# Biobased short chain fatty acid production

## Exploring microbial community dynamics and metabolic networks through kinetic and microbial modeling approaches

Atasoy, Merve; Scott Jr, William T.; Regueira, Alberte; Mauricio-Iglesias, Miguel; Schaap, Peter J.; Smidt, Hauke

**DOI**
10.1016/j.biotechadv.2024.108363

**Publication date**
2024

**Document Version**
Final published version

**Published in**
Biotechnology Advances

Biotechnology Advances 73 (2024) 108363

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

# Biobased short chain fatty acid production - Exploring microbial community dynamics and metabolic networks through kinetic and microbial modeling approaches

Merve Atasoy [a,b,c,*], William T. Scott Jr [a,d], Alberte Regueira [e,f,g], Miguel Mauricio-Iglesias [e], Peter J. Schaap [a,d], Hauke Smidt [a,c]

[a] UNLOCK, Wageningen University & Research and Delft University of Technology, Wageningen and Delft, the Netherlands
[b] Department of Environmental Technology, Wageningen University & Research, Wageningen, the Netherlands
[c] Laboratory of Microbiology, Wageningen University & Research, Wageningen, the Netherlands
[d] Laboratory of Systems and Synthetic Biology, Wageningen University & Research, Wageningen, the Netherlands
[e] CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela, Santiago de Compostela, Spain
[f] Center for Microbial Ecology and Technology (CMET), Ghent University, Ghent, Belgium
[g] Center for Advanced Process Technology for Urban Resource Recovery (CAPTURE), Frieda Saeysstraat 1, Ghent, Belgium

ARTICLE INFO

*Keywords:*
Volatile fatty acids
Open mixed culture fermentation
Microbial community
Kinetic modeling
Generalized-Lotka Volterra models
ODE-based microbe effector models
Genome-scale metabolic models

ABSTRACT

In recent years, there has been growing interest in harnessing anaerobic digestion technology for resource recovery from waste streams. This approach has evolved beyond its traditional role in energy generation to encompass the production of valuable carboxylic acids, especially volatile fatty acids (VFAs) like acetic acid, propionic acid, and butyric acid. VFAs hold great potential for various industries and biobased applications due to their versatile properties. Despite increasing global demand, over 90% of VFAs are currently produced synthetically from petrochemicals. Realizing the potential of large-scale biobased VFA production from waste streams offers significant eco-friendly opportunities but comes with several key challenges. These include low VFA production yields, unstable acid compositions, complex and expensive purification methods, and post-processing needs. Among these, production yield and acid composition stand out as the most critical obstacles impacting economic viability and competitiveness. This paper seeks to offer a comprehensive view of combining complementary modeling approaches, including kinetic and microbial modeling, to understand the workings of microbial communities and metabolic pathways in VFA production, enhance production efficiency, and regulate acid profiles through the integration of omics and bioreactor data.

## 1. Introduction

Resource recovery from waste streams has become an increasingly promising area of study in recent years, with anaerobic digestion technology emerging as a leading approach. Beyond its established role in energy recovery, this technology has expanded to include the production of carboxylic acids, particularly volatile fatty acids (VFAs) such as acetic acid, propionic acid, and butyric acid. These VFAs are of significant interest to a variety of industries and biobased product applications due to their diverse functionality (Millati et al., 2023). Despite increasing global demand, >90% of the produced VFA is currently synthetically derived from petrochemicals (Agnihotri et al., 2022). In the face of fast-depleting fossil resources, their adverse environmental effects, rising energy and material demands, a profound increase in greenhouse gas emissions and carbon footprints, and substantial waste generation from conventional processes, there is an urgent need for the industry to redirect its focus toward environmentally friendly bio-based VFA production.

Unlocking the potential of industrial-scale biobased production of VFAs from waste streams holds tremendous promise. However, several challenges need to be addressed for its successful implementation such as low production yield of biobased VFAs, unstable acid composition, complex and costly purification and separation methods, and post-processing requirements (Aghapour Aktij et al., 2020; Martinez et al.,

* Corresponding author at: UNLOCK, Wageningen University & Research and Delft University of Technology, Wageningen and Delft, the Netherlands.
*E-mail addresses:* merve.atasoy@wur.nl (M. Atasoy), william.scott@wur.nl (W.T. Scott), alberte.regueiralopez@ugent.be (A. Regueira), miguel.mauricio@usc.es (M. Mauricio-Iglesias), peter.schaap@wur.nl (P.J. Schaap), hauke.smidt@wur.nl (H. Smidt).

https://doi.org/10.1016/j.biotechadv.2024.108363
Received 7 December 2023; Received in revised form 3 April 2024; Accepted 12 April 2024
Available online 22 April 2024

2016; Reyhanitash et al., 2017; Rocha et al., 2017).

Among these challenges, production yield and product spectrum are the most significant issues. Low production yield mainly hampers economic competitiveness in both production and recovery processes. Simultaneously, the acid composition wields a substantial influence on both upstream and downstream applications of VFAs. Operational parameters, such as pH and substrate type, have a significant impact on the product spectrum.

Under acidic conditions (pH < 5), acetic acid production is often favored, whereas near-neutral pH (pH: 6.5–7.5) tends to result in a more balanced mixture of acetic, propionic, and butyric acid (Fang and Liu, 2002). However, contrasting results have been reported in other studies. For instance, Atasoy et al. (2019) showed that acetic acid dominated at pH 7, whereas the combination of acetic and butyric acid was prevalent at pH 5, and butyric acid was the primary product at pH 10 during glucose fermentation. In contrast, Jankowska et al. (Jankowska et al., 2015) reported that propionic acid prevailed at pH 4 and pH 5, with acetic acid becoming dominant at pH 10. Furthermore, the choice of substrate types, such as carbohydrates, proteins, or lipids, significantly influences the product spectrum due to variations in their chemical structures. Carbohydrate breakdown primarily yields a mixture of acetic, propionic, and butyric acids, whereas lipid degradation can produce medium and long-chain fatty acids like caproic acid (Bevilacqua et al., 2021; Ma et al., 2017). In addition to the substrate type, the physical structure of the substrate – whether it is in liquid, solid, mixed wastestream form, etc. – is another crucial parameter that influences the anaerobic degradation process. However, for the scope of this paper, our primary focus lies on the production of VFA from wastewater. Besides, different microbial species have varying pH tolerances and substrate preferences, which can further influence the VFA spectrum. Additionally, several other factors (e.g., retention time, inoculum source, bioreactor type, operation mode, etc.) affect the product spectrum as well. Therefore, it is very challenging to predict and control the acid composition.

Steering VFA production toward specific products not only eases the separation and purification of these products but also produces customized carbon sources for diverse applications, such as carbon sources for denitrification or bioplastic production (Elefsiniotis and Wareham, 2007; Zhang et al., 2023). In order to unravel the effects of several parameters on acid composition and to allow steering the acid profile, a wide range of studies have been conducted focusing on substrate selection (Jankowska et al., 2017; Shen et al., 2017; van Aarle et al., 2015), manipulation of microbial communities (Atasoy and Cetecioglu, 2021; Blasco et al., 2020; Jiang et al., 2015), optimization of operational and environmental conditions (Calero et al., 2018; Garcia-Aguirre et al., 2017; Jankowska et al., 2015), inhibitor management (Lukitawesa et al., 2020), product separation and recovery methods (Liu et al., 2020; Reyhanitash et al., 2017; Zacharof and Lovitt, 2014), metabolic engineering (Jang et al., 2014), hybrid processes and bioreactor design (Parchami et al., 2023). Even though these studies identified the effects of numerous parameters on VFA composition and production yield, steering the acid profile remains a considerable challenge due to the intricate interplay of diverse factors within the complex microbial ecosystems responsible for VFA production. Mainly, a dynamic consortium of microorganisms with varying metabolic preferences and multiple metabolic pathways as well as the exhibition of complex synergetic and competitive interactions in the microbial community contributes to the difficulty of steering VFA production toward specific products (Atasoy and Cetecioglu, 2021; Lv et al., 2022; Ramos-Suarez et al., 2021; She et al., 2020).

In summary, experimentalists have endeavored to master the VFA production process and steer its selectivity but have faced challenges due to inherent uncertainties. To address these uncertainties and optimize production yield, while controlling product profiles, a profound understanding of microbial communities is essential. This understanding should encompass metabolic networks, production kinetics, and the impacts of operational and environmental variables.

To achieve this, the combination of kinetic modeling (i.e., models for simulating at the reactor level how microorganisms or cells grow, interact, and produce desired products within the reactor based on their biological kinetics and environmental conditions) and microbial community modeling (i.e., models for simulating intercellular level complex interactions and dynamics among multiple microorganisms in a bioreactor to understand their population dynamics, metabolic processes, and overall ecosystem behavior) offers a comprehensive insight into VFA production systems, emphasizing microbial dynamics under specific conditions.

Moreover, modeling approaches enable the extrapolation and prediction of community dynamics and behavior under untested operational and environmental conditions, facilitating efficient and rapid optimization of the VFA production process. This paper offers a broad perspective on how different modeling approaches, including kinetic and microbial modeling, can be used to understand the complex dynamics of microbial communities and metabolic pathways involved in VFA production. The modeling reviewed here should be used to create a roadmap for combining omics and bioreactor data to improve production efficiency and control acid profiles precisely. Our audience includes both experimental microbiologists interested in modeling and computational biologists looking to apply their skills in practical biochemical contexts. We explore various modeling approaches, from basic Lotka-Volterra models to more detailed microbe-effector models based on ordinary differential equations, as well as comprehensive genome-scale metabolic models. By merging hands-on experimentation with computational insights, our goal is to foster a deeper and more multifaceted understanding while promoting collaborations that push the boundaries of biobased VFA production.

## 2. Comprehending microbial dynamics in the production of VFA

One of the most important parameters for bio-based VFA production is the inoculum source. It has been stated that open mixed cultures offer a stable and robust VFA production process that can adapt to varying environmental and operational conditions (Perrotta et al., 2017; van Aarle et al., 2015). Moreover, the multi-step transformations in mixed cultures allow for a shorter acclimation time, leading to increased proliferation and, ultimately, higher total production yields (Schmidt, 2021).

However, employing mixed microbial cultures for VFA production presents a major bottleneck, as their complex structure is challenging to comprehend. Microbial communities in these mixed cultures exhibit high diversity and versatile metabolism encompassing various functions for metabolic networks (Płaza et al., 2021; Sidhu et al., 2017) syntrophic interactions (McInerney et al., 2009; Stams and Plugge, 2009), and dynamic responses to operational and environmental changes (Li et al., 2015; Lv et al., 2022). These factors collectively contribute to the complexity of mixed microbial cultures, making them difficult to predict and control. Understanding microbial interactions and their functions is essential to identify key metabolic pathways and enzymes that can be targeted for process optimization. Furthermore, this understanding can facilitate the identification of strategies to mitigate inhibition and enhance process performance, which is crucial for attaining stable and efficient VFA production.

To model a microbial community, it is essential to have a comprehensive understanding of the structure of the community, as well as the functions and interactions of the microorganisms within it. Next-generation sequencing offers invaluable insights into microbial community dynamics, which is essential for identifying key metabolic pathways and enzymes for optimizing VFA production (Kim et al., 2022). While various studies have successfully identified microbial community composition in anaerobic mixed cultures, the relation between the dynamic behavior of microbial communities and their response to operational/environmental parameters for the production

and composition of VFAs remains unexplored. In this section, the application of -omics technologies for gaining insights into the microbial dynamics involved in VFA production as well as their applications for kinetic and microbial community models, are discussed.

### 2.1. Profiling microbial members and their potential activities within a community

#### 2.1.1. Who is there and potentially doing what?

DNA sequencing constitutes the primary stage of -omics technologies aimed at characterizing a given microbial community by analyzing the DNA of all its members. This approach involves recovering genetic material from the environmental matrix, commonly referred to as metagenomes i.e., the collective genome of a microbial community (Pérez-Cobas et al., 2020). Microbial DNA-targeted sequencing has been widely applied to identify microbial communities in anaerobic systems using various approaches, including amplicon-based marker gene sequencing (e.g., 16S ribosomal RNA gene), gene-centric metagenomics, and genome-centric metagenomics (Zhang et al., 2021).

Most studies investigating microbial community profiles for VFA production have relied on the 16S rRNA gene for assessing community composition and relative abundance of community members. This technique, also frequently referred to as metataxonomics, provides details on microbial taxonomy, and depending on the chosen target region of the 16S rRNA gene, can leverage information at different levels of taxonomic resolution. To this end, current standard approaches based on short read sequencing typically only allow identification up to the genus level, and can be used to some extent to predict potential metabolic functionalities (Zhang et al., 2021). Despite the microbial community profile being able to be shifted by several operational and environmental parameters, the most commonly reported dominant phyla associated with VFA production include *Bacillota* (previously *Firmicutes*), *Bacteroidota* (*Bacteroidetes*), and *Pseudomonadota* (*Proteobacteria*) (Atasoy and Cetecioglu, 2022; Feng et al., 2009; Owusu-Agyeman et al., 2022; Strazzera et al., 2021). Within these phyla dominant members at family and genus levels have been shown to differ significantly according to the experimental foundation of the studies. On the other hand, phylogenetically related species may have different metabolic capabilities, which can lead to misinterpretation of their functionalities (Wenzel et al., 2018). Furthermore, species with a relative abundance of <1% in the microbial community may be eliminated during post – analysis in terms of their functional significance, despite their substantial roles (Yin et al., 2022). While no studies have yet identified rare species exclusively involved in VFA production, uncovering such species could aid in engineering microbial communities for enhanced selective VFA production.

However, due to PCR bias and in most cases lack of species-level information, 16S rRNA gene-based methods cannot offer precise insights into microbial population functionality (Kim et al., 2022). To this end, the gene-centric metagenomics approach involves sequencing all genes present in a microbial community to gain an understanding of its metabolic potential. Although this approach yields information on overall metabolic pathways, it does not classify them into taxonomic units, thus limiting its ability to identify specific taxa and their functional roles within the community (Kleinsteuber, 2019). In contrast, the genome-centric approach enables the identification of functional potential within individual microbial members of a community. While this approach offers a more detailed analysis of the metabolic and functional potential of each taxon, it is more technically challenging than other approaches due to the large amount of data. In their study, Greses et al. (2023) identified 58 metagenome-assembled genomes and investigated the impact of pH variations on microbial dynamics and their corresponding functions in the production of VFAs through food waste fermentation. Their results revealed that a decrease in pH from 6.5 to 6.1 caused a shift in metabolite production from acetate, butyrate, and ethanol to caproate and hydrogen. However, this change did not affect the microbial community profile. Further analysis and a suitable model could have provided more profound insights into how the pH drop shifted the product profile.

Fontana et al. (2018) applied a genome-centric metagenomics approach to evaluate the performance of single and two-stage thermophilic anaerobic digesters in fermenting cheese wastewater. They have identified 50 population genomes (PGs), 22 of which were newly discovered. By correlating the presence of key metabolites with the abundance of the PGs, they estimated the metabolic pathways encoded in these genomes. Their findings revealed that a higher abundance of *Clostridium* spp. was associated with greater specialization in hydrolytic and acidogenic activities, leading to improved process efficiency (Fontana et al., 2018). Although genome-centric metagenomics can reveal the functional potential of each taxon within a community, it is essential to note that this approach sequences all genomes in a microbial community, regardless of whether they play an active role in the process studied. Therefore, while it offers valuable insights into the potential metabolic pathways of individual taxa, it may not be the most suitable approach for identifying the functional roles that microbial members actively play within the community under a specific set of conditions (Kim et al., 2022). Many studies have been conducted to profile microbial communities during anaerobic digestion (Jankowska et al., 2017; Vanwonterghem et al., 2015; Zhang et al., 2020a). Nevertheless, few studies identified the functional potential and active participation of microbial community members within the metabolic networks of VFA production (Hao et al., 2020; Luo et al., 2021; Wang et al., 2023).

### 2.2. Identification of the functional roles of microbial community members

#### 2.2.1. What are their actual functionalities?

Metatranscriptomics is a method for identifying microbial functionalities, via sequencing and analyzing the messenger RNA (mRNA) transcripts (Aguiar-Pulido et al., 2016). Identifying the expressed genes and their level of expression provides a more direct assessment of the active functional roles of individual microbial members within the community at a given moment. In addition to identifying the expressed genes and their level of expression, metatranscriptomics can provide insights into differential gene expression under various environmental conditions. In this way, it offers a more comprehensive understanding of community function and dynamics over time, beyond the simple description of active members and expressed genes (Shakya et al., 2019). The combination of metatranscriptomics and metagenomics and/or marker gene based approaches can be used to investigate the dynamics of microbial communities and their responses to changing environmental conditions. To date, a limited number of studies have employed metatranscriptomics to identify the functional roles of microbial communities for VFA production (Luo et al., 2021; Scarborough et al., 2018) due to the experimental and computational challenges (e.g., the instability of RNA molecules and the incomplete genomic information for many microorganisms in a given environment, making it complex to attribute RNA sequences to specific organisms and genes and interpret the data) in addition to financial constraints.

Luo et al. (2021) reported a 6.5-fold increase in VFA production from food waste fermentation by supplementing with linear alkylbenzene sulphonates (LAS), which enhances organic compound solubilization, at a concentration of 50 mg/L. Their findings indicated that the addition of LAS improved the hydrolysis efficiency of carbohydrates, proteins, and lipids in food waste, and facilitated membrane transport of substrates. Metatranscriptomics coupled with metagenomics revealed the expression of several functional genes, such as *accA*, *accC*, and *accD*, associated with fatty acid biosynthesis by the microbial community in LAS-treated reactors (Luo et al., 2021). Another study that employed metatranscriptomics to understand functions of the microbial community in medium-chain fatty acid (MCFA) production proposed that thioesterase instead of coenzyme A (CoA) transferase should be used as the terminal

enzyme of the reverse β-oxidation pathway for an efficient chain elongation process (Scarborough et al., 2018). Furthermore, the authors also used metabolic reconstruction in combination with metatranscriptomic analysis to predict that *Lactobacillus* and *Coriobacteriaceae* species would degrade carbohydrates and ferment sugars to lactate and acetate, while species belonging to the *Lachnospiraceae* and *Eubacteriaceae* would transform these fermentation products to MCFA (Scarborough et al., 2018). Ultimately, Scarborough et al. (2018) identified thioesterase as a pivotal enzyme in the reverse β-oxidation pathway, enhancing the efficiency of MCFA production.

While DNA-based approaches and metatranscriptomics provide valuable information about microbial communities, these approaches only provide a view of potential metabolic capabilities and gene expression under certain conditions. However, VFA production is highly dynamic, involving complex metabolic pathways involving both known and unknown microbial species. In some cases, these metabolic networks and microbial species are interdependent and regulated by a range of operational and environmental factors. Therefore, to gain a comprehensive understanding of microbial community dynamics and metabolic networks involved in VFA production, it is essential to reconstruct and integrate metabolic networks via modeling approaches, which provide a dynamic view of the metabolic products and substrates involved in VFA production.

### 2.3. Uncovering metabolic networks: an integrated approach to understanding microbial communities

To comprehensively investigate the dynamic responses of microbial communities to different environmental and operational conditions, it is necessary to integrate the identification of active community functions with the metabolic networks. Moreover, the identification of particular metabolic pathways can uncover the part played by individual microbial taxa in the fermentation process and their contribution to the production of VFAs. Metabolomics is one of the most powerful approaches to metabolic pathway analysis (Aguiar-Pulido et al., 2016). It aims to determine and quantify intracellular and extracellular metabolites, which are low-molecular-mass compounds (Lamichhane et al., 2018; Pinu et al., 2017). Recent studies showed that metabolomic analysis reveals time-dependent specific responses by the microbial community in a dynamic environment (De Sousa et al., 2018; Puig-Castellví et al., 2022; Sasaki et al., 2014). Furthermore, linking gene expression levels with metabolite production through metabolomics provides key insights into microbial interactions. Also, to determine microbial interactions, it is essential to establish a link between the gene expression level and produced metabolites.

Primarily, metabolomics is applied in environmental systems to determining biodiversity by mapping metabolic activities to specific community functions (Kyrpides, 2009), metabolic cooperation to understand the synergetic relationships in the microbial community (Raes and Bork, 2008), cell-to-cell communication between microorganisms (Bassler and Losick, 2006) and identifying novel biomarkers (Krohn et al., 2022). In this context, the application of metabolomics is pivotal in comprehending the metabolic networks associated with VFA production. Metabolomics plays a crucial role in uncovering the intricate metabolic interactions within microbial communities, offering a snapshot of their metabolite profiles (Lamichhane et al., 2018; Tang, 2011). This approach enables the identification of the metabolic pathways involved in VFA synthesis, while also facilitating the discovery of key metabolites and enzymes critical to these processes.

In contrast, the application of metabolomics for the targeted identification of the VFA production network is limited due to its complexity (e.g., preparation of samples, identification and quantification of metabolites, analysis of data). However, a few studies have successfully employed metabolomics to explore both taxonomic and metabolic profiles within anaerobic digesters, primarily focusing on biogas production (Krohn et al., 2022; Puig-Castellví et al., 2022; Sasaki et al., 2014). Mainly, these studies investigated the effects of operational and environmental conditions on a metabolic network of methanogenesis in terms of the digestibility of different substrates (Beale et al., 2016; Puig-Castellví et al., 2022; Sasaki et al., 2014). These investigations highlighted the significant role of metabolomics as a valuable tool for uncovering unknown aspects, especially concerning front-end processes (such as pretreatment of substrate). However, these studies underscore the essentiality of integrating diverse omics approaches to attain a comprehensive understanding of the entire system. Which questions can be addressed by metabolomics for VFA production is presented in Fig. 1.

Another -omics approach that plays a key role in confirming active metabolic pathways and functions of microbial communities by identifying and quantifying proteins within a given system is metaproteomics (Kleiner, 2019). While metaproteomics offers valuable insights into the functions of microbial communities and their contributions to the metabolic network, a comprehensive understanding often requires its integration with other -omics approaches. The recent study by Wang et al., (Wang et al., 2022) identified key enzymes involved in amino acid metabolism for VFA production from protein-rich waste. The combination of metaproteomics and metagenomics analyses revealed fundamental mechanisms of amino acid configuration that affect bacterial behaviors via chemotaxis and quorum sensing signals (Wang et al., 2022). Their result provided new insights into how L-AA metabolism differs from that of D-AAs, as well as why protein-rich wastes accumulate more D-AAs during VFA production (Wang et al., 2022).

Industrial-scale biobased VFA production necessitates a comprehensive understanding of microbial community dynamics and metabolic networks. The utilization of a multi-omics approach provides an exceptional opportunity to elucidate the functional roles of individual microbial members and their collective activities. By addressing these fundamental questions, we can not only enhance production yield but also optimize bioaugmentation strategies to achieve tailor-made VFA production. This approach holds enormous potential, particularly for the recovery of raw materials in the form of VFA from industrial wastewater, thereby promoting the establishment of a circular economy within the industry.

While the multi-omics approach is effective in profiling microbial communities and elucidating metabolic networks as stated in Fig. 1, it alone falls short in comprehending microbial interactions and the integration of metabolic networks with microbial community data. Consequently, the construction of a model utilizing these datasets becomes imperative for understanding the entire system. Therefore, the subsequent sections of this perspective paper will be focused on how the integration of -omics and bioreactor data into diverse models can provide insights into the entire VFA production system, thereby increasing production efficiency and manipulating acid profiles.

## 3. Interpretation of the -omics data is not efficient without modeling approaches – microbial community models

Various aspects of microbial communities can be explored through different -omics approaches, as outlined in Fig. 1. However, to establish solid connections between these datasets and unveil deeper insights into interactions, behaviors, and contributions of microbial populations to the metabolic network, microbial community models are essential.

High-throughput sequencing technologies, combined with multi-omics approaches, have significantly advanced our understanding of how microorganisms facilitate the production of VFAs, revealing the intricate metabolic pathways and regulatory networks at play. However, many of these approaches merely infer relationships and provide limited mechanistic insights about microbial physiology or community functionality because they are unable to capture the cause and direction of interactions, and/or they may not be able to determine important time-dependent properties. Moreover, the combinatorial nature of experimental testing required to investigate the myriad of abiotic and biotic factors impacting microbial communities often makes multi-omics

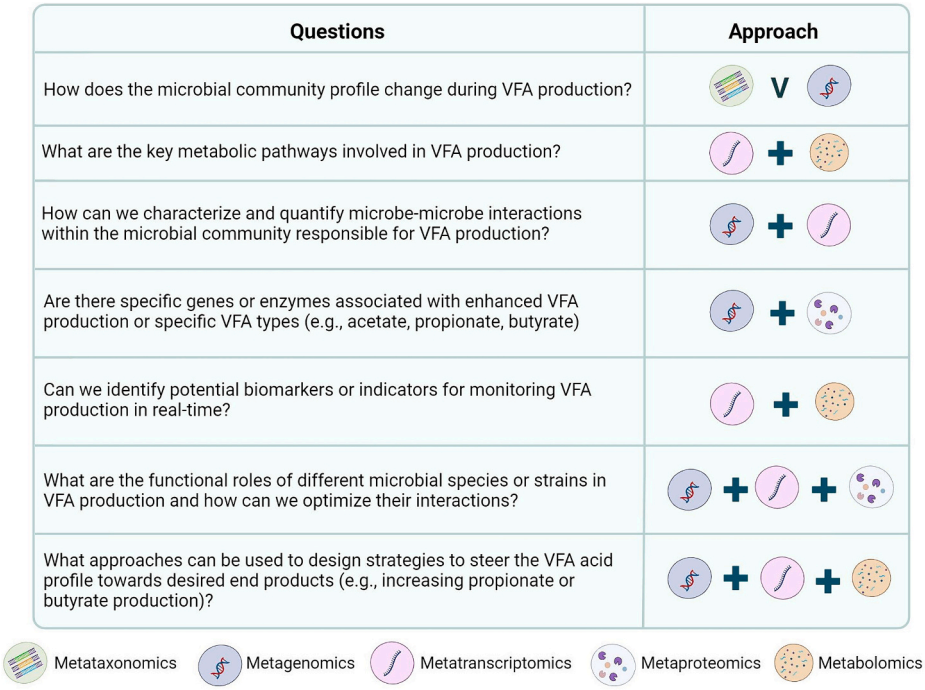


**Fig. 1.** Multi-omics Approaches in VFA Production: which questions can be addressed? (Created in BioRender.com)

strategies cost-intensive. To effectively design synthetic microbial communities or devise augmentation strategies for mixed cultures, it is crucial to understand the mechanisms underlying community behavior (Faust, 2019; Song et al., 2014).

Computational modeling of microbial communities efficiently reveals mechanistic insights into microorganisms and their impact on carboxylic acid production. For example, the role of individual members in microbial communities in the production of carboxylic acids from food waste digestion can be understood by microbial community models. Furthermore, these models can help us understand microbial interactions, predict microbial community dynamics, design microbial communities, and identify key microbial taxa and functional genes (Qian et al., 2021).

Several different types of descriptive and predictive computational models have been used to provide new insights into the functioning of microbial communities. In addition, the selection of the type of model depends on the investigation and the data available to determine essential parameters. In the intricate pursuit of understanding VFA production within microbial communities, especially in systems such as anaerobic digesters or mixed microbial communities, it is essential to discern the appropriate modeling methodology. An overview of the models along with their specifications, is summarized in Table 1.

The choice of model largely hinges on the specific objectives and the granularity of data available: **Ecological Models:** These are ideal for high-level community interactions, particularly when the primary focus is on species-level interactions that govern VFA production dynamics. Given their emphasis on broader ecological dynamics such as competition, predation, and symbiosis, they are well-suited to contexts where understanding inter-species relationships and community structures is pivotal. **Kinetic (or Process) Models:** When the data set encompasses time-series concentration values of various microbial constituents, these models are particularly apt. They are grounded in mass-action kinetics, thus making them highly effective when there is a need to model the rates of VFA formation, substrate assimilation, or microbial growth and death, especially in reactor settings. **Statistical Models:** These models shine in situations characterized by rich data sets, but perhaps a limited understanding of the underlying biological mechanics. They are predominantly data-driven, hinging on observed patterns to forecast VFA production. While they may not always capture the depth of biological mechanisms, they can swiftly identify trends and correlations in VFA production. **Metabolite-mediated models, alternatively referred to as microbe-effector models**: encompassing MacArthur consumer-resource models and trait-based models, constitute a specialized framework within microbial community modeling. These models highlight the pivotal role of specific metabolites in governing interactions and dynamics within microbial communities. **Genome-scale Metabolic Models:** For those looking to dive deep into the biochemical intricacies of microbial metabolism influencing VFA synthesis, these models are paramount. They are particularly advantageous when there is access to genome-scale data or when the objective is to predict nuanced metabolic shifts and VFA yields under varying conditions. The applications of the models outlined above, employed for VFA production or anaerobic digestion systems, are summarized in Table 2. Each example encompasses the basic equations of the model alongside its respective outputs. In summary, the optimal modeling approach is contingent upon both the specificity of the research objective and the granularity of the available data. In the domain of VFA production, striking a judicious balance between these elements can pave the way for robust and insightful analyses.

### *3.1. Generalized Lotka-Volterra (gLV) modeling and their applications for VFA production*

Ecological models represent a broad category of models that have been applied to study microbial communities using 16S rRNA-based sequence read abundance data (see recent reviews for more details (Kumar et al., 2019; Qian et al., 2021; van den Berg et al., 2022). These models are capable of predicting and analyzing population dynamics. Moreover, ecological models can assess temporal changes in the abundance of each member and can infer interactions without considering underlying molecular mechanisms (Berry and Widder, 2014). Although the boundaries among the types of ecological models can sometimes be tenuous rather than rigid, in general, model types can be broken down into four groups: generalized Lotka-Volterra (gLV) models, MacArthur consumer-resource models, trait-based models, and individual-based models. gLV models are rooted in describing predator-prey and

**Table 1**
Overview of the microbial community and kinetic models.

| | Description | Advantages | Limitations | Reference |
|---|---|---|---|---|
| Ecological Models | Predicting and analyzing population dynamics;<br>o Generalized Lotka-Voltrerra (gLV) Model<br>o Generalized Consumer – Resource Model<br>o Trait – based Model<br>o Individual – based Model | The existing time-series species abundance data can be used directly to quantify different properties of communities, including the stability of biological systems across different conditions and perturbations. | Only specific simplified aggregate interactions between organisms are feasible, often remaining static and disconnected from cellular attributes like the metabolic state of cells. | (Antoniewicz, 2020; van den Berg et al., 2022) |
| Kinetic Models | Understanding and optimizing bioreactor performance;<br>o Monod Model<br>o Andrews Model<br>o Contois Model<br>o Haldane Model<br>o Logistic Growth Model | A simplified modeling approach of the kinetic models using bioreactor data enables the prediction of growth rates, substrate utilization, and product formation under varying operational and environmental conditions. | Simplifying biological systems may not fully encapsulate their complexity, leading to estimations that may lack accuracy and precision. Additionally, adapting existing models to different systems or conditions often necessitates further adjustments. | (González-Figueredo et al., 2018) |
| Statistical Models | Analyzing bioreactor data and optimization of processes;<br>o Response Surface Methodology (RSM)<br>o Multivariate Data Analysis (MVDA)<br>o Artificial Neural Networks (ANN)<br>o Bayesian Models<br>o Time Series Analysis | For data analysis and decision-making, statistical models serve as powerful tools. They enable the inference of relationships between variables and the interpretation of results, providing valuable insights into underlying processes. | These models often depend on simplifying assumptions about the data-generating process, which can lead to misinterpretation of the results. | (Bezerra et al., 2008) |
| Genome-scale Metabolic Models | Identifying gene-protein-reaction associations in microorganisms and simulating metabolic fluxes for different system-level metabolic studies. | It offers a comprehensive understanding of cellular metabolism by mapping out the entire metabolic network of a microorganism. Additionally, they enable predictions of metabolic behaviors in diverse conditions, thereby facilitating deeper insights into cellular functions and responses. | It requires extensive and reliable data, while their computational demands can be significant. This complexity renders them challenging to interpret and analyze effectively. Moreover, the reliance on diverse assumptions and simplifications may introduce uncertainty into the predictions. | (Gu et al., 2019) |
| Metabolite Mediated Models | Focusing on the interactions and transformations of metabolites within cellular metabolism;<br>o Stoichiometric models<br>o Constraint-based models – Flux Balance Analysis (FBA)<br>o Dynamic models – Ordinary Differential Equation (ODE) | These models offer valuable insights into cellular metabolism and can predict metabolic flux distributions under varying conditions. Additionally, they serve as a useful tool for metabolic engineering, aiding in the design of microbial strains for the targeted production of specific VFAs. | It requires comprehensive and high-quality data on metabolic reactions, enzyme kinetics, and metabolites. Additionally, their applications often involve computationally intensive processes, making it challenging to interpret and analyze the resulting data. | (Ang et al., 2018; Brunner and Chia, 2019) |

competitive interactions within ecosystems (Bunin, 2017). Furthermore, gLV models use differential equations to capture the growth of populations, incorporating both cooperative and competitive interactions among species. MacArthur consumer-resource models tend to focus on the interactions between consumers and their resources emphasizing how species compete for a limited set of resources (Chesson, 1990). They are instrumental in predicting community structure and understanding the mechanisms that allow for species coexistence based on resource utilization patterns. Another approach, trait-based models, prioritizes the functional traits of organisms over their identities.

From this alternative perspective, these models can be used to predict community responses to environmental changes, focusing on how traits affect ecosystem functions (Krause et al., 2022). One more approach, individual-based models, strives to simulate ecosystems using a bottom-up approach, modeling the behavior and interactions of individual organisms. This can permit for exploring how micro-scale processes lead to emergent community-level patterns and allows for analyzing heterogeneous microbial populations (Hellweger et al., 2016).

One of the most popular types of applied ecological models is the gLV model. gLV models contain a set of non-linear, coupled first-order differential equations. This class of ecological models can describe community member growth rates as a function of time using the intrinsic growth rate as well as infer linear effects from other populations. Therefore, gLV models consist of a relatively simple set of parameters: a vector of intrinsic growth rates for each community member and a matrix interaction of coefficients for all the member pairs. gLV modeling has several benefits, including understanding the dynamics of interacting populations, predicting the effects of perturbations, and informing experimental design. However, the approach has some limitations, such as oversimplification - employing constant parameters to simulate temporally changing environments and inherent sensitivity to initial conditions – containing first-order Taylor expansion of ecological interactions near equilibrium (Letten and Stouffer, 2019).

Despite its limitations, gLV modeling has been employed in many studies to assess interactions among key species and understand dynamic growth behavior within communities (Atasoy et al., 2023; Buffie et al., 2015; Coyte et al., 2015; Marino et al., 2014; Mounier et al., 2008; Palafox-Sola et al., 2023). Originally, gLV models were applied to link species interactions to community dynamics in gut microbial communities (Fisher and Mehta, 2014; Venturelli et al., 2018). However, gLV modeling has recently been applied to model growth kinetics and community interactions to discern the impact of key taxa within anaerobic digesters (Chen et al., 2020; Schwan et al., 2020; Shaw et al., 2020). For instance, the work of Schwan and coworkers employed gLV modeling to understand how certain chemical stressors affect microbiomes within anaerobic digesters (Schwan et al., 2020). Their findings suggested that the massive resilience and stability of the methanogenic communities, coupled with the surprising flexibility of particular microbial key taxa, play a role in VFA production (Schwan et al., 2020). In general, gLV modeling can be a solid option when a coarse understanding of microbial communities is sufficient or when molecular quantitative measurements are lacking.

**Table 2**
Applications of microbial community and kinetic models in anaerobic digestion.

| | Description of the study | Equation | Notes | Outputs | Reference |
|---|---|---|---|---|---|
| Ecological Models – Generalized Lotka–Volterra (gLV) Model | Schwan et al. (2020) utilized the gLV model to explore the impact of various perturbations (nalidixic acid, γ-aminobutyric acid, and sodium phosphate) on microorganism interactions during the anaerobic digestion of glucose. | $\frac{dx_i}{t} = x_i\left(r_i + \sum_{j=1}^{n} \propto_{ij} x_j\right)$ | $\frac{dx_i}{dt}$: the rate of growth of species $x_i$, $r_i$: the intrinsic growth, $\propto_{ij}$: interaction coefficient | In this study, the outcomes derived from utilizing the Lotka–Volterra model revealed that the anaerobic digester microbiomes are both robust and flexible in their microbial interactions. Maintaining constant digester conditions could facilitate manipulation of these interactions more effectively. | (Schwan et al., 2020) |
| Kinetic Models | Regueira et al. (2021) elaborated a kinetic and stoichiometric model for VFA production from protein fermentation. | Mass balances: $\frac{dS_i}{dt} = D\cdot(S_{feed,i} - S_i) + r_i$ The protein consumption rate is described by a Monod equation: $q_s = q_{s,max} \bullet \frac{S}{K_S + S} \bullet X$ The production rate of any product (including biomass) is given by: $r_i = q_s \bullet F_i$ | $q_s$: the specific consumption rate ($g_{COD}$ Protein/ $g_{COD}$ Biomass•h), $q_{s,max}$: maximum specific consumption rate ($g_{COD}$ Protein/ $g_{COD}$ Biomass•h), S: protein concentration ($g_{COD}$ Protein/L), X: biomass concentration ($g_{COD}$ Biomass/L), $r_i$: specific production rate of the ith product ($g_{COD}$ i/$g_{COD}$ Biomass·h). $F_i$: stoichiometric factor of the ith product ($g_{COD}$ i/$g_{COD}$ Protein). In the case of biomass the stoichiometric factor is the biomass yield. | The model developed in this study, along with the simulation framework proposed, demonstrated to reproduce well VFA production from protein-rich substrate and its effectiveness in selecting optimal design parameters was shown. This led to the design of highly selective and productive processes which can be used as an stage design for converting protein-rich wastes into VFAs. | (Regueira et al., 2021) |
| Statistical Models | Nabaterega et al. (2022) used a regression model to predict VFA production from municipal sludge as well as to statistically analyze the interactions between temperature, pH and sludge retention time. | $y = \beta_0 + \sum_i X_i + \beta_{ij} X_i X_j$ | y: predicted response, $X_i$ independent variables ($X_1$:SRT; $X_2$:temperature; $X_3$:pH), $X_iX_j$: the interactions of the independent variables, $\beta_0$: the intercept, $\beta_i$ *and* $\beta_{ij}$ : regression coefficients | Their results indicated that the multiple linear regression model accurately predicted the fold increase in VFAs of acidic fermenters, achieving R-squared value of 0.9999. The contour plots constructed revealed elevated VFAs levels associated with relatively high fermenter pH (7.5–8.0), shorter SRT (2–2.2 days), and lower temperatures (45–48 °C). | (Nabaterega et al., 2022) |
| Genome-scale Models | Basile et al. (2020) built 836 genome-scale metabolic models to reveal functional capabilities of anaerobic digestion microbiome. They represented GEMs with flux balance analysis. Interactions among the dominant members and analysis of the metabolic exchanges performed using MICOM (v.0.10.0) (Diener et al., 2020). | $\mu_c = \sum_i \alpha_i \mu_i$ | $\mu_c$: the community growth rate, $\mu_i$: individual growth rates, $\alpha_i$: the relative contribution of species *i*. | The outcomes of the GEMs revealed the generation of metabolic models derived from known species identified in the environment through 16S rRNA similarity searches. However, the presence of uncultivated microbes raises unresolved questions. | (Basile et al., 2020; Diener et al., 2020) |
| Metabolite Mediated Models – Guild-Based Metabolic Models | Scarborough et al. (2020) used unicellular and guild-based metabolic models to investigate production of medium-chain fatty acids by a mixed microbial community that is fed multiple organic substrates. | ***Community Parsimonious Flux Balance Analysis (Community pFBA)*** Maximize the community-level objective function, subject to stoichiometric constraints and flux bounds: $max \sum_{j=1}^{N} w_j \bullet v_{biomass,j}$ $\sum_{j=1}^{N} S_j \bullet v_j = 0$ $v_{min,j} \le v_j \le v_{max,j}$ ***Community Flux Variability Analysis (Community FVA)*** Determine the range of possible fluxes for each reaction in each organism, maintaining | *N*: Number of organisms in the microbial community. $w_j$: Weight of organism j, reflecting its relative importance or abundance. $S_j$: Stoichiometric matrix for organism j. $v_j$: Vector of metabolic fluxes for organism j. $v_{min,j}$ and $v_{max,j}$: Lower and upper bounds on the fluxes for organism j. $v_{biomass,j}$: Flux through the | Their results from the model shed light on the metabolic pathways of three groups producing medium-chain fatty acids, unveiling potential approaches to enhance their production. | (Scarborough et al., 2020) |

(*continued on next page*)

**Table 2** (*continued*)

| Description of the study | Equation | Notes | Outputs | Reference |
|---|---|---|---|---|
| | community-level objectives: $max/min\ v_{i,j}$ $\sum_{j=1}^{N} S_j \bullet v_j = 0$ $v_{min,j} \leq v_j \leq v_{max,j}$ $\sum_{j=1}^{N} w_j \bullet v_{objective,j} \geq (1-\epsilon) \bullet v_{objective}^{opt}$ ***Community Random Flux Sampling*** Explore the feasible space of metabolic states for the community: $\sum_{j=1}^{N} S_j \bullet v_j = 0$ $v_{min,j} \leq v_j \leq v_{max,j}$ | biomass objective function for organism j. | | |

Furthermore, gLV model and other ecological models can be combined with other modeling approaches to provide more dynamic understanding of the effects of operational and environmental parameters on microbial communities and their metabolism. An integrated approach combining kinetic models with ecological models can improve ecological models' predictive accuracy by providing a mechanistic understanding of microbial metabolism within bioreactors. To our knowledge, no study has been conducted that combines ecological and kinetic models to approach specifically VFA production. However, a few studies have combined these two modeling approaches to understand microbial community interactions in anaerobic digestion under different operational conditions (Kuroda et al., 2021; Kuroda et al., 2016). In their study, Kuroda et al. (2016) conducted community and network analyses on purified terephthalic acid degradation within upflow anaerobic sludge blanket (UASB) reactors at varying temperatures. They uncovered novel networks of syntrophic metabolic interactions within different granules, attributed to distinct thermodynamic conditions. Additionally, they observed previously unseen relationships between methanogenic microorganisms at the UASB reactors (Kuroda et al., 2016). In summary, while certain ecological models may draw from elements of kinetic models, the essence of ecological modeling lies in its simplicity, enabling a direct assessment of how various parameters impact competition dynamics. Kinetic models are tailored to uncover the intricacies of processes within a reactor, which may include microbial interactions indirectly, via the utilization of shared resources like substrates. Conversely, ecological models focus on revealing the circumstances in which one microbial guild prevails over another, often tied to specific model parameters. For example, this could involve reducing the growth rate of a microbial population facing adverse conditions, such as low pH, relative to others. The primary objective is not to simulate VFA profiles within a reactor but to gain insight into the fundamental interaction dynamics among microbial populations.

### 3.2. Metabolite-mediated models and their applications for VFA production

Metabolite-mediated models, also known as microbe-effector models, which can also encompass MacArthur consumer-resource models and trait-based models, represent a specialized approach in microbial community modeling. They emphasize the mediating role of specific metabolites in dictating the interactions and dynamics within microbial communities (Qian et al., 2021). While gLV models offer a broader, ecologically-rooted perspective of microbial interactions, they often miss the detailed nuances of metabolic exchanges, making them less adept at predicting the intricacies of microbial behaviors driven by metabolites (van den Berg et al., 2022). Distinct from genome-scale metabolic (GEM) models, which aim to catalog an exhaustive representation of metabolic pathways within an individual organism, metabolite-mediated models typically encompass simpler, small-scale metabolic networks. This intentional reduction in complexity, contrasting with the more generic approach of gLV models, is designed to focus on the most pertinent metabolic interactions, prioritizing key metabolites that significantly influence microbial behaviors.

GEMs, with their comprehensive metabolic pathways, offer detailed insights into the metabolic potential and flexibility of individual organisms. They are unparalleled when predicting an organism's metabolic response to a range of environmental shifts including nutrient levels, oxygen concentrations, salinity, presence of toxins, water availability, and light conditions. For instance, a GEM modeling approach might be used to identify optimal pathways for volatile organic compound production within specific microbes such as *Clostridium kluyveri* (Benito-Vaquerizo et al., 2020; Zou et al., 2018) or *Saccharomyces cerevisiae* (Scott et al., 2023). However, when it comes to modeling interactions within a microbial community, their exhaustive nature can sometimes lead to computational challenges, making them less tractable for simulating multi-species dynamics (Bernstein et al., 2021; Fritzemeier et al., 2017). On the other hand, ODE-based microbe-effector models excel in this niche. Their targeted simplicity captures essential metabolic exchanges that drive community dynamics. For example, in a mixed culture fermentation process, these models can elucidate how the production of a specific acid by one microorganism affects the growth and metabolic activity of another. This can highlight crucial feedback loops, such as one bacterium producing lactate that is then consumed and converted into acetate by another, creating a synergistic relationship (Wang et al., 2020).

Microbe-effector models can be coupled with experimental data to simulate metabolic exchanges for a complex microbial community as has been shown for an *E. coli* community of 14 amino acid autotrophs (Liao et al., 2020) or combined with statistical modeling to design a synthetic community to steer butyrate production (Clark et al., 2021). This focused approach, while not capturing every enzymatic step as GEMs might, provides an efficient and clear representation of pivotal microbial interactions. By emphasizing these crucial exchanges, metabolite-mediated models become a powerful tool for predicting and manipulating community behaviors in complex environments. The construction of both ecological and microbe effector models follows a common workflow comprised of eight key steps as presented in Fig. 2. Ecological and microbe effector models share a common construction workflow based on defining research objectives, acquiring relevant data, and choosing an appropriate framework (Qian et al., 2021). This is followed by defining the model structure (species/strains, interactions, and environmental parameters for ecological models; key microbial effectors and targets for microbe effector models), assigning parameter values, and calibrating and validating the model. Finally, analysis and interpretation of the results yield valuable insights into microbial community in VFA production.

Metabolite-mediated models can provide insight into the dynamic interplay of select metabolites within microbial communities and present opportunities for targeted interventions. By understanding and manipulating these crucial metabolic exchanges, researchers can potentially steer microbial communities toward optimized VFA production pathways, achieving enhanced yields and more efficient bioconversion processes.

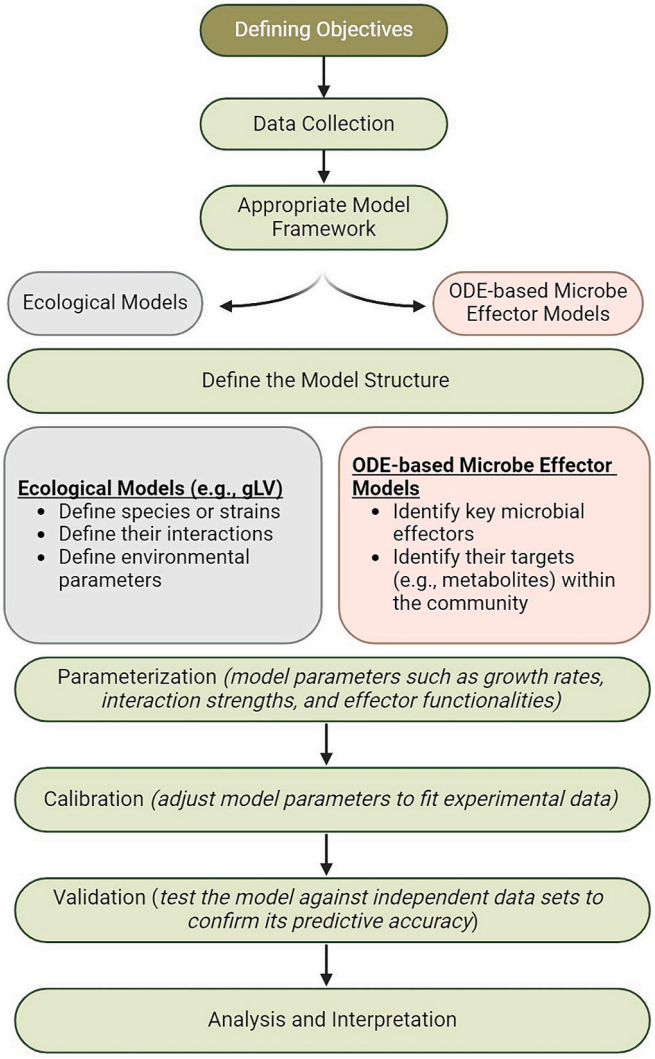


**Fig. 2.** Workflow for the construction of ecological and microbe effector models (Created in BioRender.com).

### 3.3. Genome-scale models and their applications for VFA production

While gLV and metabolite-mediate models are capable of describing the dynamics of interacting species in an ecological community, GEMs integrate various types of biological data, including genomic, transcriptomic, and metabolomic data, to create a comprehensive, genome-wide representation of the metabolic network of an organism. Modeling approaches employing GEMs of microbial communities are methods that integrate genomic data with metabolic modeling to simulate the behavior of complex microbial communities (see recent reviews for more detail, (Heinken et al., 2021; Ibrahim et al., 2021)). It involves constructing mathematical models that describe the metabolic interactions between the different microorganisms present in a community, based on their genomes. GEMs represent a particular organism's metabolic capacity and can be combined with powerful approaches such as Flux Balance Analysis (FBA) to reflect phenotypic behavior from genotypic information (Orth et al., 2010).

The first step in constructing a GEM involves assembling the genomes of the individual microbes within the community, using sequencing data (Heirendt et al., 2019). These individual genome sequences are then annotated to identify genes and metabolic pathways. Next, the metabolic networks of each microbe are integrated into a single community-level model or remain separate using a compartmentalized approach, which describes the exchange of metabolites between different microorganisms (Colarusso et al., 2021). Once the community model, either at the community or individual level, is constructed, it can be used to simulate the behavior of the microbial community under different conditions, such as changes in the availability of nutrients or changes in environmental conditions (García-Jiménez et al., 2021). These simulations can provide insights into the metabolic interactions between various microorganisms and help predict how the community will respond to changes in its environment.

Despite its many potential applications, GEMs of microbial communities also have some drawbacks that should be considered: 1) data limitations: GEMs rely on the availability and quality of genome sequences for individual microbes within the community (Heinken et al., 2021). However, not all microbial species have fully sequenced genomes, and those that do may have incomplete or fragmented sequences. This can limit the accuracy and scope of GEMs; 2) complexity: Microbial communities are complex, dynamic systems that are difficult to fully capture and model. GEMs may oversimplify the complexity of microbial interactions and may not fully account for the variability in microbial behavior under different conditions (Colarusso et al., 2021); 3) parameterization: GEMs require a large amount of experimental data to parameterize the models, such as measurements of microbial growth rates and metabolic fluxes. However, such data is often limited or challenging to obtain, particularly for complex microbial communities and computational workloads (Diener and Gibbons, 2023; Lakrisenko and Weindl, 2021); 4) constructing and simulating GEMs can be computationally intensive, requiring high-performance computing resources, and specialized software tools (Scott Jr et al., 2023). This can limit the accessibility of GEMs to researchers with limited computational resources. In essence, while genome-scale modeling of microbial communities is a promising approach, it is important to be aware of these limitations when designing and interpreting GEM studies.

GEMs can be used to identify key metabolic pathways, predict VFA production rates, optimize feedstock composition, predict VFA composition, and identify potential bottlenecks in VFA production. In a similar vein, Bauer and co-workers used genome-scale modeling of a multi-species community in the human gut to identify the key metabolic pathways involved in VFA production (Bauer et al., 2017). They identified the specific microorganisms responsible for each step in the process to understand the impact of a diet on gut health. For instance, by pinpointing optimal environmental conditions through simulations, these models can guide adjustments in an anaerobic digester to enhance or tailor VFA yields. Furthermore, GEMs can potentially inform the strategic manipulation of microbial communities, identifying key species for VFA production and suggesting ways to promote their growth or suppress competing microbes. A comprehensive approach as such, employing GEMs, would allow for combining environmental control with microbial management to create a customized system for optimizing the VFA production efficiency. Additionally, FBA employing GEMs has been used to elucidate important pairwise interactions and their associated metabolite exchanges (Basile et al., 2020). Furthermore, these simulations illustrate the positive influences of microbial dynamics, i.e., by promoting commensalism over amensalism on the rate of VFA production under different environmental conditions.

Overall, the use of GEMs of microbial communities to study VFA production can provide valuable insights into the metabolic pathways and interactions between different microorganisms involved in VFA production. Nevertheless, the microbial community models mentioned above, which offer insights into intracellular processes, should be integrated with kinetic models. This integration is crucial for a comprehensive understanding, optimization, and control of the intricate metabolic processes of VFA production.

## 4. Kinetic and statistical models for VFA production

The goal of so-called unstructured or kinetic models is to represent the whole microbial process in the bioreactor mass balances. In this

representation, the microbial metabolism is described in low detail and the cells of a given species or microbial guild are treated as a black box (Gernaey, 2015). In essence, microorganisms are seen just as compounds of the system, albeit with catalytic properties and the capacity to grow. The main aim of these models is to predict the global dynamics of the system (i.e., the rate at which the substrates are consumed or at which the different products are generated) including all the relevant processes (e.g., polymer hydrolysis, biomass settling, or gas-liquid transfer). They consist of two main parts: the definition of the process stoichiometry (often described in a Petersen matrix) and the process equation rate. Their focus is to solve the macroscopic mass balances of the system (i.e., the bioreactor). One of the most well-known kinetic models is the Anaerobic Digestion Model NO. 1 (ADM1) for the simulation of real-scale anaerobic digestion processes (Batstone et al., 2002), but models for VFA production in open culture fermentation processes of a defined substrate (e.g. glucose, fructose or proteins) or real waste (e.g. manure) are also available in literature (Alexandropoulou et al., 2018; Bai et al., 2017; Fernández et al., 2011; Infantes et al., 2012).

Kinetic models have relevant inherent advantages that have contributed to wide use and applicability. One of the most relevant qualities is their modular nature, which allows them to integrate the simulation of all the processes and measures related to bioreactor operation and which potentially affect the biological processes occurring in a bioreactor, ranging from e.g., pH determination and salt precipitation to e.g. hydraulic retention in a membrane reactor and in-situ product extraction). These phenomena can be simulated by ad hoc models and be readily integrated into the kinetic model, as they just act on the reactor mass balances. For example, the ADM1 model for anaerobic digestion was modified on different occasions to simulate particular processes of interest for a specific system. These include, e.g., Fe, P and S interactions in anaerobic digestion (Flores-Alsina et al., 2016), phosphate accumulating organism metabolism in anaerobic digestion (Wang et al., 2016), sulfate reduction (Fedorovich et al., 2003), and granular anaerobic digestion (Feldman et al., 2019). This flexibility provides a suitable way to describe the sequential conversion of a complex substrate (i.e., a real waste stream) to VFA, which may include different hydrolysis processes. Given that disintegration and hydrolysis are often critical transformation steps when dealing with complex substrates such as different organic waste streams (e.g., food waste or agrifood side-streams) these models are suitable for process engineering tasks: bioreactor design, substrate selection, definition of operating conditions. Hence, they complement the modeling approaches described in Section 3 which focus on providing an understanding of the biological transformation and are mostly used to describe the fermentation of simple and soluble monomers (e.g., glucose or individual amino acids).

Another modeling approach that uses bioreactor data is statistical modeling, which plays an important role in understanding the relationships between product formation and changing environmental and operational conditions. Many studies employed statistical models to identify correlations between operational parameters and maximum VFA production yield and/or specific VFA formation (Atasoy et al., 2019; Khatami et al., 2021; Yin et al., 2022). Some studies have even correlated specific acid formation with the abundance of specific microbial taxa to estimate the functionalities of these groups (Atasoy et al., 2020a, 2020b; Owusu-Agyeman et al., 2023; Yin et al., 2022). For instance, Owusu-Agyeman et al. (2023) showed that the total VFA production positively correlated with the relative abundance of *Lachnospiraceae* and *Atopobiaceae* (Pearson coefficients of 0.620 and 0.666, $p > 0.01$), whereas the production of caproic acid was positively correlated with the family *Atopobiaceae* (Pearson coefficient of 0.865, $p > 0.01$) (Owusu-Agyeman et al., 2023). Similarly, Yin et al. (2022) correlated the acetogenic performance with the core microbial community to predict functional pathways. Their study claimed that acetogenesis might be less dependent on the diversity of the microbial community since all alpha diversity indexes were negatively correlated with acetogenic performance (Yin et al., 2022), but obviously a negative association also indicates a possible (inverse) effect of microbial community complexity on performance. While statistical models can provide insights into system performance by identifying relationships between parameters and microbial groups, they are strictly data-driven models. This means that they are limited in capturing the complexity of biological systems.

## 5. Integrated modeling approach and future outlook

The possible application of microbial and kinetic models for VFA production has been discussed in the previous sections. Here we want to endeavor the integration of these two approaches to have a finer description of microbial metabolism and improved predictability of process dynamics for VFA production. We believe that an integrated approach would provide great progress to the production of VFA if it could i) combine different methods to represent the current knowledge brought about by models, and ii) transform the available data, including the -omics, into information that can be used to engineer the process at metabolic, cell and bioreactor level.

Microbial community models primarily emphasize VFA production at the microbial community level. The integration of microbial modeling with multi-omics data, complemented by genome-scale metabolic modeling, has emerged as a critical advancement in the biotechnology field. Such integration offers unparalleled insights into microbial interactions and their underlying functionalities, setting the stage for enhanced predictive capabilities. With the incorporation of machine learning (ML) techniques, these models can expect improvements in terms of accuracy, efficiency, and scalability. It is noteworthy to mention the importance of coupling GEMs with process simulation software, such as ASPEN (Shoabjareh et al., 2023). However, realizing the full potential of this coupling necessitates the integration of classical reactor kinetic modeling approaches. Recent studies have validated this integrative approach, showcasing its ability to accurately simulate production capabilities (Gomez et al., 2021). Nevertheless, the convergence of multi-omics data, genome-scale metabolic modeling, and ML often lacks information regarding broader aspects, such as reactor operation, effects of design, and process parameters.

Kinetic models are mainly system-specific (i.e., they have a very limited extrapolation capacity), therefore they provide low detail in metabolism description. For each set of operational conditions (i.e., substrate type, temperature, pH) experimental data should be fed into the model to have representative parameters. In the context of VFA production, the most relevant bottleneck is the inability to predict the dependency of fermentation stoichiometry on different operational parameters, among which the effect of pH is paramount (Arslan et al., 2016), or the effect of the stoichiometry of interaction among bacterial guilds of the mixed culture. While this is not an issue in anaerobic digestion, since the methane to carbon dioxide ratio in biogas can be easily predicted by substrate composition (i.e. carbon and COD concentration), it considerably limits the scope of the applicability of kinetic models for VFA production. Another limitation of kinetic models lies in predicting and even steering the acid profile. While kinetic models provide valuable insights into overall VFA production in anaerobic digestion, they often overlook the distribution of individual VFAs, from acetate (C1) to pentanoate (C5). Because the focus is on general pathways instead of the complexities of individual chains, the complexities of chain formation are overlooked. In this case, metabolic models with their more detailed description of the metabolism are better suited to address this issue and describe complex phenomena such as interactions among microbial groups. Such metabolic models have already proved that they can predict VFA production stoichiometry with respect to changes in substrate and reactor conditions while requiring minimal system information (Regueira et al., 2020b; Regueira et al., 2020a; Scarborough et al., 2020). Our proposal seeks to combine kinetic models with metabolic models, which are much more capable of predicting the

process stoichiometry due to their finer description of microbial metabolism. We aim to merge the modular nature of kinetic models and their ability to be adapted to a myriad of processes with the prediction power of the metabolic model.

Such integrated modeling approach can help us anticipate various crucial operational parameters, including but not limited to medium composition, inoculation ratio, and the retention time for specific products. In this way, the production of specific acids or total VFA can be optimized. Fig. 3 provides a visual representation of the integrated modeling approach for VFA production, showcasing the incorporation of inputs and anticipated outcomes. Given the inherent characteristics of individual modeling methods, their integration becomes imperative to attain full comprehension of VFA production.

### 5.1. *Integrated modeling approach for complex substrates*

One of the most practical challenges in modeling VFA production is the degradation of complex substrates. As mentioned in Section 3, "*Kinetic Models for VFA Production*," kinetic models have been used to describe the sequential conversion of complex substrates, including different hydrolysis processes. However, modeling the degradation of complex substrates (e.g., organic waste streams) requires a complementary approach to understand the entire transformation pathway of each fraction of the complex substrate.

One example of such an integrated approach is shown in the work of Saavedra del Oso et al. (2022), where a modeling framework was developed with the perspective of being used for the early-stage design of processes converting different complex real substrates into VFA. The framework consisted of two modules: i) a kinetic model to simulate the sequential bioconversion steps and to solve the reactor-level balances and ii) an ODE-type model to tackle the varying fermentation stoichiometry. The kinetic model has a library with information for kinetic parameters (e.g., hydrolysis rate constants, fractioning in terms of components such as sugars, proteins, etc.) for an array of common wastes with the potential to be used for VFA production. Bioenergetic models are a kind of metabolic modeling that can predict the effect of pH on the VFA fermentation stoichiometry. pH is the most relevant operational parameter in fermentation systems, on glucose, protein (i.e., any possible combination of amino acids), and their co-fermentation (González-Cabaleiro et al., 2015; Regueira et al., 2020a, 2020b). The bioenergetic model is solved dynamically and provides the kinetic model with the stoichiometry of the process at the given environmental conditions (i.e., pH and fractions of the hydrolyzed substrate to be acidified). In this way, the integral model is able to predict both the effects of changes in operational conditions on the kinetics (something most metabolic models are not able to) and on the VFA stoichiometry (something most kinetic models are not able) and stands as a sound early-stage design tool. In the case studies presented in that work, one of the conclusions is that hydrolysis is the process with a higher control over the VFA production yield and, to a lesser extent, on the process

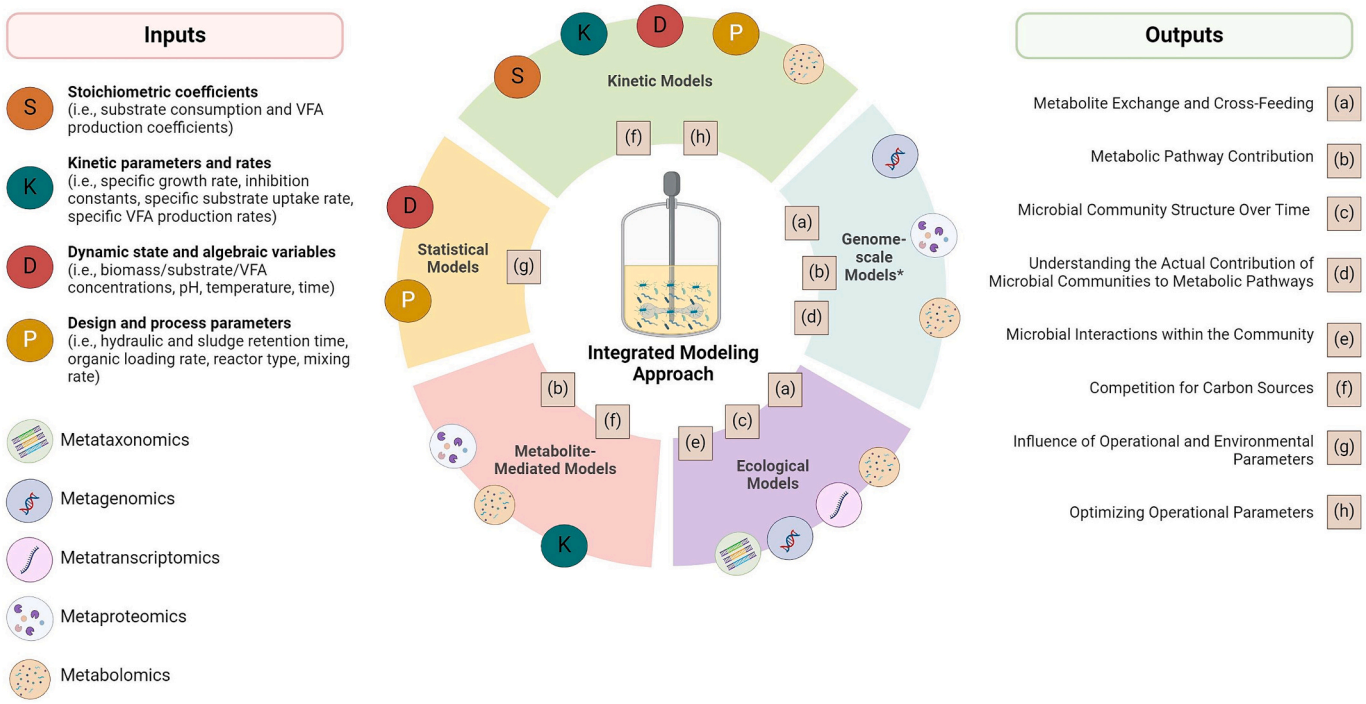


**Fig. 3.** Integrated modeling approach of VFA production including inputs, data sources, and outputs. The inputs for the models are described as stoichiometric coefficients, kinetic parameters and rates, dynamic state and algebraic variables, design and process parameters, and data from DNA-based approaches (metagenomics/metataxonomics), metatranscriptomics, metaproteomics, and metabolomics. The expected outputs from different models are (a) metabolite exchange and cross-feeding to exploring the intricate dynamics of metabolite exchange and cross-feeding within a microbial community at a genome scale, (b) metabolic pathway contribution for assessing how metabolic pathways are influenced and shaped through metabolite-mediated interactions within the microbial community, (c) microbial community structure over time for tracking the evolving ecological landscape of the microbial community structure as it changes over time, (d) understanding the actual contribution of microbial communities to metabolic pathways for determining the precise impact of microbial communities on the functionality of metabolic pathways, (e) microbial interactions within the community for investigating the various interactions and relationships among different microbial species within the community, (f) competition for carbon sources for examining the kinetics and metabolite-mediated competition for carbon sources among microbial populations, (g) influence of operational and environmental parameters for analyzing the effects of static operational and environmental factors on the microbial community, (h) optimizing operational parameters for enhancing the performance of the microbial community by optimizing kinetic operational parameters. *Here we define genome-scale models to be a comprehensive representation of the metabolic process of the organism. Furthermore, this model contains a detailed stoichiometric map of all known metabolic reactions and associated genes in the organism's genome. This figure does not allude to the application of genome-scale models to various modeling approaches. (Created in BioRender.com).

stoichiometry, reinforcing the need to describe all bioconversion steps. With this modeling framework, different substrate combinations can be tested in silico to select the best one when targeting a specific VFA(s) or the operational conditions can be selected to maximize the yield of the desired VFA composition. One advantage of this approach lies in its modularity as other models can be integrated to simulate system variations (e.g., coupling to an in-situ product removal system). Also, it can represent a module used to generate in silico data for an economic of environmental evaluation.

Dynamic Flux Balance Analysis (dFBA) emerges as another promising approach for modeling the conversion of complex substrates to VFAs. This method extends classical flux balance analysis by incorporating a dynamic framework, enabling the simulation of degradation pathways for different substrate fractions (carbohydrates, lipids, proteins) (Willemsen et al., 2015) and their conversion into VFAs. This approach holds the potential to predict VFA production profiles and degradation pathways under various conditions. However, these approaches require detailed information about individual substrate fractions, which can be challenging and resource-intensive to obtain in complex substrate matrices. Here, the integration of ML and artificial intelligence (AI) can potentially model complex organic matter directly without requiring complete fractionation, overcoming a significant bottleneck in current modeling practices.

Another significant challenge in VFA production lies in the degradation of specific substrates. For example, the degradation of amino acids leads to the production of ammonium-nitrogen in anaerobic digesters (Deng et al., 2023; Park and Kim, 2016). This not only inhibits VFA production but also complicates VFA purification and separation processes. However, with the integrated modeling approach, it becomes possible to predict the metabolic pathways of each amino acid degradation and the functional contributions of the microbial community under various operational conditions. Consequently, strategies can be devised to prevent the degradation of amino acids by inhibiting specific pathways and/or optimizing operational conditions.

### 5.2. Integrated modeling approach for inoculum type

In our previous discussion on microbial dynamics in the production of VFAs under Section 2 "*Comprehending Microbial Dynamics in the Production of VFA*", it was highlighted that open mixed cultures stand out as a preferred inoculum source for VFA production from waste streams. This approach offers numerous advantages over pure culture fermentation, including reduced sensitivity to operational and environmental conditions, higher overall VFA production yields, and the elimination of the need for substrate sterilization when utilizing waste streams. Conversely, pure culture fermentation enables the production of specific acid types and simplifies the separation and purification steps (Atasoy et al., 2020a, 2020b).

Moreover, as previously noted, modeling open mixed cultures for VFA production necessitates extensive datasets. One potential solution to address these challenges is the design of synthetic communities (Diender et al., 2021). Employing synthetic communities, or defined co-cultures, for VFA production offers the opportunity to customize acid composition, enhance substrate utilization, and improve system stability and robustness. However, this approach demands a deep understanding of the involved strains to predict their interactions, manage growth balance, and control environmental factors for various cultures. Modeling plays a crucial role in comprehending these dynamics, aiding in identifying the requirements of the cultures and elucidating their interactions (Boruta, 2023).

While constructing and applying microbial community models is more straightforward for synthetic communities compared to open mixed cultures, challenges arise when scaling up VFA production from waste streams at an industrial level. Therefore, we propose an integrated modeling approach to understand microbial communities in the bioreactor for VFA production using open mixed cultures, combined with microbial and kinetic models. Subsequently, bioaugmentation of the system with designed synthetic communities can be implemented to enhance targeted acid composition (Atasoy and Cetecioglu, 2021; Gough and Nielsen, 2016).

### 5.3. Machine learning and artificial intelligence

ML and AI offer transformative potential for enhancing and augmenting traditional modeling approaches in systems biology, including gLV, microbe-effector, and, notably, GEM modeling. The capacity of ML and AI to handle complex, high-dimensional data and uncover hidden patterns makes them invaluable tools in refining these model approaches for greater accuracy and insight.ML can augment gLV and microbe-effector models by providing methods to estimate interactions or effector functions, respectively. In gLV modeling, ML can refine interaction coefficients based on empirical data, thereby enhancing the model's ecological validity (Michel-Mata et al., 2022; Wang et al., 2024). For microbe-effector modeling, ML can predict the impacts of specific metabolites or proteins on the microbiome, informing targeted experiments or engineering strategies (Sudhakar et al., 2021).

ML and AI significantly amplify the capabilities of GEM modeling, bridging mechanistic modeling with data-driven algorithms to streamline the reconstruction process, boost predictive accuracy, and improve interpretability (Kim et al., 2021). Through automated annotation and functional prediction, ML algorithms efficiently process genomic data to predict gene functions and metabolic pathways significantly reducing manual reconstruction efforts. For instance, recent gap-filling algorithms have incorporated ML to increase the accuracy and applicability of GEM modeling to study lesser-known organisms (Chen et al., 2023). By leveraging multi-omics data integration, ML refines and validates metabolic network connections, correcting improper annotations, and filling missing links to create more accurate and comprehensive models (Zampieri et al., 2019).

The contribution of ML to GEM modeling significantly enhances their predictive power through parameter optimization and adaptive learning from experimental data. This optimization process aligns model parameters, such as reaction flux rates, more closely with experimental observations, thereby enhancing the model's ability to accurately simulate metabolic behaviors under various conditions. For example, Faure et al. (2023) demonstrated how hybrid neural-mechanistic models could improve phenotype predictions of microorganisms by integrating ML with constraint-based metabolic models, significantly outperforming traditional models with smaller training set sizes (Faure et al., 2023). By applying ML algorithms that adaptively learn, genome-scale models can continuously refine their accuracy, making precise predictions about metabolic responses to genetic or environmental changes. Furthermore, ML extends the predictive reach of GEM modeling into lesser-explored parts of metabolism by identifying patterns in known processes, thereby predicting reactions and pathways not yet fully understood. Zhang et al. (2020b) highlighted the effectiveness of combining mechanistic and ML models for predictive engineering and optimization of tryptophan metabolism in yeast, showcasing ML's ability to guide metabolic engineering efforts toward significant improvements in production (Zhang et al., 2020b). These advancements underscore ML's pivotal role in enhancing the predictive capabilities of GEM modeling, offering a promising avenue for exploring and understanding complex metabolic systems.

Improving the interpretability of GEMs is crucial for advancing our understanding of complex biological systems. ML techniques, such as feature selection and importance, play a pivotal role in identifying the most critical reactions and pathways within GEMs. This approach not only makes the intricate network of interactions more interpretable but also guides targeted experimental designs and hypothesis generation. For instance, Culley et al. (2020) demonstrated a machine-learning approach that integrates gene expression profiles with mechanistic metabolic models to characterize cell growth in *Saccharomyces*

*cerevisiae*, revealing unknown interactions between biological domains and enhancing model interpretability (Culley et al., 2020). Similarly, model simplification, achieved by focusing on significant metabolic pathways and reactions, can render GEMs more accessible without sacrificing accuracy. This simplification is facilitated by ML's ability to uncover complex relationships between genotype and phenotype, as illustrated by Askland et al. (2021), who developed an ML analytic pipeline that translates genotypic data into biologically contextualized features, thereby enhancing the interpretability of complex genetic architectures (Askland et al., 2021). Furthermore, the linkage of genotypic and phenotypic data through ML models elucidates how genetic variations influence metabolic capabilities and outcomes, providing a clearer view of how genetic information translates into metabolic functions.

### 5.4. Techno-economic aspects of an integrated modeling approach

As discussed in Section 1: *Introduction*, the production of biobased VFAs is influenced by various operational and environmental factors. Furthermore, the use of open mixed cultures as an inoculum source, comprising numerous unknown microbial groups, complicates the assessment of system performance. Consequently, prior to system operation, it is common practice to conduct batch mode tests to assess the impacts of different parameters. Despite preliminary screening, achieving reproducible results with alternative inocula or substrate sources remains a considerable challenge. Hence, adopting an integrated modeling approach offers a viable solution to minimize the number of experiments conducted, thereby significantly reducing the consumption of both single-used plastic products and chemicals (Leak et al., 2023) as well as a decreased requirement for labor. Furthermore, microbial community models offer a cost-effective way to describe and engineer microbial communities (García-Jiménez et al., 2021).

Moreover, economic parameters can be incorporated into the integrated model to provide insights into the economic feasibility of proposed systems, particularly when considering scaling up for industrial production of VFAs. For instance, BioSTEAM is a techno-economic analysis tool used to forecast the inputs and outputs of an entire system (Cortes-Peña et al., 2020), providing investment and cost analysis based on the results derived from the integrated modeling approach.

## 6. Conclusions

Volatile fatty acids stand as a remarkably promising category of biobased chemicals, holding substantial potential for resource recovery from waste streams. Despite their potential, their replacement of synthetic derivatives is currently limited by challenges in the production and recovery processes. These challenges predominantly hinge upon the need for a deeper understanding of microbial communities and bioreactor performance. In this paper, we have presented an integrated approach that combines kinetic models with microbial community models to provide a comprehensive perspective that enhances both production efficiency and the manipulation of acid profiles. Through the manipulation of acid profiles, we can ease separation and purification processes through the design of specific recovery processes tailored to each acid type based on its chemical structure. By bridging the gap between the interpretation of microbial communities with bioreactor performance together, this integrated modeling framework is expected to advance the utilization of VFAs, making significant contributions toward sustainable resource recovery and the adoption of biobased chemicals.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgement

The authors would like to sincerely thank Robbert Kleerebezem for his insightful comments and constructive feedback on the manuscript. The authors acknowledge the Dutch Research Council for its financial contribution to the UNLOCK Project (NWO:184.035.007 and NRGWI.obrug.2018.005).

## References

Aghapour Aktij, S., Zirehpour, A., Mollahosseini, A., Taherzadeh, M.J., Tiraferri, A., Rahimpour, A., 2020. Feasibility of membrane processes for the recovery and purification of bio-based volatile fatty acids: a comprehensive review. J. Ind. Eng. Chem. 81, 24–40. https://doi.org/10.1016/j.jiec.2019.09.009.

Agnihotri, S., Yin, D.M., Mahboubi, A., Sapmaz, T., Varjani, S., Qiao, W., Koseoglu-Imer, D.Y., Taherzadeh, M.J., 2022. A glimpse of the world of volatile fatty acids production and application: a review. Bioengineered. https://doi.org/10.1080/21655979.2021.1996044.

Aguiar-Pulido, V., Huang, W., Suarez-Ulloa, V., Cickovski, T., Mathee, K., Narasimhan, G., 2016. Metagenomics, metatranscriptomics, and metabolomics approaches for microbiome analysis. Evol. Bioinform. https://doi.org/10.4137/EBO.S36436.

Alexandropoulou, M., Antonopoulou, G., Lyberatos, G., 2018. A novel approach of modeling continuous dark hydrogen fermentation. Bioresour. Technol. 250, 784–792. https://doi.org/10.1016/j.biortech.2017.12.005.

Ang, K.S., Lakshmanan, M., Lee, N.-R., Lee, D.-Y., 2018. Metabolic modeling of microbial community interactions for health, environmental and biotechnological applications. Curr. Genomics 19 (8), 712–722. https://doi.org/10.2174/1389202919666180911144055.

Antoniewicz, M.R., 2020. A guide to deciphering microbial interactions and metabolic fluxes in microbiome communities. In: Current Opinion in Biotechnology, 64. Elsevier Ltd., pp. 230–237. https://doi.org/10.1016/j.copbio.2020.07.001

Arslan, D., Steinbusch, K.J.J., Diels, L., Hamelers, H.V.M., Strik, D.P.B.T.B., Buisman, C.J. N., De Wever, H., 2016. Selective short-chain carboxylates production: a review of control mechanisms to direct mixed culture fermentations. Crit. Rev. Environ. Sci. Technol. 46, 592–634. https://doi.org/10.1080/10643389.2016.1145959.

Askland, K.D., Strong, D., Wright, M.N., Moore, J.H., 2021. The translational machine: a novel machine-learning approach to illuminate complex genetic architectures. Genet. Epidemiol. 45 (5), 485–536.

Atasoy, M., Cetecioglu, Z., 2021. Bioaugmentation as a strategy for tailor-made volatile fatty acid production. J. Environ. Manag. 295, 113093 https://doi.org/10.1016/j.jenvman.2021.113093.

Atasoy, M., Cetecioglu, Z., 2022. The effects of pH on the production of volatile fatty acids and microbial dynamics in long-term reactor operation. J. Environ. Manag. 319 https://doi.org/10.1016/j.jenvman.2022.115700.

Atasoy, M., Eyice, O., Schnürer, A., Cetecioglu, Z., 2019. Volatile fatty acids production via mixed culture fermentation: revealing the link between pH, inoculum type and bacterial composition. Bioresour. Technol. 292 https://doi.org/10.1016/j.biortech.2019.121889.

Atasoy, M., Eyice, Ö., Cetecioglu, Z., 2020a. Volatile fatty acid production from semi-synthetic milk processing wastewater under alkali pH: the pearls and pitfalls of microbial culture. Bioresour. Technol. 297 https://doi.org/10.1016/j.biortech.2019.122415.

Atasoy, Merve, Eyice, O., Cetecioglu, Z., 2020b. A comprehensive study of volatile fatty acids production from batch reactor to anaerobic sequencing batch reactor by using cheese processing wastewater. Bioresour. Technol. 311, 123529 https://doi.org/10.1016/j.biortech.2020.123529.

Atasoy, M., Scott Jr., W.T., van Gijn, K., Koehorst, J.J., Smidt, H., Langenhoff, A.A.M., 2023. Microbial dynamics and bioreactor performance are interlinked with organic matter removal from wastewater treatment plant effluent. Bioresour. Technol. 372, 128659.

Bai, J., Liu, H., Yin, B., Ma, H., Chen, X., 2017. Modified ADM1 for modeling free ammonia inhibition in anaerobic acidogenic fermentation with high-solid sludge. J. Environ. Sci. 52, 58–65. https://doi.org/10.1016/j.jes.2016.03.004.

Basile, A., Campanaro, S., Kovalovszki, A., Zampieri, G., Rossi, A., Angelidaki, I., Valle, G., Treu, L., 2020. Revealing metabolic mechanisms of interaction in the anaerobic digestion microbiome by flux balance analysis. Metab. Eng. 62, 138–149. https://doi.org/10.1016/j.ymben.2020.08.013.

Bassler, B.L., Losick, R., 2006. Bacterially speaking. Cell 125, 237–246. https://doi.org/10.1016/j.cell.2006.04.001.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin, V.A., 2002. The IWA anaerobic digestion model No 1 (ADM1). Water Sci. Technol. 45, 65–73.

Bauer, E., Zimmermann, J., Baldini, F., Thiele, I., Kaleta, C., 2017. BacArena: individual-based metabolic modeling of heterogeneous microbes in complex communities. PLoS Comput. Biol. 13 (5), e1005544.

Beale, D.J., Karpe, A.V., McLeod, J.D., Gondalia, S.V., Muster, T.H., Othman, M.Z., Palombo, E.A., Joshi, D., 2016. An "omics" approach towards the characterisation of laboratory scale anaerobic digesters treating municipal sewage sludge. Water Res. 88, 346–357. https://doi.org/10.1016/j.watres.2015.10.029.

Benito-Vaquerizo, S., Diender, M., Olm, I.P., Dos Santos, V.A.M., Schaap, P.J., Sousa, D. Z., Suarez-Diez, M., 2020. Modeling a co-culture of *Clostridium autoethanogenum* and

*Clostridium kluyveri* to increase syngas conversion to medium-chain fatty-acids. Comput. Struct. Biotechnol. J. 18, 3255–3266.
Bernstein, D.B., Sulheim, S., Almaas, E., Segrè, D., 2021. Addressing uncertainty in genome-scale metabolic model reconstruction and analysis. Genome Biol. 22, 1–22.
Berry, D., Widder, S., 2014. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. Front. Microbiol. 5.
Bevilacqua, R., Regueira, A., Mauricio-Iglesias, M., Lema, J.M., Carballa, M., 2021. Steering the conversion of protein residues to volatile fatty acids by adjusting pH. Bioresour. Technol. 320, 124315 https://doi.org/10.1016/j.biortech.2020.124315.
Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. In: Talanta, 76(5). Elsevier, pp. 965–977. https://doi.org/10.1016/j.talanta.2008.05.019.
Blasco, L., Kahala, M., Tampio, E., Vainio, M., Ervasti, S., Rasi, S., 2020. Effect of inoculum pretreatment on the composition of microbial communities in anaerobic digesters producing volatile fatty acids. Microorganisms 8, 1–21. https://doi.org/10.3390/microorganisms8040581.
Boruta, T., 2023. Computation-aided studies related to the induction of specialized metabolite biosynthesis in microbial co-cultures: an introductory overview. Comput. Struct. Biotechnol. J. https://doi.org/10.1016/j.csbj.2023.08.011.
Brunner, J.D., Chia, N., 2019. Metabolite-mediated modelling of microbial community dynamics captures emergent behaviour more effectively than species–species modelling. J. R. Soc. Interface 16 (159). https://doi.org/10.1098/rsif.2019.0423.
Buffie, C.G., Bucci, V., Stein, R.R., McKenney, P.T., Ling, L., Gobourne, A., No, D., Liu, H., Kinnebrew, M., Viale, A., Littmann, E., van den Brink, M.R.M., Jenq, R.R., Taur, Y., Sander, C., Cross, J.R., Toussaint, N.C., Xavier, J.B., Pamer, E.G., 2015. Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. Nature 517 (7533), 205–208.
Bunin, G., 2017. Ecological communities with Lotka-Volterra dynamics. Phys. Rev. E 95 (4), 042414.
Calero, R.R., Lagoa-Costa, B., del Fernandez-Feal, M.M.C., Kennes, C., Veiga, M.C., 2018. Volatile fatty acids production from cheese whey: influence of pH, solid retention time and organic loading rate. J. Chem. Technol. Biotechnol. 93, 1742–1747.
Chen, W.-Y., Wu, J.-H., Chu, S.-C., 2020. Deciphering microbiomes in anaerobic reactors with superior trichloroethylene dechlorination performance at low pH conditions. Environ. Pollut. 257, 113567.
Chen, C., Liao, C., Liu, Y.-Y., 2023. Teasing out missing reactions in genome-scale metabolic networks through hypergraph learning. Nat. Commun. 14 (1), 2375.
Chesson, P., 1990. MacArthur's consumer-resource model. Theor. Popul. Biol. 37 (1), 26–38.
Clark, R.L., Connors, B.M., Stevenson, D.M., Hromada, S.E., Hamilton, J.J., Amador-Noguez, D., Venturelli, O.S., 2021. Design of synthetic human gut microbiome assembly and butyrate production. Nat. Commun. 12 (1), 3254.
Colarusso, A.V., Goodchild-Michelman, I., Rayle, M., Zomorrodi, A.R., 2021. Computational modeling of metabolism in microbial communities on a genome-scale. Curr. Opin. Syst. Biol. 26, 46–57. https://doi.org/10.1016/j.coisb.2021.04.001.
Cortes-Peña, Y., Kumar, D., Singh, V., Guest, J.S., 2020. BioSTEAM: a fast and flexible platform for the design, simulation, and techno-economic analysis of biorefineries under uncertainty. ACS Sustain. Chem. Eng. 8, 3302–3310. https://doi.org/10.1021/acssuschemeng.9b07040.
Coyte, K.Z., Schluter, J., Foster, K.R., 2015. The ecology of the microbiome: networks, competition, and stability. Science 350 (6261), 663–666.
Culley, C., Vijayakumar, S., Zampieri, G., Angione, C., 2020. A mechanism-aware and multiomic machine-learning pipeline characterizes yeast cell growth. Proc. Natl. Acad. Sci. 117 (31), 18869–18879.
De Sousa, C.S., Hassan, S.S., Pinto, A.C., Silva, W.M., De Almeida, S.S., De Castro Soares, S., Azevedo, M.S.P., Rocha, C.S., Barh, D., Azevedo, V., 2018. Microbial omics: applications in biotechnology. In: Omics Technologies and Bio-Engineering: Volume 2: Towards Improving Quality of Life, pp. 3–20. https://doi.org/10.1016/B978-0-12-815870-8.00001-2.
Deng, Z., Ferreira, A.L.M., Spanjers, H., van Lier, J.B., 2023. Anaerobic protein degradation: effects of protein structural complexity, protein concentrations, carbohydrates, and volatile fatty acids. Bioresour. Technol. Rep. 22 https://doi.org/10.1016/j.biteb.2023.101501.
Diender, M., Parera Olm, I., Sousa, D.Z., 2021. Synthetic co-cultures: novel avenues for bio-based processes. Curr. Opin. Biotechnol. https://doi.org/10.1016/j.copbio.2021.01.006.
Diener, C., Gibbons, S.M., 2023. More is different: metabolic modeling of diverse microbial communities. mSystems 8. https://doi.org/10.1128/msystems.01270-22.
Diener, C., Gibbons, S.M., Resendis-Antonio, O., 2020. MICOM: metagenome-scale modeling to infer metabolic interactions in the gut microbiota. MSystems 5 (1). https://doi.org/10.1128/msystems.00606-19.
Elefsiniotis, P., Wareham, D.G., 2007. Utilization patterns of volatile fatty acids in the denitrification reaction. Enzym. Microb. Technol. 41, 92–97. https://doi.org/10.1016/j.enzmictec.2006.12.006.
Fang, H.H.P., Liu, H., 2002. Effect of pH on hydrogen production from glucose by a mixed culture. Bioresour. Technol. 82, 87–93. https://doi.org/10.1016/s0960-8524(01)00110-9.
Faure, L., Mollet, B., Liebermeister, W., Faulon, J.-L., 2023. A neural-mechanistic hybrid approach improving the predictive power of genome-scale metabolic models. Nat. Commun. 14 (1), 4669.
Faust, K., 2019. Microbial consortium design benefits from metabolic modeling. Trends Biotechnol. 37 (2), 123–125.
Fedorovich, V., Lens, P., Kalyuzhnyi, S., 2003. Extension of anaerobic digestion model no. 1 with processes of sulfate reduction. Appl. Biochem. Biotechnol. 109, 33–45. https://doi.org/10.1385/ABAB:109:1-3:33.
Feldman, H., Flores-Alsina, X., Ramin, P., Kjellberg, K., Jeppsson, U., Batstone, D.J., Gernaey, K.V., 2019. Assessing the effects of intra-granule precipitation in a full-scale industrial anaerobic digester. Water Sci. Technol. 79, 1327–1337. https://doi.org/10.2166/wst.2019.129.
Feng, L., Chen, Y., Zheng, X., 2009. Enhancement of waste activated sludge protein conversion and volatile fatty acids accumulation during waste activated sludge anaerobic fermentation by carbohydrate substrate addition: the effect of pH. Environ. Sci. Technol. https://doi.org/10.1021/es8037142.
Fernández, F.J., Villaseñor, J., Infantes, D., 2011. Kinetic and stoichiometric modelling of acidogenic fermentation of glucose and fructose. Biomass Bioenergy 35, 3877–3883. https://doi.org/10.1016/j.biombioe.2011.06.052.
Fisher, C.K., Mehta, P., 2014. Identifying keystone species in the human gut microbiome from metagenomic timeseries using sparse linear regression. PLoS ONE 9 (7), e102451.
Flores-Alsina, X., Solon, K., Kazadi Mbamba, C., Tait, S., Gernaey, K.V., Jeppsson, U., Batstone, D.J., 2016. Modelling phosphorus (P), sulfur (S) and iron (Fe) interactions for dynamic simulations of anaerobic digestion processes. Water Res. 95, 370–382. https://doi.org/10.1016/j.watres.2016.03.012.
Fontana, A., Campanaro, S., Treu, L., Kougias, P.G., Cappa, F., Morelli, L., Angelidaki, I., 2018. Performance and genome-centric metagenomics of thermophilic single and two-stage anaerobic digesters treating cheese wastes. Water Res. 134, 181–191. https://doi.org/10.1016/j.watres.2018.02.001.
Fritzemeier, C.J., Hartleb, D., Szappanos, B., Papp, B., Lercher, M.J., 2017. Erroneous energy-generating cycles in published genome scale metabolic networks: identification and removal. PLoS Comput. Biol. 13 (4), e1005494.
Garcia-Aguirre, J., Aymerich, E., de González-Mtnez Goñi, J., Esteban-Gutiérrez, M., 2017. Selective VFA production potential from organic waste streams: assessing temperature and pH influence. Bioresour. Technol. 244, 1081–1088. https://doi.org/10.1016/j.biortech.2017.07.187.
García-Jiménez, B., Torres-Bacete, J., Nogales, J., 2021. Metabolic modelling approaches for describing and engineering microbial communities. Comput. Struct. Biotechnol. J. 19, 226–246. https://doi.org/10.1016/j.csbj.2020.12.003.
Gernaey, K.V., 2015. A perspective on PSE in fermentation process development and operation. In: Gernaey, K.V., Huusom, J.K., Gani, R. (Eds.), Computer Aided Chemical Engineering. Elsevier, pp. 123–130. https://doi.org/10.1016/B978-0-444-63578-5.50016-5.
Gomez, J.A., Höffner, K., Barton, P.I., 2021. Production of biofuels from sunlight and lignocellulosic sugars using microbial consortia. Chem. Eng. Sci. 239, 116615.
González-Cabaleiro, R., Lema, J.M., Rodríguez, J., 2015. Metabolic energy-based modelling explains product yielding in anaerobic mixed culture fermentations. PLoS ONE 10, e0126739.
González-Figueredo, C., Flores-Estrella, A., Rojas-Rejón, O.A., 2018. Fermentation: metabolism, kinetic models, and bioprocessing. In: Naofumi, Shiomi (Ed.), Current Topics in Biochemical Engineering, , 1st edn1. IntechOpen. https://doi.org/10.5772/intechopen.82195.
Gough, H.L., Nielsen, J.L., 2016. Bioaugmentation. In: McGenity, T.J., Timmis, K.N., Nogales, B. (Eds.), Hydrocarbon and Lipid Microbiology Protocols Pollution Mitigation and Waste Treatment Applications. Springer, Berlin, Heidelberg. https://doi.org/10.1007/8623_2016_205.
Greses, S., De Bernardini, N., Treu, L., Campanaro, S., González-Fernández, C., 2023. Genome-centric metagenomics revealed the effect of pH on the microbiome involved in short-chain fatty acids and ethanol production. Bioresour. Technol., 128920 https://doi.org/10.1016/j.biortech.2023.128920.
Gu, C., Kim, G.B., Kim, W.J., Kim, H.U., Lee, S.Y., 2019. Current status and applications of genome-scale metabolic models. In: Genome Biology, 20(1). BioMed Central Ltd. https://doi.org/10.1186/s13059-019-1730-3.
Hao, L., Michaelsen, T.Y., Singleton, C.M., Dottorini, G., Kirkegaard, R.H., Albertsen, M., Nielsen, P.H., Dueholm, M.S., 2020. Novel syntrophic bacteria in full-scale anaerobic digesters revealed by genome-centric metatranscriptomics. ISME J. 14, 906–918. https://doi.org/10.1038/s41396-019-0571-0.
Heinken, A., Basile, A., Thiele, I., 2021. Advances in constraint-based modelling of microbial communities. Curr. Opin. Syst. Biol. 27, 100346.
Heirendt, L., Arreckx, S., Pfau, T., Mendoza, S.N., Richelle, A., Heinken, A., Haraldsdóttir, H.S., Wachowiak, J., Keating, S.M., Vlasov, V., Magnusdóttir, S., Ng, C.Y., Preciat, G., Žagare, A., Chan, S.H.J., Aurich, M.K., Clancy, C.M., Modamio, J., Sauls, J.T., Noronha, A., Bordbar, A., Cousins, B., El Assal, D.C., Valcarcel, L.V., Apaolaza, I., Ghaderi, S., Ahookhosh, M., Ben Guebila, M., Kostromins, A., Sompairac, N., Le, H.M., Ma, D., Sun, Y., Wang, L., Yurkovich, J.T., Oliveira, M.A.P., Vuong, P.T., El Assal, L.P., Kuperstein, I., Zinovyev, A., Hinton, H.S., Bryant, W.A., Aragón Artacho, F.J., Planes, F.J., Stalidzans, E., Maass, A., Vempala, S., Hucka, M., Saunders, M.A., Maranas, C.D., Lewis, N.E., Sauter, T., Palsson, B.Ø., Thiele, I., Fleming, R.M.T., 2019. Creation and analysis of biochemical constraint-based models using the COBRA toolbox v.3.0. Nat. Protoc. 14 (3), 639–702.
Hellweger, F.L., Clegg, R.J., Clark, J.R., Plugge, C.M., Kreft, J.-U., 2016. Advancing microbial sciences by individual-based modelling. Nat. Rev. Microbiol. 14 (7), 461–471.
Ibrahim, M., Raajaraam, L., Raman, K., 2021. Modelling microbial communities: harnessing consortia for biotechnological applications. Comput. Struct. Biotechnol. J. 19, 3892–3907.
Infantes, D., González del Campo, A., Villaseñor, J., Fernández, F.J., 2012. Kinetic model and study of the influence of pH, temperature and undissociated acids on acidogenic

fermentation. Biochem. Eng. J. 66, 66–72. https://doi.org/10.1016/j.bej.2012.04.017.
Jang, Y.-S., Im, J.A., Choi, S.Y., Lee, J.I., Lee, S.Y., 2014. Metabolic engineering of *Clostridium acetobutylicum* for butyric acid production with high butyric acid selectivity. Metab. Eng. 23, 165–174. https://doi.org/10.1016/J.YMBEN.2014.03.004.
Jankowska, E., Chwiałkowska, J., Stodolny, M., Oleskowicz-Popiel, P., 2015. Effect of pH and retention time on volatile fatty acids production during mixed culture fermentation. Bioresour. Technol. 190, 274–280. https://doi.org/10.1016/j.biortech.2015.04.096.
Jankowska, E., Chwialkowska, J., Stodolny, M., Oleskowicz-Popiel, P., 2017. Volatile fatty acids production during mixed culture fermentation – The impact of substrate complexity and pH. Chem. Eng. J. 326, 901–910. https://doi.org/10.1016/j.cej.2017.06.021.
Jiang, L., Cui, H., Zhu, L., Hu, Y., Xu, X., Li, S., Huang, H., 2015. Enhanced propionic acid production from whey lactose with immobilized *Propionibacterium acidipropionici* and the role of trehalose synthesis in acid tolerance. Green Chem. 17, 250–259. https://doi.org/10.1039/C4GC01256A.
Khatami, K., Atasoy, M., Ludtke, M., Baresel, C., Eyice, Ö., Cetecioglu, Z., 2021. Bioconversion of food waste to volatile fatty acids: impact of microbial community, pH and retention time. Chemosphere 275. https://doi.org/10.1016/j.chemosphere.2021.129981.
Kim, Y., Kim, G.B., Lee, S.Y., 2021. Machine learning applications in genome-scale metabolic modeling. Curr. Opin. Syst. Biol. 25, 42–49.
Kim, N.K., Lee, S.H., Kim, Y., Park, H.D., 2022. Current understanding and perspectives in anaerobic digestion based on genome-resolved metagenomic approaches. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2021.126350.
Kleiner, M., 2019. Metaproteomics: much more than measuring gene expression in microbial communities. mSystems 4. https://doi.org/10.1128/msystems.00115-19.
Kleinsteuber, S., 2019. Metagenomics of methanogenic communities in anaerobic digesters. In: Stams, Alfons J.M., Sousa, D.Z. (Eds.), Biogenesis of Hydrocarbons. Springer International Publishing, Cham, pp. 337–359. https://doi.org/10.1007/978-3-319-78108-2_16.
Krause, S.M., Bertilsson, S., Grossart, H.-P., Bodelier, P.L., van Bodegom, P.M., Lennon, J.T., Philippot, L., Le Roux, X., 2022. Microbial trait-based approaches for agroecosystems. Adv. Agron. 175, 259–299.
Krohn, C., Khudur, L., Dias, D.A., van den Akker, B., Rees, C.A., Crosbie, N.D., Surapaneni, A., O'Carroll, D.M., Stuetz, R.M., Batstone, D.J., Ball, A.S., 2022. The role of microbial ecology in improving the performance of anaerobic digestion of sewage sludge. Front. Microbiol. https://doi.org/10.3389/fmicb.2022.1079136.
Kumar, M., Ji, B., Zengler, K., Nielsen, J., 2019. Modelling approaches for studying the microbiome. Nat. Microbiol. 4 (8), 1253–1267.
Kuroda, K., Nobu, M.K., Mei, R., Narihiro, T., Bocher, B.T.W., Yamaguchi, T., Liu, W.T., 2016. A single-granule-level approach reveals ecological heterogeneity in an upflow anaerobic sludge blanket reactor. PLoS ONE 11. https://doi.org/10.1371/journal.pone.0167788.
Kuroda, K., Narihiro, T., Nobu, M.K., Tobo, A., Yamauchi, M., Yamada, M., 2021. Ecogenomics reveals microbial metabolic networks in a psychrophilic methanogenic bioreactor treating soy sauce production wastewater. Microbes Environ. 36 https://doi.org/10.1264/jsme2.ME21045.
Kyrpides, N.C., 2009. Fifteen years of microbial genomics: meeting the challenges and fulfilling the dream. Nat. Biotechnol. 27, 627–632. https://doi.org/10.1038/nbt.1552.
Lakrisenko, P., Weindl, D., 2021. Dynamic models for metabolomics data integration. Curr. Opin. Syst. Biol. https://doi.org/10.1016/j.coisb.2021.100358.
Lamichhane, S., Sen, P., Dickens, A.M., Hyötyläinen, T., Orešič, M., 2018. An overview of metabolomics data analysis: current tools and future perspectives. Compr. Anal. Chem. 82, 387–413. https://doi.org/10.1016/bs.coac.2018.07.001.
Leak, L.B., Tamborski, J., Commissaris, A., Brophy, J.A.N., 2023. Forging a path toward a more sustainable laboratory. Trends Biochem. Sci. https://doi.org/10.1016/j.tibs.2022.09.001.
Letten, A.D., Stouffer, D.B., 2019. The mechanistic basis for higher-order interactions and non-additivity in competitive communities. Ecol. Lett. 22 (3), 423–436.
Li, L., He, Q., Ma, Y., Wang, X., Peng, X., 2015. Dynamics of microbial community in a mesophilic anaerobic digester treating food waste: relationship between community structure and process stability. Bioresour. Technol. 189, 113–120. https://doi.org/10.1016/j.biortech.2015.04.015.
Liao, C., Wang, T., Maslov, S., Xavier, J.B., 2020. Modeling microbial cross-feeding at intermediate scale portrays community dynamics and species coexistence. PLoS Comput. Biol. 16 (8), e1008135.
Liu, Hongbo, Li, Y., Fu, B., Guo, H., Zhang, J., Liu, He, 2020. Recovery of volatile fatty acids from sewage sludge through anaerobic fermentation. In: Current Developments in Biotechnology and Bioengineering: Resource Recovery from Wastes, pp. 151–175. https://doi.org/10.1016/B978-0-444-64321-6.00008-2.
Lukitawesa, Patinvoh, R.J., Millati, R., Sárvári-Horváth, I., Taherzadeh, M.J., 2020. Factors influencing volatile fatty acids production from food wastes via anaerobic digestion. Bioengineered 11, 39–52. https://doi.org/10.1080/21655979.2019.1703544.
Luo, J., Wang, F., Cheng, X., Huang, W., Zhang, Q., Fang, F., Cao, J., Wu, Y., 2021. Metatranscriptomic insights of the metabolic process enhancement during food wastes fermentation driven by linear alkylbenzene sulphonates. J. Clean. Prod. 315, 128145 https://doi.org/10.1016/j.jclepro.2021.128145.
Lv, N., Cai, G., Pan, X., Li, Y., Wang, R., Li, J., Li, C., Zhu, G., 2022. pH and hydraulic retention time regulation for anaerobic fermentation: focus on volatile fatty acids production/distribution, microbial community succession and interactive correlation. Bioresour. Technol. 347 https://doi.org/10.1016/j.biortech.2021.126310.
Ma, H., Liu, He, Zhang, L., Yang, M., Fu, B., Liu, Hongbo, 2017. Novel insight into the relationship between organic substrate composition and volatile fatty acids distribution in acidogenic co-fermentation. Biotechnol. Biofuels 10, 1–15. https://doi.org/10.1186/s13068-017-0821-1.
Marino, S., Baxter, N.T., Huffnagle, G.B., Petrosino, J.F., Schloss, P.D., 2014. Mathematical modeling of primary succession of murine intestinal microbiota. Proc. Natl. Acad. Sci. 111 (1), 439–444.
Martinez, G.A., Rebecchi, S., Decorti, D., Domingos, J.M.B., Natolino, A., Del Rio, D., Bertin, L., Da Porto, C., Fava, F., 2016. Towards multi-purpose biorefinery platforms for the valorisation of red grape pomace: production of polyphenols, volatile fatty acids, polyhydroxyalkanoates and biogas. Green Chem. 18, 261–270. https://doi.org/10.1039/C5GC01558H.
McInerney, M.J., Sieber, J.R., Gunsalus, R.P., 2009. Syntrophy in anaerobic global carbon cycles. Curr. Opin. Biotechnol. https://doi.org/10.1016/j.copbio.2009.10.001.
Michel-Mata, S., Wang, X.W., Liu, Y.Y., Angulo, M.T., 2022. Predicting microbiome compositions from species assemblages through deep learning. Imeta 1 (1), e3.
Millati, R., Wikandari, R., Ariyanto, T., Hasniah, N., Taherzadeh, M.J., 2023. Anaerobic digestion biorefinery for circular bioeconomy development. Bioresour. Technol. Rep. https://doi.org/10.1016/j.biteb.2022.101315.
Mounier, J., Monnet, C., Vallaeys, T., Arditi, R., Sarthou, A.-S., Hélias, A., Irlinger, F., 2008. Microbial interactions within a cheese microbial community. Appl. Environ. Microbiol. 74 (1), 172–181.
Nabaterega, R., Basar, I.A., Eskicioglu, C., 2022. Optimizing volatile fatty acid production from municipal sludge: linking microbial activity with reactor performance. J. Environ. Chem. Eng. 10 (3), 107827 https://doi.org/10.1016/j.jece.2022.107827.
Orth, J.D., Thiele, I., Palsson, B.O., 2010. What is flux balance analysis? Nat. Biotechnol. https://doi.org/10.1038/nbt.1614.
Owusu-Agyeman, I., Plaza, E., Cetecioglu, Z., 2022. Long-term alkaline volatile fatty acids production from waste streams: impact of pH and dominance of dysgonomonadaceae. Bioresour. Technol. 346 https://doi.org/10.1016/j.biortech.2021.126621.
Owusu-Agyeman, I., Bedaso, B., Laumeyer, C., Pan, C., Malovanyy, A., Baresel, C., Plaza, E., Cetecioglu, Z., 2023. Volatile fatty acids production from municipal waste streams and use as a carbon source for denitrification: the journey towards full-scale application and revealing key microbial players. Renew. Sust. Energ. Rev. 175 https://doi.org/10.1016/j.rser.2023.113163.
Palafox-Sola, M.F., Yebra-Montes, C., Orozco-Nunnelly, D.A., Carrillo-Nieves, D., González-López, M.E., Gradilla-Hernández, M.S., 2023. Modeling growth kinetics and community interactions in microalgal cultures for bioremediation of anaerobically digested swine wastewater. Algal Res. 70, 102981.
Parchami, M., Uwineza, C., Ibeabuchi, O.H., Rustas, B.-O., Taherzadeh, M.J., Mahboubi, A., 2023. Membrane bioreactor assisted volatile fatty acids production from agro-industrial residues for ruminant feed application. Waste Manag. 170, 62–74. https://doi.org/10.1016/j.wasman.2023.07.032.
Park, S., Kim, M., 2016. Effect of ammonia on anaerobic degradation of amino acids. KSCE J. Civ. Eng. 20, 129–136. https://doi.org/10.1007/s12205-015-0240-4.
Pérez-Cobas, A.E., Gomez-Valero, L., Buchrieser, C., 2020. Metagenomic approaches in microbial ecology: an update on whole-genome and marker gene sequencing analyses. Microb. Genom. 6, 1–22. https://doi.org/10.1099/mgen.0.000409.
Perrotta, A.R., Kumaraswamy, R., Bastidas-Oyanedel, J.R., Alm, E.J., Rodríguez, J., 2017. Inoculum composition determines microbial community and function in an anaerobic sequential batch reactor. PLoS ONE 12, 1–14. https://doi.org/10.1371/journal.pone.0171369.
Pinu, F.R., Villas-Boas, S.G., Aggio, R., 2017. Analysis of intracellular metabolites from microorganisms: quenching and extraction protocols. Metabolites 7. https://doi.org/10.3390/metabo7040053.
Płaza, G., Jałowiecki, Ł., Głowacka, D., Hubeny, J., Harnisz, M., Korzeniewska, E., 2021. Insights into the microbial diversity and structure in a full-scale municipal wastewater treatment plant with particular regard to archaea. PLoS ONE 16. https://doi.org/10.1371/journal.pone.0250514.
Puig-Castellví, F., Midoux, C., Guenne, A., Conteau, D., Franchi, O., Bureau, C., Madigou, C., Jouan-Rimbaud Bouveresse, D., Kroff, P., Mazéas, L., Rutledge, D.N., Gaval, G., Chapleur, O., 2022. Metataxonomics, metagenomics and metabolomics analysis of the influence of temperature modification in full-scale anaerobic digesters. Bioresour. Technol. 346 https://doi.org/10.1016/j.biortech.2021.126612.
Qian, Y., Lan, F., Venturelli, O.S., 2021. Towards a deeper understanding of microbial communities: integrating experimental data with dynamic models. Curr. Opin. Microbiol. 62, 84–92. https://doi.org/10.1016/j.mib.2021.05.003.
Raes, J., Bork, P., 2008. Molecular eco-systems biology: towards an understanding of community function. Nat. Rev. Microbiol. 6, 693–699. https://doi.org/10.1038/nrmicro1935.
Ramos-Suarez, M., Zhang, Y., Outram, V., 2021. Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste. In: Reviews in Environmental Science and Biotechnology. Springer Netherlands. https://doi.org/10.1007/s11157-021-09566-0.
Regueira, A., Bevilacqua, R., Lema, J.M., Carballa, M., Mauricio-Iglesias, M., 2020a. A metabolic model for targeted volatile fatty acids production by cofermentation of carbohydrates and proteins. Bioresour. Technol. 298 https://doi.org/10.1016/j.biortech.2019.122535.
Regueira, A., Lema, J.M., Carballa, M., Mauricio-Iglesias, M., 2020b. Metabolic modeling for predicting VFA production from protein-rich substrates by mixed-culture fermentation. Biotechnol. Bioeng. 117, 73–84. https://doi.org/10.1002/bit.27177.

Regueira, A., Bevilacqua, R., Mauricio-Iglesias, M., Carballa, M., Lema, J.M., 2021. Kinetic and stoichiometric model for the computer-aided design of protein fermentation into volatile fatty acids. Chem. Eng. J. 406 https://doi.org/10.1016/j.cej.2020.126835.

Reyhanitash, E., Kersten, S.R.A., Schuur, B., 2017. Recovery of volatile fatty acids from fermented wastewater by adsorption. ACS Sustain. Chem. Eng. 5, 9176–9184. https://doi.org/10.1021/acssuschemeng.7b02095.

Rocha, M.A.A., Raeissi, S., Hage, P., Weggemans, W.M.A., van Spronsen, J., Peters, C.J., Kroon, M.C., 2017. Recovery of volatile fatty acids from water using medium-chain fatty acids and a cosolvent. Chem. Eng. Sci. 165, 74–80. https://doi.org/10.1016/j.ces.2017.02.014.

Saavedra del Oso, M., Regueira, A., Hospido, A., Mauricio-Iglesias, M., 2022. Fostering the valorization of organic wastes into carboxylates by a computer-aided design tool. Waste Manag. 142, 101–110. https://doi.org/10.1016/j.wasman.2022.02.008.

Sasaki, D., Sasaki, K., Tsuge, Y., Morita, M., Kondo, A., 2014. Comparison of metabolomic profiles of microbial communities between stable and deteriorated methanogenic processes. Bioresour. Technol. 172, 83–90. https://doi.org/10.1016/j.biortech.2014.08.054.

Scarborough, M.J., Lawson, C.E., Hamilton, J.J., Donohue, T.J., Noguera, D.R., 2018. Metatranscriptomic and thermodynamic insights into medium-chain fatty acid production using an anaerobic microbiome.

Scarborough, M.J., Hamilton, J.J., Erb, E.A., Donohue, T.J., Noguera, D.R., 2020. Diagnosing and predicting mixed-culture fermentations with unicellular and guild-based metabolic models. MSystems 5 (5). https://doi.org/10.1128/msystems.00755-20.

Schmidt, S., 2021. Applications of mixed microbial cultures in industrial biotechnology. In: The Autotrophic Biorefinery: Raw Materials from Biotechnology. De Gruyter, pp. 353–384. https://doi.org/10.1515/9783110550603-013.

Schwan, B., Abendroth, C., Latorre-Pérez, A., Porcar, M., Vilanova, C., Dornack, C., 2020. Chemically stressed bacterial communities in anaerobic digesters exhibit resilience and ecological flexibility. Front. Microbiol. 11 https://doi.org/10.3389/fmicb.2020.00867.

Scott Jr., W.T., Benito-Vaquerizo, S., Zimmermann, J., Bajić, D., Heinken, A., Suarez-Diez, M., Schaap, P.J., 2023. A structured evaluation of genome-scale constraint-based modeling tools for microbial consortia. PLoS Comput. Biol. 19 (8), e1011363.

Scott, W.T., Henriques, D., Smid, E.J., Notebaart, R.A., Balsa-Canto, E., 2023. Dynamic genome-scale modeling of Saccharomyces cerevisiae unravels mechanisms for ester formation during alcoholic fermentation. Biotechnol. Bioeng. 120, 1998–2012. https://doi.org/10.1002/bit.28421.

Shakya, M., Lo, C.C., Chain, P.S.G., 2019. Advances and challenges in metatranscriptomic analysis. Front. Genet. https://doi.org/10.3389/fgene.2019.00904.

Shaw, G.T.-W., Liu, A.-C., Weng, C.-Y., Chen, Y.-C., Chen, C.-Y., Weng, F.C.-H., Wang, D., Chou, C.-Y., 2020. A network-based approach to deciphering a dynamic microbiome's response to a subtle perturbation. Sci. Rep. 10 (1), 19530.

She, Y., Hong, J., Zhang, Q., Chen, B.Y., Wei, W., Xin, X., 2020. Revealing microbial mechanism associated with volatile fatty acids production in anaerobic acidogenesis of waste activated sludge enhanced by freezing/thawing pretreatment. Bioresour. Technol. 302 https://doi.org/10.1016/j.biortech.2020.122869.

Shen, D., Yin, J., Yu, X., Wang, M., Long, Y., Shentu, J., Chen, T., 2017. Acidogenic fermentation characteristics of different types of protein-rich substrates in food waste to produce volatile fatty acids. Bioresour. Technol. 227, 125–132. https://doi.org/10.1016/j.biortech.2016.12.048.

Shoabjareh, A.S., Farhadi, F., Pishvaie, M.R., 2023. SimDFBA: a framework for bioprocess simulation and development. Comput. Chem. Eng. 169, 108073.

Sidhu, C., Vikram, S., Pinnaka, A.K., 2017. Unraveling the microbial interactions and metabolic potentials in pre- and post-treated sludge from a wastewater treatment plant using metagenomic studies. Front. Microbiol. 8 https://doi.org/10.3389/fmicb.2017.01382.

Song, H.-S., Cannon, W.R., Beliaev, A.S., Konopka, A., 2014. Mathematical modeling of microbial community dynamics: a methodological review. Processes 2 (4), 711–752.

Stams, A.J.M., Plugge, C.M., 2009. Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nat. Rev. Microbiol. https://doi.org/10.1038/nrmicro2166.

Strazzera, G., Battista, F., Tonanzi, B., Rossetti, S., Bolzonella, D., 2021. Optimization of short chain volatile fatty acids production from household food waste for biorefinery applications. Environ. Technol. Innov. 23, 101562 https://doi.org/10.1016/j.eti.2021.101562.

Sudhakar, P., Machiels, K., Verstockt, B., Korcsmaros, T., Vermeire, S., 2021. Computational biology and machine learning approaches to understand mechanistic microbiome-host interactions. Front. Microbiol. 12, 618856.

Tang, J., 2011. Microbial metabolomics. Curr. Genomics 391–403. https://doi.org/10.2174/138920211797248619.

van Aarle, I.M., Perimenis, A., Lima-Ramos, J., de Hults, E., George, I.F., Gerin, P.A., 2015. Mixed inoculum origin and lignocellulosic substrate type both influence the production of volatile fatty acids during acidogenic fermentation. Biochem. Eng. J. 103, 242–249. https://doi.org/10.1016/j.bej.2015.07.016.

van den Berg, N.I., Machado, D., Santos, S., Rocha, I., Chacón, J., Harcombe, W., Mitri, S., Patil, K.R., 2022. Ecological modelling approaches for predicting emergent properties in microbial communities. Nat. Ecol. Evol. 6 (7), 855–865. Nature research. https://doi.org/10.1038/s41559-022-01746-7.

Vanwonterghem, I., Jensen, P.D., Rabaey, K., Tyson, G.W., 2015. Temperature and solids retention time control microbial population dynamics and volatile fatty acid production in replicated anaerobic digesters. Sci. Rep. 5 https://doi.org/10.1038/srep08496.

Venturelli, O.S., Carr, A.V., Fisher, G., Hsu, R.H., Lau, R., Bowen, B.P., Hromada, S., Northen, T., Arkin, A.P., 2018. Deciphering microbial interactions in synthetic human gut microbiome communities. Mol. Syst. Biol. 14 (6), e8157.

Wang, R., Li, Y., Chen, W., Zou, J., Chen, Y., 2016. Phosphate release involving PAOs activity during anaerobic fermentation of EBPR sludge and the extension of ADM1. Chem. Eng. J. 287, 436–447. https://doi.org/10.1016/j.cej.2015.10.110.

Wang, S.P., Rubio, L.A., Duncan, S.H., Donachie, G.E., Holtrop, G., Lo, G., Farquharson, F.M., Wagner, J., Parkhill, J., Louis, P., Walker, A.W., Flint, H.J., 2020. Pivotal roles for pH, lactate, and lactate-utilizing bacteria in the stability of a human colonic microbial ecosystem. mSystems 5 (5). https://doi.org/10.1128/msystems.00645-00620.

Wang, M., Zhang, X., Huang, H., Qin, Z., Liu, C., Chen, Y., 2022. Amino acid configuration affects volatile fatty acid production during proteinaceous waste valorization: chemotaxis, quorum sensing, and metabolism. Environ. Sci. Technol. 56, 8702–8711. https://doi.org/10.1021/acs.est.1c07894.

Wang, S., Li, D., Zhang, K., Ma, Y., Liu, F., Li, Z., Gao, X., Gao, W., Du, L., 2023. Effects of initial volatile fatty acid concentrations on process characteristics, microbial communities, and metabolic pathways on solid-state anaerobic digestion. Bioresour. Technol. 369 https://doi.org/10.1016/j.biortech.2022.128461.

Wang, X.-W., Sun, Z., Jia, H., Michel-Mata, S., Angulo, M.T., Dai, L., He, X., Weiss, S.T., Liu, Y.-Y., 2024. Identifying keystone species in microbial communities using deep learning. Nat. Ecol. Evol. 8 (1), 22–31.

Wenzel, J., Fiset, E., Batlle-Vilanova, P., Cabezas, A., Etchebehere, C., Balaguer, M.D., Colprim, J., Puig, S., 2018. Microbial community pathways for the production of volatile fatty acids from CO2 and electricity. Front. Energy Res. 6 https://doi.org/10.3389/fenrg.2018.00015.

Willemsen, A.M., Hendrickx, D.M., Hoefsloot, H.C.J., Hendriks, M.M.W.B., Wahl, S.A., Teusink, B., Smilde, A.K., Van Kampen, A.H.C., 2015. MetDFBA: incorporating time-resolved metabolomics measurements into dynamic flux balance analysis. Mol. BioSyst. 11, 137–145. https://doi.org/10.1039/c4mb00510d.

Yin, Q., Wu, G., Lens, P.N.L., 2022. Characterization of the core microbial community governing acidogenic processes for the production of valuable bioproducts. NPJ Clean Water 5. https://doi.org/10.1038/s41545-022-00180-3.

Zacharof, M.-P., Lovitt, R.W., 2014. Recovery of volatile fatty acids (VFA) from complex waste effluents using membranes. Water Sci. Technol. 69, 495–503. https://doi.org/10.2166/wst.2013.717.

Zampieri, G., Vijayakumar, S., Yaneske, E., Angione, C., 2019. Machine and deep learning meet genome-scale metabolic modeling. PLoS Comput. Biol. 15 (7), e1007084.

Zhang, L., Loh, K.C., Dai, Y., Tong, Y.W., 2020a. Acidogenic fermentation of food waste for production of volatile fatty acids: bacterial community analysis and semi-continuous operation. Waste Manag. 109, 75–84. https://doi.org/10.1016/j.wasman.2020.04.052.

Zhang, J., Petersen, S.D., Radivojevic, T., Ramirez, A., Pérez-Manríquez, A., Abeliuk, E., Sánchez, B.J., Costello, Z., Chen, Y., Fero, M.J., Martin, H.G., Nielsen, J., Keasling, J.D., Jensen, M.K., 2020b. Combining mechanistic and machine learning models for predictive engineering and optimization of tryptophan metabolism. Nat. Commun. 11 (1), 4880.

Zhang, L., Chen, F.X., Zeng, Z., Xu, M., Sun, F., Yang, L., Bi, X., Lin, Y., Gao, Y.J., Hao, H.X., Yi, W., Li, M., Xie, Y., 2021. Advances in metagenomics and its application in environmental microorganisms. Front. Microbiol. https://doi.org/10.3389/fmicb.2021.766364.

Zhang, Z., Lin, Y., Wu, S., Li, X., Cheng, J.J., Yang, C., 2023. Effect of composition of volatile fatty acids on yield of polyhydroxyalkanoates and mechanisms of bioconversion from activated sludge. Bioresour. Technol. 385, 129445 https://doi.org/10.1016/j.biortech.2023.129445.

Zou, W., Ye, G., Zhang, J., Zhao, C., Zhao, X., Zhang, K., 2018. Genome-scale metabolic reconstruction and analysis for *Clostridium kluyveri*. Genome 61 (8), 605–613.