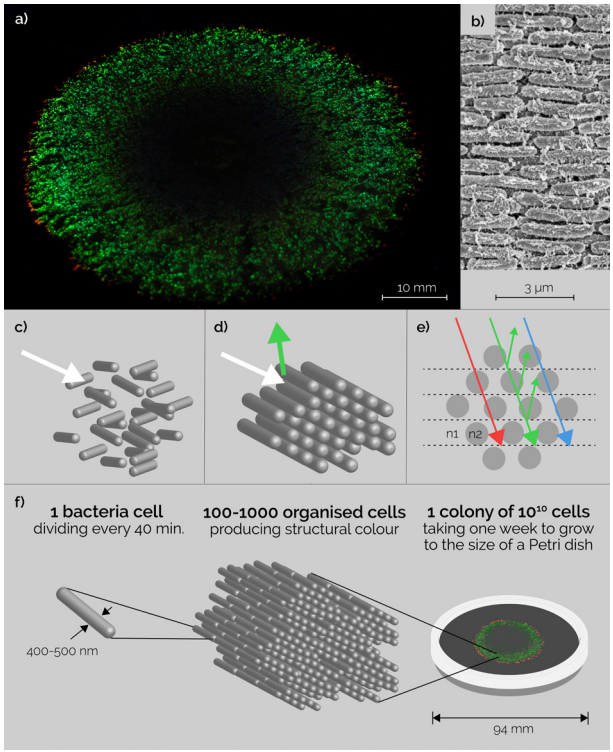
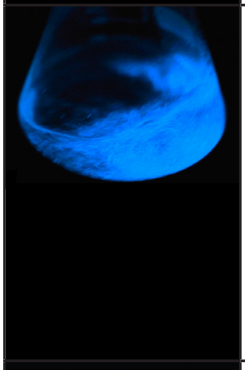
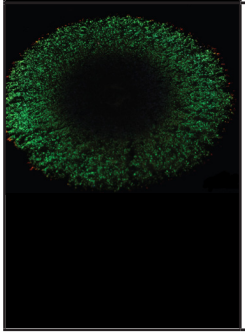


Fig. 2.2 *Flavobacteria's structural colour: a) a colony of Flavobacteria with a faded middle; b) microscopy of cell organisation by courtesy of Hoekmine B.V.; c-d) light striking, respectively, disordered and ordered bacteria; e) light interacting with ordered bacteria cells shown in cross-section with n indicating the different refractive indices of the bacteria cells and the in-between gaps; f) zooming out from one bacterium to sufficient cells to display colour to an entire colony.*

Scientists have explored Flavobacteria's ability to self-organise into 2DPCs [21–27] and how environmental factors such as nutrition, salinity and temperature can affect this. Johansen et al. [25] identified certain genes in *Flavobacterium* IR1 that are responsible for the cell organisation and the resulting structural colour. This cell organisation was later linked to the ability of IR1 to consume other bacteria for nutrients by Hamidjaja et al. [23], granting some preliminary insight as to why Flavobacteria behave in this manner. These studies suggest that Flavobacteria's structural colour is programmable through both genetics and environmental stimuli. Nevertheless, whilst the biological understanding of Flavobacteria is increasing, much remains unknown about their potential as responsive media for Living Colour Interfaces.

In Table 2.1, we compare Flavobacteria's distinct living aesthetics with three other colour-producing living media mobilised in user interfaces mentioned in the previous sections. This comparison was made based on the mechanism by which the organisms produce visible colour and the temporal qualities of the living medium, as seen in the table.

Table 2.1 *Flavobacteria's living aesthetics compared to other colour-producing organisms used in LCIs.*

	Organism	Colour Producing Mechanism	Temporal Qualities
	Pigment-producing bacteria (*Escherichia coli*) [72]	3D printed chemical signalling agents incite genetically engineered bacteria, immobilised in hydrogel, to produce various pigments.	The colour appears gradually over the course of 24 hours. When the bacteria die, the colour remains stable, but for how long is, however, unspecified.
Vespers. Series III. Designed by Neri Oxman and The Mediated Matter Group for The New Ancient Collection curated and 3D printed by Stratasys. Photo: Yoram Reshef			
	Fluorescent bacteria (*Escherichia coli*) [59]	Diffusion of chemical signalling agents triggers genetically engineered bacteria, bio-printed in a hydrogel, to produce green fluorescent proteins.	When activated, colour appears after 2 hours and is at full brightness after 8 hours. The bacteria live for up to 3 days, but how long the colour lasts and if the process is repeatable is unspecified.
	Bioluminescent algae (*Pyrocystis fusiformis*) [61, 70]	Algae grown in a liquid culture produce blue light when stimulated kinetically. This process can be repeated until the algae run out of energy. They then have to recharge through photosynthesis.	The bioluminescent algae have the ability to produce colour instantly when stimulated. Depending on the type of kinetic stimuli, the colour will last between 30 and 300 seconds.
	Iridescent Flavobacteria (*Cellulophaga lytica*) [25]	Grown on a surface and influenced by environmental stimuli, bacteria create photonic structures that refract incoming light, resulting in a wide spectrum of colour visible at specific angles.	Colour emerges 8 hours after the start of growth. The colony increases in size and changes in colour distribution over the course of days. After about 7 days, the bacteria die, and the colour fades.

Flavobacteria are unusual in the way in which they produce colour, which is influenced by environmental stimuli, the amount and the age of the cells. The result is a wide array of colours, visible at specific angles, produced by a single type of organism. In addition, this colour continuously evolves during the lifetime of the organism, the borders of the colony expand, and the colours vary across it. Thus, Flavobacteria's living aesthetics show a direct link to the passage of time, making it possible to distinguish between young, old, and dead sections of the colony. This temporal character is ill-understood for Flavobacteria as a living medium, and therefore, this paper's focal point.

2.2.5 Capturing and characterising living iridescent colour

The term ‘colour’ refers to a perceptual attribute of a material (in this case, Flavobacteria as a living medium), which is the subsequent effect of the human visual system processing a light-material interaction signal. This means that perceived colour cannot be directly linked to physical material parameters or properties of light [103]. More so, understanding iridescent colour perception is challenging, as the hue, saturation and brightness of structurally coloured materials can vary greatly, depending on viewing geometry [104].

The mechanisms responsible for producing structural colour and its function in nature have long been discussed in literature [105–110]. Existing tools and approaches to capture and characterise appearances, including iridescence, revolve around capturing a sample whilst varying illumination and viewing angle [111, 112]. Alternatively, physical parameters of the sample, such as nano-structure, layer thickness and refractive index, can be measured, serving as input to physically based appearance models [110]. The challenge of arriving at a universal approach to capturing and characterising structural colour, often with an iridescent appearance, lies in the complexity and variation of (nano-) structures found in nature. These structures lead to remarkable shifts in appearance, depending on viewing geometry, which are often difficult to model or predict. On top of that, appearances are in many cases also influenced by other light scattering effects, which need to be disentangled to enable robust modelling. Given the perceptual and technical complexity, the challenge of capturing and communicating iridescent colours unambiguously has remained to date, as highlighted by, for instance, Seago et al. [108].

The complexity of capturing and characterising the iridescent colours of Flavobacteria lies not only in the fact that the colour appearance varies as a function of the light or view angle. Due to its living nature, its appearance also varies across the surface as a consequence of being at different growth stages at different sections of a colony, and consequently, it also changes over time. In previous publications [21, 22, 25], photographs of Flavobacteria have been presented, captured from several angles, to illustrate their iridescent colour. These examples, although the photographs illustrate the presence of iridescence, remain insufficient to visualise the striking visual effects of its iridescent qualities, due to a limited set of angle configurations (i.e., changing

either the illumination or the view angle). Johansen et al. [25] also present diagrams relating the view angle to spectral power distribution at a fixed illumination angle. The diagrams, providing a very compact representation of the reflected intensity at various angles, are non-intuitive and thereby difficult to interpret when evaluating living aesthetics. Moreover, neither photographs nor diagrams show the temporal iridescent variations. In short, to our knowledge, no approach exists to capture and characterise iridescent material appearances with temporal and spatial variation at the same time.

2.3 Design Space

2.3.1 Our approach

Throughout this project, we adopted an interdisciplinary approach to biodesign, establishing a close collaborative team that includes experts from microbiology, vision and imaging sciences and design. We followed a material-driven design approach [46, 113], which motivates a back-and-forth thinking between the 'details' of this organism, i.e., the biological behaviour and characteristics, and the 'wholeness' [114], i.e., the way in which the resulting media is appraised within a composition from a design perspective.

After understanding basic protocols to cultivate Flavobacteria, we followed a hands-on tinkering process [46, 115] in which we explored the effects of different environmental factors and various inoculation techniques on the aesthetic expression of the living medium. Our design explorations, in-depth discussions on our findings within our interdisciplinary team, and the background literature helped us to identify the constraints, opportunities, and potentials (in this case, humidity) to be further explored in a systematic study. In parallel, we identified the main qualities that characterise Flavobacteria's living aesthetics.

2.3.2 Cultivating Flavobacteria

In order to form optical structures, Flavobacteria require a suitable habitat that provides the optimal salinity, nutrients, humidity, access to oxygen and a semi-solid, hydrated surface to interact and grow on. In addition, it has to maintain a sterile environment. This is to protect Flavobacteria from contaminants and potentially invasive external microbial competitors, which can compromise optimal flavobacterium growth and colour expression. Maintaining this sterility during the making process and growth phase is a vital aspect of working with Flavobacteria and any other microorganism, requiring the use of specialised lab equipment such as a laminar flow cabinet.

Flavobacteria's habitat is prepared according to the protocol shown in Figure 2.3a. It starts with mixing the medium that contains nutrients as well as agar and a black pigment (see Appendix A for the full medium recipe). The agar medium, once solidified, will form a hydrated and nutritious gel surface on which the

Flavobacteria can organise into their optical structures. The pigment will provide for a black background, contrasting with the structural colourations, so they are clearly visible. After mixing, the medium is sterilised by autoclaving at 121°C and poured under sterile conditions into a Petri dish when it is still hot and liquid. It is then allowed to cool off and solidify. After this, a small number of bacterial cells are applied with a sterile loop to the surface, called the inoculation (Fig. 2.3b). The Petri dish is then closed and sealed off by parafilm, which allows for air-permeability whilst maintaining sterility and humidity inside the Petri dish. The bacteria then grow at room temperature for a week.

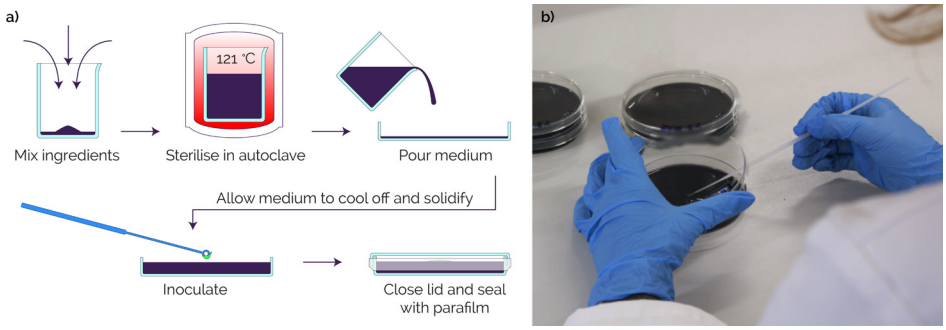


Fig. 2.3 *Cultivating Flavobacteria: a) infographic on the protocol steps; b) inoculating Flavobacteria inside the laminated airflow hood.*

2.3.3 Growth and types of change

When inoculated in the middle of a Petri dish, Flavobacteria will grow outwards at around 5 mm per day and form optical structures as shown in Figure 2.4. The colony will expand until it reaches the outer edges of the Petri dish. At this point, Flavobacteria will deplete the available nutrients and start to die, causing the iridescent colours to fade away. The total expansion and lifespan of the colony are thus limited by both the size of the Petri dish and the available nutrients. Across the colony, depending on the age, amount and arrangement of bacterial cells, the colourations will vary. Typically, the younger sections, located at the edge of the colony, will display red and purple hues. When they mature, the colour will change to green, and ultimately, the iridescent colours will fade away, as can be seen in the oldest, middle part of the colony (Fig. 2.4b).

Building upon these initial findings and the existing literature on Flavobacteria, we identified three types of change in the appearance of Flavobacteria, namely form, texture and iridescent colour (Fig. 2.4c), resulting from their growth and reproduction. While these highly interrelated changes are not instantly evident when viewing the Flavobacteria, they will reveal themselves over the course of days, resulting in their distinct living aesthetics.

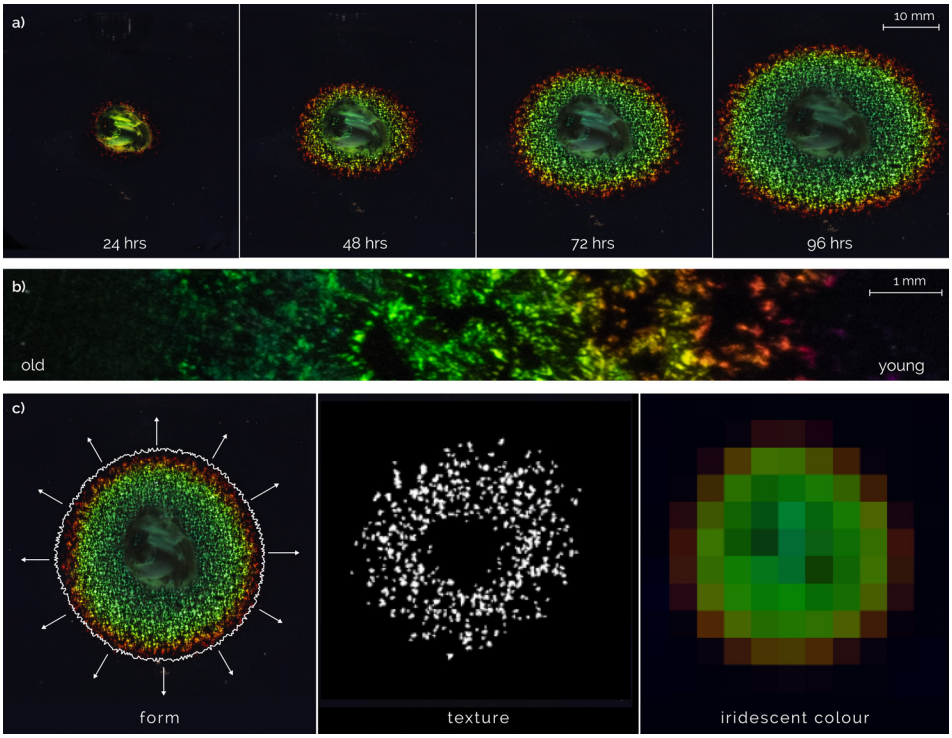


Fig. 2.4 *Growth and types of change: a) growth of Flavobacteria over 96 hours, captured and illuminated from 45°; b) close-up of the colony from its centre to the edge; c) types of change deduced from the growth.*

Form. The first type of change refers to the overall form of the colony and how it expands over time, resulting in a specific *shape* and *size* of the colony. For example, Flavobacteria's expansion rate can be evenly distributed, resulting in a *circular* shape, or the colony can take an *amorphous* shape, due to some sections expanding out faster than others. The shape of the colony can also be experienced as *hollow* or *full*, which is determined by the distribution of the iridescent sections of the colony. The overall perceived texture of the living medium plays a very important role in this.

Texture. The second type of change refers to the texture of the living medium, which is mostly determined by the distribution of iridescent sections on the surface area. Flavobacteria can form a *scattered* and *pointillistic* colour distribution, which is experienced as a *rough* texture, or a *dense* and *uniform* colour distribution, which results in a *smooth* texture.

Iridescent Colour. The third type of change refers to the iridescent colour of the colony and can be described through the qualities of *hue variation* and *brilliance*, which are dependent on the viewing angle. A colony of Flavobacteria can appear *mono coloured* or *multicoloured*, as well as *bright* or *dull*.

2.3.4 Initial design explorations

Having established a method of cultivating Flavobacteria whilst identifying the types of change in their living aesthetics, we started tinkering with the organism. We observed their temporal behaviour under different circumstances and with variations in the basic protocol. This exploration resulted in diverse expressions of form, texture and iridescent colour of the living medium, which are displayed in Figure 2.5.

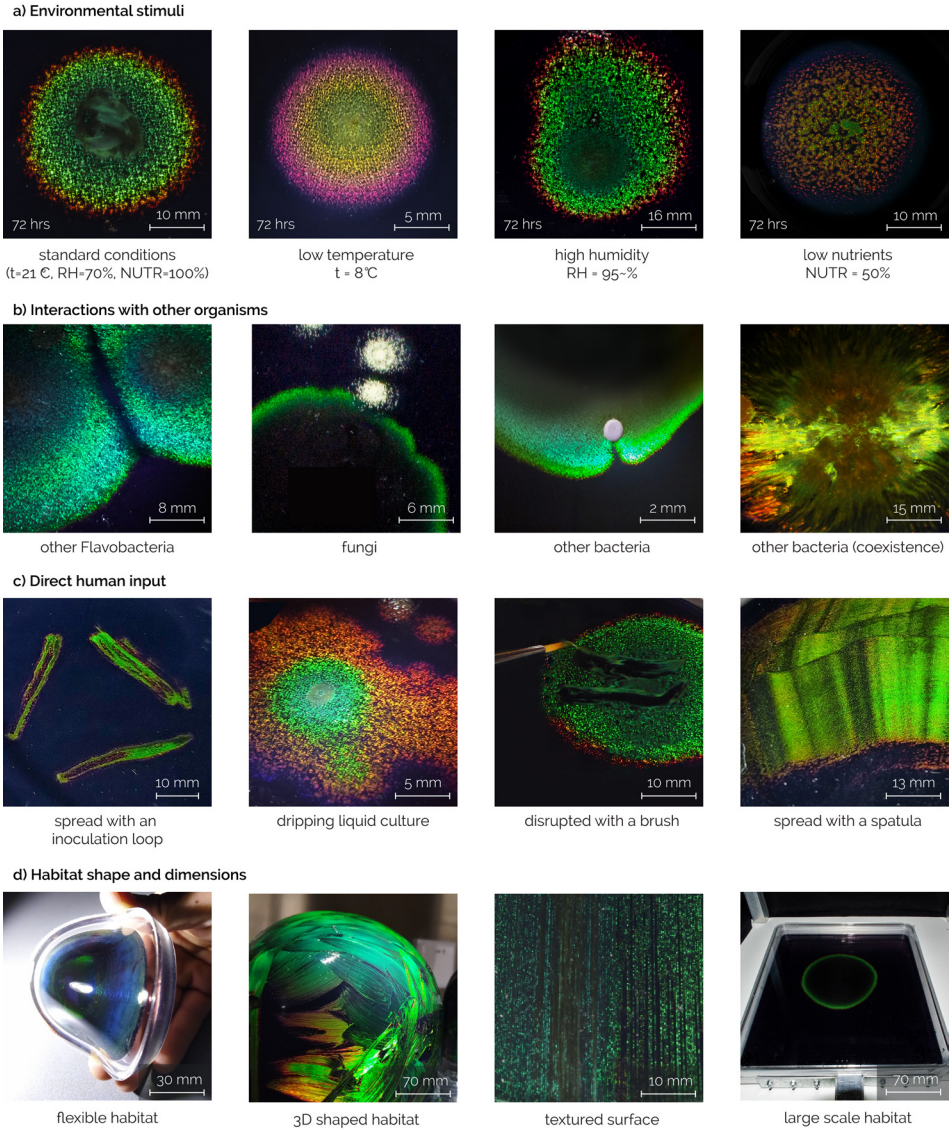


Fig. 2.5 *Initial design explorations with Flavobacteria.*

Environmental stimuli affect the living aesthetics of Flavobacteria (Fig. 2.5a). Here, influencing their growth through introducing a low temperature, high humidity or a deficiency in nutrients, will cause their form, texture and iridescent colour to vary noticeably.

Flavobacteria interact with other microbial species such as bacteria or fungi, resulting in unexpected colourations (Fig. 2.5b). When different species of Flavobacteria encounter, they tend not to combine and instead expand in the opposite direction. The presence of fungi often seemed to inhibit the brilliance of Flavobacteria's colour, whereas the presence of other types of bacteria did not. In another instance, Flavobacteria seemed to coexist with other species of bacteria, resulting in a larger expansion rate and unique texture across the colony.

It is possible to change Flavobacteria's living colour through different inoculation methods or by modifying the colonies during their growth (Fig. 2.5c). Inoculation, by means of a loop or by dripping liquid culture containing bacterial cells, will determine the starting point of the colony's growth. In a more direct manner, the colonies can also be modified during growth, altering their form expansion, texture and colour qualities. In a similar manner, it is possible to tune their living colour by varying the shape, size, flexibility and surface texture of the habitat (Fig. 2.5d). Here, Flavobacteria will arrange themselves onto any given type of surface. For example, they will grow and align themselves along fine textures faster than across flat surfaces, causing alternate forms, textures and colours to appear.

When given a larger-scale habitat, Flavobacteria also have the potential to grow for longer periods of time. Yet, after a period of one or two weeks, such large-scale habitats were found to dehydrate up to a point where Flavobacteria's expansion and colour were very much diminished (see Fig. 2.5d, large-scale habitat). This highlights a wider microbiological issue; methods are limited to the use of the Petri dish, which does not enable long-term or automated cultivation of bacteria. Through these explorations, we established an initial understanding of the organism's behaviour and how it is influenced by diverse input mechanisms.

In the next section, we describe the investigation of one of these input mechanisms, i.e., humidity, for the following reasons: Firstly, when attempting to grow Flavobacteria over a longer period, the role of humidity is deemed crucial in enabling their long-term vitality. However, there is a gap in microbiological literature when it comes to the role of humidity on the organism's vitality. Secondly, humidity influences the way in which Flavobacteria propagate, i.e., their *gliding motility* [23]. As a result, the humidity levels present in their habitat were found to be especially influential in their expansion rate, resulting in the most striking effects on the colony's ultimate form (besides colour and texture). We foresee humidity as a potential input mechanism in LCIs, for example, for more expressively embodying diverse data in bio-digital hybrid systems.

2.4 Study on Flavobacteria's Living Aesthetics

In this section, we present a controlled study in which we investigate Flavobacteria's living aesthetics in relation to humidity as an input mechanism resulting in drastic changes in Flavobacteria's living aesthetics. The temporal and iridescent aspects of this living medium require a setup to enable a relatively fast and automated acquisition in which a sample of Flavobacteria is captured and illuminated from different angles. Hence, we first designed a capture tool.

2.4.1 Development of the capture tool

Similar to existing tools developed to capture material appearances [111], our capture tool should hold a sample while varying illumination and view angle to capture its iridescent colours. Therefore, our capture tool contains a sample holder which can tilt and rotate a Petri dish. The Canon EOS 5DS camera is placed on a tripod at a fixed position. Since we are primarily interested in their appearance to the human eye, it suffices to capture their appearance via such an RGB system.

Light scattering measurements of the *Flavobacterium* IR1 performed by Schertel et al. [22] suggest an intense structural reflectance peak when placing the camera and light at the same spot (i.e., retroreflection). This was also perceived during our initial observations. Therefore, our tool contains an LED ring through which the camera can capture retroreflection in a simple, practical manner. The LED ring is mounted on a rotating arm in order to move around the sample. This allows for varying the illumination angle in relation to the view angle. Alternatively, a ring flash can be used to illuminate the sample, supposing only the retroreflection of the sample must be captured (see Fig. 2.6).

To enable an efficient acquisition of the temporal changes of Flavobacteria, we automated the activation of the light, camera shooting, and the movement of the Petri dish and the LED ring through an Arduino microcontroller. We placed the components of the capture tool in an MDF box and painted the inside of the box black to eliminate light from the environment (Fig. 2.6b,c).

Determining the suitable configurations for this study

While capturing images of Flavobacteria with the capture tool from different angle configurations, it became clear that, from all the possible configurations, placing the camera and light at the same spot resulted in the most vivid structural colour. Hence, for this study, instead of the LED ring on the rotating arm, the ring flash was used. The incident angle α (Fig. 2.7a) was varied in order to take Flavobacteria's angle-dependency into account, resulting in four different angle configurations. We set the minimum and maximum of this incident angle to 30° and 75° to generate relevant data despite the perspective and spectral reflection in the lid of the Petri dish. Accordingly, in our study, we decided to

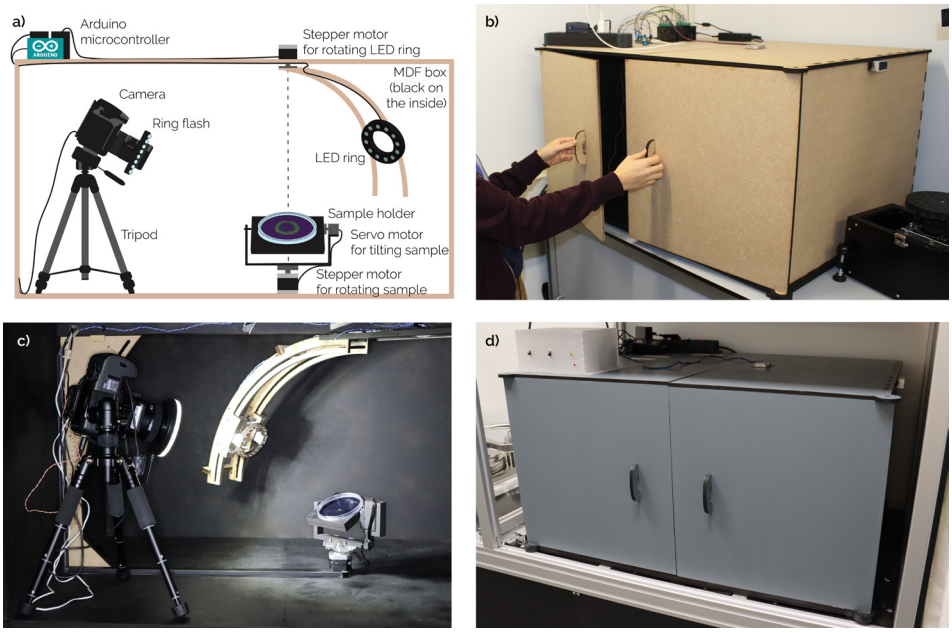


Fig. 2.6 *The capture tool: a) infographic of the capture tool; b-c) the protective MDF box from the outside and inside; d) upgraded exterior configuration adapted to biolab safety requirements*

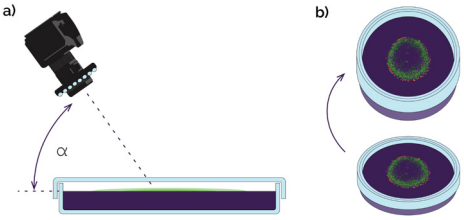


Fig. 2.7 *Data processing: a) capturing a sample with incident angle α (= angle between sample surface and the direction of camera and ring flash); b) the perspective transformation of captured data.*

place the camera and light at the same spot while capturing from incident angles of 30°, 45°, 60° and 75°.

Data processing

To easily compare captured data, images were transformed to correct the perspective, corresponding to the incident angle (Fig. 2.7b). Additionally, a Python script was created to digitally analyse the data and perform objective measurements. Based on brightness-thresholding, quantitative values were automatically extracted for the surface area of the colony, as well as for the ratio of structurally coloured areas in relation to the entire colony. These values support the analysis of the overall form of the colonies.

2.4.2 Living aesthetics of Flavobacteria in response to humidity

Procedure

During the study, Flavobacteria were grown at three different levels of humidity: low, medium and high. This was done by keeping the initial humidity present in the Petri dishes constant by fixing the amount of growth medium, pouring temperature and evaporation time. Regulation of humidity was then established by placing the Petri dishes, loosely closed, inside an incubator with varying relative humidity levels as low (35%), medium (65%) and high (95%). The samples were captured with the capture tool from the four angle configurations on the 3rd, 5th and 7th day. Based on the data, including about 360 images, the relation was explored between humidity and living aesthetics, characterised by form, texture and iridescent colour. For every humidity level, one representative sample was identified that showed the most common living aesthetics within that group. These samples are used throughout the next section to visualise the results.

The relation between humidity and the colony's **form** was explored based on the data captured from 60°. This angle maximises the intensity of structural colour but minimises loss of information due to perspective. To study the size of the colonies, a value was extracted through the digital analysis for the surface area. This value was translated into the average expansion rate for all three different levels of humidity. The shape of the colonies was analysed by comparing perspective-corrected images. Additionally, a value was extracted through digital analysis for the ratio between structural coloured areas and the entire colony in order to determine the fullness of the shape. To explore the relation between humidity and the **texture**, we again used the data captured from 60°. Binary images were created through brightness-thresholding for the three representative samples. Here, coloured sections of the colony are represented by white pixels, clearly visualising the distribution of iridescent sections. In order to explore the relation between humidity and the **iridescent colour**, the representative samples were cropped and ordered in an overview by incident angle, humidity level and day. From here, the change over time in hue variations and brilliance of the colonies could be compared between different incident angles and humidity levels.

Results

A higher humidity results in an increase in the colonies' size, i.e., a higher expansion rate. Because the samples in the most humid environment reached the edge of the Petri dish by day 5, the expansion rate was extracted from the data until day 5. Figure 2.8 shows these expansion rates for the different levels of humidity together with the representative samples and their outline on day 3. As can be seen in the overview, firstly, the colonies in a humid environment form a more amorphous shape, whereas the colonies in low humidity tend to form a refined circular shape. Secondly, the low-humidity samples appear more hollow

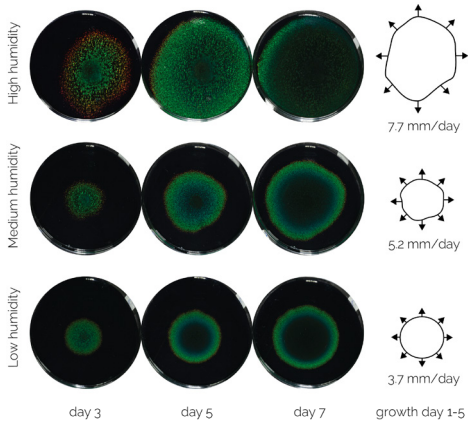


Fig. 2.8 *Representative samples and their outline illustrating the form-humidity relation.*

on day 5 than the high-humidity samples. This was validated by the automatically extracted ratios between the structural coloured area and the entire colonies (see the digital analysis results in the Supplementary Material, Section S.2). Due to this relatively low ratio and the dense distribution of the coloured sections (see also Fig. 2.9), the low-humidity sample forms a refined coloured ring with a thin wall-thickness.

The structural colour formed in high humidity appears scattered and pointillistic, which gives a rough expression in terms of texture (Fig. 2.9). Correspondingly, low humidity results in a dense and uniform distribution of colour, which gives a smooth texture.

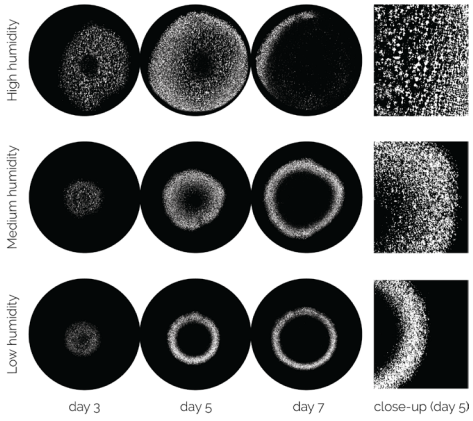


Fig. 2.9 *Binary images of representative samples illustrating the texture-humidity relation.*

The iridescent colour of the representative samples can be seen in Figure 2.10. For all the different levels of humidity, Flavobacteria appear the most brilliant and multicoloured from a viewing angle between 45° and 60°. The variations in hue seem to decrease over time (e.g., high humidity, day 3 sample vs. high humidity,

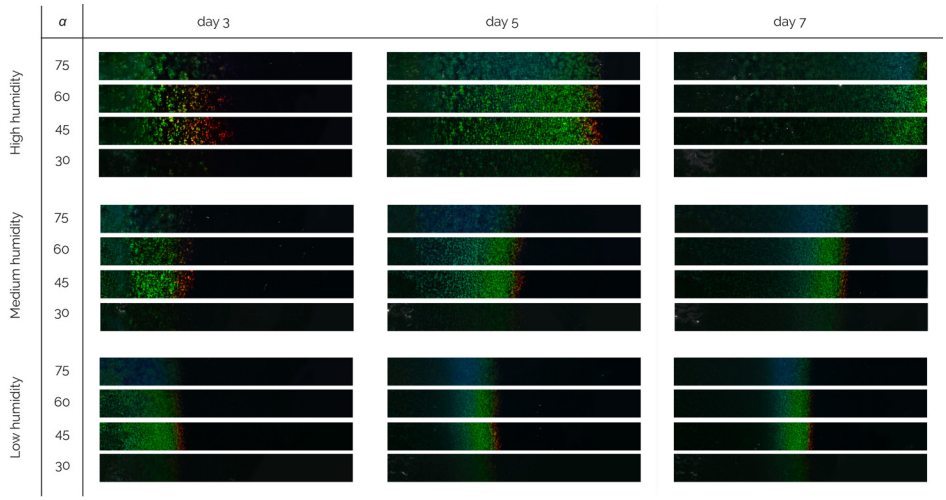


Fig. 2.10 *The representative samples, cropped from the middle of the colony to the edge of the Petri dish, illustrating the iridescent colour-humidity relations.*

day 5 sample) as well as in conjunction with the humidity (e.g., high humidity, day 3 sample vs. low humidity, day 3 sample). The brilliance of the iridescent colour appears slightly more intense at a higher humidity, but does not seem to be affected by time (e.g., low humidity sample on day 3 vs. low humidity sample on day 7).

Key takeaways

Flavobacteria's living aesthetics are affected by the humidity in multiple ways: a higher humidity results in a higher expansion rate, more amorphous shapes and more scattered and pointillistic coloured sections of a colony. The iridescent colour of the colonies appears slightly more brilliant and multi-coloured at high humidity. This study grants initial insights into how Flavobacteria's living aesthetics develop over time. However, samples grown at a high humidity reached the limits of their habitat in a matter of 5 days, making it impossible to study this behaviour over longer periods of time. In addition, capturing was done at a low frequency (i.e., every 2 days), leaving gaps of information on how Flavobacteria's living aesthetics were developing in between. To overcome these drawbacks in exploring the potential of Flavobacteria for LCIs, we developed Flavorium, which we present in the next section.

2.5 Flavorium

Flavorium is a large-scale habitat that allows for the long-term growth of Flavobacteria and automatic capturing of their growth. It grants control over the humidity present inside the habitat since dehydration was found to be the main inhibitor during the initial exploration towards up-scaling (Section 2.3.3).

Flavorium also optimises other conditions such as nutrients, sterility and access to oxygen, enabling us to study Flavobacteria's living aesthetics across new spatial and temporal scales.

2.5.1 Design and prototyping

The development of Flavorium required multiple iterations. Here, a challenge was maintaining sterility during assembly and growth whilst keeping the habitat air-permeable to supply Flavobacteria with oxygen. This permeability was also needed to allow humidified air to both enter and escape the habitat, allowing control over the humidity levels, which contrasts with the need to keep the habitat sterile.

In the final version (Fig. 2.11), this was resolved by introducing a separation between the sterile inner area and non-sterile outer area in Flavorium. The inner area is connected to the outer area using barriers that allow air to pass through whilst keeping contaminants out. The outer area was therefore not required to

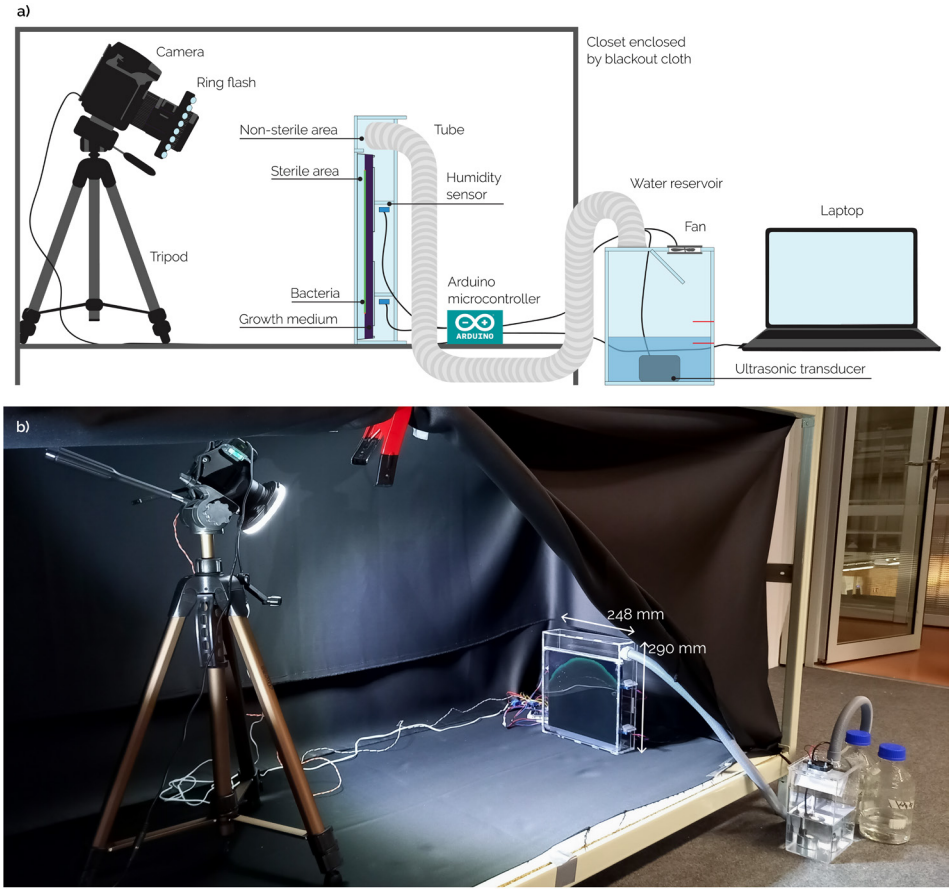


Fig. 2.11 *The Flavorium habitat: a) infographic of Flavorium; b) image of actual setup.*

be sterile and consequently used to regulate the humidity. This was done with a digitally controlled ultrasonic transducer producing mist and a fan that effectively blows the mist into the outer habitat. The resulting humidity levels are monitored by four sensors, which transfer this information to the Arduino microcontroller that, via a closed-loop system, orders the ultrasonic transducer and fan to turn on or off. The measured humidity values are also documented by a laptop. The Arduino also triggers the Canon EOS 250D camera, equipped with a ring flash and set at a 45° angle from the colony, to take a picture at a given moment. To easily divide mist inside the habitat, we adopted a vertical orientation of the colony.

2.5.2 Initial observations

As an initial test, we ran the Flavorium for a full month at a high relative humidity. This builds on the controlled study (Section 2.4.2) where the samples grown at a high humidity were restricted by the size of their Petri dish after five days. To get a better understanding of how this behaviour in humid conditions develops, the Flavorium was thus set at a relative humidity of 90%. This resulted in a large colony of Flavobacteria that was captured at hourly intervals, providing unique footage of how the striking living colour of Flavobacteria developed over longer periods of time (Fig. 2.12, see also the video footage in the Supplementary Material, Section S.3).

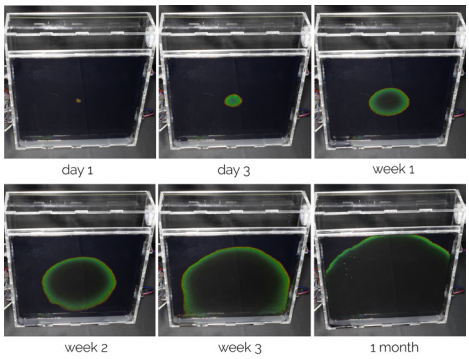


Fig. 2.12 *Growth of Flavobacteria up to one month.*

As the form of the colony developed over time, spatial variations in expansion rate were observed, with the bottom part expanding faster than the top. Also, the expansion rate appeared to accelerate and decelerate at different points in time, an observation that is made possible by the frequent capturing rate. As the weeks progressed, an increasingly larger part of the colony grew old, losing its iridescent properties and making the colony appear more hollow. Despite this, the younger, outer sections of the colony did retain their iridescent properties, showing a smooth texture at the top of the colony and a pointillistic texture at the bottom sections (Fig. 2.13a and b, respectively). This is an unexpected result which we could only obtain thanks to the scale of Flavorium.

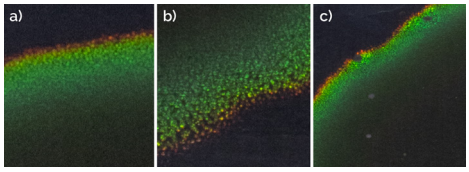


Fig. 2.13 *Initial observations with Flavorium: a) top part of the colony at 2 weeks; b) bottom part of the colony at 2 weeks; c) interaction with other bacteria in the 4th week.*

Around day 23, an interesting interaction between the Flavobacteria and an invading species of bacteria was observed (Fig. 2.13c). Due to the frequent capturing method, it was possible to observe Flavobacteria performing encircling motions around other bacteria as described by Hamidjaja et al. [23].

Our initial observations show that the enlarged spatial and temporal scale of Flavorium, as well as the frequent capturing, allowed for unveiling novel potentials of Flavobacteria and their long-term responsive behaviour. In the next section, we present possible applications of Flavorium and envision the use of other Flavobacteria-based LCIs in everyday artefacts.

2.6 Applications

We present three application directions for Flavobacteria-based LCIs. The first direction builds upon our bio-digital artefact Flavorium. The other two are informed by our controlled study and design explorations (Sections 2.3 and 2.4). In the ideation process, we were inspired by the existing body of work in biological HCI, in particular, two application domains proposed by Pataranutaporn et al. [37], namely, *embody* and *communicate*, and the notion of *material traces* in HCI to manifest time, skill, and use [95, 116].

2.6.1 Flavobacteria-based LCIs to embody digital data

The living aesthetics of Flavobacteria can embody digital data in an expressive yet ambient manner. Figure 2.14 presents a design concept, *living monitor*, that reflects users' habitual practices through its living aesthetics. The number of steps per day measured by a smartwatch is translated to the humidity level inside Flavorium. This causes the colony's form, texture, and colour to change dynamically, reflecting a person's lifestyle. Depending on the amount of physical activity measured by the smartwatch, the living monitor will either show a rapidly expanding, brightly coloured colony or one that is slowly growing and dull. The living aesthetics appeal to *a sense of shared vitality* between the user and the living medium [45], potentially motivating people to change their practices towards more sustainable and healthier directions through relatedness and empathy with living media. Similarly, such LCIs can visualise energy usage at home, data about social interactions or ecology. This could also entail more long-term applications, for example, visualising energy usage throughout the year, given a large-scale habitat that supports continuous growth.

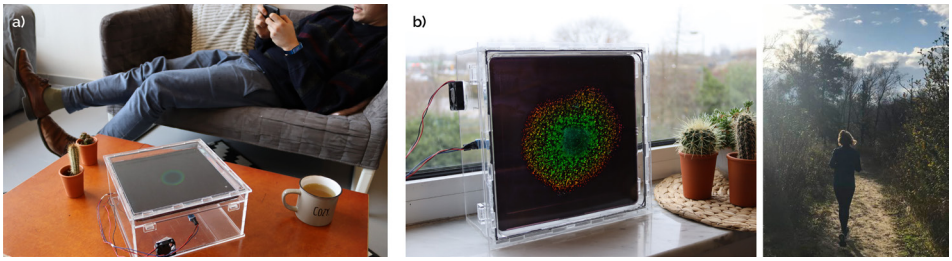


Fig. 2.14 *A living monitor embodying the amount of physical activity measured by a smartwatch of a person with an (a) inactive and (b) active lifestyle.*

2.6.2 Flavobacteria-based LCIs to communicate signals from the environment

Flavobacteria have proven sensitive to changes in environmental factors like humidity (Section 2.4) but also changes in temperature and the presence of other microbes (see Fig. 2.4, Section 2.3). This highlights the potential of these organisms to communicate such changes to us by altering their form, texture and colour, providing a living alternative for digital sensors. Such living sensors will have a relatively slow response but offer new interaction possibilities and expressions compared to digital ones. We present such a possibility with the concept of a *living label*. Given Flavobacteria's sensitivity to humidity, a living label can communicate and amplify the well-being or struggle of a houseplant to the caretaker by reacting to the humidity of the soil (Fig. 2.15). Here, Flavobacteria's living aesthetics will synergise with the well-being of the plant, allowing humans to participate in this symbiosis by providing care for both. Living labels can also communicate other factors, such as fluctuations in temperature over time, for example, relevant for the preservation of food. We envision Flavobacteria-based LCIs that can be attached to everyday artefacts, coupling environmental data to the lifetime of an artefact.

Fig. 2.15 *A living label indicating the well-being of a houseplant.*

2.6.3 Flavobacteria-based LCIs to trace skill and use

The peculiar ways in which humans interact with objects and spaces can be captured through Flavobacteria's distinct living aesthetics that enable a patina

of *living traces* to grow on things of daily use [95, 116]. A playful take on this is our concept of *living notes*. Flavobacteria can be used to leave notes that are invisible at the time the note is created but only occur over time, with specific stimuli. Based on the current possibilities of Flavobacteria, we developed a simple interaction scenario where users apply a small amount of bacteria on a substrate, barely visible to the eye (Fig. 2.16). In a matter of hours, the message will appear with the growth and reproduction of bacteria. The borders of the colony will expand in the following days, causing the original message to fade. Back and forth communication can be achieved through disrupting the living colour with a brush. The temporal aspects of these living notes offer possibilities for playful interactions, opening up a new design space where techniques to interact with such media should be further explored. We discuss some of these future research directions, as well as the limitations of our work, in the next section.

Fig. 2.16 *Living notes enabling playful back-and-forth communication.*

2.7 Discussion

This paper provides an understanding of the living aesthetics of Flavobacteria with the aim of introducing them as a novel living medium for future LCIs. We showed that the structural colourations produced by Flavobacteria are subjected to the organism's growth, reproduction and death, resulting in distinct temporal expressions, i.e., their living aesthetics [42]. We introduced form, texture and iridescent colour as the three main changes humans can experience in this living medium. Taking these three elements as a departure point, we discussed how a Flavobacteria-based LCI can be purposefully designed to be experienced as, for example, *circular* or *amorphous*, *hollow* or *full*, *smooth* or *rough*, *mono* or *multi-coloured*, *brilliant* or *dull*. We also showed how the size of the living media changes over time, and how this change can be *slower* or *faster*. Our bio-digital artefact, Flavorium, with its enlarged spatial and temporal scale, allows for the unveiling of Flavobacteria's novel potentials and their long-term responsive behaviour. Building on Flavorium and initial design explorations, we introduced potential application directions for HCI. Here, we discuss the implications and limitations of our work and challenges for HCI designers.

2.7.1 Opportunities for HCI

Flavobacteria-based LCIs can enrich human sensory experience by embodying digital information [74] as well as communicating signals across scales and environments to create observable output [37]. The delay between input and output, speed of growth and eventual disappearance of colour (i.e., the ephemerality of the medium [73]) also point out Flavobacteria's potential for playful interactions in everyday artefacts. By tapping into their potential for tracing skill, use and time in HCI [95, 116], such interactions would extend design possibilities beyond biotic games, where humans interact as active players with biological materials in a game setting [37].

Interfaces that integrate living media appeal to a sense of shared vitality [45], promoting empathy with users [65], as discussed in our living monitor concept. Karana et al. [42] explain this reciprocal and evolving relationship between humans and living artefacts with the notion of *mutualistic care*, where humans act upon a living artefact or are encouraged to perform a specific activity (e.g., workout) for it to thrive. Designers of such systems should further explore these evolving relationships between humans and living artefacts for meaningful applications that are more easily assimilated in everyday life. To that end, our paper provides an initial understanding of the experience that a living medium elicits at a sensorial level to express its well-being. Opportunities for HCI will arise when other aspects of experience at the *interpretive*, *affective* and *performative* levels [117] are explored in future studies. This will support a comprehensive understanding of how humans experience living media, for example, as *peaceful*, *turbulent* [118], *alive* [119, 120], or the extent to which living media elicits the feeling of *empathy* [45, 63].

Flavobacteria's temporal and optical properties differ from other living media used in interaction design, such as pigment-producing bacteria [72], fluorescent bacteria [59] and bioluminescent algae [61, 70]. When inoculated, Flavobacteria show the first signs of structural colouration within hours and grow continuously by expanding the size of their colonies. This growth can be affected in diverse ways, offering a broad design space for HCI researchers as discussed in Section 2.3. We tap into some of these design potentials, but we are aware that we have only scratched the surface of what novel interactions Flavobacteria could offer for HCI. For example, the angle dependence of Flavobacteria's colour offers opportunities for user interfaces where information over time can only be viewed from a certain position. In addition, HCI researchers can explore possibilities for users to activate Flavobacteria's growth or preserve their colour by preventing the optical structures from degrading after the death of the organism. We are aware that these directions are, first and foremost, a novel scientific attempt that requires an interdisciplinary approach. Yet when achieved, they will open up possibilities for novel user interfaces. Another opportunity for HCI designers is the possibility of designing both open and closed living interfaces [70] with Flavobacteria. As in the Living Notes concept, an open system allows for direct interactions with Flavobacteria during growth, before or after the appearance

of the living colour. When disrupted by such interactions, Flavobacteria will reorganise their colonies, resulting in surprising colourations. This aspect is very peculiar to Flavobacteria, which we have not seen explored with other living media in HCI. Thus, although direct interactions with Flavobacteria in an open system contrast with the need to maintain sterility, it results in unexpected emergent behaviour that can be used as a design strategy to express the livingness of a media.

Discovering and mobilising this emergent behaviour of complex biological systems, referred to as life's useful properties by philosopher Marc Bedau [121], is a challenging yet exciting future research direction for HCI. Herein, we foresee possibilities for AI-supported symbiotic systems, where algorithms learn from emergent behaviour of living media while helping the system optimise. Such bio-digital hybrid systems will play a critical role in real-life longitudinal studies, where designers can explore the way the human body and other living and non-living entities condition the livingness of the media, i.e., *habitabilities* [42].

2.7.2 Design challenges

Although our work shows that using Flavobacteria as a medium for future LCIs is possible, several practical limitations still exist for HCI designers. Flavobacteria require a suitable habitat with optimal conditions to thrive and produce structural colour. One of the practical challenges here concerns maintaining these conditions, which is not straightforward for HCI designers with no microbiology background. It requires understanding the bioprocess to sustain certain nutrients and by-products in a habitat. This calls for new interdisciplinary alliances and urges HCI designers to broaden their investigations to systems for long-term use and maintenance of bio-digital hybrids. Designers should consider to what extent such a system needs maintenance by humans or whether it can be a self-sustaining habitat [42] that adapts and evolves with digital and microbial intelligence in synergy.

Flavobacteria-based LCIs should provide a sterile environment that protects Flavobacteria from contaminants, which will affect their growth and structural colourations. Therefore, specialised biolab equipment is necessary for working with Flavobacteria. Using a standard Petri dish inside a laminar flow cabinet is a quick way to start initial design explorations whilst maintaining sterility. However, the use of a standard Petri dish can limit the growth of Flavobacteria to a maximum of 5-7 days and poses challenges in maintaining the stable conditions necessary for controlled studies. To address this and enable further studies into Flavobacteria's living aesthetics, we developed Flavorium. We envision that the design of custom-made habitats as research artefacts, therefore, will be a common first step in any biodesign process, facilitating research into the behaviour of a living medium over a longer period of time. Providing optimal growing conditions whilst maintaining sterility required multiple iterations in developing Flavorium. We hope our design process will guide other HCI designers in the development of such habitats.

2.7.3 Limitations and future work

The visual data from our characterisation study shows distinct changes in how the living medium's form, texture and iridescent colour develop over time for different humidity levels. Yet, the humidity was kept constant for each colony, thus granting no insight into how a single colony of Flavobacteria responds to variations in humidity over time. We aim to explore this in the future with Flavorium. Furthermore, although we explored many input mechanisms in our design explorations (Section 2.3), our controlled study (Section 2.4) focused on a single factor, i.e., humidity. In future studies, we aim to explore relationships between Flavobacteria's living aesthetics and different environmental factors such as temperature, air quality and the presence of other microbes, which will open up new avenues for designing LCIs.

In this paper, we focused on capturing and characterising Flavobacteria's living aesthetics by bridging the fields of biology, vision and imaging sciences, and design. We developed a tool that allows for comparing colonies' living aesthetics by systematically capturing them. Some aspects of our capture tool can be further developed, such as the implementation of a more precise directional light source with an evenly distributed spectrum. Additionally, colour calibration could be considered. While analysing the captured data, we manually defined the brightness threshold through an iterative process by comparing an actual sample with the visual outcomes of the digital analysis. Although our analysis for all samples relied on the same threshold value, in future studies, this value can be defined through digital tools for more accurate results. Our tool can be used in future research for modelling and simulating Flavobacteria's living aesthetics. Such research will support designers in understanding the microorganism's structural colour without necessarily going through all the lab experiments themselves. Scholars in computer graphics have proposed approaches focusing on rendering spatially varying appearance [122, 123] or time-varying appearance [124–127]. Approaches, although limited, also exist for rendering iridescent colours [128]. Nonetheless, researchers point out that fitting such models to measured data of real-world iridescent surfaces remains future work.

Our approach, grounded in material-driven design [46], has been collaborative in nature, letting the organism express itself and interpreting how this expression can be experienced. Here, we favoured the organism's own agency over the ability to control its behaviour and therefore preferred a wild-type organism (i.e., not genetically modified). However, recent developments at the crossovers of synthetic biology and materials science enable the development of Engineered Living Materials [129, 130], which profoundly rely on the genetic modification of organisms in biodesign. Flavobacteria's behaviour can be genetically programmed [25], offering the potential to, for example, change their sensitivity to certain stimuli, the colours they produce, or the way their colonies form. This puts forward the ethical significance and debate surrounding synthetic biology [42, 70, 131, 132] and invites us to think about future HCI studies on potential ethical concerns that such living media might elicit.

2.8 Conclusion

Through their dynamic and vivid iridescent colourations, clearly visible by the naked eye, Flavobacteria show potential as a medium for future user interfaces. In this paper, we explored Flavobacteria's potential by zooming in on their behaviour and the way in which their expression changes over time, i.e., their living aesthetics. Because Flavobacteria's appearance is highly complex, being both angle-dependent and temporal, we first created a tool to capture and characterise their living aesthetics. Secondly, to enable long-term observation, we designed Flavorium, a habitat that allows for large-scale growth of microorganisms and a means of documentation. Flavorium also allows designers to tune growing conditions in a more controlled manner, bringing forth the responsive behaviour of Flavobacteria and their applicability in future Living Colour Interfaces. We present some potential directions with Flavobacteria-based LCIs for embodying digital and environmental data, for playful interactions with living media and material traces.

Chapter 3

Towards a Digital Tool for Understanding Living Aesthetics

This chapter was motivated by the time- and resource-intensive exploration into Flavobacteria's living aesthetics presented in Chapter 2, as well as the observed complexity of their colourations that vary with viewing and illumination angles, environmental conditions, and over time. These challenges highlighted the need for tools that could support biodesigners in exploring and understanding such dynamic microbial expressions.

In response, this chapter presents the development of an interactive digital tool, *FlavoMetrics*, which simulates the growth and colourations of iridescent Flavobacteria. The tool builds on empirical insights from the controlled humidity study in Chapter 2, and detailed data on the temporality and iridescence of these colourations, which I obtained with the capture tool presented in Chapter 2. Leveraging our multidisciplinary collaboration, this data was translated into a computational framework to simulate the living colour in an interactive tool that allows users to creatively inoculate Flavobacteria and tune environmental conditions—while demonstrating how computer graphics can significantly enhance the visual fidelity and resemblance to the real-world organism.

To zoom out from our exploration with Flavobacteria to the general potential of digital tools to support biodesigners in exploring and understanding living aesthetics, we invited diverse biodesigners to engage with FlavoMetrics and reflect on its relevance for their own practices. I also presented FlavoMetrics as a demo at the Designing Interactive Systems conference 2023 and Dutch Design Week 2023, contributing to discussions on the role of digital tools in biodesign. The visual essay following this chapter documents these public and academic showcases of FlavoMetrics.

This chapter was previously published as a conference paper:
Risseeuw, C., Martinez Castro, J. F., Barla, P., & Karana, E. (2023). FlavoMetrics: Towards a Digital Tool to Understand and Tune Living Aesthetics of Flavobacteria. In Proceedings of the ACM Designing Interactive Systems Conference. https://doi.org/10.1145/3563657.3596085

This work was presented at the DIS conference 2023 in Pittsburgh.

Abstract

Integrating microorganisms into artefacts is a growing area of interest for HCI designers. However, the time, resources, and knowledge required to understand complex microbial behaviour limit designers from creatively exploring temporal expressions in living artefacts, i.e., living aesthetics. Bridging biodesign and computer graphics, we developed FlavoMetrics, an interactive digital tool that supports biodesigners in exploring Flavobacteria's living aesthetics. This open-source tool enables designers to virtually inoculate bacteria and manipulate stimuli to tune Flavobacteria's living colour in a digital environment. Six biodesigners evaluated the tool and reflected on its implications for their practices, for example, in (1) understanding spatiotemporal qualities of microorganisms beyond 2D, (2) biodesign education, and (3) the experience prototyping of living artefacts. With FlavoMetrics, we hope to inspire novel HCI tools for accessible and time- and resource-efficient biodesign as well as for better alignment with divergent microbial temporalities in living with living artefacts.

3.1 Introduction

There is a growing interest among design and HCI communities in integrating living organisms into artefacts as interactive design elements [37, 42, 45]. In these living artefacts, microorganisms, such as algae [61, 70], bacteria [53, 133], and fungi [62, 65], offer unique responsive behaviours to achieve novel functions, expressions, and interaction possibilities. Common to all, the unique temporal qualities of living microorganisms are embraced as design potentials [42]. In more recent works in biodesign, scholars have explored the complex behaviour and expression of microorganisms, for example, how Flavobacteria's living colour can be tuned with humidity [133] or how diverse kinetic stimuli affect the living light output of bioluminescent algae [61]. These explorations emphasise the role of understanding living aesthetics (i.e., the way humans experience qualities of livingness in living artefacts [42]), not only as a way to design unique expressions and functions but also to surface livingness for empathy [63] and timely noticing of microorganisms' struggles [134], towards reciprocal relationships with living artefacts [42]. However, understanding the temporal behaviour of living organisms remains a challenge in biodesign [42, 61, 70]. It requires time, resources, and knowledge, as:

- Organisms have different circadian rhythms and growth rates (e.g., the stationery and decline/death phases are different for all organisms [135]). It might take days or weeks to observe changes in microorganisms;
- Unique growing conditions, equipment, and sterility are needed;
- Stabilising conditions and exploring the effect of one stimulus at a time are needed for understanding the input and output relations. This requires multiple experiments that might take weeks (if not months) to complete.

Novel design tools are needed to mitigate these challenges in biodesign in understanding potential expressions of living organisms. To that aim, this pictorial presents an interactive digital tool, FlavoMetrics, to understand and model the temporal qualities of a specific organism, namely Flavobacteria. We explored the applicability and implications of our tool with a user study inviting six biodesigners.

3.2 Related Work

3.2.1 Digital tools to understand and model living media

Over the last decades, many digital tools have been proposed within the HCI community to support the understanding of the temporal behaviour of diverse media (e.g., for shape-changing materials [136], textiles [137], textile weaving [138]). Considering the similarities between such alive-like materials and living media, these tools serve as valuable examples while addressing the call for novel tools in the nascent field of biodesign.

Zhou et al. [139] presented an overview of digital tools used in the design of living artefacts, which aim to support biodesigners in understanding, embodying, or perpetuating the habitats where living artefacts are situated. An example is a solar radiation analysis to find the optimum form of a living artefact, taking into account the environment, desired expressions, and organisms' needs [140]. Other examples include digital tools to predict pattern formation in bacterial colonies [72, 141]. In line with this, simulations are used for bioluminescence in microarchitectures [142] and wearables from bacterial cellulose [143]. Yet, such tools do not allow for creatively exploring the possible temporal expressions of living artefacts.

In biology and bioengineering, we see various computer-aided design (CAD) tools [144, 145] that model microorganisms to accelerate robust engineering of biological systems while reducing experimental testing. Accessible, interactive tools are presented in projects such as *Scott's World* [146] and *Algae Growth Simulation* [147] (Fig. 3.1) in which users can tune organisms' behaviour (e.g., motility) or their environment (e.g., temperature). While these tools show organisms' growth and responsive behaviour in an agile manner, they lack the possibility for design intervention (e.g., how living aesthetics might change with the ways in which we inoculate living media) and visual likeness to the real organism, which is crucial for exploring their complex living aesthetics in biodesign.

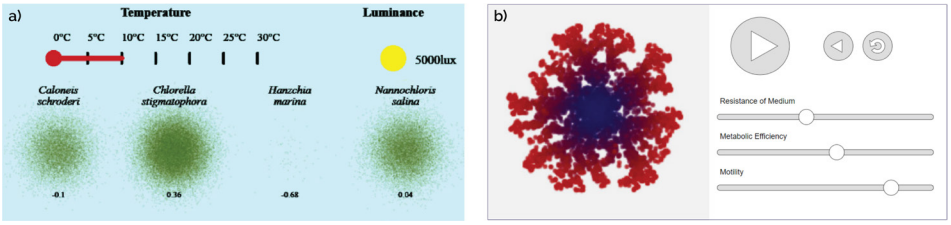


Fig. 3.1 *Interactive online tools simulating the growth of microorganisms: a) Scott's World - Microbial Pattern Formation ©Dirk Brockmann [146]; b) Algae Growth Simulation ©Tyler Sloan [147].*

3.2.2 Conveying living aesthetics through computer graphics

Computer graphics can enhance the likeness to the real-world organism by providing improved visual quality through both real-world data and computer-generated textures. Sage Jenson's [148] simulations of biological complex systems bring about many of the living aesthetics of microorganisms through high-end visualisation and simulations using algorithms based on real-world data. Other 3D artists have also showcased methods to produce realistic and convincing interactive visuals of biological and temporal phenomena using procedural modelling (e.g., for bacterial colonies [149], cobwebs [150], rusting metal [151]). Through combining real-world data of the organism

with procedural computer graphics, a convincing simulation of an organism's temporal and responsive behaviour can be created, showcasing its living aesthetics in digital media.

3.3 FlavoMetrics

In an attempt to bridge the gap in exploring living aesthetics in biodesign in an agile yet realistic manner, we developed a digital tool for a specific organism, namely Flavobacteria. Within our multidisciplinary team, we first simulated their iridescence by capturing and translating visual data. From here, we developed an interactive tool, FlavoMetrics, which aims to support biodesigners in performing quick design explorations with Flavobacteria. In this chapter, we elaborate on the development of the tool. FlavoMetrics' open-source project is available at https://github.com/josemartinez18/FlavoMetrics-Digital-Tool.

3.3.1 Challenges of Flavobacteria

Flavobacteria were recently introduced to HCI designers as a living medium [133] due to the potential they offer through their vivid, iridescent colourations when grown on a solid surface (Fig. 3.2). A colony of Flavobacteria increases in size over time and displays a wide variety of colours, ranging from violet and red to more dominant green hues. This angle-dependent optical effect is a result of their cell organisation into photonic crystals, which interact with light [21, 22, 25]. As cells multiply, glide over the surface, and organise themselves, the colony of Flavobacteria will grow outward and change colour depending on the cells' age, density, and arrangement. Their living aesthetics, concerning changes in form, texture, and iridescent colour, are affected by, for instance, various environmental factors (e.g., humidity) and the inoculation technique (i.e., method of applying bacteria to a surface), as shown by [133]. While this iridescence, temporality, and responsiveness emphasise Flavobacteria's potential as an attractive living medium, the complex combination of spatial and temporal qualities poses a challenge for understanding and predicting Flavobacteria's living aesthetics. Models in computer graphics have shown the possibilities of rendering various iridescent effects [128, 152], yet more complex effects, such as those of Flavobacteria's photonic crystals, require data-driven approaches as described in this work.

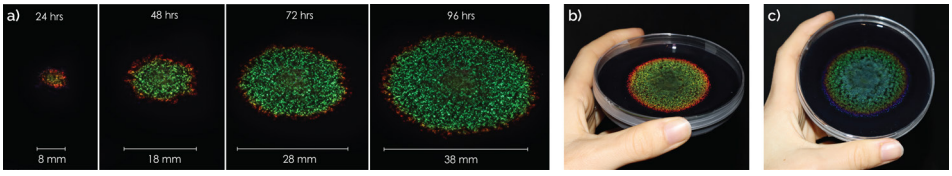


Fig. 3.2 *Colourations of Flavobacteria: a) a colony's growth over four days; b-c) the iridescent colourations of a colony seen from a viewing angle of 45° (b) and 80° (c).*

3.3.2 Collecting data on Flavobacteria's iridescence

In our procedure of data collection (Fig. 3.3), we used a custom-made tool (based on [133]) to automatically capture the full colour range (72 angle configurations) of a 5-day-old Flavobacteria colony. The images were translated through a Python script into linear colour gradients by merging the radial colour gradients across the colony. These colour gradients were then translated into bidirectional reflectance distribution function (BRDF) maps, which define how surfaces reflect light based on incoming lighting and viewing directions and are commonly used in the field of computer graphics. Each BRDF map relates to the colour of cells as a function of distance from the centre (inoculation point) of the colony for a specific light/view configuration. When implemented in material rendering software such as Blender 3D, the colour can be accessed from the BRDF map as a function of the camera view and light directions.

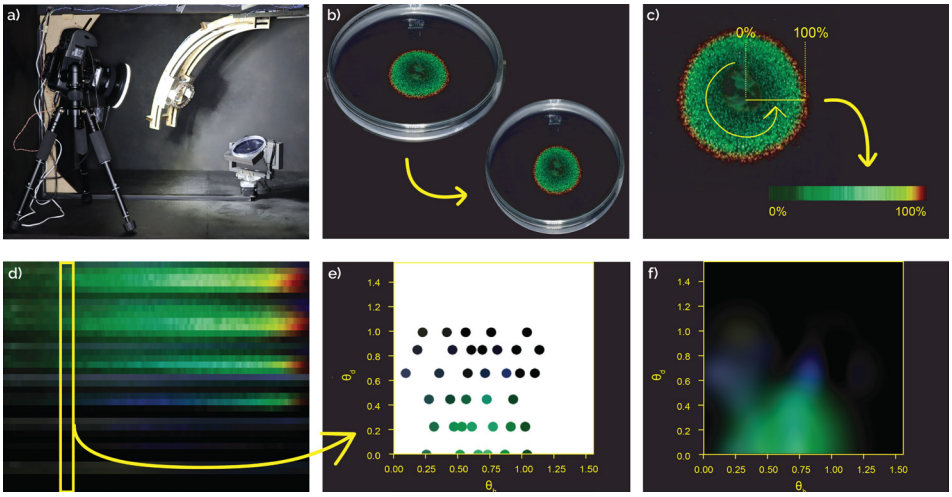


Fig. 3.3 *Procedure of data collection on Flavobacteria's iridescence: a) capture tool to collect images of Flavobacteria [133]; b) perspective transformation of images; c) fusion of radial colour gradients; d) list of representative colour gradients for different angle configurations; e) BRDF map from the colour values of a single location; f) interpolation of data per BRDF map.*

3.3.3 Translating data into a procedural texture

In order to simulate the changes in form and texture of Flavobacteria, we developed a procedural texture of the bacterial colony from the captured images (Fig. 3.4). In computer graphics, a procedural texture is a texture that is created using mathematical descriptions rather than directly stored data. We then embedded the colours from the BRDF map on the procedural texture to create the final simulation of the Flavobacteria colony.

This procedure allows for full control over the appearance of the colony, such as shape, size, and texture. Building upon the insights on how Flavobacteria's living aesthetics can be tuned with humidity [133], we manually mapped these parameters to growth (# of days) and humidity levels. Due to the procedural texture and BRDF maps, the tool can visually estimate Flavobacteria's growth, filling in the gaps of how the living aesthetics were developing in between the capture moments, which was highlighted as one of the limitations in previous work by Groutars and Risseeuw et al. [133].

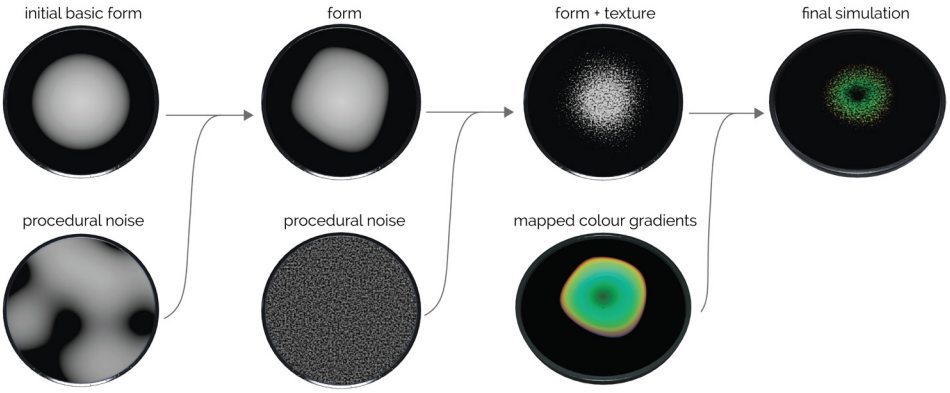


Fig. 3.4 *The multiple layers involved in the development of the procedural texture.*

3.3.4 Interactive tool

In order to engage biodesigners in a conversation about future digital tools for biodesign, we developed an interactive tool from the simulation using the Blender Game Engine (UPBGE). The simulation was enhanced with an interactive inoculation feature allowing the user to freely inoculate the bacteria anywhere on the digital Petri dish, which is then set as the origin point of the bacteria's growth. This implementation helps the user of the tool envision how their interventions affect the growth of Flavobacteria. Finally, a graphical user interface (GUI) was developed for the tool (Fig. 3.5), adding accessible control over the parameters, including time, light position, Petri dish rotation, and environmental conditions, to enable the user to explore the temporality and responsiveness of living media.

3.4 A Study with Biodesigners

We conducted a study with six biodesigners to evaluate FlavoMetrics and reflect on how such interactive digital tools could support the design process with diverse living media.

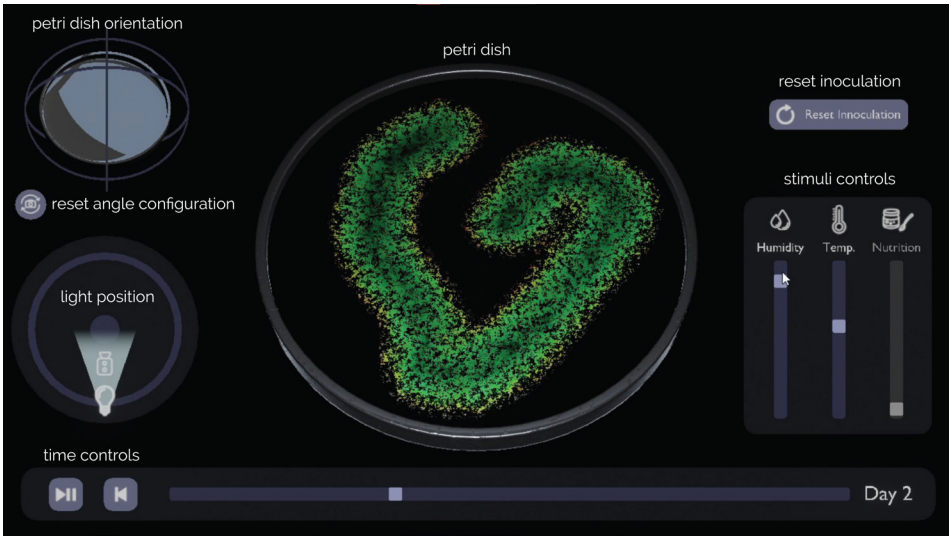


Fig. 3.5 *The interface of our interactive tool.*

3.4.1 Set-up

FlavoMetrics was displayed on an iPad, allowing the participants to interact with a pencil (Fig. 3.6) or directly with touch. To introduce the participants to Flavobacteria and enable them to compare the simulation to the real organism, we placed a sample of Flavobacteria on the same table as well as a desk light resembling the tool's interactive light. Through observing such a "static" sample, participants would become aware that temporal changes in Flavobacteria's expressions are not directly noticeable to the human eye [134]. For our analysis, we made a voice recording as well as a screen recording of the user interface.

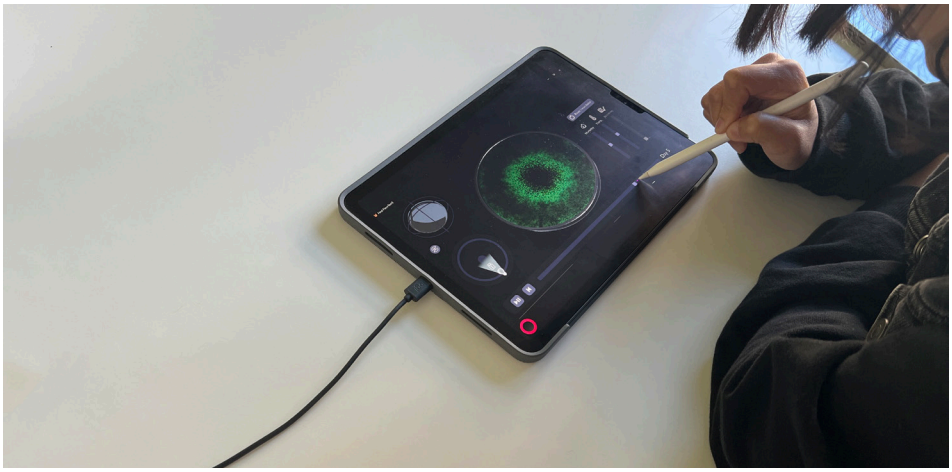


Fig. 3.6 *A participant interacting with FlavoMetrics using a pencil.*

3.4.2 Participants

We invited six biodesigners, including two artists, three design researchers, and a landscape designer. We chose biodesigners from both academia and design practice to uncover more possibilities of digital tools for biodesign, which we expected might differ based on the focus of the biodesign practice. The participants had 3-8 years of experience in biodesign, collaborating with diverse microorganisms, including cyanobacteria, algae, and fungi. One of the participants also had experience designing with Flavobacteria.

3.4.3 Procedure

The study began by introducing participants to Flavobacteria's iridescent and temporal qualities by presenting the sample and simulation. After a demonstration on how the GUI allows one to observe and tune living aesthetics, participants were given time to freely explore the tool while thinking-aloud. Each session took about an hour and was completed with an interview, discussing their experience of using the interactive tool and reflecting on how such a tool could support them in their own biodesign practice.

3.4.4 Results

The answers of the biodesigners showed great variation in the ways in which they imagined using similar digital tools in their own practice (Fig. 3.7). Overall, the participants saw great value in interactive simulations of living organisms, and all agreed to the positive implications of FlavoMetrics, including how it allows you to quickly cycle through design explorations for living aesthetics and its potential to save resources in a biolab.

As expected, the implications highlighted by biodesigners were influenced by the focus of the biodesigners' practice. For example, the landscape designer wanted to simulate living media on a 3D-formed facade, while two design researchers with teaching experience mentioned how students could benefit from the tool. Likewise, another design researcher felt the tool would restrict creativity and freedom in navigating the design space, strongly pointing towards educational purposes.

The type of microorganisms that biodesigners had experience with also influenced their experience with FlavoMetrics. For example, one biodesigner working with cyanobacteria wished to have more control over lighting conditions, as light is crucial for the organism's growth. Another biodesigner working with cyanobacteria had a hard time imagining using the tool in their own project, as this microorganism is not as expressive in colour as Flavobacteria. Nevertheless, such organisms could express their well-being in simulations through other types of change (e.g., form).

The expert on Flavobacteria especially valued the inoculation feature, through which you can quickly iterate between different patterns. Yet, they stated the

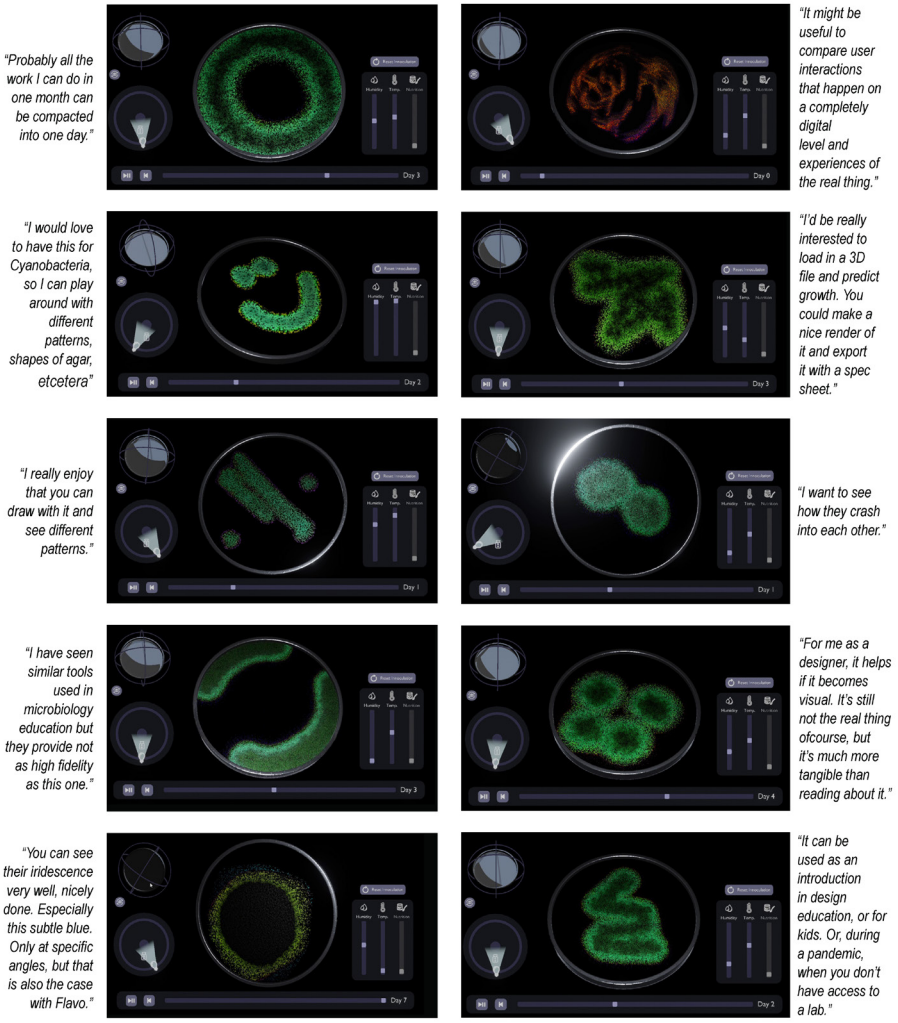


Fig. 3.7 *A collection of participant responses with a quote and corresponding screenshot of FlavoMetrics.*

tool would be more interesting for designers who are not yet familiar with Flavobacteria's living aesthetics, as an expert might already anticipate the majority of the design outcomes.

Finally, two participants stated that one could not really explore unknown relations between input and output with FlavoMetrics unless it could predict the behaviour of microbes.

From our study, we identified directions for future digital tools to support the design of living artefacts.

3.5 Biodesign Implications

Developing FlavoMetrics, we aimed to create a potential tool that could support biodesigners in navigating the design space of living aesthetics (Fig. 3.8a). The implications of FlavoMetrics that resulted from our user study were in line with these expectations. To facilitate the intended use, the tool can be further developed by including more stimuli to support biodesigners in tinkering with Flavobacteria, allowing them to explore living aesthetics in relation to various input mechanisms.

Besides, new implications were discovered that focused on understanding spatiotemporal qualities beyond 2D, biodesign education, and experience prototyping of living artefacts in biodesign (Fig. 3.8b-d). In this section, we elaborate on these implications of FlavoMetrics with diverse scenarios, specifying the required modifications to the tool's working principle and GUI.

3.5.1 Understanding spatiotemporal qualities beyond 2D with FlavoMetrics

The tool can be used to envision how Flavobacteria grow and behave on complex shapes with different scales beyond 2D Petri dishes, enabling designers to get a better understanding of Flavobacteria's spatiotemporal qualities. To that end, the tool could be further developed by allowing the import of 3D models. For example, using FlavoMetrics, one can imagine how Flavobacteria's living colour can be used as a sustainable alternative to coating for automobiles, showcasing the unique iridescence of these microorganisms through a complex 3D form.

3.5.2 Biodesign education with FlavoMetrics

FlavoMetrics can be used as a didactic tool to introduce Flavobacteria to designers. This would make biodesign more accessible, similar to, for example, affordable DIY-bio tools (e.g., [153]) and Hoissain et al.'s [154] *cloud experimentation architecture*. In addition, FlavoMetrics provides a digital environment for more time- and resource-efficient biodesign education. Designers who are novices at working with Flavobacteria can learn the basics of the microorganisms' behaviour in this digital learning environment, as well as use the tool as a generative tool to brainstorm on ideas for novel interaction scenarios. The tool could be further developed by including educational content on Flavobacteria's behaviour and allowing for virtual collaborations.

3.5.3 Experience prototyping with FlavoMetrics

FlavoMetrics can support biodesigners in conducting experiential characterisation studies with living artefacts [47, 155–157]. With such a tool, researchers can communicate how the living artefact changes over time and what it could be like for users to interact with a living artefact through the

Fig. 3.8 *FlavoMetrics supporting tinkering with iridescent Flavobacteria (a), understanding spatiotemporal qualities beyond 2D (b), biodesign education (c), and experience prototyping of living artefacts (d).*

digital representation of the artefact in context. This can mitigate some of the challenges in conducting user studies with living organisms, such as the approval of ethics committees (for biosafety) and communicating the slow changes in the living artefacts due to, for example, environmental stimuli. For this, the tool could be further developed to enable participants to digitally

interact with such artefacts and reflect on its potential implications. Long-term use scenarios in realistic contexts can be integrated into such tools to trigger further discussions on the implications of such artefacts in the everyday. Additionally, AR/VR technologies could be explored to enhance the interactive experience with the digital twin.

3.6 Reflections and Future Work

In this pictorial, we presented FlavoMetrics, a digital tool that enables biodesigners to virtually explore the living aesthetics of Flavobacteria. Through discussions with biodesigners working with diverse living media, we identified how such an interactive tool can assist in navigating the design space of living aesthetics through: understanding spatiotemporal qualities beyond 2D, biodesign education, and experience prototyping of living artefacts. In future work, we would like to delve further into the implications by conducting in-situ user studies with biodesigners to reflect on the long-term use and benefits of our tool.

Although we aim to inspire digital tools to aid biodesigners in exploring living microorganisms' temporality and responsiveness in an agile, accessible, and resource-efficient manner, we do not suggest that they should replace experiments in a tangible lab environment (Fig. 3.9). Instead, we envision a way of blended learning [158], combining digital and physical experiments. Such hands-on practice is essential for biodesigners to get a feel for the organisms' temporality and behaviour and a sense of empathy towards living organisms [63], towards more microbe-sensitive [134] biodesign practices. Additionally, novel expressions might still reveal themselves in tangible experiments due to unforeseen conditions and the microorganisms' agency, which cannot be captured in digital tools.

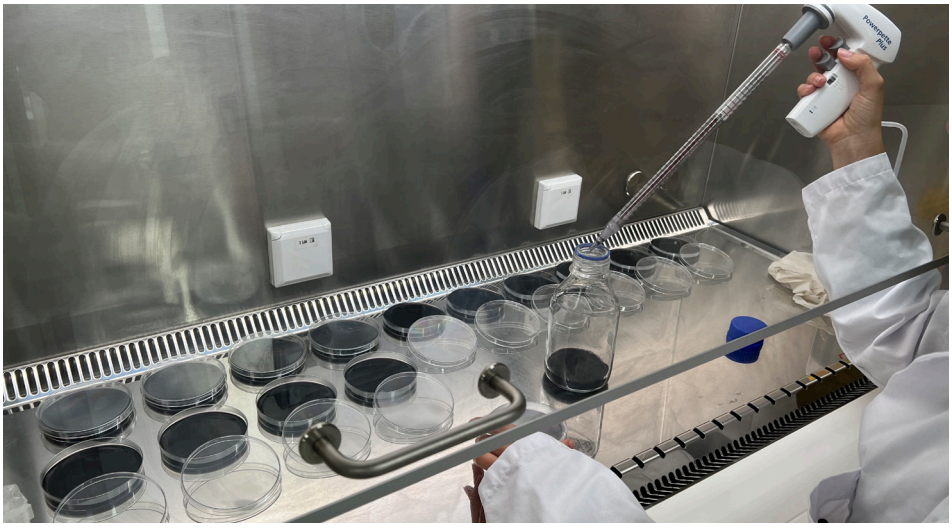


Fig. 3.9 *Hands-on practice in a lab environment.*

We designed FlavoMetrics specifically for Flavobacteria. Nevertheless, we hope that these implications can inspire multidisciplinary collaborations in developing digital tools for various kinds of microorganisms. Here, we envision digital tools being adapted to the unique temporalities and needs of other microorganisms, for example, by allowing interactions with microorganisms in liquid medium or tuning other habitat conditions such as light intensity.

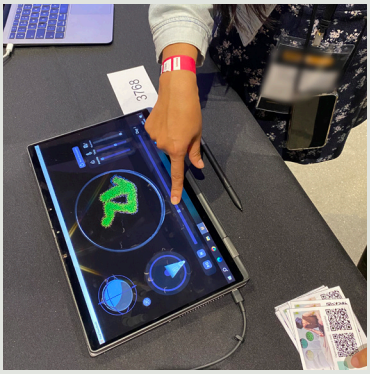
Even though we intended FlavoMetrics to be used in the design time of living artefacts, we envisage the potential use of such tools in living with microbe-embedded living artefacts. By translating complex microbial behaviour and invisible metabolic activities to humans through digital systems (e.g., [159]), we can help attune to microbes' time to establish timely care practices and elicit unique interactions and experiences toward reciprocal and evolving relationships with living artefacts. We aim to elaborate on this potential of FlavoMetrics in our future work.

Visual Essay: Showcases of our Digital Tool FlavoMetrics

IDE masterclass biodesign. March, 2023

Visit from Lululemon. July, 2023

Demo at the DIS conference. July, 2023



Dutch Design Week. October, 2023

Chapter 4

A Deep-Dive into Flavobacteria's Response to Changing Conditions

Building on the design space of living aesthetics and our concept of *Living Colour Interfaces* introduced in Chapter 2, this chapter investigates how Flavobacteria's expressions respond to environmental stimuli that fluctuate over time. Specifically, I examine whether such variations can produce repeatable changes in the bacteria's living aesthetics.

In initial experiments, using the *Flavorium* habitat introduced in Chapter 2, I observed that humidity had a lasting impact on Flavobacteria's growth and colourations: the drying of the growth medium during low-humidity periods led to permanent changes in colony growth speed and vividness. In contrast, a fellow microbiologist working with Flavobacteria suggested that temperature might have a more transient influence— an "on-off" effect, so to speak. Guided by this insight and by my curiosity about Flavobacteria's temporal expressions, I designed a study to explore how temperature shapes Flavobacteria's living colour. The striking results of this investigation prompted two deeper inquiries: a technical analysis in which I used computational methods to assess colour heterogeneity, and an interdisciplinary exploration to uncover the biological basis underlying these dynamic colour shifts. In doing so, this study offers a first empirical account of how Flavobacteria visually respond to fluctuating stimuli, strengthening their potential for future living sensors and ELMs.

Based on this interdisciplinary collaboration, I offer a personal reflection in the intermezzo following this chapter, discussing its challenges, differences between biotechnology and biodesign, and how I navigated the space in between.

This chapter was previously published as a journal article:
Risseeuw, C., Kummetha, L., Ingham, C., Karana, E., Aubin-Tam, M.E., & Martins, J. (2026). Exploring the design potential of iridescent Flavobacteria for thermochromic engineered living materials. Bio-Design and Manufacturing (Springer Nature). https://doi.org/10.1631/bdm.2500187

The supplementary material for this chapter is available online at: https://link.springer.com/article/10.1631/bdm.2500187

Abstract

By leveraging the unique qualities of microorganisms, engineered living materials (ELMs) offer functional and economic advantages in everyday applications along with notable ecological benefits. This study contributes to the growing field of biodesign by examining the potential of Flavobacteria for thermochromic ELMs. Many Flavobacteria, commonly found in marine environments, produce iridescent structural colourations as their colonies expand on semi-solid surfaces through gliding motility. In this study, we analysed the effects of temperature variations on flavobacterium *Cellulophaga lytica* PLY-A2, characterising distinct changes in colony growth and iridescent colourations at a macroscopic and microscopic scale. Using scanning electron microscopy, we investigated the relationship between iridescent colour and the underlying cell-based optical structures. By providing insights into the temperature-responsive behaviour of Flavobacteria, our findings highlight their potential for future thermochromic ELMs—with applications ranging from sustainable food packaging to smart textiles—while encouraging further characterisation studies within biodesign research.

4.1 Introduction

Inspired by the efficiency of natural living systems and driven by recent advances in synthetic biology and materials science, the field of engineered living materials (ELMs) has attracted substantial attention [129, 130, 160–162]. ELMs incorporate living cells as essential components that either construct the material itself or modulate its performance [130]. This integration can result in materials with novel functionalities, such as self-healing and biosensing, supporting a wide range of applications in healthcare, sustainable energy production, and advanced materials development [160].

In recent decades, there has been a growing interest in developing living systems in fields such as design [19, 42, 163], human–computer interaction (HCI) [37, 45, 164], and architecture [142, 165, 166]. The integration of microorganisms as design elements presents opportunities to create products that offer novel interaction possibilities, as well as more sustainable production and end-of-life processes. Contributing to these efforts, this study explores the potential of *Cellulophaga lytica* (*C*. *lytica*) PLY-A2 for sustainable thermochromic living materials. In their natural environments [24], these bacteria grow at temperatures ranging from 0 °C to above 30 °C, making them suitable for monitoring human environments.

In many living colour-changing materials, microorganisms exhibit dynamic changes in colourations in response to environmental stimuli, including temperature. This responsive behaviour may be either inherent to the organisms or the result of genetic engineering [167]. For example, a living sensor for chemical stimuli was developed by genetically modifying bacteria to achieve high levels of control, accuracy, and robustness [59]. Alternatively, biologists, engineers, and designers have leveraged microorganisms' inherent responses to develop living colour-changing materials. For instance, researchers have explored how microalgae respond to mechanical stress to develop mechanoluminescent living composites [168]. These studies highlight the advantages of unmodified biological systems over human-made alternatives in terms of sensitivity, energy efficiency, and autonomy.

When integrating microorganisms into living materials, designers must develop a thorough understanding of the complex and dynamic nature of microbial responses. This poses a challenge, as many microbial species exhibit considerable variability in their responses and are often influenced by multiple stimuli, making input–output relationships difficult to identify [169]. Characterisation studies are therefore essential in biodesign, as they offer insights into how different input mechanisms affect the spatiotemporal expressions of microorganisms. Accordingly, this study provides a detailed characterisation of the growth and colourations of *C*. *lytica* PLY-A2 colonies in response to temperature variations.

C. *lytica* belongs to the *Flavobacteriia* class, of which many strains produce structural colour as bacterial cells organise into photonic crystals during growth

on semi-solid surfaces, including agar plates [20, 28]. Flavobacteria's cell organisation has been associated with their ability to prey on other bacteria, suggesting that their photonic properties may be a secondary effect of this behaviour [23].

The iridescent colourations of Flavobacteria are easily observable and highly sensitive to environmental conditions [26, 133], sparking interest across microbiology, materials science, design, and HCI communities. Recently, Sullivan et al. [29] reported the potential of Flavobacteria's iridescent biofilm as a platform for large-scale, sustainable, structurally coloured materials, offering methods for controlling their optical, spatial, and temporal properties. Researchers have also explored genetic modifications as a way to alter the structural colourations in bacterial colonies [25, 28], underscoring Flavobacteria's potential for optical ELMs, such as living sensors. Moreover, Flavobacteria have been introduced to the design and HCI communities by Groutars et al. [133], who proposed a design space of input mechanisms and potential applications for living colour interfaces. Risseeuw et al. [170] expanded on this work by directly interacting with Flavobacteria through pressing, tilting, and swiping, fostering a personal and dynamic interplay between humans and microbes.

While some publications have addressed the effects of temperature on Flavobacteria, particularly the colourations of *C*. *lytica* (Fig. 4.1), the broader impact of temperature variation remained unknown. To bridge this knowledge gap, our study reports for the first time the effects of temperature variations on *C. lytica* growth and provides a detailed characterisation of the resulting temporal expressions (Fig. 4.2). In doing so, we aim to explore the potential of these microorganisms for thermochromic living materials.

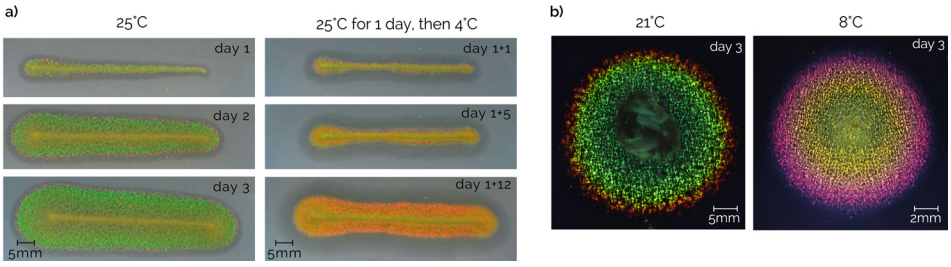


Fig. 4.1 *Effect of temperature on the growth of C. lytica on solid marine medium: a) iridescence kinetics of a colony incubated at 25 °C (left) and a colony transferred to 4 °C after 1 day (right). Reproduced from [26], with permission from the Federation of European Microbiological Societies; b) structurally coloured colonies grown at 21 °C (left) and 8 °C (right). Reproduced from [133], with permission from the authors.*

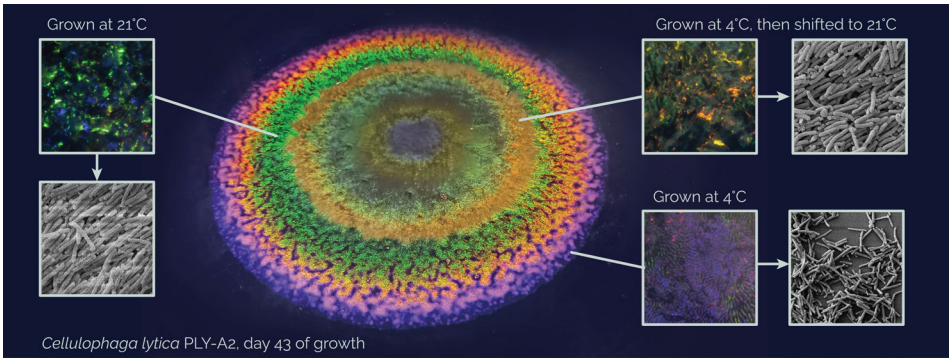


Fig. 4.2 *A visual overview of temperature-induced colour variations in C. lytica PLY-A2.*

4.2 Experiments

This study comprised three sets of experiments, with the second and third sets building on the findings of the first. All experiments focused on the species *C. lytica*, specifically the non-harmful PLY-A2 strain. This section outlines detailed procedures for each experiment.

4.2.1 Experiment 1: Capturing and characterising temporal expressions of *C. lytica* PLY-A2 in response to temperature variations

To analyse the effects of temperature variations on the growth and colourations of *C. lytica* PLY-A2, the bacteria were grown for at least two weeks in large glass Petri dishes (⌀ 200 mm). Each Petri dish contained 200 mL of marine (MAR) medium supplemented with 15 g/L agar and nigrosine pigment as a contrasting agent, as described by Groutars et al. [133]. During the incubation period, colonies were exposed to two different temperatures by placing them in a 21 °C incubator and a 4 °C refrigerator. To eliminate humidity as an influencing factor [133], the incubator was set to a relative humidity of 65%, matching the conditions inside the refrigerator. Additionally, Petri dishes were sealed with Parafilm tape to reduce moisture loss during observation.

This experiment included four groups (A–D) of replicate samples (Fig. 4.3a), all inoculated at the centre from a plate culture using an inoculation loop, and incubated upside down to safeguard colonies from condensation. Groups A and B, consisting of three samples each, served as controls and were subjected to constant temperatures for most of the experiment. Group A was incubated at 21 °C throughout, while Group B was transferred to 4 °C after 24 hours. During the first 24 hours post-inoculation, all groups were incubated at 21 °C to allow the bacteria to equally adapt to the new medium. Groups C and D alternated between the two temperatures, spending 3 days at 21 °C and 11 days at 4 °C per cycle. After two full cycles of temperature shifts, Group C remained at 21 °C (Day 31), while Group D continued undergoing temperature shifts. This approach

was intended to examine whether temperature-induced responses remained visible in older regions of the colonies under both conditions. To account for potential colony disruption due to movement between storage conditions, Groups C and D were prepared with four samples each instead of three.

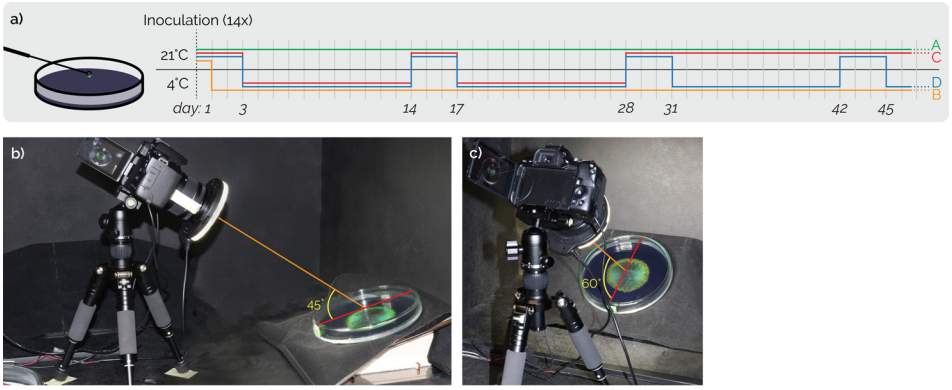


Fig. 4.3 *Procedure for Experiment 1: a) timeline showing the temperature conditions over time for Groups A–D; b-c) image capture setup with laser-cut bases for incident angles of 45° (b) and 60° (c).*

Every weekday, all samples were photographed using a camera (EOS 250D, Canon, Japan) mounted on a tripod, equipped with a macro lens and ring flash (Fig. 4.3b-c). The camera was positioned 50 cm from the samples and configured with an exposure time of 0.25 sec, ISO 800 sensitivity, an aperture of *f*/25, and a custom white balance calibrated using a colour reference card. To capture the iridescent colour, each sample was photographed at incident angles of 45° and 60° (similar to the method described in [133]). Two different laser-cut bases were used, each wrapped in dark fabric to minimise reflectivity during imaging.

Images were examined to characterise the colony colourations in terms of texture, hue, and angle dependence, and were digitally analysed to extract data on colony expansion rates. Although automated approaches—such as brightness thresholding [133]—were explored, accurately detecting colony surface areas was challenging due to the pointillistic edges of the colonies and colour similarity to the agar medium. Consequently, each colony's radius was measured using manually placed digital markers. ArUco markers, consisting of unique binary patterns [171], were generated with a Python script and imported into Photoshop as a custom brush (see Supplementary Material, Section S1.1). While this manual approach may have introduced some degree of human error and variability due to colonies not being perfectly circular, it provided a consistent and practical solution given the visual constraints. After image annotation, the fiducial markers were detected using a separate Python script (Supplementary Material, Section S1.2), allowing for the calculation and plotting of colony radii and the analysis of expansion rates across experimental groups and conditions.

4.2.2 Experiment 2: Capturing hue homogeneity across temperature-induced colour variations in *C. lytica* PLY-A2

We further analysed the response of *C. lytica* PLY-A2 to temperature variations by examining hue homogeneity across differently coloured regions. This experiment focused on the purple, orange, and green colourations induced by temperature variations, as detailed in Section 4.3.1.

Triplicate samples were inoculated and cultivated as described in Section 4.2.1, following the temperature cycling of Groups C and D (i.e., alternating between 3 days at 21 °C and 11 days at 4 °C) over a 31-day period (Fig. 4.4a). Macro and micro images were captured for each of the three coloured regions. The purple regions were photographed after 28 days of growth, while the orange and green regions were imaged after an additional 3-day incubation at 21 °C.

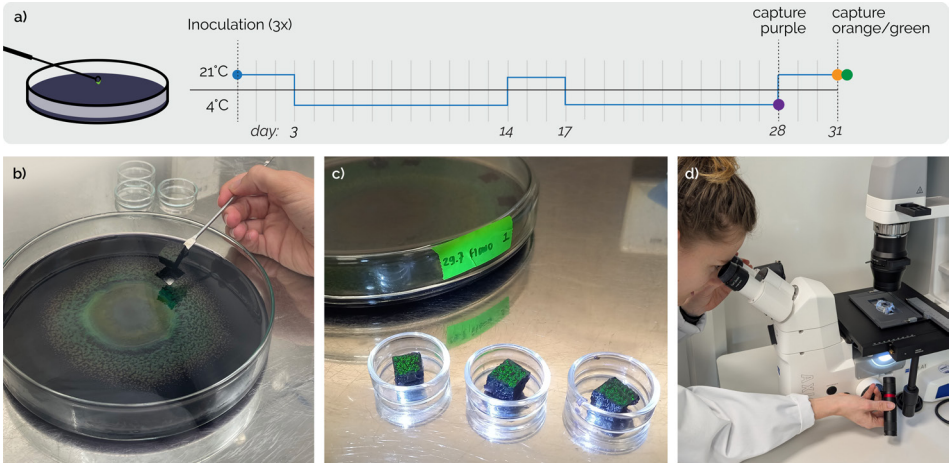


Fig. 4.4 *Procedure for Experiment 2: a) timeline showing the temperature conditions over time and the image capture points for the different colourations; b–d) steps for capturing microscopic images of the different coloured regions in C. lytica PLY-A2 colonies: (b) cutting sections from the colony, (c) placing the sections in small Petri dishes to position them close enough for proper lens focus, and (d) observing and capturing images using the microscope.*

For macro imaging, colonies were photographed at incident angles of 45° and 60° using the same setup described in Section 4.2.1, but from a distance of 30 cm. The resulting images were corrected for perspective distortion and masked to isolate the targeted colour regions.

Additionally, the coloured regions were imaged at 50× magnification to closely examine their hue characteristics (Fig. 4.4b-d) using a microscope (Carl Zeiss Axio Vert.A1, Zeiss, Germany) with a camera (Sony A6100, Sony, Japan). For each coloured region, three sections were excised from each colony, placed in small Petri dishes (⌀35 mm), and covered with plastic foil. This setup allowed the lens to focus on the colourations, with each section being photographed three times while illuminated from the side at approximately 45° using a torch.

Macro and micro images were examined to assess the hue homogeneity of the purple, orange, and green colourations, acknowledging that the RGB capture system and interpolation process may not fully represent the true characteristics of iridescent colours. Additionally, a custom Python script (Supplementary Material, Section S2) was used to digitally analyse the images and plot the average hue distribution for each colouration across the different perspectives—namely, 45° and 60° incident angles for macro images, and a 90° view with side illumination for micro images.

4.2.3 Experiment 3: Conducting SEM analysis of temperature-induced colour variations in *C. lytica* PLY-A2

The underlying optical structures of the purple, orange, and green colourations in *C. lytica* PLY-A2 colonies (as detailed in Section 4.3.1) were analysed using a scanning electron microscope (SEM), following the protocol illustrated in Figure 4.5. The entire procedure was conducted twice: first, to preserve biofilms (i.e., the surface-adhered layer of cells within the colony) for the analysis of optical structures, and second, to collect loose cells from the colonies for accurate cell size measurements. This required slight modifications to the protocol, as detailed in the following paragraphs. Photographs of the SEM sample preparation process are provided in Section S3.1 of the Supplementary Material.

C. lytica PLY-A2 was cultivated in large glass Petri dishes (⌀200 mm) using MAR medium [133] without nigrosine, as preliminary tests suggested that nigrosine dissolves in the fixative solution, potentially interfering with SEM analysis. Two groups of triplicate samples were cultivated, both subjected to temperature variations (Fig. 4.5a). The group designated for analysing the purple region remained incubated at 4 °C until fixation on Day 31. Conversely, the group intended for analysing green and orange regions was transferred to 21 °C for the final 3 days.

To prevent contamination, abnormal growth, or other inconsistencies that could compromise the study's validity, colonies were cultivated in triplicate. After cultivation, one representative colony was selected for each coloured region to proceed with the preparation process. These colonies were placed upside down overnight, with 250 mg of pulverised iodine crystals in the lids of the Petri dishes (Fig. 4.5b), allowing iodine vapour to kill the cells without altering their optical structures [25]. During this period, the colonies selected for green and orange colourations were kept at room temperature, whereas the colony designated for the purple colouration was returned to 4 °C to prevent temperature-induced colour changes. The amount of iodine crystals for this sample was increased to 500 mg to account for the reduced vapour pressure at lower temperatures. Since the iodination process temporarily alters the colour of the agar medium, the coloured regions were marked on the bottom of the Petri dishes with a permanent marker beforehand for easy recognition.

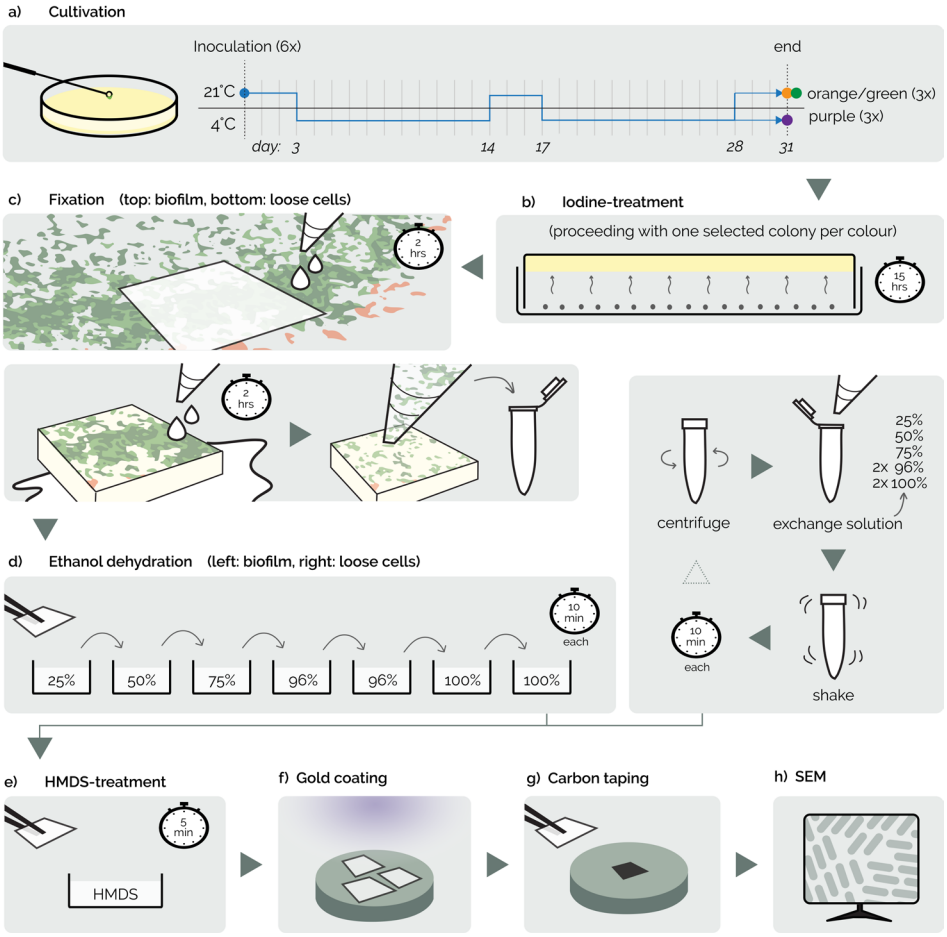


Fig. 4.5 *Sample preparation procedure for SEM analysis: a) cultivation; b) iodine-treatment; c) fixation; d) ethanol dehydration; e) HMDS-treatment (hexamethyldisilazane); f) gold coating; g) carbon taping; h) SEM.*

For SEM analysis of the biofilm, cell arrangements were preserved by placing a coverslip on top of the colony and gently pipetting a fixative solution (phosphate-buffered saline with 2% glutaraldehyde, following [25, 29]) between them (Fig. 4.5c, top). After 2 h, the coverslips—three per coloured region—were carefully removed with the biofilm adhering to them. These coverslips were then subjected to ethanol dehydration to remove water (Fig. 4.5d, left) and immersed in hexamethyldisilazane (HMDS) (Fig. 4.5e) to prevent structural collapse during drying [172].

For SEM analysis of loose bacterial cells, three cut-outs were obtained from each coloured region and submerged in the fixative solution to detach the biofilm from the growth medium in small fragments (Fig. 4.5c, bottom). The resulting suspension was carefully pipetted into an Eppendorf tube. Ethanol dehydration was then performed in a stepwise manner, with centrifugation

before each solution change and vortexing to ensure thorough mixing with each new ethanol concentration (Fig. 4.5d, right). The dehydrated sample was subsequently transferred onto a coverslip before immersion in HMDS.

A gold coating was then applied to all coverslips (Fig. 4.5f) to establish a conductive surface, preventing charging and minimising thermal damage during SEM imaging. The coated coverslips were mounted onto a sample holder using carbon tape (Fig. 4.5g) and inserted into an SEM (JSM-IT700HR, JEOL, Japan). During SEM imaging (Fig. 4.5h), all nine coverslips containing biofilm samples were imaged once at 1000× magnification to assess overall cell organisation, and at 5000× and 10,000× magnifications at three locations each for detailed observation. Similarly, the nine coverslips with loose cells were imaged at 5000× magnification at 10 different locations to obtain sufficient data for cell size measurements.

The SEM images of loose cells were analysed using ImageJ software, as described in [25], to determine the average bacterial cell width and length across the three coloured regions. For each parameter, 150 measurements were taken, plotted as histograms, and summarised using boxplots. Differences among measurements were assessed using the nonparametric Kruskal–Wallis test. When applicable, Dunn's post hoc tests with Bonferroni correction were conducted. A p-value <0.05 was considered statistically significant.

Additionally, the optical structures observed in the SEM images of biofilms were assessed for cell alignment and homogeneity of orientation. To quantify local cell alignment in the regions visible at 5000× magnification, three SEM images were analysed per colouration. Automated orientation analysis was hindered by low contrast between foreground and background cells, which impeded cell detection. Therefore, the orientation angles of at least 150 cells per image were manually measured in ImageJ by drawing straight lines from end to end of each cell. To focus on local cell alignment rather than overall orientation, the mean angle per image was subtracted from each measurement, resulting in relative angles. These were then plotted as histograms to compare local cell alignments among the different colourations.

4.3 Results

4.3.1 Effect of temperature variations on the temporal expressions of *C. lytica* PLY-A2

Temperature variations impacted both the expansion rate and iridescent colourations of *C. lytica* PLY-A2 colonies, consistent with previous studies that cultured *C. lytica* colonies at low or high temperatures [26, 133]. In our experiments, where temperature was varied during colony growth, we observed that temperature had a sustained effect over time, consistently influencing expansion rate and colourations in a similar manner.

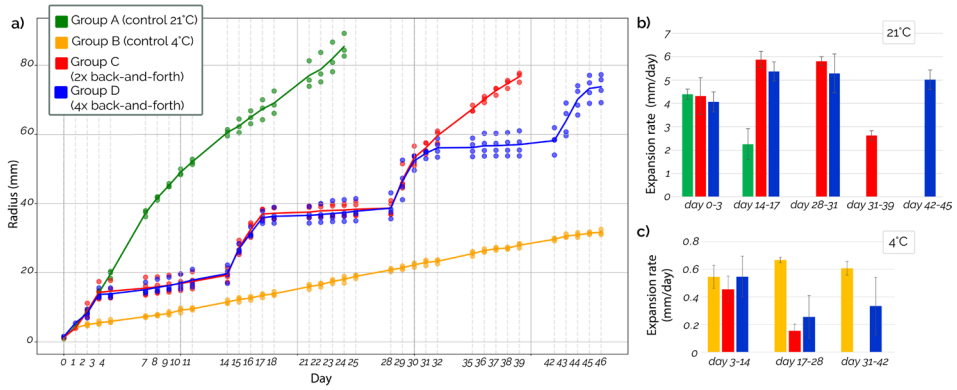


Fig. 4.6 *Growth of colonies in control Groups A (green) and B (yellow), and in groups subjected to alternating temperatures of 21 and 4 °C, i.e., Groups C (red) and D (blue): a) graph showing colony radius over time with dots representing individual samples and lines indicating averages; b-c) graphs showing average expansion rates of each group during incubation at 21 °C (b) and 4 °C (c), with error bars indicating standard deviations (n=3 for Groups A and B; n=4 for Groups C and D).*

Colony expansion over time was compared across the four groups (Fig. 4.6). Although we acknowledge that differences in the unquantified inoculum amounts may have introduced minor variations in growth rates, the data showed a clear trend: colonies grown at 21 °C expanded considerably faster than those grown at 4 °C.

In the control groups (A and B), Group A (maintained at 21 °C) expanded at a rate of 4.4 mm/d during the first 3 days but gradually slowed to about 2.3 mm/d as the colony approached the edge of the Petri dish (Fig. 4.6b). Conversely, Group B (maintained at 4 °C) exhibited a stable expansion rate of approximately 0.6 mm/d throughout the experiment (Fig. 4.6c).

Groups C and D, which were exposed to alternating temperatures, exhibited expansion rates comparable to those of the control groups during their initial 21 and 4 °C phases (i.e., Days 0–3 and 3–14, respectively) (Fig. 4.6b-c). However, their responses became more variable upon re-exposure to the same temperatures. Each time the colonies returned to 21 °C (i.e., Days 14, 28, and 42), they expanded at rates equal to or exceeding those of Group A during its initial high-growth phase (Days 0–3) (Fig. 4.6b). When 21 °C conditions were sustained for a more extended period—as in Group C between Days 31 and 39—the expansion rate slowed, mirroring the pattern observed in Group A between Days 14 and 17. During the second 4 °C phase (Days 17– 28), colonies in Groups C and D expanded more slowly than those in control Group B (Fig. 4.6c).

Colonies subjected to temperature variations over a 45-day incubation period developed distinct coloured rings (Fig. 4.7a), as new growth consistently responded to the prevailing temperature conditions. A detailed overview of the growth patterns for replicate samples from all groups is presented in Section S1.3 of the Supplementary Material.

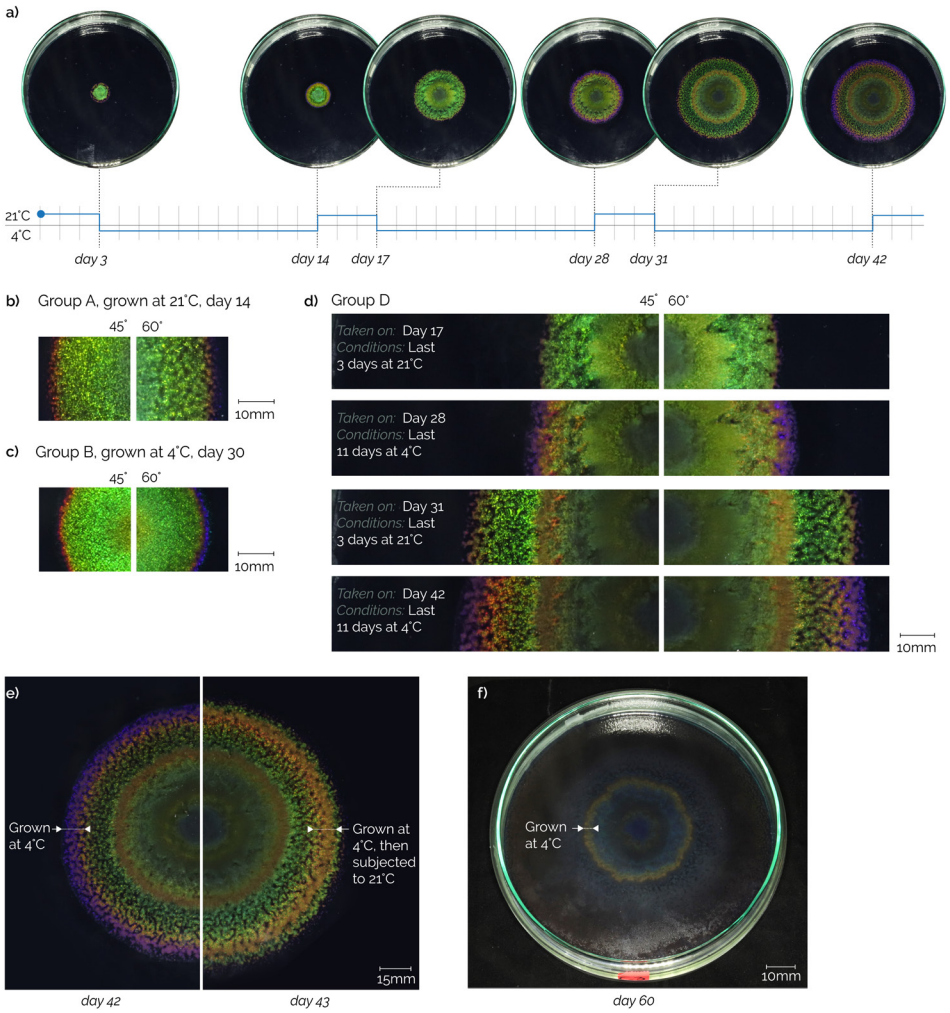


Fig. 4.7 *Perspective-corrected images of C. lytica PLY-A2 colonies from Experiment 1: a) representative colony growth over time in Group D, subjected to temperature variations, captured at 60°, showing the formation of coloured rings; b–d) close-ups of colony colourations captured at 45° and 60°: (b) edge of a Group A colony, grown at 21 °C for 14 d, (c) edge of a Group B colony, grown at 4 °C for 30 d, and (d) multiple growth stages of a Group D colony, subjected to temperature variations, with the most recent temperature condition and duration indicated for each image set; e- f) temporal changes in C. lytica PLY-A2's colony colourations, captured at 60°: (e) representative Group D sample after 42 and 43 d, showing the transition of purple colourations (formed at 4 °C), to orange within 24 hours at 21 °C, and (f) group C colony on Day 60, previously subjected to temperature variations, showing loss of iridescence but retention of lighter-coloured rings, possibly partially coloured by the inherent yellow pigment of C. lytica [173].*

As shown in Figure 4.7, *C. lytica* PLY-A2 colonies grown at 4 °C exhibited more intense purple hues than those grown at 21 °C, particularly when observed at an incident angle of 60° (Fig. 4.7b-c). Colonies grown at 4 °C also developed

a more uniform texture in their colouration, whereas those grown at 21 °C appeared more pointillistic and scattered. When the bacteria were subjected to alternating temperatures, these temperature-induced effects became apparent in the newly formed colony regions, producing concentric rings with distinct colours and textures, as observed in Group D (Fig. 4.7d).

As the colonies matured and were exposed to varying temperatures, the regions that developed under different temperature conditions retained distinct colour differences. While green colourations were relatively stable, purple regions were more temperature-sensitive, shifting noticeably to orange when exposed to higher temperatures (Fig. 4.7e), as further discussed in Sections 4.3.3 and 4.4. Over time, although the colonies gradually lost their iridescence, differences between growth at 4 and 21 °C remained visible (Fig. 4.7f), indicating that the lighter coloured rings preserved a record of past temperature variations.

4.3.2 Hue homogeneity of temperature-induced colour variations in *C. lytica* PLY-A2

The hues observed across the three coloured regions exhibited considerable variations, both from macroscopic and microscopic perspectives.

In the region designated as purple (Fig. 4.8a), cultivated at 4 °C, macroscopic images showed a colour gradient ranging from orange and red hues to purple and blue near the edges when viewed at a 45° angle. At 60°, the hues shifted toward more yellow and vivid purple, as evidenced by the hue spectrum graph. Additionally, small patches of green and red were visible at both angles. Under the microscope, purple was the predominant hue, consistent with the hue spectrum graph, where hues within the purple range exhibited the highest average normalised pixel count (approximately 0.6). Although green and red hues were also observed in these microscopic images, their peak values were significantly lower (approximately 0.1). It should be noted that achieving focus on structural colourations at higher magnifications can be challenging or even unfeasible, and that the observed hues may result from clustered points of different colours rather than a uniform structural phenomenon.

The region designated as orange (Fig. 4.8b), grown at 4 °C and subsequently exposed to 21 °C, exhibited less hue variation from a macroscopic perspective, primarily displaying orange and green hues. Conversely, microscopic examination revealed a wider array of colours, including vivid orange, yellow, and various shades of green, cyan, and blue, as evidenced by the hue spectrum graph.

Finally, the region designated as green (Fig. 4.8c), grown at 21 °C, exhibited a colour gradient at the macroscopic scale, transitioning from green and yellow to orange and red along the colony's edge. Orange hues appeared slightly more prominent at a 45° angle compared to 60°, although the overall colour profile remained stable, as evidenced by the hue spectrum graph. Microscopically, numerous blue and green dots were observed, consistent with the hue spectrum graph, while red and orange dots were sparsely distributed, and too few to be represented in the graph.

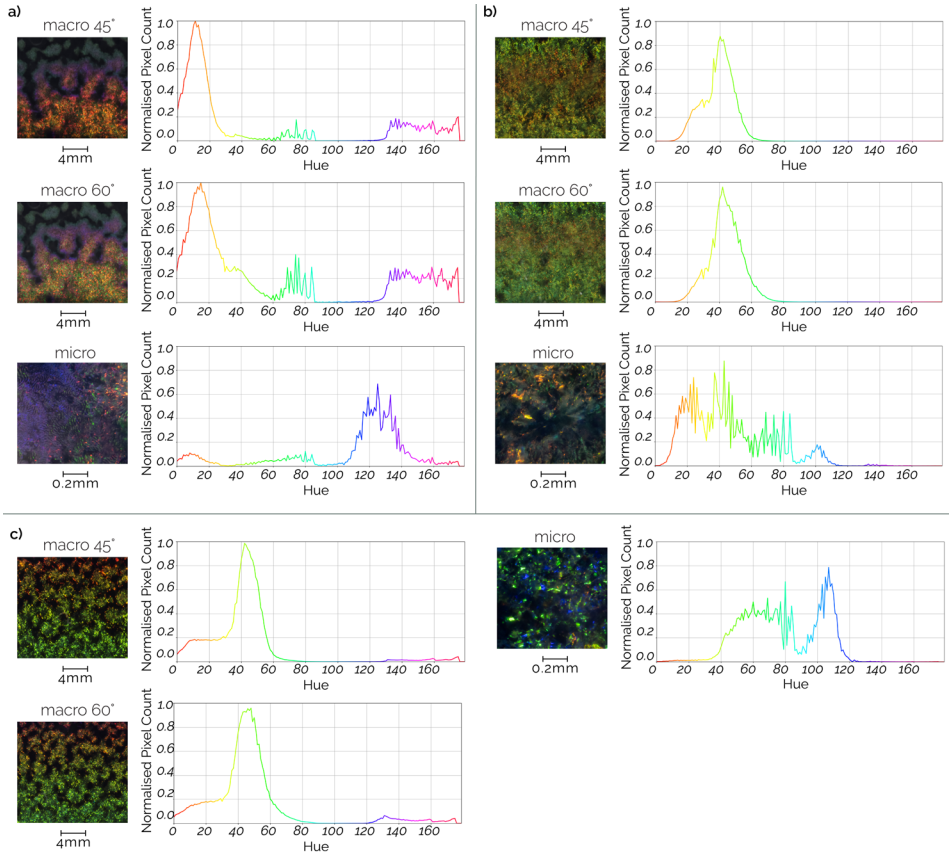


Fig. 4.8 *Colourations of the purple (a), orange (b), and green (c) regions, shown by macroscopic and microscopic images (50× magnification), along with graphs displaying the average normalised pixel counts per hue for all images. In the macroscopic images, the edges of the colonies are positioned at the top.*

4.3.3 Relationship between temperature-induced colour variations and underlying cell organisation and morphology

SEM imaging revealed distinct differences in cell organisation among samples from the three coloured regions. In green regions, well-organised cell arrangements were clearly visible (Fig. 4.9a). At higher magnifications (Fig. 4.9a, right), tightly packed cells appeared aligned in parallel, whereas lower magnifications (Fig. 4.9a, left) revealed variations in orientation, resulting in circular patterns across the colony—similar to those observed in other Flavobacteria [174] (Supplementary Material, Fig. S12). Conversely, organised cell arrangements were less frequent and less structured in the orange regions (Fig. 4.9b) and appeared absent in purple areas (Fig. 4.9c).

These differences in cell alignment were further supported by the relative angle distributions (Fig. 4.10a). In the green regions, the relative angles showed a sharp peak, indicating consistent alignment among cells within the sampled

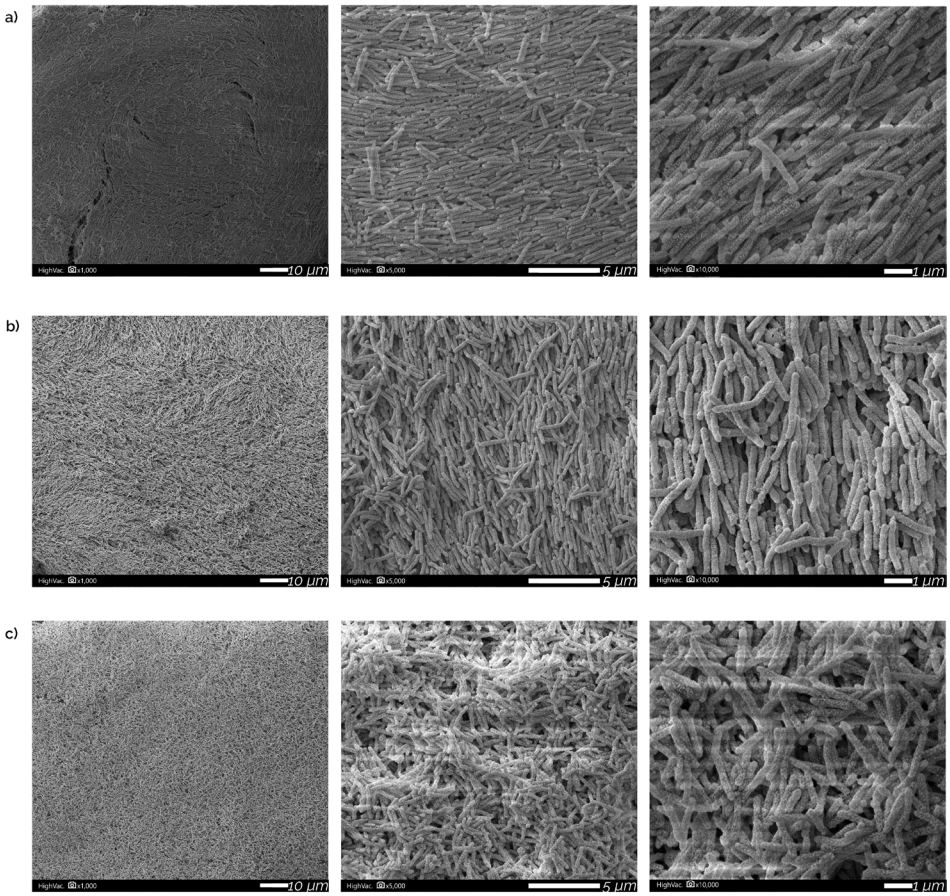


Fig. 4.9 *SEM images of biofilms showing cell arrangements from the green (a), orange (b), and purple (c) regions, captured at three magnifications: 1000× (left), 5000× (middle), and 10,000× (right). Scale bars represent 10, 5, and 1 µm, respectively.*

areas. Conversely, the distributions for the orange and purple regions were broader, suggesting more variable cell orientations within the sampled areas.

Cell size measurements obtained from SEM images of loose cells (Fig. 4.10b-f) revealed slight but statistically significant differences in cell length across the three colourations ($H(2) = 6.10$, $p = 0.047$) (Fig. 4.10b-c). Median lengths followed a consistent trend: cells from orange regions were the shortest (1.60 µm), followed by those in green (1.87 µm), with the longest observed in the purple regions (2.06 µm). Consistent with the boxplot (Fig. 4.10c), post hoc analysis revealed that cell length in the purple region differed significantly from the orange ($p = 0.014$), but not from the green ($p = 0.17$). Conversely, cell width (Fig. 4.10d-e) did not differ significantly among the colourations ($H(2) = 3.59$, $p = 0.166$), consistent with the substantial overlaps seen in the boxplot (Fig. 4.10e). Median widths showed minimal differences: green (249 nm), orange (255 nm), and purple (263 nm).

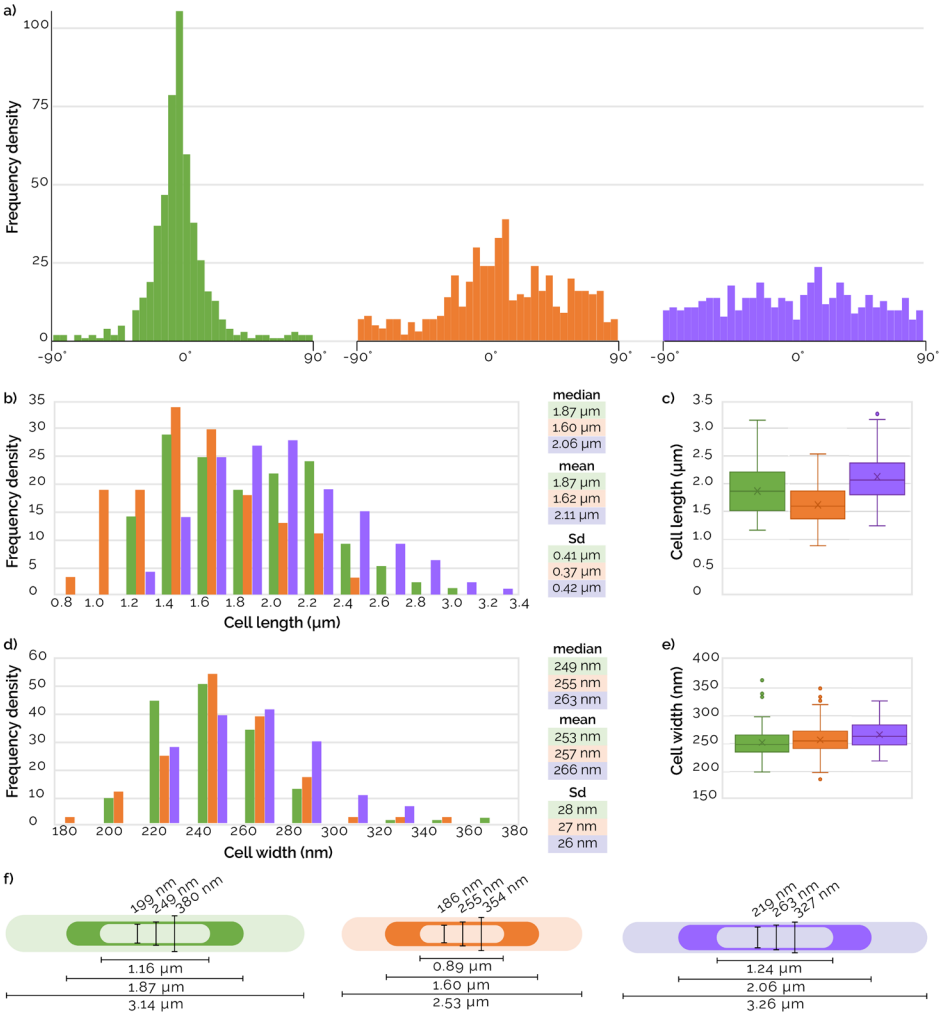


Fig. 4.10 *Cell alignment and dimensions across the different colourations. The colours used in this figure (green, orange, and purple) correspond to the visually observed colourations and are applied consistently throughout. a) histograms showing the relative angle distributions within sampled areas imaged at 5000× magnification, illustrating differences in local cell alignment among the three colourations b-c) histogram and corresponding boxplot of cell length measurements; d-e) histogram and corresponding boxplot of cell width measurements. The boxplots show the distribution of cell sizes for each region (n=150): boxes represent the interquartile range, covering the middle 50% of data; whiskers extend to capture the majority of remaining data points; individual outliers are shown as separate dots; f) infographic summarising the maximum, minimum, and median cell dimensions for the three colourations.*

Additionally, SEM imaging of loose cells revealed the presence of spherical cells within the purple regions (Fig. 4.11a) and, more prominently, in the orange regions (Fig. 4.11b). These cells were excluded from the quantitative cell size measurements due to their atypical morphology.

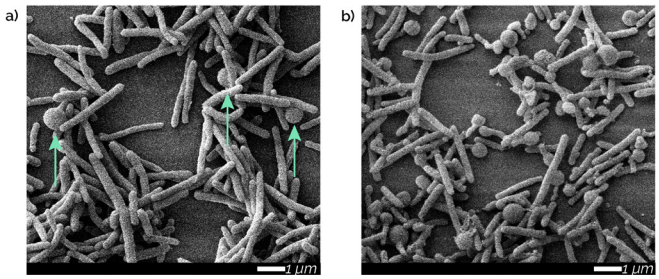


Fig. 4.11 *SEM images showing spherical cells within the purple regions of the colony, as indicated by arrows (a), and within the orange regions (b), with scale bars representing 1 µm.*

4.4 Discussion

4.4.1 Reflections and limitations

This study shows that temperature variations influence the expansion rate and colourations of *C. lytica* PLY-A2, resulting in the formation of distinct coloured rings within colonies. Previous research has provided explanations for structural colour variations in Flavobacteria. For example, green colourations observed in *Flavobacterium* IR1 (cultivated at 25 °C) have been attributed to a relatively homogeneous crystalline arrangement of cells [22]. Conversely, the purple colourations in the same strain have been associated with a more disordered structure and increased average cell width ((480±25) nm) compared to green regions ((400±20) nm) [175]. However, in that case, both colourations developed at 25 °C, and the shift to purple was triggered by the presence of polysaccharides (fucoidan), rather than temperature variations. Similarly, another study reported notable differences in cell width between the green and red colourations of *C. lytica's* DSM 7489 (310 nm vs. 428 nm), although these shifts occurred in response to salinity variations [29], with temperatures ranging from ambient to 27 °C. While these studies involved different strains and environmental factors to induce colour changes, the reported differences in the average cell width between colour variants were substantially greater than the 6–14 nm variation observed in our analysis (Fig. 4.10). This relatively small difference in cell width suggests that temperature variations have limited impact on the cell width of *C*. *lytica* PLY-A2 and that cell width is unlikely to be a significant determinant of the colour differences observed among temperature-induced rings. Instead, other structural characteristics, such as cell spacing and organisation, may play a more prominent role.

Slight differences in cell length were observed across the coloured regions, particularly between the orange and purple regions. These variations may be influenced by the cells' relative position within the colony, as the purple region is closer to the colony edge, where bacterial cells tend to exhibit more active growth. However, since cell length extends perpendicular to the plane of the two-dimensional photonic crystal lattice, it is less likely—compared to cell width

and intercellular spacing—to significantly influence the photonic interactions underlying colour production in these optical structures.

SEM analysis of the biofilm was conducted to examine the underlying cell organisation and better understand the origins of the distinct colour differences in the temperature-induced rings. However, samples of orange and purple regions exhibited minimal or no organised cell arrangements. One possible explanation for this lack of organisation is that the fixation procedure may have been less effective for the orange region and ineffective in preserving the optical structures in the purple region. This may be due to differences in cell spacing across the coloured regions, with greater spacing in the purple region potentially making the optical structures more vulnerable to disruption during sample preparation. Such variations in intercellular spacing could play a crucial role in the optical mechanisms of colour generation and may partially account for observed colour differences across the temperature-induced rings.

The presence of spherical cells within the purple region— and more prominently in the orange region (Fig. 4.11)—may indicate a stress response, as bacteria are known to undergo stress-induced adaptive morphogenesis. Under unfavourable conditions, rod-shaped cells transition to a coccoid morphology [176]. Such morphological changes may influence the colourations of *C. lytica* PLY-A2 by altering the cell spacing and organisation within its optical structures.

While SEM imaging offers valuable insights into cell spacing and morphology, it requires dehydration steps that may induce shrinkage, potentially affecting these measurements [22]. However, Schertel et al. [22] noted that such shrinkage tends to occur uniformly across bacterial samples, thereby preserving relative differences. Thus, despite the potential for altered absolute cell dimensions, the comparative analysis of the green, orange, and purple regions in our study remains valid. Nevertheless, future studies employing less invasive imaging techniques—such as environmental SEM and cryogenic SEM combined with goniometry [22]—could provide more accurate representations of the optical structures in Flavobacteria.

Furthermore, while RGB-based imaging in the first and second experiments provided valuable insights into the colourations of *C. lytica* PLY-A2, this approach has limitations in capturing the complexity of iridescent colours, such as their subtle spectral variations. Hyperspectral or multispectral imaging techniques, which offer higher spectral resolution, may provide more accurate characterisation of the optical properties of structurally coloured biofilms in future research.

Across all experimental groups, replicate samples showed strong consistency in both colour expression and growth patterns (Supplementary Material, Fig. S4), highlighting the reproducibility and robustness of our findings. This consistency was evident at both the macroscopic and microscopic levels, including the emergence of concentric coloured rings in response to temperature variations. Minor variations among replicates (e.g., in expansion rates; Fig. 4.6) likely reflect the inherent biological variability commonly associated with microorganisms.

Finally, this study shows that *C. lytica* PLY-A2 exhibits extended stability under controlled conditions, maintaining distinct and vivid colour patterns for over 42 days (Fig. 4.7). This impressive longevity suggests the potential of temperature-responsive living materials for long-term functionality—an essential consideration for practical applications. However, the durability of these properties under real-world environmental conditions remains uncertain, as all experiments were conducted in a highly controlled laboratory setting. Transitioning to real-world applications requires a deeper understanding of how interconnected environmental factors affect the behaviour of *C. lytica* PLY-A2 over time. For practical applications, thermochromic ELMs must function beyond the confines of Petri dish environments, which presents challenges related to material integration, sterility, and environmental variability. Future studies should explore strategies to enhance the resilience of Flavobacteria in applied settings, such as encapsulating bacterial colonies within protective biomaterials. Addressing these challenges will be essential to advancing the development of thermochromic ELMs.

4.4.2 Implications for biodesign

The concentric coloured rings formed in response to temperature variations underscore the potential of *C. lytica* PLY-A2 as a foundation for thermochromic living materials with applications across various domains.

Unlike conventional thermochromic materials, which only reflect current temperatures, these living colonies can record past temperature variations in their growth patterns, enabling the tracking of temperature history through *living traces* [133]. This opens up possibilities for applications where tracking past exposures is crucial, such as in the context of food safety and waste management. Additionally, these living thermochromic materials could serve as ambient temperature indicators that foster reflective engagement with environmental conditions. For instance, visualising temperature shifts in gardens may promote awareness of the behaviours, rhythms, and interactions of other living organisms. Beyond static surfaces, these materials could be integrated into smart textiles, leveraging their dynamic colour changes for temperature-responsive garments. For instance, bacteria embedded in sportswear could enable visually engaging patterns in response to body heat and ambient air, combining aesthetics and personalisation with functional monitoring. In such applications, encapsulating bacteria may mitigate potential risks associated with direct contact or unintended environmental exposure.

Since *C. lytica* PLY-A2 requires minimal resources to grow—just small amounts of salt and trace nutrients—these bacteria hold promise for producing thermochromic materials in a sustainable, resource-efficient manner, as well as for temperature sensors in areas with limited electricity. Finally, given that the bacteria are unmodified, reliant on naturally derived resources, and commonly found in marine environments [24, 173], they represent an ecologically safe and biodegradable platform for developing thermochromic living materials.

While this study focused on cultivating a single strain of *C. lytica* at two specific temperatures, the potential of thermochromic living materials extends far beyond this scope. Hundreds of iridescent bacterial strains exist, each with distinct optical properties and originating from diverse environments [20]. The variability in their growth conditions and colour responses could enable the customisation of thermochromic living sensors tailored to specific applications and contexts.

The design applications outlined above are speculative and intended to illustrate possible future directions rather than reflect current technical feasibility. Integrating these iridescent bacteria into practical biodesign applications will require further research to address real-world constraints, including material integration, robustness, and biosafety considerations. Moreover, a more comprehensive understanding is needed of how their optical properties—such as colour variability, stability, and responsiveness—are perceived and interpreted. Future work should include experiential characterisation studies [47] to explore how users perceive and interact with the dynamic colourations of thermochromic ELMs. More broadly, the acceptance of ELMs poses a social challenge, closely tied to their practical context and role in everyday interactions.

4.5 Conclusion

This study shows how temperature variations influence the expansion rate and colourations of *C. lytica* PLY-A2. Bacterial colonies alternating between 4 and 21 °C developed concentric coloured rings in orange, green, and purple, and shifted between slow and fast growth phases. The variation in colour, responsiveness, and stability highlights the potential of these iridescent bacteria for thermochromic living materials. Motivated by scientific curiosity, we further explored the homogeneity of the three coloured regions and their underlying optical structures. Our findings revealed that the coloured regions exhibit heterogeneous hues that vary with magnification. SEM imaging showed differences in cell arrangement, potentially influenced by variations in cell spacing, while cell width remained consistent across regions, and cell length exhibited only minor variation. Spherical cells, primarily observed in the orange region and, to a lesser extent, in purple, may indicate stress responses under specific conditions. Overall, our findings underscore the promise of *C. lytica* PLY-A2 for future integration into living materials, where its dynamic colourations in response to temperature variations could pave the way for innovative thermochromic ELMs.

Intermezzo: A Personal Reflection on the Interdisciplinary Research

Coming from a (bio)design background, I joined a collaborative space with colleagues from microbiology and biotechnology for the study presented in Chapter 4. This interdisciplinary environment revealed both the excitement and tensions that emerge when different epistemologies, methodologies, and disciplinary languages meet. In this personal reflection, I first discuss general challenges I encountered in this interdisciplinary collaboration, before zooming in on my experiences of how biotechnology and design research approach designing with microorganisms differently.

Navigating Differences in Interdisciplinary Collaboration

In this collaboration, I noticed how differences revealed themselves in the language we used, in our assumptions behind protocols, and in the dynamics of collaboration itself.

Every discipline develops its own vocabulary, with jargon and metaphors that make perfect sense internally but can obscure meaning across boundaries. This became particularly evident through communication challenges among collaborators. Not only did technical jargon complicate communication, but some terms also often carried subtly different meanings, even with words that might seem transparent, such as experiment, growth, and control. For my colleagues from biotechnology, an experiment was a highly controlled, repeatable procedure; for me, it could also be more explorative, even quick-and-dirty, as a way to learn through doing. Similarly, growth was for them a quantitative measure, assessed through optical density or colony counts, while I often interpreted it more qualitatively, attending to colony form and size, or other visual cues. And when they spoke of control, it referred to experimental validity with control groups and precise variables, whereas I saw it as a more ethical and relational question, one that clashed with the agency and unpredictability inherent in microbial life.

Over time, confusion and misunderstanding due to these language differences slowly gave way to a shared vocabulary through an iterative process of translation and clarification. I realised that this negotiation of meaning might not only be essential to interdisciplinary practice but also highly valuable: by clarifying language, we were also negotiating what counted as relevant and rigorous knowledge.

Besides these language-related challenges, I also realised that my expectations of the research were quickly challenged in interdisciplinary collaborations. What appeared simple in one field often turned out to be complex and iterative in practice. For instance, following microbiology protocols seemed straightforward

to me at first glance—apart from dealing with unfamiliar technical jargon, I interpreted the procedures as clear and linear, leading neatly to reported results. Only later did I realise that these protocols likely condensed many rounds of trial and error, iterations that remained invisible in the polished format of scientific publishing.

This mismatch between expectations and the reality of research seems to occur frequently in interdisciplinary collaborations, as one rarely understands the full complexity of another discipline in great detail. Such misalignment often surfaces in collaboration dynamics, where we might ask or expect unrealistic things from one another. For example, I initially intended to cultivate the Flavobacteria and then simply bring the samples over so that my colleagues could capture their cell organisation. Little did I know how much preparation this actually required, and how we would eventually go through a long iterative process together to fine-tune the protocol (Section 4.2.3), finally succeeding only on the seventh attempt after many long days in the lab.

I was reminded that the opposite also occurred in the interdisciplinary collaboration presented in Ch. 3, when I was considered the "expert" on the microbiology and cultivation side. In this collaboration with computer graphics specialists, I was asked to ensure that the bacterial colonies grew in perfect circles to ease data extraction on their colourations. While this request might have been logical from their perspective, it was impossible to meet when working with living microorganisms that have their own agency and variability.

Beyond false expectations, I noticed how an incomplete understanding of the full complexity also challenged the interpretation of results. It becomes difficult to understand what a validated outcome looks like if one is not aware of the sources of variation within a process. In the next part of this reflection, I explore this tension between precision and creative interpretation in the context of biodesign.

Looking back, these differences in language, expectations, and approach were not necessarily obstacles, but rather indicators of the distinct ways in which each field constructs and validates knowledge. Working in an interdisciplinary environment showed me how collaboration across disciplines is as much about negotiating meaning as it is about sharing expertise, and that it deserves more than mutual respect. It requires a willingness to dwell in uncertainty, to slow down when meaning slips, and to remain open to being misunderstood.

At the same time, working across disciplinary differences was intellectually stimulating and opened up new insights and perspectives that I would not have reached alone— something I return to later when reflecting on what these different approaches within biodesign can offer one another.

Contrasting Approaches to Designing with Microorganisms

I now turn to the differences I observed specifically between biodesign as practised within design research communities and biotechnology. In reflecting

on my experiences in this interdisciplinary collaboration, I began to notice how these two fields often approach biodesign research in distinct ways. I first explore these contrasts through the lens of research practice, before briefly reflecting on their broader relational orientations towards microorganisms.

One of the clearest differences between biotechnology research practice and biodesign research as practised within design research lies in how research is communicated. Biotechnology publications are precise, highly technical, and focused on reproducibility. They tend to be concise, merging introduction and background into a single section and maintaining a strict separation between methods and results. In contrast, biodesign publications within design research often read as more narrative and contextual. They seem to be written for a multidisciplinary audience rather than only for specialists in the field. Rather than masking complexity in rigidly presented biotechnology protocols, the writing often foregrounds the messy process, uncertainty, and reflection as integral to the research itself. As a result of this difference, I might have initially underestimated the technical parts of the research, which seemed easier to me on paper than they turned out to be in practice.

Differences also became evident in how results are interpreted and discussed. In biotechnology, conclusions are drawn with great caution and remain closely tied to the specific conditions of an experiment. Findings are validated through repetition and framed within strict methodological boundaries, leaving little room for creative speculation beyond the data. I noticed how this caution often tempered the tone of enthusiasm when observing the fascinating colourations produced by the microorganisms. Where I expressed excitement about the vivid colours and their design potential, my colleagues from biological sciences were carefully noting the specific conditions under which these effects occurred. In contrast, design research allows greater freedom in framing and interpreting results. Outcomes are often treated as proof of concept rather than generalisable or replicable evidence, and small-scale experiments can become starting points for wider reflection or speculation. I also noticed this difference when deciding which colonies to photograph: while I instinctively wanted to select the brightest, most vivid colonies, captivated by their striking appearance, my collaborators from biological sciences wanted to account for the variation in colour intensity across samples. Their approach was arguably more “honest” to the biological data, yet it also felt constraining to the creative imagination that drives design exploration. I realised that both fields offer valuable ways of interpreting and discussing results, and can nicely complement each other to balance the imagination and methodological precision within biodesign research. In achieving this complementarity, I felt proud and increasingly excited about the potential impact this synthesis might hold. It is also a skill I now recognise as a meaningful achievement of this collaboration.

Finally, I noted differences in research practices related to how each field positions expertise, which shaped many moments in this interdisciplinary collaboration. In biotechnology, researchers often work within a narrowly defined domain, mastering highly specific tools and methods. Design researchers, by

contrast, tend to draw on broader, cross-domain knowledge, and may claim familiarity or expertise more quickly. In my experience, these differences became visible in several moments of this collaboration. At times, I felt that my knowledge of Flavobacteria—built through hands-on experimentation and literature review—was undervalued by microbiology experts, as if it did not count as legitimate expertise compared to their disciplinary specialisation. Conversely, I assumed that my colleagues from biological sciences would be familiar with technical imaging techniques like scanning electron microscopy (SEM), only to realise that such expectations overlooked how expertise in their field is distributed: deep but narrow.

I also noticed how my colleagues from biotechnology placed strong value on established domain expertise: methods and interpretations grounded in prior validated work carried significant weight, and protocols tended to emerge by extending or refining existing approaches rather than departing radically from them. While this practice fosters rigour and reliability, in our interdisciplinary collaboration, I sometimes felt restricted when methods needed to be validated through existing literature before being considered viable. In contrast, in design research the stance toward methods is often more flexible—especially in exploratory or proof-of-concept work—although studies with quantitative aims might also rely on strict protocols, just as biotechnology scholars may be open to novel techniques. Still, in our interdisciplinary collaboration, these different orientations occasionally became tangible. For instance, when I presented hue-spectrum graphs to quantify and visualise the hue homogeneity of the iridescent colourations, the approach felt intuitive and like a promising creative solution. Yet I immediately received the question of whether this had been done before in this way. These hesitant responses and questions about validation made me less confident about whether my divergence reflected innovation or stubbornness.

Beyond the challenges, these two different research practices also complement each other in valuable ways. While biotechnology offers deep, precise insights into microbial behaviour, biodesign in design research brings a broader, interaction-level perspective and allows us to speculate on potential futures. Where biotechnology can unpack the technical conditions under which such possibilities become feasible, designers further explore how these possibilities might be meaningfully implemented. In our collaboration, these orientations came together productively: together, we were able to unpack the design potential of Flavobacteria as a thermochromic living material—both by understanding microbial behaviour in technical detail and by exploring their living aesthetics and temporality to consider their potential for interaction design.

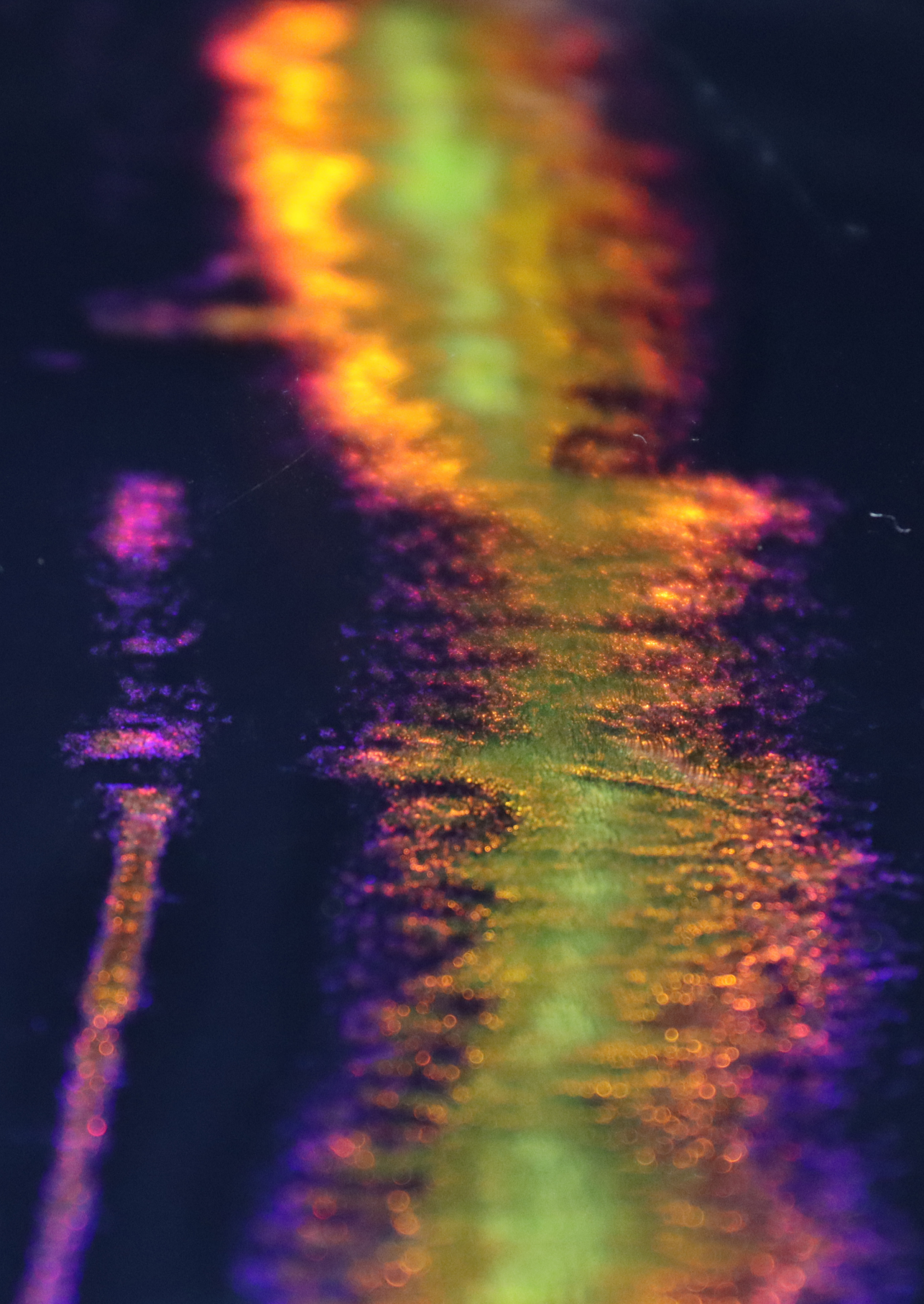
Beyond differences in research practice, biotechnology and design research often reflect different relational orientations towards microorganisms. In the biotechnological work I encountered, microorganisms were often approached in functional terms—studied or modified to achieve particular outcomes, with an emphasis on reliability and understanding the conditions under which they

behave in predictable ways. In design research, the stance can vary: at times it is also instrumental, but in strands informed by more-than-human thinking, researchers increasingly foreground relational perspectives, exploring what engaging with microorganisms might reveal about interconnectedness, care, and reciprocal relationships, rather than focusing on what they can produce.

Even within a shared project, these differences became apparent. For example, I recall being surprised, or honestly a bit shocked, by the language one of my collaborators used, referring to "exploiting microbes" and Flavobacteria's "exploitable visual effects." To me, this phrasing felt uncomfortable, as if the bacteria's aesthetic qualities were simply resources to be harnessed. I also noticed how casually collaborators spoke about killing the bacteria, without any emotional response. In contrast, my perspective was much more about attending to their expressions and the possibilities of living with the microorganisms. Through these differences, this interdisciplinary collaboration prompted me to reflect more consciously on my own relational orientation towards the Flavobacteria and how this has shifted throughout the PhD trajectory. It also helped me articulate my position within the biodesign field, which I discuss further in Chapter 7.

To conclude, this interdisciplinary collaboration enabled a rich exchange of expertise and allowed me to gain hands-on technical skills in the lab. It provided me with a deeper understanding of Flavobacteria's colourations and their responsive behaviour, while also sharpening my appreciation for methodological caution. It also taught me to communicate both more precisely and more creatively, ensuring that my contributions were legible and meaningful across disciplinary boundaries. Interdisciplinary collaboration proved to be a space of tension, learning and mutual growth, where value lies in learning to navigate different ways of knowing, valuing, and acting, and in embracing the complexity and possibilities that this process brings.

Through this collaboration, I came to see how the strengths of these fields can meaningfully extend one another and how both fields—with their distinct ways of knowing, doing, and communicating—offer essential contributions to biodesign, whether independently or in combination. Neither perspective alone would have allowed us to explore Flavobacteria's thermochromic qualities in the same way. The technical work made it possible to understand and characterise the microbial behaviour in depth, while the design perspective shaped how we attended to its visual, aesthetic, and temporal qualities and how these might translate into meaningful applications. It also informed the ways we communicated these technical findings visually. In that sense, the interdisciplinary setting enabled work that neither discipline would likely have arrived at alone.



<< Cellulophaga lytica PLY-A2

manually spread shortly after inoculation

Part II

Exploring Human-Flavobacteria Engagement

Chapter 5

Direct Interactions with Flavobacteria

This chapter marks the transition from understanding Flavobacteria's temporal expressions to exploring human-Flavobacteria engagement by examining direct interactions with these vividly coloured bacterial colonies. These explorations build on the concept of living traces and expand on the design space outlined in Chapter 2 by investigating in more detail how Flavobacteria's expressions respond to direct human input.

While Chapter 2 primarily frames Flavobacteria as a living medium for interaction design, this chapter moves beyond that instrumental framing toward a more relational orientation. Although I still describe human actions as 'input' and microbial responses as 'output', the emphasis here lies on cultivating a personal and dynamic interplay between humans and microbes—towards living artefacts that foster attentiveness and care for microbial worlds and beyond. I also reflect on my personal experience of relationality with Flavobacteria in this chapter, which inspired the longitudinal study presented in Chapter 6.

To illustrate the interplay between humans and microbes, this chapter presents three detailed studies that explore direct interactions with Flavobacteria through pressing, tilting, and swiping. Two of these actions emerged from Chapter 2: swiping emerged from the design-space explorations, and tilting from the unexpected observation that plate orientation affects colony growth. The third action, pressing, builds on a method to induce dormancy in Flavobacteria, presented by an MSc. graduation student I supervised during this PhD[2].

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This work was presented at the CHI conference 2024 in Honolulu. The supplementary material for this chapter is available online at: https://dl.acm.org/doi/10.1145/3613904.3642262

[2] See thesis of Evy Murraij at: https://repository.tudelft.nl/record/uuid:84b6a75c-c367-496c-bb84-d5fedf9153a6

Abstract

HCI designers increasingly engage in the integration of microbes into artefacts, leveraging their distinct biological affordances for novel interactions. While in many explorations the interaction between humans and microbes is mediated, scholars also highlight the potential of direct interactions, such as visualising mechanical distortions or fostering a sense of relationality with nonhumans through eliciting intimate encounters. Seizing upon this potential, our study delves into the realm of direct interactions involving Flavobacteria, recently introduced as a colour-changing interactive medium in HCI. We present a design space for direct interactions where humans can (re)activate, (re)direct, and (re)arrange Flavobacteria's colourations, thereby fostering a personal and dynamic interplay between humans and microbes. With our work, we aspire to provide pathways and ignite inspiration among HCI designers to create living artefacts that cultivate active engagement and heightened attentiveness towards microbial worlds and beyond.

5.1 Introduction

Driven by multiple factors, such as increased mediation via technology, a lack of access to natural spaces, and diminishing ecological literacy [177], human-nature interactions are increasingly scarce [178–180]. Ironically, this alienation comes at a time when human connectedness to nature is presented as a leverage point for two of the most pressing issues of modern times: the environmental crisis [181, 182] and human well-being [183].

Within HCI, the importance of designing for human well-being has long been recognised [184], while more recently scholars have also called for the widening of practices to navigate the complex challenge of sustainability [11–13]. Demonstrating the potential to attend to both, biodesign approaches in HCI [44, 51] facilitate interactions with and draw our attention to the usually hidden world of microorganisms (e.g. [185]), which may yield benefits for ourselves and the wider natural world. The integration of living microbes into interactive systems is a growing area of interest for HCI and design researchers. Organisms have, for example, been embedded in sensing devices [59, 186], ambient displays [63, 65], games [57, 58], and interactive installations [54, 55], in which novel functionalities and interaction possibilities are achieved through organisms' distinct biological affordances. In most of these interactive systems, interaction between humans and microorganisms is indirect, as human input is translated into a specific stimulus that is known to affect living microorganisms. Such mediation helps tackle challenges of control, accuracy, longevity, and bio-safety for interactive living systems [37, 45]. Yet, HCI designers show increasing interest in exploring more direct interactions with microbes, stressing their significance for establishing reciprocal relationships [42] towards a *culture of life* [159] instead of using microorganisms strictly as *controlled media* [70].

5.1.1 Direct interactions with living microbes

The potential for profound connections with nonhuman entities through direct interactions between humans and living microorganisms has been widely acknowledged [70, 185, 187, 188]. These interactions foster intimate experiences that, in turn, amplify relational dynamics and empathy [17, 189], particularly when the intricate living aesthetics of microbes (i.e., their temporal expressions [42]) are perceivably influenced by human interactions. This relationality holds the potential to develop sensibilities for non-human temporalities and needs [164, 190], while affording reflection on our own everyday practices through a patina of *living traces* [133]. Ultimately, these reciprocal engagements hold potential for increased ecological awareness and a deeper appreciation for the intricate web of life that surrounds us [18]. Acknowledging the potential for intimate engagements facilitated by direct interactions with living microorganisms, our objective is to explore and dissect this design realm further. We initiate this exploration by explaining what we mean by 'direct interactions with microbes'. Given the relatively nascent nature of

microbial-HCI [51] as a research endeavour, we adopted a broader perspective to screen through literature pertaining to direct interactions involving other living entities (i.e., plants) or entities with lifelike attributes (e.g., smart materials). This approach aided us in refining our description.

Looking first within the field of microbe-HCI, Ofer et al. [70] describe direct interactions as unmediated and physical interactions in which microbes respond to kinetic stimuli within designed environments. At the larger scale of living plant interfaces, Chang et al. [191] consider other inputs, in addition to touch, through which humans can directly interact with living organisms, for example, gestures and voice. Yet, by their definition, a direct interaction between plants and humans can be both locally situated in close proximity and occur remotely through a connected interface. In the context of shape-changing interfaces, Rasmussen et al. [118] classify direct interactions as those that employ shape-change as both input and output, which can again be experienced locally or remotely. Finally, we looked into Lim et al.'s [192] paper on interaction gestalt, which helped us get a better understanding of how interaction attributes, such as *directness* and *proximity*, can define direct interactions with microbes.

Drawing from these existing works in HCI and extensive discussions within our team, we have formulated the following definition to serve as a guiding framework for our research, as presented in this paper: **Direct interactions with microbes refer to instances where humans act upon living artefacts through their own bodies (e.g., touch, movement, voice, etc.), thereby inputting signals to microorganisms in an unmediated way. In response, these microorganisms adapt their behaviour, often manifesting changes in their living aesthetics (e.g., alterations in colour) that are perceivable by humans in close proximity.**

Given the immense potential inherent in direct interactions with microbes, it becomes evident that HCI stands to benefit from a design space outlining a comprehensive spectrum of possibilities within this context. With that aim, this research contributes to the HCI community by inspiring and outlining the possibilities of direct interactions with living microorganisms through our explorations with Flavobacteria. Recently introduced as a colour-changing interactive medium in HCI [133, 134], these bacteria show great variety in their colony's form, texture, and iridescent colour as well as in their stimulus response time (i.e., immediate and delayed) and are affected by direct human input. Specifically, our goal is to further explore direct interactions with Flavobacteria by (re)activating, (re)arranging, or (re)directing their living colour. Through this exploration and our in-depth discussion, including a reflection from an ecological perspective, we aim to provide inspiration to HCI designers, opening up new and innovative avenues for interacting not only with Flavobacteria but also with other microorganisms.

5.2 Related Work

5.2.1 Human-nature engagement in HCI

Humans possess an innate affiliation with living systems—biophilia [193], which fosters a deep sense of connection linked to improved mental health, stress reduction, and enhanced cognitive abilities [194–197]. In addition to these positive effects on humans' well-being and functioning, nature engagement can increase our ecological awareness and sense of responsibility towards the natural world [198]. Yet, technological development in the industrialised world seems to have gradually removed people from nature [199, 200]. Digital technologies play a significant role in this phenomenon and therefore contribute to catastrophic effects such as human depression, loss of emotional affinity to nature, and a decline in pro-environmental attitudes and behaviours [179, 180].

Aiming to restore this balance, HCI researchers have identified many possibilities for public (re)engagement with nature through technologies [201], ranging in distance from nature (i.e., in situ versus ex situ) and in their directness of experience (i.e., directly or indirectly, e.g., through mediation). Mediated nature experiences allow for temporal compression of ecological processes and the capture of otherwise imperceptible phenomena, as, for example, Gaver et al.'s [202] DIY wildlife camera, which makes humans more aware of and ultimately concerned with local wildlife. Direct experiences, on the other hand, might have a greater capacity for enriching encounters, fostering a deeper sense of connection and appreciation for nature. Researchers have, for example, explored how computation might help us connect to and care for nature through inspiring a new wave of forest technology [203].

Challenging the ingrained idea of an ontological separation between humans and nature, Haraway [189] introduced the notion of *natureculture*, which recognises that humans are deeply entangled in complex relationships with other species and ecosystems. Puig de la Bellacasa [204] further explores this idea by emphasising the ethical responsibilities and actions that arise from these interconnected naturecultures. Likewise, Escobar's work on pluriversal approaches [205] emphasises the significance of diverse worldviews and engagement with non-human entities. Tsing [206] amplifies the conversation by advocating for the recognition of agency within the realms of non-human actors—be they plants, animals, or microbes. Through this lens, we can develop more sustainable and inclusive approaches to environmental stewardship.

These concepts of ongoing interspecies entanglements have been adopted by HCI communities, shifting attention from a human-centred agenda to a multispecies worldview [207]. Researchers within this community challenge us, for example, to design for cohabitation [69, 208], engage in symbiotic encounters [209], and become more attentive towards the more-than-human world [11]. Rodgers et al. [210] presented an approach to imagining such sustainable futures, seeking to highlight relations between people and nonhuman stakeholders. Reflecting how interactive artefacts can open new

pathways for noticing and engaging with other species, Liu et al. [211] have introduced the concept of *collaborative survival* within the context of mushroom foraging, which brings us to the field of microbe-HCI.

5.2.2 Human-microbe interaction in HCI

Focusing on the microscopic lifeforms of our natural world, designers and researchers within the field of bio-HCI [37, 45] explore the unique temporal qualities of microorganisms for novel interaction possibilities between humans and microbes. In the case of Liu et al.'s [211] multisensory tools for noticing fungi, humans are invited to interact with microbes in situ (i.e., at the microbe's original location). Numerous other concepts within microbe-HCI [51] concentrate on engaging with microbes outside their natural setting and incorporating them into interactive artefacts, which Merritt et al. [45] characterise as living media interfaces. *Rafigh* [65], for example, encompasses a living mushroom colony, motivating children to do speech exercises as data on usage of a digital app is used to regulate the interface's irrigation system.

Even though, in many of these instances, living microorganisms are mainly presented as controlled media [70], researchers increasingly promote the establishment of a culture of life, raising critical questions about mutualism, care, and cohabitation [42, 70, 159]. Zhou et al. [164] have, for example, introduced a temporal-aligning interface for timely noticing cyanobacteria behaviour, fostering reciprocal human-microbe relations in everyday scenarios. Ofer et al. [70] have delved into unmediated and physical interactions with bioluminescent algae while introducing an organism-centred approach to preserve the organism's livingness. Both of these works extend invitations for humans to engage in direct interactions with microbes, emphasising opportunities for more intimate and reciprocal relationships with them. This is particularly evident in the context of how humans can influence the living aesthetics [42] of microbes. Harnessing this opportunity, researchers have, for example, explored possibilities for visualising direct interactions (e.g., bending, twisting, and stretching) through encapsulating bioluminescent algae in soft chambers [212] and developed a DIY shaking device to explore diverse ways humans can provide direct input for living light output [61].

Zooming further into the design of living artefacts for direct interactions with microbes, Ofer et al. [70] distinguished open, porous, and closed environments to physically interact with bioluminescent algae. While a closed environment might be beneficial in safeguarding the well-being of microorganisms and enhancing our sense of safety and acceptance towards such living artefacts, it may potentially diminish the intimacy of the experience. Open environments, on the other hand, such as *Nukabot* [62], which invites humans to stir a symbiotic culture of microbes by hand on a daily basis, foster affective relationships between humans and microbes through physical contact. Along the same lines, a number of researchers have delved into the kombucha fermentation practices, wherein humans and nonhumans engage in a notably direct manner with one

another in open environments. Researchers have, for example, investigated this practice as a platform to recognise relationality with nonhumans through collective reflection with kombucha brewers [188] or presented a range of probes for direct sensory engagement with this biofilm made by bacteria and yeast, aiming to reveal the interconnectedness between human- and nonhuman-designers [187].

Exploring another facet of the potential within direct interactions with microbes, Groutars and Risseeuw et al. [133] discussed how structurally coloured Flavobacteria can capture the peculiar ways in which humans interact with objects and spaces through their distinct temporal expressions, namely *living traces*. Besides insights into our own behaviour, such a patina of living traces offers a unique perspective on the intricate relationships between humans and the microbial world. Highlighting such relationships can aid in understanding that humans and nonhumans are inextricably interconnected.

Overall, the perceptibility and promptness of response have been discussed by researchers at times (e.g., [70]), highlighting the need to mediate the “output" of some microorganisms to effectively unveil shifts in their behaviour [134]. For example, the behaviour of cyanobacteria was manifested through colour shifts in electrochromic material triggered by their photosynthetic activity [164]. Adopting digital avenues, the well-being of fermentation bacteria was translated into voice interactions and blinking patterns [62], while the electrons generated by microbial fuel cell biofilms enabled the creation of artistic animations [159]. Intervening in microbial responses can serve to address ’temporal dissonance’ [164] between humans and microorganisms, aiding in exposing the well-being or struggles of microorganisms [134] for timely care practices. Nevertheless, excessive mediation might detract from the experience, much like the way unmediated interactions with nature in general tend to offer more enriching encounters than mediated ones [213].

5.2.3 Flavobacteria as an interactive medium

Flavobacteria species, in particular *Cellulophaga lytica* (*C. lytica*), have recently been introduced as an interactive living medium for HCI [133] due to their ability to produce dynamic colourations when growing on a surface (Fig. 5.1). These bacteria can organise their cells into so called photonic crystals [21, 22, 25], which interact with light and create striking visual effects akin to butterfly wings. While the exact reason behind this behaviour is, to date, unknown, microbiologists have revealed a lot of insights over the last few decades on Flavobacteria's ability to self-organise. Those include the factors that affect this organisation, such as temperature [21, 175], different substrates [29] and the presence of other microbes [23], as well as the associated genetic pathways [25]. Doing so, they identify opportunities for engineering living optical materials such as sustainable paints and living sensors [25].

In the context of HCI, Groutars and Risseeuw et al. [133] explored Flavobacteria's temporal qualities and responsive behaviour, focusing on the

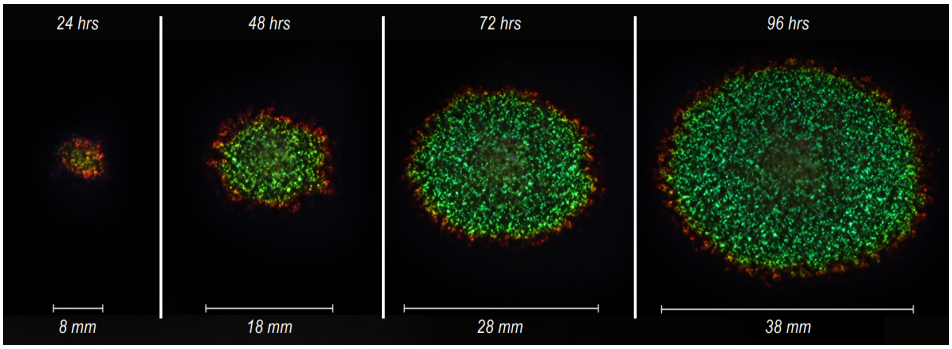


Fig. 5.1 *C. lytica's growth over four days, expanding around 5mm in radius a day and displaying a variety of colours, ranging from violet and red to more dominant green hues. Image credits: [169].*

changes in their expression, i.e., living aesthetics. They introduced various ways to tune Flavobacteria's living aesthetics, such as through environmental stimuli and "direct human input"—in which they explored different techniques of applying bacteria and physically altering the colonies' growth. This work highlights the potential of Flavobacteria for Living Colour Interfaces (LCIs), which can embody digital and environmental data as well as enable playful interactions with living media.

To further support HCI designers in exploring Flavobacteria's living aesthetics, Risseeuw et al. [169] presented *FlavoMetrics*, a digital tool that enables designers to virtually inoculate Flavobacteria and manipulate stimuli to tune the living colour in a digital environment. Kim et al. [134] explored how different mechanisms could aid in surfacing Flavobacteria's livingness towards timely noticing Flavobacteria's changes in behaviour and enriching how we interact with them.

Building upon the foundation laid by these HCI studies, we aim to elaborate on different ways to influence Flavobacteria's living aesthetics as well as further unpack direct interaction possibilities with these vividly coloured bacterial colonies. We believe Flavobacteria provides an exceptional case for exploring the landscape of direct interactions with microorganisms due to the striking transformations in their colony's form, texture, and iridescent colour. Furthermore, Flavobacteria exhibit a spectrum of response times to stimuli, including both immediate and delayed reactions, offering a rich design space for HCI designers, which we aim to commence with the initial set of explorations in our paper.

5.3 Design Space of Direct Interactions with Flavobacteria

5.3.1 Our approach

Focusing on the distinct qualities of Flavobacteria, our research is guided by our curiosity and fascination with these microorganisms. Over the course of three

years, we embraced a material-driven design approach [46], which prompted us to centre our attention on the distinct characteristics and affordances of these microorganisms while exploring potential user experiences and interactions. We started our explorations by carefully considering the microorganisms' needs and exploring their habitat elements [42, 70, 214]. In an attempt to open up the design space of directly engaging with these microorganisms, we then proceeded to explore Flavobacteria's unique colour-producing mechanism (i.e., structural colour as a means of cell organisation) and elements that affect their living aesthetics.

Our exploratory studies were conducted in our biolab at the TU Delft Faculty of Industrial Design Engineering, where we had access to specialised equipment for cultivating living microorganisms. We worked with *C. lytica* PLY A2, which we cultivated at room temperature. This non-pathogenic bacterium originates from marine environments and is known for its brilliant structural colourations [24, 133]. The habitat we designed to support Flavobacteria's growth included a semi-solid surface created with marine agar medium [133], enabling them to thrive and form optical structures. All the experiments were conducted with sample triplets for reliable results, except for one of our studies in Section 5.4 (as elaborated on in the discussion).

We used photography and videomaking to capture the living aesthetics of Flavobacteria in response to our direct interactions with them. This allowed us to reflect on the particular effects of these interactions on the microbes' behaviour and analyse the living aesthetics in terms of form, texture, and iridescent colour, leveraging the vocabulary introduced by Groutars and Risseeuw et al. [133]. We used a Canon EOS 250D camera equipped with a macro lens and ring flash and aimed for capturing the colonies with an incident angle of 45°, from which they have been shown to appear the most brilliant and multi-coloured [133].

We documented events in the biolab as well as our experiences when interacting with and observing the microorganisms. Through this documentation of the first author, we were able to record the nuances and intricacies of our personal experience while interacting with Flavobacteria in the three presented studies (see Appendix B), which we used to support our application concept ideation.

5.3.2 Basic habitat architecture of Flavobacteria artefacts

Living artefacts that integrate Flavobacteria consist of a habitat enclosure, the microorganism(s), and growth medium (Fig. 5.2). The habitat enclosure can vary in *shape* and *size* as well as in properties related to the material, such as *flexibility*, *porosity*, and *transparency*. Considering these properties and the desired interactions, designers can, for example, create a more open or closed environment [70] for the microorganism or tap into materials' performative qualities [47], for example, by creating a flexible habitat from silicone [133] to invite humans to bend the artefact. The microorganism(s) can vary in their *genotype* (i.e., which species or strain(s)), *culture condition* (i.e., active or

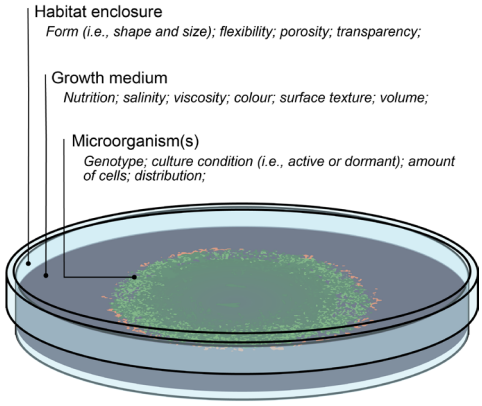


Fig. 5.2 *Basic habitat architecture and properties of Flavobacteria artefacts.*

dormant), as well as their *amount* and *distribution* within the habitat. Regarding the growth medium, the ideal *nutrition*, *salinity*, and *viscosity* highly depend on the species. The agar medium [133] used in this work, for example, provides the optimal amount of nutrients, salt, and agar for *C*. *lytica* to thrive and produce structural colour. Additionally, designers can vary the growth medium's *colour* (e.g., adding black pigment to highlight Flavobacteria's colour), *surface texture* (e.g., creating texture to steer Flavobacteria's growth [133]), and *volume*, which mainly influences the temporal scale of the living artefact.

5.3.3 Interaction primitives for direct interactions with Flavobacteria

Acting upon Flavobacteria artefacts through their own bodies, humans can directly interact with Flavobacteria through different input mechanisms (i.e., stimuli that affect microorganisms' living colour). These include salinity, oxygen, nutrients, humidity, temperature, exposure to other microbes, and mechanical force (Fig. 5.3a). The first five were derived from considering the microorganisms' needs, resulting in a range for each of these stimuli that is essential for Flavobacteria's livingness and/or structural colour. The last two factors, exposure to other microbes and mechanical force, were deduced by considering Flavobacteria's responsive behaviour. Mechanical force, in particular, boasts a broad spectrum as an input mechanism due to its capacity to physically interfere with Flavobacteria's optical structures as well as guide the bacteria to different conditions (e.g., nutrients).

Several examples of human input are included in the design space (Fig. 5.3b), which are categorised into body liquid and airflow, touch, and movement. The relationships between these human inputs and the input mechanisms are highly dependent on the basic habitat architecture. The porosity of the habitat enclosure, for example, influences whether touch allows Flavobacteria to interact with other microbes. We speculated on Flavobacteria's potential response to the examples of human input (visualised in Figure 5.3b) based on

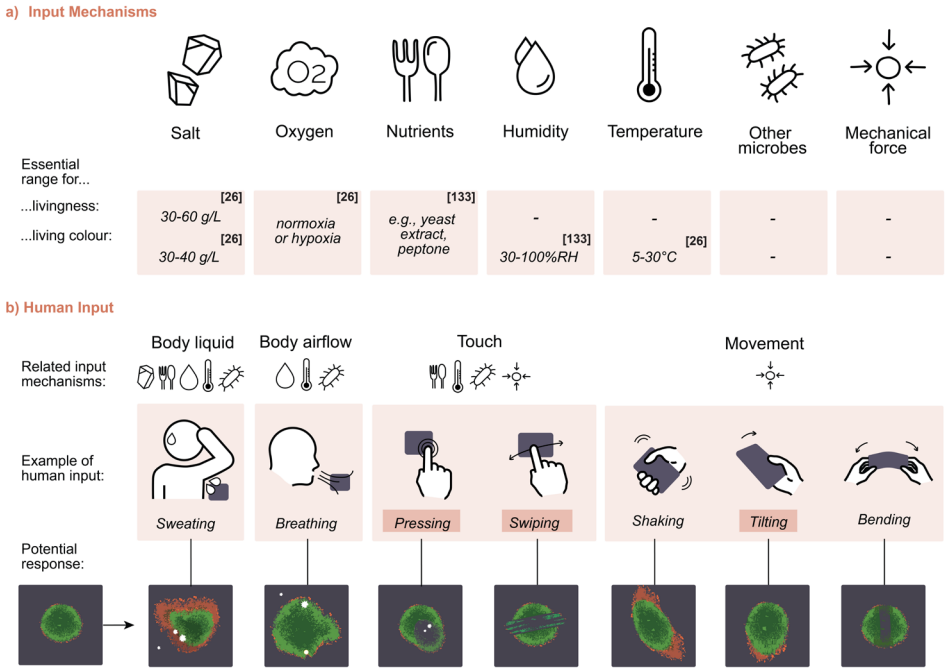


Fig. 5.3 *Interaction primitives for direct interactions with Flavobacteria: a) input mechanisms, including the essential range of conditions for C. lytica's livingness and living colour; b) examples of human input with icons indicating their relation to the input mechanisms, red markings to show human inputs selected for further exploration, and Flavobacteria's potential response, in which white dots indicate other microbes.*

prior works [26, 133] and our extensive experience with these microbes, yet none of these outputs had at this time been shown in a systematic study. This opens up an exciting research avenue for HCI, which we aim to inaugurate with the initial set of explorations in our paper.

5.3.4 Interaction mechanisms

We distinguish three ways in which humans can interact with Flavobacteria's living colour: (re)activate, (re)direct, and (re)arrange. These are presented in Figure 5.4.

Through the first interaction mechanism, humans can activate Flavobacteria's living colour as their input triggers the bacteria's cells to organise into optical structures that interact with incident light. This occurs when the necessary conditions for living colour are created as a result of the input, for example, when inoculating Flavobacteria from liquid freezer stock (-80°C) to moist, nutritious agar medium at room temperature. On a macro level, the resulting cell organisation over time culminates in the observable emergence of living colour. Upon the fading of iridescence over time, Flavobacteria's living colour can be

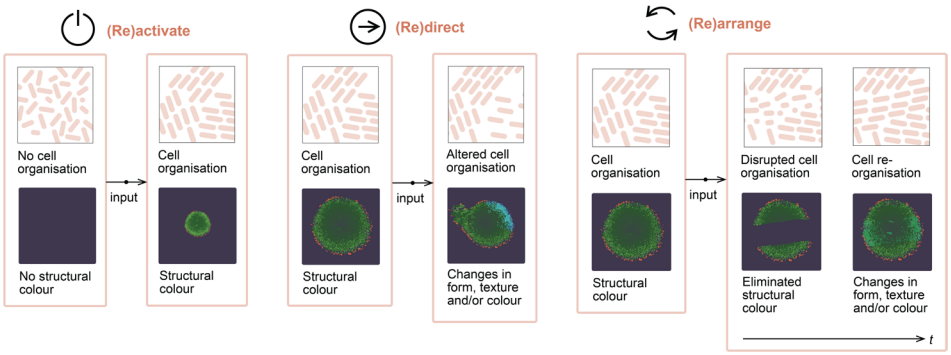


Fig. 5.4 *Mechanisms for interacting with Flavobacteria's living colour: (re)activate, (re)direct, and (re)arrange, showing changes on the micro (top) and macro (bottom) levels.*

reactivated by once again establishing the necessary conditions, in contrast with the prior assumption that Flavobacteria are dead as the iridescence fades away [133].

Through the second interaction mechanism, Flavobacteria's living colour is (re) directed as input changes conditions, such as humidity [133], that affect how the cells organise themselves into optical structures. These changes prompt Flavobacteria to arrange their cells in a different way and/or expand their colony in a particular direction, leading to distinct changes in the colony's form, texture, and iridescent colour. Thirdly, humans can interact with Flavobacteria by (re) arranging their living colour. Here, the input physically interferes with the optical structures of the microorganisms, disrupting their cell organisation, upon which the cells will reorganise themselves in a different way over time. On a macro level, this results in the elimination of living colour and, later on, surprising colourations [133].

5.3.5 Selection of interaction primitives for further exploration

To illustrate how this design space can inspire a broad array of direct interactions, we selected three human actions to be further explored in our study, to either (re)activate, (re)direct, or (re)arrange Flavobacteria's living colour. We opted for the human actions of pressing, tilting, and swiping as they closely mirror the ways in which humans engage with everyday objects in HCI interactions (e.g., pressing buttons, tilting laptop screens, swiping touchscreens). Groutars and Risseeuw et al. [133] touched upon the effect of swiping movements that interfere with Flavobacteria's optical structures. Nevertheless, the microorganisms' response had not been fully explored, triggering our interest and curiosity. Besides, we were inspired by previous observations of Flavobacteria growing in a static vertical habitat [133], prompting our interest to investigate how tilting as a human action can (re)direct Flavobacteria's living colour.

5.4 Three Studies into Direct Interactions with Flavobacteria

In this section, we present our three lab studies in which we explored direct interactions to (re)activate, (re)direct, and (re)arrange Flavobacteria's living colour. We investigated Flavobacteria's response in relation to the human inputs of *pressing*, *tilting*, and *swiping*, aiming to identify the changes in the microbes' living aesthetics. For bio-safety reasons, we focused on the input mechanism of mechanical force only (i.e., not including the effect of other microbes). Focusing on one input mechanism at a time enabled us to get a better understanding of the input and output relations. Although the following three studies all revolve around the same input mechanism, they distinctly showcase the diverse spectrum of direct interactions it facilitates with Flavobacteria. We discuss the interaction procedure and the response of Flavobacteria for each study in detail, as well as potential HCI applications.

5.4.1 Study 1: (Re)activating through pressing

Procedure

For our explorations into (re)activating Flavobacteria's living colour through pressing, a standard-sized Petri dish with agar medium was used as a habitat. We designed a flexible lid by laser cutting a hole in the standard rigid lid and sealing it with thin plastic foil. After sterilising the flexible lid with UV light, Flavobacteria were applied to the inside surface of the plastic foil. To extend bacterial survival in this environment without nutrients, Flavobacteria were first induced into a dormant state through lyophilisation. This method, also called freeze-drying and commonly used in microbiology, inhibits microbial growth by removing water from the culture under low pressure. To protect the bacterial cells during this process, we first suspended them in a solution with a protective agent (i.e., sucrose).

The experiment of the *pressing* study consisted of two parts (Fig. 5.5). In the first part, aimed at *activating* Flavobacteria's living colour, dormant bacteria were exposed to agar medium to rehydrate and revive them. To do so, pressure was applied to the flexible lid until the Flavobacteria touched the nutritious, semi-solid surface (Fig. 5.6), mimicking the gentle touch of the inoculation process. In the second part, seven days later, Flavobacteria's living colour was *reactivated* by providing new territory to the colonies as they began to lose their

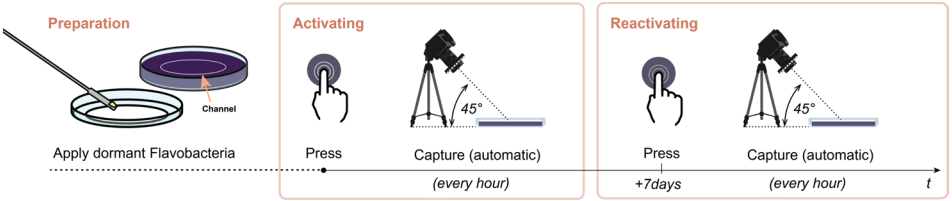


Fig. 5.5 *Illustrated procedure of the pressing study.*

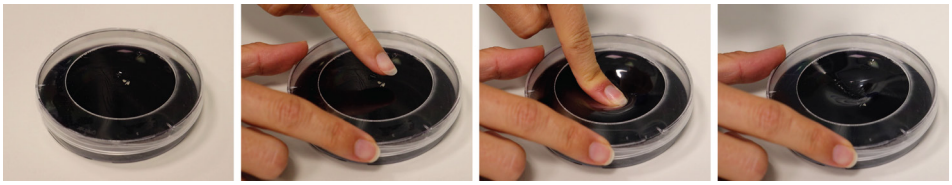


Fig. 5.6 *Applying pressure to the lid to activate Flavobacteria's living colour.*

iridescence. When preparing the habitat for this study, different compartments were made in the agar medium using circular cookie cutters. This allowed us to build a barrier, created by a small channel, ensuring that the bacteria initially only expanded within the first area. When the first area was colonised and faded in colour, pressure was once again applied to the centre of the flexible lid, allowing the bacteria to cross the channel and produce structural colour again in the new territory.

Response of Flavobacteria

After activation, Flavobacteria produced a familiar colony, i.e., expanding in a circular shape, ranging in colour from violet and red to more dominant green hues. However, whereas structural colour typically becomes apparent within 24 hours after inoculation, the colourful colony did not emerge until two days later (Fig. 5.7). This is probably due to adaptation to new conditions. The colony expanded until it reached the small channel in the agar medium on day 5 and obtained a more hollow shape over time as its iridescence gradually faded away from the centre. After applying pressure for the second time, seven days after activation, we could see Flavobacteria adhering to the plastic foil through their inherent yellow pigment [173] (Fig. 5.8).

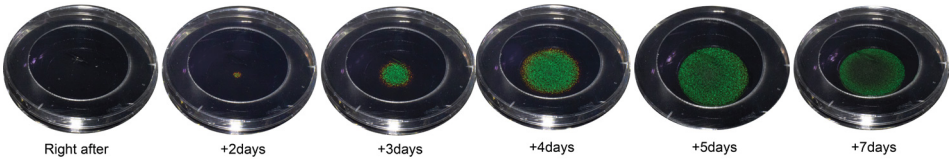


Fig. 5.7 *Overview of Flavobacteria's living colour after activation. See Supplementary Material, Section A.2, for details on the camera setup and the complete overview of images.*

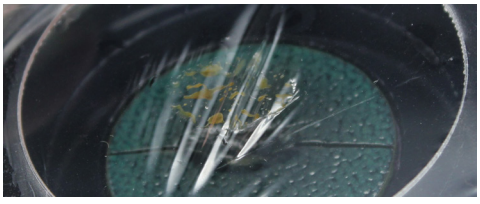


Fig. 5.8 *Flavobacteria on the plastic foil after applying pressure to reactivate their colour.*

Five hours after the reactivation, it became apparent that Flavobacteria started producing structural colourations in new areas (Fig. 5.9). Again, the colony's texture and iridescent colour developed in familiar ways. However, the form was no longer circular because the bacteria were irregularly pushed to the new area, initially forming separate colonies that seemed to merge over time.

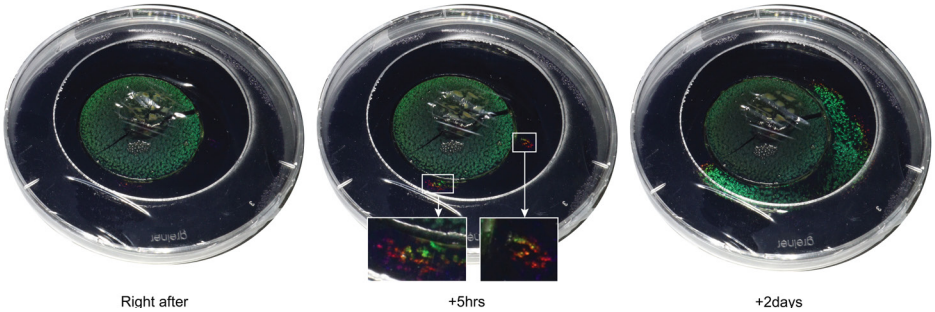


Fig. 5.9 *Overview of Flavobacteria's living colour after reactivation, with close-ups showing first signs of structural colour after five hours. See Supplementary Material, Section A.2, for details on the camera setup and the complete overview of images.*

5.4.2 Study 2: (Re)directing through tilting

Procedure

Flavobacteria's living colour is affected by the orientation of the agar medium, as can be concluded from initial observations of growth in a vertical habitat presented in [133]. Yet, this responsive behaviour only comes to light on a large spatial and temporal scale. For our explorations into (re)directing Flavobacteria's living colour through tilting, we therefore created an environment for the microorganisms to thrive for up to one month. The habitats (Fig. 5.10) offered a 240 mm by 240 mm surface of agar medium and a relative humidity of 95% to ensure hydration. In prior work [133], the habitat was tilted 90 degrees, yet we wondered whether a smaller tilt—which could be the case in everyday interactions with HCI objects—would be adequate to affect Flavobacteria's growth. We opted for 45 degrees and laser-cut a base to support tilting the habitat.

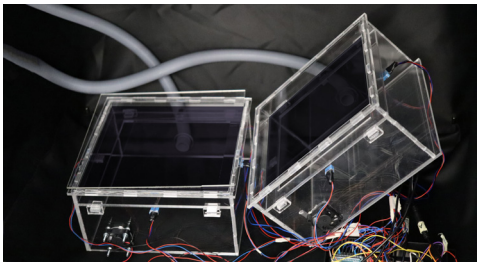


Fig. 5.10 *Two custom-made habitats for the tilting study. The figure shows the setup of the first experiment on day 1.*

The first experiment of this exploration (Fig. 5.11a) evolved around *directing* Flavobacteria's living colour in a certain direction. The bacteria were inoculated in the designed habitat, which, one hour later, after allowing the Flavobacteria culture to settle, was tilted 45 degrees by carefully placing it on the base. Flavobacteria were also inoculated in a second habitat, which was placed in a horizontal position, as a control group.

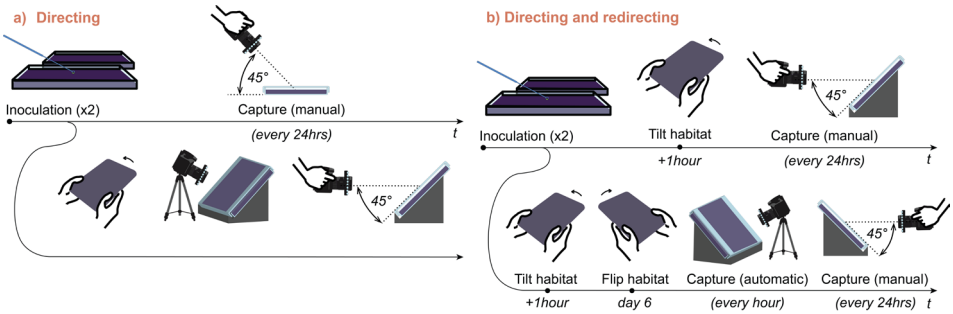


Fig. 5.11 *Illustrated procedures of the tilting study's first (a) and second (b) experiments.*

In the second experiment (Fig. 5.11b), our aim was to *redirect* Flavobacteria's living colour. This time, both habitats were tilted 45 degrees one hour after inoculation. After six days of growth—when there was still plenty of available agar medium for bacterial growth—one of the habitats was carefully flipped the other way (Fig. 5.12).

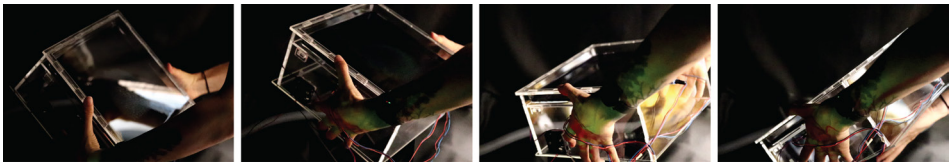


Fig. 5.12 *Flipping the habitat on day 6 during the second experiment, captured with the automated camera.*

As it proved challenging to capture Flavobacteria's iridescent colour in both habitats simultaneously, the automated camera was only used to extensively capture the microorganisms that we interacted with (in experiment 1) or those we interacted with repeatedly (in experiment 2). It was positioned at an approximate 45° angle with the habitat surface (see left image of Figure 5.12) to capture the distinctively brilliant colours of the colony. To analyse differences between the colonies, both habitats were also captured manually from the same angle and distance (i.e., circa 30cm) every weekday.

Response of Flavobacteria

In the first experiment, colonies in the tilted and horizontal habitats displayed remarkable differences in their texture and form, proving that a tilt of 45 degrees was adequate to affect Flavobacteria's growth. As can be seen in Figure 5.13,

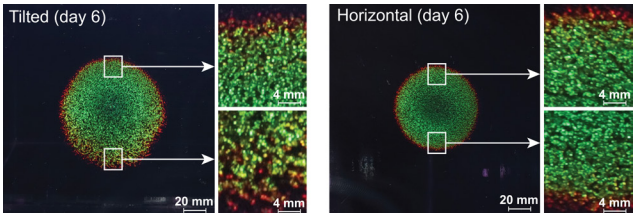


Fig. 5.13 *Texture differences between Flavobacteria's living colour in the tilted (left) and horizontal (right) habitats on the sixth day of growth. Pictures are perspective-corrected and 5x magnified in closeups on the right.*

Flavobacteria in the tilted habitat created different colony textures in the top and bottom areas, whereas bacteria in the horizontal habitat demonstrated a more uniform texture. Only six days after the tilting movement, these subtle differences in texture became noticeable.

The overview in Figure 5.14 shows how the colonies further developed over time, illustrating the difference in the colonies' form, which becomes noticeable around days 7/8. Whereas bacteria in the tilted habitat managed to extend their colourful colony to the bottom edge of the habitat in nine days, bacteria in the horizontally orientated habitat only started to reach the edges around day 15. At this time, the bacteria in the tilted habitat also reached the top part of their habitat. These differences in colonies' texture and form are probably caused by the moisture that escapes from the agar medium [215], easing the microbes' gliding motility in a downward direction.

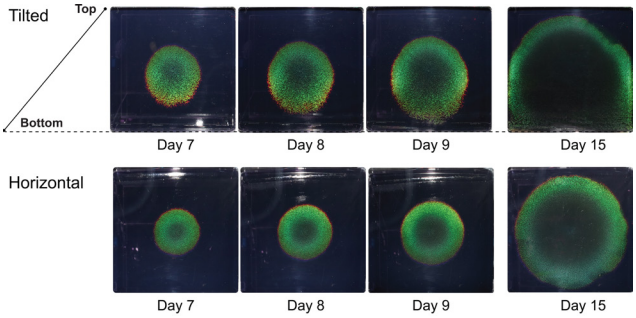


Fig. 5.14 *Overview of Flavobacteria's growth in the tilted (top) and horizontal (bottom) habitats, showing perspective-corrected images. See Supplementary Material, Section A.3, for details on the camera setup and the complete overview of images.*

In the second experiment, both colonies demonstrated similar differences in texture between the top and bottom areas (as seen in the first experiment) until one habitat was flipped on day 6. Over the following six days, new bacteria in the flipped colony started to form structural colour correspondingly: the (now) bottom area started to show a more scattered texture, while the (now) top area transformed to a dense texture (Fig. 5.15).

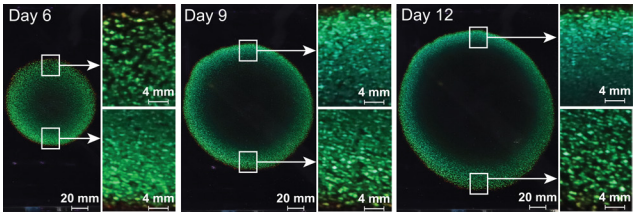


Fig. 5.15 *Adjustment of texture differences within the flipped colony. The picture on day 6 was taken immediately after flipping. Pictures are perspective-corrected and 5x magnified in closeups on the right.*

Whereas the static-tilted colony was the first to hit the bottom of its habitat, the flipped colony started to adjust its form to its new orientation in a similar manner over time (Fig. 5.16).

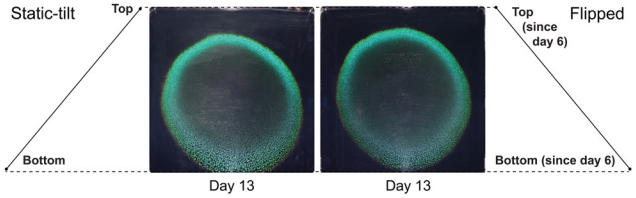


Fig. 5.16 *Flavobacteria's perspective-corrected growth in the static-tilted (left) and flipped (right) habitats, illustrating how the static-tilted colony (left) hit the bottom of its habitat first. See Supplementary Material, Section A.3, for details on the camera setup and the complete overview of images.*

5.4.3 Study 3: (Re)arranging through swiping

Procedure

For these explorations into (re)arranging Flavobacteria's living colour through swiping movements, we used a standard-sized Petri dish with agar medium as a habitat. Contrary to the first study, we did not opt for a flexible plastic foil as a lid, as bacteria adhering to it might compromise our observations of Flavobacteria's response. Instead, we performed the swiping in a sterile environment within the biolab (i.e., a laminar airflow cabinet), wearing a sterile glove to separate Flavobacteria from the human microbiome.

The first experiment of this exploration (Fig. 5.17a) evolved around *arranging* Flavobacteria's living colour. Here, we interacted with the bacteria by swiping only once (Fig. 5.18), three days after inoculation.

In the second experiment (Fig. 5.17b), the same swiping movement was repeated five times with an interval of 30 minutes. Again, this interaction took place three days after inoculation.

Iridescent Flavobacteria are known to reorganise their cells when their organisation, producing structural colour, is interfered with [133]. However, we

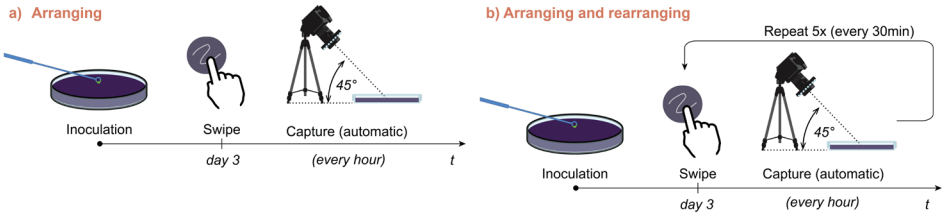


Fig. 5.17 *Illustrated procedure of the swiping study's first (a) and second (b) experiments.*

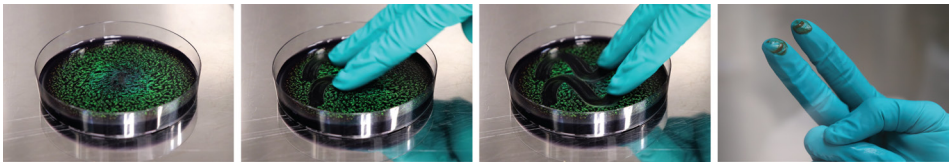


Fig. 5.18 *Swiping the bacteria on day 3. As can be seen in the left image, the colony's growth is remarkably advanced for the third day, which was caused by a heat wave over the weekend. The right image shows some transference of Flavobacteria to the glove.*

were unsure how quickly this response could be observed by the naked eye. In our first attempt to understand this response time, capturing Flavobacteria with a commonly-used time interval of one hour proved, to our surprise, not to be frequent enough to capture the first signs of response. With further testing, a time interval of 10 minutes was selected for the rest of our experiments.

To get a better understanding of the effect of swiping on the colony's iridescent properties, we additionally captured a sample from several angles—before and 30 minutes after swiping—using a custom-made tool (based on [133]).

Response of Flavobacteria

Flavobacteria's iridescent colour is instantly eliminated when swiping through the optical structures (Fig. 5.18), as the cell organisation is disrupted. Colourations in these areas noticeably reappear after 20 minutes and become clearer over time (Fig. 5.19). After swiping, the affected areas no longer exhibit a pointillistic texture but instead show a very uniform, remarkable colour, which

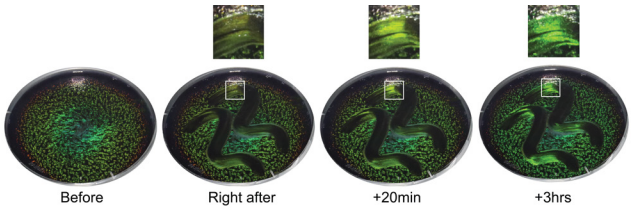


Fig. 5.19 *Overview of Flavobacteria's response to swiping movements over time, including a close-up showing the uniform texture after swiping. See Supplementary Material, Section A.4, for details on the camera setup and a complete overview of images.*

is observable from specific angles (Fig. 5.20). This observed phenomenon likely results from the swiping movement aligning all cells in the direction of the motion, thereby influencing their reorganisation.

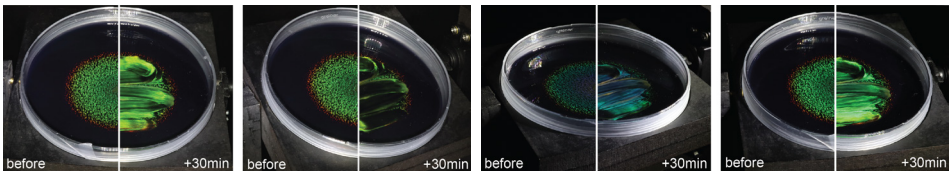


Fig. 5.20 *A colony of Flavobacteria captured from different angles before and 30 minutes after a swiping movement, demonstrating the effect of swiping on the colony's texture and iridescent colour. See Supplementary Material, Section A.4, for details on the camera setup.*

During the second experiment, in which we interacted with Flavobacteria up to five times, the bacteria persistently reorganised into optical structures, recolouring the swiped areas (Fig. 5.21). Yet, some areas (as indicated in Figure 5.21) exhibited less vivid colour over time.

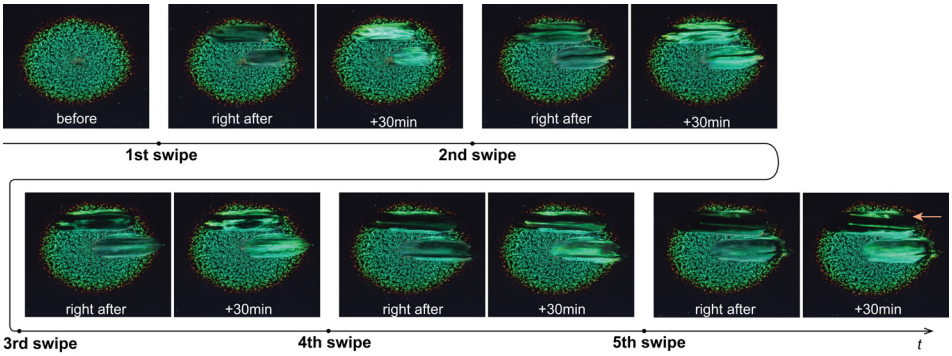


Fig. 5.21 *Overview of Flavobacteria's response to repeating swiping movements, showing a colony before, right after, and 30 minutes after each swiping. The arrow indicates the area that exhibited less structural colour over time. See Supplementary Material, Section A.4, for details on the camera setup.*

5.4.4 Application concepts

Our explorations into direct interactions with Flavobacteria through pressing, tilting, and swiping highlight their potential to act as a *qualitative display* [216] through a diverse range of grown patina affected by human input, i.e., living traces [133]. This potential can be harnessed to invite humans to reflect on everyday practices while building awareness and sensitivities towards Flavobacteria's unique temporalities, needs, aesthetics, and conditions in shared habitats. Below, we present three application concepts to ignite novel research directions in this context.

In our concept, *FlavoToile* (Fig. 5.22), Flavobacteria are integrated into a dress, foregrounding the history between humans and their garments. This could enhance emotional attachment as well as provide insights into our body posture for better self-care practices through empathy towards microbes. Additionally, Flavobacteria's living colour can elevate the aesthetic appeal of these garments, adding a unique and artistic element to the wearables while also encouraging individuals to develop sensibilities towards microbes and embrace the integration of nature in their daily lives.

Fig. 5.22 *Visualisation of our concept FlavoToile: a) a kid asking for attention from the mother by touching her dress, thereby unintentionally (re)arranging Flavobacteria's living colour; b) Flavobacteria revealing the touch as their living colour is instantly eliminated.*

On a larger scale, direct interactions with Flavobacteria could capture the peculiar ways that people interact with their surroundings. In our concept, *FlavoTread* (Fig. 5.23), the pressure from the footsteps of people walking through a public space activates Flavobacteria's living colour. Similar to the *Drift Table* [217], such an artistic, interactive surface could support ludic engagement in the everyday, driven by curiosity, exploration, and reflection rather than predefined tasks. It will bring a highly artistic element to public spaces and encourage people to notice, acknowledge, and reflect upon the temporal beauty that the microbes of our natural world have to offer. Additionally, the performative, dynamic interplay in which humans can activate and reactivate Flavobacteria's colour in FlavoTread invites humans to seek out unexplored areas in shared habitats while providing new territory to the bacteria, which prompts reflection on their caregiving practices for nonhumans.

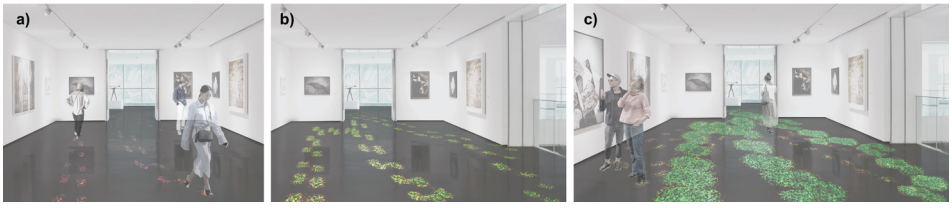


Fig. 5.23 *Visualisation of our concept FlavoTread: a) people activating Flavobacteria's living colour through their pressing footsteps; b) Flavobacteria's growth overnight; c) Flavobacteria revealing how people interact with a public space and inviting people to explore new areas, as well as provide new territory to them. The colonies in these visualisations were simulated using FlavoMetrics [169].*

Flavobacteria's temporal response to humans' direct input could also foster mindful interactions. For example, by marking the start of a work break with a swipe that disrupts the structural colour, mental well-being could be supported as Flavobacteria's response over the next twenty minutes is patiently observed. This intentional interaction with Flavobacteria's optical structures could serve as a meditative practice, allowing individuals to focus their attention and find a sense of calm in the mesmerising colourations. In contrast to the relatively fast response to swiping, Flavobacteria respond to tilt in a particularly slow manner, highlighting their potential to enhance mindfulness by revealing the passing of time in an abstract manner. In our concept *FlavoTempo* (Fig. 5.24), the slow changes in Flavobacteria's living aesthetics in response to tilt create a unique and intriguing visual representation of time passing, akin to an hourglass, allowing individuals to develop a deeper understanding of the concept of time and its relationship to their daily activities.

Fig. 5.24 *Visualisation of our concept FlavoTempo: a) a person tilting the artefact, thereby directing Flavobacteria's living colour; b) people observing how Flavobacteria reveal the passing of time in an abstract manner in response to tilting movements.*

5.5 Discussion

This paper expands on the notion of direct interactions with microorganisms in living artefacts, providing HCI designers with a design space that explores (re)activating, (re)directing, and (re)arranging Flavobacteria's colourations in this context, supported by application concepts. In this section, we reflect on the complexities and opportunities that emerged from this research, discuss the further implications of direct interactions with microbes for HCI, and address the limitations of our study.

5.5.1 The nature of direct interactions with microbes

While our explorations diversified the range of possible interactions with Flavobacteria, we struggled to define what we meant by direct interactions with microbes. Our comprehension of the boundary separating indirect from direct interactions in this context evolved as we explored related definitions and existing living artefacts. Yet, some cases left us with more confusion than clarity, such as the *Living Light* [218]. Here, humans physically touch a plant to activate a light, which is powered by microbes in the soil that release electrons

while interacting with the plant. In this case, human input is mediated as motion sensors detect humans' touch, and the behaviour of microbes is not affected by this input. Yet, the interaction can be experienced as a direct one, as humans might not be aware of the living artefact's working principle. Such examples lead us to believe that the threshold between indirect and direct interactions with microorganisms in the real world is, in fact, blurred, and linked to human experience rather than discrete boundaries. Nevertheless, to narrow the scope of this work and guide our explorations, we have formulated a definition that aims to differentiate indirect and direct interactions with microorganisms. While our scope for direct interactions excludes both mediating human input to affect microorganisms and the remote experiencing of microorganisms' responses, there still exists a spectrum of direct interactions in which both mediation and proximity play important roles.

Mediation-related opportunities and dilemmas

Considering our definition of direct interactions, microbes adapt their behaviour in response to unmediated human input. Nevertheless, mediation is sometimes needed to effectively reveal shifts in their behaviour. This is especially relevant when microbial responses remain entirely imperceivable to humans or occur so slowly that we either lose interest or are too late to prevent harm to the microorganisms. The methods of mediating microbial responses exhibit significant variation. For instance, mediating these responses may involve extensive translation, such as when fermentation bacteria's activity is conveyed through voice interaction [62] or electrons generated by biofilms bring forward artistic animations [159]. In contrast, other cases might offer more natural ways to reveal the responses of living organisms through, for example, the magnification of cell movements (e.g., [134]) or the temporal alignment of colour-changes in the living interface through non-digital mediation, which humans may not even be aware of [164]. As Flavobacteria have distinct living aesthetics, exhibiting visual changes in their colony's form, texture, and iridescent colour, mediation of their output is not strictly *necessary*, allowing us to position our studies at the least mediated end of the spectrum.

Proximity-related opportunities and dilemmas

Within our scope of direct interactions, humans provide input to microorganisms by acting upon living artefacts through their own bodies. Yet, the distance between humans and microbes themselves can vary extensively. The closest proximity of input for these interactions would involve humans touching microorganisms without any barrier in-between. Such direct interactions with microbes can facilitate microbial transfer, which, in the case of Nukabot [62], personally enriches the flavour of pickles, or can allow for the decomposition of human remains in the case of a living mycelium coffin [219], promoting biodiversity and circularity. However, due to bio-safety reasons and to maintain the integrity of microorganisms (e.g., by protecting them from dehydration or

contamination), physical barriers may be necessary. In the *tilting* study, we used such a barrier and interacted with Flavobacteria by tilting their rigid habitat, as also shown in the FlavoTempo concept. Alternatively, touching microbes through a thin glove or plastic foil retains close proximity and provides a sense of intimacy (like in our concept FlavoToile)- perhaps so intimate that people are resistant to interacting with microorganisms in such a direct manner. *C. lytica*'s non-pathogenicity allowed us to move towards the close-proximity end of the spectrum. However, not all microorganisms are suitable for this [220], and close proximity with microbes is not always desired by default. Therefore, we call for designers to carefully consider both microorganisms and human needs and tune proximity as well as mediation of microbial responses for the desired outcome.

5.5.2 Challenges and opportunities of direct interactions with microbes for HCI

Diverse temporalities of microbes

Microorganisms exhibit a wide spectrum of temporal behaviours, ranging from immediate to gradual responses, presenting both challenges and opportunities for HCI designers. Slow microbial responses can encourage human mindfulness and reflection, but, in extreme cases, may be considered a technical constraint [134], demanding excessive patience from humans to attend to microorganisms' temporality. They can even diminish the interaction effect [70] and the sense of reciprocity [42], as humans might no longer be aware that microbes are adapting their behaviour in response to their input. However, to a certain extent, delayed responses can enhance human curiosity, captivation, and emotional investment in microorganisms, fostering deeper engagement and creating a strong sense of connection towards profoundly meaningful relationships, as instantiated in our application concepts.

Comprehending these distinct temporal behaviours is crucial for designers of living artefacts, allowing them to tailor the temporal scale of the artefact and consider aligning human-microbe temporalities for timely care practices [164]. Yet, understanding these diverse temporal behaviours is challenging due to the different circadian rhythms and growth rates among them [135], as well as their ability to adjust these temporal behaviours based on various inputs. As demonstrated in our studies, Flavobacteria take six days to respond noticeably to tilting movements, while swiping elicits an immediate change in their living aesthetics. While offering a rich design space for HCI, this diversity makes predicting response times to certain inputs challenging, even with years of expertise in a specific microorganism, as shown by our initial attempt to capture Flavobacteria's response to swiping movements with a one-hour time interval.

Complex and unpredictable microbial behaviour

As we move further away from interactions in lab environments and the mediation of input to regulate living systems, we enter a realm of (semi)open

habitats [70] with less human control. Here, multiple input mechanisms can be in play, complicating our understanding of microbes' responses to certain inputs. For example, as illustrated in our design space, sweating can potentially affect salinity, nutrition, humidity, temperature, and the presence of other microbes. Such a single human input can lead to a wide variety in microbes' responses, as even subtle differences in microbes' environments can drastically affect their growth. For instance, in one of our studies, Flavobacteria's growth occurred twice as fast as usual due to high temperatures over the weekend. Whereas the interconnectedness of input mechanisms complicates the understanding of microbial responses, designers can also tap into this as an opportunity to create a dynamic interplay between human input and the diverse and unexpected living aesthetics of microbes.

Even in relatively controlled habitats, unexpected behaviour by microbes may occur. Our *tilting* study revealed unanticipated microbial behaviour in our first attempt to explore the effect of tilt (Fig. 5.25). Though grappling with the explanation of emergent behaviour was initially challenging, it sparked a fruitful discussion within our team about the level of control we should aim for. The emergent behaviour that comes from working with living entities poses challenges and questions for designers regarding how we should design in light of the unpredictabilities of microbes and how this unpredictability is experienced by humans interacting with living artefacts.

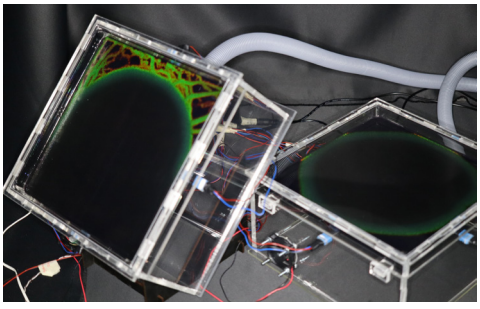


Fig. 5.25 *Flavobacteria's unanticipated behaviour in the first attempt to explore the effect of tilt. On day 14 of the experiment, bacteria in the colony created different paths that went in all directions across the available agar medium in the top part of the habitat.*

Bio-safety of direct interactions

As discussed by diverse scholars in bio-HCI (e.g., [70, 133]), it is challenging to design an appropriate habitat for microorganisms that preserves their livingness outside lab environments, as these controlled environments offer sterility and ways to stabilise growing conditions. Especially in this case of direct interactions, safeguarding the microbes' well-being becomes more challenging as inputs to living systems are not regulated and could potentially harm the microorganisms. In this regard, decisions regarding proximity and open habitats for direct interactions with microbes, while considered a means to foster intimate

experiences [70], should be meticulously evaluated in consideration of these bio-safety aspects.

Besides maintaining a safe environment for nonhumans, designers should consider safety concerns for humans in their direct interactions with microbes. Even when dealing with non-pathogenic microbes (e.g., *C. lytica*), direct physical contact might raise concerns when encountering a large quantity of the same microorganisms, which should be further explored in designing for direct interactions with living artefacts.

The additional risk living artefacts bring due to their potential to distribute foreign living microorganisms to ecosystems is difficult to anticipate and, therefore, important to consider. Living artefacts designed as closed systems could, in theory, address this risk by collecting or destroying the microorganism after use; however, it seems prudent that all living artefacts should utilise endemic, benign, or beneficial microorganisms. Living artefacts designed using such approaches would reduce environmental impacts and might even enrich ecosystems [18, 219].

Sustainability and ethics of direct interactions

Whether facilitating direct or indirect interactions, all living artefacts, if deployed within existing linear models of design, production, utilisation, and waste, may pose a threat to ecosystems through the utilisation of unsustainable materials and production processes [18]. To address this, living artefacts should either use materials that can be recycled or reused within a product return system or align the diverse temporalities of materials and microbes for the safe and timely decomposition of artefacts.

The use of microorganisms within HCI raises distinct ethical concerns [159], in part because we struggle to relate to them, often resulting in ambivalence towards their welfare [134]. Organism-centred [70] and more-than-human [164, 187, 190, 221] approaches in bio-HCI prioritise the safeguarding of microbial welfare through designing appropriate habitats and interactions centred on care practices (e.g., [42, 164]). In line with these, we call for HCI designers to give attention to microbial sensitivities during design time, which should further encourage them to extend the nurturing of relationality and livingness beyond use time and throughout the whole life cycle of the organism. Whereas we often struggled to move further away from our human-centred approach of considering Flavobacteria as a living *medium* for HCI, there was a strong sense of what could be characterised as kinship, caregiving, and responsibility throughout the experiments conducted, as expressed below by the first author: "*I felt worried about Flavo when they were in the freeze-dryer and afterwards, to see them as a powder, in a dormant state. Therefore, I was happy to provide them access to the nutritious agar medium by pressing the super-thin plastic foil. Oftentimes, I felt extremely guilty that they didn't have more space at the moment, which I hadn't really experienced before when cultivating them in a standard Petri dish. In that sense, seeing the bacteria touching the new area in*

the Petri dish was very satisfying. Ready to explore new territory! I would have loved to keep this interaction going, redirecting them again and again to fresh nutrients. Through the swiping experiment, at some point, some parts of the colony became less bright, which made me feel worried and guilty—had there been some leftover alcohol on the glove through which I was trying to keep them safe from other microbes? Was I exhausting them by interacting with them so frequently? Or were there simply not enough cells left at the moment to form structural colour?"

We argue that these questions and concerns, coupled with the experience of relationality—or kinship [222], provide rich ground for further research and bode well for dynamic and reciprocal relationships with living artefacts.

Challenges concerning microbial semantics during use time

This study also revealed specific concerns related to microbial semantics [134] in direct interactions with microorganisms. For example, it might feel as though interacting with Flavobacteria by touching them through a pliable membrane is more 'disruptive' to them than leaving them to 'grow in peace' within Flavorium [133]. Therefore, even if a habitat provides Flavobacteria with their desired conditions, designers should consider to what extent the directness of interaction gives humans the idea that they contribute to microbes' wellbeing. At the same time, we need to better understand what is gained and lost from direct interactions. Even though our intention was to offer HCI designers a way to nurture human-nature connectedness through direct interactions with Flavobacteria, it is possible that direct interactions with microorganisms further cultivate feelings of disgust (e.g., [223]), resulting in a corresponding reduction in empathy. With the FlavoToile concept, we confront such stigma and semantic dilemmas that warrant deeper exploration in the field of HCI research concerning human-microbe interactions.

5.5.3 Limitations and future work

In this paper, we showcase three distinct studies. Among them, two involved meticulous design and prototyping of experimental setups and research artefacts tailored for bio-HCI experiments, specifically focused on investigating direct interactions. In the *pressing* and *swiping* studies, we opted for a standard-sized Petri dish as a habitat to quickly iterate, allowing us to conduct these studies with sample triplets for more reliable results. In the *tilting* study, we employed a larger habitat because Flavobacteria's gradual response to tilt is only evident on a large spatial scale, as initially shown by Groutars and Risseeuw et al. [133]. The experiments of the *tilting* study were therefore not conducted with sample triplets, as the larger habitats were custom-made, and this would require six (three times a control and an experimental). Even though they differentiated, all habitat sizes in the studies limited Flavobacteria's ability to expand their colonies further, as well as the number of direct interactions possible within that time frame.

While the team has diverse backgrounds across HCI, design, and microbiology, providing expertise and alternative insights, it was the first author—a researcher with many years of experience working with Flavobacteria—who provided the initial interpretations of the study results. We acknowledge that the personal and long-term relationship the first author has with Flavobacteria may have caused a positive bias and awe towards the interpretation of these results. Therefore, in the future, studies with other designers and human users of living artefacts are important to explore the temporal nature of such relationalities with microbes.

Our endeavour was driven by a commitment to enrich the ongoing discourse in HCI concerning human-nature engagement and connectedness, with the goal of fostering greater comprehension and empathy towards microbes. Although we presented the technological potential for designing such engagements, we have not yet investigated whether we have successfully established the intended connections between humans and microbes over an extended period. This marks the focus of our next research endeavour. To that end, even though our explorations were oriented towards future experiments outside the lab, we acknowledge the need for additional consideration regarding the viability of the living artefacts when they are provided to human users. Moreover, a more comprehensive understanding of the interdependencies among habitat, microorganisms, and interaction variables in real-world settings is required. We know it can already be difficult for designers to understand and work with Flavobacteria's particular temporal expressions [133], and our studies add new temporal rhythms and real-world variables to further complicate this. Therefore, incorporating the outcomes of these and future studies—possibly exploring additional suggested human inputs—into digital tools that support design practices with Flavobacteria (e.g., [169]) would be a valuable addition to this design space.

5.6 Conclusion

This paper delves into the notion of direct interactions with microorganisms, particularly emphasising the potential of such interactions through the lens of structurally coloured Flavobacteria. We introduce a design space that encompasses the basic habitat architecture of Flavobacteria artefacts, various input mechanisms for directly tuning their living colour, and examples of human input. Our three studies, which involve (re)activating, (re)directing, and (re)arranging Flavobacteria's colourations through pressing, tilting, and swiping, showcase the intricate interplay between human input and microbial responses. These interactions exhibit variations in proximity between humans and microorganisms and underscore the diverse response times of Flavobacteria, ranging from immediate to gradual. As well as the design space, proximity and mediation are presented as dimensions of variety to explore in direct interactions. Through our work, we seek to support the development of living artefacts that cultivate a sense of kinship and care, extending human relationality towards the microbial world and the ecosystems we all belong to.

Chapter 6

Human-Flavobacteria Engagement in Everyday Life

This last chapter presents a longitudinal study, through which I extend and integrate the insights from previous chapters by situating human–Flavobacteria engagement in everyday life. It moves from brief interactions in lab environments to long-term engagement with a Flavobacteria living artefact in domestic settings. In doing so, it also expands on earlier lab-based insights on Flavobacteria's living aesthetics by exploring them in real-world contexts, where interconnected stimuli and emergent, unpredictable behaviours come into play. Building on my personal experience from Chapter 5 on relationality, this study provides in-depth experiential insights into how people without prior experience with this microorganism perceive, interact with, and make sense of its temporal expressions.

While Chapter 5 framed human action as input and Flavobacteria's responses as output, this chapter takes a more intricate and relational perspective, investigating how human and microbial expressions continually shape one another. In this view, the emphasis is not only on how microbial expressions respond to human action, but also how they, in return, shape people's observation, interactions, and affective and reflective responses.

The iterative development of the living research artefact that enabled this exploration of human-Flavobacteria engagement in everyday life is documented in the visual essay following this chapter.

This chapter is currently under review as a journal article:
Risseeuw, C., McQuillan, H., & Karana, E. (2026). The Role of Microbial Aesthetics in Everyday Engagement with Living Artefacts: A Case of Flavobacteria. Under review with ACM Transactions on Computer-Human Interaction – **not for sharing**.

The supplementary material for this chapter is available online at: https://doi.org/10.4121/498fd36d-ff97-4d18-8b44-e501a14b04b9

Abstract

Biodesign researchers increasingly explore microbial living artefacts in everyday life to foster awareness and understanding of multispecies interactions. Within these artefacts, microorganisms exhibit diverse temporal expressions that vary in type, rhythm, and intensity. Biodesign scholars argue that the way humans experience these expressions (i.e., living aesthetics) shapes how we engage with microorganisms. Yet, little is known about how microbial aesthetics influence human–microbe engagement over time in daily life, and about the role of interaction techniques in shaping these engagements. Addressing this gap, our paper presents a four-week study in which six participants lived with a Flavobacteria living artefact in their homes. Colonies of the non-harmful marine flavobacterium *Cellulophaga lytica* exhibit rich temporal expressions, including gradual and sudden colour and shape changes. We introduced two distinct ways for participants to interact with the organism, each eliciting different microbial responses. We analysed human engagement in relation to these changes through diary entries, semi-structured interviews, and sensor data. Our findings provide an in-depth empirical perspective on how microbial aesthetics shape observation, interaction, emotional response, and reflection, contributing to biodesign practices that foster more regenerative ways of coexisting with nonhuman life.

6.1 Introduction

In the past decade, designers have increasingly turned to the living world, not just for inspiration, but as an active collaborator in design and HCI, largely framed under the notion of 'biodesign' [32]. Integrating living organisms into everyday artefacts is a growing practice within biodesign, resulting in what is known as *living artefacts* [42]. These design outcomes maintain the viability of organisms such as fungi, algae, bacteria, and plants during use-time, enabling novel expressions, radically sustainable alternatives for energy and material use, and new functions and interactions in daily life. Researchers across domains, including architecture [36, 165], design [34, 35], and human-computer interaction (HCI) [37, 45], have explored a wide range of possibilities, including grown materials with self-healing capabilities (e.g., [224]) and living sensors that monitor environmental changes (e.g., [59]). As the field has matured, its initial human-centred focus on interaction possibilities—often tied to ecological benefits through materiality and fabrication—has progressively shifted toward broader posthuman perspectives that decentre the human and embrace multispecies interactions [159, 225, 226]. In line with this expanded view, Karana et al. [18] have extended the concept of living artefacts by articulating their potential to contribute to regenerative ecologies—ecological systems characterised by mutualism and co-evolution.

In line with this thinking, a significant body of work has focused on human–microbe engagement in biodesign and bio-HCI. These explorations (e.g., [170, 185, 211]) underscore the potential of engaging with microbial life to foster greater awareness and multispecies understanding—particularly toward the often-overlooked microbial entities that are, in fact, vital to life on Earth. Yet, scholars also highlight challenges of engaging with microbial life given their distinct temporalities, scales, and aesthetics [134, 185, 227]. Their unfamiliar, subtle, or emergent expressions may go unnoticed or be misinterpreted, which may hinder the development of awareness and relationality that regenerative design strives for. In response, biodesign researchers have called for the cultivation of microbial sensibilities [18, 227], so that microbial life may become more noticeable, relatable, and ultimately, engageable, thereby enabling cohabitation and coevolution between humans and non-humans within shared ecologies.

When microbial life is sustained and made visible through living artefacts, it can reveal a wide variety of living aesthetics [42]—the dynamic, temporal expressions that emerge from the organisms' livingness. These may include subtle shifts in colour, the gradual emergence of patterns, or immediate responses to environmental changes. As these expressions unfold over time, they can trigger our attention, potentially inviting us to act or reflect upon them. In this way, living aesthetics become crucial to shaping how humans engage with microbial life—not as passive matter, but as living beings that are present, responsive, and evolving alongside us. Yet, while both the diversity of microbial living aesthetics and the importance of human-microbe engagement within

regenerative ecologies are increasingly acknowledged, it remains underexplored how these aesthetics shape human-microbe engagement in practice. In particular, little is known about how these aesthetics play a role in engagement with everyday microbial living artefacts.

To address this gap, we conducted a longitudinal study with a living artefact (Fig. 6.1) designed to sustain Flavobacteria, a microorganism known for its dynamic and temporal colourations. Flavobacteria proved particularly fitting collaborators in this context, given their rich expressions, characterised by shifts in colony form, texture and colour over varying timescales. Furthermore, prior studies have demonstrated their feasibility for embedding in artefacts and sustained their viability over a course of a month without strict maintenance conditions (e.g., light/dark cycles required by bioluminescent algae) [133, 170]. We developed a research artefact that safeguarded Flavobacteria's viability for four weeks in a domestic setting while enabling direct interactions via tilting and swiping, thereby eliciting diverse microbial expressions. Six participants lived with the artefact in their homes for four weeks, during which they were introduced to the two distinct modes of interaction across two stages of the study. Drawing on qualitative insights from interviews along with data from embedded sensors, we provide a detailed account of how these living aesthetics shaped participants' observation, interaction, emotions, and reflection in everyday life, what we refer to as *everyday engagement*.

Fig. 6.1 *The living artefact Flaviri and its evolving expressions in response to tilting and swiping interactions.*

In addition, as the first in-home study of a Flavobacteria living artefact, this work offers insights into how these microorganisms behave and express themselves over time in everyday environments, complementing previous lab-based explorations on their living aesthetics [133, 170, 228]. With our approach and design considerations, we aim to provide guidance and inspiration for longitudinal, in situ studies within biodesign, as well as for biodesign practitioners in developing interactive and meaningful living artefacts. Furthermore, our mixed-method approach—combining qualitative and quantitative techniques—offers a methodological contribution for studying human–microbe engagement in situ.

Taken together, this work contributes an empirical perspective on how living aesthetics shape human–microbe engagement in daily life—supporting broader

conversations on the possibilities living artefacts open for more regenerative ways of coexisting with nonhuman life.

6.2 Related Work

6.2.1 Human-microbe engagement in HCI

In recent years, there has been growing interest in human–microbe engagement within Human–Computer Interaction (HCI) and related design disciplines. Explorations of living bits [37] and living media interfaces [45]—where microorganisms are integrated into novel interactive systems—often seek to extract human-beneficial resources from microbial life. While posthuman approaches explore how we might cohabit and relate to nonhuman life forms (e.g., [69, 159, 225, 229, 230]). These posthuman approaches challenge human-centred design paradigms by positioning living microorganisms as active participants that invite new ways of noticing, relating, and designing. In line with this, Groutars et al. [19] proposed an ecological approach in which living artefacts are designed to support multispecies interactions, emphasising the interconnectedness and reciprocity between humans, other organisms, and computational systems.

Aiming to foster human-microbe engagement, HCI researchers have examined microorganisms situated within their natural environments. Liu et al. [211], for instance, designed interactive artefacts for mushroom foraging that foster direct engagement with fungi in the forest, seeking to encourage attentiveness and compassionate connections with other species. Similarly, several studies have recently investigated engagement with microorganisms living on or within the human body, such as in our guts [231], on our skin [185], and within intimate bodily fluids [232]. These endeavours challenge us to rethink our relationship with our unseen inhabitants, fostering awareness and care.

Beyond these in situ contexts, researchers have also explored intimate encounters with microbial life through cultivation and designed artefacts. Examples include interactions with bioluminescent algae [70], Flavobacteria [170], and bacterial cellulose [233], sometimes using probes to enhance engagement—for instance, enabling felt experiences with growing bacterial cellulose [187] akin to Liu's [211] wearable artefacts in the forest. Risseeuw et al. [170] unpacked the notion of 'direct interactions with microorganisms', emphasising how living artefacts might cultivate a sense of kinship and care through explorations with structurally coloured Flavobacteria. Taken together, these works highlight the potential of direct interactions to foster new forms of engagement between humans and microbial life.

Researchers have begun to examine how such engagements unfold over time in everyday contexts through longitudinal studies with living artefacts (e.g., [62, 234–236]), offering early insights into how encounters and relationships with microbial life can evolve within daily life. Lu et al. [235] explored human engagement with a living smartwatch, where caretaking practices for the

integrated slime mould directly influenced the artefact's functionality, fostering new forms of responsibility and relationality. Zhou et al. [234] conducted a study with an air-purifying cyanobacterial living artefact, investigating how its materiality can dynamically shape routines of care and open space for creative, situated ways of attending to microbial life. Yet, these empirical insights into long-term, everyday engagements with microbial life remain limited. These prior studies, typically lasting one or two weeks, have focused on care practices, emphasising responsibilities or functional benefits that motivate interaction. Even though Zou et al. discuss, albeit briefly, how changes in microbial expression can influence care practices in cyanobacteria-based artefacts, much less is known about how people engage with living artefacts whose primary invitation is their dynamic expressions rather than care obligations or functional benefits. In other words, we still lack an understanding of how to design for such dynamic changes in ways that meaningfully unfold human–microbe engagement in everyday life. To explore this, we designed a self-sustaining living artefact without a predefined function—one that foregrounds microbial expressions—and studied engagement with it over four weeks.

6.2.2 Microbial living aesthetics

The dynamic expressions of microorganisms have increasingly captured the interest of researchers within HCI and design communities. Living aesthetics was proposed in 2020 by Karana et al. [42] as a principle for designing living artefacts, emphasising how aspects of livingness come to expression within these artefacts and can, therefore, be experienced. Analysing existing artefacts, they demonstrate how these expressions vary widely in type of change, intensity, and temporality. Meanwhile, Merrit et al. [45] elaborated on the temporal aspects of life while introducing living media interfaces to the HCI community, stressing the importance of acknowledging organism-specific characteristics and exploring the impact of design interventions on these.

Recent work by Ikeya et al. [237] provides a systematic review of perspectives on more-than-human aesthetics, critiquing traditional design aesthetics for their continued human-centred orientation. They argue that tying aesthetics primarily to human experience risks neglecting nonhuman experiences and the ecological and ethical complexities of more-than-human entanglements. Karana et al. [18] seek to foreground more-than-human sensibilities in biodesign practices rooted in human experiences. They advocate for crafting living artefacts that help humans become attuned to the needs, scales, temporalities, and expressions of non-human species, fostering ecological awareness and responsibility.

More-than-human sensibilities can be especially challenging to cultivate in relation to microorganisms, given their distinctive scales and temporalities, which can hinder humans from observing changes in their expressions, or even recognising their livingness. To address this, Kim et al. [227] proposed an approach to develop microbial sensibilities, inviting designers to imagine

the world through the lens of a microbe by providing tangible manifestations of expressions that extend beyond human boundaries. Interestingly, while Ikeya et al. [237] critique centring of human sensory experience in designing with living organisms, Kim et al. leverage such experience—for example, through digital tools—to evoke empathy and understanding, using human perception as a means to foster attentiveness to microbial life. In line with this approach and focusing more directly on microorganisms within artefacts, HCI researchers offered a taxonomy of six mechanisms to surface livingness in microbial displays [134]. While varying widely in the level of human intervention, these mechanisms are framed to address challenges around perceptibility with the aim to enrich how we might notice, interact and empathise with the microbial world.

Several design concepts within HCI and design communities have addressed such challenges. Bell et al. [233], for instance, translated kombucha SCOBY growth into sound to foster multispecies attunement, while Zhou et al. [164] used electrochromic materials to reveal subtle photosynthetic shifts in cyanobacteria, highlighting the temporal dissonance between microbial activity and human senses. These works underscore the need to bridge sensory and temporal gaps in human-microbe engagement for timely noticing of microbes and attentive engagement with living artefacts.

Crafting living artefacts to foster microbial sensibilities requires an enhanced understanding of microbial behaviour and their accompanying temporal expressions, as articulated in characterisation studies within biodesign. Such studies provide valuable insights regarding the diversity, responsiveness, and temporality of more-than-human aesthetics by outlining detailed characteristics of, for example, bioluminescent algae [61], bacterial cellulose as microbial habitat [238], and iridescent Flavobacteria [133], as well as fungal fruiting bodies [239]. Tools have been developed to support designers in understanding microorganisms' temporal expressions by, for example, enabling detailed mapping of temporal aspects [240], and streamlining the learning process through digital simulations (e.g., [169]), intended as a complement to hands-on work. While prior characterisation studies on microbial living aesthetics offer valuable insights into microorganisms' diverse and temporal expressions, they are primarily based on lab-controlled explorations. This raises questions about how such expressions evolve in everyday contexts, where interconnected stimuli and emergent, unpredictable behaviours might come into play.

Besides increasingly outlining the diversity of microbial living aesthetics, scholars have also discussed associated challenges and opportunities. For example, regarding temporal dynamics of microbial expression, prolonged microbial responses have been discussed to cultivate attentiveness, patience and observational skills [58, 233], while also risking reducing perceived responsiveness [70]. Yet, despite such exploratory discussions, empirical insights into how these expressions are experienced in practice remain limited, particularly in the context of everyday life. Specifically, it is unclear how engagement with microbial living artefacts in everyday life unfolds over time across varied temporal expressions.

Our study addresses this gap by deploying a microbial living artefact in a domestic setting and examining how its diverse temporal expressions are perceived, interpreted, and acted upon in daily life, thereby shaping human-microbe engagement over time. Understanding these dynamics is essential for designing living artefacts that foster microbial sensibilities and cultivate active engagement with microbial life and broader ecologies. As inspiration, we turn to research on non-microbial temporal expressions within interaction design to examine how temporal dynamics have been empirically explored in relation to interaction, experience, and engagement.

6.2.3 Engaging with temporality in interaction design

Temporality has long been recognised in HCI and design as an inherent quality of interactive artefacts, with early notions such as slow technology [241], ephemerality [73], and temporal form [97] articulating how it shapes the unfolding of interactions and experiences. Bergström et al. [92] introduced the concept of becoming materials, emphasising that aesthetic expressions only emerge over time and in context. Building on this, other works have explored temporal expressions through the lens of material ageing and decay [242–245], and material traces [95, 246]. These and related studies frame temporality as a rich, expressive dimension through which designers can craft meaningful, affective, and reflective experiences. More recently, researchers reflect on limitations of seeing time as a universal and human-centred notion [247] and call for more pluralistic temporal understandings that draw on more-than-human temporalities [190] and include the social and cultural dimensions of time [248].

Building on these foundations, several studies have empirically explored how temporal dynamics shape user engagement, perception, and interaction. Wakkary et al. [249] conducted a three-month field study in which six philosophers lived with the *Tilting Bowl*—a ceramic artefact that unpredictably yet gently tilts throughout the day [250]. Its autonomous temporal expressions invited reflection on everyday relations with technology, revealing the mediations that emerge when living with digital artefacts. Similarly, Zhong et al. [251] deployed *DeformTable*, a shape-changing artefact that adjusts its height according to the weight placed upon it [252]. Over a year, its temporal expressions triggered rich creative actions across the five different homes, as well as critical reflections on long-term interactions and entanglements with the artefact. Buso et al. [253] provided an empirical account within the context of multimorphic textiles, inviting eight households to live with *AnimaTo*, a shape-changing textile presented as a tea towel, which morphed drastically in response to water and heat [254]. Building on the Materials Experience Framework [117], they collected in-depth accounts of participant experiences over the two weeks, revealing that the irreversible transformations triggered creative repurposing, frustration, and interrupted use—highlighting the need to balance function, temporality, and materiality.

While the studies above address non-living artefacts, empirical accounts have also explored engagement with temporality in the context of human-plant interaction (HPI), drawing on the temporal rhythms inherent to plants as they grow, decay, and respond to their environments. A recent study, for example, examined how humans engaged with a living textile artefact fabricated by embedding microgreen seeds into biodegradable yarns [236]. Over a short, three-day deployment, wearers responded to the sprouting fabric through care-based interactions, revealing new forms of intimacy and attentiveness in human–plant relations. Moreover, Ben-Meir et al. [255] conducted a two-week field study in which eleven households lived with a sensor-equipped moss terrarium, chosen for its expressive responsiveness to environmental conditions. Analysing engagement with the living artefact, they explored how personal and sociocultural factors shape care practices and identified five types of domestic cultures of plant care.

These empirical accounts offer valuable insights into evolving human-artefact relations in everyday life and the role of temporal expressions in these. Aiming to explore these dynamics for microbial living artefacts, our study investigates human-microbe engagement through a longitudinal deployment of a Flavobacteria living artefact that displays distinct temporal expressions.

6.2.4 Flavobacteria's living colour

Many bacterial species within the class *Flavobacteriia* produce vivid colourations when growing on semi-solid surfaces (see Figure 6.2a-b; [20, 27, 28]). These bacteria are commonly found on marine flora (e.g., seaweed) and fauna (e.g., molluscs and jellyfish) [24]. Their optical effects, similar to those observed in butterfly wings and peacock feathers, emerge from the microscopic arrangement of bacterial cells (Fig. 6.2c). Through self-organising behaviour linked to surface motility [27] and predation [23], the cells assemble into highly ordered structures known as photonic crystals [20, 22], which interact with light, resulting in shimmering, iridescent colourations.

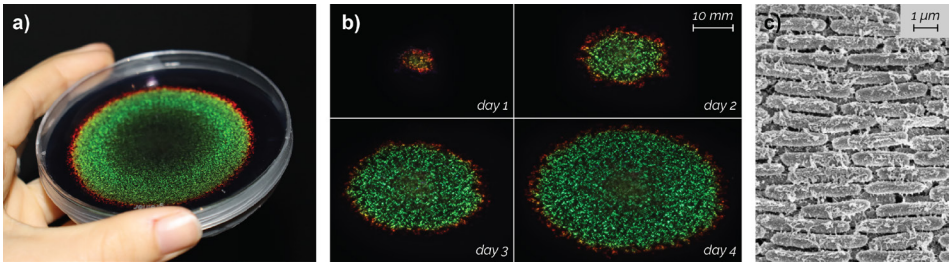


Fig. 6.2 *Structural colourations of Flavobacteria cultivated in a Petri dish (a) and growing over time (b), with its underlying cell organisation (c). Images adapted with permission: a-b from [169] and c from [133].*

Over the last decades, research has revealed many insights into their self-organisation and environmental responsiveness, with factors such as

temperature and salinity shown to influence colour expressions [26, 228]. Studies have also reported alterations in Flavobacteria's colourations due to interspecies interactions, in which Flavobacteria can even promote the growth of neighbouring organisms [256, 257].

These striking optical effects, accompanied by their strong responsiveness to environmental stimuli, have sparked growing interest across materials science, design, and HCI. In materials science, Sullivan et al. [29] highlighted the potential of Flavobacteria's iridescent biofilms as a platform for sustainable structurally coloured materials, offering new ways to control their optical, spatial, and temporal characteristics. More recently, Risseeuw et al. [228] examined Flavobacteria's temperature-responsive behaviour, highlighting their potential for thermochromic living materials. In parallel, Groutars and Risseeuw et al. [133] introduced Flavobacteria to the design and HCI communities, exploring their temporal qualities and responsive behaviour within interaction design. They presented a design space of input mechanisms that affect the bacteria's vivid colourations and highlighted their potential for Living Colour Interfaces. Since then, efforts have focused on supporting HCI designers in, for example, exploring their living aesthetics through a digital tool [169] and surfacing their livingness to encourage noticing [134]. Recent explorations emphasised direct human-Flavobacteria engagement—particularly through pressing, tilting and swiping—to foster a personal and dynamic interplay between humans and microbes [170].

Iridescent Flavobacteria have also featured in artistic explorations, for instance, within an installation that mapped diverse microbial life within the city to foster appreciation for urban nature [258]. More recently, the artwork *Gâteau Vivant* [259] staged living Flavobacteria on cake-shaped growth medium as a symbolic gift, drawing attention to biotechnology's tendency to take without necessarily giving back and inviting reflection on the ethical implications of harnessing bacterial life for art and design.

Together, these works reveal the rich aesthetics of Flavobacteria, whose changes in colony form, texture and iridescent colourations exhibit diverse temporal behaviours, ranging from subtle and gradual to immediate and pronounced. Yet, little is known about how these microbial expressions unfold in everyday settings and shape human-Flavobacteria engagement beyond the laboratory.

6.3 Research Design

In this study, we explored how living aesthetics shape everyday engagement with living artefacts in practice. Specifically, we asked: how do the living aesthetics of microbial artefacts shape human-microbe engagement over time? To address this, we designed an artefact with Flavobacteria and invited six participants to live with it in their homes for four weeks. Throughout this longitudinal study, we analysed how participants engaged with the artefact and

its emerging expressions through a mixed-methods approach combining diaries, interviews, and sensor data.

6.3.1 Understanding engagement

Our understanding of engagement draws on the experiential characterisation levels in materials experience (i.e., sensorial, performative, affective, and interpretive [47]) and engagement literature in behavioural sciences (see, for instance, [260–262]). We define engagement as the ways—and the frequency with which—participants attended to, interacted with, felt about, and made sense of the living artefact over time. Accordingly, we examined engagement across four complementary modes: (1) how participants observed the changes in the artefact, (2) how they acted upon the artefact, (3) how they felt upon these interactions, and (4) how they interpreted and reflected on the changes in the living artefact.

This understanding shaped the study at multiple levels, including research inquiries, data collection and the design of the artefact. The four modes structured our focused inquiries, articulated as the following research questions:

- What visual changes do participants observe in the artefact, and how does their attention to these changes evolve?
- How do participants physically interact with the living artefact—how often, for how long, and in what ways—and how is this shaped by observed visual changes and their temporality?
- How do participants emotionally respond to the living artefact and the microorganisms within it?
- How do participants' perceptions, understandings, and reflections on the living artefact develop throughout the study?

To address these inquiries, we adopted a mixed-methods approach. Diaries and semi-structured interviews provided qualitative insights into observations, interactions, feelings, and thoughts, while embedded sensors recorded physical interactions (i.e., the frequency, duration, and type of contact [253]) to complement and strengthen these subjective accounts with objective behavioural data.

The closely related aspects of observation and interaction were often intertwined in practice, and so we discuss these together in the results. Drawing on the notion of embodiment in interaction design [263, 264], we refer to these combined practices as an embodied form of engagement to emphasise their bodily and situated nature.

Our understanding of engagement also guided the design of interaction mechanisms, ensuring that participants could engage physically with the artefact through contrasting gestures that elicited diverse microbial responses. Tilting and swiping were selected because, together, they offered qualities beneficial across all four modes of engagement, as prior studies have shown that they differ significantly in their characteristics and effects (see Fig. 6.3;

[170]). For observation, these gestures triggered different microbial responses—tilting elicited slower, subtler changes, while swiping produced faster and more pronounced transformations—creating varied temporal rhythms and intensities for participants to notice and interpret. For action, both gestures provided embodied affordances for interaction, yet differed in their level of directness and interference: tilting as a gentle, low-interference movement and swiping as a more direct, high-interference gesture. This contrast allowed us to explore how interaction mechanisms—varying in their sense of directness and perceived proximity to the microorganisms—might shape engagement. Finally, the two modalities were expected to evoke distinct senses of intimacy, control, and proximity to microbial life. By offering contrasting gestures, we aimed to investigate how embodied interaction mediates emotional and cognitive engagement.

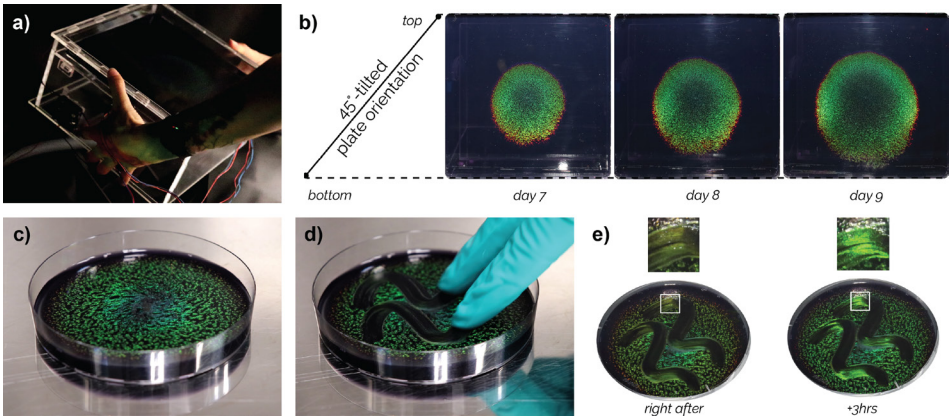


Fig. 6.3 *Direct interactions with Flavobacteria and their effect, adapted from Risseeuw et al. [170] with permission: a-b) tilting and its slow, subtle impact on colony form; c-e) swiping and its immediate, more severe impact, alongside recolourations that gradually appear in the hours following interaction.*

6.3.2 Designing the research artefact: Flaviri

The research artefact, named *Flaviri*, incorporated the iridescent, nonharmful Flavobacteria species *Cellulophaga lytica* (strain PLY-A2), selected for its resilience and expressive visual qualities [21]. The artefact was designed to support the viability of these microorganisms while enabling and tracking direct interactions that evoke diverse microbial expressions. Designed to sustain microbial viability outside the laboratory for four weeks, Flaviri allowed us to observe participants' engagement without the confounding influence of care obligations. Additionally, the artefact was intentionally designed to have no specific functionality, ensuring that engagement was not driven by instrumental motivations.

Central to the design was an oversized Petri dish (⌀250 mm), which provided a suitable habitat for the microorganisms and sufficient surface area for hosting

the Flavobacteria for four weeks. We re-engineered this dish from a passive container into an interactive artefact, enabling participants to engage with the colony through two distinct interaction modalities.

Flaviri supported two interaction modalities—tilting and swiping—selected because they elicit distinct microbial responses: tilting encouraged colonies to expand downward, with effects typically visible after about a week; swiping disrupted the optical structures responsible for colouration, instantly eliminating colours and triggering altered colourations within hours as bacteria reorganised. To expose participants to these contrasting expressions, tilting was available from the start of the study, while swiping was introduced after two weeks, enabling comparison of participant responses to changes that differed in timing and intensity.

Flaviri was developed through an iterative process over several months to optimise microbial viability, support these direct interactions in domestic settings, and ensure safety. The artefact was built around a Petri dish, which was integrated into a mechanical structure with embedded electronics, as illustrated in Figure 6.4a and detailed in the following paragraphs. Additional information about the design can be found in Section S.1 of the Supplementary Material and in the visual essay following this chapter.

The Flavobacteria were hosted in the centre of the artefact in an oversized glass Petri dish, custom-made by a glass manufacturer and filled with solid growth medium. To enable direct interaction through tilting, this Petri dish was integrated into a mechanical structure, consisting of Petri dish holders, a rotation disc, and a metal frame with a tilt mechanism. The Petri dish was secured within 3D-printed holders that slotted into multiple layers of black-painted laser-cut plywood discs. This infrastructure allowed the dish to rotate without slipping out of the structure. The rotating unit (Fig. 6.4b) was mounted into the metal frame via a mechanism that allowed the entire inner assembly to tilt (Fig. 6.4c). Together, these two degrees of freedom made it possible to orient the colony in any direction. A tilt-lock was added to prevent unintended movement caused by imbalance or external disturbance. To increase stability of the artefact—and therefore its safety in domestic settings—the metal frame was reinforced with a weight in the base and a horizontal beam to prevent spinning of the rotation disc.

To enable direct interaction through swiping without opening the Petri dish, a magnetic aquarium cleaner was repurposed (Fig. 6.4d): the inner magnet was extended with a small tip that gently contacted the growth medium, allowing participants to swipe the colourations by moving the paired outer magnet. Because swiping was introduced only in the second half of the study, a small 3D-printed magnetic insert held the inner magnet securely in place until it was replaced by the outer magnet after two weeks.

An Arduino Nano 33 BLE equipped with a gyroscope and magnetometer was embedded in the artefact. Tracking the orientation of the Petri dish and the position of the magnet, these sensors monitored participants' acts of tilting and

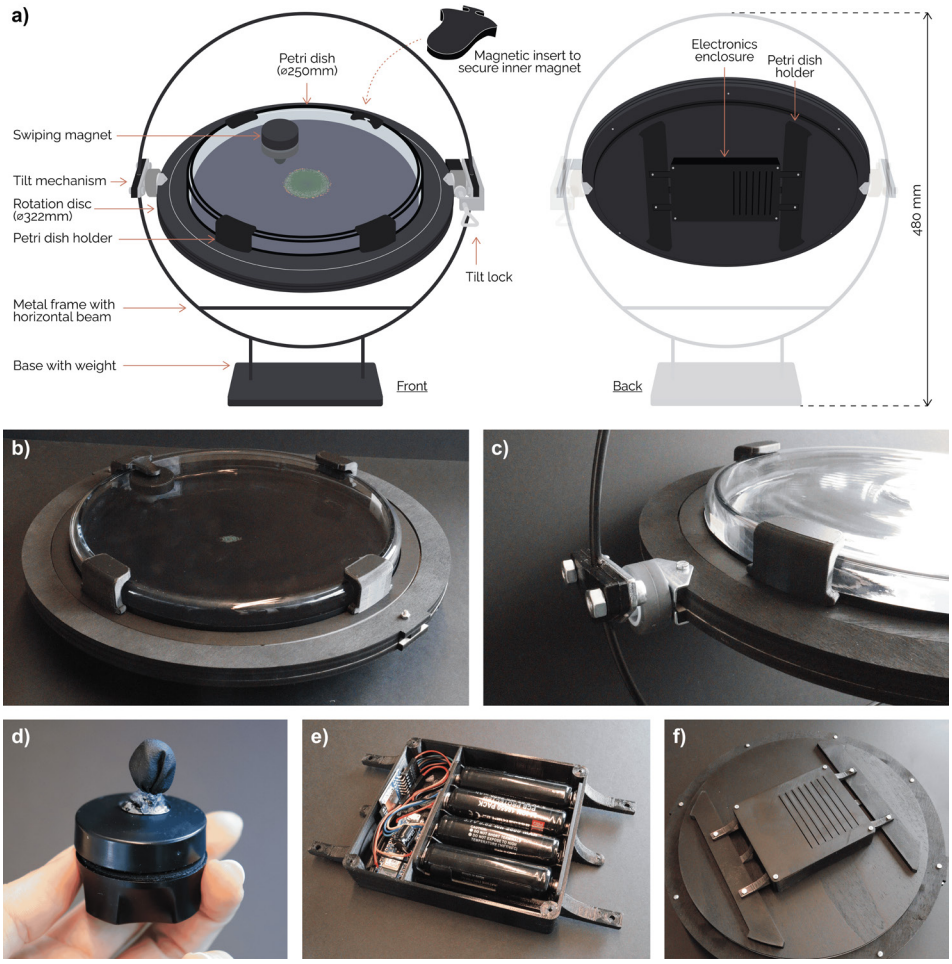


Fig. 6.4 *Details of Flaviri: a) infographic showing the artefact from the front and back with the different components indicated; b) Petri dish in the holders and multi-layer rotation disc; c) rotation unit secured in metal frame through tilt mechanism; d) swiping magnet with a tip made out of nitrile laboratory gloves; e) electronics housed in the 3D-printed enclosure; f) enclosure locking the Petri dish holders in the multi-layer rotation disc.*

swiping. These electronics, supplemented with an SD card module for data storage and four rechargeable 18650 batteries for power supply, were housed in a 3D-printed enclosure (Fig. 6.4e). This enclosure also locked the layers of the rotation disc in place and served as a counterbalance for the Petri dish (Fig. 6.4f).

The artefact was designed with sustainability in mind, reusing materials where possible and ensuring that the assembly could be disassembled for reuse or recycling after the study. For example, the Petri dish size was selected to be compatible with standard laboratory equipment, such as an autoclave, enabling reuse by fellow and future researchers.

Biolab preparations

The artefact was prepared with 400 ml of solid MAR medium to provide Flavobacteria with the required nutrition and salinity, as well as an ideal viscosity for the formation of their colourations. Nigrosine was added as a contrast agent and nystatin to reduce the chances of fungal contamination. After mixing all ingredients, the medium was autoclaved and poured into a sterilised Petri dish under sterile conditions in a laminar airflow (LAF) cabinet (Fig. 6.5a). To minimise excess condensation, the dish was left uncovered for 20 minutes during solidification and, after four hours, stored vertically in an incubator (12°C, 90% RH), with residual moisture later removed by pipette.

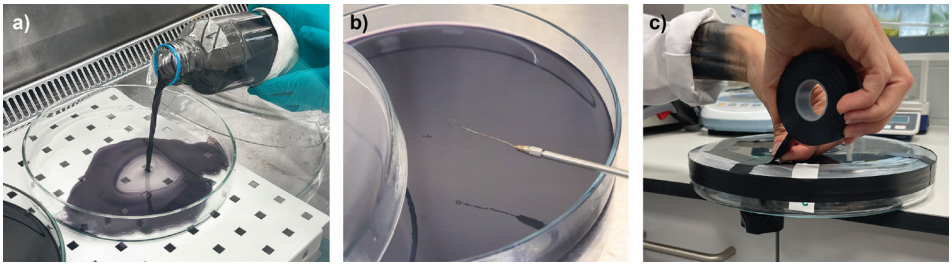


Fig. 6.5 *Biolab preparations of the living artefact: a) pouring the growth medium into a horizontally levelled Petri dish; b) inoculating Flavobacteria into the dish by making a small incision into the growth medium to avoid spreading; c) applying the waterproof tape in the sealing process.*

After three days, and again under sterile conditions in the LAF cabinet, *Cellulophaga lytica* PLY-A2 was inoculated into the centre of the dish using an inoculation loop (Fig. 6.5b). Next, the aquarium magnet (sterilised with UV and 70% ethanol) was added, and the dish was sealed with Parafilm. The inoculated dish was then placed horizontally in the incubator for three days to allow the colony to establish without unintended spreading. Finally, the dish was sealed with waterproof tape (Fig. 6.5c) and aquarium sealant. The sealant was left to cure for at least four hours under an extraction hood before the dish was assembled into the rotation disc. The full MAR recipe and a step-by-step protocol can be found in Appendices A and C, respectively.

As the Petri dish was taken out of the biolab and integrated into Flaviri for deployment in domestic settings, we iterated on the design with particular attention to biosafety. We explored, for instance, different sealing strategies for the oversized Petri dish to prevent leakage during handling and movement—conditions very different from static laboratory samples—while still allowing sufficient airflow for the microorganisms.

Interaction mechanisms of Flaviri

As shown in Figure 6.6, participants could interact with Flaviri in three ways: rotating the inner disc with the Petri dish, tilting the assembly after releasing the

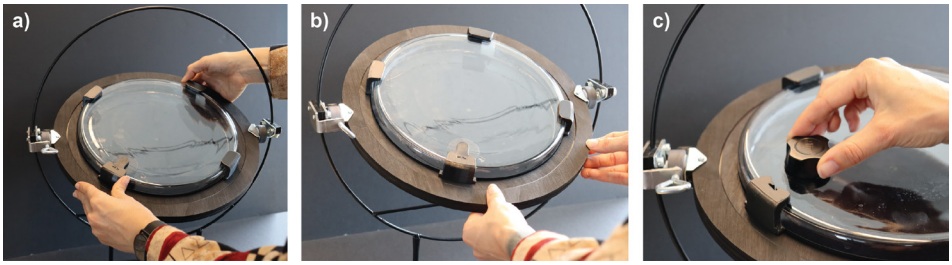


Fig. 6.6 *Interactions with Flaviri: a) rotating the Petri dish within its holder; b) tilting the entire disc assembly within the frame; c) swiping the colony by moving the outer magnet.*

lock, and swiping the colourations by moving the outer magnet. In this paper, we refer to the first two actions as *tilting*, as both involved reorienting the colony through physical movement.

6.3.3 Pilot study

To refine our procedure and interview protocol, we conducted a pilot study with two participants, each keeping a standard Petri dish (⌀90 mm) with Flavobacteria at home for 8–14 days. While these participants were acquainted with the responsible researcher, they were unfamiliar with the study's inquiry and the biodesign research field. Participants were not assigned any specific tasks; their role was simply to host the Petri dish in their homes and report any observations. They were encouraged to share photos and comments via WhatsApp at their discretion. Semi-structured interviews were conducted at the end of the study to reflect on participants' experiences and engagement, despite the absence of explicit interaction mechanisms such as tilting or swiping.

The pilot study provided several insights that informed the main study design. Participants raised unanticipated questions, such as "Can I take the bacteria outdoors?", which highlighted the need to define handling boundaries and to be prepared to provide clear guidance when such questions arise. One participant's cracked Petri dish underscored the importance of having backup colonies available for substitution and of providing clear emergency instructions to ensure participant safety and prevent unintended release of Flavobacteria. Participants also struggled to observe and capture the structural colourations, largely because these colours depend on light interacting with the optical structures. To address this, we provided background information on structural colour, practical documentation tips regarding illumination, and a torch for each participant. Finally, pilot feedback also led to minor clarifications in participant instructions.

6.3.4 Testing Flaviri in a home environment

In addition to the pilot study with external participants, we conducted a two-week self-deployment of the complete living artefact at the first author's home to

test its practical feasibility in a domestic setting (Fig. 6.7). We observed that the location of the artefact, particularly in relation to surrounding light, was critical for visibility of its iridescent colourations, and so we decided to coordinate with participants to select an initial placement during drop-off. Capturing the iridescence using a mobile phone's built-in flash proved inadequate at typical viewing distances under diffused home lighting (Fig. 6.7b), so, in the main study, participants were explicitly advised to use the torch during documentation. Additionally, we observed that even after 1.5 weeks, tilting had little effect on microbial growth, potentially because the growth medium dried out more quickly due to the increased height of the custom-made Petri dish compared to the thinner standard Petri dishes used previously. To achieve visible effects within a week as intended, we slightly increased the incubator humidity during sample preparation to reduce moisture loss and thereby accelerate changes in expansion rate through tilting.

Fig. 6.7 *Flaviri during test deployment at the first author's home: a) observation of colourations using the torch; b-c) photographs of the artefact illuminated respectively by the mobile phone's built-in flash and the torch.*

6.3.5 Main study participants

The main study was conducted with six participants (table 6.1), aged 25–60, with diverse nationalities and occupations and a balanced gender distribution. Participants varied in professional background and prior experience with living or microbial systems, which was noted during initial interviews to help contextualise their engagement patterns in the study. Our selection criteria aimed to ensure practical feasibility, safety and reliable data collection: 1) living within a 15 km radius of the research institute to minimise transport risks for the fragile artefact and reduce periods during which participants missed the colony's development; 2) absence of young children and pets that could access or damage the artefact, to minimise potential artefact damage; and 3) living alone to allow clear attribution of sensor data and reduce external influence on interview responses.

Participants were recruited through colleagues' acquaintances, ensuring they were unfamiliar with the research topic and unknown to the responsible researcher (first author). All joined voluntarily and were pseudonymised.

Table 6.1 *Overview of participants.*

Participant	Age	Gender	Nationality	Occupation	Familiarity with living/microbial systems
P1	31	f	Dutch	Primary school teacher	Considerable experience with bakery and home fermentation
P2	33	m	American	Architect	Moderate experience with home fermentation
P3	60	m	Dutch	CEO of biotech startup	Extensive knowledge about microbes in biodesign practices
P4	25	f	French	Receptionist / Teacher assistant	Moderate experience in human–plant interaction research
P5	41	m	Dutch	Project manager / Ecologist researcher	Extensive experience in ecology-related research
P6	39	f	Macedonian	Associate professor, Mechanical Engineering	Limited experience with microbes in a research context

6.3.6 Procedure

In the longitudinal study, participants lived for four weeks with the Flaviri artefact in their homes. The midway interview and staggered introduction of interaction modalities divided the study into two phases. Participants were not assigned any specific functional tasks with the artefact; their sole responsibility was to host Flaviri and report any changes they noticed during the study. Unlike the pilot study, in which participants lived with a Petri dish that offered little opportunity for interaction, the living artefact in the main study allowed participants to engage actively through tilting and swiping, while still providing complete freedom in how and when they interacted.

At the start of the study, the responsible researcher visited each participant at home to drop off the living artefact and explain the study. Participants signed the informed consent form—shared with them before the drop-off date—after which they took part in a brief verbal questionnaire to provide demographic information. The researcher then assembled the living artefact, which had been wrapped in reused bubble plastic for transport: batteries were connected to initiate tracking, and the rotation unit containing the Petri dish was inserted into the frame. Meanwhile, participants read through the provided instructions.

They were informed that it was a self-sustaining living artefact with structurally coloured, non-harmful Flavobacteria. Participants were invited to interact with the artefact through tilting to direct the Flavobacteria to more space and nutrients. They were told that the artefact would change in colour over time, though no specific details were given about what changes to expect. They were instructed to avoid exposing the artefact to direct sunlight, water, or heat and were reassured that, other than that, there was no correct or incorrect way to engage with the artefact. These instructions were provided verbally and written on an instruction card for reference (Appendix D). They were also provided with a torch to highlight the colourations and facilitate in-depth observation. The study materials can be seen in Fig. 6.8.

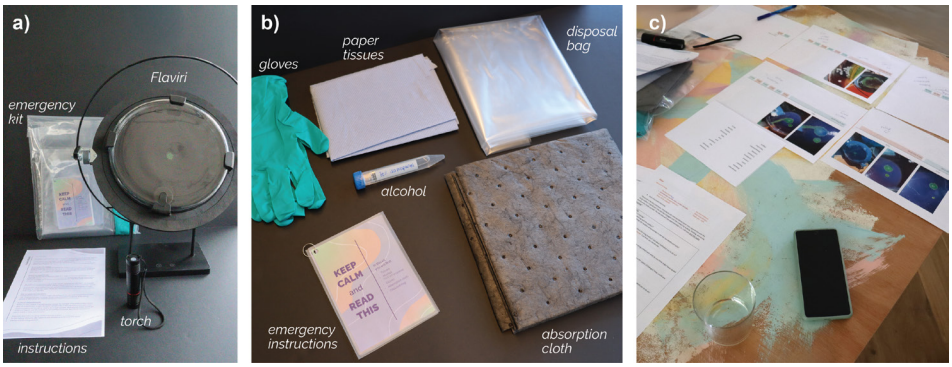


Fig. 6.8 *Study materials: a) Flaviri with accompanying materials; b) content of emergency kit; c) interview materials used during the final interviews.*

After two weeks, the responsible researcher revisited each participant to conduct a semi-structured interview (Fig. 6.8c), reflecting on their experiences and interactions with the artefact up to that point. They were then introduced to the second form of interaction, swiping, as a way to both direct the bacteria toward more space and nutrients and alter existing colourations. They were reminded that responses could range from subtle to extreme and that there was no correct or incorrect way to engage with the artefact. The study continued for another two weeks, allowing participants to interact with Flaviri through both tilting and swiping. At the end of the four weeks, a final interview was conducted to reflect on their overall experience, building on responses from the previous interview.

For one participant, the colony had expanded so rapidly during the first phase of the study that its structural colourations had fully faded by the time of the midway interview. To enable continued engagement and inclusion in the second study phase, the original colony was replaced with one of the backup colonies after the midway interview.

Throughout the entire study, participants were invited to share photos and observations with the responsible researcher via WhatsApp or Signal whenever they felt like it. These logbooks supported the discussions in both interviews

by helping participants recall observations, interactions, and their evolving feelings and thoughts about the artefact. This approach helped reveal evolving experiences that might otherwise not emerge in single-time-point interview discussions. All interviews were audio-recorded and typically lasted around 25 minutes (range: 10-50 minutes).

Quantitative data on participant interactions with Flaviri were collected via embedded sensors, which recorded the frequency and duration of tilting and swiping gestures. This data was retrieved at the mid-point visit, when the batteries were replaced, and again at the final visit.

For safety purposes, participants were given an emergency kit containing gloves, alcohol, and disposal materials (Fig. 6.8b), and the responsible researcher remained available throughout the study for issues such as contamination or artefact damage. The study protocol was approved by the research institute's Human Research Ethics Committee.

6.3.7 Interview questions

Our interview questions (table 6.2) aimed to document everyday engagement with the living artefact and how this engagement evolved in response to the living aesthetics. We drew inspiration from other longitudinal studies on engagement with artefacts [234, 249, 253] in formulating them and structured the questions around our four analytical lenses. To support participants in articulating their emotional engagement, we provided a list of emotions (Supplementary Material, Section S.4) inspired by the Experiential Characterisation Toolkit [47] and a vocabulary for the impact of living materials [157]. This list was refined and slightly extended based on insights from the pilot study. For example, emotions such as guilt and pride were added, as they came up in discussions with pilot participants. Additionally, one new interview question was included based on pilot feedback, to capture how participants introduced the artefact to others, as this topic arose naturally in discussions with a pilot participant and offered insight into how they related to the artefact.

In the final interview, questions were used cumulatively: after receiving new responses, we revisited answers from the midway interviews to compare engagement over time. Additional reflective questions were included at the end to explore how participants' engagement, understanding of the artefact, and broader perceptions of microorganisms had potentially changed during the study.

6.3.8 Data analysis

The study produced six participant logbooks containing 100 shared images and 43 written comments, 5 hours and 10 minutes of interview recordings, and multiple data logs with a total of 135 registered interactions. Diary entries, submitted at intervals of 1–13 days, were collated into participant-specific timelines that supported subsequent interviews.

Table 6.2 *Interview questions.*

Introduction	• How has it been living with the artefact? • Did you change the location of the artefact in the last two weeks, and why?
What and how participants observed	• How often did you check the living artefact? And what motivated you to do so? • What changes did you notice in the living artefact, and what do you think might have caused this? • How would you describe these changes? What about the speed of changes? How would you describe it?
What they did	• How often did you interact with the living artefact? And what motivated you to do so? • Did your living artefact affect your daily routines, and if so, in what manner?
How they felt	• How did you feel when you interacted with the living artefact? • *[Presenting logbook and list of emotions]* Can you describe how your overall experience with the artefact changed over time by selecting a couple of descriptions from this set?
How they interpreted or reflected	• Did you find yourself thinking about the artefact during your daily activities? If so, what were your thoughts? • Did you show the living artefact to any guests? If so, how did you introduce the artefact? • Did you have any other discussions about the living artefact with anyone? Additionally, during the final interview: • Looking back over these four weeks, have your thoughts on the Flavobacteria changed over time, and in what way? • Have your thoughts on microorganisms in general changed in any way?
Closing	Additionally, during the final interview: • Will it make a difference to you and your space if the living artefact is no longer here from this afternoon/evening? • How do you imagine living with a living artefact in the long term? • Do you have any recommendations regarding the design of the living artefact? • Do you have any other comments, suggestions or things you want to share?

Interview recordings were transcribed using the Google Pixel Recorder application and manually corrected. Transcripts were then subjected to reflexive thematic analysis [265], selected for its flexibility and suitability to capture evolving perceptions and interactions in a longitudinal context. This approach emphasised iterative engagement with the data and allowed us to remain attentive to unexpected insights rather than relying on predefined categories.

Analysis proceeded in several stages. First, the first author engaged in familiarisation by reading and annotating the entire dataset. Next, coding was conducted in Atlas.ti using a hybrid coding approach: the four analytic lenses informed the top-level code categories, under which inductive codes were developed. Coding was initially conducted by the first author, reviewed with the second author, and refined iteratively. Codes were consolidated by merging overlaps and discarding low-relevance codes, resulting in 214 detailed codes refined into subcategories across the four main categories (see codebook in Supplementary Material, Section S.5).

Finally, the first author analysed the codes using network views and co-occurrence tables to identify patterns, relations, and tensions, and to trace how these evolved over time and across participants. These analyses were later discussed in a collaborative session with all authors to critically review and refine interpretations. Through this process, we developed both concrete answers to the sub-research questions within each engagement lens and higher-level thematic reasoning about broader patterns. Candidate themes were initially developed by the first author and subsequently reviewed and refined in discussion with all authors, capturing shared and divergent experiences while remaining sensitive to temporal dynamics.

Our reported findings were also informed by the analysis of visual and quantitative data. Images shared in the logbooks were examined to identify variations in the temporal expressions of Flavobacteria and the placement of Flaviri within participants' homes. Where relevant, these images were anonymised and included in this results section. Quantitative sensor data was visualised to trace interaction frequency and duration over time, revealing how changes in the artefact's living aesthetics invited or discouraged tilting and swiping interactions.

6.4 Results

Throughout the study, participants exhibited diverse responses, while the living artefacts adapted in distinct ways to their respective home environments and participant inputs. This section begins by outlining the distinct microbial expressions that emerged across artefacts over time. Building on these variations, we then detail how participants engaged with the artefacts as microbial expressions evolved. In presenting these results, we move from observation and interaction practices to more affective and cognitive forms of engagement. Finally, we also reflect on the different roles that participants adopted in relation to their living artefact.

Before describing the microbial expressions of Flaviri, we first note the initial placement of the artefacts across households. The artefacts were given an initial placement in the six households at drop-off, considering available space and the need to avoid direct sunlight. Their positions, and thus their visual presence in the households, varied widely: in some homes they occupied marginal spots,

such as a kitchen corner near the trash (Fig. 6.9a), while in others they were placed more prominently, among houseplants with abundant light (Fig. 6.9b) or centrally in the living room, blending with surrounding artworks (Fig. 6.9c).

Fig. 6.9 *Examples of initial artefact placements in the homes of participants: a) unused kitchen corner; b) among houseplants in bright daylight; c) central living room location.*

6.4.1 Diverse microbial expressions of Flaviri

While all artefacts began from identical preparation, their trajectories diverged considerably during deployment in the homes of participants, resulting in distinct microbial expressions (Fig. 6.10).

Some artefacts displayed rich, shifting iridescent hues that gradually faded, while others underwent more rapid transformations, with colours suddenly turning dull, reviving at later stages, or fading completely (Fig. 6.10a-g).

Growth patterns also varied (Fig. 6.10h-k). Initially, most colonies expanded in a circular form, though the duration of this phase differed across artefacts. Over time, colonies began to grow more irregularly, and in some cases, fragmented into multiple separate, colourful sections.

More unexpectedly, some artefacts accumulated condensation on the inside of the Petri dish (Fig. 6.10l), and in several cases, contamination emerged towards the end of the study (Fig. 6.10m). Both phenomena visibly affected the microbial expressions, with contamination clearly accelerating colour decline.

Main drivers of changing expressions

Several factors contributed to the divergent trajectories observed among the living artefacts, including participant interactions, differences in household environments, and the inherent variability of living organisms. The participant interactions directly influenced the living aesthetics, with variations in frequency and intensity resulting in diverse microbial expressions among artefacts.

The artefacts were also shaped by environmental factors such as temperature, relative humidity, and sunlight exposure, which presumably differed

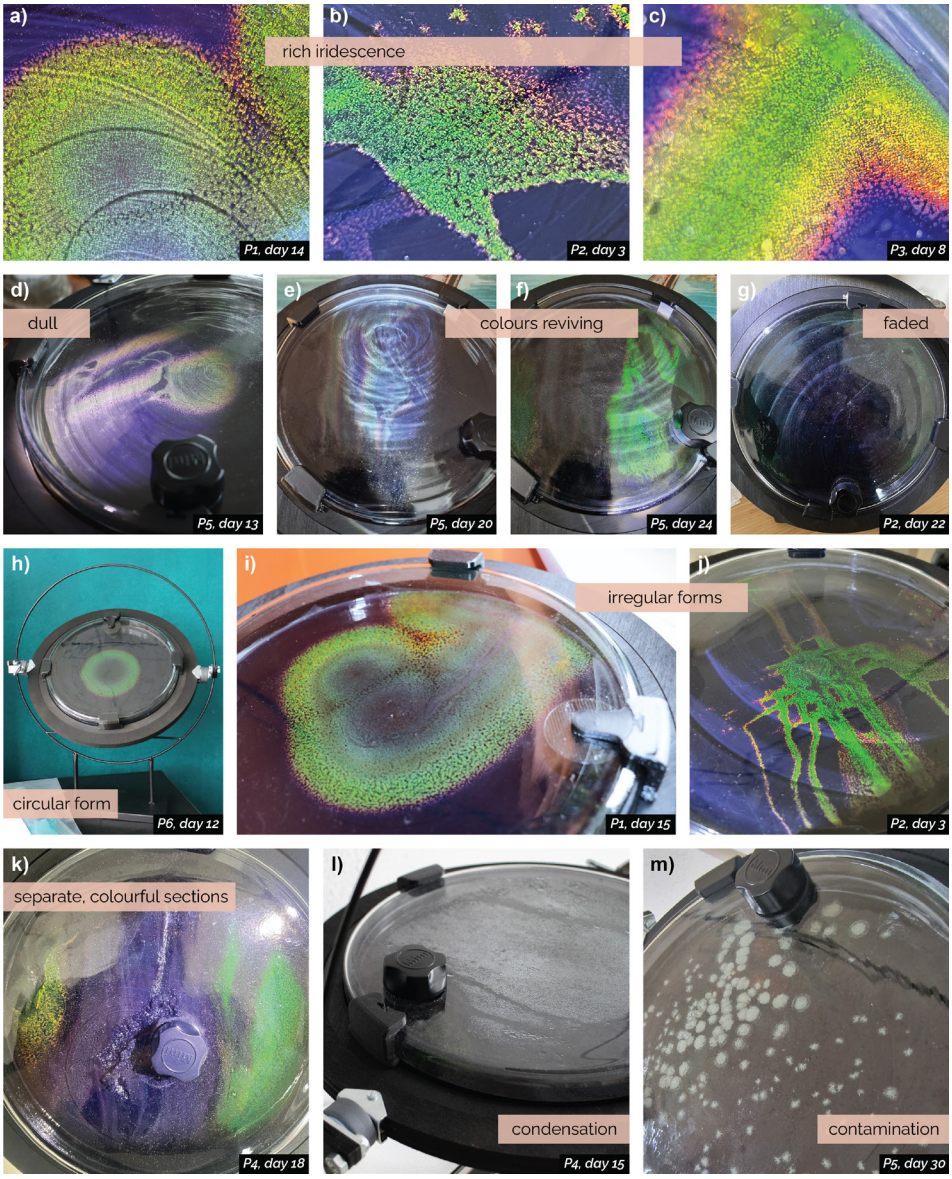


Fig. 6.10 *Examples of deployed artefacts showing diverse expressions: a-c) close-ups of iridescent colourations, with red edges merging into each other (a), rich colour gradients from green to yellow, orange and purple (b), and extreme brightness (c); d) an artefact which colours suddenly turned dull towards the midway interview; e-f) the same artefact reviving its colour in new areas after a swiping event; g) an artefact which colours permanently faded after extensive repeated swiping; h) a colony still expanding radially on day 12 due to lack of interventions with its centre gradually fading; i-j) colonies developing more irregular forms over time due to tilting acts, with minor degree of intervention and effect (i), and extreme irregular growth patterns following extensive intervention the previous day (j); k) a colony fragmented into separate, colourful sections upon reviving its colour; l) extensive condensation in an artefact located in a sunlight-rich environment; m) contamination after repeated swiping events.*

considerably across households. These conditions, for instance, most likely relate to the unexpected amounts of condensation in some plates. This moisture amplified the effect of tilting as it spread the colony into irregular forms and thereby accelerated growth beyond the intended pace for the first two weeks of the study. Moreover, the longitudinal study was conducted in May-June, when ambient temperatures were higher than during the home-deployment test. These elevated temperatures likely further increased microbial growth and condensation inside the artefacts. As both the amount of moisture in the plates and the intensity and frequency of tilting varied, microbial expressions became progressively more diverse.

Finally, contamination was likely caused by the swiping magnet, as mould growth often emerged close to it and shortly after swiping. Participants may have unintentionally accelerated its spread through repeated swiping.

Noticed and unnoticed expressions

Participants primarily commented on changes in the colour and shape of the Flavobacteria colony, with colony expansion and colour decline reported most frequently. For example, P4 observed, "*Definitely on the sides, there are spots that appear in the morning, and then in the evening, they're bigger,*" indicating awareness of daily changes. Similarly, P5 described rapid but more severe changes in form: "*It was just growing in a circle, and then suddenly it had this streak to one side and then to the other side, very quickly and maybe even overnight.*"

Five out of six participants noted that the artefact's growth and colour changes occurred at varying speeds, with phases of faster and slower development. P2 reflected, "*Within two or three days, it had expanded very rapidly, then the decrease of colour was much more gradual, and then, all of a sudden, within a day, the colour really fully dissipated.*" P5 similarly noted, "*Speed-wise, [the changes were] super variable. Because in the beginning, nothing happened, and then suddenly, apparently, [the artefact changed] pretty quickly.*"

During the second phase of the study, some participants noted that colony growth became less noticeable due to its scale or shape, as P6 reflected, "*It was no longer a very concrete shape, so then you cannot really tell [that it is expanding].*"

Certain nuanced changes in Flavobacteria's expressions—such as recolourations in swiped areas or subtle shifts in texture and form due to tilted orientations (Fig. 6.11a-b)— remained unnoticed. Interestingly, although recolourations in swiped areas were not verbally acknowledged, some participants visually recorded this phenomenon in their logbook images, such as P1, while noting the appearance of white specks within the colony (Fig. 6.11c).

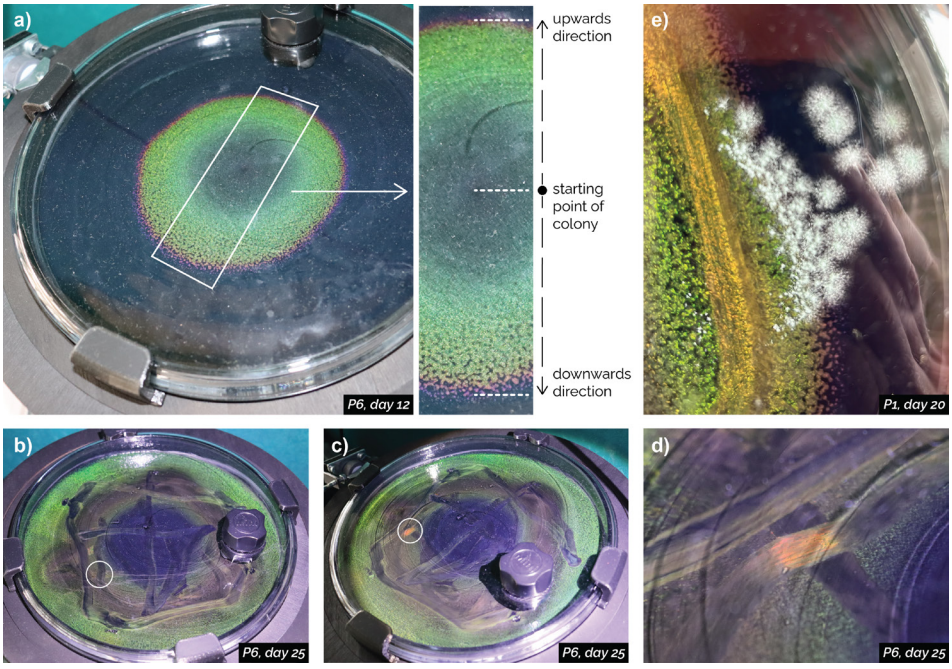


Fig. 6.11 *Microbial responses that were unnoticed by participants: a) a subtle and slow response of the Flavobacteria colony to a slightly tilted orientation on day 12, with a perspective-corrected close-up showing differences in texture and expansion; b-c) a swiped colony captured from different angles, with the same swiped region encircled and the orange colourations visible in (c); d) a close-up of these colourations; e) an image shared by P1 to report contamination, showing yellow-orange recolourations in the swiped area on the left.*

6.4.2 Embodied engagement with Flaviri

Participants actively engaged with the living artefact through observation and physical interactions, such as tilting, swiping, and relocating the artefact. Observation was sometimes passive, yet often intertwined with physical interaction—participants reported looking closely while interacting with Flaviri, and often interacted because something caught their eye. As P4 put it, "*When I observe, I tilt.*" This embodied engagement occasionally extended to visitors, who sometimes interacted playfully with the artefact. P2 recalled, "*I was showing it to them, like, look how cool this is!*"

Engagement also involved documentation, sometimes meticulous, by using microlenses to capture colourations in detail (Fig. 6.12), and often leading to a mismatch between lived and documented experiences. P3 expressed, "*I have some pictures, but it's just not working out.*" Similarly, P1 noted, "*It is super nice to look at, but then in the picture it is less nice.*" To improve photo quality, some adapted their interaction techniques. P2 explained, "*The magnet helped with*

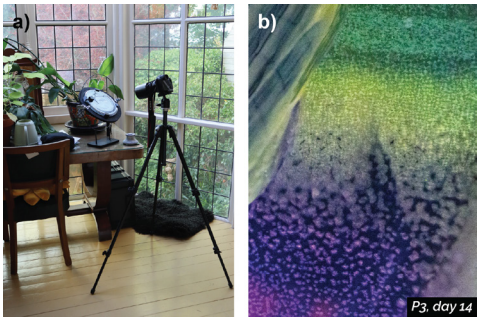


Fig. 6.12 *Participant P3's setup to capture details of the colourations (a) and the corresponding result (b).*

cleaning off the humidity on the inside. When I took photos of the mould, I did the whole surface to get a better photo."

These photos were also shared remotely, positioning the artefact as a shared object of attention. P4 noted, "*I shared pictures with friends in other cities, and then they wanted updates. I find it interesting that people feel intrigued, even though they don't see it physically.*"

Routines of embodied engagement

Observation frequency was high, at least at the start of the study, with all participants checking the artefact daily, though the nature and intensity of observation varied. P5, for instance, noted, "*With its location, you pass by a lot, so I see it a lot when I'm at home. Like, 10 times a day at least.*" P3 described a more measured approach: "*I checked it daily or every other day, at least, because I wanted to see the colours.*"

As the study progressed, some participants reduced the frequency of observation as visible changes diminished or stagnated. P2 noted, "*When the humidity started getting really noticeable, I became less interested in looking at it because I couldn't see anything.*" Similarly, P1 reflected, "*Then at some point I started to look less. I think it was already quite big. Then the growth was less interesting.*"

Participants reported both structured observation routines and spontaneous glances while passing the artefact. P4 shared, "*I think [checking] it was like a little ritual at some point. I wake up in the morning and check if it's looking good, and then in the evening, the same.*" P6 noted, "*I checked it every morning when we were having coffee here.*" P5 reflected, "*It was one of the first things that I checked after I was out for a couple of days.*"

The frequency and duration of tilting and swiping are illustrated in Fig. 6.13, which plots the physical interactions detected by the sensors. In some cases, data was missing due to power issues; these gaps were reconstructed from participant comments and are shown in grey.

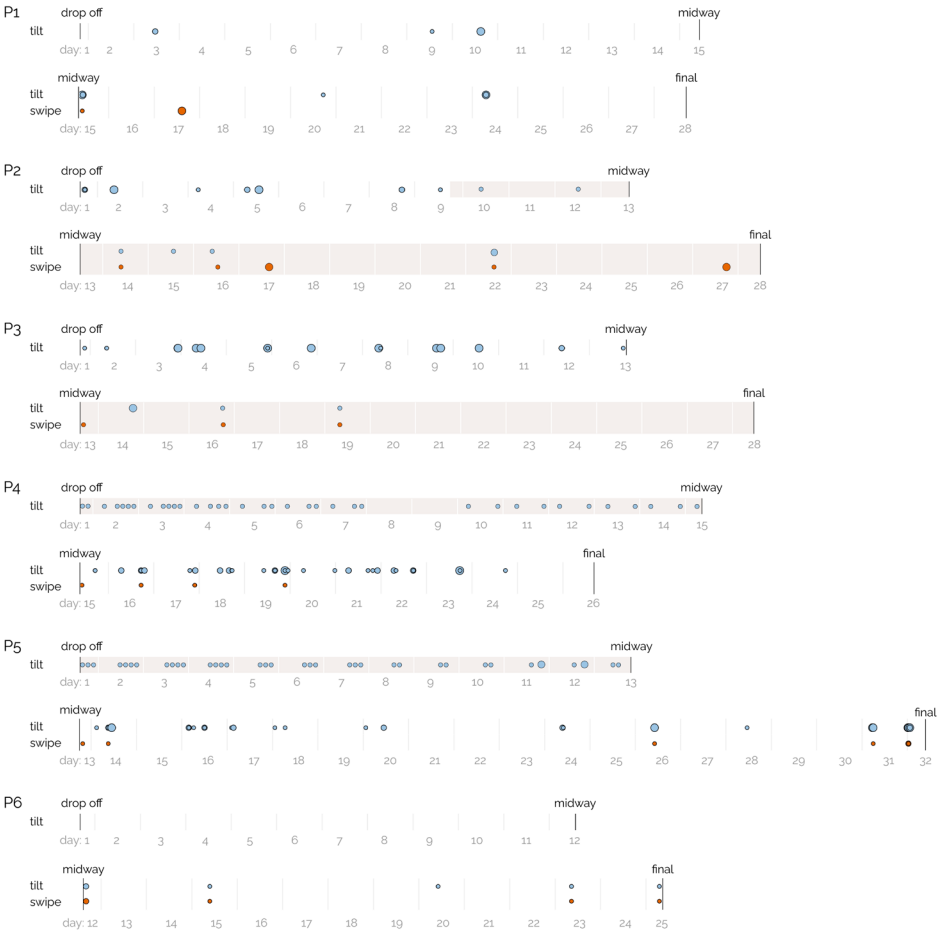


Fig. 6.13 *Interactions through tilting and swiping per participant, with duration indicated by dot size (small = up to 10 seconds, large = more than 30 seconds). A gap threshold of 30 seconds was used to separate registered interaction events. Interaction data shown in grey areas represents reconstructed events based on participant comments, due to missing sensor data in certain phases of the study.*

Interaction frequency varied widely across participants. For instance, P6 did not touch the artefact at all during the first two weeks, while P4 interacted extensively throughout the entire study. Sensor data of P4 showed on average 3.6 interaction events per day, peaking at seven on some days, aligning closely with her self-report: "*When I'm at home, it's like five to six times a day.*"

Over time, interaction patterns shifted. Some participants reduced physical contact, often following events such as colour decline or contamination. P2 noted, "*As far as like physically touching it, dropped off dramatically actually, because the colours went away.*" Others maintained or resumed interaction, hoping for recovery based on prior experiences where the artefact regained colour (Fig. 6.10e-f). P5 recalled, "*I thought I killed them all, and nothing was*

going to happen anymore. Then I came back, and suddenly it was all different—regenerated in different spots."

The duration of these interactions also ranged considerably, with the sensors tracking brief one-second gestures as well as extended sessions exceeding five minutes. P2 reflected, "*I interacted with it for longer periods in the beginning. As I kind of understood what was happening, I interacted for shorter periods of time.*"

Some participants developed structured rituals for these physical interactions, such as daily tilting at set times, while others acted more casually when passing by or noticing changes. P5 described, "*Every time that I passed by, I would move it a little bit.*" P4 established a more structured routine: "*It became regular, like, in the morning, I would tilt it and then again in the evening, when I came back.*"

Furthermore, relocating Flaviri ranged from a few instances during the study to multiple times per day, and sharing and documentation practices evolved alongside these physical interactions. Over time, these practices became increasingly selective, often limited to the artefact's 'best' states. Many participants stopped sharing once the decline set in, and some hesitated to document the artefact when it appeared less appealing. P6 explained: "*We didn't send too much. My friend said, 'Let's try to make it pretty first.'*"

Motivations for embodied engagement

All participants noted curiosity as a primary motivation for observing Flaviri. In some cases, unexpected changes renewed attention and increased observation. P1 described, "*Then I suddenly had another form, and then I checked it more often. That was interesting.*" P5 similarly remarked, "*And now that something [in the artefact] is becoming liquid, it's like, oh, what's happening? You notice that instantly you're invested again.*"

Curiosity also drove physical interaction through tilting and swiping. For some, these acts were further motivated by a wish to influence or control the growth patterns of the artefact. P3, for instance, explained, "*I was trying to influence a growth pattern. I wanted to grow this smiley.*"

Tilting was additionally motivated by the perception that it encouraged growth by circulating liquid and by its ability to reveal iridescence. P4 recalled, "*I tilted, thinking maybe it would bring the colours back by moving the material around.*" P5 explained, "*I move it a little bit to get the different light angles and to see the colours.*"

Other reported motivations for swiping, in addition to curiosity, included its playfulness, tactility and directness. P1 noted, "*I liked the feeling of making stripes, like the physical feeling.*" P2 reflected, "*I like that I could interact with it directly. In a sense, I was touching the colours.*"

Relocating the artefact was, for some participants, motivated by care. P4 shared, "*Sometimes it ends up behind a cupboard, so it doesn't get direct sunlight, or in the kitchen. He has been moving around, you know?*" Others relocated it to facilitate observation. P5 remarked, "*On days that I was working at home, I put it next to me to see the colours a bit and move it. It's a nice distraction.*" He also noted, "*I put it on the table when people were coming over so they could have a proper look.*"

Barriers to embodied engagement

Observing and interacting with Flaviri was limited by practical and social factors, including work obligations, being away from home, or having guests. For example, P6 had a guest staying with her and imagined how her engagement might have differed without this social constraint: "*I probably would have noticed it more […] I would have looked differently and tried different things, but because we were both trying, it was difficult to understand the pattern.*"

Observation was further hindered by artefact-related challenges, such as glare, dust on the glass, and extensive humidity inside the Petri dish (Fig. 6.14), which impacted participants' ability to see and document microbial growth. P2 described, "*There's a lot of humidity within the artefact, and it made it hard to see what was happening, to photograph and to really feel the colourful experience,*" noting a rhythm in visibility: "*In the morning, the humidity was the lowest, and I could really see what was happening inside of it.*"

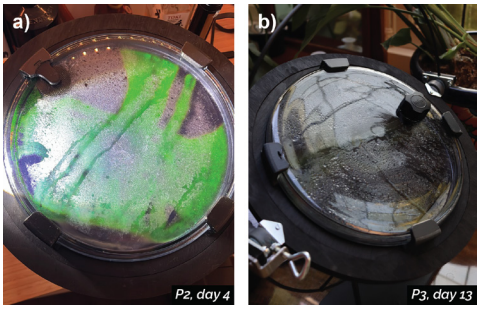


Fig. 6.14 *Humidity and reflections hindering visibility in the living artefacts of P2 (a) and P3 (b).*

Physical interactions with Flaviri were, first of all, limited by motivational barriers. P6 reflected on the lack of tilting in the first two weeks: "*I just didn't think of it. I didn't, maybe, get that it was maybe part of the assignment, or it's not…*", indicating a lack of intrinsic drive to interact. Another participant gradually stopped interacting as his actions failed to influence growth patterns. P3 noted, "*I tried to influence the growth pattern, but I couldn't. And I love to be in control.*"

Swiping, in particular, was quickly avoided by some participants as it was linked to artefact decline or disrupted microbial aesthetics. P1 reflected: "*I didn't like the stripe. I tried more round forms, but I felt like I was only ruining it.*"

Furthermore, mismatches between expected and actual microbial responses limited physical interactions, as participants often postponed interventions while waiting for expected delayed effects. P4 remarked, "*I didn't move the magnet a lot because I wanted to see the change, and nothing happened, so I was like, maybe I just need to be more patient.*" Similarly, P2 noted, "*I let the magnet sit to see if it would regrow or change colours. I was thinking it was a delayed effect.*"

Styles of embodied engagement

Participants reported both casual observation and focused, in-depth viewing, using a flashlight or carefully comparing old and new parts of the colony. P1 noted, "*When you look with the flashlight, it has another colour than without,*" while P2 mentioned, "*Whenever I took photos, I would use the torch on it and illuminate, trying to get the right colour.*"

In terms of physical interactions through tilting and swiping, participants varied in their style, ranging from gentle gestures to more playful experimentation or forceful attempts to control growth. Even when motivated by the same intention—such as recovering faded colours—participants differed in approach: some acted carefully, while others engaged in more forceful interventions (Fig. 6.15). P4 explained, "*I carefully moved the magnet to the middle to bring colour to the centre,*" whereas P2 described, "*I wanted the colour to come back, and so, I did another really intense playing around.*"

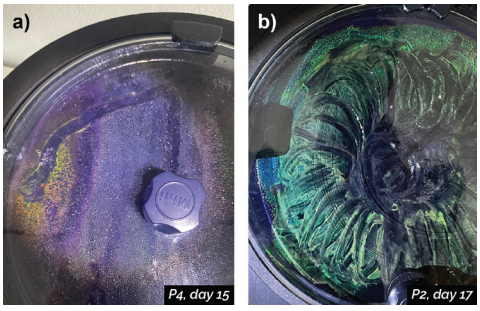


Fig. 6.15 *Swiping to recover colourations: careful intervention by P4 (a) versus bold, expressive gestures by P2 (b).*

Over time, gentle interaction sometimes gave way to more forceful gestures in the absence of observable changes. P1 described this shift: "*I was a bit bored because nothing happened, and the stripes stayed. I was, like, okay?! Frustration. I'm going to make more stripes, and I was also a bit less careful.*"

6.4.3 Affective engagement with Flaviri

Beyond embodied engagement, participants engaged with their living artefacts on an affective level, expressing a rich and diverse range of emotional responses towards the artefact and the microorganisms within it. Reported emotions were often a direct response to the artefact's expressions—its colour, growth pattern, and other transformations.

Curiosity was a common emotion, frequently accompanied by fascination, especially when participants noticed microbial growth, unexpected colour shifts, or even contamination. P4 noted, "*I'm intrigued because it changes quite a lot sometimes throughout the day.*" P1 described her reaction to contamination: "*Then, the spots came, and there was curiosity and a bit of joy… When I looked closely, they looked nice. Fascinating*" (Fig. 6.16a).

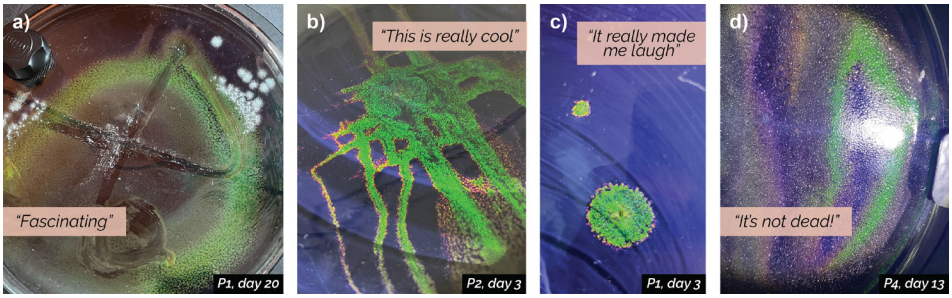


Fig. 6.16 *Changes in living artefact sparking curiosity and excitement: a) white spots appearing; b) spreading patterns; c) emergence of a second colony; d) recovery of colourations.*

Excitement was commonly sparked by visible change, responsiveness, or signs of recovery (Fig. 6.16b-d). P2 shared, "*I was, like, oh, this is really cool. It's spreading. I can tell it's following the liquid that I moved around.*" P1 reflected on an unexpected change: "*First, I had one spot, and then suddenly there were two. It really made me laugh.*" P4 described a moment of recovery: "*I saw that it was shining again, and I was really happy about that. I was, like, 'It's not dead. It's still there!'*"

These emotional responses were closely tied to microbial temporality. Rapid changes commonly sparked excitement, while slower growth often evoked anticipatory joy. Conversely, stagnation or deceleration led to frustration, disappointment, or worry. P3 captured this duality (Fig. 6.17) by first noting, "*I was excited here because… look at that, right? From day one to day four, a dramatic change,*" and later, "*Total disappointment here because it didn't grow anymore.*"

Negative emotions also emerged in response to fading colours or the perceived effect of participants' own interventions. Whereas P3 noted insufficient effect: "*I was disappointed because there was hardly any effect,*" P6 felt they had interfered too much: "*We messed it up. The prettiness was lost with our*

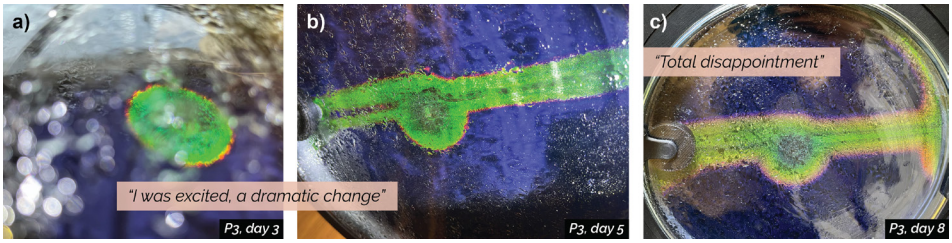


Fig. 6.17 *Emotional responses linked to microbial growth: a-b) extensive growth during the first five days; c) stagnation of growth on day 8.*

intervention" (Fig. 6.18a). Frustration was reported when participants were unable to observe the colourations due to obstructed views or absence, sometimes leading to missed crucial moments. As P5 expressed upon his absence: "*Now I'm frustrated that I didn't see it happening and wondering how and why all of a sudden [it changed].*"

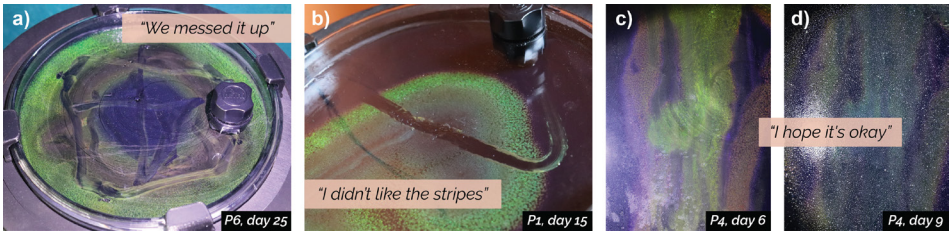


Fig. 6.18 *Decline and aesthetic discomfort: a) perceived loss of prettiness; b) unwanted stripes; c-d) murky colours after potential incidental sunlight.*

Temporal changes in affective engagement

Over time, emotional trajectories began to reveal themselves. Excitement and curiosity peaked when changes were new or vivid, but tended to decline as microbial growth slowed or transformations became less noticeable. While most participants remained curious and engaged throughout the study, some began to distance themselves as the artefact declined or became less appealing to them. As P1 reflected, "*I didn't like the stripes, it didn't change that much, and the colours were less bright*" (see Figure 6.18b).

In the second phase of the study, negative emotions—including aesthetic discomfort, disappointment, frustration, and worry—were reported almost twice as often. Even highly engaged participants noted a slight reduction in their interest or fascination. P4 observed, "*The sense of wonder was kind of lost because nothing was really happening, and it was not really shining anymore.*" Similarly, P5 explained, "*The last couple of days I paid less attention to it because it was not multiplying or moving anymore.*"

In terms of the two interaction modalities, participants expressed more diverse emotional responses to swiping. Initial reactions were positive, driven by

tactility, immediate visual feedback, and intense curiosity, as P4 reflected, "*It really brought a new level of curiosity. It left me wondering a lot how it would look.*" However, reactions shifted to confusion, disappointment, and ultimately reluctance when the intervention was linked to the decline of the artefact.

Emotional orientations towards the Flavobacteria

At a more relational level, participants' responses towards the artefact and the microorganisms within it ranged from deep concern and guilt to indifference. For some, their responses reflected care, including worry about harming the Flavobacteria through interventions, relocating the artefact to protect it from sunlight, and sadness upon its decline. As P4 shared, "*I was gone for two days, and then it got a bit murky here, and I was like, I hope it's okay. I was very concerned about the health*" (Fig. 6.18c-d). Similarly, P2 reflected, "*I think, actually, I feel some guilt as well. Once I noticed the green wasn't really repopulating the areas where the magnet had gone, it was like, 'Am I killing what is in here?'*"

Others expressed more indifference toward the microorganisms, as P3 explained, "*It didn't do a lot to me. No, it's a few bacteria. It's okay, come on.*" Yet despite this emotional distance, these participants still showed emotional investment in the artefact itself—adjusting its position to recover colourations, and expressing pride or frustration in response to changes. As P5 reflected, "*I don't have an emotional bond with them, so it's not that I think, 'Oh, sad, bacteria,' but more like, 'Oh, I'm killing the experiment.'*"

6.4.4 Understanding and reflecting upon Flaviri

Finally, participants' engagement with the living artefact unfolded on a cognitive level, as they interpreted visual changes in the moment while also developing broader ways of understanding, reasoning about, and reflecting on the living artefact.

Reasoning about viability and the impact of actions

Participants' cognitive engagement was initially sparked by visible changes, as they sought to make sense of microbial behaviour through momentary reflection on their observations. They primarily interpreted the colour within the artefact as signs of microbial viability or decline. As P4 explained, "*I was, like, if it's coloured, it's healthy. If it's not, then not.*" Faded colour was interpreted as the death of the colony, as P3 noted, "*The colour is gone. It was like, everybody died in there.*" Additionally, colour shifts in the liquid within the Petri dish were associated with microbial viability, as P2 remarked, "*The colours started to fade, and I assumed that correlates with the liquid turning brown and becoming less of a sustenance for the colours.*"

These interpretations not only reflected participants' attempts to understand microbial viability but also shaped how they reasoned about the consequences of their own actions. Tilting, which initially appeared to spread colour, was cognitively linked to microbial recovery or wellbeing, whereas swiping, often followed by colour fading or contamination, came to signify disturbance or decline. As P4 reflected, "*There definitely was a reluctance to interact with the magnet because I was like, 'It is probably the source of my problems.'*"

Mental presence beyond observation

Beyond immediate sense-making, participants' cognitive engagement also involved a mental presence of the artefact, as it occasionally surfaced in their thoughts even when they were not directly observing it. The artefact occupied participants' minds in uneven ways: for some, it gradually faded into the background as novelty diminished and rarely entered thought during absence. As P2 noted, "*After two weeks, it really started to disappear into the background of the home, like, it became an object on a shelf.*" For others, the artefact maintained a more active mental presence. It became a pleasant distraction within the home environment and a topic of conversation elsewhere. Some also found themselves thinking about it during their absence, wondering how it might have changed. As P5 described, "*Some days in the office, I thought, 'Oh, I wonder if something happened today.'*" Similarly, P4 remarked, "*When I was away for two days, I was also thinking about it… I wish I could just take it with me, you know?*"

Conceptualising the artefact and its livingness

Over time, participants' understanding of the artefact's living nature and their position in relation to the artefact also evolved. Participants varied in how they conceptualised the artefact, with perceptions of its livingness shifting over time. While most recognised the artefact as a living entity, some continued to describe it in casual or vague terms, reflecting its unfamiliarity. P2 described it as "*this cool thing in my home that is changing colours.*" Similarly, P4 remarked, "*I tried my best? I'm like, 'It's algae that looks like butterfly wings.' But then I admit, I cannot go too much into detail. I'm like, 'It grows, spreads or something.' I don't even know what the word is for it,*" reflecting uncertainty about how to describe the artefact.

Occasionally, participants used biological language, describing the artefact as growing, dying, or reproducing as they made sense of observed visual changes. P2 employed such biological terms but, interestingly, only truly recognised the artefact as living once mould appeared: "*I don't know if in the first two or three weeks, I ever really thought of it as a living thing until the mould appeared. It was really more of an object before,*" reflecting a clear shift in perception. In contrast, for others, the artefact's responsiveness to its environment served as a clear reminder of its living qualities. As P4 described, "*It is very alive in the sense that,*

if there's less sunlight, it shines a little less. But if it's very sunny, then at the end of the day, I see more shimmer. So, it really lives with the environment around it."

Participants variously described the living artefact as fragile and sensitive or resilient and flexible, and frequently drew analogies to familiar entities to make sense of it—seeing it as a living artwork, a roommate, or a pet, or comparing it to their household plants. Some described a sense of cohabitation, actively sharing their space with it, while others experienced it as a more passive entity, as P6 noted, "*It's not supposed to do anything; it's just supposed to be there.*"

Reflections on the impact and value of the artefact

Beyond sense-making of microbial behaviour and the artefact in general, participants also shared reflections on its impact, meaning, and anticipated futures. Some participants reported a heightened environmental attentiveness as a result of living with the artefact. P5 reflected, "*You're kind of less mindless walking through the house, so more aware of your surroundings, maybe.*" Similarly, P4 described, "*I felt like, oh, hey! Now I know that the sun comes from this direction and ends at this line at this period of the year. It felt like it connected me more with my environment around me, which I think is really powerful, actually.*" In some cases, such heightened awareness extended beyond the immediate environment into broader reflections on life, growth, and the presence of microorganisms in daily life. As P4 reflected, "*It definitely made me more aware that microorganisms are part of a lot of things in life.*" She continued, "*To me, it really brought, like, a feeling of reflection about life, I guess, and like how things grow.*"

Other participants struggled to understand the value and purpose of the artefact and the broader research context. P1 wondered, "*What's the purpose and urge of it? The value for society… What can it bring to us?*" Despite such struggles in meaning-making, most participants valued the artefact's presence and the routine of engagement and could imagine long-term engagement with the living artefact if practical constraints were manageable. P1 noted, "*It could be really nice, but it shouldn't get mouldy.*" Similarly, P2 remarked, "*If there is a way for it to continuously stay in this growing, dissipating, growing state, it'd be really cool to have at home.*" They also reflected on what they considered an acceptable level of effort in living with the artefact in the long term. For some, occasional care was acceptable as long as maintenance remained simple, as P5 remarked, "*If this is an easy thing to keep it going, I think it would be fun to have in the house, and I think lots of people would enjoy it.*" Others expressed a more passive attitude, as P6 noted, "*If it's self-sustainable, and I don't need to do anything, it'd probably be very cool.*" In contrast, P3 expressed less interest in long-term engagement, pointing to the artefact's low responsiveness: "*I would never buy one, because they don't really interact with you.*"

Together, these findings illustrate how participants' cognitive engagement developed through momentary interpretation of microbial behaviour and

broader reflections on impact and meaning. This cognitive engagement was deeply intertwined with participants' observations, interactions, and affective responses—an interrelation that the following section explores in greater depth.

6.4.5 Adopted roles in relation to Flaviri

Lastly, throughout our study, we observed that participants varied in how they positioned themselves in relation to the artefact, ranging from observers and carers to experimenters and controllers. For example, P4 explained, "*I started being like, this is 'my plant' as well, so I need to take care of it. I need to be sure that it's looking healthy, even though I'm not sure what's supposed to look healthy with this.*" Some approached the artefact more experimentally, yet they varied in the perception of microbial agency. Whereas P5 explained, "*It is not that I'm trying to make them do anything like in a circus or something,*" P3 clearly stated, "*I love to be in control.*" Other participants adopted a more observant stance, interfering very little with microbial growth, as P6 reflected after the study, "*I think maybe people shouldn't interact and just let it grow.*"

These adopted roles shaped engagement across different modes, revealing distinct patterns in observation, interaction, emotions, and thoughts of participants, as illustrated in Figure 6.19.

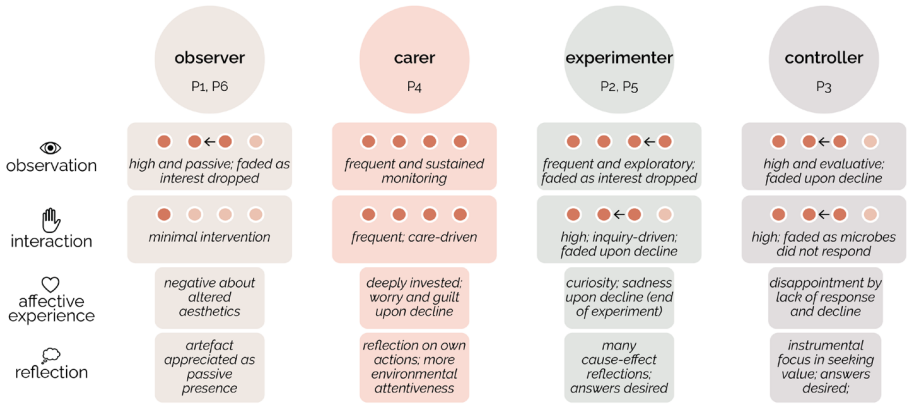


Fig. 6.19 *Identified roles that participants adopted and their engagement patterns across observation, interaction, affective experience, and reflection.*

While these roles capture dominant patterns, they were not static. For instance, P1, primarily acting as an Observer, briefly shifted to a Controller when frustrated by stagnation in the second half of the study, intervening out of boredom and frustration despite earlier regret and disappointment about disrupting the colony's organic shape.

6.5 Discussion

This study examined how the diverse temporal expressions of microorganisms shape human–microbe engagement in everyday life. Over four weeks of deploying the living artefact Flaviri in six households, we observed diverse patterns of engagement across the four modes. Participants frequently observed the artefact, though the frequency of these observations declined as visible microbial changes became less pronounced. Their physical interactions with the artefact ranged from cautious handling to more exploratory actions, and in some cases, participants deliberately paused to see whether the organism would respond. Emotional reactions, such as fascination or concern, were closely tied to visible microbial transformations. Participants' reflections centred around understanding the microbial aesthetics, situating them within their everyday routines, and considering the artefact's broader purpose and impact in their lives. The following discussion interprets these findings within individual modes of engagement and across them, before turning to limitations and broader reflections.

6.5.1 The seen and unseen

The fact that some microbial expressions—such as those shown in Figure 6.20—were likely overlooked by participants, even though they appeared quite evident in previous lab studies and our test deployment, raises a question: which microbial expressions can actually be noticed and appreciated when participants are not yet attuned to what they might look like? Flaviri's temporal expressions were deeply unfamiliar to the participants and sometimes clashed with participants' expectations as they varied both in timing and intensity. More time and repeated exposure may therefore be needed to develop the sensibilities required to detect subtler changes. Future studies might extend the duration of deployment to explore whether noticing emerges over time or include guided observation sessions that progressively introduce participants to microbial dynamics, helping them attune to subtler changes.

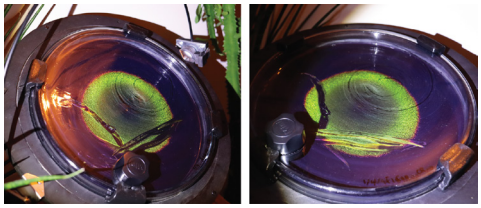


Fig. 6.20 *Microbial expressions in response to swiping during the test-deployment. Two photos show the same colony at the same moment from a different angle, revealing bright recolourations that are only visible from certain viewpoints.*

Unseen microbial expressions in living artefacts might sometimes be inevitable and inconsequential, but can also carry risk: they can lead to confusion—participants assumed nothing was happening—and, in turn, disengagement or

even compromised care practices. As Zhou et al. [164] note, aligning microbial and human temporalities might be crucial for timely care actions. Moreover, perceptibility also shapes our emotional investment and sense of responsibility. As Rosen [225] argues, 'By expanding the sensible we expand the thinkable, and hence our scope of responsibility and care.'"

To increase noticeability, biodesigners can scaffold perception through cues, prompts, or interpretive aids that help participants recognise and interpret these dynamics, or surface microbial expressions through visible manifestations [134]. Yet, in doing so, they must remain critical: how can expressions be made noticeable enough to invite engagement without instrumentalising microbial life or compromising its ecological and aesthetic integrity?

This question points to a deeper issue: engagement with living artefacts requires more than amplified microbial expressions. It is not simply about making microbial expressions visible, but about attuning ourselves to microbial temporalities, scales, and modes of existence, ultimately expanding what we notice and respond to. Sensibilities-oriented perspectives argue for developing better ways of sensing, reading, and observing, to cultivate familiarity with a world that is not easily perceptible on human terms [18, 227]. Surfacing techniques alone cannot achieve this. Rather, humans must be willing to invest time, energy, and effort to attune themselves—supported by new tools and forms of guidance that help us re-learn how to look, observe, and notice, and build the sensitivities required to perceive these subtle forms of life.

6.5.2 Between detachment and investment

As discussed earlier, some participants approached the artefact from an instrumental or experimental stance, which may have encouraged a sense of detachment. Yet, despite this stated distance, they expressed strong emotions—fascination, pride, and frustration—and even tried to recover fading colourations, suggesting a degree of investment directed not at the microorganisms themselves but at the artefact and the interaction possibilities it afforded.

Given their professional backgrounds—a biotech CEO and an ecological researcher—the term "feelings" in relation to microorganisms may have clashed with disciplinary norms around objectivity. Although the questions concerned how they felt *during the interactions*, both participants quickly emphasised their lack of affection for the microbes. This made us wonder whether they were not invested, or if the phrasing simply triggered them to reaffirm their professional stance. Future studies exploring affective engagement with microbial living artefacts might want to carefully reassess the vocabulary used to probe affective engagement, opting for terms that feel less conceptually loaded. Beyond verbal descriptions, alternative approaches—such as observational methods and physiological measures [266, 267], or creative elicitation techniques (e.g., drawing, or storytelling) [268]—could offer richer insights into how emotional investment manifests in practice. Emotional engagement may not always align

with participants' conscious self-reports—especially when notions of caring for microorganisms feel conceptually unfamiliar and challenge disciplinary values.

In any case, indifference towards the well-being of the microorganisms does not seem to out-rule meaningful interactions with the living artefact. Not caring about the microorganisms themselves does not necessarily imply a lack of care for the system they inhabit or for the interactions it enables. For example, despite expressing little emotional investment in the microorganisms, P5 reported strong curiosity, fascination, and heightened attentiveness to his surroundings. This suggests that even without establishing—or explicitly self-reporting—relationality with the microorganisms themselves, engagement with living artefacts can still reinforce attentiveness to more-than-human life and support appreciation for other living entities.

Moreover, initial indifference does not mean that affective engagement cannot emerge or evolve. As participants continue to value and interact with the artefact, feelings of care toward the microorganisms within it may gradually develop—a trajectory that resonates with the first author's growing sense of connection and relationality that emerged through sustained engagement with Flavobacteria. Future work could explore longer-term deployments of living artefacts to examine how engagement evolves once the initial novelty diminishes and humans continue to share their space with the artefact. Ongoing interaction and care practices may deepen engagement, as attending to or caring for a system can enhance investment and appreciation over time (e.g., [269–271]).

6.5.3 Ambiguity of livingness

Knowing that microorganisms are present does not always translate into a sense of aliveness, meaning the recognition of livingness depends not solely on the information provided but also on how microbial aesthetics are interpreted. Microbial expressions, while dynamic and responsive, do not always register as "life" within everyday interpretive frameworks. The fact that mould—an organism and phenomenon far more familiar in domestic contexts—served as a stronger cue of livingness than Flavobacteria's responsive and temporal expressions highlights how recognition can be compromised by unfamiliarity. As participants lacked prior experience with life forms that behaved and expressed themselves like Flavobacteria, their interpretations sometimes defaulted to non-living categories already available in their world, such as colour-changing objects like liquid motion toys, in which coloured oil and water offer a dynamic colour play. Our focus on 'our world' [272], including forms of life that behave in recognisable and relatable ways, makes some microbial life more difficult to perceive as *alive*, even when it is relatively expressive. In this sense, what "counts" as alive is not only a biological fact but also a culturally and perceptually shaped construct.

Our study also showed how recognising the artefact as alive carried moral implications. As P2 reflected, had he more fully applied the idea that "this is living", intervening so forcefully might have evoked a stronger sense of guilt. The

ambiguity of livingness appears to have shaped how participants navigated care, responsibility, and attachment in their interactions with Flaviri.

Taken together, these observations suggest a tension between technical knowledge ("I know it is alive") and perceptual or affective recognition ("it feels alive"), as the artefact's microbial aesthetics did not always bridge this gap. Biodesigners may need to scaffold this recognition or actively remind us to support emotional engagement and responsible interactions with microorganisms. In doing so, they should consider avoiding oversimplifying or anthropomorphising microbial life to not merely make the livingness visible, but also cultivate sensibilities and attentiveness to unfamiliar life forms.

6.5.4 Fragility of engagement

Engagement with living artefacts can be remarkably fragile, easily disrupted by fading novelty, stagnation of microbial growth, colour decline, or aesthetic discomfort. This pattern resonates with broader human tendencies shaped by our fast-paced, consumption-oriented world, where interest often depends on continuous stimulation and rewards. Within such frameworks, living artefacts risk being interpreted through an instrumental lens—valued either for clear functional benefits (e.g., air purification) or, in their absence, treated as entertainment that should deliver constant visual change, as observed with Flaviri. Even when people approach these artefacts as relational entities rather than functional or decorative objects, slow microbial expressions can still be difficult to attend to in a culture accustomed to immediacy and instant feedback.

This raises a critical design challenge: how can we sustain meaningful engagement with living artefacts when their expressions slow down or stop changing? Designers might consider amplifying changes or introducing interpretive cues to reinforce connections. However, doing so risks reinforcing human-centred expectations of constant stimulation and aesthetic control, masking the qualities that make these artefacts living—such as their cycles of growth, decline, and transformation. Rather than concealing pauses or decay, designers could embrace these phases as opportunities for reflection on life cycles and ecological rhythms. Such an approach could cultivate acceptance of temporal variability and less conventionally appealing aesthetics, reshaping expectations to align with the living nature of these artefacts.

At the same time, integrating living artefacts into a society accustomed to immediacy and instrumental value remains challenging. While the world in its current cultural context may not be ready for sustained engagement with living artefacts that lack clear utility or conventional aesthetics, this does not diminish their potential. Even transient encounters can matter: they can spark curiosity, foster more-than-human sensibilities, and gradually expand our understanding of what constitutes 'our world,' encouraging attentiveness to unfamiliar life forms and their rhythms.

6.5.5 Limitations and broader reflections

A key limitation of this study lies in its small sample size and its duration of four weeks. While the study yielded rich qualitative insights, it only involved six participants, given the complexity and labour involved in creating the living artefacts. Together with the limited duration, which is in fact relatively long for deployments of living artefacts in domestic settings, these aspects constrain the generalisability of our findings. As such, the participant roles we identified, for example, should not be taken as generalisable categories but as early indications of possible stance-taking in human–microbe encounters.

Interestingly, however, we see parallels between our roles and patterns reported in a longitudinal study with a moss living artefact [255]. For example, our observer stance overlaps with their 'previous living ornaments' culture of care, characterised by brief, consistent checks and low exploration, while our carer role resonates with their 'interconnectedness' culture, marked by attentiveness and respect. These similarities suggest that certain stance-taking patterns may recur across different living artefact contexts, pointing to opportunities for further investigation in varied contexts.

We also observed intriguing patterns regarding these roles that could prompt further investigation. Participants' professional backgrounds appeared to shape how they engaged with Flaviri: those working in educational roles approached it as observers, those in support roles as carers, participants with research or design backgrounds approached the artefact experimentally, and the participant with a biotech leadership role engaged with it in a more controlling manner. These alignments suggest that disciplinary logics may inform how people position themselves in relation to the microorganisms within living artefacts.

We also noted a tentative pattern related to gender: in our study, observers and carers were women, while experimenters and controllers were men. While our sample size is far too small to support generalisations—and gender itself is neither binary nor biologically deterministic—this pattern raises questions about how gendered norms and care practices, even if socially constructed, emerge and intersect in situated engagements with living artefacts. This points to a valuable area for future work that could contribute to existing discussions about the entanglements of gender and care [271, 273].

Reflecting on Flaviri in practice

Although our study involved only six living artefacts, a wide diversity of microbial expressions emerged. We had intended to evoke subtle and slow responses at first, followed by faster and more diverse expressions through the staggered introduction of interaction modalities. In practice, however, this sequence proved difficult to realise. Differences in environmental conditions across households, combined with the unexpectedly intense interactions of some participants, led to substantial variation from the very start of the study—well before the intended transition into the more expressive phase. This variability

complicated the formulation of concrete answers to our research questions. Yet the resulting diversity was compelling and highlighted how strongly the bacteria respond to their surroundings. Although we did not anticipate expressions diverging so widely and early, this unexpected variation ultimately enriched our understanding of how living artefacts behave under "real-world" conditions.

Furthermore, the deployment of Flaviri revealed a material constraint: the agar-based growth medium degraded over time, causing cracks and crumbling. This highlights the need for alternative substates that can better support long-term use, scaling-up, and mobility, both for everyday living artefacts and for research contexts.

Finally, we designed Flaviri to support interaction through tilting and swiping, which differed in terms of directness [170]. We anticipated that these differences might shape participant responses and their willingness to physically interact with Flaviri and intended to investigate this. However, in practice, it proved difficult to disentangle the influence of the interaction modality from the influence of the microbial aesthetics themselves. The effects of the modalities, the diverse microbial aesthetics, and the roles participants adopted became intertwined, making it impossible to isolate the contribution of the interaction mechanisms alone. Moreover, the differences in directness were not always immediately apparent to participants; some, for instance, realised only through doing that moving the magnet caused the inner attachment to touch the colony. Future research is therefore needed to better understand how the directness and nature of interaction mechanisms shape human–microbe engagement over time.

Reflecting on the implications for regenerative ecologies

Through our study, we explored human–microbe engagement with a living artefact in daily life by outlining emerging microbial expressions and detailing engagement from the human perspective. In doing so, we generated insights into which microbial expressions are noticed and experienced as performative, emotionally engaging, or thought-provoking. We found that these insights not only illustrate nuances of enriched human experiences, but, importantly, also help designers understand how to invite, prompt, and support attentiveness to non-human life.

In living with Flaviri, participants described becoming attuned to microbial presence, needs, and diverse temporalities. Such forms of attentiveness reflect what Karana et al.'s framework [18] describes as *cultivating more than human sensibilities*. Notably, this attentiveness extended beyond the artefact itself. In addition to attending to the Flavobacteria, several participants reported a heightened awareness of microorganisms and environmental dynamics in their surroundings. Encounters with the artefact also provided opportunities for informal learning about microbial behaviour and responsiveness, possibly enhancing our understanding of the intricate dynamics within everyday life. Such shifts gesture toward living artefacts' potential to contribute to the

development of ecological literacy, culture change and more holistic worldviews [14], suggesting that they can foster ecological sensitivity and support everyday practices of attention and care that reach beyond the immediate system.

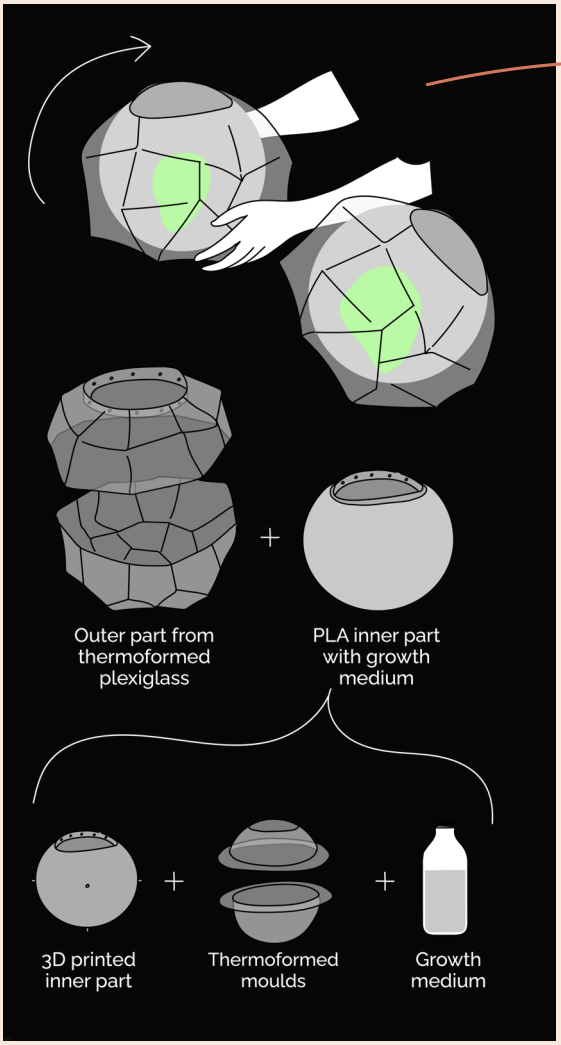
Taken together, the findings position living artefacts as relational mediators that can foster care, reciprocity, and ecological responsibility by expanding how humans notice and relate to microbial worlds and surrounding ecosystems.

6.6 Conclusion

This study explored how the living aesthetics of an everyday microbial artefact shape human–microbe engagement in practice by designing and deploying a Flavobacteria living artefact. By bringing Flaviri into our six participants' homes, we gained insight into how people perceive, interpret, and respond to microbial expressions as part of their daily routines. Our study revealed a wide variety of microbial expressions across the six artefacts, and rich insights into how participants' engagement evolved in relation to this. Findings show that microbial aesthetics can foster curiosity, attentiveness, and attempts at sense-making of microbial behaviour, yet engagement proved fragile—highly sensitive to microbial decline, stagnation, fading novelty, and mismatches between expectations and expression. Analysing participants' engagement with the living artefact through their observations, interactions, affective experiences, and reflections, we also identified four different roles that participants adopted in relation to the artefact: observer, carer, experimenter, and controller. Furthermore, the living artefact prompted several participants to actively attend to their environment and reflect on the presence of microorganisms in their surroundings. This supports the idea that living artefacts, when carefully designed, can contribute to more regenerative ways of coexisting with nonhuman life—not through functionality, but through attention, respect, and an expanded sense of the worlds we share.

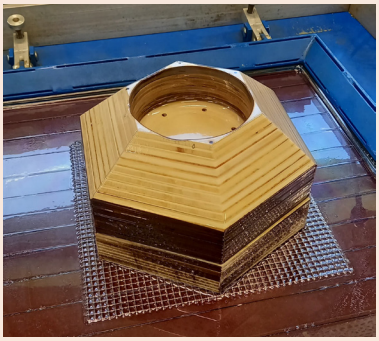
Visual Essay: The Development of the Living Artefact Flaviri

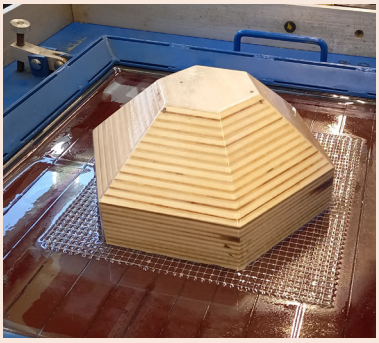
Version 1.0

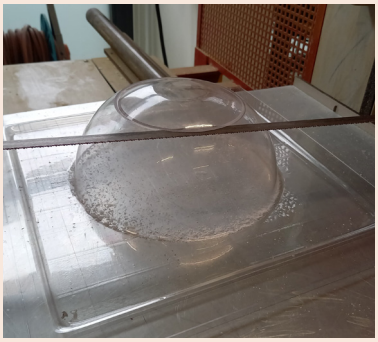


Design sketches

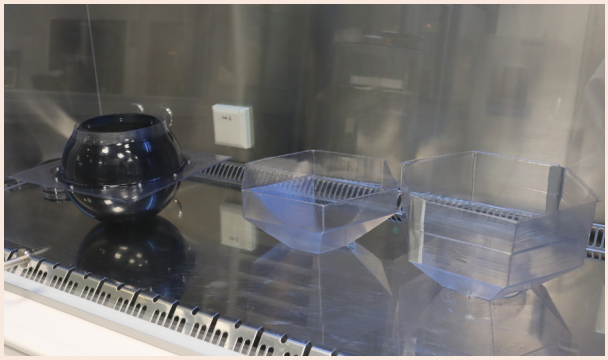
Simple interaction to elicit slow, subtle microbial response



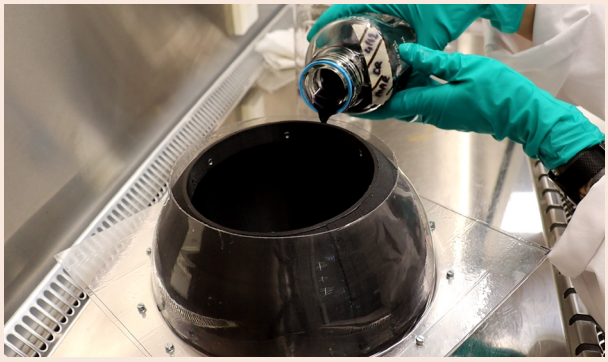




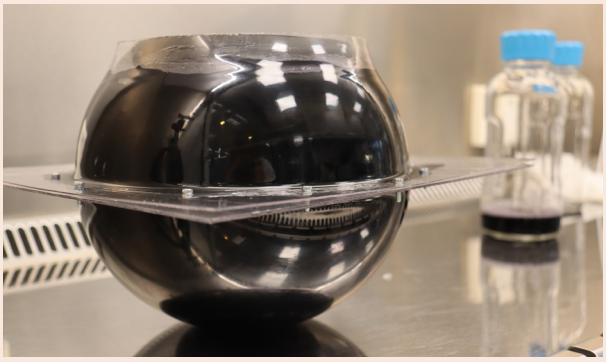
Thermoforming and cutting moulds



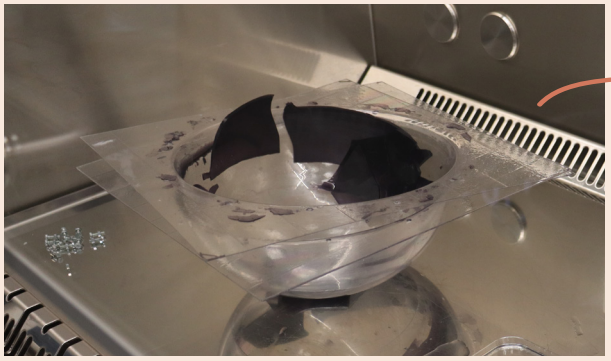
Sterilisation with UV light



Pouring growth medium into the moulds

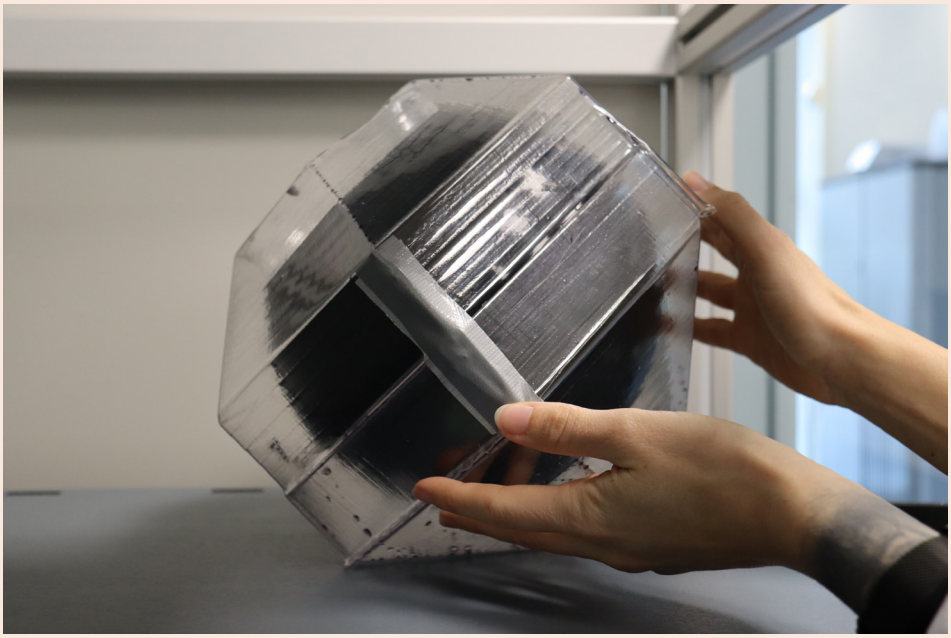


The solidifying growth medium

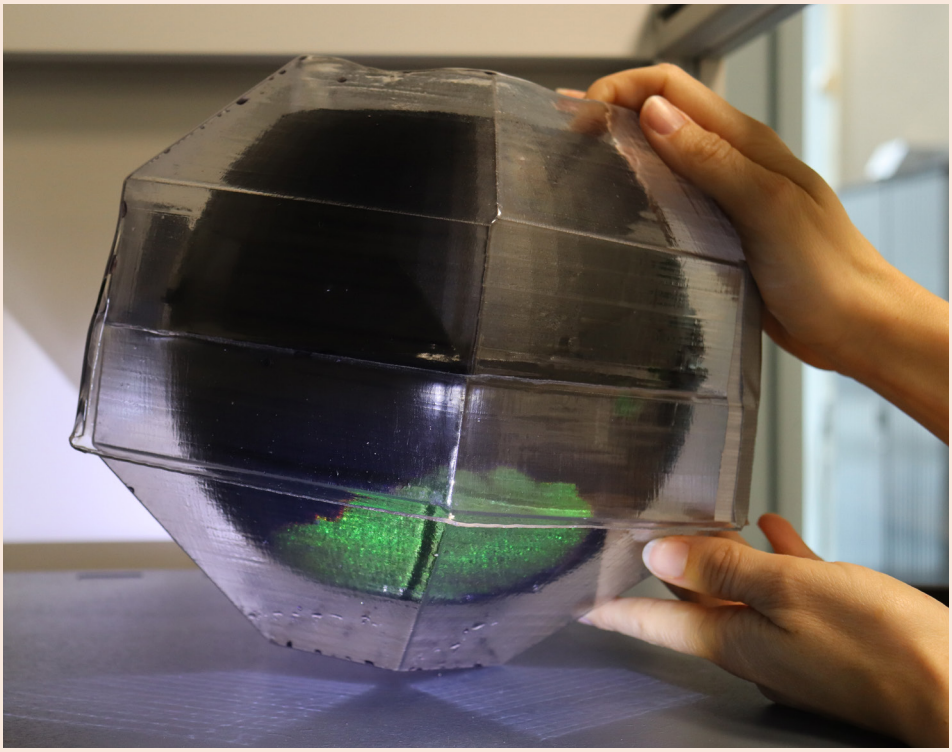


Challenging to fabricate

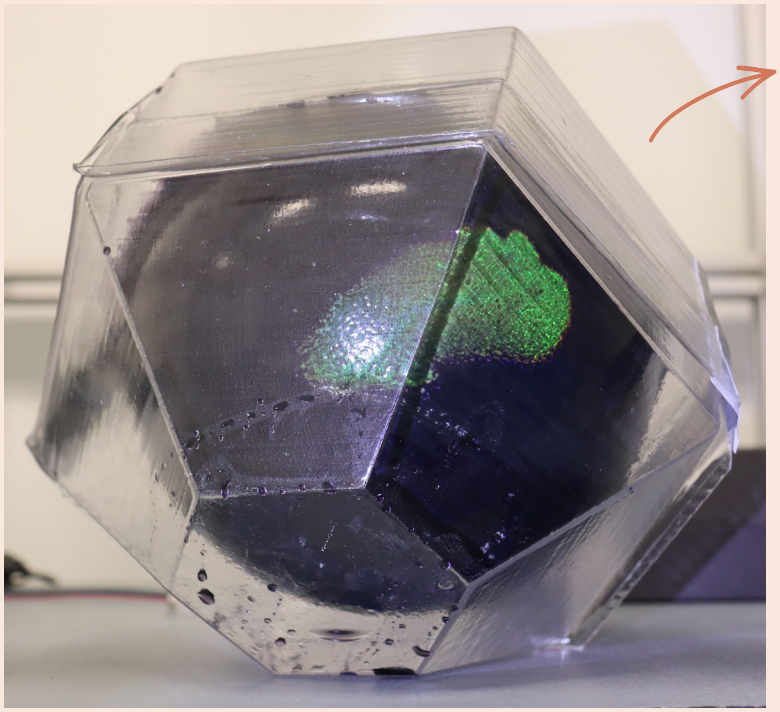
Struggles of removing moulds: the growth medium tears and sticks to the moulds



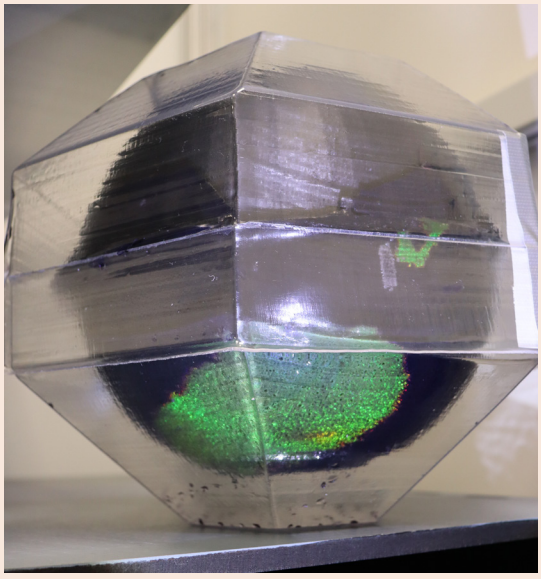
The assembled prototype after inoculation



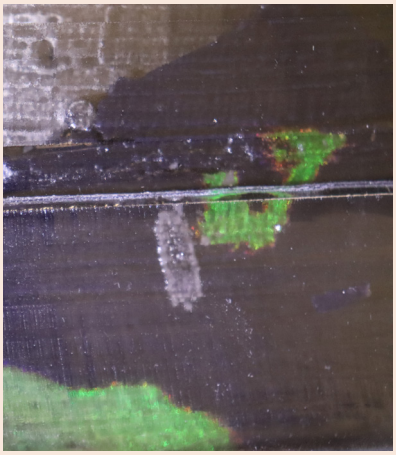
Flavobacteria's living colour within the artefact



Extensive moisture collecting in the bottom of the artefact

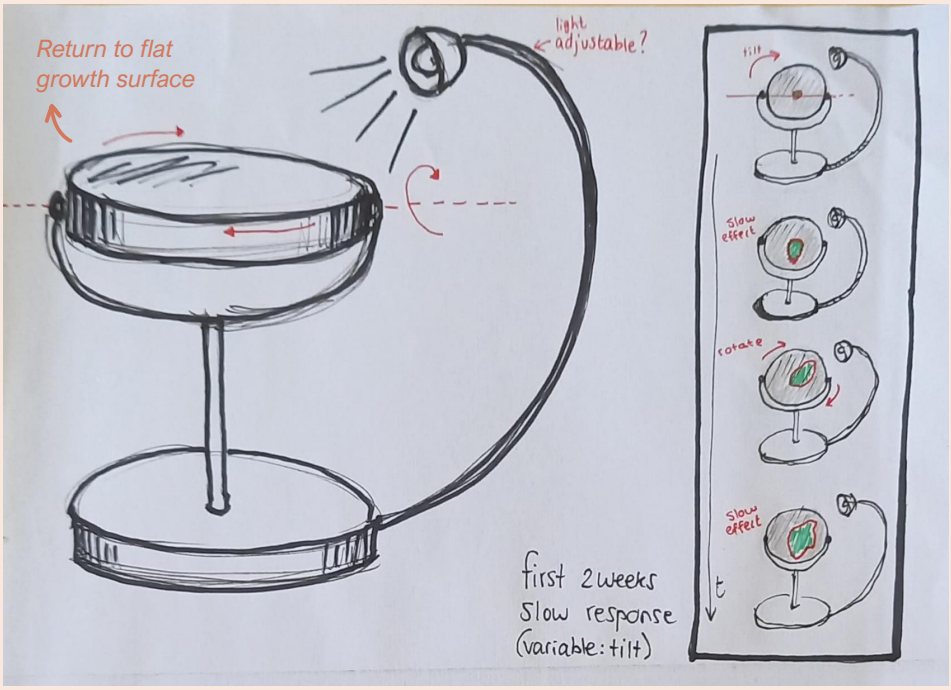


Bacterial contamination



Close-up of contamination

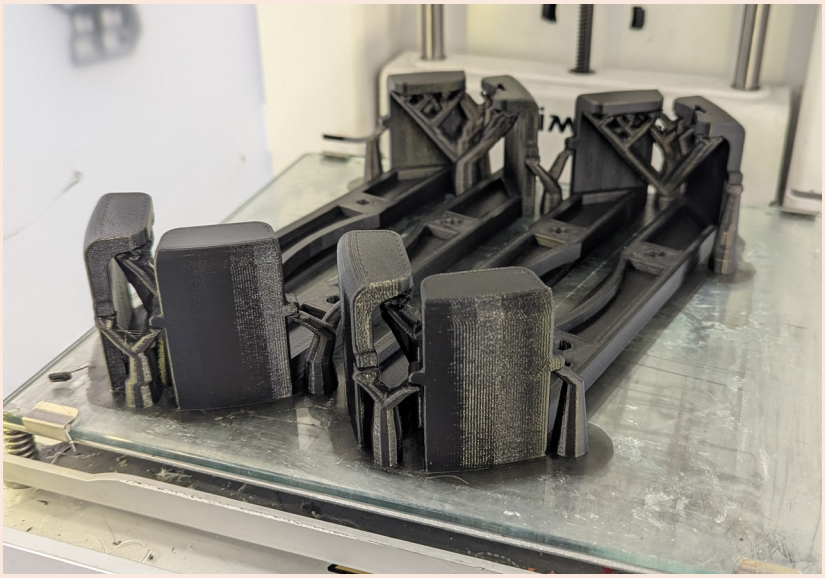
Final Design Configuration



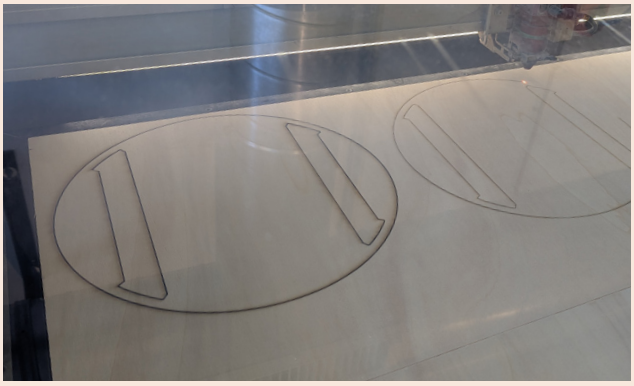
Early design sketches of the iteration supporting tilting and swiping interactions

Two interaction modalities

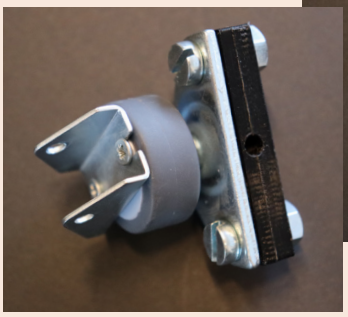
- *differing in interaction directness*
- *eliciting diverse microbial responses*



3D-printing Petri dish holders



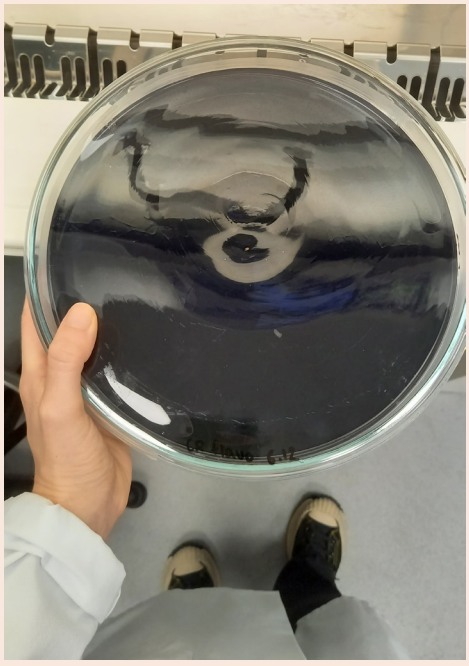
Laser-cutting a multi-layered rotation disc



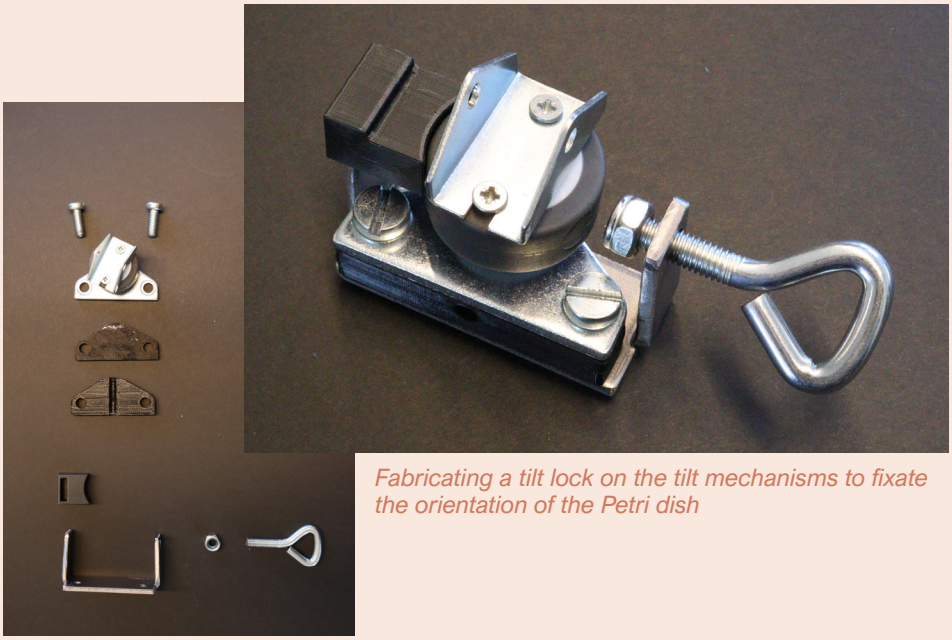
Fabricating a tilt mechanism with 3D prints and a wheel

The first prototype with the Petri dish on top of the rotation disc, resulting in balance issues

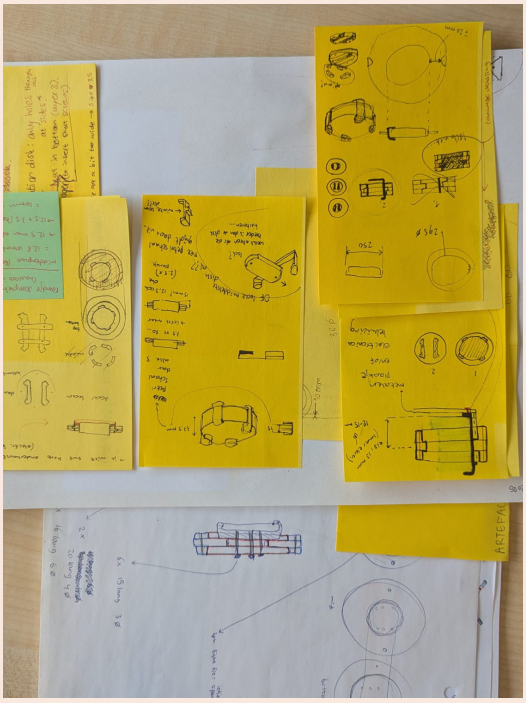
↘ *Safety concerns*



The bigger and even heavier (approx. 1kg) custom-made Petri dish



Fabricating a tilt lock on the tilt mechanisms to fixate the orientation of the Petri dish

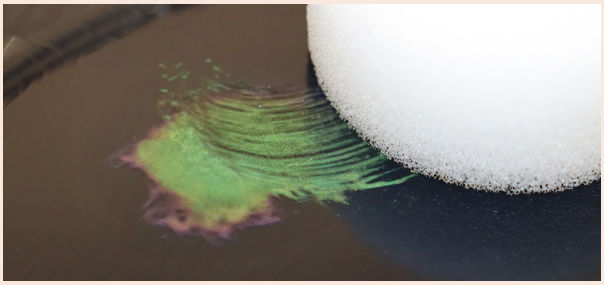


Redesigning the rotation disc to resolve balance issues by centring its centre of gravity

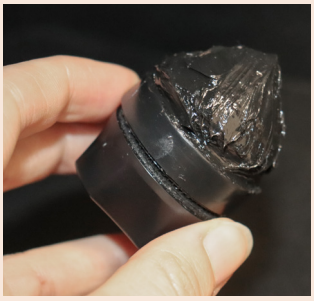
Improved stability

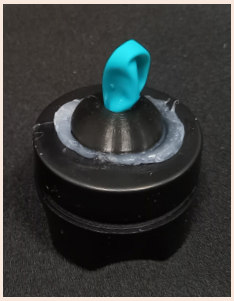
The layers of the rotation disc, enabling rotation while securing the Petri dish

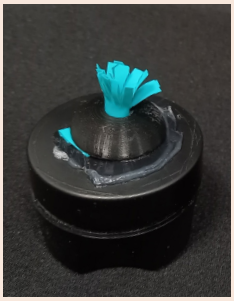
Iterating the Swiping Technique



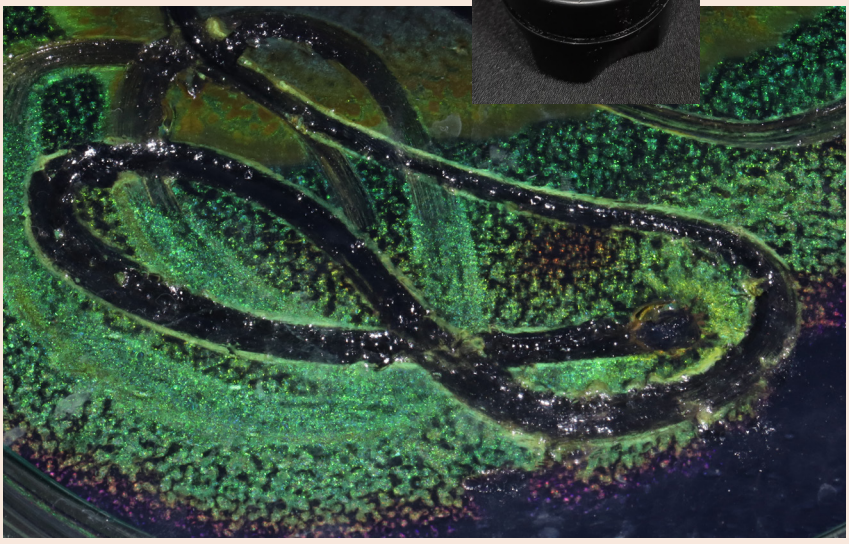
Early tests with laboratory foam, sometimes making insufficient contact to affect colouration





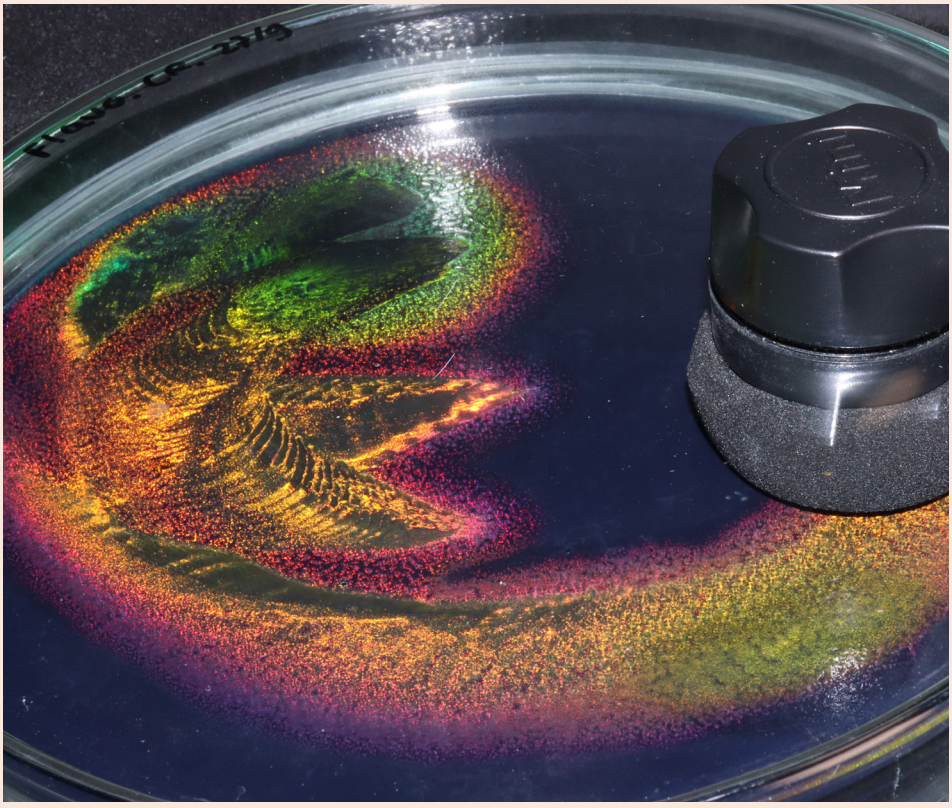


Exploring various tips for the aquarium magnet to gently swipe

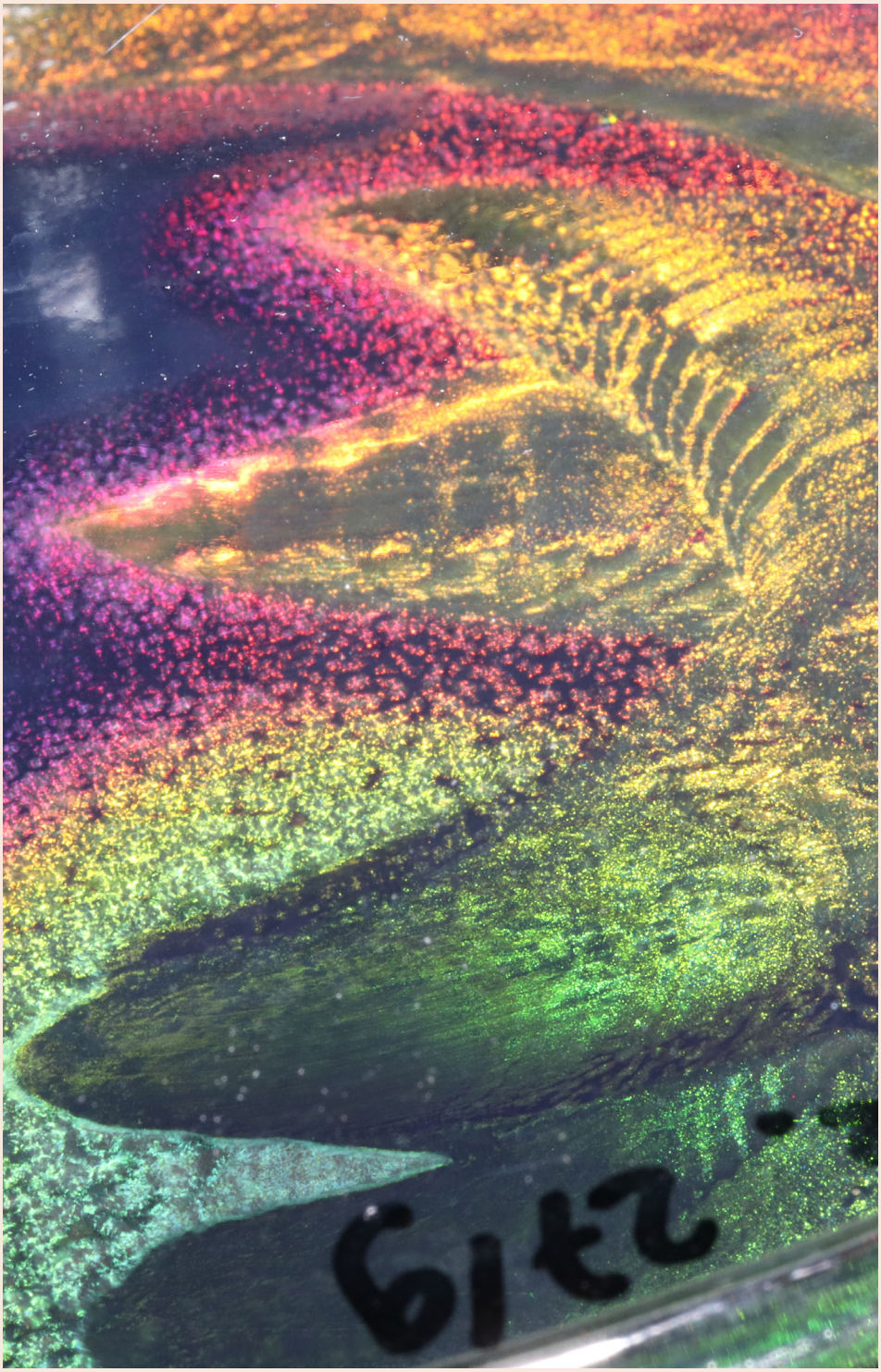


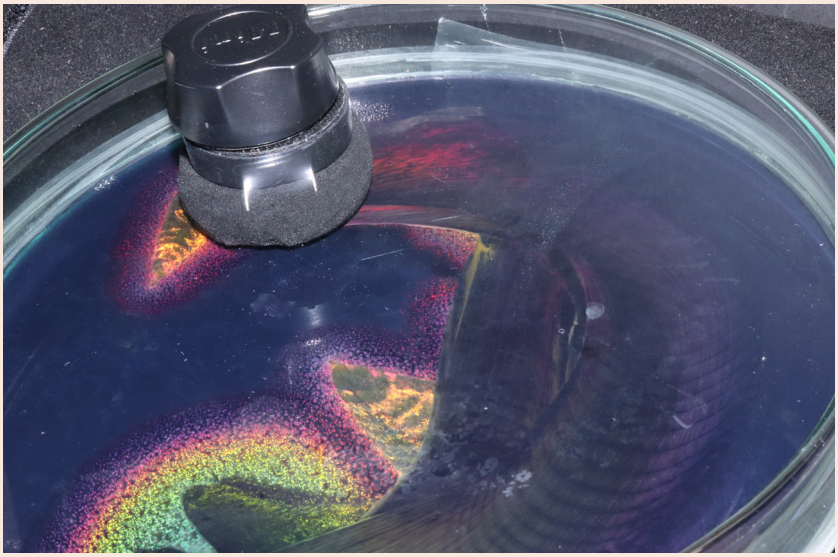
Damaged growth medium due to the aquarium magnet fitted with a brush tip

Exploring a make-up sponge as swiping tip



Vivid recolourations one day after swiping


Bitz



Reduced microbial viability

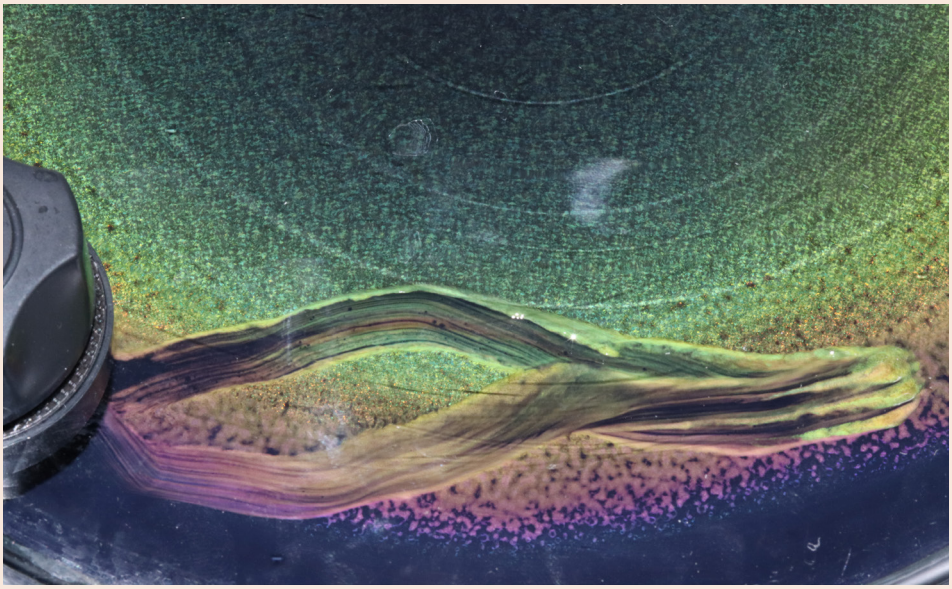
Stagnation of growth and decline in colour during the week following swiping

Signs of moisture absorption and potential contamination

A dent in the growth medium caused by pressure exerted by the sponge

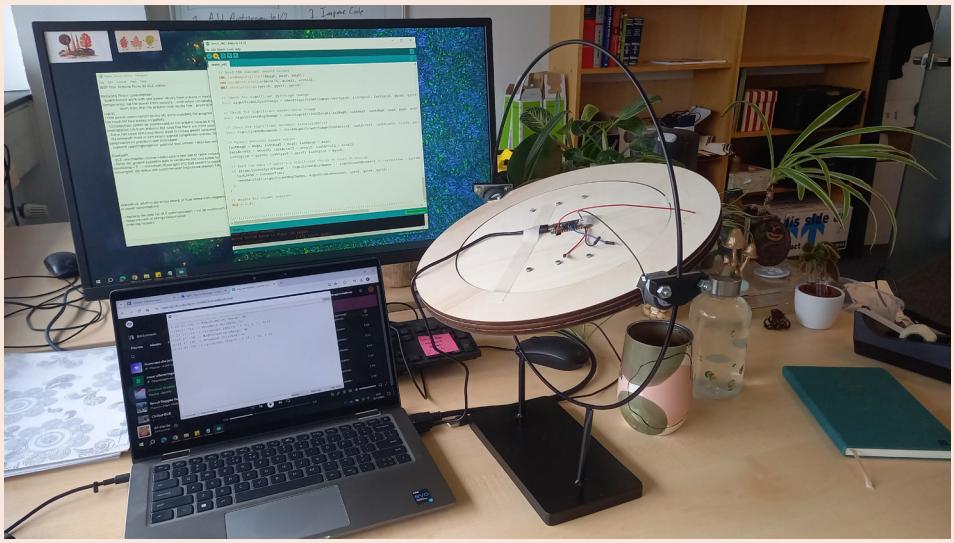
Redesign: soft, non-absorbing tips

Fabricating swiping tips using gloves, laboratory foam, and kit

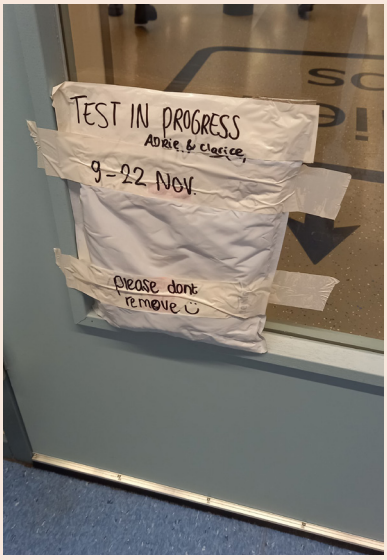


Successful swiping with the final glove-based tip

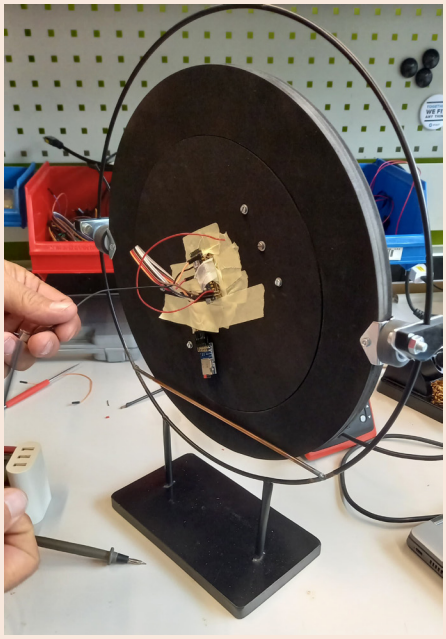
Embedding Electronics to Track Interactions



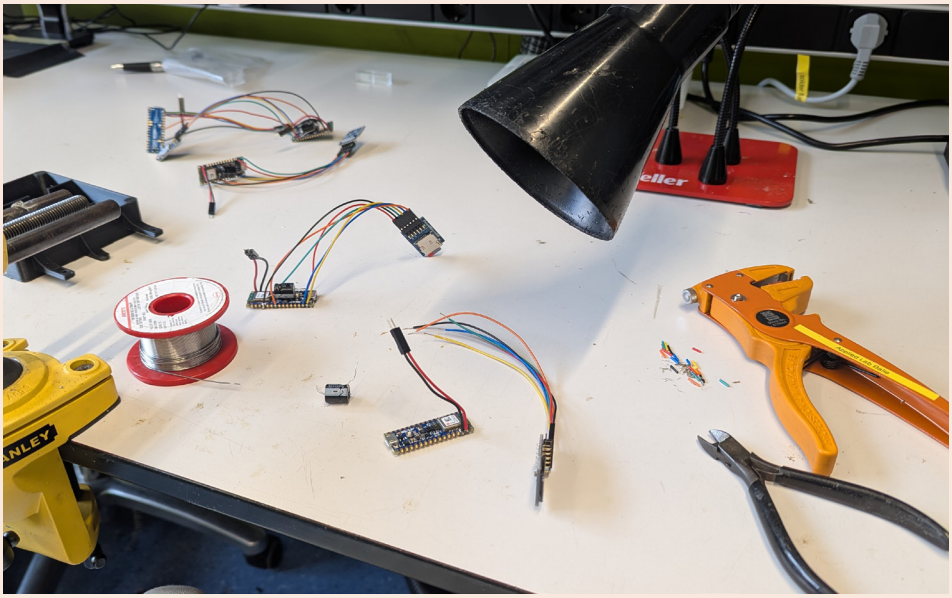
Testing the 9-axis IMU outputs upon movement of the artefact and the swiping magnet



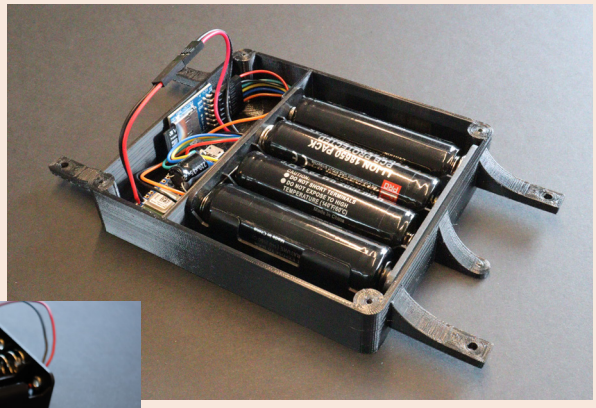
Long-term battery testing using incidental door movement for sensor activation

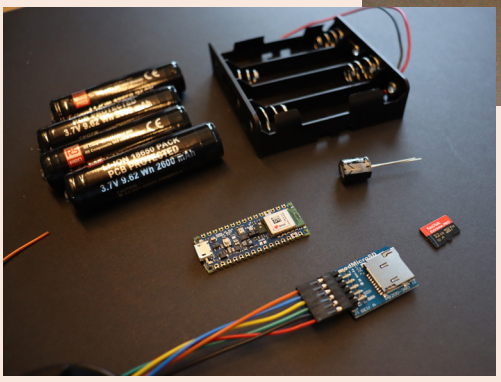


Measuring battery consumption

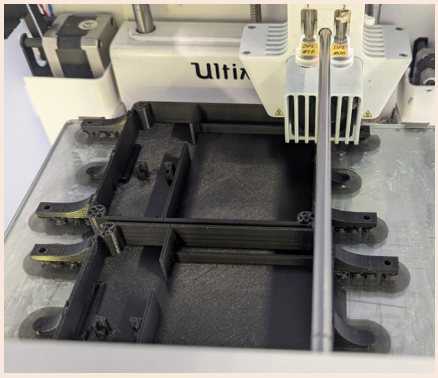


Soldering electronics components for tracking interactions



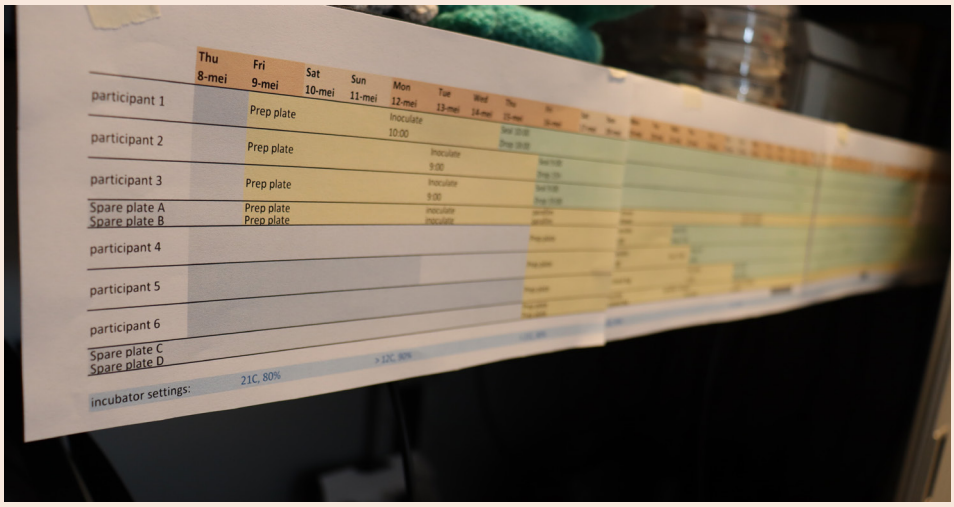


The electronic components

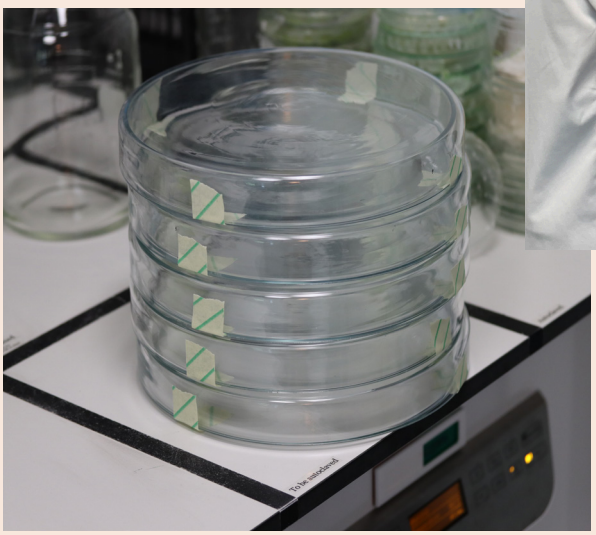


3D-printing the electronic enclosure

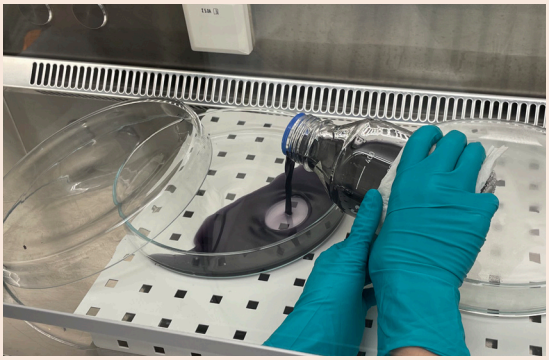
Lab Preparation of Flaviri



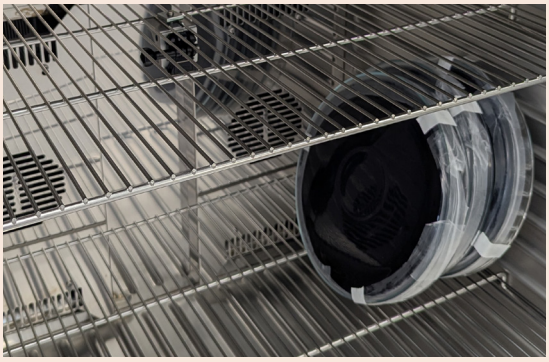
Planning all steps for preparing and distribution of the six Flaviri artefacts



Sterilisation of Petri dishes in the autoclave



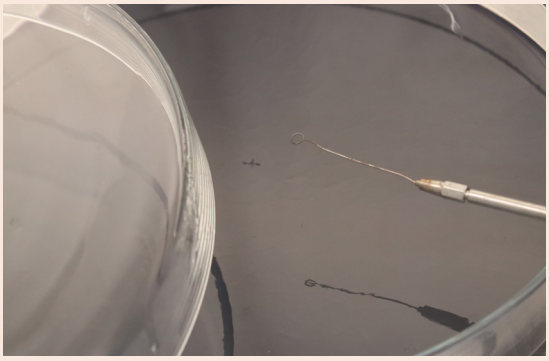
Pouring growth medium into horizontally-levelled Petri dishes to ensure consistent swiping



Optimising the procedure to prevent colony spreading prior to biofilm formation:

1. *Vertically positioned Petri dishes to collect extensive moisture prior to inoculation*

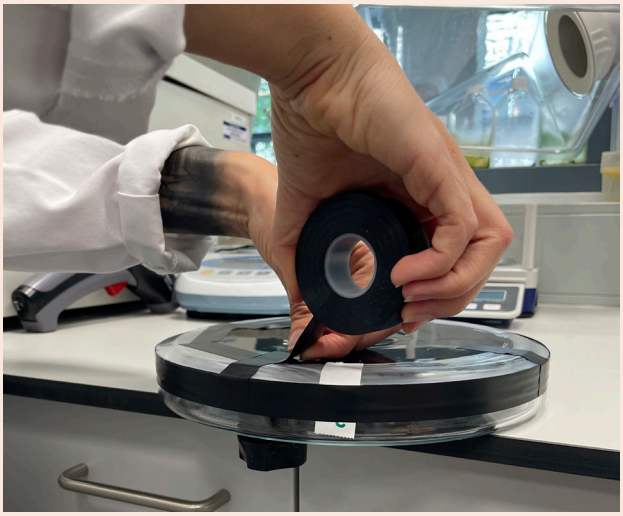
2. *Collecting accumulated moisture using a sterile pipette*



3. *Making a small incision with the inoculation loop to limit spreading of the inoculum*

The incubator reserved for Flavobacteria

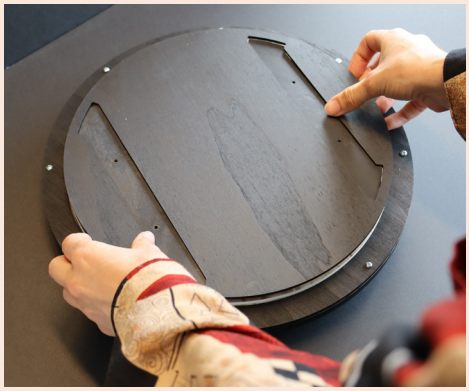
Optimised sealing for everyday contexts



Applying waterproof tape to prevent leakage from Parafilm

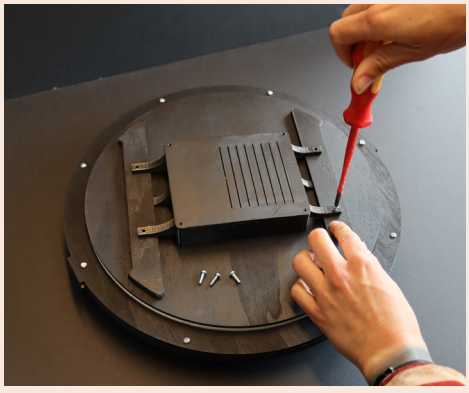
Assembly and Transportation

Placing the holders around the Petri dish

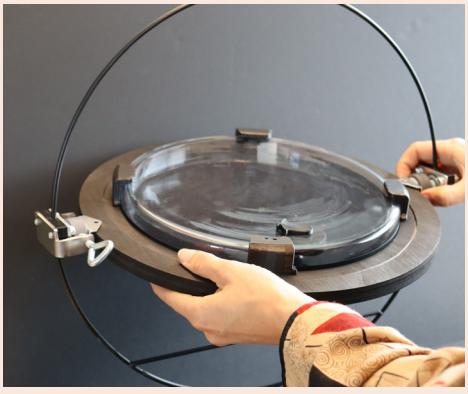


Placing the rotation disc around the dish

Locking the rotation disc



Fastening the electronics enclosure



Placing the disc within the frame

Securing disc within tilt mechanism

The six fabricated Flaviri artefacts

Ready for transportation and assembly on location

Chapter 7

Conclusions and Reflections

7.1 Addressing Research Questions

This dissertation began with the ambition to explore whether iridescent Flavobacteria could offer a sustainable alternative to conventional colour-changing and sensing materials in interaction design. While the ecological benefits of such living alternatives still require evaluation, this work reveals clear design potential: Flavobacteria exhibit colourations that change over time and respond directly to external conditions, opening possibilities for living artefacts that exhibit stimulus-responsive colour changes.

In this concluding chapter, I return to this initial aim by answering the research questions that guided the inquiry. I first address the three sub-questions through concise responses grounded in the empirical and conceptual studies, and then synthesise how the findings collectively respond to the main research question. These answers set the stage for the discussion of the dissertation's contributions and the reflections that follow.

7.1.1 RQ1: How do Flavobacteria's temporal expressions unfold over time and in response to external conditions?

Flavobacteria's temporal expressions unfold through changes in colony form, texture, and iridescent colour. As documented in Chapter 2, form varies in shape and size; textures range from scattered and pointillistic to dense and uniform; and iridescent colourations shift in hue, brightness, and degree of angle-dependence.

Over time, each colony expands outward and displays a wide variety of colours. Colour transitions follow gradual gradients rather than abrupt shifts: younger edge regions typically display purple and red hues, maturing zones transition into greens, and the iridescence in central regions eventually fades. As shown in Chapter 4, colour is rarely uniform but emerges from clustered points of different colours, producing the characteristic multi-coloured speckling and glitter-like iridescence.

These expressions unfold in response to external conditions. For example, environmental factors such as humidity significantly affect the growth and colourations of colonies (Ch. 2). Chapter 4 further shows that temperature fluctuations can produce repeatable shifts in colouration, resulting in concentric coloured rings that reflect past conditions—functioning as living traces of environmental history.

Flavobacteria's temporal expressions also respond to other external conditions, such as biological and designed aspects of their environment (Ch. 2). The presence of other microorganisms can prompt directed growth associated with predatory behaviour and shifts in colour brightness. Habitat architectures shape living colour through features such as, for example, surface texture, which can alter the isotropy of colourations, and plate orientation, which influences growth direction and texture. Direct human input also affects evolving expressions, with,

for instance, touch instantly eliminating colour yet leading to renewed, often surprising, and highly angle-dependent colourations as the cells reorganise (Ch. 5). In domestic settings, Flavobacteria give rise to a wide diversity of expressions due to fluctuating household conditions and varied human inputs, further demonstrating how strongly living colour is shaped by its environment (Ch. 6).

Overall, Flavobacteria display highly dynamic and temporal expressions (Fig. 7.1). Their living colour changes continuously over time, responds sensitively to environmental, biological and human inputs, and can reveal both present conditions and past events through evolving colouration patterns.

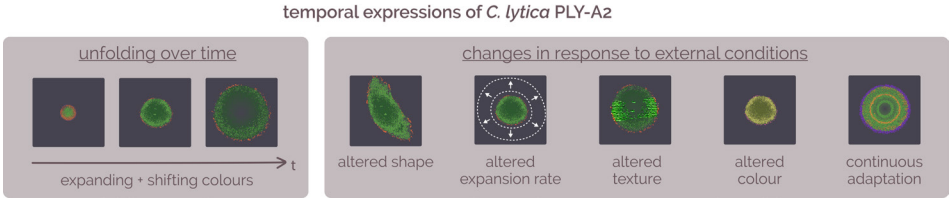


Fig. 7.1 *An overview of the temporal expressions of C. lytica PLY-A2, illustrating how these expression unfold over time and in response to external conditions.*

7.1.2 RQ2: How can direct human-Flavobacteria interactions be designed within living artefacts to reveal responsive microbial expressions?

Direct human-Flavobacteria interactions can be designed within living artefacts by establishing conditions in which bodily inputs act as signals that elicit and reveal responsive microbial expressions. Because there is no single method for achieving this, I propose both conceptual and practical frameworks that guide how such interactions can be designed.

In Chapter 5, I first define direct human–microbe interaction as an encounter in which human bodily actions act upon a living artefact in ways that trigger changes in microbial behaviour. I frame these interactions as a spectrum shaped by degrees of proximity and mediation. Second, I demonstrate that designing such interactions requires identifying relevant input mechanisms (e.g., humidity, salinity, temperature) and mapping them to human inputs such as tilting, pressing, or swiping. Third, I show that designing these interactions also requires attention to the habitat architecture, including properties of the habitat enclosure (such as its size, flexibility, and porosity) and characteristics of the microorganisms and their growth medium. These design parameters determine how human input affects the microorganisms, how microbial expressions become visible and encounterable, and what forms of interaction are possible without compromising microbial viability.

Lastly, in Chapters 5 and 6, I demonstrate practical techniques for enabling interaction in everyday or long-term contexts, particularly where sterility must

be maintained to support microbial viability and expression and to address biosafety considerations. Techniques such as flexible lids or swiping with repurposed aquarium magnets enable bodily input without direct touch, making direct interaction feasible outside controlled laboratory settings. This was exemplified by the living artefact Flaviri, which enabled sustained encounters with Flavobacteria's living colour in domestic settings.

Taken together, these elements show that direct human-microbe interaction can be intentionally designed by aligning microbial responsiveness, input modalities, habitat architectures, and interaction tools in ways that reveal responsive microbial expressions within living artefacts.

7.1.3 RQ3: How do humans experience and engage with these microbial expressions in everyday life?

Humans respond to Flavobacteria's living colour with curiosity and fascination, as seen in initial encounters during workshops, exhibitions, and public demonstrations. These microbial expressions capture attention even through mediated formats such as images and videos, and become especially compelling when experienced firsthand. My engagement with Flavobacteria in the laboratory further indicated how sustained, direct interactions with the organisms can foster attentiveness and an emerging relational orientation (Ch. 5).

In everyday contexts, engagement with Flavobacteria's living colour is highly diverse and shaped by what expressions people notice, how they interpret them, and the roles they adopt in relation to the living artefact (Ch. 6). People often observe the organism most when expressions are vivid or changing, with attention declining as transformations become less pronounced. Some microbial responses, such as subtle changes or highly angle-dependent colourations, were easily overlooked, suggesting that certain expressions require more time and familiarity to notice. Physical interaction ranges from cautious handling to more exploratory gestures, and emotional responses such as fascination or concern are closely tied to visible microbial activity. Sustained engagement proved fragile, sensitive to microbial decline or stagnation, fading novelty, and mismatches between expectations and observed expression. Participants' reflections centre on making sense of the expressions and situating the artefact within their homes and daily lives, including considerations of its purpose and impact. At the same time, living with the artefact often prompted heightened attentiveness to the surrounding environment, increased awareness of microbial presence, and reflection on human–microbe interconnectedness.

Overall, humans approach Flavobacteria's living colour with curiosity, fascination, and attentiveness. Yet, engagement remains fragile—shaped by the legibility of microbial expressions and the individual perspectives and roles through which people relate to the artefact.

7.1.4 Main RQ: How can biodesign facilitate human-microbe engagement in everyday life?

Biodesign can facilitate everyday human–microbe engagement by designing living artefacts in which microbial expressions become visible and encounterable. In order to do so, designers need a detailed understanding of microorganisms, including their needs, temporal dynamics, and responsiveness. Such understanding can be developed through designers' own explorations, interdisciplinary collaborations, and existing tools or frameworks for understanding living aesthetics (e.g., design spaces and digital tools (Ch. 3)). Building on this understanding, biodesigners can configure habitat architectures that support microbial viability, address biosafety considerations, and open up space for human–microbe interactions in everyday life (Ch. 5-6).

Biodesign can further facilitate engagement by designing opportunities for direct interaction, which can offer living traces and foster a sense of relationality with nonhumans. Designing these interactions requires aligning input mechanisms with human actions and establishing techniques to enable interaction in ways that are safe for humans, embedded microorganisms, and the environment. In some cases, sterile forms of interaction may be needed—for instance, to ensure microbial viability and the legibility of expressions within everyday living artefacts (Ch. 6).

Taken together, organism understanding, careful habitat configuration, and considered interaction pathways provide a practical route for designing human–microbe engagement in everyday life. At the same time, designing such engagement remains complex and necessarily diverse, as different microbial behaviours and everyday contexts call for different design strategies. Biodesign research can help to navigate this complexity by offering shared resources—from design spaces and conceptual frameworks to digital tools and interaction techniques—that support designers in exploring, shaping, and sustaining meaningful encounters with microbial life across contexts.

7.2 Discussion of Contributions

This section unpacks the contributions of this dissertation in detail. Building on the key contributions outlined in Chapter 1, I elaborate on the scope and significance of contributions across four areas: theoretical and conceptual contributions, empirical and experiential insights, methodological contributions, and design instances.

7.2.1 Theoretical and conceptual contributions

Living colour as communicative matter

This dissertation introduces iridescent Flavobacteria into the domains of HCI and design, conceptualising living colour as communicative matter.

This conceptualisation expands interaction design's understanding of communication modalities by showing how the colour of living organisms can convey information through dynamic changes. Building on this perspective, we conceptualise Living Colour Interfaces as a form of Living Media Interfaces, positioning colour as a dynamic and expressive quality through which organisms can embody digital data, signal environmental conditions, or leave a patina of living traces (Ch. 2). This contribution refines and extends Living Media Interfaces by identifying temporal colouration as a distinct communicative modality, opening new design possibilities grounded in the colour-changing behaviour of living organisms.

Vocabulary of Flavobacteria's living aesthetics

We introduce a vocabulary to analyse and communicate Flavobacteria's living aesthetics (Ch. 2), identifying how humans can experience changes in colony form (circular↔amorphous; hollow↔full), texture (rough↔smooth), and iridescent colour (mono- or multi-coloured; brilliant↔dull), and how these expressions unfold at different temporalities. Such vocabularies are essential for biodesign practice because they support systematic documentation of microbial expressions and offer a common language to describe these, facilitating collaboration in multi-disciplinary teams.

Design space of Flavobacteria's living aesthetics

Through the extensive explorations in Chapter 2, this dissertation articulates a design space that illustrates how Flavobacteria's living colour can be tuned via environmental stimuli, interactions with other microorganisms, direct human input, and habitat shape and dimensions. This design space foregrounds the organism's diverse living aesthetics and their responsive behaviour to different input mechanisms, forming a conceptual scaffold for unpacking the potential of their living colour as communicative matter and designing living colour interfaces.

Design spaces play an important role in HCI and design by making possibilities visible, structuring further exploration, and providing generative frameworks that can be adapted to other contexts. As a visual overview, they are particularly important in biodesign, where designers often depend on technical insights from microbiology to understand microbial behaviour. By visually foregrounding microbial expressions, the design space supports biodesigners in attending to the richness and variability of living aesthetics and in speculating how these might translate into meaningful applications.

Unpacking direct interactions with microbes

This dissertation contributes a conceptual framework for understanding and designing direct human–microbe interactions (Ch. 5). First, it offers a definition of direct human–microbe interaction, conceptualising it as an exchange in which

humans act upon microbial living artefacts through their own bodies (e.g., touch, voice), and microorganisms adapt in response, often manifesting changes in their living aesthetics. Rather than treating directness as a binary condition, this conceptualisation frames it as a spectrum shaped by degrees of mediation and proximity. Clarifying this notion is important, as the term "direct interaction" is frequently used ambiguously in biodesign and bio-HCI—variously referring to temporal immediacy, physical proximity, or the absence of technological mediation—risking conceptual misalignment across research efforts.

Building on this definition, I articulate a design space that encompasses direct interactions with living microorganisms. It identifies key input mechanisms (e.g., salt, oxygen, humidity) alongside possible human input, demonstrating how bodily actions can function as signals that shape microbial expression. By specifying these interaction primitives, the design space provides a structured foundation for exploring and designing interactive living artefacts that foster intimate engagement with microbial life. Together, the definition and articulated design space enable researchers to reason more precisely about design choices, compare approaches, and make explicit how human actions and microbial responses are brought into relation.

Habitat architectures for Flavobacteria

In Chapter 5, I articulate the basic habitat architecture for Flavobacteria artefacts, identifying key properties (e.g., size, flexibility and porosity) that shape both microbial viability and how human input can affect microbial behaviour. Across the dissertation, application concepts and fabricated habitats and artefacts demonstrate variation in these properties, revealing how different architectural choices condition microbial growth, responsiveness, and expressive potential, while simultaneously shaping the possibilities for human–microbe interaction. This contribution provides a conceptual and practical foundation for designing viable living artefacts, particularly in relation to crafting habitabilities. By articulating concrete habitat properties, it supports biodesigners in reasoning what to take into account when designing habitats, while also framing these properties as design variables to be explored and played with—helping them navigate the challenge of creating suitable habitats while enabling human–microbe interaction.

7.2.2 Empirical and experiential insights

Insights into Flavobacteria's living colour

The dissertation provides novel technical insights into the temporality and responsiveness of Flavobacteria's living colour, with relevance for both microbiology and biodesign. The study presented in Chapter 2 was the first to demonstrate and analyse long-term growth through extended observation periods within a large-scale habitat, revealing temporal patterns overlooked in earlier short-term studies. Across the dissertation, I further document the

effect of fluctuating environmental conditions, as well as the influence of habitat architecture and direct human input—dimensions not thoroughly explored in microbiology. In addition, the work in Chapter 6 shows how living colour evolves in less-controlled, everyday environments, where varying environmental conditions and human inputs shape microbial expression. By characterising Flavobacteria's living colour in detail, this dissertation offers foundational knowledge for future biodesign practices involving Flavobacteria. More broadly, these insights may inspire the design of colour-changing living artefacts and encourage biodesign explorations with other microorganisms. The relevance of this contribution for the biological sciences becomes particularly apparent in Chapter 4, which offers technical detail on the behaviour and cell organisation of Flavobacteria that may be of interest to researchers investigating iridescent bacteria and their underlying optical structures.

Insights into Flavobacteria's living aesthetics

This dissertation contributes experiential insights, grounded in both my own experience and participants' accounts, into how humans perceive and respond to Flavobacteria's living colour and direct human–microbe interactions (Ch. 5-6). It examines these experiences across sensorial, performative, affective, and interpretive dimensions, detailing what microbial changes people notice, how these expressions invite bodily action, what emotions they evoke, and how participants interpret and reflect upon them. In doing so, this dissertation provides grounded knowledge that supports the design of living artefacts whose expressions can be meaningfully perceived and engaged with over time. By extending from personal engagement to participant accounts, it strengthens experiential insight through multiple situated perspectives, supporting biodesigners in making more informed design decisions about expression legibility and interaction—particularly for everyday contexts, where people may have limited familiarity with microorganisms and their expressions.

Empirical insights into human-microbe engagement in the everyday

The dissertation contributes in-depth empirical insights into how human–microbe engagement unfolds in everyday contexts and how living aesthetics and interaction modalities shape this engagement over time (Ch. 6). It demonstrates how Flavobacteria's living colour—its temporality, variability, and responsiveness—along with differing degrees of interaction directness, shape how people attend to, interact with, feel about and make sense of microbial living artefacts in domestic environments. These insights highlight the importance of attending to living aesthetics and carefully designing interaction modalities—particularly their degree of directness—when designing for sustained human–microbe engagement in the everyday.

The findings further reveal that human–microbe engagement is diverse, fragile and contingent, shaped by factors such as novelty, aesthetic comfort, and individual personalities. From recurring patterns in participants' observations,

actions, emotional responses, and reflections, the dissertation identifies distinct roles that participants adopted—as carer, observer, experimenter, and controller. These roles illuminate how people position themselves in relation to living microorganisms, enriching ongoing discussions in biodesign about how humans negotiate curiosity, care, hesitation, and responsibility toward microbial life.

Together, these insights support biodesign practices that seek to design for long-term engagement with living artefacts. By revealing how engagement evolves, fluctuates, and sometimes fades, this contribution helps designers reason about the conditions under which human–microbe relations can be sustained in everyday life, and how living artefacts might be designed to support attentiveness, care, and coexistence over time.

7.2.3 Methodological contributions

An in-depth, microorganism-specific design research approach

This dissertation demonstrates an in-depth, microorganism-specific design research approach that integrates controlled laboratory characterisation, interdisciplinary collaborations, interaction-centred design explorations, and a field-based study. It combines detailed investigation of Flavobacteria's temporal expressions—grounded in laboratory experiments and interdisciplinary collaborations—with design explorations examining direct interactions and everyday human-microbe engagement.

This approach shows how biodesigners can unpack the design potential of a specific microorganism by bridging two perspectives: the microorganisms—their expressions and environmental responsiveness—and the human—how people perceive and respond to these microbial expressions. A dual orientation of great value within biodesign.

By building on biological and technical knowledge developed through laboratory work and carrying these insights forward into interaction and experiential design explorations, this dissertation demonstrates how biodesign research can be both exploratory and deeply informed. Few design-led biodesign studies pursue this level of microorganism-specific depth. By doing so, this dissertation highlights the value of thoroughly understanding a microorganism's behaviour and resulting expression to generate feasible and grounded opportunities for designing living artefacts. The interdisciplinary collaborations—both within biodesign and beyond it—played a key role in enabling a deeper, organism-specific understanding.

Overall, this holistic approach offers a model for future biodesign research that seeks to engage deeply with living microorganisms while fostering meaningful human-microbe engagement.

A mixed-method approach to investigating human-microbe engagement in everyday life

This dissertation develops a mixed-method, in-situ approach for studying human–microbe engagement in everyday life (Ch. 6). Combining diary methods, semi-structured interviews, and embedded sensor data within a longitudinal study, this approach enables a detailed account of how engagement unfolds over time and how participants experience microbial expressions.

It demonstrates how qualitative methods and quantitative sensor data can be meaningfully combined to understand human experience with living artefacts—an approach that is particularly powerful given that microbial expressions change gradually and irregularly over time, and human engagement may fluctuate. Rather than offering a momentary snapshot, this methodology supports the investigation of temporal dynamics, fragile engagements, and subtle, evolving relational dynamics.

Overall, this methodological contribution offers guidance and inspiration for future longitudinal, real-world studies in biodesign, providing a framework for researching everyday human–microbe engagement—an area that remains under-articulated yet increasingly relevant for establishing future regenerative ecologies.

Reflexive insights from interdisciplinary collaboration

Building on the collaboration with biotechnology researchers (Ch. 4), this dissertation contributes reflexive insights into navigating interdisciplinary work within biodesign, as outlined in the intermezzo reflection. The collaboration revealed how differences in language, expectations, and research practices can be both challenging and generative, and how biotechnology and design research communities often approach biodesign research in distinct yet complementary ways. These insights offer guidance for researchers entering or navigating interdisciplinary collaborations and highlight how bridging disciplinary perspectives can meaningfully enrich biodesign practice.

7.2.4 Design instances

Tools and protocols for characterising living colour

Supporting inquiry across both biology and design, this dissertation presents a set of custom-designed tools, techniques, and laboratory protocols for documenting and characterising Flavobacteria's living colour. These include a capture setup that enables automatic and consistent documentation of temporal and iridescent colour; a habitat designed to support long-term microbial growth and documentation; laboratory protocols for exploring temporality and responsiveness to different stimuli; and computational analysis scripts for quantifying colony growth, texture, and colour intensity and homogeneity.

Together, these design instances were, and continue to be, critical in enabling the systematic characterisation of Flavobacteria's living colour, directly supporting the novel technical insights presented in this dissertation. More broadly, they offer similar possibilities for characterisation in the context of other temporal, dynamic, or iridescent microorganisms and materials.

Tools and protocols for direct human-microbe interaction

This dissertation also contributes a set of custom-designed tools, techniques, and laboratory protocols for designing and exploring direct human–microbe interactions, both within and beyond laboratory settings. These include laboratory protocols that enable safe, sterile exploration of direct interaction, adapted habitat designs (e.g., flexible lids that allow touch-based input), and tools for interacting directly with the bacteria through swiping without contaminating (e.g., aquarium magnets with attached sponges that allow colour to be swiped through a glass habitat).

By enabling direct interaction in a sterile manner, these tools make it possible to explore sustained and repeated interactions over time, rather than one-off encounters that might compromise microbial viability. This is particularly important when considering biosafety and long-term deployment, especially as living artefacts move from laboratory contexts into everyday domestic environments. More broadly, these tools expand what direct interaction with microorganisms can entail, opening up new possibilities for intimate engagement with microbial life. As such, they are relevant both as research instruments and as enabling components for future living artefacts that seek to foster human–microbe engagement.

Digital tools for understanding living aesthetics

Through a collaboration with computer graphics experts, the dissertation contributes FlavoMetrics, a digital tool that simulates Flavobacteria growing in a Petri dish (Ch. 3). By modelling the organism's temporal qualities, iridescent colouration, and responsive behaviour, the tool supports biodesigners in understanding and exploring Flavobacteria's living aesthetics. FlavoMetrics demonstrates that convincing simulations of microorganisms can be created by combining empirical data from biological experiments with procedural computer graphics techniques, offering a model for developing realistic digital tools in biodesign.

More broadly, this dissertation also outlines the potential of digital tools within biodesign to support tinkering, biodesign education, experience prototyping, and the exploration of spatiotemporal qualities beyond two-dimensional representations. Such tools can lower barriers to engaging with biodesign by increasing accessibility, while enabling more time- and resource-efficient ways of imagining, learning about, and designing with living microorganisms.

A living artefact for human-microbe engagement

The dissertation introduces Flaviri, a living artefact designed to enable long-term, everyday human–microbe engagement (Ch. 6). Flaviri sustained Flavobacteria's viability for four weeks in a domestic setting while enabling direct interactions through tilting and swiping that elicited diverse microbial expressions. In doing so, it enabled longitudinal exploration of how people relate to Flavobacteria's living colour in everyday contexts.

Flaviri functioned as a research artefact: embedded sensors recorded physical interactions over time, demonstrating how living artefacts can simultaneously support human–microbe engagement and generate quantitative data about interactions. Research artefacts are particularly valuable in biodesign, as they allow real-world probing of engagement with living artefacts—capturing temporal dynamics, routines, and evolving relationships that are difficult to observe in short-term laboratory studies.

As a design instance, Flaviri illustrates how interactive living artefacts can be made viable and encounterable outside the lab, thereby offering inspiration and practical insights both for future biodesign research exploring human–microbe engagement and for the design of everyday living artefacts.

Application concepts with living colour

Finally, the dissertation presents a set of application concepts that illustrate how Flavobacteria's living colour might be integrated into everyday contexts. These range from functional biosensing artefacts (e.g., thermochromic wearables (Ch. 4)) to less functional designs, such as FlavoTempo (Ch. 5), which invites people to attend to the slow changes in Flavobacteria's living aesthetics in response to tilt. These concepts function as design exemplars that illustrate the diverse design potential of living colour. They expand the imagination of what living colour in everyday life might entail, enable critical reflection on future forms of human–microbe engagement, and serve as inspiration for biodesign practices. As exemplars, these concepts play a key role in design research by opening space for discussion, critique, and future innovation, supporting both reflective inquiry and the exploration of design directions for living artefacts.

7.3 Reflections

7.3.1 Reflections on my position as a biodesign researcher

This dissertation began with a strong instrumental focus: exploring Flavobacteria as a sustainable living medium for interaction design. Over time, my perspective broadened to consider how such interfaces might not only serve functional goals but also foster attentiveness and appreciation toward microbial life. This trajectory was informed by biodesign literature but mostly shaped through my personal experiences of working with these fascinating microorganisms over time. Throughout the 5.5 years of working with Flavobacteria, I developed

a sense of connection with the organisms through observation, moments of surprise, and care practices. These experiences informed my research intentions, broadening from designing living artefacts framed around functional roles towards a desire to deeply understand the microbial behaviour and also explore more open-ended living artefacts that create space for reflection on interconnectedness and relationality with microbial life.

Intervention, agency, and autonomy

While I designed artefacts aimed at supporting Flavobacteria's needs, design decisions were made in parallel with my intention to elicit vivid colourations and enable particular interaction modalities. As my connection to the microorganisms grew, I became increasingly aware of the tensions between caring for them and intervening in ways that could be disruptive. Swiping interactions, explored in Chapters 2, 5, and 6, made this tension particularly tangible. A rather forceful intervention by one of my students made me aware of how cautiously I had been interacting through swiping with the colonies myself. Yet, her assertive gesture—contrary to my own intuitive hesitance—still resulted in vivid recolourations, revealing the organisms' resilience and prompting me to explore such direct interactions more intentionally (Fig. 7.2).

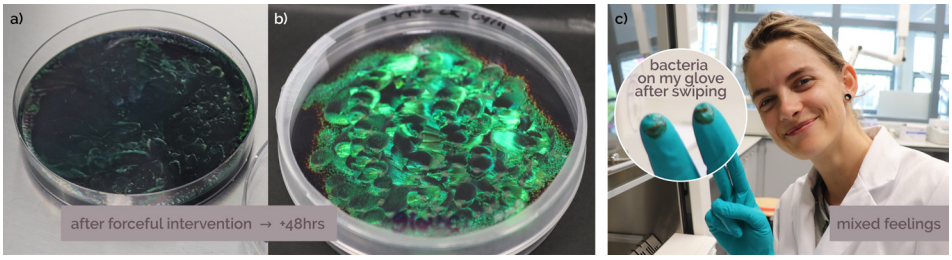


Fig. 7.2 *Interactions through swiping: a) a Flavobacteria colony after a student's forceful intervention and the resulting recolourations 48 hours later; b) me after swiping with Flavobacteria on my gloves and mixed feelings about the interaction.*

Alongside the joy and fascination I experienced in my subsequent experiments, this hesitance resurfaced. Disrupting the optical structures felt counterintuitive and, when parts of a colony stopped manifesting recolourations after a swipe, I experienced a sense of guilt. These moments made me realise that although I never intended harm, the interactions I designed might affect the microorganisms in ways I could not yet predict or fully assess. Later, a similar uncertainty emerged when I encountered stress-induced cell morphology (Ch. 4), reminding me again that my interactions might affect the microorganisms in ways that remain difficult to interpret. Triggering in me the question: what does stress mean to these organisms?

Although swiping was a highly interfering gesture, it also opened space for expressions that were not under my control. After each swipe, the reorganisation of the colonies seemed entirely "up to the bacteria," resulting in

colourations and patterns that I could not predict. This unpredictability became one of the most compelling aspects of the swiping for me. It highlighted both a form of interplay in which human intervention and microbial response co-shaped the outcome, and the fact that Flavobacteria are a complex living system whose colourations I cannot fully control. This strengthened my sense of working with a living entity and deepened my connection to the microorganisms, particularly when observing their unexpected colourations. These moments underscored the relational nature of the interaction and highlighted for me the added value of living artefacts: their potential for relationality and evoking reflection on the interconnectedness between microbial life, humans and beyond.

At the same time, these reflections made visible the extent to which the microorganisms were shaped by the designed contexts. The bacteria were not growing in their natural habitats but within artefacts designed to support specific interactions and tune their colourations. While these configurations offered limited autonomy for the microorganisms, they seem inherent to developing viable interfaces with Flavobacteria, as designed environments make their colourations visible and their responses encounterable in ways that allow interaction and reflection to take shape. Yet even within these engineered conditions, the microorganisms retained moments of agency—expressed through unexpected growth dynamics, surprising reorganisations, and responsive colourations—revealing the value and richness of working with living microorganisms in design.

Navigating technical inquiry and relational engagement

During the second half of my PhD trajectory, I found myself moving between two different modes of engagement with Flavobacteria. On one hand, the biotechnology-oriented collaboration of Chapter 4 foregrounded technical inquiry: controlled experiments, in-depth characterisation, and application concepts with strong functional grounding. While unfolding partly in parallel (Fig. 7.3), the research presented in Chapters 5 and 6 focused on more relational engagement, in which attentiveness and emotional involvement increasingly shaped my thinking. Navigating these orientations was not always straightforward; sometimes it felt contradictory. Yet the convergence of multiple perspectives proved intellectually generative, offering insights that helped me articulate my position as a biodesigner.

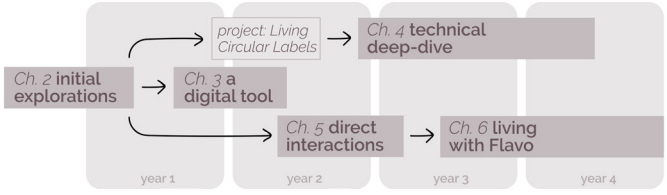


Fig. 7.3 *Timeline of research activities per chapter, illustrating the overlap between the technical work in Chapter 4 and the more relational-focused research in Chapters 5 and 6.*

At times, tension arose while performing these different research activities in parallel. For instance, we intentionally killed the bacteria to examine their cell organisation (Ch. 4)—an act that felt like the most human-centred moment of my PhD—while I was increasingly reflecting on emotional engagement, attentiveness, and appreciation for microbial life (Ch. 5 and 6). This duality forced me to confront uncomfortable questions: Why did I suddenly feel less involved with these bacteria in the context of the experimental work described in Chapter 4? What gave me the right to kill them for gaining knowledge? And yet, I wondered, how different is experimental killing from everyday acts like washing one's hands? Where should I draw boundaries?

I realised that research in biolabs may always be human-centred to some degree: knowledge is often gained by cultivating, manipulating, and ultimately killing microorganisms, even when the broader intention is to support their livingness and regenerative ecologies. While in Chapter 4, we intentionally killed the bacteria to analyse their cell organisation, it was also true that Flavobacteria were dying throughout my other studies and in everyday biolab practices. Even when carefully maintaining the cultures by transferring them to a new Petri dish each week, only a fraction of the bacterial population survived—the cells that happened to be collected on the inoculation loop. Such routine losses had rarely felt emotionally charged. In the technical inquiry of Chapter 4, however, the killing was explicit and purpose-driven. We needed fixated colonies for the analysis, and so we actively introduced chemicals into a colourful, thriving colony with the intention of ending its viability. This intentional act felt more confronting than the day-to-day activities within the biolab.

These reflections also made me realise that acting in line with my relational intentions was easier said than done. During moments of curiosity-driven, goal-oriented technical work, my reflective stance slipped into the background almost without me noticing. For example, when fixating those colourful colonies, I carried out the procedure without pausing to consider its implications. Only afterwards, when I remarked to a colleague that "the bacteria were dying as we spoke," did I fully register that I was ending their viability to gain information. In that moment, I felt positioned far above these organisms—blinded, in a sense, by my curiosity and technical focus. The fact that these thoughts only surfaced *after* the act reminded me how easily I could slip back into a functional, human-centred mode of inquiry.

At the same time, these reflections made me return to the broader sustainability ambitions that initially motivated this dissertation. Research in biolabs—including technical inquiries—is essential for exploring the potential of living microorganisms in new material paradigms that reduce environmental impact and rethink relationships between technology, living systems, and the environment. To understand the potential of iridescent Flavobacteria as sustainable alternatives to dynamic and sensing materials in HCI and interaction design, we need detailed knowledge of their needs, behaviours, and expressions. Such understanding can only emerge through systematic experimentation and, at times, interventions that can sometimes feel at odds

with more relational orientations. A relational lens can broaden what designers notice, value, and foreground in living artefacts, but it cannot replace the technical investigations required to realise viable living artefacts.

Finally, I realised how difficult it is to draw a clear boundary between what is justifiable within biodesign and what is not. Bacteria outside the lab also die—death is an inevitable part of life—yet how much intervention are humans *allowed* to provide? At what point do our actions exceed our accepted role within the interconnected web of life? The intentional killing of Flavobacteria with iodine vapour felt wrong, in a sense, but if the knowledge gained ultimately contributes to developing living artefacts that foster appreciation and awareness of microbial life, could the act then be justified?

As a more extreme example, in the work of one of my collaborators, Flavobacteria are intentionally killed to extract non-toxic colourants—a process that is indeed exploitative toward the bacteria. Yet, it also aims to address sustainability challenges in the colouring industry, such as energy and water waste, pollution, and the reliance on petroleum products and heavy metals, potentially benefiting many other species in the broader ecological picture. So, while this process might feel as though it positions humans above the Flavobacteria, can we truly say that the act is wrong, even though it aims to advance the polluting colouring industry toward more ecologically responsible practices? Or does the potential broader ecological benefit complicate such a judgment? These questions made me realise how complex ethical reasoning becomes when intentions, consequences, and scales of impact intertwine.

Not only did these experiences prompt internal reflection, but the way I combined technical inquiry with relational engagement also provoked discussion within interdisciplinary collaborations and the research communities I engaged with. For instance, when I found myself feeling concerned about killing the microorganisms I worked with, my collaborators from biological sciences framed my concern as unnecessary and a bit ridiculous, noting that “they are just microorganisms; they do not have emotions or anything.” At the same time, including the technical work of Chapter 4 in my dissertation often made me anticipate critique from the more-than-human design research community, where human-centred research activities and highly functional applications are often critiqued. For example, when the living-monitor concept (Section 2.6.1) was presented, a few design researchers wondered whether enclosing microorganisms in a plastic container was appropriate. Moving between these expectations was sometimes challenging and a bit uncomfortable: I sometimes felt like I was being judged by one community for being too human-centred, and by another for being overly reflective.

While I believe attending to non-human perspectives is crucial in biodesign, I do not think it is realistic—or even necessary—for every researcher within the field to actively reflect or act on the relational dynamics of human-microbe engagement. Different orientations foreground diverse aspects of working with living microorganisms, and biodesign benefits from their interplay: technical

inquiries deepen our understanding of microorganisms and enable viable living artefacts for sustainable futures, while relational and reflective forms of engagement help cultivate the attentiveness and affective shifts that may support truly regenerative futures. In turn, such efforts may invite technical inquiries to attend to microbial expressions that would otherwise remain unexplored, gradually expanding what is considered valuable and research-worthy within technical biodesign.

For those of us navigating both orientations, however, it becomes important to adopt a pluralistic stance and decide deliberately where to place attention. While the position can feel exposed or even intimidating, as decisions are visible and open to critique from multiple communities, it is also crucial for bridging fields, enabling in-depth knowledge and broader contextual perspectives that inform and enrich each other. With that in mind, it is worth remembering that while it may be impossible to do everything perfectly, every thoughtful step matters in moving practice forward.

Positioning my studies and my biodesign practice

To articulate my position and the diversity among the chapters in this dissertation, I mapped my studies according to the degree to which their proposed interactions and living artefacts emphasise functional versus relational aims. Figure 7.4 presents the chapters as distinct regions within a shared design space, highlighting how each foregrounds different design emphases. Chapter 3 spans the entire spectrum: although it introduces a *functional* tool, it can support the development of living artefacts with both functional and relational orientations, depending on how it is taken up in practice.

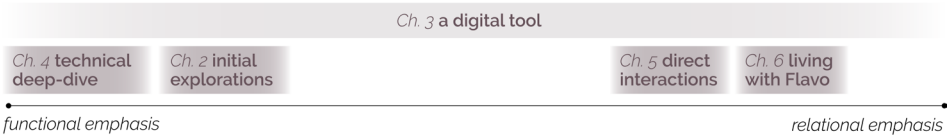


Fig. 7.4 *Positioning of dissertation chapters within a shared design space, based on their dominant design emphasis (functional versus relational). Note: Chapter 3 is shown in a lighter colour because it introduces a tool that supports biodesign practice rather than proposing a living artefact or specific human–microbe interactions.*

Both Chapter 4 and Chapter 6 were finalised within the last year of my PhD, yet they differ substantially in the degree to which they emphasise functional versus relational aims. The application concepts building on Flavobacteria's thermochromic qualities (Ch. 4) were framed in ways that solely emphasised functional benefits for humans, such as visually expressive sportswear that communicates body temperature. This framing aligned with the expectations of my colleagues from biological sciences and the technical journal to which we aimed to publish the work. Yet the relational potential of such applications remained present in my thinking, even if it was not emphasised in the chapter. I realised that, compared to the start of my PhD, I found it increasingly difficult to

imagine design possibilities without also considering the relational dynamics of human-microbe engagement.

At the same time, I recognised that application concepts with a clear human benefit do not preclude meaningful engagement with microbial life. Functional living artefacts can draw many people in to interact with microorganisms, creating space for attentiveness, curiosity, and reflection on our interconnectedness with microbial life. In this sense, highly functional living artefacts and relational engagement need not be in opposition; rather, they can be mutually supportive modes that together enrich the affordances of living artefacts.

I also recognise that, while the studies vary in their functional and relational emphasis, they are all planet-focused. Initially motivated by sustainability concerns at the material and resource level, I began exploring how interfaces with Flavobacteria can be created under realistic conditions towards living artefacts that respond expressively to environmental cues and human touch. Through this practice-grounded process, I came to understand Flavobacteria's potential not only as an environmentally responsible alternative to colour-changing materials and sensors, but also as an opportunity for meaningful encounters. By revealing the bacteria's striking colourations and responsiveness, the artefacts allowed people to directly experience the beauty and responsiveness of microbial life. Such encounters often sparked amazement, fostered attentiveness, and prompted reflection on interconnectedness as microbial behaviour unfolded in relation to human actions—qualities that I see as central to cultivating ecological awareness and pro-environmental orientations in everyday life. In this sense, what began as a pursuit of material-level ecological benefits has broadened into a recognition of Flavobacteria's potential to support wider ecological sensibilities and relational understandings.

Together, these efforts position my work within biodesign as a pragmatic, design-led, and planetary-focused practice: one that realistically explores how we can create everyday living artefacts with Flavobacteria, supporting both functional purposes and affective connections between humans and microbial life. With this, I position myself as a design researcher who works pragmatically yet imaginatively with living microorganisms—investigating how real-world living artefacts can balance functional aims while also opening space for relational dynamics and reflection on interconnectedness. This orientation carries the broader intention of fostering more holistic understandings of "our world", encouraging ecological responsibility, and opening possibilities for pro-environmental behaviour in everyday life.

7.3.2 Limitations and future work

In this section, I reflect on the constraints encountered throughout the dissertation and outline directions for future research that address these limitations.

Constraints of designing everyday living artefacts

Designing living artefacts for everyday human-microbe engagement brings practical challenges that were directly encountered in this dissertation. Supporting microbial viability and living colour within these artefacts proved difficult: iridescent Flavobacteria required a semi-solid surface with sustained humidity and protection from contamination. Even under these constraints, their living colour could only be preserved for a limited time, as colonies eventually outgrew the habitat and their iridescence faded as regions matured. This limitation directly constrains the lifespan of colour-changing artefacts with Flavobacteria: once colonies exceed the available surface, iridescence diminishes and ultimately disappears. In practice, this means that smaller habitats support only short-term engagement, while more sustained expression requires larger growth surfaces, which may not always be feasible, desired, or practical for everyday settings. Maintenance practices, such as transferring colonies to fresh media, could potentially prolong living colour, but introduce additional design challenges, human responsibility, and biosafety considerations that require further exploration.

The studies also revealed limitations in the stability and, therefore, the legibility of microbial expressions. Flavobacteria's living colour is highly sensitive to subtle environmental fluctuations, and noticeable variability was observed both within and across individual samples. As a result, expressions occasionally diverged significantly from expectations. While such variability and emergent behaviour can be particularly compelling, they may challenge functional design intentions; for instance, when colour is used as an indicator (e.g., within temperature-responsive applications), unexpected shifts can undermine clarity or reliability. Furthermore, despite efforts to foreground the organism's expressions within the artefact, some remained too subtle, unfamiliar, or angle-dependent to catch participants' attention.

Biosafety considerations further constrained the design space. To ensure safe handling in domestic environments, the artefacts required closed configurations and durable materials, limiting the range of interaction modalities and openness that could be explored. Together, these constraints shaped what kinds of microbial living artefacts were feasible within the scope of this dissertation and highlight the practical boundaries of designing with living microorganisms for everyday life.

A microorganism-specific inquiry

Focusing on a specific group of microorganisms enabled in-depth exploration across laboratory, design, and field settings, supporting rich investigation into direct interaction and human–microbe engagement. However, this focus also limits the generalisability of the findings. Many insights—such as how people engage with living colour—were closely tied to Flavobacteria's particular aesthetics and behavioural characteristics. Likewise, the direct interaction techniques developed throughout the research were grounded in

this organism's distinct responsiveness and interaction-relevant needs (e.g., the requirement for sterility to preserve microbial viability and living colour). These approaches may not translate directly to microorganisms with different characteristics and requirements.

The microorganism-specific approach also generated valuable technical and practical insights into Flavobacteria's habitat requirements, temporal dynamics, and responsiveness. Although I use the term "Flavobacteria" throughout this dissertation for readability and ease of communication, these insights do not apply uniformly to all iridescent Flavobacteria. Rather, they are closely tied to the specific strain used in this work (*C. lytica* PLY-A2). Even within this single strain, noticeable differences emerged across batches and individual samples during cultivation and experimentation. Such variation further limits the extent to which technical and experiential findings can be extrapolated beyond this particular species and the specific conditions investigated here.

Methodological constraints of a practice-grounded, in-depth biodesign inquiry

This dissertation also encountered methodological constraints that shaped both the scope and character of findings. Working with living microorganisms requires time-intensive cycles of cultivation and experimentation, which inherently limited the number of studies that could be conducted within the timeframe of the PhD. Combining laboratory exploration, interaction design, and an in situ field study further increased the time demands: constructing and refining a Flavobacteria living artefact required iterative rounds of testing, troubleshooting, and adaptation. While this process was essential for developing a viable artefact for everyday deployment, it also limited the number of design explorations, artefacts, and participant deployments that could be realised within this study. The artefact for this small-scale study was carefully designed to support biosafety, microbial viability, and expression legibility for specific modes of interaction, yet this engineering also narrowed the exploratory space, limiting opportunities to investigate more open-ended or evolving forms of engagement.

This inquiry was also shaped by its practice-grounded orientation, in which iterative design explorations and engagement with the organism formed an integral part of the research process. While this approach deepened my familiarity with the organism and sensibilities toward its expressions—thereby supporting a nuanced understanding of its living aesthetics and interaction possibilities—it also shaped which expressions were foregrounded and how design decisions unfolded, introducing a degree of methodological subjectivity.

Subjectivity also arose within the longitudinal user study. Participant accounts reflected individual lived experiences, and the thematic analysis required interpretive judgement in identifying patterns and meanings. While such subjectivity is inherent to all qualitative research, the small-scale nature of the user study will have amplified this, thereby constraining the extent to which the findings can be generalised.

The use of living microorganisms imposed further methodological constraints, particularly in terms of replicability and comparability. Microorganisms are sensitive to subtle environmental differences and exhibit inherent variability, meaning that even under highly controlled conditions, outcomes may not be perfectly reproducible. In real-world contexts—such as domestic environments—even minor variations in household conditions influenced the expression of Flavobacteria, resulting in a wide range of microbial expressions that complicated direct comparison of participant experiences. Moreover, not all insights from the laboratory studies translated directly to domestic settings. Everyday environmental conditions and patterns of human handling shaped microbial behaviour and expression in ways that could not be fully anticipated from controlled experiments, limiting the transferability of results between the laboratory and real-world contexts.

Finally, the four-week study offered rich insights into early trajectories of everyday human-microbe engagement, but did not capture longer-term relational developments. As such, it remains unclear how people's interactions, sensibilities, and forms of attentiveness might evolve over extended periods of living with microbial artefacts, including how engagement may shift once initial novelty fades. As longer-term patterns may differ substantially from early responses, understanding these dynamics is important for biodesigners aiming to foster sustained and meaningful human–microbe engagement in everyday life.

Practical limitations of the approaches

This dissertation also encountered practical limitations in the techniques, materials, and analytical approaches that supported the work. Automated methods for analysing microbial behaviour and expressions were not always reliable. In several cases, computational scripts required manual correction due to difficulties detecting the colony or cell boundaries against the background. These manual steps introduced additional labour and the potential for human error, limiting the scalability of the analysis.

Certain laboratory techniques presented further constraints for the analysis of microbial behaviour. Efforts to fixate Flavobacteria's temperature-induced purple colourations for SEM imaging proved unsuccessful, as the biofilm's optical structure was disrupted during preparation, preventing a deeper analysis of the underlying cell organisation.

The digital tool FlavoMetrics, developed as part of this dissertation, also introduced practical limitations. As a simulation-based visualisation, it inevitably simplified complex microbial behaviour and could not capture all nuances of Flavobacteria's living colour, providing only a partial view of the organism's living aesthetics. While the tool included slight randomisation factors—for example, varying colony shape, and doing so to a greater extent under high-humidity settings—it did not reflect the more emergent and unexpected behaviours observed during hands-on experimentation. As a result, the tool could lead to

expectations of deterministic outcomes, which might not always be the case with real cultures.

Furthermore, material limitations constrained the robustness and scalability of the living artefacts. Agar-based growth medium provided a suitable substrate for Flavobacteria to produce iridescent colourations but exhibited poor long-term hydration and mechanical fragility. Larger-scale or three-dimensional habitat configurations proved especially challenging, as the growth medium tended to dry out or tear under movement. These constraints present a bottleneck for everyday living artefacts, particularly when aiming for longer-term growth or artefacts that involve handling or movement, as the material does not reliably support sustained viability or structural stability.

Contamination occurred in several artefacts during the everyday deployment despite careful sterilisation procedures and the development of sterile direct interaction techniques. While not uncommon in microbial research, contamination constrained the lifespan of artefacts and the consistency of microbial expressions over time. It also influenced how participants perceived and engaged with the artefacts, and raised biosafety concerns regarding safe handling and disposal.

Finally, attempts to quantify participants' observation practices within the longitudinal study through more advanced sensors were constrained by privacy considerations and by the need for subtle and low-power sensing. As a result, observation practices were captured primarily through participants' qualitative accounts rather than through integrated sensing, making the data more subjective and likely less precise than sensor-based measurements. This also limited opportunities to capture fine-grained patterns of interaction that may not have been reported by participants.

Future research directions

Building on the constraints related to microorganisms and the materiality of living artefacts, future research could explore new habitat configurations that support long-term microbial viability and enable more open or varied forms of interaction. This includes investigating alternatives to the agar-based growth medium, integrating Flavobacteria into different material systems, and exploring fabrication strategies—such as bioprinting—that allow living colour to be embedded in more durable or flexible formats. In material sciences, Sullivan et al. [29] reported initial experiments growing *C. lytica* on porous substrates and 3D-printing the organism as iridescent bio-ink, opening new avenues for exploring material integration and fabrication strategies for living colour within design contexts. Further work could also examine how Flavobacteria respond to the more varied and fluctuating stimuli present in everyday environments, and how other interaction modalities might surface different temporal expressions. Extending this line of inquiry to other colour-producing microorganisms would broaden the understanding of living colour and its possibilities. Together, such work could support the development of more durable, expressive, and context-

responsive living artefacts for longer-term deployment in research contexts and eventually in everyday life.

Opportunities also arise for future research on the human experience of microbial expressions in everyday life, particularly in relation to the methodological constraints encountered in the longitudinal study. Longer-term deployments and studies situated in more diverse households or cultural settings could reveal how engagement unfolds over months or years, including how familiarity, sensibilities, and forms of attentiveness develop over time. Insights from related work support the value of such extended timelines; for example, in the *tilting bowl* study [249], participants' attention declined after four to five weeks, indicating that engagement trajectories with responsive artefacts evolve over extended periods. How such temporal dynamics would unfold with living artefacts remains an open question for future work. Beyond domestic contexts, long-term observation in public settings—such as the exhibition of the living artefact *Nukabot* across Japanese venues, including science and design museums and a fermented-food store [274]—could surface diverse interaction patterns at larger scales. Further work might also explore richer approaches to data collection, such as advanced sensing, mixed-method strategies, or alternative qualitative techniques. These could offer valuable insights into the full complexity of human-microbe engagement, including the subtle, fleeting, or affective dimensions that may remain unarticulated in interviews. Multimodal approaches such as *UserSense* [275] illustrate how combining physiological measurements with qualitative data can offer a more nuanced understanding of situated experience. In addition, studies involving less engineered or more open-ended artefacts, or deploying different artefacts within the same household, could help illuminate how varying configurations and expressions shape interaction and interpretation. Finally, artefacts building on other interaction modalities and stimuli—such as Flavobacteria presented as temperature-responsive indicators—could further expand understanding of how people interpret and respond to microbial expressions in everyday life.

Finally, there are opportunities to advance the tools and infrastructures that support biodesign practice. More sophisticated digital tools and analysis pipelines could help designers interpret complex living aesthetics, while refined lab protocols and diverse open-source design spaces could offer inspiration and methodological guidance for designing with microorganisms. In this regard, publications such as Crawford's *Designer's Guide to Lab Practice* [276] illustrate how field-specific lab guidance can support designers entering biological work, suggesting the value of comparable resources tailored more specifically to interaction design, living colour, or even iridescent Flavobacteria. Further research could also articulate guidelines for constructing viable living artefacts for everyday contexts, taking into account long-term microbial viability, expression legibility, biosafety and ecological considerations. Taken together, these research directions could support richer, more sustained, and more meaningful forms of human–microbe engagement across everyday contexts.

7.4 Conclusion

This dissertation responds to the accelerating environmental crisis, marked by climate change, pollution, and biodiversity loss. It proposes Flavobacteria as a promising direction for sustainable, living alternatives to colour-changing and sensing materials within HCI and interaction design. Two challenges became central to this inquiry. First, designing with Flavobacteria required a thorough understanding of the organism's needs, colour-producing mechanism, sensitivities, temporal qualities, and responsive behaviour to assess what kinds of interactions and applications are realistically possible. Second, it required an understanding of how humans experience and engage with these living microorganisms and their dynamic colourations. To address these challenges, this dissertation integrates laboratory characterisation, interdisciplinary collaborations, interaction-centred design explorations, and a field-based study.

The findings emphasise the potential of Flavobacteria's living colour to capture external conditions through diverse and dynamic changes in colony form, texture, and iridescent colour. As the research unfolded, it also demonstrated how living artefacts with Flavobacteria can open space for relational dynamics and reflection on interconnectedness between microbial life, humans, and their surroundings, which may support more ecological ways of thinking in everyday contexts.

The dissertation contributes to the field of biodesign and relevant HCI communities by offering conceptual, empirical, and methodological frameworks for human-microbe engagement, grounded in diverse design instances situated around living colour. In particular, it demonstrates an in-depth, microorganism-specific design research approach through a pragmatic yet imaginative exploration of iridescent Flavobacteria. In doing so, it opens pathways for both technically informed and experientially attuned enquiries, and invites further exploration into how designers can facilitate rich, sustained, and meaningful forms of human–microbe engagement in everyday life.

Epilogue

It is April 3rd, 2025, and I am about to go to bed. I feel like I have butterflies in my stomach: tomorrow I will take Flaviri home. I ask my partner for the second time if he is ready, though I suspect he does not share quite the same excitement. The next day, I open the incubator. I am as curious as always, but this tiny flavobacterium colony looks even cuter than usual. Carefully, I pick up the Petri dish and seal it. It has to stay in the lab a little bit longer, but then I can finally take it home.

Before I began working in the biolab and learned standard lab practices, I had taken Flavobacteria home at least a few times. I realised in this moment that I missed that. Working with Flavobacteria in the lab feels very different from having them in my home. It feels more intimate, more personal, like an adventure we are embarking on together. I can see more of the dynamic colourations, something that is also visible in the timelapses from the capture tool, but experiencing it first-hand feels far more exciting. The microorganisms become part of my personal environment, present when I am not working, sitting on the table while we eat, like a quiet companion in the house.

Working with living microorganisms over these five and a half years has changed me in ways I did not quite anticipate. As my practice unfolded, I developed a sense of connection with the Flavobacteria. Even when my approach was practical and "down to earth," I found myself becoming emotionally invested and experiencing a sense of relationality with the microorganisms. Through this experience, I came to recognise the capacity of microbial living artefacts to open up space for new relational dynamics: attunement, sensitivities, and forms of engagement that extend beyond technical interaction alone.

As I close this dissertation, I am aware that my relationship with these microorganisms does not end here. The questions they raised, and the modes of attunement they invited, will continue shaping how I experiment, design, observe, and care. If this work offers anything, I hope it is an invitation to approach living microorganisms not merely as tools or materials, but as living entities capable of fostering active engagement and heightened attentiveness towards microbial worlds and beyond.


1/4 flavo CR

<< Cellulophaga lytica PLY-A2

within Flaviri, after three weeks in my home

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Appendices

A. The Recipe of the MAR Growth Medium

The ingredients of the growth medium used throughout this dissertation:

- 1 g peptone (nutrient)
- 100 mg yeast extract (nutrient)
- 13 g sea salt (salt)
- 50 mg $MgSO_4$ (salt)
- 10 mg KNO_3 (salt)
- 6 g agar (for solid medium)
- 100 mg nigrosine (pigment to highlight iridescence)
- 400 ml demineralised water

For the Flaviri artefact, I additionally added 4 ml nystatin (10,000units/ml) to reduce the chances of fungal contamination.

B. Personal Reflections on Direct Interactions with Flavobacteria

B.1 Pressing Study (Delft, Augustus 15, 2023)

"I felt worried about Flavo when they were in the freeze dryer and afterwards, to see them as a powder, in a dormant state. Therefore, I was happy to provide them access to the nutritious agar medium by pressing the super-thin plastic foil. This step felt really exciting and intimate; it was like I was touching them with my bare hands. I was really happy to interact with them in a less lab-like setting- it finally felt more like "interacting-with" than performing microbiology experiments. While I was waiting for their colour to appear, I felt a bit impatient and worried- It took surprisingly long. After two days, I saw the first signs of structural colour. I was relieved and felt way more excited and proud than usual, as the activation process felt like such an intimate journey.

For the second experiment, I created channels in the agar medium so that the bacteria would initially only grow until that point, enabling me to reactivate their colour later on. I was satisfied to see that this border in fact worked, yet at the same time, I felt extremely guilty that they didn't have more space at the moment, which I hadn't really experienced before when cultivating them in a standard Petri dish.

During the reactivation, I was scared to break the foil as I didn't want to contaminate Flavo with my microbiome- instead, I wanted them to thrive and create structural colour to their fullest potential. The interaction was very satisfying, as the agar medium immediately broke, and I could see the bacteria touching the new area in the petri dish. Ready to explore new territory! I was glad to see that the colour returned the next day, super bright and vivid!"

B.2 Tilting Study (Delft, July 7, 2023)

"The week I spent waiting for Flavo's response felt like an eternity. Even though I knew their response would be slow and the camera was capturing every moment, I found myself frequently visiting the biolab to check whether something had changed within the colonies, to make sure the bacteria weren't contaminated, and just because I was extremely excited to see an effect. Even though I expected these results, I was still amazed by the changes in the colony's texture and form. I felt quite proud of the bacteria, to be honest.

During the first experiment, I felt tempted to interact with the bacteria again after seeing the subtle changes in their living aesthetics after six days. So, it was truly satisfying and exciting to flip the habitat the other way in the second experiment and direct the bacteria to more space and nutrients. I was excited to see that four days later, the different textures of the top and bottom areas had swapped, and, later on, also to see an effect on the form. It was sad to see the microorganisms running out of space in this habitat. I would have loved to keep this interaction going, redirecting them again and again."

B.3 Swiping Study (Delft, July 3, 2023)

"At first, I was a bit hesitant to touch Flavo's optical structures. The glove and microbiology setting, like the Petri dish and the laminar airflow cabinet, made me feel like I shouldn't be doing this. Also, I simply didn't want to disturb Flavo. But then, as soon as I touched Flavo, it felt exciting and playful. While I felt a bit of disgust when looking at the smutchy bacteria on the glove, it also made me feel like a kid playing joyfully in the mud. I was excited to see Flavo's response!

When I saw their reorganisation for the first time, I was flabbergasted - it was so bright! Yet, when I tried to catch their response on camera, I didn't see much happening. I felt sad, disappointed, and confused - I have seen them do it, and I know they are able to show these amazing traces! Then I realised that their colour became even more angle-dependent than usual and that the camera was simply not taking pictures from the right angle. Yet, this angle-dependent colour is so pretty and fascinating to look at!

During the second experiment, I felt excited to see whether Flavo would rearrange themselves again and again, yet also a bit sad as I didn't want to erase the beautiful traces of the first interaction. But Flavo created beautiful traces again! However, at some point, some parts of the colony became less bright and made me feel worried and guilty- had there been some leftover alcohol on the glove through which I was trying to keep them safe from other microbes? Was I exhausting them by interacting with them so frequently? Or were there simply not enough cells left at the moment to form structural colour?"

C. Laboratory Protocol for Preparing the Flaviri Artefact

Day 1:

- If needed: start a fresh culture of *Cellulophaga lytica* PLY-A2 from the -80 °C freezer.
- Prepare 400 ml of growth medium.
- Apply a few tapes on the side of a clean Petri dish to prevent it from opening after autoclaving.
- Sterilise the growth medium and Petri dish in the autoclave for 30 minutes at 121 °C.
- Level a flat plate in the laminar airflow (LAF) cabinet to ensure the final growth medium surface is perfectly horizontal.
- Clean the LAF cabinet with 70% ethanol and activate sterile mode.
- Retrieve the autoclaved growth medium and Petri dish.
- Put on sterile gloves to minimise contamination risk.
- Carefully open the Petri dish and remove condensation by briefly holding it upside down.
- Pour the 400 ml of growth medium into the Petri dish.
- If necessary, remove bubbles using a sterile pipette.
- Leave the plate uncovered for 20 minutes, then close the lid and let it solidify further for another 20 minutes.
- Without tilting the Petri dish, apply Parafilm and a few tapes on the side to prevent it from opening.
- Place the Petri dish horizontally in the incubator at 12°C, 90% RH.
- After 4 hours, position the Petri dish vertically in the incubator to allow excess moisture to collect at the bottom.

Day 4:

- Clean the LAF cabinet with 70% ethanol and activate sterile mode.
- Put on sterile gloves to minimise contamination risk.
- Sterilise the inner swiping magnet using alcohol and UV light.
- Remove the tape from the Petri dish and place it slightly tilted upside down.
- Remove residual moisture from the lid using a sterile pipette.
- Sterilise a metal inoculation loop with a torch and allow it to cool.
- Carefully make a cross-shaped incision in the centre of the growth medium (to prevent the inoculum from dripping until the biofilm formation begins).
- Inoculate *C. lytica* PLY-A2 from the plate culture onto the centre cross.
- Insert the inner swiping magnet into the Petri dish (on the side) and keep it in place by positioning the outer magnet on the lid.
- With minimal movement, wrap the Petri dish with Parafilm and apply a few tapes on the side to prevent it from opening.
- Place the inoculated Petri dish horizontally in the incubator.

Day 7:

- Apply waterproof tape along all edges of the Parafilm, ensuring not to move the swiping magnet.
- Apply aquarium sealant along the taped edges.
- Allow the sealant to cure for at least 4 hours under an extraction hood.
- Assemble the sealed Petri dish into the rotation disc, and connect the swiping magnet to the Petri dish holder to prevent accidental movement.

D. Participant Instructions

D.1 Instruction Card Provided at Study Start

Instructions

Dear participant,

This object is a self-sustaining, colour-changing living artefact that will remain in your home for the next four weeks. The living artefact embeds Flavobacteria, a species of marine bacteria, which produces vivid colourations similar to butterfly wings. These colourations change over time and in response to their environment, including your interactions with the living artefact. Some changes might appear more gradually and need time to reveal themselves.

Where to place the artefact?
We recommend you restrain from putting the artefact in direct sunlight or exposing it directly to water (e.g., rain) or heat (e.g., heater or heat-producing light source). To highlight colourations, it is best to place the artefact somewhere with light coming from the front – either natural light or a desk lamp positioned in front of it.

What do we expect from you?
The living artefact can be tilted and rotated as a nudge to direct the Flavobacteria to more space and nutrients. There is no correct or wrong way of interacting with the living artefact. Feel free to engage with it however and whenever you like. Over time, subtle or extreme changes might appear.

We kindly ask you to share a picture of the artefact whenever you feel like it – for example, when you see something changing, nothing is happening and you think something is wrong, or at any other given moment! You can send the pictures to me (+316 81792584) through WhatsApp or, if preferred, Signal. Please also mention briefly why you shared the image or what caught your attention.

How to capture the colourations?
Flavobacteria's colour is a result of light interacting with the structure of organised bacteria cells, meaning that light is essential for the vivid colourations to appear. When taking pictures of the colony, we therefore advise you to use the torch provided.

In case of unexpected events
Whilst it is very unlikely, if something goes wrong—for example, if the artefact leaks, shows signs of contamination, or gets damaged—please refer to the instructions included in the small "emergency kit". It outlines what to do in each scenario. Rest assured, these events are unlikely to happen, and the bacteria are not harmful. Of course, feel free to contact us if you're unsure or have any concerns.

Enjoy your time with your living artefact, and see you on _____
for the midway interview, after which we will introduce you to a second way to interact with the living artefact!

D.2 Instruction Card Provided at Study Midpoint

Additional instructions

Dear participant,

For the final two weeks of living with the artefact, we introduce a second way to interact with the Flavobacteria: swiping their colourations by gently moving the magnet over the glass. Just like tilting, this allows you to direct the Flavobacteria toward more space and nutrients, but it also provides a way to alter their existing colourations. As a reminder, subtle or extreme changes may appear; there is no right or wrong way to interact with the living artefact. Please continue to share pictures of the artefact whenever you feel like it!

Enjoy, and see you during the final interview on _____

D.3 Emergency Instructions

KEEP CALM and READ THIS

In this kit, you will find:

- Tissues
- Alcohol (70% iso-propanol)
- Gloves
- Absorption cloth
- Disposal bag

What to do in case of
leakage

Clean the affected surfaces with tissues and alcohol while wearing gloves. If any liquid from the artefact touched your skin, wash with soap and water.

Contact me so we can assess the situation and decide whether I need to pick the artefact up.

If leaking continues or you feel uncomfortable: Place the artefact upright in the **disposal** bag, with absorption cloth at the bottom and the rotating part in a vertical position to prevent liquid from reaching the electronics. I will pick it up as soon as possible.

Note: The artefact does not contain any harmful substances, so any leakage does not pose a direct health risk.

What to do in case of
contamination

Contact me so we can assess the situation and decide whether I need to pick the artefact up.

If there is serious contamination (e.g., fuzzy growth by moulds), or you feel uncomfortable: Place the artefact in the **disposal** bag. I will pick it up as soon as possible.

Note: Most contamination is non-harmful. The artefact is sealed with waterproof tape and silicone kit, which forms a strong barrier to keep any contamination inside. Therefore, it does not pose a direct health risk.

What to do in case of
breakage

Contact me as soon as possible so we can assess the situation. I will come to repair the artefact (if possible), or pick it up.

If the glass is broken: **carefully** place the artefact in the **disposal** bag, wrapped in absorption cloth and with the electronics facing up to prevent liquid from reaching them. Please be cautious of sharp edges - you can use the absorbent cloth to protect your hands while handling the object - and avoid handling the broken glass more than necessary. I will pick it up as soon as possible.

Summary

The accelerating environmental crisis demands new material paradigms that minimise ecological harm and rethink how technologies interact with living systems. Both colourant production and electronic sensing contribute significantly to pollution, resource depletion, and waste, underscoring the need for more sustainable dynamic materials. This dissertation explores iridescent Flavobacteria as a living alternative to conventional colour-changing materials, focusing on the temporality and responsiveness of their vivid structural colourations. However, realising the potential of living microorganisms in design remains challenging, requiring a detailed understanding of microbial needs, behaviours, and expressions, as well as insight into how humans perceive and engage with them. These broader biodesign challenges became central to this exploration, framing the scope of the dissertation and motivating its direction.

Addressing these wider challenges, this dissertation investigates how biodesign can facilitate human–microbe engagement in everyday life through an in-depth, design-led exploration of iridescent Flavobacteria, focusing specifically on the marine strain *Cellulophga lytica* PLY-A2. Guided by three sub-questions concerning their temporal expressions, possibilities for direct human–microbe interaction, and the lived experience of engaging with Flavobacteria's living colour, the research combines laboratory experimentation, design explorations, and a field study. Working with a single microorganism enabled a deep and iterative investigation across laboratory, design, and field settings. Through mixed methods—including temporal characterisation, interaction prototyping, and experiential studies—the dissertation develops knowledge, tools, and frameworks that support designers in working with living microorganisms.

This dissertation provides a detailed characterisation of Flavobacteria's temporal expressions, offering conceptual, methodological, and technical insights into how their living colour unfolds over time. It outlines how the microorganisms display dynamic changes in colony form, texture, and iridescent colour as they grow, move through gliding motility, and organise their cells into optical structures. Through exploratory laboratory studies supported by custom-made tools—such as a setup for consistent multi-angle imaging and a habitat for longterm growth—the dissertation shows how these expressions evolve and respond to environmental conditions and external input (Ch. 2). To support designers in understanding and exploring these dynamic behaviours, it introduces *FlavoMetrics*, a digital tool that simulates Flavobacteria's living colour (Ch. 3). The work further provides technical insight into responsive behaviour, including temperature-induced coloured rings within colonies and the underlying cell organisation, and a methodological approach for analysing colour homogeneity, revealing that the glitter-like iridescence arises from clusters of differently coloured points (Ch. 4). Together, these contributions form a robust foundation for understanding the organism's temporal and responsive qualities.

Next, this dissertation defines direct human-microbe interaction as interaction in which bodily action upon a living artefact triggers changes in microbial behaviour, conceptualised as a spectrum shaped by mediation and proximity. Building on this, it provides a design space for direct human-Flavobacteria interactions that encompasses habitat architecture, relevant input mechanisms (e.g., humidity, salinity, temperature) and possible human input (Ch. 5). Three laboratory studies show how pressing, tilting, and swiping can (re) activate, (re)direct, and (re)arrange Flavobacteria's colourations, revealing the intricate interplay between bodily input and microbial responsiveness. To support interactions beyond the lab, the dissertation introduces refined techniques and artefacts that preserve microbial viability and address biosafety, culminating in the living artefact *Flaviri* (Ch. 6). Flaviri demonstrates how sustained engagement with Flavobacteria's living colour can be achieved in domestic settings and how fluctuating household conditions and varied human inputs yield diverse unfolding expressions. Collectively, these contributions demonstrate how direct human–microbe interaction can be intentionally designed by aligning microbial behaviour, bodily input, habitat design, and interaction tools.

Finally, this dissertation advances understanding of how humans experience and engage with Flavobacteria's living colour. My personal experiences in laboratory experiments showed that sustained interaction can cultivate attentiveness, emerging microbial sensibilities, and a relational orientation toward the organisms (Ch. 5). To investigate these dynamics in everyday settings, the dissertation presents a detailed methodology for studying human–microbe engagement in daily life through a longitudinal, mixed-methods inquiry in which participants lived with the Flaviri artefact (Ch. 6). Diaries, sensor-tracked interactions, and semi-structured interviews revealed diverse engagement, shaped by what expressions people notice, how they interpret them, and the roles they adopt. Engagement also proved fragile, influenced by microbial decline, fading novelty, and mismatches between expectations and observable change. At the same time, living with the artefact heightened environmental attentiveness and reflection on microbial presence and interconnectedness between humans, microbial life, and their surroundings. Together, these insights provide an empirical foundation for understanding how microbial expressions are perceived, interpreted, and acted upon in everyday life, and show how living aesthetics and interaction modalities shape human–microbe engagement over time.

This work offers several contributions to biodesign and relevant HCI communities. Theoretically, it conceptualises living colour as communicative matter and develops a vocabulary and design space for Flavobacteria's living aesthetics and direct human–microbe interactions. Empirically, it provides technical insight into the temporality and responsiveness of Flavobacteria's living colour, alongside an understanding of how humans perceive and engage with these expressions in everyday life. Methodologically, it demonstrates a microorganism-specific design research approach and presents a detailed

methodology for studying everyday human–microbe engagement. Finally, it contributes custom tools, protocols, and research artefacts that enable exploring and engaging with living colour within and beyond laboratory settings. The concluding chapter (Ch. 7) synthesises these contributions in detail.

In the concluding chapter, I also reflect on my position as a biodesign researcher, describing how an initially instrumental focus broadened into a more relational approach to human–microbe engagement. I reflect on the intervention within designed interactions and how the studies varied in their emphasis on functional versus relational aims. This reflective stance is complemented by an earlier intermezzo (after Ch. 4), which examines the interdisciplinary collaboration with biotechnology researchers and highlights how biotechnology and design offer distinct but complementary ways to biodesign. The concluding chapter also identifies key limitations of the work and outlines directions for future research aimed at developing robust living artefacts for everyday contexts and deepening understandings of human–microbe engagement.

Taken together, this dissertation demonstrates how Flavobacteria's living colour can not only capture external conditions, but also open space for relational dynamics and reflection on interconnectedness between microbial life, humans, and their surroundings. Through this pragmatic yet imaginative exploration of iridescent Flavobacteria, the work returns to its initial motivation: to explore sustainable, living alternatives to colour-changing and sensing materials that respond to ecological concerns. More broadly, the dissertation establishes a foundation for technically informed and experientially attuned biodesign enquiries through its in-depth, microorganism-specific design research approach, integrating laboratory characterisation, design-led experimentation, and a longitudinal everyday study. In doing so, it invites future work that deepens and expands the possibilities for meaningful human–microbe engagement in everyday life.

Samenvatting

De versnellende ecologische crisis vraagt om nieuwe materiaalparadigma's die milieuschade minimaliseren en heroverwegen hoe technologieën in wisselwerking staan met levende systemen. Zowel de productie van kleurstoffen als de elektronische sensortechnologie dragen aanzienlijk bij aan vervuiling, afvalstromen en uitputting van grondstoffen, wat de noodzaak van duurzamere dynamische materialen onderstreept . Dit proefschrift onderzoekt structureel gekleurde Flavobacteriën als een levend alternatief voor conventionele kleurveranderende materialen, met specifieke aandacht voor de tijdsdynamiek en responsiviteit van hun levendige kleur. Het realiseren van het potentieel van levende micro-organismen in ontwerppraktijken blijft echter uitdagend en vereist diepgaand begrip van de groeicondities, het gedrag en de expressies van deze organismen, evenals inzicht in hoe mensen deze waarnemen, ervaren en ermee omgaan. Deze bredere biodesign-vraagstukken kwamen centraal te staan in dit onderzoek en vormden zowel het kader als de richting van het proefschrift.

Voortbouwend op deze bredere uitdagingen onderzoekt dit proefschrift hoe biodesign kan bijdragen aan interacties en ervaringen tussen mens en micro-organismen in het dagelijks leven, aan de hand van een diepgaande, ontwerpgedreven verkenning met iriserende Flavobacteriën, gericht op de mariene bacteriestam *Cellulophga lytica* PLY-A2. Geleid door drie deelvragen—met betrekking tot hun temporele expressies, de mogelijkheden voor directe interactie tussen mens en micro-organisme en de ervaring van de levende kleur van Flavobacteriën—combineert het onderzoek laboratoriumexperimenten, ontwerpexploraties en een praktijkstudie. Het werken met één specifiek micro-organisme maakte een diepgaande en iteratieve onderzoeksbenadering mogelijk in zowel laboratorium-, ontwerp- als dagelijkse omgevingen. Het proefschrift maakt gebruik van een combinatie van methoden, waaronder temporele karakterisering, interactieprototyping en ervaringsgerichte studies. Hiermee ontwikkelt het kennis, tools en ontwerpkaders die ontwerpers ondersteunen bij het werken en ontwerpen met levende micro-organismen.

Het proefschrift biedt een gedetailleerde karakterisering van de temporele expressies van Flavobacteriën en verschaft conceptuele, methodologische en technische inzichten in hoe hun levende kleur zich in de tijd ontvouwt. Het beschrijft hoe deze micro-organismen dynamische veranderingen vertonen in hun kolonievorm, textuur en hoeksafhankelijke kleur, terwijl zij groeien, zich verplaatsen via glijdende motiliteit en hun cellen organiseren in optische structuren. Aan de hand van exploratieve laboratoriumstudies, ondersteund door zelfontwikkelde tools—zoals een opstelling voor consistente beelddocumentatie vanuit meerdere hoeken en een habitat voor langdurige groei—laat dit proefschrift zien hoe deze expressies zich ontwikkelen en hoe zij worden beïnvloed door omgevingscondities en externe input (H. 2). Om ontwerpers te ondersteunen bij het begrijpen en verkennen van deze dynamiek, introduceert het proefschrift *FlavoMetrics*, een digitale tool die de levende kleur van Flavobacteriën simuleert

(H. 3). Daarnaast biedt het werk technisch inzicht in de responsiviteit, bijvoorbeeld hoe temperatuurvariaties gekleurde ringen binnen kolonies genereren en hoe celorganisatie ten grondslag ligt aan deze patronen (H. 4). Het bevat bovendien een methodologische aanpak voor het analyseren van kleurhomogeniteit, waaruit blijkt dat de glinsterende, iriserende kleur van Flavobacteriën ontstaat uit clusters van verschillende kleuren. Samen vormen deze inzichten een stevig fundament voor het begrijpen van de temporele en responsieve eigenschappen van het organisme.

Vervolgens definieert dit proefschrift directe mens–microbe-interactie als een interactie waarbij lichamelijke handelingen op een levend artefact veranderingen in microbieel gedrag teweegbrengen, opgevat als een spectrum gevormd door gradaties van afstand en tussenkomst. Hierop voortbouwend biedt het proefschrift een ontwerpraamwerk voor directe mens–Flavobacteria-interacties dat de habitatarchitectuur, relevante inputmechanismen (zoals vochtigheid, zoutgehalte en temperatuur) en mogelijke menselijke input omvat (H. 5). Drie laboratoriumstudies tonen hoe acties als drukken, kantelen en vegen Flavobacteria's kleur kunnen (her)activeren, (her)sturen en (her)ordenen en onthullen de verfijnde wisselwerking tussen lichamelijke input en microbiële responsiviteit. Om interactie buiten het lab te ondersteunen, introduceert het proefschrift technieken en artefacten die de microben in leven houden en aan bioveiligheidsvereisten voldoen, met als culminatie het levende artefact *Flaviri* (H. 6). Flaviri laat zien hoe langdurige omgang met de levende kleur van Flavobacteria in alledaagse omgevingen mogelijk wordt en hoe wisselende omgevingscondities en diverse menselijke handelingen leiden tot uiteenlopende expressies. Gezamenlijk tonen deze bijdragen hoe directe mens–microbe-interactie intentioneel kan worden vormgegeven door microbieel gedrag, lichamelijke input, habitatontwerp en interactietools op elkaar af te stemmen.

Ten slotte verdiept dit proefschrift het begrip van hoe mensen de levende kleur van Flavobacteriën ervaren en ermee omgaan. Mijn eigen ervaringen in laboratoriumexperimenten lieten zien dat langdurige interactie kan leiden tot aandachtigheid, de ontwikkeling van microbieel-gerichte sensibiliteit en een relationele houding ten opzichte van de organismen (H. 5). Om deze dynamieken in alledaagse contexten te onderzoeken, presenteert het proefschrift een gedetailleerde methodologie voor het bestuderen van mens–microbe-omgang in het dagelijks leven via een longitudinale studie waarin deelnemers leefden met het artefact Flaviri (H. 6). Dagboeken, sensorgegevens over interacties en semigestructureerde interviews brachten uiteenlopende vormen van omgang aan het licht, gevormd door welke expressies mensen opmerken, hoe zij deze interpreteren en welke rollen zij aannemen ten opzichte van het artefact. De omgang bleek bovendien kwetsbaar, beïnvloed door waargenomen achteruitgang, afnemende nieuwigheid en discrepanties tussen verwachtingen en waarneembare veranderingen. Tegelijkertijd versterkte het leven met het artefact het omgevingsbewustzijn en de reflectie op de aanwezigheid van micro-organismen, evenals op de onderlinge verbondenheid van mensen, microbieel leven en hun omgeving. Gezamenlijk vormen deze inzichten een empirische basis

voor het begrijpen van hoe microbiële expressies in het dagelijks leven worden waargenomen, geïnterpreteerd en beantwoord, en laten zij zien hoe levende esthetiek en interactiemodaliteiten mens–microbe-omgang in de tijd vormgeven.

Dit werk levert verschillende bijdragen aan biodesign en relevante gemeenschappen binnen mens–computerinteractie (HCI). Theoretisch conceptualiseert het levende kleur als communicatiemedium en ontwikkelt het een vocabulaire en ontwerpraamwerk voor de levende esthetiek van Flavobacteriën en directe mens–microbe-interacties. Empirisch biedt het technisch inzicht in de temporaliteit en responsiviteit van Flavobacteria's levende kleur, naast een begrip van hoe mensen deze expressies in het dagelijks leven waarnemen en ermee omgaan. Methodologisch demonstreert het een micro-organisme-specifieke ontwerponderzoeksaanpak en presenteert het een gedetailleerde methodologie voor het bestuderen van mens–microbe-omgang in alledaagse contexten. Ten slotte draagt het werk zelfontwikkelde tools, protocollen en onderzoeksartefacten bij die het mogelijk maken levende kleur te verkennen en ermee te interageren binnen én buiten laboratoriumomgevingen. Het afsluitende hoofdstuk (H. 7) vat deze bijdragen in detail samen.

In het afsluitende hoofdstuk reflecteer ik ook op mijn positie als biodesign-onderzoeker, waarbij ik beschrijf hoe een aanvankelijk instrumentele focus geleidelijk verbreedde tot een meer relationele benadering van mens–microbe-omgang. Ik bespreek de rol van interventie binnen de ontworpen interacties en hoe de studies verschilden in hun nadruk op functionele versus relationele doelen. Deze reflectieve houding wordt aangevuld door een eerder intermezzo (na H. 4), waarin de interdisciplinaire samenwerking met biotechnologie-onderzoekers wordt besproken en wordt benadrukt hoe biotechnologie en design elk eigen maar complementaire benaderingen van biodesign bieden. Het afsluitende hoofdstuk identificeert daarnaast belangrijke beperkingen van het werk en schetst richtingen voor toekomstig onderzoek, gericht op het ontwikkelen van robuuste levende artefacten voor alledaagse contexten en het verdiepen van inzichten in mens–microbe-omgang.

Alles samengenomen laat dit proefschrift zien hoe de levende kleur van Flavobacteriën niet alleen externe condities en input kan weerspiegelen, maar ook ruimte kan openen voor relationele dynamieken en reflectie op de onderlinge verbondenheid van microbieel leven, mensen en hun omgeving. Met deze pragmatische én verbeeldingsrijke verkenning van iriserende Flavobacteriën keert het werk terug naar zijn oorspronkelijke motivatie: het onderzoeken van duurzame, levende alternatieven voor kleurveranderende en sensormaterialen die inspelen op ecologische vraagstukken. In bredere zin legt het proefschrift een fundament voor technisch onderbouwde en ervaringsgeoriënteerde biodesignbenaderingen, door middel van een diepgaande, op één micro-organisme gerichte ontwerponderzoeksaanpak die laboratoriumkarakterisering, ontwerpexperimentatie en een longitudinale studie in het dagelijks leven integreert. Daarmee nodigt dit werk uit tot vervolgonderzoek dat de mogelijkheden voor betekenisvolle mens–microbe-omgang in het dagelijks leven verder verdiept en verruimt.

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About the Author

Clarice Risseeuw was born on February 7th, 1997, in Vlissingen, a coastal city in the southwest of the Netherlands. She obtained a Bachelor's degree in Industrial Design Engineering at Delft University of Technology in 2018. She continued her studies at the same university through the MSc program in Industrial Design Engineering, during which she began working with iridescent Flavobacteria as part of her graduation project. She obtained her Master's degree cum laude in early 2021.

After graduating, she joined the Centre of Applied Research for Art, Design and Technology (CARADT) at Avans University of Applied Sciences in Den Bosch, the Netherlands, where she continued her biodesign research. One year later, in March 2022, she returned to Delft University of Technology as a PhD candidate. Her doctoral research was conducted within the Materials Experience Lab, which later evolved into the Centre of Design Research for Regenerative Material Ecologies (DREAM).

In her free time, Clarice enjoys spending time outdoors, whether in the garden, at the beach, in forests, or in mountain areas while travelling. She also takes pleasure in caring for her indoor plants and in creative activities such as painting, drawing, and personal design projects.

List of Publications

- Groutars, E. G.*, **Risseeuw, C. C.***, Ingham, C., Hamidjaja, R., Elkhuizen, W. S., Pont, S. C., & Karana, E. (2022). Flavorium: An Exploration of Flavobacteria's Living Aesthetics for Living Color Interfaces. In *Proceedings of the ACM Conference on Human Factors in Computing Systems*. Association for Computing Machinery. https://doi.org/10.1145/3491102.3517713

  * shared first authorship

- Kim, R., **Risseeuw, C.**, Groutars, E.G., & Karana, E. (2023). Surfacing Livingness in Microbial Displays: A Design Taxonomy for HCI. In *Proceedings of the ACM Conference on Human Factors in Computing Systems*. Association for Computing Machinery. https://doi.org/10.1145/3544548.3581417

- **Risseeuw, C.**, Martinez Castro, J. F., Barla, P., & Karana, E. (2023). FlavoMetrics: Towards a Digital Tool to Understand and Tune Living Aesthetics of Flavobacteria. In *Proceedings of the ACM Designing Interactive Systems Conference*. Association for Computing Machinery. https://doi.org/10.1145/3563657.3596085

- **Risseeuw, C.**, McQuillan, H., Martins, J., & Karana, E. (2024). (Re)activate, (Re)direct, (Re)arrange: Exploring the Design Space of Direct Interactions with Flavobacteria. In *Proceedings of the ACM Conference on Human Factors in Computing Systems*. Association for Computing Machinery. https://doi.org/10.1145/3613904.3642262

  Award: Best Paper Honourable Mention

- **Risseeuw, C.**, Kummetha, L., Ingham, C., Karana, E., Aubin-Tam, M.E., & Martins, J. (2026). Exploring the design potential of iridescent Flavobacteria for thermochromic engineered living materials. Bio-Design and Manufacturing. Springer Nature. https://doi.org/10.1631/bdm.2500187

- **Risseeuw, C.** (2026). Designing with Flavobacteria: Tools and Artefacts for Exploring and Engaging with Living Colour. Accepted for publication, Biodesign Conference (Demo track). Cambridge University Press.

- **Risseeuw, C.**, McQuillan, H., & Karana, E. (2026). The Role of Microbial Aesthetics in Everyday Engagement with Living Artefacts: A Case of Flavobacteria. Under review with ACM Transactions on Computer-Human Interaction.

  [under review]

Academic and Professional Activities

Presentations, Demos, and Workshops

- Studium Generale Caradt (2021): livestream presentation
- Bioday TU Delft (2022): pitch and poster presentation
- CHI (2022): conference presentation, New Orleans (USA)
- IDE Masterclass Biodesign (2023): presentation and biolab workshop
- DIS (2023): conference presentation and demo, Pittsburgh (USA)
- Dutch Design Week (2023): exhibition and workshop
- CHI (2024): conference presentation, Honolulu (USA)
- Additional dissemination activities (2022–2026): project presentations and biolab demonstrations for visiting professors, industry partners, and internal advisory boards.

Teaching and Mentorship

- Lecturer, workshop facilitator, and/or coach in the following MSc courses at Delft University of Technology: Material-Driven Design, Fundamentals of Biodesign, Designing Living Artefacts, Animated Materials, Making and Prototyping Skills, and Product Futures (2022-2026)
- Supervisor of an MSc graduation project at Delft University of Technology (2023)

Research Funding

- Lead author and project lead of the successful KIEM-CE funding proposal *Living Circular Labels*, funded by Regieorgaan SIA (part of the Dutch Research Council, NWO). The project secured €25,000 in research funding and €25,000 in industry partner contributions.
- Contributed to the writing and preparation of the successful RAAK-PRO funding proposal *ChromoFlavo*: *Advancing Sustainable, Bacterial-Derived Structural Colours for Design, Architecture, and Beyond*, funded by the Dutch Research Council, NWO.

Academic Reviewing

- Reviewer for CHI 2023
- Reviewer for DRS 2024
- Reviewer for DIS Pictorials 2024
- Associate Chair (AC) reviewer for DIS Pictorials 2025
- Reviewer for the Biodesign Conference 2026