Extracellular Polymeric Substances from Aerobic Granular Sludge: Implications for Agricultural Use in Foliar Fertilization

CIE5060-09 MSc Thesis

Presented by:
Sofía Velásquez Posada (5491835)
Supervised by:
Philipp Wilfert (Postdoc)

Assessment Committee:
Prof. Dr. Ir. Merle de Kreuk
Dr. Yuemei Lin
Prof. Dr. Ir. Jules van Lier

Faculty of Civil Engineering
Department of Sanitary Engineering

TU Delft

Acknowledgements

This thesis marks the end of my two-year journey as a master's student at TU Delft. Throughout this period, I have faced numerous challenges that have tested my skills and pushed me to my limits every day. Yet, I can confidently say that it has been one of the most transformative experiences of my life. I have undergone tremendous personal growth and gained invaluable knowledge along the way. Now, reflecting on this journey so far, I am truly amazed at finding myself in a beautiful country, surrounded by remarkable people, and uncovering my true passions. It is a feeling of immense satisfaction and a sense of belonging that makes it feel like home.

First and foremost, I would like to express my heartfelt gratitude to my daily supervisor, Philipp. I am immensely grateful for his support throughout this entire endeavor. As a mentor, he has been exceptional, providing guidance not only in academic matters but also on a personal level. He consistently stood by me, offering assistance with any challenges I faced and reassuring me during moments of anxiety. With his constant presence, I always felt supported and accompanied in this academic pursuit. I would also like to extend my gratitude to my other supervisors: Yuemei, Merle, and Jules. Yuemei deserves special thanks for offering me such an intriguing topic and for welcoming me to the Applied Sciences building. Moreover, Merle and Jules have provided me with invaluable feedback and fresh perspectives that have greatly enhanced my thesis. In addition, I would like to thank the PhD candidates Lemin, Javier, Stefan, and Anand, as well as the lab technicians Zita and Ben from Applied Sciences, and Judith and Armand from the WaterLab. Their invaluable assistance in conducting the laboratory tests and their willingness to address my inquiries have been immensely valuable. I also want to acknowledge and thank Harald Mikkelsen and Stephen Picken for their generous support and guidance.

Additionally, I want to wholeheartedly thank those who have turned Delft into my second home: Nikoletta, Max, Marianna, Mostafa, Carolina, Akhilesh, Mike, Alessandro, Nick, and Amalia. Your unwavering support, shared laughter, cherished memories, and constant presence during both the highs and lows mean the world to me. I would also like to extend my appreciation to the other master's students, from both Civil Engineering and Applied Sciences, for their companionship. Thank you for contributing to an unforgettable experience!

I also want to dedicate this closing paragraph to my beloved family: my mother, my sister, and Lucas. Their constant uplifting presence, encouraging words, and unconditional love and support have been invaluable to me. They have played an integral role in shaping the person I am today and continue to help me grow as a better individual. Lastly, but certainly not least, I want to express my deepest gratitude to my father. Although he is no longer with us, I am forever grateful that he motivated me to study abroad in order to pursue my dreams. He always believed in me, and without him, none of this would have been possible. He remains my eternal role model.

Sofía Velásquez Posada

Delft, July 2023

Abstract

This study explores the potential use of extracellular polymeric substances (EPS), commercially known as Kaumera Nereda Gum®, extracted from aerobic granular sludge (AGS), for foliar fertilization in agriculture. Koppert, a company specializing in sustainable agricultural products, is interested in using Kaumera Nereda Gum® for its biostimulating effects, water-absorbing capacity, biofilm formation, and adhesive properties. They are currently spraying leaves with a solution mixed with Kaumera Nereda Gum®, water, and other fertilizers.

Nonetheless, the utility of using Kaumera Nereda Gum® for foliar fertilization can be affected by several factors, such as seawater intrusion in AGS projects like Faro-Olhão in Portugal and the type of acid utilized for the precipitation of the polymer. In certain places like Utrecht, hydrochloric acid is used, whereas in Faro, sulfuric acid is employed. The latter acid is more favorable when considering agricultural applications.

Thus, the chemical composition of Kaumera samples from Faro and Utrecht was compared, and their suitability for foliar fertilization assessed. Parameters such as total solids, volatile solids, carbohydrate content, and protein content were quantified, and FTIR-ATR analysis was conducted to gain a better understanding of each polymer. Conductivity and pH levels were also measured and compared to expert recommendations against the ideal values for foliar fertilization. Additionally, nutrient levels were quantified and compared to regulatory guidelines and nutritional recommendations. An adhesion protocol was also designed to compare the adhesive properties between Kaumera Utrecht, Kaumera Faro, and Kaumera Zutphen (established benchmark).

Results indicated that Kaumera Faro has 0.114 ± 0.005 grams of carbohydrate per gram VS, whereas Kaumera Utrecht has 0.168 ± 0.002 grams of carbohydrate per gram VS (47% more). On the other hand, Kaumera Utrecht has 0.323 ± 0.009 grams of protein per gram VS, while Kaumera Faro has 0.456 ± 0.004 grams of protein per gram VS (41% more). High carbohydrate content could be positively correlated with strong adhesion properties, whereas a high protein content might demonstrate enhanced biostimulating effects. In addition, the pH and salinity adjustments required for applying the Kaumera solution to leaves are determined by its mixing ratios. When preparing the solution, it is essential to consider the low pH of Kaumera Nereda Gum® and evaluate whether washing the polymer to reduce salinity is necessary. Heavy metal concentrations in samples of Kaumera Utrecht and Kaumera Faro remained within permissible limits according to regulatory thresholds when considering the applied dosage of 35 liters per hectare per year (as suggested by Koppert). However, with such dosage, nutrient levels in the Kaumera samples were lower than recommended, confirming that additional fertilizers are to be mixed with Kaumera Nereda Gum®. Furthermore, the adhesion protocol proved to be a valuable screening tool, showing that Kaumera Utrecht has superior adhesive properties compared to the benchmark. In summary, this study confirms that Kaumera Utrecht and Kaumera Faro exhibit advantageous properties in foliar fertilization, but their effective use requires pH adjustment, salinity reduction, and nutrient supplementation.

Table of Contents

List of Figures

List of Tables

Abbreviations

AGS	Aerobic Granular Sludge
WWTP	Wastewater Treatment Plant
EPS	Extracellular Polymeric Substances
TS	Total Solids
VS	Volatile Solids
EC	Electrical Conductivity
DM	Dry Matter
CV	Crystal Violet
RSD	Relative Standard Deviation
FTIR-ATR	Fourier-Transform Infrared Spectroscopy – Attenuated Total Reflection
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IC	Ion Chromatography

1. Introduction

1.1. Aerobic Granular Sludge (AGS) Technology

Aerobic Granular Sludge (AGS) technology is an innovative and sustainable solution to wastewater treatment that could replace the conventional Activated Sludge (AS) process in the near future. By using sequencing batch reactor technology, highly diverse microbial communities are able to grow and inhabit granules (Nancharaiah et al., 2019). As a result, this technology effectively removes organic carbon, nitrogen, phosphate, and other pollutants from wastewater simultaneously in a single tank. Meanwhile, AS requires multiple process units (anaerobic, anoxic, and aerobic tanks) to obtain such nutrient removal. Moreover, the compact, spherical granular sludge found in AGS technology has a higher settleability than the flocculated sludge found in AS. As a result, secondary clarifiers (settling tanks) which are essential in AS are not required in AGS. Therefore, AGS technology could be the preferred choice as it minimizes land footprint (up to 75% lower), has approximately 30% less energy consumption, and has a significant reduction in total annual costs (approximately 20%) compared to the conventional AS process (De Kreuk et al., 2005; De Kreuk, 2006; Pronk et al., 2015).

AGS technology under the Nereda® brand has gained enormous popularity in both domestic and industrial sectors. Currently, there are 100 Nereda® plants in over 20 countries worldwide (Royal HaskoningDHV, n.d.).

1.2. Extracellular Polymeric Substances (EPS)

Currently, excess sludge is the primary waste product generated by various types of wastewater treatment plants (WWTPs), including AGS technology (Feng et al., 2021). However, ongoing research is being conducted to explore resource recovery from the excess sludge produced by AGS technology (Nancharaiah et al., 2019). One approach involves extracting structural extracellular polymeric substances (EPS) from the surplus granular sludge. EPS are biopolymers released by bacteria during their metabolic processes to facilitate granule formation and provide structural stability (Feng et al., 2021). Particularly, successful extraction of structural EPS from AGS-EPS has been achieved. Structural EPS is one crucial constituent of EPS identified as the gel-forming constituent in AGS (Lin et al., 2010; Felz et al., 2016). It has been observed that the extraction of structural EPS allows for the recovery of approximately 30% of organic matter, 20% of total phosphorous (TP), and 30% of total nitrogen (TN) from sludge (Bahgat et al., 2023). This extracted product became commercially known as Kaumera Nereda Gum® and has already been used for diverse applications, contributing to the development of a circular economy.

Currently, EPS-based biomaterials are a viable alternative to synthetic polymers and are used as gel-forming materials for the paper industry, in cement curing, and as biosorbents or flame-retardant (Feng et al., 2021). Still, applications are abundant and more of them are yet to be discovered.

1.3. Kaumera Nereda Gum® for Foliar Fertilization

One plausible application for Kaumera Nereda Gum® is agriculture, especially for foliar fertilization. This is an alternative or compliment to soil fertilization, where plants absorb mineral nutrients from liquid fertilizer applied to the leaves (Fernández & Brown, 2013). Foliar fertilization

facilitates the rapid absorption of mineral elements, especially in the growing seasons when there is a high nutrient demand. With foliar fertilization, there is no interaction with soil components and hence the problems of nutrients leaching out via precipitation, being lost by erosion, or being absorbed onto soil surfaces are avoided.

Kaumera Nereda Gum® exhibits several characteristics that make it a suitable candidate to be used for foliar fertilization. To begin with, Kaumera Nereda Gum®, specifically derived from Zutphen, has exhibited remarkable adhesive properties that are comparable to existing products in the market, such as alginate (H. Mikkelsen from Koppert, personal communication, May 30, 2023). In the context of foliar fertilization, alginate serves as the adhesive agent that attaches liquid fertilizers onto leaves. Secondly, there is evidence suggesting that Kaumera Nereda Gum® possesses biostimulating effects, which can enhance plant growth and development (STOWA, 2019). Additionally, its hydrophilic properties aid in improving water uptake, while its chelating properties facilitate nutrient uptake, thereby promoting overall plant health. Lastly, when Kaumera Nereda Gum® dries, it forms a protective film on the leaves, serving as a barrier against pathogens and potentially reducing the risk of disease (H. Mikkelsen from Koppert, personal communication, May 30, 2023). These combined factors make Kaumera Nereda Gum® a favorable choice for foliar fertilization in agricultural practices.

Currently, the company Koppert, which focuses on selling sustainable agricultural products, are using Kaumera Nereda Gum® as a sustainable replacement for seaweed products (i.e., alginate) that serve as raw material for several agricultural products (Koppert, 2021). Over the past two years, Koppert has been evaluating the use of Kaumera Nereda Gum® in agriculture and has observed significant stimulation of plant growth and soil life compared to its seaweed counterpart.

However, several challenges can arise when using Kaumera Nereda Gum® for foliar fertilization. One particular obstacle faced in worldwide Nereda® projects, such as the Faro-Olhão WWTP in Portugal, is the issue concerning seawater intrusion. The close proximity of wastewater treatment plants to the ocean, combined with the challenges of blocking seawater intrusion, in most cases result in the entry of seawater into the Nereda® tank, influenced by the tides. This intrusion has the potential to hinder the agricultural usability of Kaumera Nereda Gum®, as high chloride levels can create problems when considering its application for foliar fertilization. To address this issue, users in Faro chose to precipitate Kaumera Nereda Gum® using sulfuric acid, instead of the most commonly used hydrochloric acid, employed in other locations such as Utrecht, Zutphen, and Epe (refer to figures 1 and 2). Therefore, it is essential to thoroughly examine the differences in the properties of Kaumera Nereda Gum® obtained from different sites, considering the varying climates, wastewater compositions, and specific conditions such as the presence of seawater intrusion and different acids used for precipitation. This becomes particularly important if the intention is to commercialize Kaumera Nereda Gum® from multiple locations.

The research on using Kaumera Nereda Gum® for agricultural purposes is an ongoing effort. This study constitutes a step towards a better understanding of the biopolymer, its components and properties, and its feasibility in agriculture, specifically in foliar fertilization.

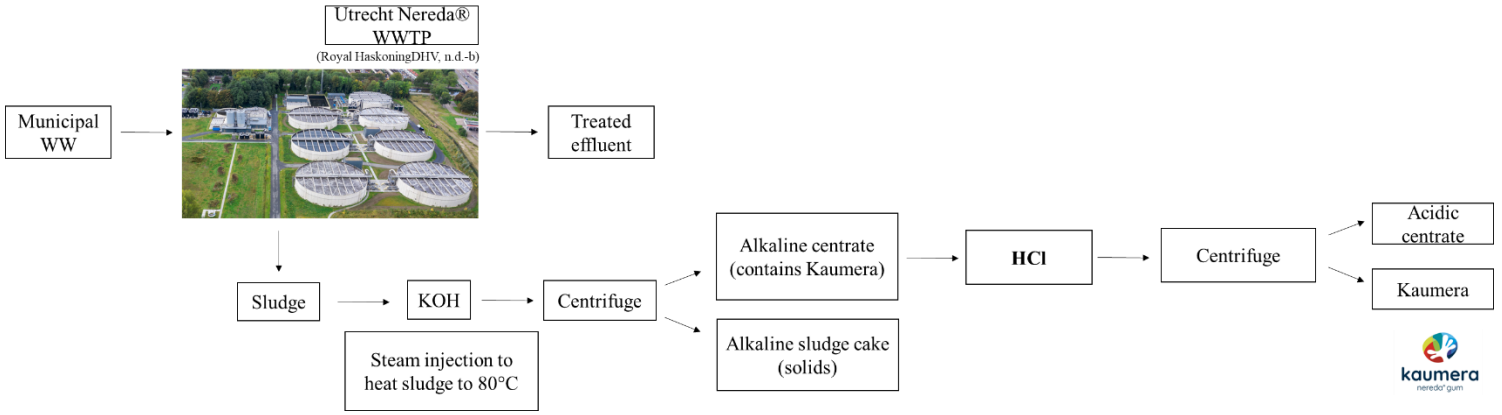


Figure 1: Kaumera Nereda Gum® production line in Utrecht Nereda® WWTP.

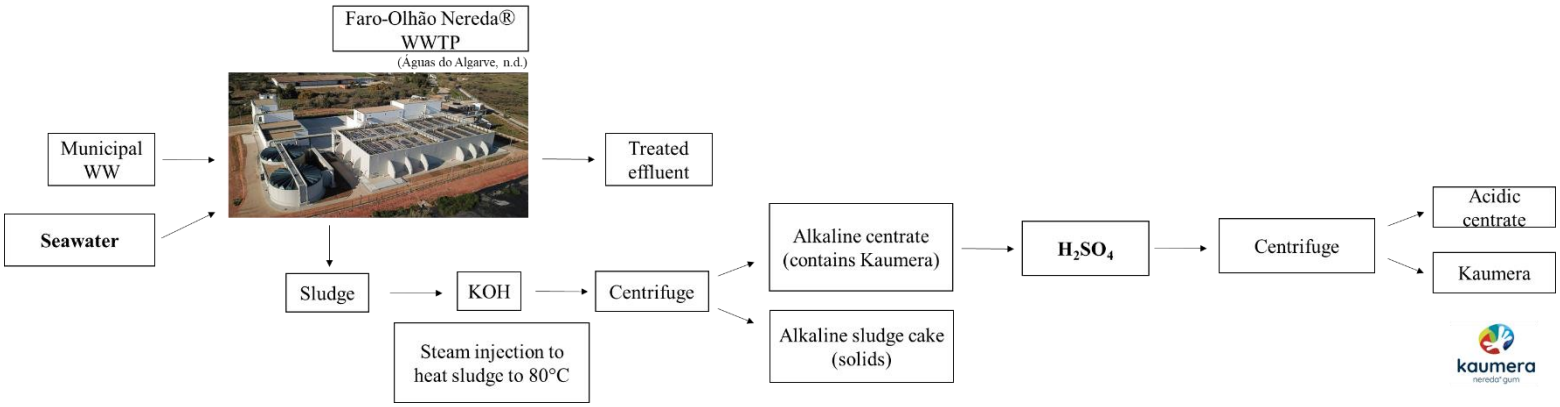


Figure 2: Kaumera Nereda Gum® production line in Faro-Olhão Nereda® WWTP.

2. Literature Review

2.1. Sludge Treatment and Resource Recovery

The management of excess sludge from all types of WWTPs, including Nereda®, is a highly complex and costly activity, ranging from 20% to 60% of the total operating costs of the wastewater treatment plant (Andreoli et al., 2007). If poorly accomplished, it can create sanitary and environmental hazards, mainly related to the final destination of disposal. Sludge treatment currently consists of the following steps:

1) Thickening: involves the reduction of volume required for digestion, as excess water has negative effects on such process. This can be accomplished through the use of gravity thickeners, dissolved air flotation units, or sedimentation tanks.

2) Digestion: sludge is biologically stabilized under anaerobic conditions and converted into stable substances. In digesters, anaerobic microorganisms are able to convert organic matter into carbon dioxide (CO_2) and methane (CH_4), where the latter is collected to generate power.

3) Dewatering: remaining sludge is dewatered to decrease volume before final disposal. This step helps to lower transportation costs to the disposal site, enhance sludge management, increase the sludge's heating capacity prior to incineration, and reduce the volume intended for landfill disposal or land application, thereby minimizing leachate production.

4) Disposal: the process of incineration, landfill disposal, or land application as fertilizer.

With the projected rapid urbanization, particularly in developing countries, it is anticipated that the number of wastewater treatment plants (WWTPs) will rise, leading to an increase in the volume of sludge generated (Tay & Show, 1997). Additionally, in heavily urbanized regions, the option of landfill disposal for sludge may become limited due to land scarcity and stricter environmental regulations aimed at preventing soil contamination. Consequently, future sludge management strategies are focused on minimizing waste and maximizing the reuse of valuable resources, particularly for agricultural purposes.

One way to obtain value from waste is by extracting EPS from sludge. Biopolymers derived from renewable resources have gained significant attention due to their advantages over oil-based synthetic polymers (Kreyenschulte et al., 2012). These bio-based alternatives are not only biodegradable but also offer a simpler and more cost-effective production process. In addition, EPS accounts for a significant portion of the dry weight of sludge (Feng et al., 2019; Lin et al., 2015), so recovering it would reduce the volume and costs required for sludge treatment substantially.

2.2. Extracellular Polymeric Substances (EPS)

Extracellular polymeric substances (EPS) are biopolymeric substances secreted by bacteria during metabolism (Feng et al., 2021). EPS is a complex matrix consisting of proteins, exopolysaccharides, DNA, lipids, glycoproteins, S-layer, and humic-like substances (Seviour et al., 2019). Other constituents from AGS-EPS can be seen in figure 3. Moreover, AGS-derived EPS serves multiple functions. It assists in the formation of granules, contributes to structural stability,

acts as a nutrient source, and serves as a protective barrier against harmful compounds or adverse environmental conditions that could potentially harm the bacteria (Feng et al., 2021).

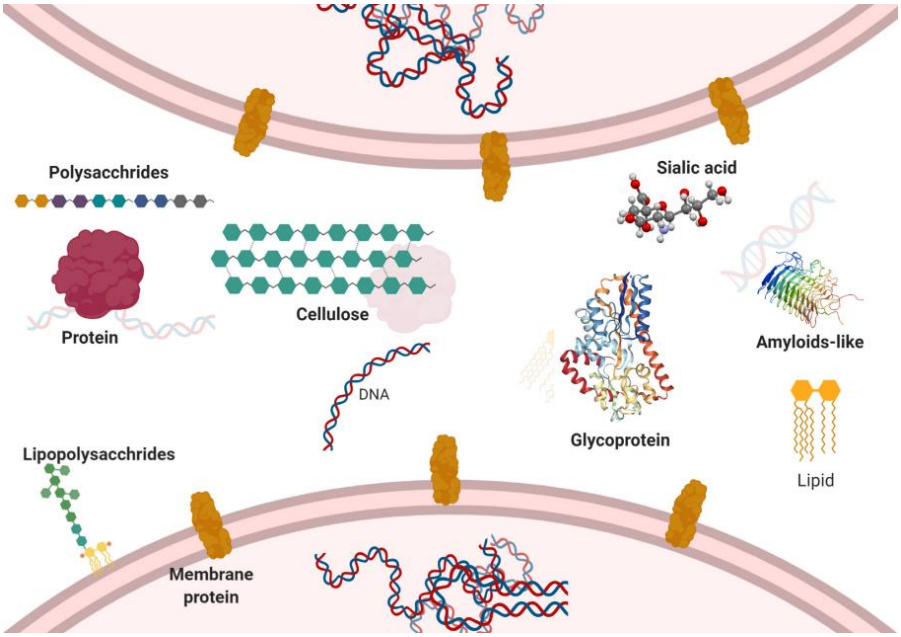


Figure 3: components of EPS in granular sludge (Feng et al., 2021).

2.3. Structural EPS (Kaumera Nereda Gum®)

Structural EPS is a subset and the main structure of the total EPS that allows the formation of hydrogels (Lin et al., 2010; Felz et al., 2016). Due to its gel-forming property, structural EPS started being commercialized for diverse industrial applications as Kaumera Nereda Gum®.

Structural EPS derived from granular sludge was first referred to as alginate-like extracellular (ALE) polymers due to the various similarities it has with alginate, a natural polysaccharide that exists in brown seaweeds (Lin et al., 2010). Both structural EPS and alginate have the ability to form hydrogels with calcium ions, precipitate as a gel at low pH, and contain carboxyl groups (Lin et al., 2010; Draget et al., 1994). In addition, their extraction methods are very similar (McHugh, 2003). However, it was determined via Fourier-Transform Infrared Spectroscopy (FTIR) analysis that structural EPS is far more complex than pure alginate. Structural EPS, for example, is also composed of proteins, neutral sugar, amino sugars, uronic acids, and polyphenolic compounds (Felz et al., 2019).

2.3.1. Structural EPS Extraction

The aggregation of the EPS matrix is caused by the interaction between the polymers forming it (Nielsen & Jahn, 1999; Monique et al., 2008; Pfaff et al., 2021). The main forces that maintain this structure are Van der Waals forces, hydrogen bonds, electrostatic linkages, hydrophobic interaction, and covalent bonds. To solubilize these polymers, breaking these interactions are necessary, and this is done by using extraction methods that can focus on one or multiple types of EPS bonds in the matrix (D'Abzac et al., 2009). To recover EPS from granular sludge, harsh extraction methods are needed. Physical methods (heating, centrifuging, sonication), chemical methods (alkaline extractions, chelators, detergents), or a combination of both can be used to

achieve the maximal extraction yield. Hence, extraction techniques are diverse and there is no standardized extraction protocol.

Utilizing alkaline compounds, such as potassium hydroxide, sodium hydroxide, or sodium carbonate, is commonly used due to their ability to achieve high extraction yields (Felz et al., 2016). Since the isoelectric points of EPS (the pH at which the net charge is zero) generally fall below 6 (Nielsen & Jahn, 1999), elevated pH levels cause the deprotonation of carboxylic groups in proteins and polysaccharides (Sheng et al., 2010; Novák & Havlíček, 2016). As a result, the surface of proteins and polysaccharides become predominantly negatively charged, leading to strong repulsion between EPS molecules and providing higher solubility of the substance. High temperatures of 80°C have also been used in combination with sodium carbonate to enhance solubilization (Lin et al., 2010).

After AGS-EPS is extracted, structural EPS (Kaumera Nereda Gum®) is recovered from the EPS matrix by acid dosage (Felz et al., 2016). Structural EPS precipitation consists of reaching the isoelectric point, where the negative and positive charges are balanced, and the attraction forces predominate, causing aggregation and precipitation (Novák & Havlíček, 2016). Acids such as hydrochloric or sulfuric acid are commonly used as precipitants in the Kaumera pilots.

To prevent any potential confusion with other publications discussing EPS, and to facilitate the clarity of this study, from now on the term "EPS" will exclusively refer to structural EPS.

2.3.2. Chemical Composition

2.3.2.1. Dry Matter Content

EPS samples extracted from communal granular sludge from the Vroomshoop WWTP and excess sludge from Epe WWTP in the Netherlands were analyzed to determine the total dry and organic matter content (STOWA, 2019). Both extractions were carried out in the laboratory following the procedure described in Felz et al. (2016).

The content of total solids (TS) in the EPS samples varied within the range of 5-12%. This percentage is primarily influenced by the extraction conditions and the settings of the centrifuges (STOWA, 2019). As a result, the TS% can be adjusted to an optimal value based on specific applications and transportation costs.

2.3.2.2. Organic Matter Content

On average, the EPS obtained from the Vroomshoop and Epe WWTPs had an organic matter content of approximately 70% of the TS (STOWA, 2019). Analysis also revealed that the main constituents of EPS were proteins followed by carbohydrates.

In a study conducted by Felz et al. (2019), the protein and carbohydrate content of EPS obtained from the Dinxperlo WWTP were measured. The EPS was also extracted in the laboratory following the procedure described in Felz et al. (2016). In this case, sodium carbonate and hydrochloric acid were employed. To measure both proteins and carbohydrates, EPS was dissolved in 0.02 M NaOH.

When quantifying the total protein content, Felz et al. (2019) used two different colorimetric methods: the Lowry method and the BCA assay. For the Lowry method, bovine serum albumin

(BSA) and cytochrome C from equine heart were used as standards. Measurements were performed in a 96-well plate with the absorbance measured at 750 nm. For the BCA assay, the same standards were used but the absorbance was measured at 562 nm. Using these methods, the protein standard equivalents were 38-62 wt% in total organic matter.

Additionally, Felz et al. (2019) measured carbohydrates using another colorimetric method known as the phenol sulfuric method (Dubois et al., 1956). The standards included: glucose, xylose, and a sugar mixture (equal amounts of fucose, rhamnose, galactose, glucose, xylose, mannose, and ribose). Measurements were performed in cuvettes at absorbance maxima of the sugars standard. The corresponding wavelengths used were 480 nm (xylose), 482 nm (sugar mixture), and 487 nm (glucose). With these methods the carbohydrate standard equivalents vary from 11 to 15 wt% of the organic matter.

2.3.2.3. Others

Table 1 shows the concentrations of inorganic components in Kaumera Nereda Gum® (STOWA, 2019). The data below was obtained from both laboratory and pilot research from Vroomshoop, Epe, and Dinxperlo WWTPs.

Table 1: average elemental composition of EPS from Vroomshoop, Epe, and Dinxperlo WWTPs (STOWA, 2019).

Element	*Unit*	*Value*
Total Phosphorus	% TS	2-3%
Total Nitrogen	% TS	6-9%
Iron	mg/kg TS	7000-10000
Calcium	mg/kg TS	4000-5000
Aluminum	mg/kg TS	3000-5000
Zinc	mg/kg TS	400-1000
Copper	mg/kg TS	100-500
Lead	mg/kg TS	28-71
Cadmium	mg/kg TS	0.5-1
Arsenic	mg/kg TS	2-3

2.3.3. Current Applications

Compared to other synthetic polymers, EPS-based materials are prevalent, bio-based, biodegradable and have a higher productive rate and an easier extraction procedure (Feng et al., 2021). Hence, they are a feasible and sustainable alternative to the former.

Currently, EPS-based biomaterials are used for several applications:

- Coating material for the paper industry: due to its hydrogel-forming capacity (insoluble in water), EPS can be used as paper coating to increase impermeability and resistance to grease (Lin et al., 2015; Lotti et al., 2019).
- Curing of concrete: due to its hydrophilic property, AGS-based EPS helps concrete retain moisture to keep gaining strength and resist shrinkage cracking (Zlopasa et al., 2014).
- Biosorbent material: AGS-derived EPS is a cost-effective biosorbent material for water treatment applications (Li et al., 2017). It is able to remove heavy metal ions or organic

pollutants via physical contact, electrostatic attraction, ion-exchange function, binding sites, and chemical precipitation.
- Flame retardant material: due to its self-extinguishing properties, AGS-based EPS is an alternative to halogenated fire retardants that can be used for flax fabrics (Kim et al., 2020).
- Membranes: since EPS has ion exchange properties, it can be used to form a selective membrane towards the transport of monovalent ions (Feng et al., 2021).

2.4. Foliar Fertilization

Foliar fertilization is a valuable tool to sustain or increase plant nutrient levels throughout the growth periods (Johnson, 2022). It is particularly beneficial in preventing or correcting deficiencies, especially when the functioning of the root system is impaired, such as during prolonged rainy conditions leading to waterlogged soils or when the soil exhibits a low pH, causing nutrient immobilization and limiting uptake by the roots. By targeting specific growth stages, foliar fertilizers can be utilized to enhance vegetable nutrition, improving characteristics such as color, appearance, quality, and yield.

Foliar fertilizers are applied as liquid solutions of dissolved fertilizers in the form of ions or small molecules in water (Johnson, 2022). Primarily, nutrients enter the leaf surface through the waxy cuticle, which protects the epidermal cells of leaves (see figure 4). Even though the waxy cuticle controls water loss from leaf surfaces, it does contain very small pores that allow water and small solute molecules to penetrate into the underlying leaf cells. These pores are lined with negative charges. Thus, fertilizer nutrients in cation form (e.g., NH_4^+, K^+, Mg^{2+}) or with neutral charges enter most readily through these pathways. In comparison, negatively charged nutrients (e.g., phosphate-P, sulfate-S) are much slower to move through the cuticle and they must be paired with a cation. Movement in the cuticle is also dependent on molecular size, nutrient concentration, the time the nutrient is in solution on the leaf, weather the nutrient is in ionic or chelated form (see section 2.5.2), and the thickness of the leaf cuticle. Another important factor to consider is what happens after the nutrient enters the leaf area. Small molecules are those with less of a charge (e.g., NH_4^+, K^+, Mg^{2+}) and are readily transported in the vascular system to other areas of the plant. Larger molecules or those with more strongly positive charges (e.g., Ca^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}, Cu^{2+}) stay close to the entrance of the leaf since they bind to negatively charged walls in the intercellular area. So, since these nutrients do not mobilize as much once they pass the cuticle, these nutrients are best applied as chelated forms. Table 2 provides recommended nutrient rates for foliar fertilization (Johnson, 2022). The number of applications per year strongly depends on the plant's needs, growth stage, and environmental conditions (WorkingLeaf, 2023). Assuming an average of five applications per year as suggested by H. Mikkelsen from Koppert (personal communication, May 30, 2023), these nutrient rates were calculated in terms of kg per hectare per year.

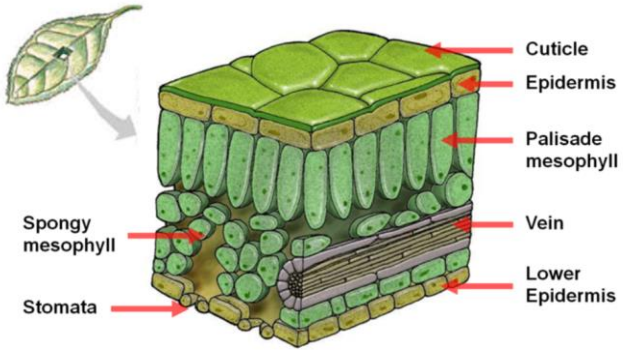


Figure 4: cross section of a leaf (Himme, n.d.).

Table 2: recommended nutrient rates (kg DM/ha) for foliar fertilization (Johnson, 2022). The last column was calculated assuming five applications per year.

Element	*Best Forms*	*Crop Type*	*Recommended Rate (kg DM/ha)*	*Recommended Rate (kg DM/ha/year)*
Nitrogen	Urea, methylene urea, triazones, ammonium sulfate	Benefit most vegetables if plant is low in N	1.12-11.21	5.60-56.04
Potassium	Potassium sulfate, potassium nitrate	Fruiting vegetables (e.g., tomatoes, melons)	4.48	22.42
Magnesium	Magnesium sulfate	Tomatoes, melons, beans	0.56-2.24	2.80-11.21
Iron	Iron sulfate or chelated forms	-	1.12-2.24	5.60-11.21
Manganese	Manganese sulfate or chelated forms	-	1.12-2.24	5.60-11.21
Zinc	Zinc sulfate or chelated forms	-	0.28	1.40

2.5. Kaumera Nereda Gum® for Foliar Fertilization

At present, Koppert is diluting Kaumera Nereda Gum® with additional fertilizers and water (H. Mikkelsen from Koppert, personal communication, May 30, 2023). Subsequently, they spray the resulting solution onto the leaves. However, the specific effects of these other ingredients on the properties of Kaumera Nereda Gum® have not been explored. Furthermore, the exact mixing ratio of all the ingredients is not known. Nonetheless, it has been established that approximately seven liters of Kaumera Nereda Gum® are utilized per hectare, with an average of five applications per

year (H. Mikkelsen from Koppert, personal communication, May 30, 2023). This corresponds to a dosage of 35 liters per hectare per year.

Kaumera Nereda Gum® possesses several advantageous properties that make it a potential candidate for foliar fertilization. These properties include its high adhesiveness, biostimulating effects, capacity to retain water, and ability to form a protective biofilm, guarding plants against pathogens (STOWA, 2019; H. Mikkelsen from Koppert, personal communication, May 30, 2023). In addition, it is expected that a large portion of heavy metals and pathogenic microorganisms will be removed during its production line, making it a safe choice for agricultural use. In the context of foliar fertilization, it is essential to take into account various properties of Kaumera Nereda Gum®, along with important guidelines and expert opinions that need to be met when utilizing Kaumera Nereda Gum® for this purpose. These properties will be discussed further in the following sections.

2.5.1. Biostimulation

Biostimulants are organic mixtures applied to plants to enhance growth, reduce stress, or improve nutrient uptake (STOWA, 2019). They can be applied to plants via seed coating, root bath, dipping the cuttings in biostimulants, or by foliar fertilization. Examples of biostimulants include seaweed extract, humus, and protein hydrolysate. Moreover, the presence of specific amino acids, such as tryptophan, are closely related to biostimulation as they can significantly enhance the growth and yield of cultivated crops (Chiaiese et al., 2018). Tryptophan, which has been detected in substantial amounts within AGS-EPS as reported by Zhang et al. (2019), plays a fundamental role in plant metabolism (Chiaiese et al., 2018). It serves as a crucial building block for proteins, acts as a precursor for plant hormones like auxin and salicylic acid, and contributes to the formation of aromatic secondary compounds with multiple biological functions.

In 2016, the biostimulating effects of Kaumera Nereda Gum® on perennial rye grass and cattail were examined (STOWA, 2019). It was observed that there was an increase in above-ground biomass, comparable to using alginate. Even though there was a clear positive effect on the plants, the mechanism of action has not been explained. Other studies have also shown how EPS present in soil and in plant roots promote an efficient uptake of water and nutrients (Alami et al., 2000; Bezzate et al., 2000; Sandhya et al., 2009).

2.5.2. Chelating Property

Kaumera Nereda Gum® can also make micronutrients more available to plants by acting as a sustainable chelator (STOWA, 2019). Besides making sure that sufficient quantities of micronutrients are present, they must also be chemically available to the plants. Chelators are organic molecules that enable nutrients to be available for plant uptake (Liu et al., 2022). Figure 5 shows how the organic chelators work. The chelator (brown) encircles the micronutrient (orange), such as iron or zinc, and protects it from oxidation, precipitation, and immobilization. Then, it moves the ion through the wax layer (which repels water and charged substances) of the leaves (dark green) and into the mesophyll (light green), where the micronutrient is released. Without chelation, micronutrients would stay on the leaf surface. Some plants have their own chelation system, and these are less likely to succumb to nutrient deficiencies (Trees, 2023). However, in other plants, chelators are not naturally released, and thus external chelating agents can be used as supplement.

Furthermore, using Kaumera Nereda Gum® is an excellent alternative compared to other commercial chelators as it is biodegradable (STOWA, 2019).

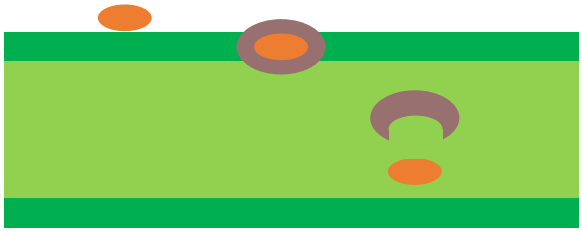


Figure 5: scheme of chelated fertilizer in foliar fertilization. Chelator (brown) encircles the nutrient (orange) and moves it through the wax layer (dark green) and into the mesophyll (light green) (Liu et al., 2022).

2.5.3. Pathogenic Microorganisms

Kaumera Nereda Gum® is derived from municipal or industrial AGS. In the case of municipal sludge, it often contains pathogens commonly encountered in municipal waste, such as *E. coli*, *Enterococci*, and *Clostridia* (STOWA, 2019). Nonetheless, it has been proven that these microorganisms are effectively killed during the Kaumera Nereda Gum® production process due to the addition of high-temperature water and/or chemical dosage.

The killing of the pathogens *E. coli*, *Enterococci*, and *Clostridium Perfringens* were recently studied under different extraction conditions (STOWA, 2019). It was concluded that these pathogens were killed within the standard extraction time and caustic doses to below the detection limit (N=10 cfu/g). *E. coli* was killed when the temperature reached above 55°C, while the killing of *C. Perfringens* (the hardest pathogen to kill) took approximately an hour, well within the standard extraction time.

The staff at Águas do Algarve, the company responsible for operating the Faro-Olhão WWTP, also conducted measurements of *Sulphite-reducing Clostridia*, *E. coli*, and *Salmonella* in their Kaumera Nereda Gum®. Results revealed the absence of *Sulphite-reducing Clostridia* and *Salmonella*, while the concentration of *E. coli* was found to be below $1.0x10^{-1}$ cfu/g. In Portugal, regulations exist for *E. coli* and *Salmonella* concentrations in sewage sludge intended for agricultural purposes (Hudcová et al., 2019). *Salmonella* should not be detected in a 50-gram sample and the concentration of *E. coli* must be less than 1000 cfu/g. Therefore, Kaumera Faro already complies with these guidelines.

2.5.4. pH

To ensure effective foliar fertilization, it is crucial that the pH of the sprayed solution containing Kaumera Nereda Gum® is slightly acidic, as this facilitates easy penetration of the solution through the cuticle and absorption by the leaves (Sela, 2021). However, the optimal pH level will differ depending on the specific nutrient being applied. For instance, for phosphorus application, the ideal pH range is 3.0-3.7, whereas for zinc, it falls within 4.1-4.9.

The pH of the solution plays a significant role in foliar fertilization as it impacts the solubility of applied fertilizers and nutrient penetration (Sela, 2021). Nutrients need to be in their soluble form

for effective absorption by the leaves. Typically, the solubility of most nutrients increases in slightly acidic pH, preventing precipitation and allowing them to remain in solution.

2.5.5. Salinity or Conductivity

Salinity is also a crucial property to consider when using Kaumera Nereda Gum® for agricultural purposes. With a high salt content, soil salinization, a land degradation process that decreases soil fertility, can occur (Thomas & Middleton, 1993). Additionally, salt deposits can cause direct foliar injury, leaf burn, and foliar fruit staining, mainly associated with boron, sodium, and chloride (WateReuse Foundation, 2007).

If sodium hydroxide (NaOH) and hydrochloric acid (HCl) are used to produce Kaumera Nereda Gum®, the resulting product may contain a notable amount of sodium chloride (STOWA, 2019). Therefore, if Kaumera Nereda Gum® is intended for agricultural applications, it is recommended to extract it with potassium hydroxide (KOH) and/or use sulfuric acid (H_2SO_4) for precipitation. Nevertheless, in cases where chloride levels are significantly high, a washing process can be employed to reduce the chloride content in Kaumera Nereda Gum® if necessary. This washing procedure involves preparing an acid solution (HCl) with the same pH as the Kaumera Nereda Gum®, in order to maintain the polymer's charge during washing. Then, the Kaumera Nereda Gum® is diluted by a factor of two using the acid solution, thoroughly mixed, and centrifuged at the desired RPM for a specified duration. The resulting supernatant is discarded, and the Kaumera Nereda Gum® is once again diluted by a factor of two using the acid solution. These steps are repeated until the desired level of conductivity is achieved.

Electrical conductivity (EC) measures the ability of water to conduct an electrical current. Therefore, EC is related to the concentration of dissolved ions, including salt ions, and thus it is commonly used as an indicator of salinity that can be employed for quality control in agricultural practices (De Oliveira et al., 2002). In general terms, an EC of more than 3 mS/cm for foliar fertilizers already presents a high risk to most crops (H. Mikkelsen from Koppert, personal communication, May 30, 2023). Thus, it is essential to closely monitor and manage conductivity levels to ensure the well-being and productivity of the crops.

2.5.6. Nutrients

To determine if Kaumera Nereda Gum® complies with the Netherlands' guidelines regarding heavy metal concentrations and to conclude if it can be used as a standalone foliar fertilizer without supplementation from other fertilizers to meet nutrient requirements (as shown in table 2), it is necessary to measure and analyze its nutrient content.

The macronutrients and micronutrients essential for plant growth are listed in table 3 (Cornell University, n.d.). Note that macronutrients are elements which plants require in relatively large amounts whereas micronutrients are those required in smaller amounts. Furthermore, it is important to highlight that foliar fertilization is primarily employed for micronutrients rather than macronutrients (Trinklein, 2019). This is due to the fact that applying macronutrients through foliar fertilization is not as economically viable as using soil fertilization methods.

Table 3: essential elements for plant growth (Cornell University, n.d.).

Essential Elements for Plant Growth	
Macronutrients	
Primary	Nitrogen (N)
	Phosphorous (P)
	Potassium (K)
Secondary	Calcium (Ca)
	Magnesium (Mn)
	Sulfur (S)
Micronutrients	
Main	Boron (B)
	Chloride (Cl-)
	Copper (Cu)
	Iron (Fe)
	Manganese (Mn)
	Molybdenum (Mo)
	Zinc (Zn)
Others	Cobalt (Co)
	Nickel (Ni)

2.5.6.1. Macronutrients

As can be seen from table 1, macronutrients such as phosphorus (P) and nitrogen (N) are present in Kaumera Nereda Gum® in the same magnitude as sludge: 2-3% P and 6-9% N as a percentage of dry matter (STOWA, 2019). Moreover, since potassium hydroxide is dosed in the Kaumera Nereda Gum® production line (see figure 1 and 2), potassium (K) is also expected to be present in Kaumera Nereda Gum® in a high concentration.

2.5.6.2. Micronutrients

Many micronutrients such as boron, chloride, copper, iron, manganese, and zinc are essential to plant growth but become toxic in high concentrations and are then referred to as “heavy metals” (Rengel, 1999).

EPS derived from AGS have a large capacity of adsorption of heavy metal ions (Liu et al., 2015). The large quantity of carboxyl and hydroxyl groups located in protein surfaces are the main adsorption sites. The adsorption capacity involves valence forces through sharing of electrons between metals ions and EPS (electrostatic forces). As pH increases, functional groups are deprotonated and thus become negatively charged, attracting heavy metals which are mostly cations. Meanwhile, at low pH, functional groups are protonated and positively-charged, so adsorption capacity is unfavorable.

According to a study conducted by Liu et al. (2015), it was observed that under acidic conditions, metal ions can be desorbed from AGS-EPS due to the replacement of adsorbed metal cations by H+ ions. For instance, at a pH of 1, the desorption efficiencies for Pb(II), Cd(II), and Zn(II) were observed to be 90.6%, 92.4%, and 95.3%, respectively. Hence, when an acid is used to precipitate

Kaumera Nereda Gum® towards the end of its production line, heavy metal ions previously adsorbed during biological treatment are released into the solution, and later separated from the final product in the centrifugation step. It has already been proven that a significant amount of heavy metals ends up in the sludge cake and the acidic centrate during production (see figure 1 and 2), and thus most of these compounds in Kaumera Nereda Gum® are lower than in waste sludge (STOWA, 2019).

2.5.6.3. Guidelines

Currently, within the European Union, there are no specific regulations for foliar fertilizers other than those developed for fertilizers as a whole, which mainly focus on soil fertilization (Ciavatta & Benedetti, 2002). Additionally, it is important to highlight that fertilizer regulations can vary among different countries.

In the Netherlands, The Fertilizers Act Implementation Decree (2021) has established maximum concentrations for heavy metals in fertilizers across three different categories: “sewage sludge and other inorganic fertilizers”, “compost”, and “other organic fertilizers”. Since Kaumera Nereda Gum® originates from sewage sludge, it will be analyzed as a fertilizer falling under the "sewage sludge and other inorganic fertilizers" category. Table 4 displays the maximum allowable concentrations for heavy metals in this category (Fertilizers Decree, 2021). In addition, there is a maximum dosage of two tons dry matter per hectare per year permitted for sewage sludge on arable lands (land under temporary crops).

Table 4: maximum permitted concentrations of heavy metals in sewage sludge fertilizer in the Netherlands (Fertilizers Decree, 2021).

Heavy metals	*Maximum limits for sewage sludge (mg/kg DM)*
Cd	1.25
Cr	75
Cu	75
Hg	0.75
Ni	30
Pb	100
Zn	300
As	15

2.5.7. Adhesion

As mentioned earlier, Kaumera Nereda Gum® shows promise as a substitute for alginate, which is commonly used in the market to enhance the adhesive properties of foliar fertilizers. In the context of foliar fertilization, adhesion plays a vital role as it directly impacts the fertilizers' capability to adhere to leaves and resist being washed away by rain.

In this study, adhesion was defined as the ability of a wet complex fluid to remain on the surface even after experiencing force or stress; in other words, its resistance to debonding.

When a polymer, such as a hydrogel, is brought into contact with the surface of another material, an adhesive bond is formed in most cases (Zosel, 1985). An effective adhesion requires a favorable interaction (energy) at the interface and depends on several factors such as wettability, hydrophobicity, roughness, topography, and chemistry (Zheng et al., 2021). Additionally, wettability and rheology play crucial roles in the adhesion process and will be explored in greater detail in the upcoming sections.

2.5.7.1. Wettability

Wettability is defined as the ease at which an adhesive can contact and spread over a given surface (Agrawal et al., 2017). In addition, it determines the adhesion of coatings (Laurén, 2019). When the Kaumera solution is applied to leaves, it forms a coating similar to when paint is applied to walls.

To achieve a uniform coating that effectively covers the surface, such as a leaf, a low contact angle is desirable (refer to figure 6) (S. Picken, personal communication, June 23, 2023). Attaining a low contact angle also requires a favorable interaction energy at the surface. Once this favorable condition is met, the consistency, viscosity, and yield stress of the fluid will determine the amount of fluid that adheres to the surface.

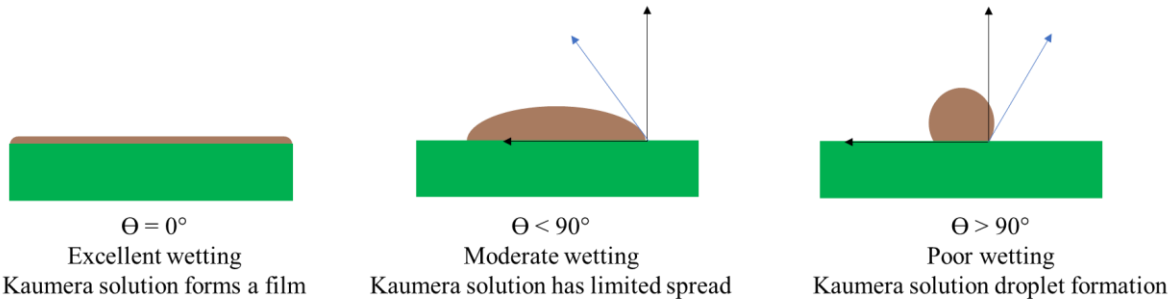


Figure 6: wetting capabilities depending on contact angle between Kaumera solution (brown) and leaf surface (green).

2.5.7.2. Rheology

In the field of rheology, the critical parameter of yield stress for non-Newtonian fluids is measured. Kaumera Nereda Gum®, like other polymer solutions, exhibit non-Newtonian fluid properties, which means that its viscosity depends on how hard the force applied to it is (its magnitude) and how long the force is applied (its duration) (Zheng & Zhang, 2017). Moreover, yield stress is the minimum stress required for a material to undergo permanent deformation or exhibit plastic flow (Hosch, 2023). In other words, it quantifies the transition from elastic to plastic behavior in a material. Hence, the yield stress can give important information on the polymer's characteristics (fluid-like vs. solid-like) and behavior when it is in contact with a surface.

For a polymer to be an effective adhesive, the polymer must combine liquid-like characteristics to form good molecular contact and solid-like characteristics to resist an applied stress once the bonds have already been formed (Zosel, 1985). These characteristics require a high molecular weight polymer to form the backbone of the adhesive, and a low molecular weight fraction which improves flow and deformation (Roos et al., 2002).

2.5.7.3. Measuring Adhesion

In order to compare the adhesion properties of Kaumera Nereda Gum® from different sources and assess its suitability for foliar fertilization, it is necessary to quantify its adhesion. However, there is currently no established protocol specifically designed to quantify the adhesion of Kaumera Nereda Gum®. The following sections delve into the methods used to measure adhesion of gel polymers (section 2.5.7.3.1.) and biofilms (2.5.7.3.2.), providing a foundation for the development of an adhesion protocol tailored to Kaumera Nereda Gum®.

2.5.7.3.1. Measuring Adhesion of Polymers

Adhesion between soft polymeric materials and a surface are typically measured by two methods: peel testing or probe (tack) testing (Grillet et al., 2012). Peel testing is conducted by curing a polymer film on a surface. Once it is cured, one edge of the film is gripped by a mechanical pulling device that peels the polymer from the surface at a constant velocity and at a constant peel angle (commonly 90° is used). During this test, the force required to peel the polymer from the surface is recorded, as well as the maximum force measured during the process. This maximum force can then be compared across different polymers. Some disadvantages of using this test are the challenging interpretation of results due to the complex stress distribution near the peel front, as well as the significant influence of peel angle, peel velocity, and interface formation process on the outcomes. On the other hand, a probe test is done by bringing a probe into contact with the surface of the polymer being tested under a given force for a specified period of time. Afterwards, the probe is raised at a constant velocity while measuring the force required to do so. The force versus distance curve that results from this process provides valuable information on the adhesion properties of the polymer. These measurements are also highly dependent on the probe speed, contact time, force, probe shape, and surface characteristics. Furthermore, a significant disadvantage of both methods is the high cost of equipment.

2.5.7.3.2. Measuring Adhesion of Biofilm

Taking a closer look at the adhesive properties of EPS, it is crucial to emphasize that EPS plays a significant role in promoting the attachment of biofilms (collections of bacteria encapsulated by EPS) to both biotic and abiotic surfaces (Wolfaardt et al., 1999).

Several procedures exist that measure biofilm attachment. The Microtiter Plate Biofilm Assay is one of the most frequently used methods to assess bacterial attachment to abiotic surfaces by measuring the staining of the adherent biomass (Merritt et al., 2005; Negri et al., 2010; O'Toole, 2011). Also known as the 96-well dish plate assay, this method consists of growing bacteria in microtiter dishes for a desired period of time, and then rinsing the wells to remove planktonic bacteria. Bacteria that remain adhered to the wells are then stained with crystal violet (CV) dye, as the positive ions of the dye are attached to the negatively charged elements of the cell wall such as DNA, lipopolysaccharides, and peptidoglycan, allowing for visualization of the attachment pattern. Therefore, if the quantity of bacteria increases, the color's opaqueness will increase as well. The color's opaqueness is determined by measuring the optical density or absorbance with a spectrophotometer. In contrast with the former methods, this test does not require expensive equipment and it is easy to use.

3. Problem Statement & Knowledge Gaps

There is currently a growing interest in using Kaumera Nereda Gum® for agricultural purposes, particularly for foliar fertilization. However, it is important to consider the potential challenges that may arise from giving it such use. One of these challenges could be seawater intrusion, which occurs in Nereda® projects worldwide, including the Faro-Olhão WWTP in Portugal. If excessive chloride concentrations are found in the Kaumera Nereda Gum®, this could potentially hinder its use. Another factor to consider is the variation in acids used for the precipitation of Kaumera Nereda Gum®, which differs at each site. In Faro-Olhão, sulfuric acid is employed, whereas hydrochloric acid is commonly used in other locations, such as Utrecht. If, for instance, hydrochloric acid is employed to precipitate Kaumera Nereda Gum®, this will also lead to an increase in its chloride content. The impact of various parameters, including the influent wastewater, the Nereda® operation, and the chemicals used during the extraction of the polymer, on the composition of Kaumera Nereda Gum® has not yet been extensively investigated. As a result, there is limited understanding of the variations that may exist among Kaumera Nereda Gum® from different origins, particularly those obtained from outside of the Netherlands. Thus, it is uncertain whether these differences could potentially impede the suitability of Kaumera Nereda Gum® for specific applications, such as foliar fertilization.

In summary, this investigation aimed to assess the suitability of using Kaumera Nereda Gum® from Utrecht and Faro for foliar fertilization. It is necessary to highlight that this investigation studies solely the Kaumera Nereda Gum® product and not the solution created by Koppert with Kaumera Nereda Gum® (section 2.5). To achieve this purpose, this investigation was further divided into two tasks:

The first task involved determining the major components of Kaumera Utrecht and Faro, specifically the percentage of organic matter, with a focus on protein and carbohydrate content to gain a better understanding of each polymer. Additionally, properties relevant to foliar fertilization such as pH and EC were measured and compared to recommended values provided by experts in the field. The concentration of nutrients were also quantified to determine if they meet the desired requirements for plant growth, while also ensuring they do not exceed the toxicity limits outlined by the Netherland's Fertilizers Decree to avoid potential risks to human health. This analysis aimed to evaluate whether the current dosage of Kaumera (35 liters per hectare per year, as suggested by Koppert [section 2.5]), needed to be reduced to avoid toxicity thresholds or if it needed to be mixed with other liquid fertilizers to enhance nutrient content.

The second task consisted of analyzing an important parameter in Kaumera Nereda Gum® for foliar fertilization: adhesion. Adhesion is a critical property in foliar fertilization as the product must be able to adhere to the leaves instead of washing out with precipitation. Consequently, it was necessary to quantify and compare the adhesion properties of Kaumera Utrecht and Faro with Kaumera Zutphen, which served as a benchmark due to its highly regarded adhesive properties by Koppert. However, there is no current methodology that quantifies adhesiveness of Kaumera Nereda Gum®. To ensure the utilization of Kaumera Nereda Gum® from different locations worldwide for this application, it was essential to establish a standardized protocol. This protocol

would serve as a means to quantitatively measure adhesion and enable a comparison between the adhesion properties of different Kaumera Nereda Gum®.

To develop a standardized protocol, it is fundamental to achieve accurate, repeatable, and reproducible results. Since this study was conducted independently, the reproducibility across different laboratories or users could not be evaluated. Moreover, a desired outcome was to have a low relative standard deviation (RSD) between replicates. For this study, an RSD of 5% or lower was considered acceptable. The protocol also aimed to be time-efficient and cost-effective, providing advantageous qualities for its implementation. Table 5 outlines the established requirements for the protocol, along with the corresponding measures that were taken to fulfill those specific requirements.

Table 5: established requirements for creating a standardized protocol.

Requirements	*How?*
Accurate	Compared with another complementary technique
Repeatable	≤ 5% relative standard deviation between replicates
Time-effective	Short time test (1 day)
Economical	Inexpensive materials

Hence, the objectives of this research were:

1. Conduct an analysis of the chemical composition of Kaumera Utrecht and Kaumera Faro to assess their suitability for agricultural purposes in foliar fertilization.
2. Compare the adhesion properties of Kaumera Utrecht and Kaumera Faro to Kaumera Zutphen (benchmark) by developing a standardized protocol that quantifies the adhesion properties of Kaumera Nereda Gum® to abiotic surfaces.

The two research questions were as follows:

RQ1: Does the chemical composition of Kaumera Utrecht and Kaumera Faro present any (regulatory or practical) limitations for its use in foliar fertilization?

H1: No, the chemical composition of Kaumera Utrecht and Kaumera Faro do not present any limitations for its use in foliar fertilization.

RQ2: Is Kaumera Utrecht and Kaumera Faro comparable to Kaumera Zutphen (benchmark) in terms of its adhesion properties?

H2: Yes, Kaumera Utrecht and Kaumera Faro adhesive's property are equal or higher to those of Kaumera Zutphen.

4. Method

4.1. Pilot Extraction

To produce a batch of Kaumera Faro (550 L), 4.5 m^3 of sludge is brought to a pH of 9.5 and to a temperature of 80°C by dosing 25 L of 40% KOH and 0.45 m^3 of pure water coming from the steam injection. Afterwards, this stream is centrifuged, and the centrate (4.5 m^3) is separated from the alkaline sludge cake. The centrate is then cooled overnight to a temperature of 30°C. The next day, 40 L of 40% H_2SO_4 is dosed to precipitate Kaumera Faro. The final step is to centrifuge again and separate Kaumera Faro from the acidic centrate. For Kaumera Utrecht, the same process is conducted but 50 L of 25% KOH and 35 L of 30% HCl are used instead. It is also important to highlight that the settings of the centrifuges also vary between the sites. A scheme of the production line for Kaumera Utrecht and Kaumera Faro can be observed in figure 1 and 2.

The Kaumera Faro analyzed in this study was obtained on November 30, 2022, while the sludge it originated from was from the previous day. Specifically, on November 29, 2022, the conductivity of the influent at the Faro-Olhão Nereda® plant was measured at 5500 uS/cm. Assuming a homogenous mixing of seawater in the tank and a negligible conductivity from the municipal wastewater, seawater intrusion could represent a maximum value of 10% for this day (see table 6). During the Kaumera Faro pilot operation period, the conductivity varied between 3000-18000 uS/cm, representing a seawater intrusion of 6-34% of the influent. Moreover, the extraction yield of Kaumera Faro during the pilot operation ranged from 31-42% (expressed as % of VS from feed sludge ending up in Kaumera Nereda Gum®). Meanwhile, the Kaumera Utrecht assessed on this study was obtained on July 18, 2022, and the conductivity of the influent wastewater could be approximated to that of drinking water in Utrecht. The Netherlands' guidelines for drinking water states that the EC must be less than 250 uS/cm (European Union, 2015). Furthermore, the extraction yield of Kaumera Utrecht ranged between 20% and 22%.

Table 6: EC of influent Faro-Olhão Nereda® compared to EC of typical seawater.

Type	*Typical seawater*	*Influent Faro-Olhão Nereda®*		
		29/11/2022	*Min*	*Max*
EC in mS/cm	53.9	5.5	3.0	18.0
Contribution	-	10%	6%	34%

4.2. Methodology

The properties or tests performed on Kaumera Utrecht and Kaumera Faro for this study are depicted in Figure 7. The properties under the basic characterization refer to control parameters that must be determined to have a better understanding of the chemical composition of each polymer, while the properties under advanced characterization are mostly related to the application of foliar fertilization.

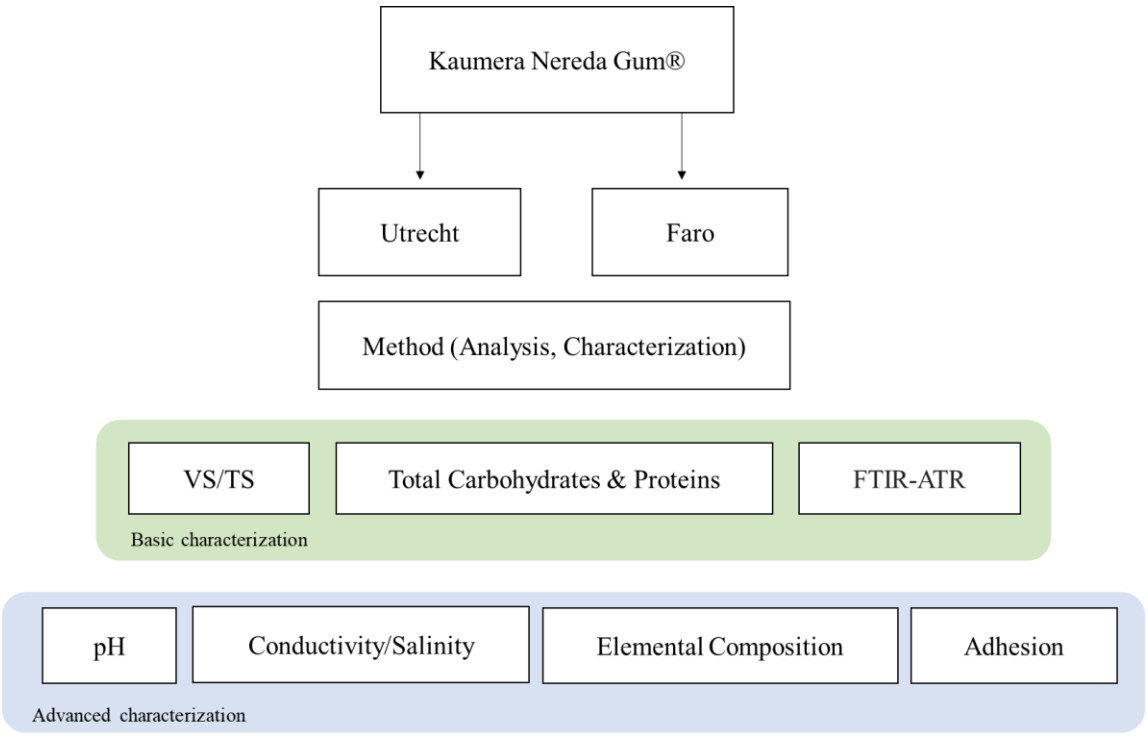


Figure 7: summary of measured properties or tests conducted on Kaumera Utrecht and Kaumera Faro.

4.2.1. Basic Characterization

4.2.1.1. VS/TS Analysis

The VS/TS analysis serves as an indicator of how much organic matter is in Kaumera Nereda Gum® by quantifying the volatile solids (VS) in respect to the total solids (TS). This test was done in triplicates for each Kaumera Nereda Gum®.

First, aluminum cups were pre-ashed for 2 hours at 550°C. Then, they were cooled down by placing them in the dessicator for 15 min. These cups were then weighed (empty weight, EW). The Kaumera samples were then added to the cups and weighed (wet weight, WW). Afterwards, the samples were placed in a 105°C oven overnight. The next day, the samples were taken out and cooled in the desiccator for 15 minutes. Afterwards, they were weighed again (dry weight, DW). Next, they were placed in a 550°C muffle furnace for 2 hours. The samples were then taken out, cooled in the desiccator for 15 minutes, and then reweighed. This weight corresponds to the ash (A).

The following equations were applied to obtain TS(%), VS(%), and A(%).

$$TS\ (\%) = \frac{DW-EW}{WW-EW}$$

$$VS\ (\%) = \frac{DW-A}{DW-EW}$$

$$A(\%) = \frac{A-EW}{DW-EW}$$

4.2.1.2. Total Carbohydrates

To prepare the Kaumera samples for the total carbohydrates test, Kaumera Utrecht and Kaumera Faro samples were first dialyzed overnight against Milli-Q water in a dialysis bag (MWCO 3.5 kDa) to decrease the salt content. This was done since the salt content can interfere with the reactions, as well as to have a proper comparison between Kaumera Utrecht and Kaumera Faro. Afterwards, they were frozen at -80°C for 3 hours and freeze dried. Next, both freeze-dried Kaumera Utrecht and Kaumera Faro samples were dissolved in 0.1 M NaOH in Milli-Q water to obtain a concentration of 1000 mg/L. The solutions were left stirring overnight.

To determine the total carbohydrate content for both Kaumera samples, the methodology used was adjusted from the phenol-sulfuric acid method established by Dubois et al. (1956). This test is a colorimetric method that determines the total carbohydrates in a sample. The concentrated sulfuric acid breaks down any polysaccharides, oligosaccharides, and disaccharides to monosaccharides (Nielsen, 2010). Pentoses (5-carbon compounds) are then dehydrated to furfural, while hexoses (6-carbon compounds) to hydroxymethyl furfural. Moreover, the phenol is added to react with these compounds and produce a yellow-gold color. Although the method detects all types of carbohydrates, the absorptivity of the different carbohydrates varies, and thus the result is expressed in terms of one carbohydrate.

To create the standard calibration curve, standard samples of 1500 µL were prepared with different concentrations of glucose (0-200 mg/L) diluted in 0.1 M NaOH. For the Kaumera samples, different concentrations were tested to make sure the absorbance values were inside the range of the calibration curve. At the end, the Kaumera solutions utilized were diluted to 500 mg/L. 300 µL of each standard solution (triplicates) and 300 µL of each Kaumera sample (triplicates) were pipetted into glass borosilicate tubes. For each tube, 300 µL of 5% w/v phenol was added. Immediately after, 1500 µL of sulfuric acid was dosed and the tube vortexed. Afterwards, the samples were incubated at room temperature for 20 minutes. Then, 2 ml of each solution was placed into a 2.5 ml cuvette. The absorbance was then measured for each cuvette at the wavelength of 490 nm in the spectrophotometer. After the absorbance was measured for the carbohydrate standards, a calibration curve was made. Note that the average 490 nm absorbance measurement of the blank standard was subtracted from all the individual standards and unknown Kaumera samples. With the known absorbances for the Kaumera samples, the carbohydrate concentration for both Kaumera Utrecht and Kaumera Faro were determined with the equation of the line that resulted from the calibration curve. Afterwards, the carbohydrate concentrations in mg/L were normalized into grams carbohydrates/gram VS.

4.2.1.3. Total Proteins

To determine the total protein content for Kaumera Utrecht and Kaumera Faro samples, the same solutions prepared for the carbohydrate test were used (500 mg/L in 0.1 M NaOH). In this case, the Pierce® BCA Protein Assay kit was utilized. This test is also a colorimetric method in which proteins are quantified by a color development reaction with a working reagent, which turns the samples into different shades of purple depending on the protein concentration. In this case, different dilutions of BSA standards were prepared to construct the calibration curve. Three replicates were performed for each standard and Kaumera sample.

The preparation of the BCA working reagent (WR) was done by mixing 50 parts of BCA reagent A with 1 part of BCA reagent B (50:1, Reagent A:B). 25 µL of each standard and unknown sample was pipetted into a microplate well. 200 µL of the WR were then added to each well and the plate was mixed on a plate shaker for 30 seconds. The plate was then covered and incubated for 2 hours at room temperature. As final step, the absorbance was measured in a plate reader at 562 nm. The average 562 nm absorbance measurement of the blank standard was subtracted from the 562 nm measurements of all the individual standards and unknown samples. The calibration line was created and the protein concentration for the Kaumera samples determined with the equation of the line constructed with the calibration curve. Like the total carbohydrates test, the protein concentrations in mg/L were normalized into grams proteins/gram VS.

4.2.1.4. FTIR-ATR

FTIR-Attenuated Total Reflection (ATR) consists of applying infrared radiation (IR) to samples of materials in order to measure the sample's absorbance of infrared light at various wavelengths with the aim of determining the material's molecular composition and structure (i.e., functional groups) (Mathias, 2022).

The sampling methodology used for this test was attenuated total reflectance (ATR) with a *PerkinElmer Spectrum 100*. Dialyzed freeze-dried Kaumera samples were placed on a crystal surface and pressed down using the swivel press to ensure optimal contact between the sample and the surface. Afterwards, infrared light in the wavenumber range of 4000-600 cm^{-1} travelled through the crystal surface, and part of the infrared light was absorbed by the sample. The sample's ability to absorb the infrared light at different wavelengths determines the material's molecular composition.

Before placing the sample on the crystal surface, the background was scanned in order to subtract the background spectrum from the sample spectrum to eliminate unwanted residual peaks. Moreover, eight scans were conducted per sample.

4.2.2. Advanced Characterization

4.2.2.1. pH

The pH of Kaumera Utrecht and Kaumera Faro was measured with a calibrated pH probe.

4.2.2.2. Electrical Conductivity

The EC of both Kaumera Utrecht and Kaumera Faro, along with their corresponding supernatant (refer to section 4.2.2.3.3.2), was measured using an EC meter at a temperature of 25°C. Furthermore, the EC values were divided by the TS% of the respective Kaumera Nereda Gum® for better comparison.

4.2.2.3. Elemental Composition

4.2.2.3.1. Element Analyzer

An element analyzer was used to quantify the macronutrients carbon (C), hydrogen (H), and nitrogen (N) in samples of Kaumera Utrecht and Kaumera Faro. The analysis was conducted on one gram of dried Kaumera sample, which had been subjected to overnight drying in an oven at 105°C. These prepared samples were then sent to a laboratory for testing.

To measure the remaining elements in the Kaumera samples and their respective supernatants (collected after centrifugation), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used. Ions present in the supernatant were measured using Ion Chromatography (IC). The purpose of testing the supernatant was to examine how much percentage of each element or ion was found in the supernatant in respect to the total Kaumera, in order to assess the feasibility of washing the Kaumera Nereda Gum® in order to reduce salinity (see section 2.5.5.).

4.2.2.3.2. Microwave Assisted Acid Digestion

Before measuring the elements in the Kaumera samples via ICP-OES, they first had to go through microwave assisted acid digestion. This procedure consists of exciting nearby water molecules to tear sample materials apart (De Palma, 2020). Adding acids, in this case nitric acid and hydrochloric acid, speeds up homogenization. At the end, elements are left in solution and with uniform oxidation states suitable for ICP-OES.

The method used was adapted from "U.S. EPA Method 3051A: Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils" (U.S. EPA, 2007) and performed in duplicates for each Kaumera Nereda Gum®.

First, the vessels for digestion and glassware were acid-washed to remove any contaminants. Next, for each sample, approximately 2.5 grams of Kaumera Nereda Gum® were placed in a digestion vessel and weighed. Afterwards, 9 mL of 69% nitric acid and 3 mL of 30% hydrochloric acid were added to each vessel. Then, the digestion vessels were placed in the microwave digester tray for digestion. Samples were fully digested if the solutions were translucid green and there were no visible remains or solids. When this was confirmed, the digestion broth was transferred into an acid-cleaned volumetric flask. With a solution of 2% HNO_3 in Milli-Q water, the vessels were rinsed to remove all remanent of the digestion broth. Finally, the volumetric flask was filled with solution of 2% HNO_3 in Milli-Q water to the desired volume in order for the concentration of the elements to be inside the range of the ICP-OES.

4.2.2.3.3. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

4.2.2.3.3.1. Kaumera Samples

Once digested and diluted, the samples were sent to the ICP-OES in the WaterLab in the Civil Engineering Faculty of TU Delft. Nevertheless, as a result of technical difficulties encountered with the ICP-OES at the WaterLab, the measurement of elements was performed by Gustav Simoni, PhD at Aalborg University. His tests were performed in triplicates. However, the Kaumera Faro tested by Gustav Simoni was not the same as the one studied in this investigation. The Kaumera Faro used in his testing was obtained on a different date, specifically December 21, 2022, and it was extracted from sludge collected the day before. On December 20, 2022, the conductivity of the influent measured 3.2 mS/cm.

Figure 8 provides a summary of the procedure implemented to measure the element concentration in the Kaumera samples.

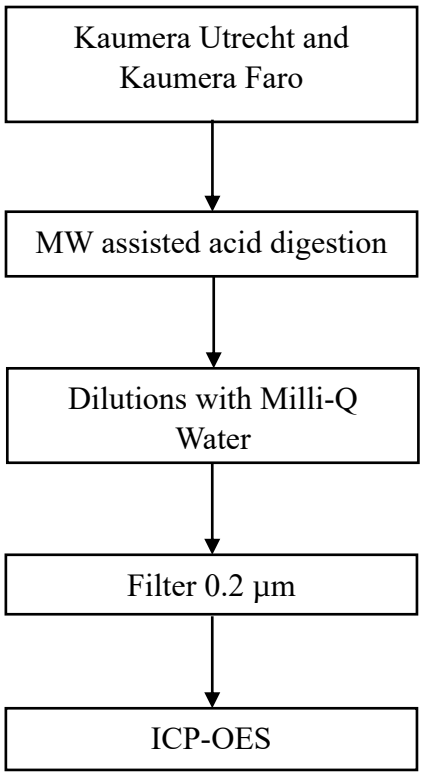


Figure 8: scheme for measuring elements in the Kaumera samples.

4.2.2.3.3.2. Supernatant Samples

Approximately 20 ml of each Kaumera Nereda Gum® was centrifuged for 10 minutes at 13,300 x g in a *Heraeus Pico 17 Centrifuge* to obtain roughly 18 ml of supernatant. These samples were then diluted with Milli-Q water to ensure detectable levels of the elements in the ICP-OES. The diluted samples were filtered through a 0.2 µm syringe filter, and 9.9 ml of each supernatant sample was mixed with 0.1 ml of nitric acid to reach a 1(v/v)% HNO_3 concentration for the ICP-OES. The nitric acid helps keep the analytes in solution and stabilizes the plasma of the ICP-OES device. The final solutions were sent to the ICP-OES in the WaterLab in TU Delft.

4.2.2.3.4. Ion Chromatography (IC)

For the IC, 3 ml of each Kaumera supernatant were collected and sent to the WaterLab to test for ions. The summarized procedure for testing the Kaumera supernatant samples can be seen in figure 9.

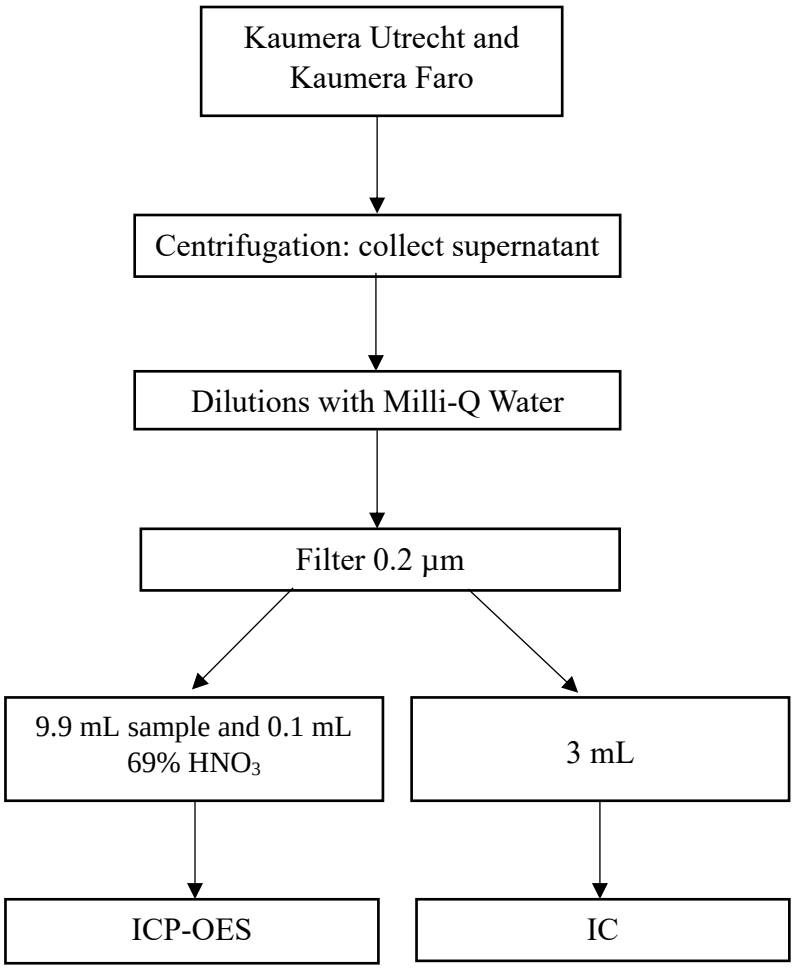


Figure 9: scheme for measuring elements and ions in the Kaumera supernatant samples.

4.2.2.4. Adhesion

To address **RQ2**, a protocol was developed to quantify the adhesion of Kaumera Utrecht, Kaumera Faro, and Kaumera Zutphen. The protocol requirements (listed in table 5) closely matched the advantages offered by the Microtiter Plate Biofilm Assay (section 2.5.7.3.2.), such as time efficiency and cost-effectiveness, making it the chosen test for designing the Kaumera adhesion protocol. Thus, an attempt was made to modify the Microtiter Plate Biofilm Assay to measure the adhesive properties of Kaumera Nereda Gum® on abiotic surfaces, focusing on testing EPS instead of biofilms. The Kaumera adhesion protocol can be seen in section 5.2.1.

During the development of the protocol, certain parameters and modifications were considered, which will be elaborated upon in the following sections.

4.2.2.4.1. Calibration Curve

If the protocol proved to be effective, it could be used to evaluate the adhesiveness of Kaumera samples obtained from diverse locations, which could aid in comparing their potential for foliar fertilization. However, the output values (absorbances) can vary greatly across different models,

manufacturers, and users. Therefore, a calibration curve or standard curve would be necessary to compare the outputs of different laboratories. Hence, the absorbances of different concentrations of CV dye were measured and a calibration curve was constructed.

When studies conduct the Microtiter Plate Biofilm Assay, the creation of a calibration curve is not a common practice, making it hard for microtiter plate data to be compared across research groups (Allkja et al., 2021). In this case, a calibration curve was indispensable in order to create a standardized protocol. This was also supported by a study conducted by Allkja et al. (2021), where they compared the results of CV staining across five different laboratories. In this research, it was concluded that each laboratory should calculate their own calibration curve as an attempt to compare "raw" data affected repeatability and reproducibility.

4.2.2.4.2. Testing Different Concentrations

In the establishment of this protocol, it was essential to test different concentrations of Kaumera samples to verify that with increasing concentration, the absorption increased as well. It was hypothesized that the samples with higher concentrations of Kaumera Nereda Gum® would have more negatively charged biomass attached to the surface and hence the absorbance (or color opaqueness) would be higher. Therefore, it was necessary to verify that there was a gradient color change between samples of different concentrations.

4.2.2.4.3. Control Experiment

A control experiment was also conducted in which only CV dye was added to the surface. This would guarantee that Kaumera Nereda Gum® was adhering to the surface and contributing to the solution's color.

4.2.2.4.4. Comparison with a Complimentary Technique

The Microtiter Plate Biofilm Assay is commonly employed as a screening tool, often complemented by additional experimental methods such as microscopic observations to validate observed patterns. Thus, it was also necessary to device a method that could also measure adhesion of Kaumera Nereda Gum® and serve as a comparison to the designed adhesion protocol. A straightforward and cost-effective approach would consist of immersing a basic plastic surface into Kaumera Nereda Gum®, removing it, allowing it to dry, and subsequently measuring the resulting weight changes. This method would enable the identification and comparison of patterns with the designed adhesion protocol in order to validate results.

4.2.2.4.5. Context: Foliar Fertilization

Since we are interested in quantifying adhesion in the context of foliar fertilization, it was also necessary to modify certain parameters in the protocol to simulate real-life conditions. To begin with, the pH of the Kaumera samples needed to be adjusted, as a slightly acidic pH is used in foliar fertilization, as mentioned in section 2.5.4. Thus, the pH of the Kaumera samples were increased with a base, in this case potassium hydroxide (KOH), to a pH of 6.5 ± 0.1. In addition, Kaumera Nereda Gum® needed to be diluted to a suitable concentration that would allow it to be effectively sprayed while maintaining its adhesive properties. Nonetheless, excessive dilution had to be avoided to prevent the loss of its adhesive characteristics. Therefore, Kaumera Nereda Gum® was diluted with Milli-Q water to obtain three different concentrations: 5%, 3.5%, and 2% TS. These

values were selected as Kaumera Nereda Gum® with a TS range of 2-5% can still be sprayed and maintains a homogenous film if placed on a surface (S. Picken, personal communication, April 5, 2023).

Furthermore, in order to determine whether Kaumera Utrecht and Kaumera Faro are comparable to the adhesive products currently available in the market, a benchmark comparison was necessary. At first, alginate was selected as reference material. However, after conducting the adhesion protocol on alginate, it was clear that it behaved very differently from Kaumera Nereda Gum® (see Appendix A), and thus Kaumera Zutphen was chosen instead. Since Koppert is conducting adhesion testing of Kaumera Zutphen on leaves and reporting positive feedback, the adhesiveness of Kaumera Zutphen was measured with the designed protocol and compared to Kaumera Utrecht and Kaumera Faro.

5. Results

5.1. Chemical Composition

5.1.1. VS/TS Analysis

After conducting the VS/TS analysis, it was concluded that Kaumera Faro contains a slightly higher average TS% and VS% than Kaumera Utrecht. In table 7, these results are presented as well as their respective standard deviation (SD). More detailed information of this analysis can be found in Appendix B.

Table 7: VS/TS results for Kaumera Utrecht and Kaumera Faro.

Kaumera Sample	*Average TS (%)*	*SD*	*Average VS (%)*	*SD*	*Average A (%)*	*SD*
Utrecht	5.97%	0.01%	81.38%	0.06%	18.62%	0.06%
Faro	8.63%	0.01%	84.05%	0.04%	15.95%	0.04%

5.1.2. Total Carbohydrates

After conducting the phenol-sulfuric method to measure the total carbohydrates in the Kaumera samples, it was observed that Kaumera Utrecht has 0.168 ± 0.002 g carbohydrates/g VS, while Kaumera Faro has 0.114 ± 0.005 g carbohydrates/g VS. This means that on average, Kaumera Utrecht has approximately 47% more carbohydrates than Kaumera Faro. This can be better observed in figure 10. Appendix C contains additional detailed data for this test.

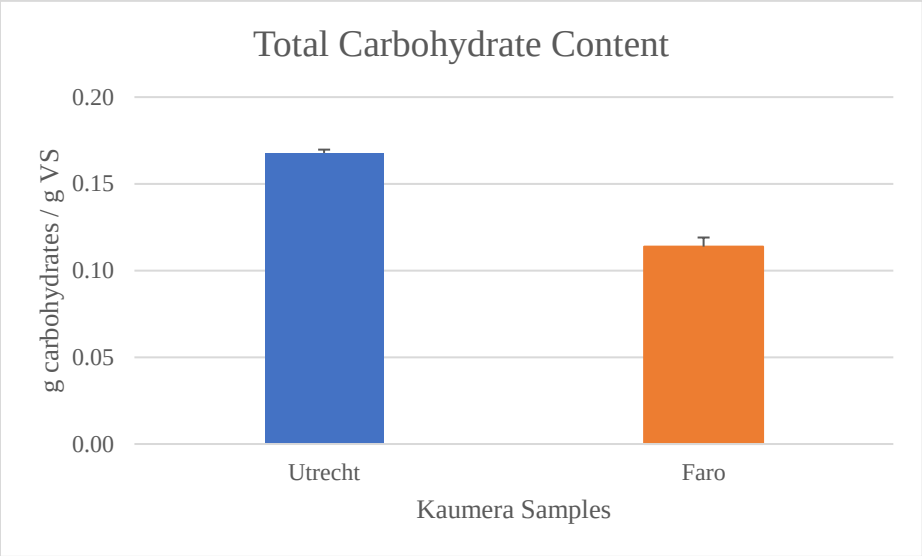


Figure 10: total (average) carbohydrates in Kaumera Utrecht and Kaumera Faro.

5.1.3. Total Proteins

When quantifying the total proteins in the Kaumera samples, it was observed that Kaumera Utrecht has 0.323 ± 0.009 g proteins/g VS while Kaumera Faro has 0.456 ± 0.004 g proteins/g VS (figure 11). With this method, Kaumera Faro has approximately 41% more proteins than Kaumera Utrecht. For more detailed data, see Appendix D.

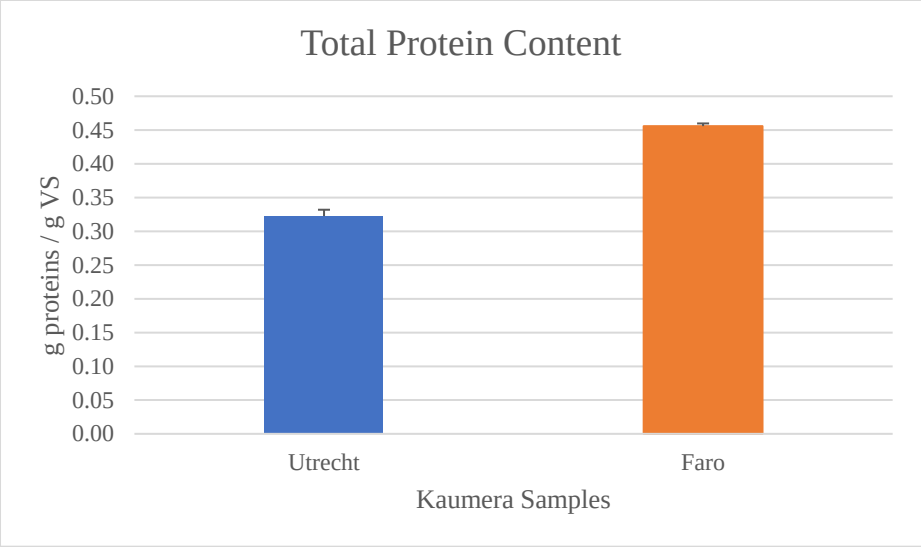


Figure 11: total (average) proteins in Kaumera Utrecht and Kaumera Faro.

5.1.4. FTIR-ATR

The FTIR-ATR analysis generated a graph showing absorption on the y-axis and frequency on the x-axis. Each peak in the graph, also known as absorbance band, represents the vibrations of atoms in the sample when exposed to infrared light at different frequencies. Thus, each peak signifies a particular functional group present in the sample. Each peak is assigned according to literature. In this case, "Advances in Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues" (Talari et al., 2017) was used.

The FTIR-ATR analysis identified ten significant functional groups in both Kaumera samples, as shown in Figure 12 and listed in Table 8. The alignment of the peaks for both samples indicates that there were no additional functional groups present in one sample compared to the other.

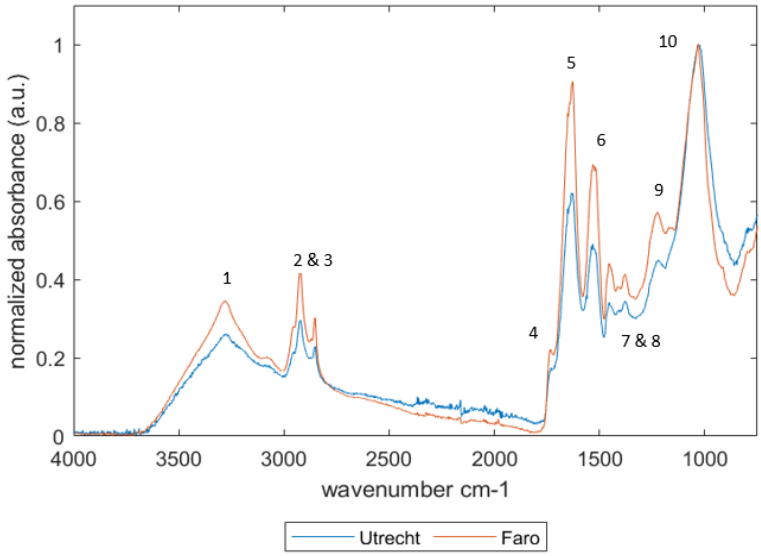


Figure 12: FTIR-ATR results for Kaumera Utrecht and Kaumera Faro.

Table 8: functional groups in Kaumera Utrecht and Kaumera Faro based on wavelength.

Peak #	*Wavelength (cm^{-1})*	*Functional Groups*
1	3300	Amide A (N-H stretch) in proteins and nucleic acids
2	2925	C-H stretch
3	2850	C-H stretch
4	1730	Ester
5	1630	Amide I
6	1530	Amide II
7	1455	Methyl (proteins)
8	1380	Methyl (proteins)
9	1220	Tertiary amine (C-N stretch)
10	1030	Primary alcohol (C-O stretch)

5.1.5. pH

The pH of both Kaumera Nereda Gum® is acidic and approximately the same (see table 9).

Table 9: pH of Kaumera Utrecht and Kaumera Faro.

Kaumera Sample	*pH (-)*
Utrecht	2.63
Faro	2.62

5.1.6. Electrical Conductivity

When comparing the EC of Kaumera Faro and Kaumera Utrecht, it is evident that Kaumera Faro has a slightly higher EC with 14.13 mS/cm, whereas Kaumera Utrecht has an EC of 10.08 mS/cm. However, when normalizing the values in relation to the total solids percentage (TS%), the EC of Kaumera Utrecht becomes higher than Kaumera Faro, as shown in Table 10. In contrast, the EC of the supernatant of Kaumera Faro is higher than that of Kaumera Utrecht, regardless of normalization, as shown in Table 11.

Table 10: EC of Kaumera Utrecht and Faro at 25°C. The last column shows electrical conductivity normalized with the respective TS% for better comparison.

Kaumera Sample	*EC (mS/cm) at 25°C*	*EC (mS/[cm*TS%]) at 25°C*
Utrecht	10.08	168.75
Faro	14.13	163.71

Table 11: EC of supernatant Kaumera Utrecht and Faro at 25°C. The last column shows electrical conductivity normalized with the respective TS% for better comparison.

Kaumera Supernatant Sample	*EC (mS/cm) at 25°C*	*EC (mS/[cm*TS%]) at 25°C*
Utrecht	11.44	191.52
Faro	17.15	198.70

5.1.7. Elemental Composition

5.1.7.1. Kaumera Samples

Table 12 displays the concentrations of the tested elements in the Kaumera Utrecht and Kaumera Faro samples. As previously stated, the measurement of elements other than C, H, and N was carried out by Gustav Simoni, PhD, at Aalborg University. Furthermore, the sulfur and chloride concentrations of Kaumera Faro were obtained from testing conducted by the staff at Águas do Algarve. Similar to Gustav Simoni's sample, the sample tested by Águas do Algarve also originates from the Kaumera Faro batch acquired on December 21, 2022 (section 4.2.2.3.3.1).

When considering the fundamental macronutrients for agriculture, namely nitrogen (N), phosphorus (P), and potassium (K), both Kaumera samples demonstrate similar nitrogen levels. Nonetheless, it is notable that Kaumera Utrecht exhibits considerably higher concentrations of phosphorus in comparison to Faro, while Faro has a higher concentration for potassium.

Table 12: average elemental concentrations in Kaumera Utrecht and Kaumera Faro.

*Element/Ion**	*Unit*	*Utrecht*	*SD*	*Faro*	*SD*
C	% TS	40.24	-	42.92	-
H	% TS	6.15	-	6.36	-
N	% TS	6.61	-	7.36	-
S	mg/g DM	-	-	34.00	-
K	mg/g DM	28.18	2.64	40.82	0.43
Fe	mg/g DM	23.33	0.31	1.93	0.08
P	mg/g DM	20.76	0.09	5.95	0.13
Cl^-*	mg/g DM	-	-	9.36	-
Na	mg/g DM	1.36	0.25	7.06	1.04
Ca	mg/g DM	4.70	0.54	1.71	0.03
Al	mg/g DM	1.68	0.07	2.86	0.35
Mg	mg/g DM	0.97	0.13	1.52	0.04
Zn	mg/g DM	0.26	0.01	0.57	0.05
Cu	mg/g DM	0.28	0.02	0.14	0.02
Ba	mg/g DM	0.07	0.01	<0.01	-
Pb	mg/g DM	0.06	0.01	<0.01	-
Ti	mg/g DM	0.04	0.01	0.03	0.01
Sr	mg/g DM	0.04	0.01	<0.01	-
Mn	mg/g DM	0.02	0.01	<0.01	-
Cd	mg/g DM	<0.01	-	<0.01	-
Cr	mg/g DM	<0.01	-	<0.01	-
Ni	mg/g DM	<0.01	-	<0.01	-
As	mg/g DM	<0.01	-	<0.01	-

5.1.7.2. Supernatant Kaumera Samples

Table 13 and 14 display the concentrations of tested elements and ions in the supernatant of Kaumera Utrecht and Kaumera Faro. It is worth noting that the supernatant of Kaumera Faro has 89 times more sulfate, 17 times more sodium, and 3 times more potassium than the supernatant of Kaumera Utrecht.

Using the concentrations listed in table 12, it was possible to determine the percentage of elements and ions present in the supernatant relative to the "total" Kaumera (refer to Appendix E for details). Specifically, for sulfate, phosphate, and ammonium, the percentages were calculated with respect to the total sulfur, phosphorus, and nitrogen. These percentages provide an estimate of the potential removal of each element or ion if the washing step is included to reduce salinity. Nevertheless, it is important to note that the percentages calculated for Kaumera Faro are based on the supernatant results obtained from a sample taken on November 30, 2022, while the results for the "total" Kaumera Faro are derived from a sample taken on December 21, 2022.

Table 13: average elemental concentrations in the supernatant of Kaumera Utrecht and Faro and relative concentrations in supernatant in respect to total Kaumera.

Element	*Average Concentration (mg/L)*		*Percentage (%) in supernatant*	
	Utrecht	*Faro*	*Utrecht*	*Faro*
K	1858.0	2760.0	104%	72%
Na	83.0	1304.0	96%	196%
P	296.4	150.0	22%	27%
Ca	225.7	140.5	76%	87%
Fe	194.0	84.6	13%	46%
Mg	47.6	129.3	77%	90%
Zn	10.2	38.6	62%	72%
Mn	1.7	0.7	121%	-
Cu	1.0	0.2	6%	1%

Table 14: average ionic concentrations in the supernatant of Kaumera Utrecht and Faro and relative concentrations in supernatant in respect to total Kaumera.

Ion	*Average Concentration (mg/L)*		*Percentage (%) in supernatant*	
	Utrecht	*Faro*	*Utrecht*	*Faro*
SO_4^{2-}	52.5	4682.5	-	49%
Cl^-	2868.8	2120.7	-	240%
K^+	1797.3	2619.9	100%	68%
Na^+	70.1	1252.3	81%	188%
PO_4^{-3}	795.0	273.0	20%	16%
NH_4^+	202.2	205.7	4%	2%
Ca^{2+}	195.1	103.1	65%	64%
Mg^{2+}	31.8	109.1	52%	76%

5.2. Kaumera Adhesion Protocol

5.2.1. Method Development

The following protocol was adjusted from the Microtiter Dish Biofilm Formation Assay (Merritt et al., 2005 and O'Toole, 2011) to be used for Kaumera Nereda Gum®. The goal of this protocol is to quantify adhesion of Kaumera Nereda Gum® to abiotic surfaces by determining the CV concentration (mg/L). This protocol can be used for any Kaumera Nereda Gum® in order to compare adhesiveness between samples. A more detailed protocol can be found in Appendix F.

It is important to highlight that cuvettes were used instead of the commonly used microtiter dish as the latter resulted in higher contamination between samples, especially during rinsing, as the wells are adjacent to each other. As a result, cuvettes, which are the same material as the microtiter dish (i.e., polystyrene), were used instead to isolate each sample and prevent contamination.

Kaumera Adhesion Protocol

Materials

- Spectrophotometer
- 1.5 mL cuvettes
- 2 waste trays: one for Kaumera samples and one for CV
- 50 mL Kaumera Nereda Gum®
- 2 M potassium hydroxide (KOH)
- 100 mL, 0.1% (w/v in Milli-Q water) Crystal Violet (CV)
- 400 mL, 30% acetic acid in Milli-Q water

Constant variables

Perform protocol under 25°C. Before starting Phase III, measure pH and electrical conductivity (EC) of each Kaumera sample. The pH should fall within the range of 6.5 ± 0.1. The electrical conductivity within the same concentration (5%, 3.5%, or 2% TS) might be similar across different Kaumera samples but can also show variations depending on Kaumera composition and type of sludge. To improve accuracy, Kaumera samples can be washed and later dosed with KCl to bring the conductivity to the desired value.

Safety measures

When handling the CV dye, wear goggles and chemical-resistant, impervious gloves. Handle inside the fume hood.

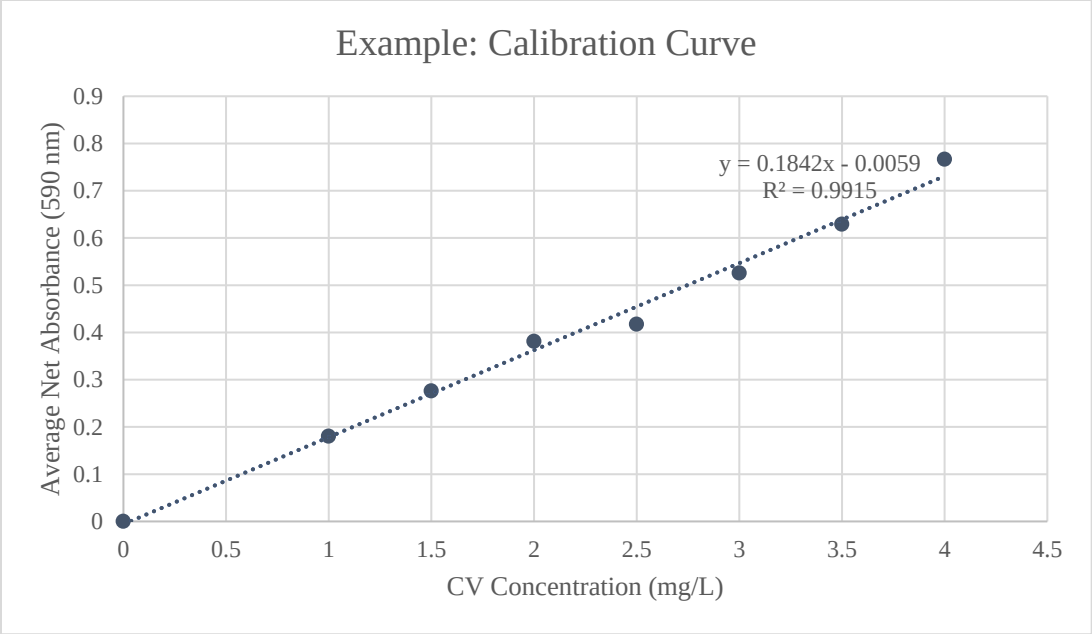
Method

Phase I: creation of calibration curve

1. Prepare the following CV standards with concentrations 0-4 mg/L for the calibration curve in triplicates. Note: for the blank, directly transfer 1.5 mL to a 1.5 mL cuvette.

CV Concentration (mg/L)	*0 (blank)*	*1*	*1.5*	*2*	*2.5*	*3*	*3.5*	*4*
Volume 0.1% (w/v) CV (mL)	0	0.025	0.030	0.030	0.025	0.030	0.035	0.040
Volume of 30% acetic acid (mL)	1.5	24.975	19.970	14.970	9.975	9.970	9.965	9.960

2. Transfer 1.5 mL of each standard to a 1.5 mL cuvette.
3. Read the blank at a wavelength of 590 nm in the spectrophotometer. Then, measure the net absorbance of each standard at the same wavelength.



Phase II: Preparation of samples

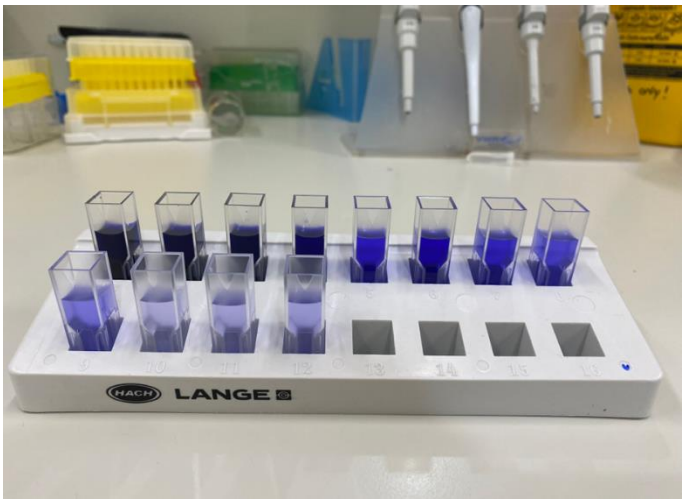
1. Add 2 M KOH to 50 mL Kaumera Nereda Gum® while monitoring the pH changes constantly with a pH probe to obtain a final pH of 6.5 ± 0.1. Note: add the 2 M KOH in small increments to avoid drastic changes in pH (e.g., 0.1 or 0.2 mL).
2. Prepare 3 different concentrations of Kaumera solution: 5%, 3.5%, and 2% TS with Milli-Q water. Since each concentration is tested in 4 replicates, each concentration requires a total of 15 mL of Kaumera solution.

Phase III: testing samples

1. Fill each 1.5 mL cuvette completely to the top with the respective Kaumera sample. Note: pipette gently so bubbles are not formed.
2. For Kaumera to stick to the cuvettes wait 1 minute.
3. Turn over the cuvettes and remove Kaumera by shaking gently over the Kaumera waste tray. Continue to do so until there is no more dripping.
4. Rinse the cuvettes by adding Milli-Q water until it reaches the top, and then remove over the Kaumera waste tray. Repeat this step twice. This step helps remove unattached Kaumera.
5. Tap on paper towels to remove excess liquid. Allow plates to air-dry for approximately 2 hours at room temperature.
6. Fill the cuvettes completely with 0.1% (w/v) CV in Milli-Q water. Let it stain for 15 min.
7. Shake each cuvette over the CV waste tray to remove excess crystal violet solution.
8. Wash the cuvettes by adding Milli-Q water until it reaches the top, and then remove over the CV waste tray. Repeat this step twice.
9. Turn the cuvette upside down and tap on paper towels to remove excess liquid. Allow plates to air-dry for approximately 2 hours at room temperature.

10. Fill each cuvette with 30% acetic acid in Milli-Q water to solubilize the CV. Pipette up and down three times for better mixing. Wait 15 minutes until it solubilizes completely.
11. Transfer 1.5 mL of CV/acetic acid solution from each cuvette to a separate, optically clean 1.5 mL cuvette.
12. Using 30% acetic acid in Milli-Q water as blank, measure the net absorbance of each cuvette at a wavelength of 590 nm. Note: if absorbance is greater than 1, the sample must be diluted to be inside the range of the calibration curve.
13. With the known absorbances, the CV concentrations (mg/L) can be determined via the calibration curve. These values can be used to compare the adhesion of different Kaumera Nereda Gum®.

Representative results:



5.2.2. Results: Kaumera Comparison

The adhesion protocol was tested on samples of Kaumera Utrecht, Faro, and Zutphen with the EC and pH showcased in table 15. Kaumera Utrecht and Kaumera Faro showed similar EC and pH values for each concentration, whereas Kaumera Zutphen exhibited slight differences. These variations could be attributed to the fact that Kaumera Zutphen is extracted from industrial wastewater sludge, which may result in some variability in its properties.

Table 15: EC and pH for Kaumera samples in adhesion protocol.

Kaumera Sample	*EC*			*pH*		
	5% TS	*3.5% TS*	*2% TS*	*5% TS*	*3.5% TS*	*2% TS*
Utrecht	10.98	8.26	5.12	6.44	6.46	6.51
Faro	11.10	8.28	5.14	6.42	6.42	6.48
Zutphen	13.78	10.73	6.62	6.57	6.62	6.64

During the experiment, visual observations were made at the different steps of the protocol. After shaking the cuvettes to remove the Kaumera solution, and particularly after shaking out the CV, a noticeable trend was observed. Across all Kaumera, it became evident that the attachment of Kaumera to the cuvettes exhibited a patchier pattern when the concentration increased, as can be observed in figure 13.

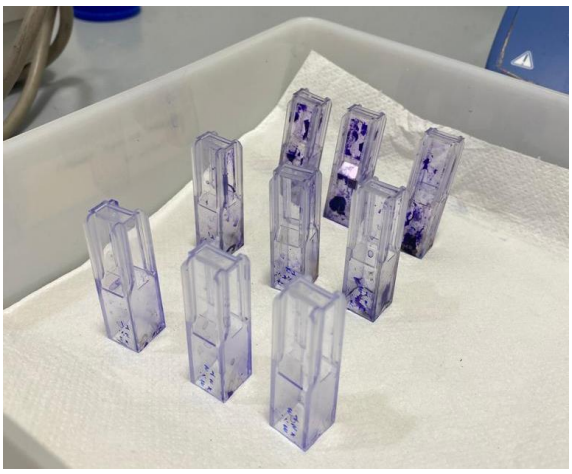


Figure 13: CV staining pattern of Kaumera Utrecht. Concentration (%TS) increases from left to right.

The resultant solutions for Kaumera Utrecht, Faro, and Zutphen after completing the protocol can be seen in figure 14. In general terms, a color gradient can be observed between the different concentrations (TS%). Note that each concentration had four replicates. However, it is also clear that some samples within the same concentration differ considerably in color opaqueness. Across all Kaumera samples, the RSD between replicates ranged from 7 to 36%.

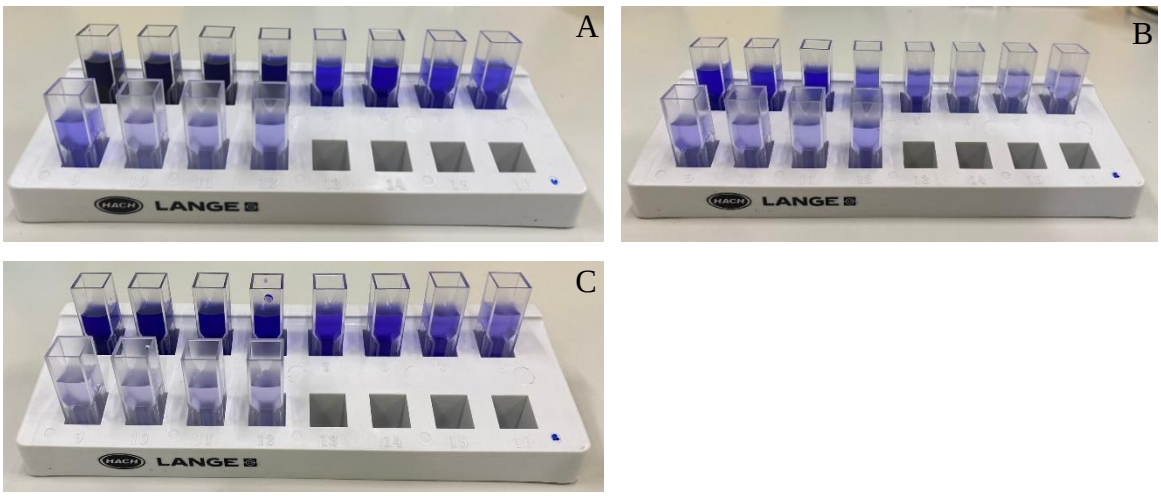


Figure 14: output of CV protocol for (a) Kaumera Utrecht, (b) Kaumera Faro, (c) Kaumera Zutphen.

When plotting the CV concentration (mg/L) vs. total solids percentage (TS%), it was observed that as the TS% decreased, indicating higher dilution, there was a clear decrease in the adhesion property of Kaumera Utrecht, Faro, and Zutphen (figure 15, 16, and 17). This decrease appeared to follow an exponential trend, but further data points are needed to confirm this observation.

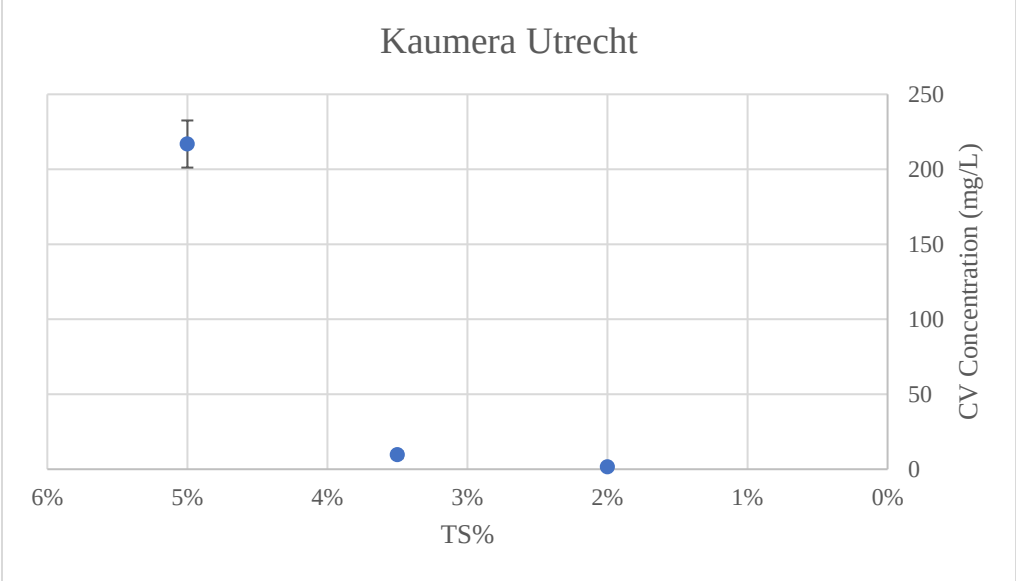


Figure 15: CV concentration vs. TS% for Kaumera Utrecht.

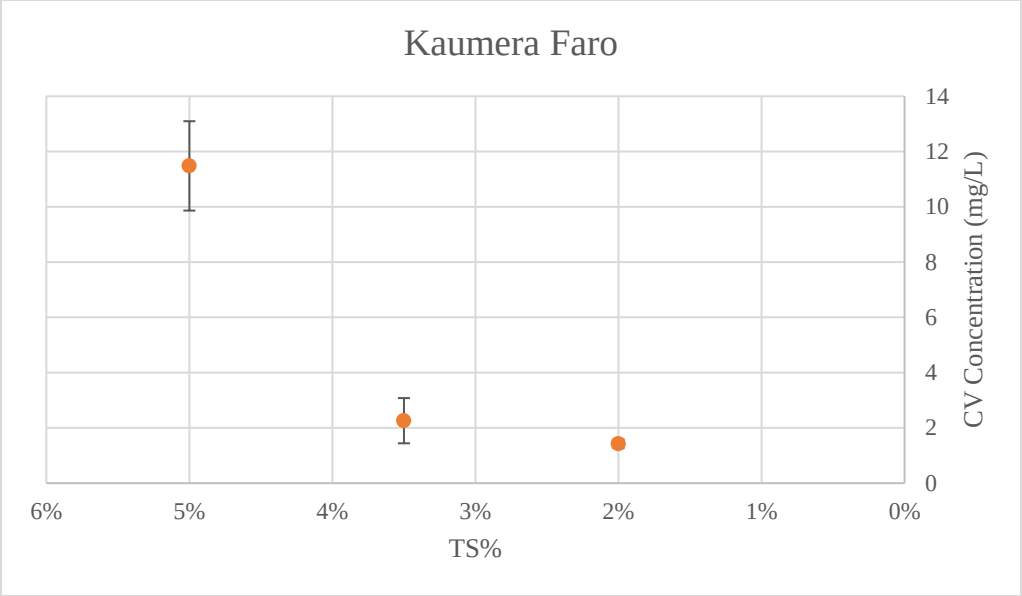


Figure 16: CV concentration vs. TS% for Kaumera Faro.

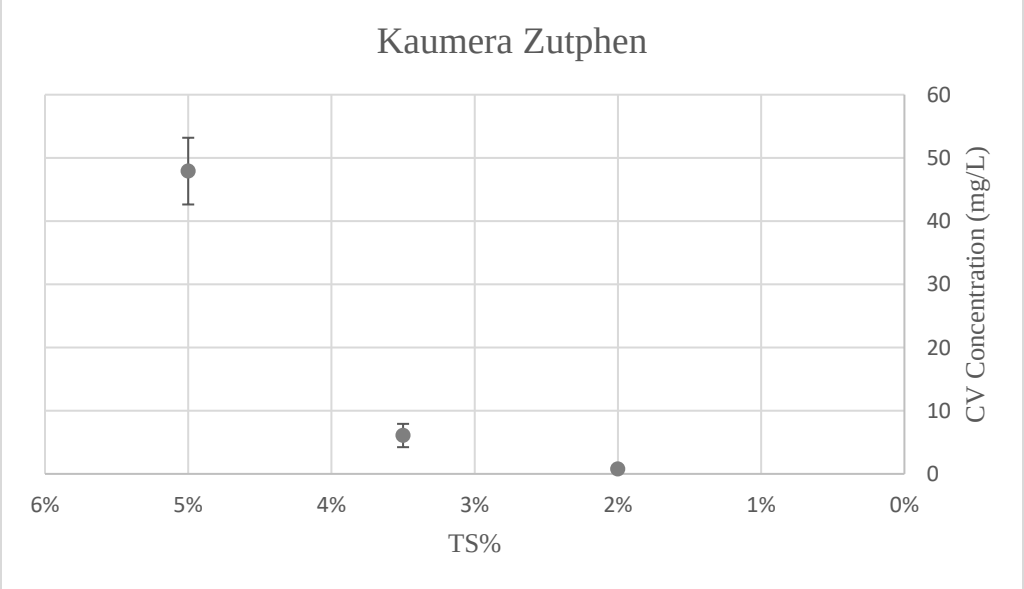


Figure 17: CV concentration vs. TS% for Kaumera Zutphen.

Furthermore, the different Kaumera samples were compared to each other at the different concentrations (TS%) (see figures 18, 19, and 20). The data obtained at 5% TS exhibited the most significant statistical differences among the Kaumera samples, making it the most representative result. At this concentration, Kaumera Utrecht demonstrated the highest measured adhesion, followed by Kaumera Zutphen and Kaumera Faro. Kaumera Utrecht exhibited an adhesive property 4.5 times higher than Kaumera Zutphen (the benchmark). In contrast, the adhesive property of Faro was only one-fourth that of Kaumera Zutphen.

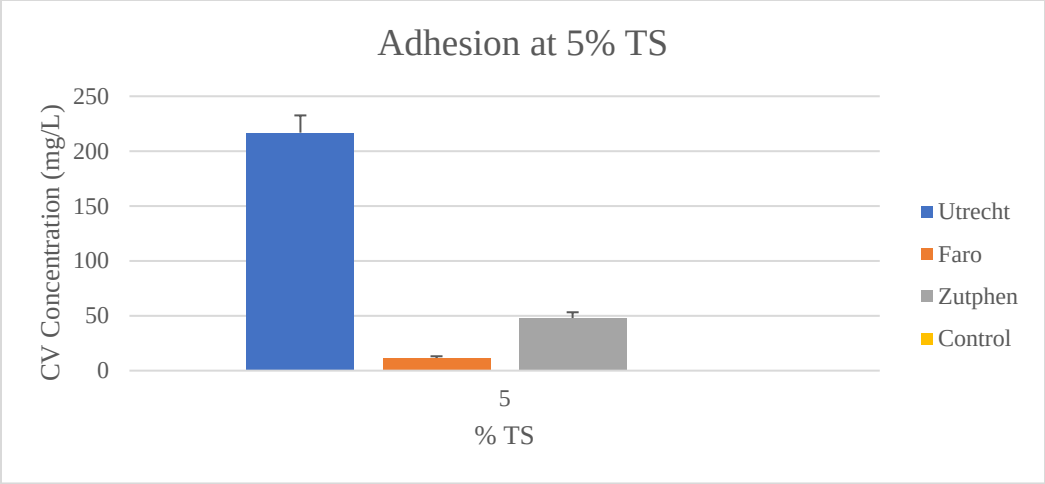


Figure 18: adhesion for the different Kaumera at 5% TS.

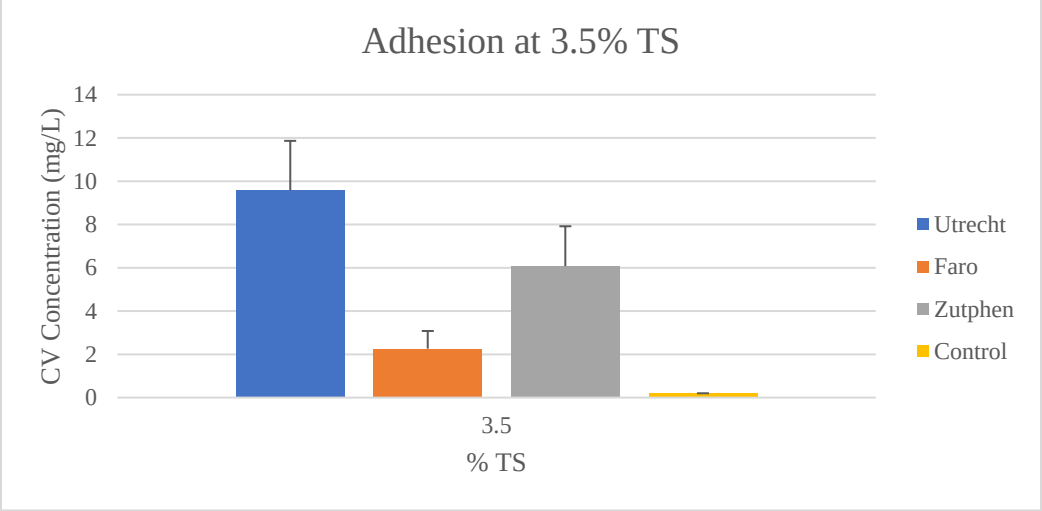


Figure 19: adhesion for the different Kaumera at 3.5% TS.

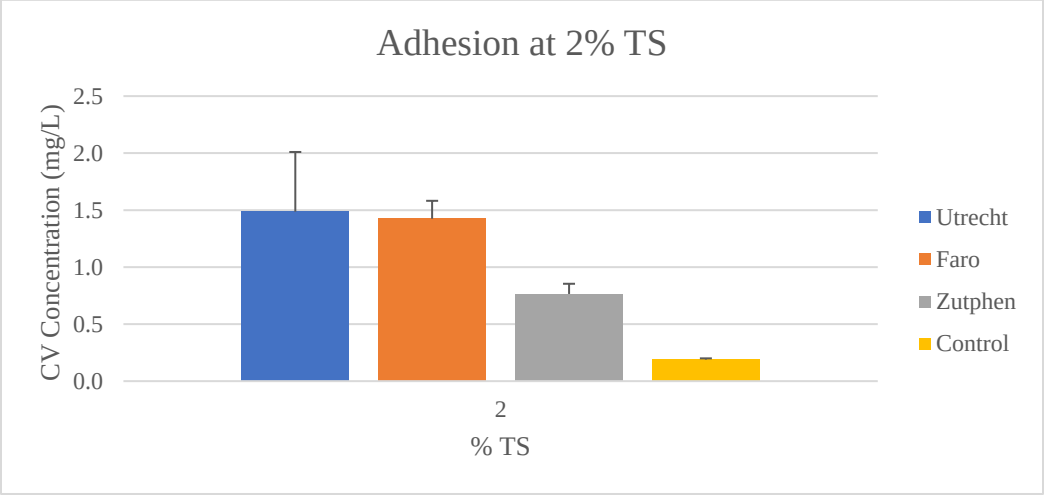


Figure 20: adhesion for the different Kaumera at 2% TS.

6. Discussions

6.1. Chemical Composition

6.1.1. VS/TS Analysis

The results outlined in table 7 indicate that Kaumera Faro exhibits slightly higher values for total TS% and VS% compared to Kaumera Utrecht. Still, these findings align with the observations reported by STOWA (2019), where it was noted that the total TS% of Kaumera generally falls within the range of 5-12% (see section 2.3.2.1.). In terms of organic matter, both Utrecht and Faro surpassed the presented value of 70% VS as presented in the same study. Variations in TS% and VS% can be attributed to differences in the influent wastewater, operation of the Nereda® reactor (e.g., SRT/HRT), sludge composition, or the specific acids utilized for the Kaumera Nereda Gum® precipitation (see section 6.1.2). Additionally, the different centrifuge settings employed also contribute to the TS% and conversely VS%, as highlighted in the STOWA report of 2019.

6.1.2. Total Proteins and Total Carbohydrates

Proteins were found to be the dominant component over carbohydrates in both Kaumera samples. Considering the results from the colorimetric assays, the protein-to-sugar ratio for Utrecht and Faro was 1.92:1 and 4.00:1, respectively.

A notable difference in protein content was observed when comparing Kaumera Utrecht and Kaumera Faro, with Kaumera Faro showing a significantly higher value. Several factors could account for this discrepancy. One hypothesis that could explain this difference is related to the choice of acid used for precipitating Kaumera Nereda Gum®. In the Faro pilot, sulfuric acid is employed, while hydrochloric acid is utilized in Utrecht. The use of an acid results in the protonation of carboxyl and amine groups present in amino acids, leading to the occurrence of hydrogen bonding (S. Picken, personal communication, May 6, 2023). Nonetheless, the sulfate ions in Faro could also be acting as crosslink reagents, covalently linking proteins by forming bonds between amino acid functional groups (i.e., amine, carboxyl). Sulfur and sulfur compounds possess the ability to facilitate crosslinking, which results in the creation of a three-dimensional network that interconnects multiple chains of the polymer (Akiba & Hashim, 1997). Consequently, this phenomenon could result in a significant increase in protein concentration and could contribute to higher percentages of TS, VS, and extraction yield, which was also observed for Kaumera Faro.

When comparing the protein concentrations of Kaumera Utrecht and Kaumera Faro with the findings of Felz et al. (2019), it was observed that the concentrations were within a similar range as the reported values. Their study showed that when using the same BCA assay kit and albumin as standard, proteins constituted approximately 38 wt% of the organic matter (VS) in Kaumera Dinxperlo. In contrast, Kaumera samples from Utrecht and Faro showed protein contents of approximately 32 wt% VS and 46 wt% VS, respectively. However, the study of Felz et al. (2019) used EPS samples extracted in the lab with sodium carbonate, while the samples used in this study were extracted in the pilot with potassium hydroxide. Additionally, a different concentration of NaOH was used as diluent.

The protein content obtained with the BCA assay can also be compared to an estimated protein content calculated by using a nitrogen-to-protein conversion factor. Assuming that all inorganic

nitrogen is present in the form of ammonium (since nitrate and nitrite concentrations in AGS are already negligible [Bahgat et al., 2023]), the organic nitrogen in Kaumera samples can be multiplied by a suitable conversion factor to determine the protein content. These conversion factors vary depending on the type of biomass, but a conversion factor of 6.25 has previously been utilized for EPS (Frølund et al., 1996). Thus, based on the total nitrogen levels provided in table 12 and the ammonium concentrations presented in table 14 for Kaumera Utrecht and Kaumera Faro, the organic nitrogen content was determined to be 78 mg organic N/g VS and 86 mg organic N/g VS, respectively. By applying a conversion factor of 6.25 to these values, the protein content for Kaumera Utrecht was calculated to be 49 wt% VS, while for Kaumera Faro it was determined to be 53 wt% VS. The protein content values obtained from this method showed a similar magnitude compared to the BCA assay results. Nevertheless, the protein concentrations determined by the BCA assay were lower for both Kaumera samples. There was also a significant difference in the relative comparison between Kaumera Faro and Kaumera Utrecht between the two methods. The BCA assay showed a relative difference of 41% between the two samples, while this method resulted in a relative difference of just 10%.

In his study, Felz (2019) concluded that results obtained with colorimetric methods have to be interpreted very carefully, as the BCA assay (Avella et al., 2010; Zhou et al., 2016; Ras et al., 2008) and the phenol-sulfuric acid method (Zhou & Stuckney, 2016) can produce inaccurate results when analyzing EPS. Several drawbacks of using colorimetric methods to analyze EPS include the high dependency on the standard compound selection, a lack of suitable standards which feature a similar composition with the analyzed sample, and cross-interference among EPS compounds in the measurements (e.g., humic acids and galacturonic acid). Therefore, it was determined that more advanced methods are needed in order to draw conclusions about EPS composition.

Furthermore, concerning the foliar application, proteins can be a significant factor to consider due to their association with biostimulation (section 2.5.1.). Therefore, a Kaumera Nereda Gum® with a substantial protein content holds considerable promise for further investigation. Apart from examining the impact of sulfuric acid on the protein content of Kaumera Nereda Gum®, another parameter worth exploring is the sludge retention time (SRT) of the Nereda® system. In a study conducted by Zhang et al. (2019), AGS reactors with varying SRTs were evaluated, leading to the conclusion that a short SRT of 6 days facilitated the formation of dense aerobic granules containing higher levels of tryptophan and protein-like substances within the EPS. On the other hand, an SRT of 12 days resulted in loosely formed granules with lower amounts of tryptophan and protein-like substances. Another study observed that tightly-bound EPS in AGS reactors contained nearly twice the amount of proteins compared to sugars, whereas loosely-bound EPS contained more sugars than proteins (Basuvaraj et al., 2015). This suggests that there is potential to modify parameters within the Nereda® or Kaumera Nereda Gum® process in order to achieve the desired characteristics of the product.

In respect to carbohydrate content, Kaumera Utrecht showed a significantly higher concentration than Kaumera Faro. There are several factors that can affect carbohydrate content of EPS, such as the microbial species, carbon substrate, nutrients (N, P), the extraction method used, and the AGS

operation process as mentioned above (Nouha et al., 2017). Additionally, in the context of foliar application, carbohydrates can be an interesting component as it can be related to adhesion. Further details on this topic can be found in section 6.2.2.

When comparing the carbohydrate content of Kaumera Utrecht and Kaumera Faro with the findings reported by Felz et al. (2019), it can be observed that the concentrations of carbohydrates were of the same magnitude. When they used glucose as a standard to measure carbohydrates, a content of 15 wt% VS was reported. Meanwhile, Kaumera Utrecht and Faro exhibited carbohydrate contents of approximately 17 wt% VS and 11 wt% VS, respectively. However, it is important to consider the differences in method development as mentioned earlier.

In another study, it was determined that Kaumera Utrecht had a carbohydrate content of 8.8% (w/w TS), which corresponds to approximately 11 wt% VS (De Roos, 2022). This low carbohydrate content compared to the 17 wt% VS reported in this study could be attributed to the use of Milli-Q water as a diluent instead of (0.1 M) NaOH. Since a high pH is necessary for complete dissolution of Kaumera Nereda Gum®, using Milli-Q water results in incomplete dissolution. Moreover, De Roos (2022) also used a mix of C5 and C6 sugars to construct the calibration curve as opposed to just using glucose, and measured absorbances at 482 nm. Thus, differences in method development can account for variations in results.

Furthermore, De Roos (2022) employed a standard consisting of a mixture of sugars, which may prove to be a more effective approach compared to the utilization of a single saccharide as used in this study. Felz et al. (2019) proposed that incorporating a mixture of sugars, rather than a single saccharide, is a more suitable choice due to the diverse array of sugars found in EPS. Consequently, a broader representation in the standard could increase the accuracy of the carbohydrate measurement in EPS.

Table 16 presents a summary of the carbohydrate and proteins results measured in this research, as well as the values presented in literature.

Table 16: comparison of carbohydrate and protein content in Kaumera with literature values.

Kaumera Sample	*Carbohydrates (wt% in total organic mass)*	*Proteins (wt% in total organic mass)*	*Notes (Differences)*
Utrecht	17, 11 (De Roos, 2022)	32	De Roos (2022) used a different diluent (Milli-Q water), standard (mix of C5 and C6 sugars), and wavelength (482 nm). There was also incomplete dissolution of Kaumera Nereda Gum®.
Faro	11	46	-
Dinxperlo	15 (Felz et al., 2019)	38 (Felz et al., 2019)	Lab-extracted EPS with sodium carbonate. Used a different concentration of diluent (0.02 M NaOH). For carbohydrates, same standard (glucose) was measured but different wavelength (487 nm). For proteins, same standard (albumin) and wavelength (562 nm).
Zutphen	6 (De Roos, 2022)	-	Same as above (Kaumera Utrecht)

6.1.3. FTIR-ATR

The FTIR-ATR results showed that Kaumera Utrecht and Faro have similar spectra. Moreover, these results confirmed that carbohydrates and proteins are present in Kaumera Nereda Gum®. Polysaccharides are composed of alcohol, aldehyde, ketone, ether, and carboxylic acid functional groups (Harimawan & Ting, 2016). In contrast, proteins are associated with amine, amide, and carboxylic acid functional groups. Therefore, the intense absorbance for alcohol, amine, and amide groups confirmed that carbohydrates and proteins are the predominant components. Furthermore, when peak #5 (Amide I) is qualitatively compared to peak #10 (primary alcohol) for both Kaumera Utrecht and Faro, it can be concluded that the protein-to-carbohydrates ratio is higher for Faro than for Utrecht, confirming the previous results.

One drawback encountered when analyzing FTIR results is the potential for discrepancies among different literature sources when assigning peaks to specific functional groups. Additionally, there can be instances of overlapping functional groups, wherein two or more functional groups may be present at the same wavelength. It can also occur that individual functional groups fall outside the quoted ranges due to the influence of other functional groups within a molecule, the impact of preferred spatial orientations, and environmental effect on the molecule (Coates, 2006).

6.1.4. pH

The pH levels of both Kaumera Utrecht and Kaumera Faro were highly acidic, approximately 2.6. This acidity is achieved by introducing an acid during the final stage of the production process to precipitate the Kaumera Nereda Gum®. Across all pilots, it is customary to lower the pH of Kaumera to a range of 2-3. As discussed in section 2.5.4, it is generally preferred for the solution to have a slightly acidic pH when mixed with other fertilizers and water. Nonetheless, since the optimal pH varies across nutrients, the pH of the Kaumera solution would have to be adjusted based on the specific target nutrient being considered.

6.1.5. Electrical Conductivity

The EC levels of both Kaumera Utrecht and Kaumera Faro exceed the recommended threshold of 3 mS/cm for foliar fertilizers. Therefore, using Kaumera Utrecht or Kaumera Faro in its original form is likely to pose problems. However, the potential risk of salinity-related issues is already minimized by Koppert's practice of diluting Kaumera Nereda Gum® with other fertilizers and water. Still, Koppert should evaluate whether a washing step (as described in section 2.5.5) is necessary as the final ionic concentrations in the Kaumera solution depend on the specific recipe used to prepare the fertilizer solution.

Furthermore, as depicted in table 11, the supernatant of Kaumera Faro has a higher EC than the supernatant of Kaumera Utrecht. This is also corroborated with the high ionic concentrations obtained from the IC. However, when the EC was measured and then normalized by the TS% for the total Kaumera, Kaumera Utrecht exhibited an even higher conductivity. This indicates that, overall, seawater intrusion did not have a significant impact on the salinity of the analyzed Kaumera Faro. Nevertheless, it is essential to examine other batches of Kaumera Faro from different dates, as seawater intrusion can vary considerably on a daily basis. Understanding whether the salinity of Kaumera Faro is truly influenced by the salinity of the influent or not becomes imperative, especially considering the goal of year-round Kaumera production. It is also

fundamental to assess how seasonal fluctuations can impact the properties of Kaumera Nereda Gum®.

6.1.6. Elemental Composition

6.1.6.1. Kaumera Samples

Table 17 allows for a comparison of the elemental composition of Kaumera Utrecht and Kaumera Faro with the elemental composition reported in STOWA (2019) for Kaumera Epe, Vroomshoop, and Dinxperlo. In general terms, the concentrations of elements appear to fall within a similar range for all samples, except for iron and phosphorus. Notably, Kaumera Utrecht exhibits a concentration of iron that is 12 times higher than that in Faro and approximately 2.7 times higher than the average concentration found in Epe, Vroomshoop, and Dinxperlo. This could be explained by the iron dosing that occurs at several locations in Utrecht WWTP to aid in phosphorous binding or to differences in wastewater influent (P. Wilfert, personal communication, May 26, 2023).

Table 17: elemental composition comparison between Kaumera Utrecht, Faro, Epe, Vroomshoop, and Dinxperlo. The last column was obtained from STOWA, 2019.

*Element/Ion**	*Unit*	*Utrecht*	*Faro*	*Epe, Vroomshoop, Dinxperlo*
C	%TS	40.24	42.92	-
H	%TS	6.15	6.36	-
N	%TS	6.61	7.36	6-9
S	mg/g DM	-	34.00	-
K	mg/g DM	28.18	40.82	-
Fe	mg/g DM	23.33	1.93	7-10
P	mg/g DM	20.76	5.95	20-30
Cl^-*	mg/g DM	-	9.36	-
Na	mg/g DM	1.36	7.06	-
Ca	mg/g DM	4.70	1.71	4-5
Al	mg/g DM	1.68	2.86	3-5
Mg	mg/g DM	0.97	1.52	-
Zn	mg/g DM	0.26	0.57	0.4-1
Cu	mg/g DM	0.28	0.14	0.1-0.5
Ba	mg/g DM	0.07	<0.01	-
Pb	mg/g DM	0.06	<0.01	0.028-0.071
Ti	mg/g DM	0.04	0.03	-
Sr	mg/g DM	0.04	<0.01	-
Mn	mg/g DM	0.02	<0.01	-
Cd	mg/g DM	<0.01	<0.01	0.0005-0.001
Cr	mg/g DM	<0.01	<0.01	-
Ni	mg/g DM	<0.01	<0.01	-
As	mg/g DM	<0.01	<0.01	0.002-0.003

To determine whether Kaumera Utrecht and Kaumera Faro comply with the fertilizer regulations in the Netherlands, it is necessary to consider the intended dosage. Currently, Koppert estimates that approximately 35 liters of Kaumera is used per hectare per year (H. Mikkelsen from Koppert, personal communication, May 30, 2023). When this value is converted to a dry matter basis, this equals 2.09 kg per hectare per year for Kaumera Utrecht and 3.02 kg per hectare per year for Faro. These quantities are significantly lower than the permitted dosage established in the Fertilizers Decree, which stands at two tons per hectare per year. It is worth noting that the Fertilizers Decree primarily focuses on soil fertilization rather than foliar fertilization, which explains the relatively high limit.

Next, the heavy metal concentrations of both Kaumera samples were compared to the maximum limits presented in the Fertilizers Decree of the Netherlands. This comparison can be observed in table 18. No definitive conclusions could be made regarding cadmium or lead. Cadmium levels were found to be below the detection limit of the ICP-OES, making it challenging to make accurate comparisons against the maximum allowable limit. Additionally, the ICP-OES does not measure mercury. Moreover, the concentrations of chromium, nickel, lead, and arsenic in Kaumera samples from Utrecht and Faro were found to be below the established maximum limits. However, the concentration of copper surpasses the established limit in both Utrecht and Faro, and Faro does not comply with the guidelines for zinc. Nevertheless, these limit values primarily pertain to soil fertilization, making it an unfair comparison since foliar fertilization typically involves significantly smaller dosages.

Table 18: comparison of heavy metal concentrations in Kaumera Utrecht and Faro to the maximum permitted concentrations presented in the Fertilizers Decree (2021).

Heavy metals	*Maximum limits for sewage sludge (mg/g DM)*	*Kaumera Utrecht (mg/g DM)*	*Satisfies: Yes/No*	*Kaumera Faro (mg/g DM)*	*Satisfies: Yes/No*
Cd	0.00125	<0.01	-	<0.01	-
Cr	0.075	<0.01	Yes	<0.01	Yes
Cu	0.075	0.28	No	0.14	No
Hg	0.00075	-	-	-	-
Ni	0.03	<0.01	Yes	<0.01	Yes
Pb	0.1	0.06	Yes	<0.01	Yes
Zn	0.3	0.26	Yes	0.57	No
As	0.015	<0.01	Yes	<0.01	Yes

Therefore, to ensure a fair comparison, the dosages of Kaumera Utrecht and Kaumera Faro (2.09 kg per hectare per year and 3.02 kg per hectare per year, respectively) were taken into account, and the maximum limits for heavy metals were adjusted based on a dosage of two tons per hectare per year (assuming the maximum dosage is used). This adjustment involved multiplying the concentrations of heavy metals by their respective doses, resulting in concentrations expressed in grams per hectare per year, which allows for a more meaningful comparison (see table 19). In this case, it was found that all the concentrations of heavy metals in both Kaumera samples were below

the maximum allowed concentration. For cadmium, an initial concentration of 0.01 mg/g DM was assumed.

Table 19: comparison of heavy metal concentration of Kaumera Utrecht and Faro to the maximum permitted concentrations presented in the Fertilizers Decree, considering dosage.

Heavy metals	*Maximum limits for sewage sludge (g/ha/year)*	*Kaumera Utrecht (g/ha/year)*	*Satisfies: Yes/No*	*Kaumera Faro (g/ha/year)*	*Satisfies: Yes/No*
Cd	2500	0.02	Yes	0.03	Yes
Cr	150	0.02	Yes	0.03	Yes
Cu	150	0.59	Yes	0.42	Yes
Hg	1.5	-	Yes	-	Yes
Ni	60	0.02	Yes	0.03	Yes
Pb	200	0.13	Yes	0.03	Yes
Zn	600	0.54	Yes	1.72	Yes
As	30	0.02	Yes	0.03	Yes

The concentration of nutrients in Kaumera Utrecht and Faro were also compared to the recommended rates for appropriate plant nutrition depicted in table 2. Assuming five applications per year, it becomes apparent that all the concentrations for both Kaumera fall significantly below the recommended levels (see table 20). As a result, it can be concluded that the primary purpose of Kaumera Nereda Gum®, given its low dosage, is not to serve as a nutrient source. Instead, its main focus lies in providing other advantageous properties such as biostimulation, water-holding capacity, adhesion, and the formation of a protective biofilm. This statement was reaffirmed by H. Mikkelsen from Koppert on May 30, 2023.

Table 20: comparison of nutrient content in Kaumera Utrecht and Faro to the recommend concentrations for foliar fertilization.

Nutrient	*Recommended Rate (kg/ha/year)*	*Utrecht (kg/ha/year)*	*Faro (kg/ha/year)*
Nitrogen	5.6-56.0	0.01	0.02
Potassium	22.4	0.06	0.09
Magnesium	2.8-11.2	<0.01	<0.01
Iron	5.6-11.2	0.05	<0.01
Manganese	5.6-11.2	<0.01	-
Zinc	1.4	<0.01	<0.01

6.1.6.2. Supernatant Kaumera Samples

As mentioned before, the elevated EC of the supernatant of Kaumera Faro indicates a higher concentration of dissolved salts compared to the supernatant of Kaumera Utrecht. The increased conductivity can be attributed primarily to the prominent levels of sulfate, sodium, potassium, and magnesium ions, which are commonly found in seawater, along with calcium and chloride. However, the supernatant of Kaumera Utrecht has higher concentrations of calcium and chloride ions, though only slightly higher for calcium. The addition of hydrochloric acid in the production

process of Kaumera Utrecht explains the expected high chloride levels. Similarly, the high concentration of sulfates in the supernatant of Kaumera Faro can also be attributed to the introduction of sulfuric acid in its production process.

By analyzing the relative proportions of ions or elements in the supernatant compared to the total Kaumera (as presented in tables 12 and 13), it was possible to estimate the potential removal that could occur during a washing step, where the supernatant is discarded. Nevertheless, certain percentages exceeded 100%. In the case of Kaumera Utrecht, this discrepancy could potentially be attributed to equipment inaccuracies. Meanwhile, the elevated percentages observed for Kaumera Faro, particularly for chloride concentration, can be explained by the fact that the Kaumera sample from which the supernatant was taken was different from the one utilized for the "total Kaumera". Thus, an additional attempt was made to calculate these ratios more accurately for Kaumera Faro (refer to table 21).

The initial assumption was that the influent of the Faro Nereda® behaved like seawater in terms of conductivity. The influent exhibited a conductivity of 3.2 mS/cm on December 20, 2022, whereas typical seawater conductivity is 53.9 mS/cm. By using the known ionic concentrations contributing to the seawater conductivity (see Appendix G), it was possible to calculate the estimated ionic concentrations associated with a conductivity of 3.2 mS/cm. These estimated ionic concentrations were then presumed to be present in the liquid phase or supernatant of Kaumera Faro. Subsequently, these ionic concentrations were divided by the measured elemental composition of the total Kaumera Faro of that same day (December 20, 2022) to derive the percentages. Furthermore, it is important to emphasize that the ions used for these calculations were not introduced into Kaumera during the extraction process.

Table 21: relative concentration in supernatant in respect to total Kaumera Faro calculated via conductivity measurements.

Ions	*Percentage (%) in supernatant Faro relative to total Kaumera Faro*
Cl^-	127%
Na^+	94%
SO_4^{2-}	33%
Mg^{2+}	76%
Ca^{2+}	16%

The fact that chloride still exceeds 100% can be attributed to the limitations of the assumptions made. It should also be noted that other components present in the wastewater, such as solid materials, can impact the conductivity measurements, causing the influent's conductivity to deviate from that of seawater.

Nevertheless, taking into account the results obtained from both Kaumera Utrecht and Kaumera Faro, it can be concluded that washing has the potential to remove sodium up to 96%, and a significant reduction in chloride concentration is also achievable. However, other beneficial

nutrients would be removed as well. Since the goal is not to utilize Kaumera Nereda Gum® for nutritional purposes, washing the Kaumera Nereda Gum® can be a viable option to reduce salinity if the solution remains highly saline after dilution.

6.2. Kaumera Adhesion Protocol

6.2.1. Method Development

The developed protocol represented a significant advancement in quantifying the intricate property of adhesiveness in Kaumera samples. An indication that the protocol worked properly was how adhesion decreased as the concentration of the Kaumera solution decreased. This occurs because at lower concentrations, there may be lower molecules available to form strong intermolecular bonds with the surface. Nonetheless, the relationship between adhesion and concentration was not linear, suggesting that the relationship is not as straightforward and can be influenced by a variety of factors. Moreover, this protocol is easy to use and time-effective, as it only takes one day to complete. In addition, it is inexpensive since the required materials can be found commonly in labs and no expensive equipment is needed. This protocol can certainly be used as a screening tool to get a notion of how adhesive a Kaumera sample is, but it is still advised to use another method (i.e., peel or probe testing) if more accurate results are required.

A significant drawback of the protocol was the high RSD between replicates, which ranged from 7-36% when testing Kaumera Utrecht, Faro, and Zutphen. Therefore, the goal of achieving an RSD of 5% or less was not met. For a protocol to be accepted as a standard method, the RSD needs to be low, indicating that the measurements are precise and reproducible (Allkja et al., 2021). The high RSD found in the results could perhaps be explained by the formation of bubbles in each individual cuvette, varied amount of force and speed to shake out the Kaumera solution, among others. Still, a high RSD was expected, as the original Microtiter Dish Biofilm Formation Assay suggests using up to 8 replicates (O'Toole, 2011).

Furthermore, it was observed that there are more statistical differences among Kaumera samples for the concentration of 5% TS. The relative standard deviation (RSD) for the 5% TS concentration is also significantly lower compared to the other concentrations. It reaches up to 14%, whereas for the 2% and 3.5% TS concentrations, it goes up to 36%. Therefore, comparisons between different Kaumera samples should be done for this specific concentration.

Unfortunately, time limitations prevented the execution of the additional method described in section 4.2.2.4.4. to corroborate patterns with the adhesion protocol. The details on how this test could be conducted in the future are discussed in section 7.2.

Table 22 presents the initial goals set for the adhesion protocol and indicates whether or not they were successfully accomplished.

Table 22: summary of requirement compliance: yes/no assessment.

Requirements	*How?*	*Was the goal achieved?*
Accurate	Compared with another complementary technique	No: due to time constraints, it was not possible to carry out a complementary technique.
Repeatable	≤ 5% relative standard deviation between replicates	No: Up to 14% RSD for 5% TS and up to 36% RSD for 3.5% TS and 2% TS.
Time-effective	Short time test (1 day)	Yes
Economical	Inexpensive materials	Yes

In addition, it is important to highlight that it is challenging to correlate the results from the adhesion protocol which were conducted on polystyrene to the surface of leaves. Adhesion on foliage depends on several factors, such as the contact angle, interfacial energy, chemical affinity, roughness, ductility of the film, and possibly physical interlocking (S. Picken, personal conversation, May 24, 2023). Moreover, it also depends on the type of leaf, as they differ in texture and level of hydrophobicity. The results in the adhesion test could be representative for leaves with smooth surfaces and high hydrophobicity, as well as leaves with the same surface energy and contact angle. However, since there are so many types of leaves, that is why a simpler surface was chosen for the protocol, especially one that could be easily found in a laboratory. Furthermore, other studies have employed CV staining and utilized plate readers to extend their findings to other types of surfaces. These studies have applied this approach to diverse contexts such as medical and industrial settings (Shanks et al., 2005; Djordjevic et al., 2002).

In the context of foliar fertilization, it is essential to employ an effective application technique when administering Kaumera solution, specifically to the leaves, as it significantly impacts the outcome. Currently, Kaumera Nereda Gum® is being diluted with other fertilizers and water. When sprayed, Kaumera experiences a high deformation, which enables it to overcome the yield stress (S. Picken, personal conversation, June 13, 2023). As a result, the Kaumera solution has the ability to flow, allowing the formation of a homogeneous film (low contact angle, high surface energy) that covers the leaf (see figure 6). Thus, spraying leads to the most desired outcome, as drops or patches would limit surface coverage. In the case of the protocol, force was applied when shaking out the Kaumera solution from the cuvettes, allowing it to flow out. Indeed, it was harder to shake out the higher concentration Kaumera solution as physical connections of solid parts gave it a higher strength to resist flow (Simonovich, 2017). Additionally, it was observed that the higher concentration of Kaumera solution resulted in a patchy pattern of adhesion to the surface (figure 15). Therefore, in the case that Kaumera Nereda Gum® is placed on a surface, and a uniform film is desired, it is necessary to dilute it accordingly. Nevertheless, if Kaumera Nereda Gum® is diluted too much, it risks losing its adhesive properties. As a result, it is necessary to find an optimal concentration or dilution factor in which Kaumera Nereda Gum® is able to spread in a homogenous pattern but also has high adhesion capabilities.

6.2.2. Results: Kaumera Comparison

As can be seen in figure 18, Kaumera Utrecht shows superior adhesive properties on abiotic surfaces than Kaumera Zutphen (benchmark).This leads to the conclusion that Kaumera Utrecht

shows promise as an effective adhesive agent for foliar fertilization. On the other hand, Kaumera Faro exhibited a lower adhesive property than Kaumera Zutphen. However, before discarding the option of using Kaumera Faro as adhesive agent, it should still be tested on leaves as the surfaces can differ significantly.

One reason that could explain the higher adhesive property of Kaumera Utrecht compared to Kaumera Faro is due to its higher carbohydrate content. In recent studies, it was concluded that polysaccharides promote the adhesion strength of the EPS while proteins have lesser adherence effects (Harimawan & Ting, 2016). In a study conducted by Harimawan & Ting (2016), the adhesion of EPS coming from *B. subtilis* and *P. aeruginosa* were measured. EPS from *B. subtilis* had more proteinaceous compounds, while EPS from *P. aeruginosa* had greater carbohydrate components. It was observed that the high-carbohydrate EPS had superior adhesion, and the lack of adhesion of the high-protein EPS was correlated with the absence of exopolysaccharides. Other studies have also supported this claim. A study by Herald & Zottola (1989) showed that compounds that disrupt carbohydrates decrease adherence of EPS of *P.fragi* to stainless steel, while compounds specific for proteins had less effect on adherence. Another study showed that lipopolysaccharides and alginate polysaccharides of EPS from *P. aeruginosa* were important components influencing initial surface attachment (Mai et al., 1993; Shi & Zhu, 2009). To better prove this hypothesis in this study, measuring the carbohydrate content of Kaumera Zutphen under the same conditions as Kaumera Utrecht and Faro would have been optimal in order to see if there exist a direct relationship between carbohydrate content and adherence among all Kaumera samples. Moreover, fixing VS%, carbohydrate content, or protein content across all Kaumera samples instead of TS% could also give valuable insights on how the adhesion changes.

Furthermore, adhesion can be examined from a rheological perspective. Across all concentrations (2%, 3.5%, and 5% TS), Kaumera Faro had visually more fluid-like characteristics compared to Kaumera Utrecht and Kaumera Zutphen. This can also be confirmed by its low yield stress (0.13 Pa) compared to Kaumera Utrecht (0.54 Pa) and (washed) Kaumera Zutphen (0.16 Pa) at 2.4% TS (A. Raja, unpublished data). In order for Kaumera Faro to behave similarly like Kaumera Utrecht or Kaumera Zutphen in terms of adhesion under the predefined concentrations, it is hypothesized that the concentration (TS%) of Faro would have to be increased significantly, allowing for the build-up of yield stress.

7. Recommendations

7.1. Carbohydrate and Protein Quantification

To improve the characterization of EPS for future studies, the utilization of more advanced methods is necessary. As mentioned previously, employing colorimetric assays such as the phenol-sulfuric method and the BCA assay may yield results that are potentially inaccurate (Felz, 2019). Therefore, it is advisable to utilize advanced techniques such as chromatographic methods. One such method is high-performance anion exchange chromatography (HPAEC) combined with pulsed electrochemical detection (PED), which has proven highly valuable for the determination and quantification of carbohydrates (Corradini et al., 2012). HPAEC-PED enables effective separation of mixtures containing simple sugars, oligosaccharides, and polysaccharides, and allows their quantification using standard curves. Other studies have also employed reversed-phase high-performance liquid chromatography (RP-HPLC) or HPLC coupled with evaporative light scattering detector (ELSD) to measure proteins with high precision and selectivity (Grotefend et al., 2012; Hussain et al., 2019).

7.2. Adhesion Analysis

To further explore the adhesive properties of Kaumera Nereda Gum®, an additional technique that can be employed is size-exclusion chromatography (SEC). As mentioned in section 2.5.7.2, superior adhesive polymers typically contain both high and low molecular weight fractions (Roos et al., 2002). Consequently, conducting SEC analysis on Kaumera Utrecht and Faro samples would possibly enable more conclusions to be drawn.

Moreover, there are several steps that can be implemented to improve the formulated adhesion protocol in this study. Initially, increasing the number of replicates from four to eight is recommended to reduce the observed high RSD. Considering that the 5% TS concentration exhibited the most significant statistical differences among the Kaumera samples as well as the lowest RSD among the replicates, it is suggested to exclusively test this concentration. Consequently, only eight samples would be tested, minimizing the manual labor required to conduct the test. Additionally, another method that could reduce human error can include using a motored device to shake out the Kaumera samples. This would enable the Kaumera samples to be shaken out of the cuvettes at a constant speed and force. Furthermore, it is worth considering the exploration and utilization of alternative dyes apart from CV. Several researchers have successfully employed dyes such as safranin, Congo red, or calcofluor white to quantify the overall biomass of biofilms (Sung et al., 2021). Safranin, in particular, offers several advantages, such as improved reproducibility, lower toxicity, and greater ease of use compared to CV staining methods (Ommen et al., 2017). In general, this protocol should also be conducted by other users, preferably in other labs, to observe how reproducibility is affected.

As previously discussed, it was not possible to conduct the complimentary technique to corroborate the results from the adhesion protocol. For future testing, different types of plastic surfaces (e.g., polystyrene [PS], Polyethylene terephthalate [PET], polypropylene [PP]) could be immersed into different Kaumera Nereda Gum®, and their weight measured before the immersion

and after the film has dried to assess changes in weight. This would give a notion of how much substance has adhered to the surface. To make all Kaumera Nereda Gum® comparable in terms of weight, a parameter such as TS% should be fixed across all samples.

7.3. Kaumera Nereda Gum® for Foliar Fertilization

Currently, Kaumera Nereda Gum® for foliar fertilization is just in its initial stages. For now, a low dosage of 35 liters per hectare per year is applied and there is evidence of its benefits, including biostimulation, biofilm formation, among others (H. Mikkelsen from Koppert, personal communication, May 30, 2023). However, it is still necessary to optimize the dosage, as increasing the dosage could perhaps enhance its water-holding capacity or be able to provide more nutrients in order to decrease fertilizer use. For the latter, measuring and controlling that heavy metal content do not exceed the guidelines would be imperative. Thus, controlled experiments in which Kaumera Nereda Gum® is applied in different doses on different leaves could be conducted in order to see how the effects vary.

Moreover, it is recommended to conduct further studies to investigate the potential impact of salinity or acid selection on the composition and properties of Kaumera Nereda Gum®. Regarding salinity, it would be valuable to extract and analyze EPS from a laboratory bioreactor fed with hyper-saline water to observe any changes in chemical composition and potential alterations in properties relevant to agriculture. Concerning acid selection, laboratory extractions utilizing both sulfuric acid and hydrochloric acid to extract EPS from the same sludge (e.g., sludge from Utrecht) could be carried out. The extracted EPS can then be analyzed to determine any compositional changes and investigate whether any agriculture-relevant properties are influenced by the choice of acid.

Furthermore, it is important to assess the presence of Per- and Polyfluorinated Substances (PFAS) in Kaumera Nereda Gum®, as they pose significant health risks. PFAS are synthetic chemicals that are hazardous, persist in the environment for a long time, and are poorly biodegradable (RVIM, n.d.). In a monitoring campaign conducted in the Netherlands to measure PFAS levels in eight WWTPs, the concentrations of PFAS in sludge ranged from 10 to 100 µg/kg DM (STOWA, 2021). However, it is important to highlight that none of these WWTPs were Nereda® projects. Currently, it remains unknown how the Kaumera Nereda Gum® production process may affect PFAS, but chemical and thermal treatments have not proven effective in treating them (ITRC, 2022). Additionally, the Netherlands currently does not have a quality standard for PFAS in sewage sludge intended for fertilization (STOWA, 2021). Thus, if PFAS are measured in Kaumera Nereda Gum®, a comparison can be made but with guidelines from other countries. For instance, Norway has proposed a standard of 100 µg/kg DM for total PFAS in sewage sludge applied on land (Eggen et al., 2019). In Germany, the fertilizer regulation (Düngemittelverordnung, 2012) has set a threshold limit of 100 µg/kg DM for the combined levels of Perfluorooctanoic Acid (PFOA) and Perfluorooctane sulfonic acid (PFOS) in sewage sludge.

Another important aspect to consider is the presence of organic micropollutants (OMPs). OMPs, including pharmaceuticals, personal care products, and stimulants, have raised concerns due to their toxicity and ability to accumulate in organisms (Abbasi et al., 2022). Nonetheless, there is limited information available regarding the concentrations of OMPs in AGS. Similar to PFAS,

advanced treatment methods are necessary to effectively address OMPs (Guillossou et al., 2019). Therefore, there is a risk that OMPs are present Kaumera Nereda Gum®. In addition, the Fertilizers Decree of the Netherlands has regulations for OMPs for the category “other organic fertilizers”, but interestingly they do not apply for sewage sludge (Ehlert et al., 2013).

To ensure the safe use of Kaumera Nereda Gum® in fertilization, levels of PFAS and OMPs could be measured in several Kaumera Nereda Gum® and compared to existing guidelines. By doing so, any potential risks can be identified and addressed. However, it is worth mentioning that the current dosage of Kaumera Nereda Gum® used in foliar fertilization is kept very low, which helps to minimize any potential problems.

8. Conclusions

The findings of this study have shed a light on several important aspects regarding the composition and application of Kaumera Nereda Gum® for foliar fertilization.

Regarding pH and EC, there are no limitations in utilizing Kaumera Utrecht and Kaumera Faro for foliar fertilization, as long as appropriate adjustments are implemented. When preparing the spraying solution by mixing Kaumera Nereda Gum® with water or other fertilizers, it is crucial to ensure the pH is appropriately adjusted due to the acidic pH (2.6) of Kaumera Nereda Gum®. For foliar fertilization, achieving a slightly acidic pH is essential for optimal nutrient absorption. Nevertheless, since the ideal pH varies depending on the specific nutrient being targeted, the Kaumera solution must be adjusted accordingly. Koppert also needs to evaluate if washing Kaumera Nereda Gum® to reduce salinity is necessary, considering that the final ionic concentrations depend on the specific mixing ratios used to create the fertilizer solution.

Moreover, it was observed that when comparing the levels of heavy metals in Kaumera Utrecht and Kaumera Faro with the regulations outlined in the Netherlands' Fertilizers Decree, neither Kaumera Utrecht nor Kaumera Faro adhered to the specified copper (Cu) concentration limit. In addition, Kaumera Faro also exceeded the recommended threshold for zinc (Zn). However, when considering the concentrations in relation to the applied dosage, which is significantly low for Kaumera Nereda Gum® (35 liters per hectare per year), the heavy metal concentrations in both Kaumera Utrecht and Kaumera Faro remain well below the set limits. Therefore, it was concluded that the concentrations of heavy metals in Kaumera Nereda Gum® are not a cause for concern and do not pose a threat to human health when this low dosage is used. Additionally, the nutrient levels of Kaumera Utrecht and Kaumera Faro were not superior to the recommended values for effective foliar fertilization under this low dosage. So, in this scenario, it is recommended to combine Kaumera Nereda Gum® with other fertilizers to ensure that the desired levels of nutrition are met.

It was also noted that protein and carbohydrate concentrations hold significant importance for foliar fertilization. The elevated carbohydrate content of Kaumera Utrecht (47% higher compared to Kaumera Faro) could potentially account for its superior adhesive properties. Similarly, the higher protein concentration in Kaumera Faro (41% more than Kaumera Utrecht) may contribute to strong biostimulating effects. Hence, additional testing is necessary to explore the operational mechanisms of these factors specifically within the context of foliar fertilization.

Furthermore, the designed protocol showcased in this study proved to be a valuable screening tool for measuring adhesion in a general sense. Nonetheless, this method should be validated using actual leaves to establish more robust conclusions regarding the adhesion levels of Kaumera Nereda Gum® to foliage. In addition, the results indicated that adhesion decreases significantly with increasing dilution, suggesting that a more concentrated Kaumera solution is advised if adhesion is the primary goal. Importantly, the study demonstrated the potential of Kaumera Utrecht as an adhesive agent, as it exceeded the adhesion levels of Kaumera Zutphen, the established benchmark. On the other hand, the adhesive property of Kaumera Faro was found to be inferior to

that of Kaumera Zutphen. Still, before disregarding Kaumera Faro as a potential adhesive agent for foliar fertilization, it is advisable to conduct testing specifically on the desired type of leaf.

In summary, this research has provided valuable insights into the composition and properties of Kaumera Nereda Gum® in order to assess its use for foliar fertilization. The results contribute to the broader understanding of the potential of Kaumera Nereda Gum® in agriculture and serve as a foundation for further exploration and optimization of its usage in sustainable farming practices.

9. References

Abbasi, N. A., Shahid, S. M., Majid, M., & Tahir, A. (2022). Ecotoxicological risk assessment of environmental micropollutants. In *Elsevier eBooks* (pp. 331–337). https://doi.org/10.1016/b978-0-323-90555-8.00004-0

Agrawal, G. P., Negi, Y. S., Pradhan, S. K., Dash, M., & Samal, S. (2017). Wettability and contact angle of polymeric biomaterials. In *Elsevier eBooks* (pp. 57–81). https://doi.org/10.1016/b978-0-08-100737-2.00003-0

[Águas do Algarve]. (n.d.). Retrieved May 18, 2023. https://www.acciona.com/projects/wwtp-faro/?_adin=02021864894

Akiba, M., & Hashim, A. S. (1997). Vulcanization and crosslinking in elastomers. *Progress in Polymer Science*, *22*(3), 475–521. https://doi.org/10.1016/s0079-6700(96)00015-9

Alami, Y., Achouak, W., Marol, C., & Heulin, T. (2000). Rhizosphere Soil Aggregation and Plant Growth Promotion of Sunflowers by an Exopolysaccharide-Producing *Rhizobium* sp. Strain Isolated from Sunflower Roots. *Applied and Environmental Microbiology*, *66*(8), 3393–3398. https://doi.org/10.1128/aem.66.8.3393-3398.2000

Allkja, J., Van Charante, F., Aizawa, J., Reigada, I., Guarch-Pérez, C., Vazquez-Rodriguez, J. A., Cos, P., Coenye, T., Fallarero, A., Zaat, S. a. J., Felici, A., Ferrari, L., Azevedo, N. F., Parker, A. E., & Goeres, D. M. (2021). Interlaboratory study for the evaluation of three microtiter plate-based biofilm quantification methods. *Scientific Reports*. https://doi.org/10.1038/s41598-021-93115-w

Andreoli, C. V., Von Sperling, M., Fernandes, F., Ronteltap, M., & Von Sperling, M. (2007). *Sludge Treatment and Disposal* (Vol. 6). Van Haren Publishing.

Avella, A., Görner, T., & De Donato, P. (2010). The pitfalls of protein quantification in wastewater treatment studies. *Science of the Total Environment*, *408*(20), 4906–4909. https://doi.org/10.1016/j.scitotenv.2010.05.039

Bahgat, N. T., Wilfert, P., Korving, L., & Van Loosdrecht, M. C. (2023). Integrated resource recovery from aerobic granular sludge plants. *Water Research*, *234*, 119819. https://doi.org/10.1016/j.watres.2023.119819

Basuvaraj, M., Fein, J., & Liss, S. N. (2015). Protein and polysaccharide content of tightly and loosely bound extracellular polymeric substances and the development of a granular activated sludge floc. *Water Research*, *82*, 104–117. https://doi.org/10.1016/j.watres.2015.05.014

Bezzate, S., Aymerich, S., Chambert, R., Czarnes, S., Berge, O., & Heulin, T. (2000). Disruption of the Paenibacillus polymyxa levansucrase gene impairs its ability to aggregate soil in the wheat rhizosphere. *Environmental Microbiology*, *2*(3), 333–342. https://doi.org/10.1046/j.1462-2920.2000.00114.x

Chiaiese, P., Colla, G., Colla, G., Colla, G., & Rouphael, Y. (2018). Renewable Sources of Plant Biostimulation: Microalgae as a Sustainable Means to Improve Crop Performance. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.01782

Ciavatta, C., & Benedetti, A. (2002). Foliar Fertilizers: Legislative Aspects in Europe. *Acta Horticulturae*, *594*, 269–276. https://doi.org/10.17660/actahortic.2002.594.31

Coates, J. D. (2006). Interpretation of Infrared Spectra, A Practical Approach. *Encyclopedia of Analytical Chemistry*. https://doi.org/10.1002/9780470027318.a5606

Cornell University. (n.d.). *Nutrient Management*. Retrieved January 14, 2023, from https://nrcca.cals.cornell.edu/soilFertilityCA/CA1/CA1_print.html

Corradini, C., Cavazza, A., & Bignardi, C. (2012). High-Performance Anion-Exchange Chromatography Coupled with Pulsed Electrochemical Detection as a Powerful Tool to Evaluate Carbohydrates of Food Interest: Principles and Applications. *International Journal of Carbohydrate Chemistry*, *2012*, 1–13. https://doi.org/10.1155/2012/487564

D'Abzac, P., Bordas, F., Van Hullebusch, E., Lens, P. N. L., & Guibaud, G. (2009). Extraction of extracellular polymeric substances (EPS) from anaerobic granular sludges: comparison of chemical and physical extraction protocols. *Applied Microbiology and Biotechnology*, *85*(5), 1589–1599. https://doi.org/10.1007/s00253-009-2288-x

De Kreuk, M., Heijnen, J. J., & Van Loosdrecht, M. C. (2005). Simultaneous COD, nitrogen, and phosphate removal by aerobic granular sludge. *Biotechnology and Bioengineering*, *90*(6), 761–769. https://doi.org/10.1002/bit.20470

De Kreuk, M. K. (2006). *Aerobic Granular Sludge: Scaling-up a New Technology*.

De Oliveira, R. B., Georgieva, P., & De Azevedo, S. F. (2002). Plant and Equipment | Instrumentation and Process Control: Instrumentation. In *Elsevier eBooks* (pp. 234–241). https://doi.org/10.1016/b978-0-12-374407-4.00412-x

De Palma, A. (2020, March 10). *How to Achieve Consistency with Microwave Digestion*. Lab Manager. https://www.labmanager.com/product-focus/how-to-achieve-consistency-with-microwave-digestion-21854#:~:text=Microwave%20digestion%20works%20by%20exciting,base%2C%20speeds%20up%20sample%20homogenization.

De Roos, B. (2022). Extracellular polymeric substances: the link between chemical composition and gel formation [unpublished].

Djordjevic, D., Wiedmann, M., & McLandsborough, L. (2002). Microtiter Plate Assay for Assessment of *Listeria monocytogenes* Biofilm Formation. *Applied and Environmental Microbiology*, *68*(6), 2950–2958. https://doi.org/10.1128/aem.68.6.2950-2958.2002

Draget, K., Skjåk Bræk, G., & Smidsrød, O. (1994). Alginic acid gels: the effect of alginate chemical composition and molecular weight. *Carbohydrate Polymers*, *25*(1), 31–38. https://doi.org/10.1016/0144-8617(94)90159-7

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. H. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, *28*(3), 350–356. https://doi.org/10.1021/ac60111a017

Ehlert, P. A. I., Posthuma, L., Römkens, P. F. A. M., Rietra, R. P. J. J., Wintersen, A. M., Van Wijnen, H., Van Dijk, T. A., Van Schöll, L., & Groenenberg, J. E. (2013). *Appraising Fertilisers: Origins of current regulations and standards for contaminants in fertilisers* (WOT-04-008-023). Statutory Research Tasks Unit for Nature & the Environment (WOT Natuur & Milieu). Retrieved June 15, 2023, from https://edepot.wur.nl/262256

Düngemittelverordnung [Fertilizer Ordinance]. (2012, 5 December). Retrieved June 20, 2023, from https://www.gesetze-im-internet.de/d_mv_2012/

Eggen, T., E.S. Heimstad, V. Nikiforov & C. Vogelsang (2019). Maximum limit values for selected hazardous organic contaminants (HOCs) in secondary raw materials used in fertilisers and soil products. Norwegian Institute of Bioeconomy Research (NIBIO). NIBIO Report 5/110/2019. Commissioned by the Norwegian Environmental Agency.

European Union. (2015, October 27). *Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption*. European Union Law. http://data.europa.eu/eli/dir/1998/83/2015-10-27

Felz, S. (2019). Structural Extracellular Polymeric Substances from Aerobic Granular Sludge. https://doi.org/10.4233/uuid:93e702d1-92b2-4025-ab57-6d2c141ed14d

Felz, S., Al-Zuhairy, S., Aarstad, O. A., Van Loosdrecht, M. C., & Lin, Y. M. (2016). Extraction of Structural Extracellular Polymeric Substances from Aerobic Granular Sludge. *Journal of Visualized Experiments*, *115*. https://doi.org/10.3791/54534

Felz, S., Vermeulen, P., Van Loosdrecht, M. C., & Lin, Y. M. (2019). Chemical characterization methods for the analysis of structural extracellular polymeric substances (EPS). *Water Research*, *157*, 201–208. https://doi.org/10.1016/j.watres.2019.03.068

Feng, C., Lotti, T., Canziani, R., Lin, Y., Tagliabue, C., & Malpei, F. (2021). Extracellular biopolymers recovered as raw biomaterials from waste granular sludge and potential applications: A critical review. *Science of the Total Environment*, *753*, 142051. https://doi.org/10.1016/j.scitotenv.2020.142051

Feng, C., Lotti, T., Lin, Y., & Malpei, F. (2019). Extracellular polymeric substances extraction and recovery from anammox granules: Evaluation of methods and protocol development. *Chemical Engineering Journal*, *374*, 112–122. https://doi.org/10.1016/j.cej.2019.05.127

Fernández, V., & Brown, P. H. (2013). From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Frontiers in Plant Science*, *4*. https://doi.org/10.3389/fpls.2013.00289

Frølund, B., Palmgren, R., Keiding, K., & Nielsen, P. H. (1996). Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Research*, *30*(8), 1749–1758. https://doi.org/10.1016/0043-1354(95)00323-1

Grotefend, S. B., Kaminski, L., Wroblewitz, S., Deeb, S. E., Kühn, N., Reichl, S., Limberger, M., Watt, S., & Wätzig, H. (2012). Protein quantitation using various modes of high-

performance liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis*, *71*, 127–138. https://doi.org/10.1016/j.jpba.2012.08.024

Guillossou, R., Roux, J. L., Mailler, R., Vulliet, E., Morlay, C., Nauleau, F., Gasperi, J., & Rocher, V. (2019). Organic micropollutants in a large wastewater treatment plant: What are the benefits of an advanced treatment by activated carbon adsorption in comparison to conventional treatment? *Chemosphere*, *218*, 1050–1060. https://doi.org/10.1016/j.chemosphere.2018.11.182

Grillet, A.M., Wyatt N.B., Gloe L.M. (2012). Polymer Gel Rheology and Adhesion, Rheology, Dr. Juan De Vicente (Ed.), ISBN: 978-953-51-0187-1, InTech, retrieved from: http://www.intechopen.com/books/rheology/rheology-and-adhesion-of-polymer-gels

Harimawan, A., & Ting, Y. (2016). Investigation of extracellular polymeric substances (EPS) properties of P. aeruginosa and B. subtilis and their role in bacterial adhesion. *Colloids and Surfaces B: Biointerfaces*, *146*, 459–467. https://doi.org/10.1016/j.colsurfb.2016.06.039

Herald, P. J., & Zottola, E. A. (1989). Effect of Various Agents Upon the Attachment of Pseudomonas fragi to Stainless Steel. *Journal of Food Science*, *54*(2), 461–464. https://doi.org/10.1111/j.1365-2621.1989.tb03106.x

Hosch, W. L. (2023, June 11). Stress. Encyclopedia Britannica. https://www.britannica.com/science/stress-physics#ref285382

Hudcová, H., Vymazal, J., & Rozkošný, M. (2019). Present restrictions of sewage sludge application in agriculture within the European Union. *Soil and Water Research*, *14*(No. 2), 104–120. https://doi.org/10.17221/36/2018-swr

Hussain, M. R., Forbes, N. S., & Perrie, Y. (2019). Comparative Analysis of Protein Quantification Methods for the Rapid Determination of Protein Loading in Liposomal Formulations. *Pharmaceutics*, *11*(1), 39. https://doi.org/10.3390/pharmaceutics11010039

Interstate Technology Regulatory Council [ITRC]. (2022, June). *12 Treatment Technologies*. PFAS — Per- and Polyfluoroalkyl Substances. Retrieved June 15, 2023, from https://pfas-1.itrcweb.org/12-treatment-technologies/

Johnson, G. (2022, April 29). *Foliar Fertilization for Vegetable Crops, Revisited*. Weekly Crop Update. Retrieved May 29, 2023, from https://sites.udel.edu/weeklycropupdate/?p=20114

Kim, N. K., Mao, N., Lin, R., Bhattacharyya, D., Van Loosdrecht, M. C., & Lin, Y. (2020). Flame retardant property of flax fabrics coated by extracellular polymeric substances recovered from both activated sludge and aerobic granular sludge. *Water Research*, *170*, 115344. https://doi.org/10.1016/j.watres.2019.115344

Koppert. (2021, December 20). *Koppert verduurzaamt grondstoffeninzet met Kaumera*. Koppert Nederland. https://www.koppert.nl/nieuws-pers/2021/koppert-verduurzaamt-grondstoffeninzet-met-kaumera/

Kreyenschulte, D., Krull, R., & Margaritis, A. (2012). Recent Advances in Microbial Biopolymer Production and Purification. *Critical Reviews in Biotechnology*, *34*(1), 1–15. https://doi.org/10.3109/07388551.2012.743501

Laurén, S. (2019, July 19). *Wettability determines the adhesion of coatings*. Biolin Scientific. https://www.biolinscientific.com/blog/wettability-determines-the-adhesion-of-coatings

Lenntech. (n.d.). *Composition of seawater*. Retrieved May 4, 2023, from https://www.lenntech.com/composition-seawater.htm

Li, N., Wei, D., Wang, S., Hu, L., Xu, W., Du, B., & Wei, Q. (2017). Comparative study of the role of extracellular polymeric substances in biosorption of Ni(II) onto aerobic/anaerobic granular sludge. *Journal of Colloid and Interface Science*, *490*, 754–761. https://doi.org/10.1016/j.jcis.2016.12.006

Lin, Y., De Kreuk, M., Van Loosdrecht, M., & Adin, A. (2010). Characterization of alginate-like exopolysaccharides isolated from aerobic granular sludge in pilot-plant. *Water Research*, *44*(11), 3355–3364. https://doi.org/10.1016/j.watres.2010.03.019

Lin, Y., Nierop, K., Girbal-Neuhauser, E., Adriaanse, M., & Van Loosdrecht, M. (2015). Sustainable polysaccharide-based biomaterial recovered from waste aerobic granular sludge as a surface coating material. *Sustainable Materials and Technologies*, *4*, 24–29. https://doi.org/10.1016/j.susmat.2015.06.002

Liu, G., Li, & Hanlon, E. A. (2022, June 20). *Understanding and Applying Chelated Fertilizers Effectively Based on Soil pH*. University of Florida. Retrieved January 14, 2023, from https://edis.ifas.ufl.edu/publication/HS1208

Liu, W., Zhang, J., Jin, Y., Zhao, X., & Cai, Z. (2015). Adsorption of Pb(II), Cd(II) and Zn(II) by extracellular polymeric substances extracted from aerobic granular sludge: Efficiency of protein. *Journal of Environmental Chemical Engineering*, *3*(2), 1223–1232. https://doi.org/10.1016/j.jece.2015.04.009

Lotti, T., Carretti, E., Berti, D., Montis, C., Del Buffa, S., Lubello, C., Feng, C., & Malpei, F. (2019). Hydrogels formed by anammox extracellular polymeric substances: structural and mechanical insights. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-47987-8

Mai, G. T., McCormack, J. G., Seow, W. K., Pier, G. B., Jackson, L. E., & Thong, Y. (1993). Inhibition of adherence of mucoid Pseudomonas aeruginosa by alginase, specific

monoclonal antibodies, and antibiotics. *Infection and Immunity*, *61*(10), 4338–4343. https://doi.org/10.1128/iai.61.10.4338-4343.1993

Mathias, J. (2022, December 20). *A Beginner's Guide to FTIR Analysis: Interpreting & Analyzing Results*. Innovatech Labs. https://www.innovatechlabs.com/newsroom/1882/interpreting-analyzing-ftir-results/

McHugh, D. J. & Food and Agriculture Organization of the United Nations. (2003). *A Guide to the Seaweed Industry*. Food and Agriculture Organization of the United Nations.

Merritt, J. H., Kadouri, D. E., & O'Toole, G. A. (2005). Growing and Analyzing Static Biofilms. *Current Protocols in Microbiology*, *00*(1). https://doi.org/10.1002/9780471729259.mc01b01s00

Monique, R., Elisabeth, G. N., Etienne, P., & Dominique, L. (2008). A high yield multi-method extraction protocol for protein quantification in activated sludge. *Bioresource Technology*, *99*(16), 7464–7471. https://doi.org/10.1016/j.biortech.2008.02.025

Nancharaiah, Y. V., Sarvajith, M., & Krishna Mohan, T. V. (2019). Aerobic Granular Sludge:The Future of Wastewater Treatment. *Current Science*, *117*(3), 395. https://doi.org/10.18520/cs/v117/i3/395-404

Negri, M., Gonçalves, V., Silva, S., Henriques, M., Azeredo, J., & Oliveira, R. (2010). Crystal violet staining to quantify Candida adhesion to epithelial cells. *British Journal of Biomedical Science*, *67*(3), 120–125. https://doi.org/10.1080/09674845.2010.11730308

Nielsen, P. H., & Jahn, A. (1999). Extraction of EPS. *Microbial Extracellular Polymeric Substances*, 49–72. https://doi.org/10.1007/978-3-642-60147-7_3

Nielsen, S. S. (2010). Phenol-Sulfuric Acid Method for Total Carbohydrates. *Springer eBooks*, 47–53. https://doi.org/10.1007/978-1-4419-1463-7_6

Nouha, K., Kumar, R. L., Balasubramanian, S., & Tyagi, R. D. (2017). Critical review of EPS production, synthesis, and composition for sludge flocculation. *Journal of Environmental Sciences-china, 66*, 225–245. https://doi.org/10.1016/j.jes.2017.05.020

Novák, P., & Havlíček, V. (2016). Protein Extraction and Precipitation. In *Elsevier eBooks* (pp. 51–62). https://doi.org/10.1016/b978-0-444-63688-1.00004-5

Ommen, P., Zobek, N., & Meyer, R. L. (2017). Quantification of biofilm biomass by staining: Non-toxic safranin can replace the popular crystal violet. *Journal of Microbiological Methods, 141*, 87–89. https://doi.org/10.1016/j.mimet.2017.08.003

O'Toole, G. A. (2011). Microtiter Dish Biofilm Formation Assay. *Journal of Visualized Experiments, 47*. https://doi.org/10.3791/2437

Pfaff, N. M., Kleijn, J. M., Van Loosdrecht, M. C., & Kemperman, A. J. (2021). Formation and ripening of alginate-like exopolymer gel layers during and after membrane filtration. *Water Research, 195*, 116959. https://doi.org/10.1016/j.watres.2021.116959

Pronk, M., De Kreuk, M., De Bruin, B., Kamminga, P., Kleerebezem, R., & Van Loosdrecht, M. C. (2015). Full scale performance of the aerobic granular sludge process for sewage treatment. *Water Research*, *84*, 207–217. https://doi.org/10.1016/j.watres.2015.07.011

Ras, M., Girbal-Neuhauser, E., Paul, E. S., Spérandio, M., & Lefebvre, D. (2008). Protein extraction from activated sludge: An analytical approach. *Water Research*, *42*(8–9), 1867–1878. https://doi.org/10.1016/j.watres.2007.11.011

Rengel, Z. (1999). Heavy Metals as Essential Nutrients. *Heavy Metal Stress in Plants*, 231–251. https://doi.org/10.1007/978-3-662-07745-0_11

Rijksinstituut voor Volksgezondheid en Milieu [RIVM]. (n.d.). *PFAS*. RIVM. Retrieved June 15, 2023, from https://www.rivm.nl/pfas

Roos, A., Creton, C., Novikov, M. B. & Feldstein, M. M. (2002). Viscoelasticity and Tack of Poly(Vinyl Pyrrolidone)-Poly(Ethylene Glycol) Blends. Journal of Polymer Science: Part B: Polymer Physics, Vol. 40, pp. 2395-2409, ISSN 1099-0488

Royal HaskoningDHV. (n.d.). *Nereda Technology*. Retrieved December 15, 2022, from https://nereda.royalhaskoningdhv.com

Royal HaskoningDHV. (n.d.-b). *The Netherlands - Utrecht*. Retrieved May 18, 2023, from https://nereda.royalhaskoningdhv.com/en/projects/the-netherlands-utrecht

Sandhya, V., SK. Z., A., Grover, M., Reddy, G., & Venkateswarlu, B. (2009). Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing Pseudomonas putida strain GAP-P45. *Biology and Fertility of Soils*, *46*(1), 17–26. https://doi.org/10.1007/s00374-009-0401-z

Sela, G. (2021). Foliar Feeding. *Cropaia*. https://cropaia.com/blog/foliar-feeding/

Seviour, T., Derlon, N., Dueholm, M. S., Flemming, H. C., Girbal-Neuhauser, E., Horn, H., Kjelleberg, S., Van Loosdrecht, M. C., Lotti, T., Malpei, M. F., Nerenberg, R., Neu, T. R., Paul, E., Yu, H., & Lin, Y. (2019). Extracellular polymeric substances of biofilms: Suffering from an identity crisis. *Water Research*, *151*, 1–7. https://doi.org/10.1016/j.watres.2018.11.020

Seviour, T., Pijuan, M., Nicholson, T., Keller, J., & Yuan, Z. (2009). Gel-forming exopolysaccharides explain basic differences between structures of aerobic sludge granules and floccular sludges. *Water Research*, *43*(18), 4469–4478. https://doi.org/10.1016/j.watres.2009.07.018

Shanks, R. A., Donegan, N. P., Graber, M. L., Buckingham, S., Zegans, M. E., Cheung, A. L., & O'Toole, G. A. (2005). Heparin Stimulates *Staphylococcus aureus* Biofilm Formation.

Infection and Immunity, *73*(8), 4596–4606. https://doi.org/10.1128/iai.73.8.4596-4606.2005

Sheng, G. P., Yu, H. Q., & Li, X. Y. (2010). Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. *Biotechnology Advances*, *28*(6), 882–894. https://doi.org/10.1016/j.biotechadv.2010.08.001

Shi, X., & Zhu, X. (2009). Biofilm formation and food safety in food industries. *Trends in Food Science & Technology*, *20*(9), 407–413. https://doi.org/10.1016/j.tifs.2009.01.054

Simonovich, S. (2017, September 14). Understanding Viscosity and Non-Newtonian Fluids Will Help Your Ketchup Game. *Medium*. https://medium.com/petroleum-service-company/understanding-viscosity-and-non-newtonian-fluids-will-help-your-ketchup-game-584c1724a609

STOWA. (2019). *Kaumera Nereda Gum.* (Rapport 14). https://www.stowa.nl/publicaties/kaumera-nereda-gum-samenvatting-naop-onderzoeken-2013-2018

STOWA. (2021). *PFAS in Influent, Effluent, and Sewage Sludge Results of a Monitoring Campaign at Eight WWTPs* (Rapport 46E). https://www.stowa.nl/sites/default/files/assets/PUBLICATIES/Publicaties%202021/STOWA%202021-46%20PFAS.pdf

Sung, K., Park, M., Chon, J., & Khan, S. R. (2021). Methods to Grow and Measure In Vitro Static Biofilms. In *Elsevier eBooks* (pp. 408–429). https://doi.org/10.1016/b978-0-12-818731-9.00167-1

Talari, A. C. S., Martinez, M. a. G., Movasaghi, Z., Rehman, S., & Rehman, I. U. (2017a). Advances in Fourier transform infrared (FTIR) spectroscopy of biological tissues. *Applied Spectroscopy Reviews*, *52*(5), 456–506. https://doi.org/10.1080/05704928.2016.1230863

Tay, J.-H., & Show, K.-Y. (1997). Resource recovery of sludge as a building and construction material — a future trend in sludge management. *Water Science and Technology*, *36*(11). https://doi.org/10.1016/s0273-1223(97)00692-6

Thomas, D., & Middleton, N. (1993). Salinization: new perspectives on a major desertification issue. *Journal of Arid Environments*, *24*(1), 95–105. https://doi.org/10.1006/jare.1993.1008

Trees. (2023, February 23). *Chelated Iron for Plants - What You Need to Know*. Trees.com. https://www.trees.com/gardening-and-landscaping/chelated-iron

Trinklein, D. (2019, April 16). *Foliar Feeding Revisited*. University of Missouri - Integrated Pest Management. Retrieved November 5, 2023, from https://ipm.missouri.edu/MPG/2019/4/foliarFeeding/

Uitvoeringsbesluit Meststoffenwet [Fertilizers Act Implementation Decree]. (2021, 20 February). Retrieved May 15, 2023, from https://wetten.overheid.nl/BWBR0019031/2021-02-20#origineel-opschrift-en-aanhef

U.S. EPA. 2007. "Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils," Revision 1. Washington, DC.

WateReuse Foundation. (2007). *Salinity Management Guide: Learn about the effects of salt on plants*. Retrieved May 25, 2023, from https://watereuse.org/salinity-management/le/le_5.html#:~:text=Most%20plants%20will%20typically%20suffer,from%20a%20saturated%20soil%20paste.

Wolfaardt, G. M., Lawrence, J. R., & Korber, D. R. (1999). Function of EPS. *Microbial Extracellular Polymeric Substances*, 171–200. https://doi.org/10.1007/978-3-642-60147-7_10

Working Leaf. (2023). Timing Your Foliar Fertilizer for Maximum Results. *Working Leaf*. https://working-leaf.com/timing-your-foliar-fertilizer-for-maximum-results/

Zhang, Z., Yu, Z., Wang, Z., Ma, K., Xu, X., Alvarezc, P. J., & Zhu, L. (2019). Understanding of aerobic sludge granulation enhanced by sludge retention time in the aspect of quorum sensing. *Bioresource Technology*, *272*, 226–234. https://doi.org/10.1016/j.biortech.2018.10.027

Zheng, L. S., & Zhang, X. Y. (2017). Exact Analytical Solutions for Fractional Viscoelastic Fluids. In *Elsevier eBooks* (pp. 279–359). https://doi.org/10.1016/b978-0-12-811753-8.00007-4

Zheng, S., Bawazir, M., Dhall, A., Kim, H. S., He, L., Heo, J., & Hwang, G. (2021). Implication of Surface Properties, Bacterial Motility, and Hydrodynamic Conditions on Bacterial Surface Sensing and Their Initial Adhesion. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.643722

Zhou, Y., Kunacheva, C., & Stuckey, D. C. (2016). "Protein" Measurement in Biological Wastewater Treatment Systems: A Critical Evaluation. *Environmental Science & Technology*, *50*(6), 3074–3081. https://doi.org/10.1021/acs.est.5b05261

Zhou, Y., & Stuckey, D. C. (2016). Colorimetric measurement of carbohydrates in biological wastewater treatment systems: A critical evaluation. *Water Research*, *94*, 280–287. https://doi.org/10.1016/j.watres.2016.03.008

Zlopasa, J., Koenders, EAB., & Picken, SJ. (2014). Using bio-based polymers for curing cement-based materials. In EAB. Koenders, & K. van Breugel (Eds.), Proceedings of the 1st

international conference on ageing of materials and structures, AMS'14 (pp. 220-226). DCMat Ageing Centre.

Zosel, A. (1985). Adhesion and tack of polymers: Influence of mechanical properties and surface tensions. *Colloid and Polymer Science*, *263*(7), 541–553. https://doi.org/10.1007/bf01421887

Appendices

Appendix A

Kaumera Adhesion Protocol conducted on alginate

This appendix presents the results of the adhesion protocol conducted on alginate. In the CV concentration versus TS% curve, it is important to observe the contrasting convex shape compared to the concave shape observed in all Kaumera samples.

Table A1: EC and pH for tested alginate concentrations.

Alginate Concentration (%TS)	*EC at 25°C*	*pH (-)*
5	8.25	5.81
3.5	6.21	5.87
2	3.83	5.99

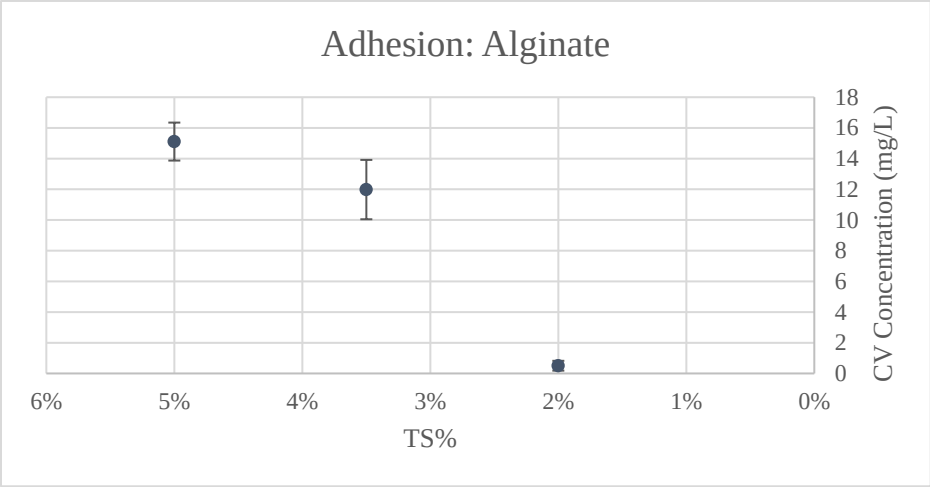


Figure A1: CV concentration vs. TS% for alginate.

Figure A2: output of CV protocol for alginate.

Appendix B
Detailed VS/TS analysis

This appendix contains more detailed information on the VS/TS analysis conducted on Kaumera Utrecht and Kaumera Faro.

Table B1: detailed VS/TS analysis for Kaumera Utrecht.

Kaumera Utrecht									
Sample	Empty Weight (EW)	Wet Weight (WW)	Dry Weight (DW)	Ash (A)	TS	VS	TS	VS	A
	g	g	g	g	g	g	%	%	%
R1	2.1991	8.3535	2.5672	2.2674	0.3681	0.2998	5.98%	81.45%	18.55%
R2	2.2049	7.1682	2.5008	2.2601	0.2959	0.2407	5.96%	81.35%	18.65%
R3	2.2265	7.2509	2.5268	2.2825	0.3003	0.2443	5.98%	81.35%	18.65%
Average:							5.97%	81.38%	18.62%
SD:							0.01%	0.06%	0.06%

Table B2: detailed VS/TS analysis for Kaumera Faro.

Kaumera Faro									
Sample	Empty Weight (EW)	Wet Weight (WW)	Dry Weight (DW)	Ash (A)	TS	VS	TS	VS	A
	g	g	g	g	g	g	%	%	%
R1	2.2141	7.3532	2.6585	2.2848	0.4444	0.3737	8.65%	84.09%	15.91%
R2	2.2147	8.6050	2.7659	2.3027	0.5512	0.4632	8.63%	84.03%	15.97%
R3	2.1937	9.8640	2.8549	2.2994	0.6612	0.5555	8.62%	84.01%	15.99%
Average:							8.63%	84.05%	15.95%
SD:							0.01%	0.04%	0.04%

Appendix C

Calibration curve for total carbohydrates

This appendix contains the data used to construct the calibration curve to determine the total carbohydrates in the Kaumera samples via the phenol sulfuric method (Dubois et al., 1956).

Table C1: volumes required to prepare the different concentrations of glucose standards.

Glucose concentration (mg/L)	*0 (blank)*	*30*	*60*	*90*	*120*
Volume of glucose solution (µL)	0	225	450	675	900
Volume of 0.1 M NaOH (µL)	1500	1275	1050	825	600

Table C2: absorbances measured at 490 nm for the glucose standards.

Glucose Concentration (mg/L)	*Absorbances (490 nm)*				
	R1	*R2*	*R3*	*Average*	*SD*
0	0.047	0.039	0.113	0.066	0.04
30	0.315	0.342	0.423	0.360	0.06
60	0.428	0.463	0.489	0.460	0.03
90	0.766	0.798	0.814	0.793	0.02
120	1.018	0.932	0.969	0.973	0.04

Table C3: net absorbances obtained by subtracting the average blank for the glucose standards.

Glucose Concentration (mg/L)	*Net Absorbances (490 nm)*				
	R1	*R2*	*R3*	*Average*	*SD*
0	-	-	-	0.000	-
30	0.249	0.276	0.357	0.294	0.06
60	0.362	0.397	0.423	0.394	0.03
90	0.700	0.732	0.748	0.726	0.02
120	0.952	0.866	0.903	0.907	0.04

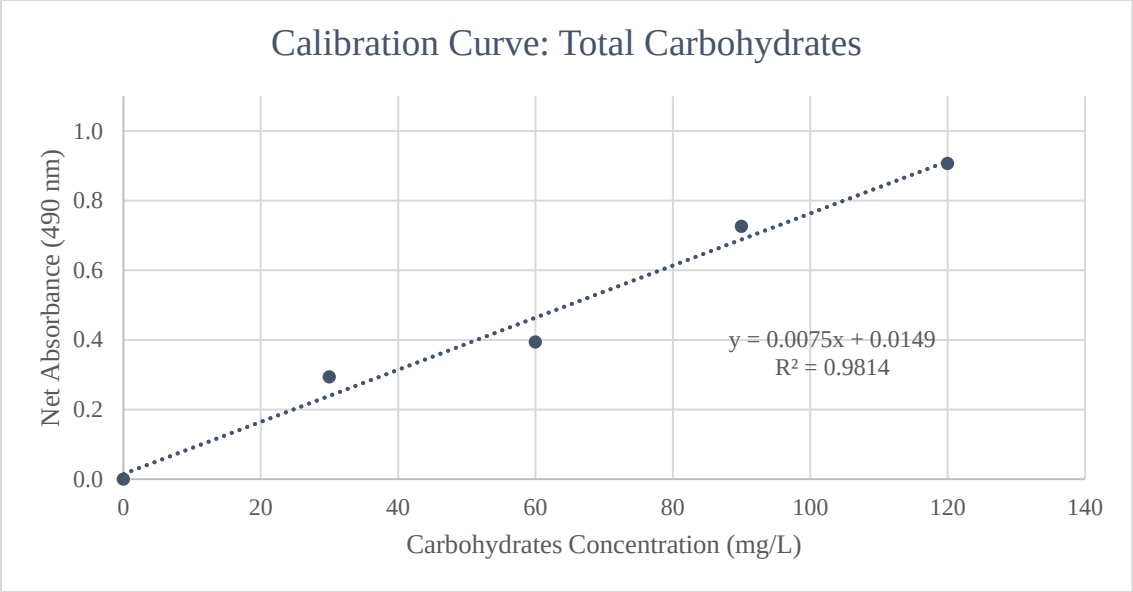


Figure C1: calibration curve to determine the total carbohydrates in a sample.

Appendix D

Calibration curve for total proteins

This appendix contains the data used to construct the calibration curve to determine the total proteins in the Kaumera samples via the Pierce® BCA Protein Assay.

Table D1: volumes required to prepare the different concentrations of BSA standards.

Vial	*Volume of 0.1 M NaOH (uL)*	*Volume of BSA (uL)*	*BSA Concentration (ug/mL or mg/L)*
A	0	300 of stock	2000
B	125	375 of stock	1500
C	325	325 of stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C solution	500
F	325	325 of vial E solution	250
G	325	325 of vial F solution	125
H	400	100 of vial G solution	25
I (blank)	400	0	0

When measuring the absorbances at 562 nm for the different standards, concentrations above 500 mg/L were discarded as their absorbances were higher than 1.

Table D2: absorbances measured at 562 nm for the BSA standards.

BSA Concentration (mg/L)	*Absorbances (562 nm)*				
	R1	*R2*	*R3*	*Average*	*SD*
0	0.099	0.096	0.099	0.098	0.002
25	0.152	0.136	0.137	0.142	0.009
125	0.337	0.344	0.33	0.337	0.007
250	0.547	0.553	0.54	0.547	0.007
500	0.877	0.975	0.958	0.937	0.05

Table D3: net absorbances obtained by subtracting the average blank for the BSA standards.

BSA Concentration (mg/L)	*Net Absorbances (562 nm)*				
	R1	*R2*	*R3*	*Average*	*SD*
0	-	-	-	0.000	-
25	0.054	0.038	0.039	0.044	0.009
125	0.239	0.246	0.232	0.239	0.007
250	0.449	0.455	0.442	0.449	0.007
500	0.779	0.877	0.86	0.839	0.05

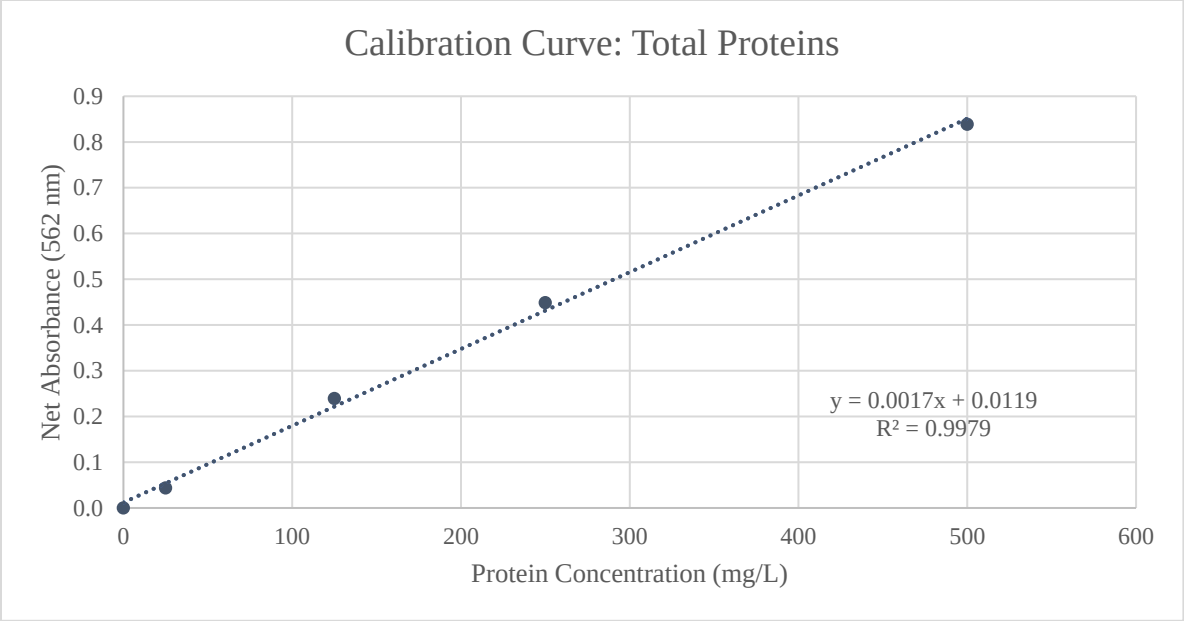


Figure D1: calibration curve to determine the total proteins in a sample.

Appendix E

Calculations: Percentage of elements and ions in respect to total Kaumera

The appendix provides detailed calculations to determine the extent to which elements and ions can be removed from the total Kaumera by implementing a washing step that involves discarding the supernatant.

Initially, the concentrations of elements in the supernatant, measured using ICP-OES, were examined. These results were given in units of "mg/L," with "L" representing the supernatant volume. However, to facilitate a more accurate comparison between the supernatant and the total Kaumera, it was necessary to convert this unit to "mg/(L Kaumera)." To achieve this, the results from the VS/TS analysis were used. In one liter of Kaumera Utrecht (assuming a density of 1 kg/L), there are 59.73 grams of total solids and 940.27 grams of liquid, equivalent to 0.94 liters. For Kaumera Faro, there are 86.31 grams of total solids and 913.69 grams of liquid, equivalent to 0.91 liters, in one liter of Kaumera Faro. Assuming the weight of the supernatant is equivalent to the liquid phase, the concentrations for Kaumera Utrecht and Kaumera Faro in "mg/(L supernatant)" were multiplied by 0.94 and 0.91, respectively, to obtain "mg/(L Kaumera)".

Next, the concentrations of the "Total Kaumera" were evaluated. In this case, the ICP-OES provided concentrations in "mg/g DM". Therefore, the concentrations for Kaumera Utrecht and Kaumera Faro were multiplied by 59.73 grams/(L Kaumera) and 86.31 grams/(L Kaumera), respectively, to obtain concentrations in "mg/(L Kaumera)." As the units were now the same, the concentrations of the supernatant could be divided by the concentrations of the "Total Kaumera."

Table E1: calculations for determining the percentage of elements in the supernatant relative to total Kaumera Utrecht.

Kaumera Utrecht					
Element	*Supernatant*		*Total Kaumera*		*Percentage (%)*
	mg/(L supernatant)	*mg/(L Kaumera)*	*mg/g DM*	*mg/(L Kaumera)*	
K	1858.0	1747.0	28.18	1683.47	104%
Na	83.0	78.0	1.36	81.33	96%
P	296.4	278.7	20.76	1240.08	22%
Ca	225.7	212.2	4.70	280.95	76%
Fe	194.0	182.4	23.33	1393.42	13%
Mg	47.6	44.7	0.97	57.96	77%
Zn	10.2	9.6	0.26	15.52	62%
Mn	1.7	1.6	0.02	1.31	121%
Cu	1.0	0.9	0.28	16.65	6%

Table E2: calculations for determining the percentage of elements in the supernatant relative to total Kaumera Faro.

Kaumera Faro					
Element	*Supernatant*		*Total Kaumera*		*Percentage (%)*
	mg/(L supernatant)	*mg/(L Kaumera)*	*mg/g DM*	*mg/(L Kaumera)*	
K	2760.0	2521.8	40.82	3523.26	72%
Na	1304.0	1191.5	7.06	609.35	196%
P	150.0	137.1	5.95	513.15	27%
Ca	140.5	128.4	1.71	147.35	87%
Fe	84.6	77.3	1.93	166.50	46%
Mg	129.6	118.4	1.52	131.40	90%
Zn	38.6	35.3	0.57	48.92	72%
Mn	0.7	0.6	<0.01	-	-
Cu	0.2	0.2	0.14	12.04	1%

The same calculations were made for the ionic concentrations obtained by IC:

Table E3: calculations for determining the percentage of ions in the supernatant relative to total Kaumera Utrecht.

Kaumera Utrecht					
Ion	*Supernatant*		*Total Kaumera*		*Percentage (%)*
	mg/(L supernatant)	*mg/(L Kaumera)*	*mg/g DM*	*mg/(L Kaumera)*	
Cl^-	2868.8	2697.4	-	-	-
K^+	1797.3	1689.9	28.18	1683.47	100%
Na^+	70.1	65.9	1.36	81.33	81%
Ca^{2+}	195.1	183.4	4.70	280.95	65%
Mg^{2+}	31.8	29.9	0.97	57.96	52%

Table E4: calculations for determining the percentage of ions in the supernatant relative to total Kaumera Faro.

Kaumera Faro					
Ion	*Supernatant*		*Total Kaumera*		*Percentage (%)*
	mg/(L supernatant)	*mg/(L Kaumera)*	*mg/g DM*	*mg/(L Kaumera)*	
Cl^-	2120.7	1937.6	9.36	807.87	240%
K^+	2619.9	2393.7	40.82	3523.26	68%
Na^+	1252.3	1144.2	7.06	609.35	188%
Ca^{2+}	103.1	94.2	1.71	147.35	64%
Mg^{2+}	109.1	99.7	1.52	131.40	76%

The calculation of percentages for sulfate, phosphate, and ammonium ions was based on the total sulfur (S), phosphorus (P) and total nitrogen (N) content in the "Total Kaumera," respectively. To facilitate the calculation, the concentrations were converted to "mmol/L" using the atomic masses of 32.07 g/mol (sulfur), 96.06 g/mol (sulfate), 16.00 g/mol (oxygen), 30.97 g/mol (phosphorus),

94.97 g/mol (phosphate), 14.01 g/mol (nitrogen), and 18.04 g/mol (ammonium). By using the same unit, the percentages could then be accurately determined.

Table E5: calculations for determining the percentage of sulfate, phosphate, and ammonium in the supernatant relative to total Kaumera Utrecht.

Kaumera Utrecht							
Ion	*Supernatant*			*Total Kaumera*			*Percentage (%)*
	mg/(L supernatant)	*mg/(L Kaumera)*	*mmol/L*	*mg/g DM*	*mg/(L Kaumera)*	*mmol/L*	
SO_4^{2-}	52.5	49.4	0.5	-	-	-	-
PO_4^{3-}	795.0	747.5	7.9	20.76	1240.08	40.04	20%
NH_4^+	202.2	190.1	10.5	66.12	3949.45	281.96	4%

Table E6: calculations for determining the percentage of sulfate, phosphate, and ammonium in the supernatant relative to total Kaumera Faro.

Kaumera Faro							
Ion	*Supernatant*			*Total Kaumera*			*Percentage (%)*
	mg/(L supernatant)	*mg/(L Kaumera)*	*mmol/L*	*mg/g DM*	*mg/(L Kaumera)*	*mmol/L*	
SO_4^{2-}	4682.5	4278.3	44.5	34.00	2934.57	91.52	49%
PO_4^{3-}	273.0	249.4	2.6	5.95	513.15	16.57	16%
NH_4^+	205.7	187.9	10.4	73.63	6354.70	453.68	2%

Appendix F
Detailed Kaumera Adhesion Protocol

This appendix contains a more detailed protocol to quantify adhesion of Kaumera Nereda Gum® to abiotic surfaces by determining the CV concentration (mg/L).

Materials

- Falcon 50 mL Conical Centrifuge Tubes
- Falcon 15 mL Conical Centrifuge Tubes
- 5 mL plastic pipettes
- 1 Balloon
- 100-1000 µL adjustable-volume micropipette
- 1.5 mL cuvettes
- 2 (waste) trays: one for Kaumera samples and one for CV
- pH probe
- Spectrophotometer
- 50 mL Kaumera Nereda Gum®
- 2 M potassium hydroxide (KOH)
- 100 mL, 0.1% (w/v in Milli-Q water) Crystal Violet (CV)
- 100 mL, 30% acetic acid in Milli-Q water

Constant variables

Perform protocol under 25°C. Before starting Phase III, measure pH and electrical conductivity (EC) of each Kaumera sample. The pH should fall within the range of 6.5 ± 0.1. The electrical conductivity within the same concentration (5%, 3.5%, or 2% TS) might be similar across different Kaumera samples but can also show variations depending on Kaumera composition and type of sludge. To improve accuracy, Kaumera samples can be washed and later dosed with KCl to bring the conductivity to the desired value.

Safety measures

When handling the CV dye, wear goggles and chemical-resistant, impervious gloves. Handle inside the fume hood.

Method

Phase I: creation of calibration curve

1. Prepare the following CV standards with concentrations 0-4 mg/L for the calibration curve in triplicates in the Falcon 50 mL Conical Centrifuge Tubes and the Falcon 15 mL Conical Centrifuge Tubes (depending on the total volume required). Note: for the blank, directly transfer 1.5 mL to a 1.5 mL cuvette with a 5 mL plastic pipette and a balloon.

Table F1: volumes to prepare the different concentrations of CV standards.

CV Concentration (mg/L)	*0 (blank)*	*1*	*1.5*	*2*	*2.5*	*3*	*3.5*	*4*
Volume 0.1% (w/v) CV (mL)	0	0.025	0.030	0.030	0.025	0.030	0.035	0.040
Volume of 30% acetic acid (mL)	1.5	24.975	19.970	14.970	9.975	9.970	9.965	9.960
Total volume (mL)	1.5	25	20	15	10	10	10	10

2. With a 5 mL plastic pipette (non-reusable) and a balloon, transfer 1.5 mL of each standard to a 1.5 mL cuvette.
3. Read the blank at a wavelength of 590 nm in the spectrophotometer. Then, measure the net absorbance of each standard at the same wavelength.

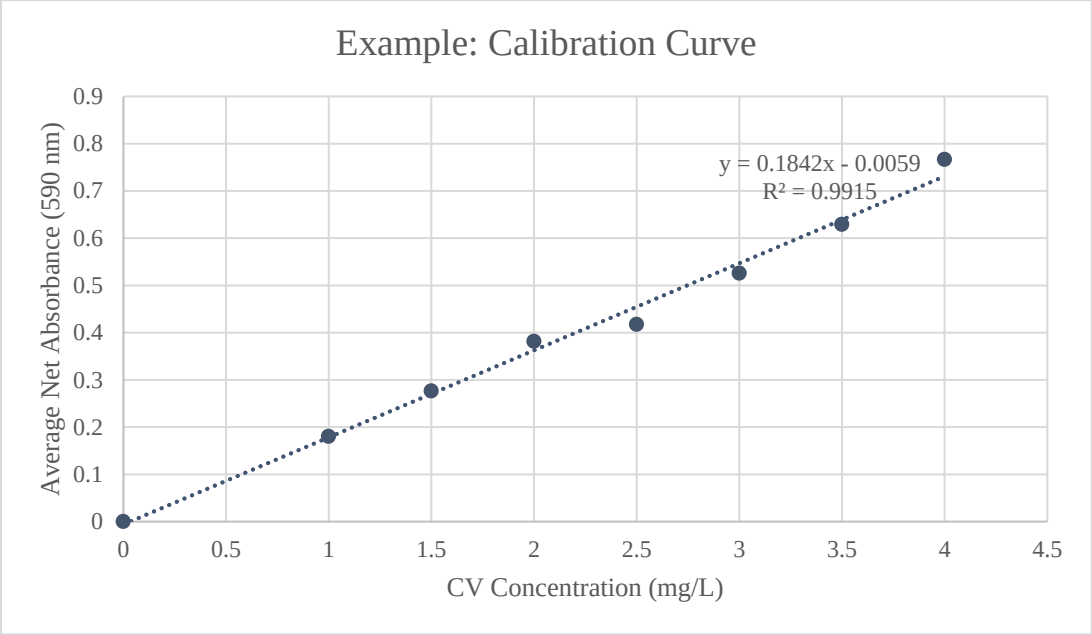


Figure F1: example of calibration curve for CV standards.

Phase II: Preparation of samples

1. Add 2 M KOH to the 50 mL Kaumera Nereda Gum® while monitoring the pH changes constantly with a pH probe to obtain a final pH of 6.5 ± 0.1. Note: add the 2 M KOH in small increments to avoid drastic changes in pH (e.g., 0.1 or 0.2 mL).
2. Prepare 3 different concentrations of Kaumera solution: 5%, 3.5%, and 2% TS with Milli-Q water. To calculate this, the original TS% of the sample is required. Since each concentration is tested in 4 replicates, each concentrations requires a total of 15 mL of Kaumera solution.

Phase III: testing samples

1. If Kaumera is stored in the fridge/cold room, take it out and wait approximately 1.5 hours until it reaches room temperature.
2. Label the 1.5 mL cuvettes with the corresponding name.
3. Use a 100-1000 µL adjustable-volume micropipette to fill each 1.5 mL cuvette completely with the respective Kaumera sample. Note: Pipette gently so bubbles are not formed.
4. For Kaumera to stick to the cuvettes wait 1 minute.
5. Turn over the cuvettes and remove Kaumera by shaking gently over the Kaumera waste tray. Continue to do so until there is no more dripping.
6. Rinse the cuvettes by adding Milli-Q water until it reaches the top, and then remove over the Kaumera waste tray. Repeat this step twice. This step helps remove unattached Kaumera.
7. Tap on paper towels to remove excess liquid. Allow plates to air-dry for approximately 2 hours at room temperature.
8. With a balloon and a 5 mL plastic pipette (reusable) fill the cuvettes completely with 0.1% (w/v) of crystal violet in Milli-Q water. Let it stain for 15 min.
9. Shake each cuvette over the CV waste tray to remove excess crystal violet solution.
10. Wash the cuvettes by adding Milli-Q water until it reaches the top, and then remove over the CV waste tray. Repeat this step twice.
11. Turn the cuvette upside down and tap on paper towels to remove excess liquid. Allow plates to air-dry for approximately 2 hours at room temperature.
12. With a balloon and a 5 mL plastic pipette (non-reusable), fill each cuvette with 30% acetic acid in Milli-Q water to solubilize the CV. Pipette up and down three times for better mixing. Wait 15 minutes until it solubilizes completely.
13. With a balloon and a 5 mL plastic pipette (non-reusable) transfer 1.5 mL of CV/acetic acid solution from each cuvette to a separate, optically clean 1.5 mL cuvette.
14. Using 30% acetic acid in Milli-Q water as blank, measure the net absorbance of each cuvette at a wavelength of 590 nm. Note: if the absorbances are greater than 1, the samples must be diluted to be inside the range of the calibration curve.
15. With the known absorbances, the CV concentrations (mg/L) can be determined via the calibration curve. These values can be used to compare the adhesion of different Kaumera Nereda Gum®.

Appendix G

Calculations: Percentage of ions in respect to total Kaumera Faro via conductivity measurements.

This appendix presents the detailed calculations performed to determine the potential removal of ions in the supernatant through the implementation of a washing step for Kaumera Faro. The calculations involved estimating the presence of elements and ions in the supernatant by assuming that the electrical conductivity of the influent of the Nereda® was primarily influenced by seawater intrusion, while the wastewater itself had negligible electrical conductivity.

For typical seawater, the electrical conductivity is 53.9 mS/cm, and the ionic concentrations are the following:

Table G1: major ion composition of seawater in mg/L (Lenntech, 2005).

Major ion composition of seawater	*Concentration (mg/L)*
Chloride (Cl^-)	18980
Sodium (Na^+)	10556
Sulfate (SO_4^{2-})	2649
Magnesium (Mg^{2+})	1262
Calcium (Ca^{2+})	400
Potassium (K^+)	380
Bicarbonate (HCO_3^-)	140
Strontium (Sr^{2+})	13
Bromide (Br^-)	65
Borate (BO_3^{3-})	26
Fluoride (F^-)	1
Silicate (SiO_3^{2-})	1
Iodide (I^-)	<1
Others	<1

On December 20, 2022, sludge was collected with the intention of extracting Kaumera Faro the following day. On that same day, the influent of Nereda® exhibited an electrical conductivity of 3.2 mS/cm. Since the ionic concentrations are proportional to the conductivity, it was feasible to estimate the approximate concentrations of ions present in the liquid phase or supernatant of Kaumera. This estimation was based on the assumption that the ions remain dissolved in the solution. Thus, by linear association, the concentration in milligrams per liter was calculated. However, it was also necessary to account that Kaumera Nereda Gum® also has a solid phase, and these concentrations are only related to the liquid phase. It is known that for one liter of Kaumera Faro, there are 86 grams of TS and thus 914 grams of liquid or 0.914 liters of liquid (assuming a density of 1 kg/L). Therefore, the concentrations of ions in milligrams per liter were multiplied by the fraction 0.914 liters of liquid per liter of Kaumera Faro to obtain ionic concentrations in milligrams per liter of Kaumera Faro.

Table G2: estimated ionic concentrations in the supernatant of Kaumera Faro.

Ions	*Concentration (mg/L)*	*Concentration (mg/L Kaumera)*
Cl^-	1127	1030
Na^+	627	573
SO_4^{2-}	157	144
Mg^{2+}	75	68
Ca^{2+}	24	22

As Gustav Simoni and the staff at Águas do Algarve had previously measured the elements in a sample from Kaumera Faro taken on December 21, 2022, it became feasible to compare and calculate the ratios of the ionic concentrations obtained from Table G2 with those measured by them. The external results were originally provided in milligrams per gram of total solids (mg/g TS), but they were converted to milligrams per liter (mg/L Kaumera) by applying a conversion factor of 86.3 grams of total solids per liter of Kaumera Faro.

Table G3: values used to calculate the percentage of ions in supernatant relative to total Kaumera Faro via conductivity measurements.

Ions	*Supernatant (mg/L Kaumera)*	*Total Kaumera (mg/L Kaumera)*	*Percentage (%) in supernatant Faro relative to total Kaumera Faro*
Cl^-	1030	807.87	127%
Na^+	573	609.35	94%
SO_4^{2-}	144	2934.57*	33%
Mg^{2+}	68	131.4	76%
Ca^{2+}	22	147.35	16%

* This value stands for total sulfur. For this calculation, the atomic masses were considered for an accurate comparison.