FACULTY OF BIOSCIENCE ENGINEERING

ANAEROBIC DIGESTION AT THE AXIS OF CIRCULAR FOOD PRODUCTION

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Preface

Finishing this master's thesis, brings also an end to five beautiful years of being a student at Ghent University, more specifically at the faculty of Bioscience Engineering. I would never have succeeded in writing this thesis without the support and help of many.

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Abstract

The global aquaculture production is rapidly increasing, primarily achieved by intensification of the process. However, efficient management of aquaculture waste remains a critical challenge, as improper handling can contribute to environmental degradation. This study investigates the anaerobic digestion of aquaculture solids, focusing on the impact of salinity concentration on the methane yield. Aquaculture can also be combined with the cultivation of plants in aquaponics, which can even be made more circular by the cultivation of black soldier fly larvae (BSFL) to produce fish meal. The BSFL can feed on the solid fraction of the food waste, another waste stream. The byproduct of BSFL cultivation, known as BSFL frass, includes larvae waste, exoskeleton sheds, and remaining digested feed. This study also explores the potential of co-digesting BSFL frass with food waste leachate, the leftover material unsuitable to feed the BSFL larvae.

Fed-batch reactors were used to perform the conversion of the feedstocks into a methane-rich biogas stream. Treatments included the anaerobic digestion of freshwater aquaculture solids, saltwater aquaculture solids (12 g/L salinity), and a mix of food waste leachate and BSFL frass. Next, the maximum salinity tolerance of the anaerobic digestion of the aquaculture sludge was investigated from an economic perspective, and the recovery capacity of the microbial community after salinity toxicity was evaluated.

The co-digestion of BSFL frass and food waste leachate failed, due to sudden acidification of the reactor. Rapid acid generation of the easily biodegradable matter in the leachate caused the pH to drop to 5, inhibiting the methanogens. Therefore, the decision was made to shut down the reactor after two weeks.

The results of the anaerobic digestion of the aquaculture solids highlight the critical role of salinity in influencing methane yields. Freshwater and brackish water (12 g/L) treatments demonstrated stable biogas production of around 0.3 Nm^3/gVS, with no significant differences in methane yields between both treatments. However, significantly higher levels of volatile fatty acids, volatile solids, and total solids were observed in the digestate of the saltwater treatment, indicating the higher amount of biodegradable matter that remained unconverted into biogas. A higher methane purity was observed in the biogas produced by the anaerobic digestion of the saltwater aquaculture solids, which can suggest an increased relative abundance of the hydrogenotrophic methanogenic archaea.

Aquaculture sludge with higher salinity levels (20 g/L) led to reduced methane yields, due to osmotic stress and potential sulfide inhibition. Yields of around 0.1 NL CH_4/gVS were obtained, resulting in a potential electricity production of 0.004 kWh per litre incoming sludge, which is too low to be economically viable, given the operational and capital costs associated with biogas installations. However, recovery from high salinity levels was observed upon reintroduction of freshwater aquaculture sludge, indicating only temporarily inhibition of the methanogens by the high salinity levels.

This studies highlights the viability of anaerobic digestion as a method for managing aquaculture waste. Promising results for biogas yield and long-term process stability were obtained from the anaerobic digestion of both freshwater aquaculture solids and those with a sea salt salinity of 12 g/L. This shows the potential of using the waste stream of aquaculture as a resource, which can make aquaculture operations more sustainable by using renewable energy and making fish production more circular. However, alternative strategies need to be explored for managing aquaculture solids at a salinity of 20 g/L, as this didn't result in economically feasible biogas production.

Samenvatting

De wereldwijde aquacultuurproductie groeit snel, voornamelijk door procesintensivering. Efficiënt beheer van aquacultuurafval blijft echter een belangrijke uitdaging, omdat onjuiste behandeling ervan kan bijdragen aan milieuvervuiling. Deze studie onderzoekt de anaerobe vergisting van slib afkomstig van aquacultuur, waarbij de invloed van zoutconcentratie op de methaanopbrengst wordt onderzocht. Aquacultuur kan worden gecombineerd met plantenteelt in aquaponics. Dit systeem kan nog meer circulair gemaakt worden door de cultivatie van larven van de zwarte soldaatvlieg (BSFL) die vervolgens verwerkt kunnen worden in visvoeder. Deze larven kunnen zich voeden met de vaste fractie van het voedselafval, een andere afvalstroom. Het bijproduct van de BSFL-kweek, bekend als BSFL-frass, bestaat uit larvenafval, exoskeletresten en faeces. Deze studie onderzoekt ook het potentieel van co-vergisting van BSFL-frass met percolaat afkomstig van voedselafval, dat bestaat uit het deel van het voedselafval dat niet wordt geconsumeerd door de larven.

Fed-batch reactoren werden gebruikt om de afvalstromen om te zetten in methaanrijk biogas. De behandelingen omvatten de anaerobe vergisting van zoetwater aquacultuur slib, aquacultuur slib (12 g/L zoutgehalte), en een combinatie van voedselafvalpercolaat en BSFL-frass. Daarnaast werd de maximale saliniteitstolerantie van de anaerobe vergisting van aquacultuurslib onderzocht vanuit een economisch perspectief, evenals de herstelcapaciteit van de microbiële gemeenschap na zouttoxiciteit.

De co-vergisting van BSFL-frass en voedselafvalpercolaat mislukte door plotse verzuring van de reactor. De snelle zuurvorming van het gemakkelijk biologisch afbreekbare materiaal in het percolaat verlaagde de pH tot 5, wat leidde tot de inhibitie van de methanogenen. Daarom werd besloten de reactor na twee weken stop te zetten.

De resultaten van de anaerobe vergisting van slib, afkomstig van aquacultuur, benadrukken de cruciale rol van het zoutgehalte op de methaanopbrengst. Zoetwater- en brakwaterbehandelingen (12 g/L) leidden tot stabiele biogasproductie van ongeveer 0,3 NL CH_4/gVS, zonder significante verschillen in methaanopbrengst tussen beide behandelingen. Hogere concentraties van vluchtige vetzuren, vluchtige vaste stoffen en totale vaste stoffen werden echter waargenomen in het digestaat van de zoutwaterbehandeling, wat duidt op een grotere hoeveelheid biologisch afbreekbaar materiaal dat niet werd omgezet in biogas. Bovendien werd een hogere methaanzuiverheid waargenomen in het biogas dat geproduceerd werd door de anaerobe vergisting van vaste stoffen uit de zoutwater aquacultuur (12 g/L), wat kan wijzen op de aanrijking van hydrogenotrofe methanogene archaea.

Aquacultuur slib met hogere zoutgehaltes (20 g/L) leidde tot lagere methaanopbrengsten door osmotische stress en mogelijke sulfide-inhibitie. De opbrengst bedroeg ongeveer 0,1 NL CH_4/gVS, wat resulteert in een potentiële elektriciteitsproductie van 0,004 kWh/L slib, te laag om economisch haalbaar te zijn, gezien de hoge operationele en kapitaalkosten van biogasinstallaties. Er werd echter herstel van de micro-organismen waargenomen na herintroductie van zoetwater aquacultuur slib, wat erop wijst dat de methanogenen slechts tijdelijk geïnhibeerd werden door de hoge zoutgehaltes.

Deze studie benadrukt het potentieel van anaerobe vergisting voor het beheer van aquacultuurafval. De resultaten toonden aan dat anaerobe vergisting van zowel zoetwater aquacultuur slib als aquacultuur slib met een zeezoutgehalte van 12 g/L veelbelovende biogasopbrengsten en langdurige processtabiliteit opleverde. Dit wijst op het potentieel van het gebruik van aquacultuur slib als grondstof, wat de duurzaamheid van aquacultuur kan verhogen door hernieuwbare energie te gebruiken en de visproductie meer circulair te maken. Voor het beheer van aquacultuur slib met een zoutgehalte van 20 g/L zijn echter alternatieve strategieën nodig, aangezien dit niet leidde tot economisch haalbare biogasproductie.

List of abbreviations

AD	Anaerobic digestion
bCOD	Biodegradable chemical oxygen demand
BIGH	Brussels Integrated Green Houses
BOD	Biological oxygen demand
BSF	Black soldier fly
BSFL	Black soldier fly larvae
CEA	Controlled environment agriculture
CHP	Combined Heat and Power
COD	Chemical oxygen demands
FM	Fresh matter
GHG	Greenhouse gases
HRT	Hydraulic retention time
OLR	Organic loading rate
RAS	Recirculation aquaculture system
VFA	Volatile fatty acids
SRB	Sulphate reducing bacteria
TAN	Total ammonia nitrogen
WSPs	Waste stabilization ponds


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1. LITERATURE REVIEW

1.1 Introduction

The global population has increased over the last centuries from one billion in 1800 to more than eight billion people today (Ritchie et al., 2023), exerting a strenuous demand on Earth's resources. The Earth overshoot day fell in 2023 on the 2nd of August; for Belgium specifically on the 26th of March. This day marks the point which humanity has surpassed a sustainable level of consumption of the natural resources generated by the Earth annually (Geneva Environment Network, 2023). Six out of the nine planetary boundaries have been transgressed, including the biogeochemical flows of phosphorus and nitrogen. These flows reflect anthropogenic perturbation of global element cycles (Steffen et al., 2015). Two important sources of nitrogen are the intensive livestock production (emission of NH_3) and the combustion of fossil fuels (NO_x), whereas phosphorus mainly enters the environment via runoff from land-based fertilisation. Nitrogen and phosphorus can also enter the marine environment through marine aquaculture as waste products from the fish are discharged directly in the environment. In 2018, global salmon aquaculture - totalling 2.2 million tonnes - released an annual waste discharge of 889 kilotonnes of carbon, 1.13 million tonnes of nitrogen, and 20.6 kilotonnes of phosphorus into coastal regions (Lobanov et al., 2023). Nitrogen is highly mobile in soils, leaching into groundwater or running off directly to surface waters. Both nitrogen and phosphorus cause eutrophication with major impacts on ecosystems. Thus, high nutrient-use efficiency in agricultural systems is needed. These difficulties have resulted in the rise of controlled environment agriculture (CEA), encompassing the cultivation of plants in fully controlled environments, which may be combined with aquaculture in the form of aquaponics. Systems like these enhance the circularity required to reduce the human footprint on our planet.

1.2 Circular food production

To increase the sustainability of human activities on Earth, it's important to increase the circularity of our food production systems. Beside agriculture, the circularity of animal production also needs to be improved. Currently, animal production falls far short of being circular, due to inefficient resource utilisation and high waste generation (Pikaar et al., 2017). Over 58 million tonnes of food waste is generated annually, which is approximately a third of all food produced for human consumption (European Commission, 2023). Of this food waste, 54% is generated by households, whereas 21% is produced during the manufacturing of the food itself (Eurostat, 2023b). The other 25% of generated food waste was from the primary production sector, restaurants and food services, and retail and other distribution of food sectors (Eurostat, 2023b). A more circular food production is needed to optimize the use of resources, and this can be achieved in multiple food sectors, whereby animal husbandry, aquaculture, and aquaponics are discussed below.

1.2.1 Animal husbandry

Animal-based foods contribute to 40% of the protein and account for 18% of the total calorie intake in the human diet (Harchaoui et al., 2023). Scarlat et al. estimated the livestock and poultry population in Europe based on data from Eurostat for years 2009-2013 (Eurostat, 2023a). This total livestock population in Europe counts approximately 2260 million animals from which 4% are cattle, 7% pigs, 5% sheep and goats, and 83% poultry. However, animal production is currently not sustainable. Despite being one of the oldest and most traditional industries worldwide, animal farming generates a significant quantity of waste in the form of manure, which consists of urine and faeces and may contain livestock bedding and wasted feed. These waste streams include a wide spectrum of essential nutrients including nitrogen, phosphorus, potassium, as well as micronutrients like copper, manganese, and zinc (Manitoba, 2015).

Manure can be thought of as a renewable resource from which energy may be recovered or from which nutrients may be used as fertiliser for subsequent plant cultivation. However, untreated, manure can cause severe environmental impacts. Poor management of manure contributes towards global climate change via the emissions of methane (CH_4) and nitrous oxide (N_2O), while ammonia (NH_3) emissions can also negatively affect the air, soil, and air quality (Z. He et al., 2016).

Addressing excessive nutrient discharge is a matter of concern regardless the source. The natural levels of phosphorus and nitrogen in the environment are below the established environmental quality standards (Emis Vito, 2023). For instance, in rivers these standards specify a summer half-year average of 0.14 mg/L for total phosphorus and a 90^{th} percentile limit of 10 mg/L for nitrogen. In European rivers, nitrogen concentrations below 0.3 mg/L and total phosphorus concentrations between 5 and 50 µg/L are considered natural (Nilja et al., 2000). However, the concentrations of nitrogen and phosphorus can exceed these standards due to the eutrophication of water bodies.

A substantial amount of feed is required for the animals, which in turn demands a significant amount of land. Deforestation is connected to the land needed for agriculture through the direct causality of forests being destroyed to increase cultivatable land. For example, in Brazil an annual conversion of 785-2150 km² of tropical forest into cropland took place between 2001 and 2004 (Assunção et al., 2016). This cropland is mainly used to produce soybean, sugarcane, and maize (Morton et al., 2006). Considering that 98% of the soybean production ends up in animal feed, animal production indirectly causes deforestation (Anderson international corp, 2023).

1.2.2 Aquaculture

Aquaculture is the farming of aquatic organisms. It covers the farming of both animals (including molluscs and crustaceans) and plants (including freshwater macrophytes and seaweeds) and occurs in both freshwater and saltwater (brackish water and seawater) (Food and Agriculture Organisation of the United Nations, 1998). Aquaculture is the controlled cultivation of aquatic organisms, whereas fisheries primarily involve the capture of wild aquatic organisms from natural habitats. Figure 1 illustrates the increasing quantities of animals cultured in aquaculture systems over time, due to the increase in demand for fish by the growing world population. In 2020, the majority of finfish production in aquaculture (85%) occurred through inland aquaculture, while the remaining 15% was attributed to marine and coastal aquaculture (FAO, 2022). The increase of aquaculture production can be achieved by intensification of aquaculture. Nonetheless, intensification necessitates higher inputs, including fish and feed per unit of culture area, causing a rise in waste production within aquaculture production systems. This high amount of waste produced in aquaculture systems may result in negative economic externalities and its improper treatment may lead to the decline of water quality, environmental pollution, and an increased prevalence of aquatic diseases (Y. Wu & Song, 2021).

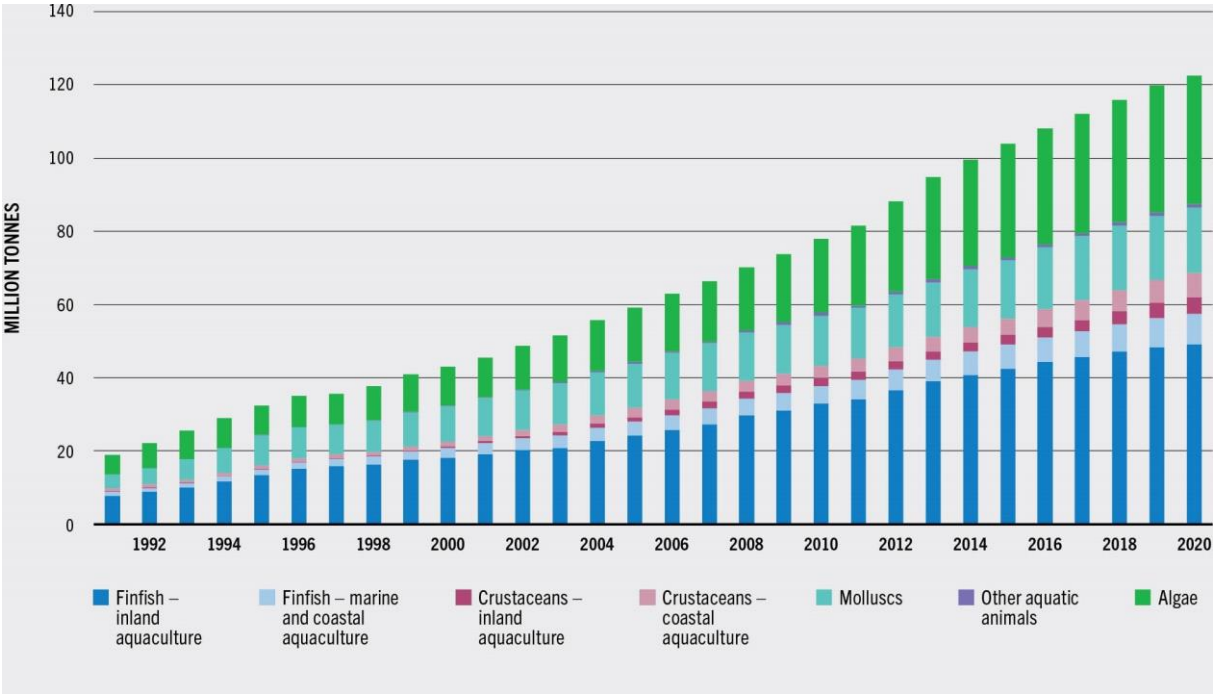

Figure 1: Global aquaculture production from 1990 until 2020 (FAO, 2022).

Finfish aquaculture waste includes aquaculture sludge and fish processing waste (Y. Wu & Song, 2021). The processing of fish results in substantial quantities of by-products, often disposed of as waste. Mechanically processing fish for fillets typically yields 30-40% of fillets, with the remaining 60-70% by weight consisting of discarded by-products (Gehring et al., 2009). Aquaculture sludge includes residual feed, and fish excreta. The quantity of waste generated by feeding is influenced by numerous variables, such as nutrient composition, the method of production (extrusion or pelleting), the ratio of feed size to fish size, the amount of feed provided per unit of time, the feeding method, and the duration of storage (Dauda et al., 2018).

Aquaculture waste can generally be categorized in two groups: dissolved waste and solid waste. The solid waste consists of uneaten feed and fish faeces. Around 30% of the feed utilized will be converted into solid waste in a properly managed farm (Miller & Semmens, 2002). However, the amount of feed wasted can be decreased by providing slightly suboptimal rations to fish, i.e. feeding the fish at a level below their maximum consumption capacity, ensuring that they receive enough feed to support their nutritional needs for optimal

growth but not providing the maximum digestible amount that would lead to excess waste (Ali et al., 2010; Mizanur et al., 2014). It was observed that the proportion of wasted feed could be reduced to 10.64% by maintaining an initial maintenance feeding period lasting 14 days, followed by 28 days of daily feeding at 75% of the control ration. In contrast, conducting a treatment involving an initial 14-day maintenance feeding period followed by 28 days of daily feedings at 100% of the control ration resulted in a 3.84% reduction in feed wastage. The control group, which entailed feeding at the maximum ration, led to a 38.34% of feed wastage. (Ali et al., 2010).

Solid waste can be classified as suspended solids and settled solids. Suspended solids are fine particles that are suspended in the water unless coagulation or sedimentation is performed. They are the most difficult particles to remove. The settled solids are larger particles that settle within a short time period, and can be easily removed (Dauda et al., 2018). Solid waste removal should be done as fast as possible to reduce waste fragmentation, which can cause leaching of nutrients in the water (Miller & Semmens, 2002).

The other category of waste is the dissolved waste. This includes decomposed uneaten feed and products of food metabolism in fish, such as ammonia. Nitrogen and phosphorus products are the major components of concern in dissolved waste. Phosphorus is mainly excreted as particulate matter, whereas nitrogen is mostly excreted in dissolved form as ammonia (Dauda et al., 2018). Ammonia is harmful for fish and is the most toxic in its un-ionized form. It's converted into nitrite and nitrate by nitrifying microbial communities in specially designed bioreactors called biofilters. The concentrations of nitrate in both natural environments and aquaculture systems normally remain below toxic levels (Hamlin, 2006). However, it can cause eutrophication when present in high concentrations. Phosphorus is mainly released in the water as phosphate and can also contribute to eutrophication (Dauda et al., 2018; Miller & Semmens, 2002). In addition to dissolved nutrients, dissolved waste also includes dissolved organic matter, quantifiable as chemical oxygen demand (COD) and biological oxygen demand (BOD). The BOD is the dissolved oxygen used by microorganisms in the biochemical oxidation of organic matter, whereas COD is the amount of oxygen required to oxidize by chemical means organic carbon completely to CO_2 (Rabaey & Wang, 2021).

The circularity in aquaculture systems can be improved by recirculating the water of the fish tanks to a biofilter and back - standard practice in recirculating aquaculture systems (RAS). The water is filtered to remove the solids, and a biofilter is used to convert the ammonia to nitrate. However, there are also some challenges accompanied with RAS. Extensive knowledge is required to operate RAS at commercial scale (Badiola et al., 2012). The buildup of nutrients and dissolved organic materials, originating from unconsumed feed and fish excrement, can create a conducive environment for various microorganisms, including some pathogens. These microorganisms have the potential to influence water quality, ultimately affecting the well-being of the fish stock (Badiola et al., 2012).

RAS may encounter pathogens despite strict biosecurity measures. Even the most efficiently operated farms may encounter contamination over time (Badiola et al., 2012). Vibriosis is a disease that can lead to significant economic losses and the mortality of cultured shrimp, fish, and selfish (Novriadi, 2016). Vibriosis is caused by bacteria of the genus *Vibrio*, which include many species that are recognized as pathogens affecting both freshwater and saltwater fish (Novriadi, 2016). *Vibrio parahaemolyticus*, *Vibrio alginolyticus, Vibrio harveyi*, *Vibrio owensii*, and *Vibrio campbellii* are the most common species infecting farmed aquatic animals. The occurrence of diseases can be reduced by improving the water quality, providing high-quality feed, breeding of disease-resistant broodstocks, establishment of a vaccination program, etc. (Ina-Salwany et al., 2019).

1.2.3 Controlled environment agriculture (CEA)

The CEA has emerged as a strategy to tackle the global issue of long-term decline in the availability of agricultural land per capita. This decreasing trend is expected to persist, primarily due to the impact of climate change, the increase of dry regions, diminishing freshwater resources, and population growth (Benke & Tomkins, 2017). Controlled environment agriculture may take many forms from simple greenhouse techniques to urban, indoor, climate-controlled structures (Benke & Tomkins, 2017). The CEA is a method in which a fully controlled environment is created to grow plants using intensive growing conditions throughout the year with advanced technologies (Congressional Research Service (CRS), 2023). Multiple CEA production systems exist. There are hydroponic systems in which plants are grown in a water-based nutrient solution. In aeroponic systems, plants are grown by suspending their roots in the air. Their roots are misted regularly with a water and nutrient solution. Another CEA system is a vertical system in which crops grow in vertically stacked layers on top of each other or in tall towers. It can use soil or soilless techniques such as hydroponics or aeroponics (Congressional Research Service (CRS), 2023).

Plants in CEA are often grown with artificial lights accompanied by water-based nutrient delivery systems. Mineral nutrients are provided through inorganic chemicals. Most of the fertilizers used in CEA are chemical fertilizers, which involve an energy cost and monetary expense (Masabni & Niu, 2022). Environmental parameters, such as temperature, light (intensity and quality), air movement, humidity, and carbon dioxide levels, are controlled to create optimal conditions, which results in higher crop yield (Congressional Research Service (CRS), 2023; Dantherm Group, n.d.). According to analysis of 18 literature papers, it was determined that the average rise in soybean biomass and seed yield following a doubling of ambient CO_2 concentrations was 39% and 29%, respectively (Lawlor & Mitchell R.A.C., 1991). Plants can be grown year-round and are safe from outside contamination. This likewise results in less fertilizers, herbicides, pesticides, land usage, and the water needed can be reduced by 70 - 95% (Dantherm Group, n.d.).

1.2.4 Aquaponics

To enhance the sustainability and circularity of CEA systems, plant cultivation can be integrated with aquaculture, resulting in aquaponics. In this system, the nutrient-rich water from the fish tank is fed to the plants. These plants absorb the nutrients and, subsequently, the water is returned to the fish tanks. Fish excrete ammonia, either through their gills or urine, which can be highly toxic to them, even at concentrations as low as 1.2 to 2.0 mg/L (Masabni & Niu, 2022). Ammonia is converted to nitrate by nitrifying bacteria (nitrification). It is first converted to nitrite by ammonia oxidising bacteria (AOB), represented principally by members of the genera *Nitrosomonas* and *Nitrosospira*. Nitrite is then further oxidised to nitrate by the nitrite oxidising bacteria (NOB), represented by members of the genera *Nitrobacter* and *Nitrospira* (Rabaey & Wang, 2021). However, the nitrification intermediates -nitrite and hydroxylamine- can produce some nitrogen-containing, gases such as nitric oxide (NO) and nitrous oxide (N_2O), which have negative environmental consequences (Heil et al., 2016). The nitrate is absorbed by the plants and the treated water returns to the fish. Chemical fertilizers are no longer needed or at least reduced, as the nutrients are delivered by the fish and bacteria.

Traditional aquaponics is a coupled system where the water is recirculated between the fish tank and the hydroponic system. It's also called closed loop, single loop, or conventional aquaponics. There are some challenges accompanied with these kind of systems as every component of the aquaponic system (fish, plants, bacteria) has its own set of optimal parameters to achieve maximum production. A compromise needs to be made in overall conditions, thus reducing productivity and efficiency (Masabni & Niu, 2022). A schematic representation of the key components of a coupled aquaponics system is shown in Figure 2.

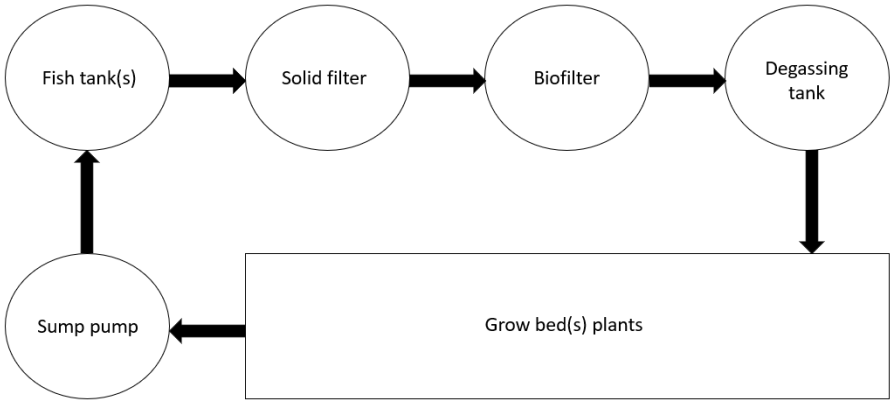

Figure 2: The key components of a coupled aquaponics system, adapted from (Masabni & Niu, 2022).

The solid filter removes the larger settleable solids and a biofilter is used as a secondary stage of waste removal of smaller suspended solids. A degassing tank is employed for removing excessive gases, like carbon dioxide, resulting from anaerobic conditions within the filtration system. It also has the capability to provide the system with essential nutrients, such as calcium, and adjust the pH as needed (Masabni & Niu, 2022).

The other type of system that can be implemented is a decoupled aquaponics system. In this case, the aquaculture and hydroponic system can operate independently from each other to ensure optimal growth for both plants and fish. There are a lot of variations, but it typically consists of two independent recirculating units: a recirculating aquaculture system for fish and a hydroponic system for the plants. The two units are linked via a one-way valve, permitting the solution stored in the tank to flow into the grow beds if required (Masabni & Niu, 2022). A schematic diagram of a decoupled aquaponic system is depicted in Figure 3.

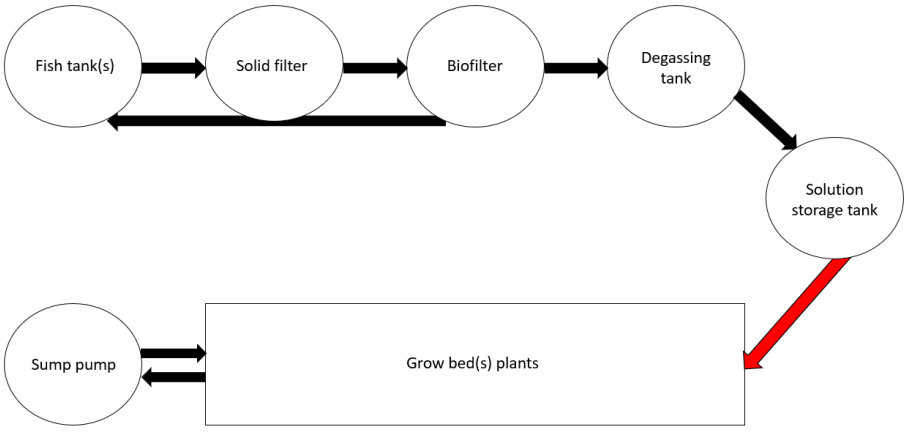

Figure 3: Schematic representation of a decoupled aquaponic system. The red arrow indicates that the flow of the nutrient solution from the storage tank to the grow beds is not permanent. The connecting valve is normally closed and is only opened when a nutrient solution is needed to refill the grow beds. Adapted from (Masabni & Niu, 2022).

1.2.5 Insect cultivation

To make the food production more circular, insect-based bioconversions can be implemented as a solution to reduce food waste, with the insect protein usable as a component of animal feed. According to the UNEP, 50 kg food waste is produced per capita in households in Belgium per year, which is on the low side compared to other European countries. The global food waste from households, retail establishments and the food service industry is estimated to be 931 million tonnes each year (UNEP, 2021).

The use of insects to make food production more sustainable and circular has gained a lot of interest worldwide, due to (1) their capacity to convert organic matter into proteins, (2) their ability to use food waste as feed so they can help to tackle the food waste problem, (3) reduced amount of space, water and often energy use needed compared to their livestock counterparts, (4) they can serve as substitutes for fishmeal due to its similar nutrient profile as fish meat (Derler et al., 2021). The food waste can be converted to valuable products through insect production, such as human and animal food, fertilisers, and biofuels. The insects protein originating from Black soldier fly (*Hermetia illucens),* common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus)*, and field cricket (*Gryllus assimilis*) can be used in the feed for aquaculture, poultry, and swine animals in the EU (Commission Implementing Regulation (EU) 2021/882, 2021; Commission Regulation (EU) 2017/893, 2017). Veldkamp et al. (2012) state that black soldier fly, common housefly, and yellow mealworm are the most promising for industrial production in the Western world, due to their short lifecycle and their ability to convert low-quality organic side-streams efficiently to valuable proteins.

The production of Black soldier fly larvae (BSFL) in particular is gaining momentum through companies like Innovafeed, Protix, and Wastech. Their lifecycle is depicted in Figure 4. It goes through four main phases throughout its life, namely the larval stage, the pupal stage, the adult stage and the egg stage, with a total duration of around 40 days. BSFL can feed on food processing waste. The substrate reduction and bioconversion rate of food waste into BSFL biomass varies with the BSF strain, the feeding rate and larval density. Surendra et al. (2020) performed a literature review about the bioconversion rates of different waste streams by BSFL. They found that when using Black Soldier Fly Larvae in the bioconversion process, the dry matter content of fruit and vegetables is reduced by 46.7% to 60%, with a bioconversion rate ranging from 4.1% to 10.8%. This rate is calculated as the weight of larval biomass divided by the weight of substrate added. Additionally, the overall reduction in food waste was 55.3%, achieved through a bioconversion rate of 13.9%. BSFL can be used for a lot of applications. High protein larvae meal can be produced by dehydration of the larvae, followed by pressing so that the fat is separated from the flour. The BSFL meal contains 54% protein, which is quite similar to the protein content found in fish meal, which is between 58% and 70%. As aquaculture is one of the fastest growing industries, the use of BSFL meal as replacement of fish meal gained a lot of interest. The BSFL meal has a lower production cost, reduces the use of raw materials and the environmental footprint is much lower (Mohan et al., 2022).

The model of the company Wastech relies on food processing waste and produces two waste streams: food waste leachate and BSFL frass. The frass is a mixture of insect faeces, waste residue and exoskeleton sheds (Dzepe et al., 2022). It can be used for multiple applications, such as organic fertiliser, soil amendments, growing medium for plants, biochar, and the production of biogas by anaerobic digestion (Basri et al., 2022). The other waste stream produced by Wastech is food waste leachate, which is the liquid that leaches out from decaying food waste. In a study by Yoon et al. (2018), anaerobic digestion was conducted on wastewater treatment sludge from various sources, including brewery, dairy factory, bread factory, and sewage sludge, in combination with food waste leachate. The methane production from the wastewater treatment sludge from the brewery, dairy factory, bread factory, and sewage sludge was respectively 149.4, 80.2, 246.81, and

200.39 mL/g VS. Through the combination of these feedstocks with food waste leachate in a 9:1 ratio, there was an observed increase in methane production by 18%, 47%, 9%, and 16%, respectively.

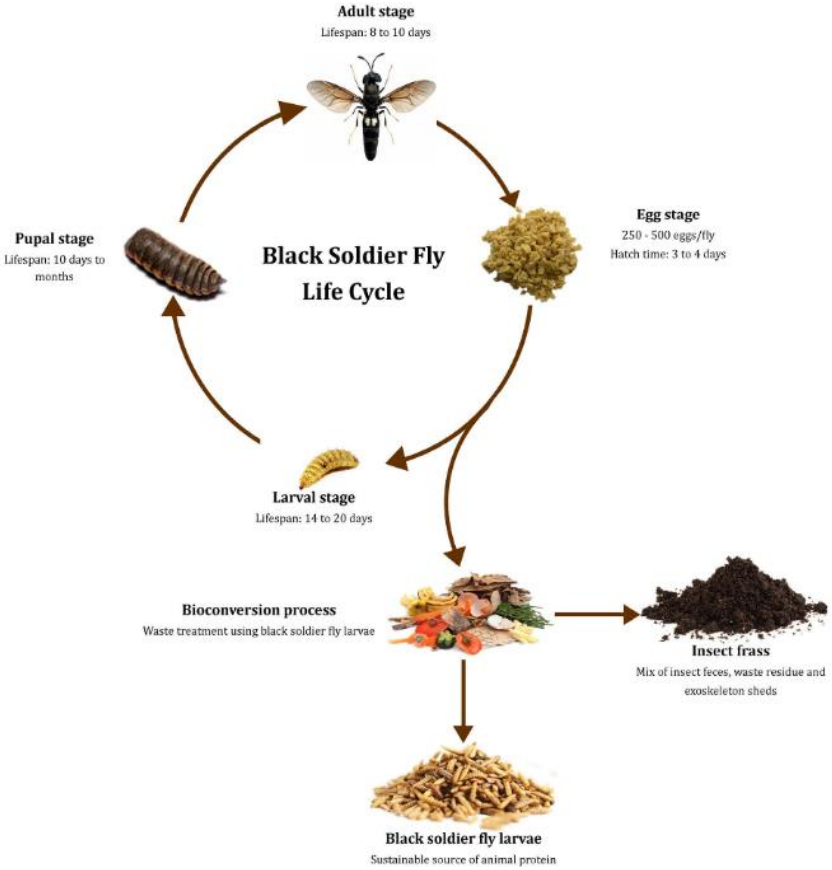

Figure 4: Lifecycle of black soldier flies with integration of the bioconversion process of biowaste (Dzepe et al., 2022).

1.2 Anaerobic digestion

Waste streams, such as manure, food waste, agricultural waste, and sludge, can be used to produce biogas by anaerobic digestion. This conversion increases the circularity and reduces its environmental impact. Anaerobic digestion is an energy recovery technology. It can be applied as pre-treatment step to reduce the waste volume and control the odour and pathogens or as primary treatment of biomass to generate renewable energy and fertilizer (Rufai & Rufai, 2010).

1.2.1 Relevance

Biogas, a mixture of mostly methane and carbon dioxide, is produced by anaerobic digestion. The raw biogas can be burnt directly to produce thermal energy (Kaparaju & Rintala, 2013). Nevertheless, purification is often necessary, requiring processes like desulphurisation to prevent corrosion. The treated biogas, once cleaned, can be utilized for the generation of electricity and heat using for example a Combined Heat and Power (CHP) system (Pavičić et al., 2022). Further enhancing the methane content in the biogas is achievable by reducing the carbon dioxide fraction. In Belgium, biomethane with a methane concentration of at least 97.5% is eligible for injection into the gas grid (Fluvius, n.d.). The production of biogas increased with ~90% over the past 10 years (120 GW in 2019 compared to 65 GW in 2010) due to climate change awareness, reasonable energy prices, etc (Abanades et al., 2022). Renewable energy accounted for 13% of Belgium's gross final energy consumption, 25% of electricity generation, and 8% of heating and cooling demand (International Energy Agency, 2022). Europe contributed in 2019 to over 70% of the world biogas generation (Abanades et al., 2022). The production of biogas is very promising, it's a renewable energy source that can contribute to the goal of Europe to become climate neutral in 2050 by reducing the need of fossil fuels.

Biogas can be produced from waste. The most common waste streams used to produce biogas are organic wastes, which include domestic waste (food, vegetables, and fruits) or public moist wastes (daily markets, cafes and restaurants, and biological waste), due to their high moisture content and degradability. These waste streams are classified as the organic fraction of municipal solid waste (OFMSW) (Abanades et al., 2022). The high concentration of solids produced in aquaculture systems can also be digested and produce biogas instead of being discharged into receiving water bodies or the local sewer system, or into a decentralized treatment unit, most commonly waste-stabilisation ponds (WSPs) (Mirzoyan et al., 2010).

1.2.2 Process

Anaerobic digestion (AD) is a biological conversion process in which organic matter is degraded by microbes under anaerobic conditions (Mirzoyan et al., 2010). Biogas, composed of methane and carbon dioxide, is produced during this process together with small levels of hydrogen sulphide and ammonia (Mirzoyan et al., 2010). The residual product of anaerobic digestion is digestate and is often used as fertilizer. The digestate is a concentrated inorganic/organic mixture with a high moisture content and is highly dependent on the type of system that is used (Wang & Lee, 2021). The wet anaerobic digestion process (<10-20% TS) involves the introduction of a significant quantity of water for digestion process, resulting in a higher water content in the produced digestate (90-98%) (Karunanithi et al., 2018; Luning et al., 2003). According to a literature review performed by Lu & Xu (2021), the moisture content of food waste digestate ranged from 92.2 to 98.6%. The amount of biogas produced depends on several factors, such as the pH, digestibility of the feedstock, salinity, loading rate, hydraulic retention time (HRT), etc (Mirzoyan et al., 2010). The major processes of AD can be classified in four stages: hydrolysis, acidogenesis, acetogenesis, methanogenesis (Figure 5).

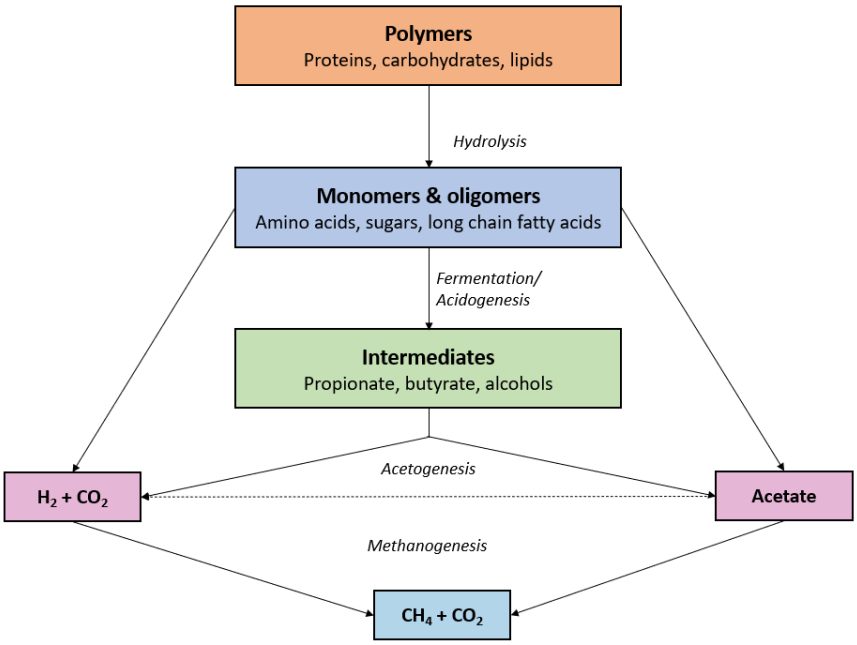

Figure 5: Schematic overview of the anaerobic digestion process (Angenent et al., 2004).

Hydrolysis is the first step in AD. Water-insoluble molecules are decomposed into small compounds: proteins, carbohydrates and lipids are converted by hydrolytic bacteria into their monomers, namely amino acids, sugars and long chain fatty acids (Atelge et al., 2018). These bacteria produce extracellular hydrolytic enzymes, such as protease, lipase, cellulase, etc. to degrade the polymers (Mara & Horan, 2003). The more carbohydrates present in the substrate, typically the higher the fraction of CO_2 in the biogas. Carbohydrates produce biogas with 50% methane and 50% CO_2, whereas lipids and proteins produce a higher fraction of CH_4, as they are more reduced compared to carbohydrates (Atelge et al., 2018).

The monomers, produced during hydrolysis, are converted into secondary metabolites, such as volatile fatty acids (VFA), alcohols, hydrogen, and CO_2 during acidogenesis, also called fermentation. The fraction of CO_2 and H_2 in the products are approximately 70% whereas the VFA and alcohols are around 30% (Atelge et al., 2018). Most substrates are, under stable conditions, converted to hydrogen and acetate directly, rather than through the reduced products. However, larger amounts of less oxidized products such as propionate, butyrate, and ethanol are produced when the reactor is overloaded, which can be through excessive production of acetate and hydrogen, or pH extremes (Angelidaki & Batstone, 2010). The pH will then decrease, due to the formation of protons (Ganigue et al., 2023).

During the acetogenesis, the VFA and alcohols, produced during the acidogenic phase, are converted into acetate with H_2 and CO_2 as by-products (Angelidaki & Batstone, 2010). It also includes the potential conversion of CO_2 and H_2 into acetate or the other way around, depending on local conditions (Angenent et al., 2004). The conversion processes during acetogenesis are performed by obligate hydrogen producing acetogens and syntrophic acetogenic bacteria (Ganigue et al., 2023).

The last phase is methanogenesis, which is performed by methanogens belonging to the domain of archaea. Methane and CO_2 are produced via two pathways, dependent on multiple factors. Typically, 70% of the methane is produced from acetate (acetoclastic methanogenesis). The other 30% is produced from the conversion of H_2 and CO_2 (hydrogenotrophic methanogenesis). The conversion of acetate to methane can only be performed by two genera of archaea, namely *Methanosarcina* and *Methanothrix*, whereas the *Methanosarcina* have the highest maximum growth rate and *Methanothrix* have the highest substrate affinity for acetate (Mara & Horan, 2003). The *Methanosarcina* are also capable to use the hydrogenotrophic methanogenesis pathway, so they are considered to be mixotrophic (Ganigue et al., 2023). Methanogenesis

is the most critical phase because the methanogenic archaea are the most sensitive group. Operating conditions, such as substrate type, pH, temperature, and feeding rate have significant effects on methanogenic archaea. The anaerobic digestion can be terminated due to overloading of the digester, temperature fluctuation of more than 3 °C and large amounts of oxygen, because of the high sensitivity of the methanogenic archaea (Angelidaki & Batstone, 2010; Atelge et al., 2018). The acetogenic microorganisms, producing hydrogen, live in syntrophic association with the hydrogenotrophic methanogens, which consume the hydrogen, keeping the partial pressure of hydrogen sufficiently low to allow acetogenesis to be thermodynamically favourable (Angenent et al., 2004).

1.2.3 Process factors

There are several factors that influence the performance of the anaerobic digestion, which are discussed below.

1.2.3.1 Temperature

Temperature significantly influences the metabolic activities of microbial communities and has subsequently an effect on the efficiency and stability. Nie et al. (2021) state that the temperature is one of the most important parameters that affects both the AD microbial ecosystem and the digester performance. The AD process can occur at various temperature levels, categorized into three temperature ranges: psychrophilic (< 20 °C), mesophilic (20-43 °C, optimal between 35 °C and 37 °C), and thermophilic (50-60 °C, optimal at 50 °C) (Nie et al., 2021).

The effect of the temperature is different according to the different stages of AD. The substrate hydrolysis rate increases with temperature (Donoso-Bravo et al., 2009; Hao & Wang, 2015; Veeken & Hamelers, 1999). Hao & Wang (2015) found that the extracellular enzyme activity was nearly twice as high under thermophilic conditions (55°C) compared to mesophilic conditions (35°C). The relation between temperature and hydrolytic microorganisms can be described by the Arrhenius equation: the hydrolytic activity increases until an optimal temperature after which the activity decreases. The low activity at low temperature can be explained by the fact that the uptake of substrates into the cell and the membrane functions will be inhibited at lower temperatures (Nie et al., 2021). As enzymes are very thermally sensitive, the enzymatic activity will also be lower at lower temperatures (Donoso-Bravo et al., 2009).

Methanogenic microorganisms are more sensitive than hydrolytic and acidogenic bacteria. Most studies show that acetoclastic methanogens largely dominate at mesophilic temperatures while hydrogenotrophic methanogens dominate at thermophilic temperatures (Nie et al., 2021). This suggests that the predominant pathway for methanogenesis changes according to the temperature (Nie et al., 2021). Methanogenic species are also more sensitive to temperature fluctuations. They are already sensitive to changes of 1 °C in thermophilic conditions whereas hydrolytic and acidogenic species can tolerate changes of 3 °C without significant changes in the biogas production (Atelge et al., 2018).

The solubility of several products is also influenced by the temperature. Gases as H_2S, CH_4, and NH_3 will be less soluble when the temperature increases, which can diminish their inhibitory effect on the AD process (Atelge et al., 2018).

In general, the anaerobic digestion rate will be higher at thermophilic conditions compared to mesophilic conditions (Mara & Horan, 2003). Thermophilic microorganisms exhibit elevated substrate utilisation and growth rates, along with a higher decay rate, in comparison to mesophilic bacteria (Kim et al., 2002). Thermophilic systems also result in lower quantities of sludge production (Mara & Horan, 2003). However, there are some disadvantages accompanied with these higher temperatures, such as the lower reactor

stability and the higher energy requirements (Mara & Horan, 2003). The lower reactor stability has been associated with the accumulation of VFA, potentially arising from the temperature sensitivity of acetolactic methanogens in elevated thermophilic conditions (Wilson et al., 2008). Dissociation of these VFA can cause the release of free hydrogen, lowering the reactor pH (Wilson et al., 2008). Another challenge in thermophilic anaerobic digestion is ammonia inhibition, presenting an increased risk at higher temperatures due to the observed correlation between rising temperatures and free ammonia concentrations (Ryue et al., 2019).

1.2.3.2 pH

The pH has also an impact on the performance of the reactor as anaerobic microorganisms, and especially methanogens, are sensitive to pH extremes (Mara & Horan, 2003). Fermentative bacteria are the least influenced by the pH, demonstrating a good activity within the pH-range of 4.0 to 8.5. However, the products formed during the fermentation are influenced by the pH. The butyrate and acetate concentrations increase in the pH-range 4.0 to 8.0, whereas the propionic acid level is elevated at pH values higher than 8.5 (Appels et al., 2008). Methanogens are the most pH sensitive microorganisms in the anaerobic digestion. Their pH range is between 6.5 and 7.8 (Mara & Horan, 2003). In mildly acidic pH conditions, *Methanosarcina* is favoured above *Methanothrix*, as *Methanothrix* are inhibited at pH-levels lower than 6 (Ganigue et al., 2023).

During anaerobic digestion, the pH is influenced by four types of reactions, namely (1) ammonia consumption and release, (2) production and consumption of VFA, (3) release of sulphides by dissimilatory reduction of sulphite or sulphate, (4) conversion of neutral carbonaceous organic carbon to methane and carbon dioxide (Mara & Horan, 2003). In a well working reactor, the pH reduction, due to the production of VFA, can be countered by the activity of the methanogens, which produce CO_2, ammonia, and bicarbonate (Appels et al., 2008). However, if the pH is decreased too much, the feeding of the reactor can be stopped to give the methanogens enough time to consume the VFA, or alkali (NaOH, Na_2CO_3) can be added to the reactor to increase the pH albeit this latter is not a viable long-term solution (Mara & Horan, 2003).

1.2.3.3 Salinity

The salinity has also an effect on the biogas production. Low concentrations of NaCl improve both the hydrolysis and acidification. The addition of Na^+-ions can enhance enzyme activity, sustain biofilm equilibrium, and regulate osmotic pressure during microbial growth (Zhang et al., 2014). An increasing salinity causes a decreasing specific activity of the methanogens. The extent of inhibition resulting from NaCl varies with the dosage. In a study conducted by Zhao et al. (2016), it was observed that the degradation rate of acetate decreased from 91.6% (without any salt addition) to 42.7% and 26.1% after six days with the addition of 8 g NaCl/L and 16 g NaCl/L, respectively. Another study by Zhao et al. (2017) revealed a decline in the acetate degradation rate from 53.9% to 12.6% after three days as the NaCl concentration increased from 0 g/L to 15 g/L. The toxicity to methanogens may be caused by an increase of the osmotic pressure, which affects the metabolically active intracellular enzymes in living cells (Zhang et al., 2014). However, Zhang et al. (2014) still observed methanogenic activity at salt concentrations of 44 g/L, probably due to the presence of some halophilic methanogens, whereas Rinzema et al. (1988) observed inhibition at a NaCl concentration of 36 g/L. They also found that an increase of the salt-concentration from 13 g/L to 25 g/L caused a decrease of the methane fraction in the biogas from 50% to 10%.

1.2.3.4 Inhibitory factors

Speece (1983) defined inhibition in anaerobic digestion as the impairment of microbial function. There are a lot of possible inhibitors in the anaerobic digestion process.

Hydrogen is produced during the acidogenesis and acetogenesis. Hydrogen can only be formed during acetogenesis when it's also consumed by the methanogenesis or used for the acetate formation, so it doesn't accumulate. The conversion of propionate and butyrate is only thermodynamically favourable when the partial pressure of hydrogen (pH_2) is lower than 10^{-4} for butyrate and 10^{-5} atm for propionate. When the pH_2 is higher than 10^{-4}, CO_2 will be reduced instead of acetate as its Gibbs free energy change is higher.

Long chain fatty acids (LCFA) can inhibit the metabolism of the methanogens at high concentrations by the solubilisation of the lipid bilayer of membrane proteins causing cell lysis, inhibition of the enzyme activity or disruption of the electron transport chain. This causes the accumulation of volatile fatty acids and a decreased methane production (Ma et al., 2015). Angelidaki & Ahring (1992) found that LCFA, like oleate and stearate, are inhibitory at concentrations of respectively 0.5 g/L and 1.0 g/L in thermophilic conditions.

Even short chain volatile fatty acids, produced during the fermentation and acetogenesis, can be toxic for microorganisms, especially to methanogens at a concentration of 1.15-1.55 g/L. The accumulation of VFA can occur as a result of system imbalances, which can be caused by temperature variations, toxic compounds, etc., so that the methanogens cannot remove the VFA fast enough (Appels et al., 2008). The accumulation of VFA can cause such a strong decrease in pH, thus, also inhibiting the hydrolysis or acetogenesis (Appels et al., 2008).

Ammonia is produced during the degradation of nitrogen containing molecules, such as proteins. The two most predominant forms of inorganic nitrogen are ammonium ion (NH_4^+) and free ammonia (NH_3) from which free ammonia is the most toxic, as it can pass through the cell membrane. The concentration of free ammonia is dependent on the temperature, pH, and total ammonia concentration. A higher pH results in a higher toxicity, as more nitrogen is present in the form of free ammonia (Appels et al., 2008). The concentration of free ammonia also increases slightly with increasing temperature (Ganigue et al., 2023). The free ammonia is most toxic for methanogens, as it alters their potassium-influx, so that they become less performant both in terms of methane production rate and residual VFA removal (Ganigue et al., 2023).

1.2.3.5 Nutrients

The balance of carbon sources together with other macronutrients like nitrogen, phosphorus and sulphur are important process parameters for the anaerobic digestion. Additionally, certain micronutrients, such as iron, nickel, and magnesium, are essential for a variety of chemical, biochemical and microbiological reactions related to VFA utilization, methane generation and cell lysis (Menon et al., 2017).

1.2.3.5.1 Macronutrients

Optimal nutrient supply in the anaerobic digester is essential for efficient biogas production. Atelge et al. (2018) stated that the balance of macronutrients in an AD reactor should be 1000:5:1:1 (biodegradable COD (bCOD):N:P:S) for substrate to be more suitable for methanogenic archaea, whereas this ratio should be around 350:5:1:1 (bCOD:N:P:S) if the substrate will be used for the hydrolysis phase (Atelge et al., 2018).

The C/N ratio is a critical parameter for characterizing feedstock. It should be in a range between 20/1 and 30/1 for methanogenesis, and between 16/1 and 45/1 for hydrolysis. If this ratio is high, the available nitrogen is consumed quickly by the microorganisms for their cell synthesis, whereas the carbon utilisation

will be limited. Hence, the anaerobic digestion process may come to a halt. A low C/N ratio can cause the conversion of nitrogen to ammonia, which can inhibit the AD process (Atelge et al., 2018). However, the requirements of nitrogen during anaerobic digestion are minimal since the cell yields under anaerobic conditions are quite low (0.05-0.1 gram cell dry weight (CDW) per gram of COD) compared to aerobic conditions (0.15-0.5 g CDW/g COD, depending on the type of microorganism and substrate) so the C/N ratio of the substrate is rarely a limiting factor (Angelidaki & Batstone, 2010; Sakarika et al., 2020; W. M. Wu et al., 1998).

1.2.3.5.2 Micronutrients

Micronutrients are essential to maintain the biological activity of the microorganisms. These nutrients are used for synthesis, replication, stabilisation, enzymatic processes, and transcription of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The prolonged utilisation of single-feedstock sources, such as agricultural waste, food waste, and industrial wastewater, can impede the stability of anaerobic digestion, due to the low concentration of micronutrients in these feedstocks (Bardi et al., 2023). This can be overcome by performing co-digestion with other feedstocks with higher concentrations of these micronutrients (Bardi et al., 2023).

According to Bardi et al. (2023), iron is the most important micronutrient in anaerobic digestion following the order of trace element usage: Fe >> Zn > Ni > Cu ≈ Co ≈ Mo > Mn. In research conducted by Feng et al. (2014), it was noted that the introduction of 20 g/L zerovalent iron resulted in a 43.5% rise in methane production over a period of 20 days. Iron can enhance the hydrolysis and acidification stages, leading to increased volatile fatty acid production. Iron can also accelerate the production rate of methane by increasing the activity of essential enzymes in methanogenic archaea. It can function as electron donor: zerovalent iron can act as an electron donor in the hydrolysis and acidogenesis. In addition, oxidized iron species, such as hematite (Fe_2O_3) and magnetite (Fe_3O_4) can act as a bridge for electron exchange between bacteria and methanogens due to their (semi-)conductive characteristics. Lastly, iron has the ability to precipitate sulphides and phosphates, which might otherwise facilitate the precipitation of other important trace elements (Bardi et al., 2023).

Calcium, sodium, magnesium, and potassium can be inhibitory in high concentrations, but they can stimulate the acidogenesis when they are present in low concentrations. Angelidaki & Batstone (2010) state that these cations improve the anaerobic digestion at concentrations between 0.005 M and 0.01 M. Many micronutrients can optimise process rates, especially nickel and cobalt are important for the growth of anaerobic microorganisms. Nickel is one of the most important additives used to improve the performance of the anaerobic digestion as it plays a key role in the enzymatic pathways: it's used by the methanogenic archaea for the synthesis of Ni-dependant enzymes. Nickel, just like iron, enhances the hydrolytic enzymatic activity (Angelidaki & Batstone, 2010).

1.3 Anaerobic co-digestion

Anaerobic co-digestion is the anaerobic digestion of two or more different feedstocks or waste streams in the same digester to overcome drawbacks in mono-digestion as ammonia inhibition, lack of micronutrients, and imbalanced C/N ratio to achieve improvements in the biogas yield, methane content, and process stability (Karki et al., 2021; Netshivhumbe et al., 2022). Food waste, for example, can give rise to a pH drop, as the rapid hydrolysis rate can cause VFA accumulation. In addition, some forms of food waste may be scarce in trace elements, as nickel and iron, making it inadequate for efficient mono-digestion (Karki et al., 2021). However, food waste can act as a co-substrate in anaerobic digestion in which the hydrolysis rate is reduced due to other, recalcitrant feedstocks. On the other hand, waste streams rich in proteins result in a low C/N ratio and will produce high amounts of ammonia during the fermentation (Esposito et al., 2012). By digesting these together with waste streams with high C/N ratios, the biogas yield can be improved.

1.4 Implementation of a biogas-system into circular food production systems

Biogas-systems can be implemented in circular food systems, such as aquaponics (Figure 6). The waste streams produced in the aquaponics system, can be anaerobically digested to produce biogas. Nutrient-rich water from the fish tank is fed to the plants, which absorb the nutrients. The aquaculture solids can be mechanically separated by the use of a drum filter, swirl separator, etc., after which they can be digested to produce biogas (Lobanov et al., 2023). Biogas can also be produced from the agricultural waste of the aquaponics system. The digestate can be utilised as fertilizer for the growing plants (nutrient remineralisation). However, most of the nitrogen is present as NH_4^+, which can be used as nitrogen source for plants, but it can be toxic when it's present in too high concentrations. Some studies suggest that the fraction of ammonia should be at maximum 50% of the total nitrogen present and therefore the digestate should first undergo nitrification (Bergstrand et al., 2020). In addition, the salt concentration should not be too high, as Na^+ and Cl^- ions can be toxic, decreasing the uptake of for example Ca^{2+}, K^+, and NO_3^-. They also exhibit a direct toxicity by reducing the water potential, increasing the ionic strength, and causing an impaired water and nutrient uptake (Bergstrand et al., 2020). The CO_2 present in the biogas can be utilized to enrich the plant crops, increasing their net photosynthesis (Mortensen, 1987).

The agricultural waste can also, in combination with for example food waste, be used to grow insects, such as BSFL. Insect meal, derived from the BSFL, can serve as feed for the fish, whereas biogas can be produced from the frass. The food waste leachate can, together with the frass, agricultural waste, and aquaculture solids be anaerobically digested. This circular process is depicted in Figure 6.

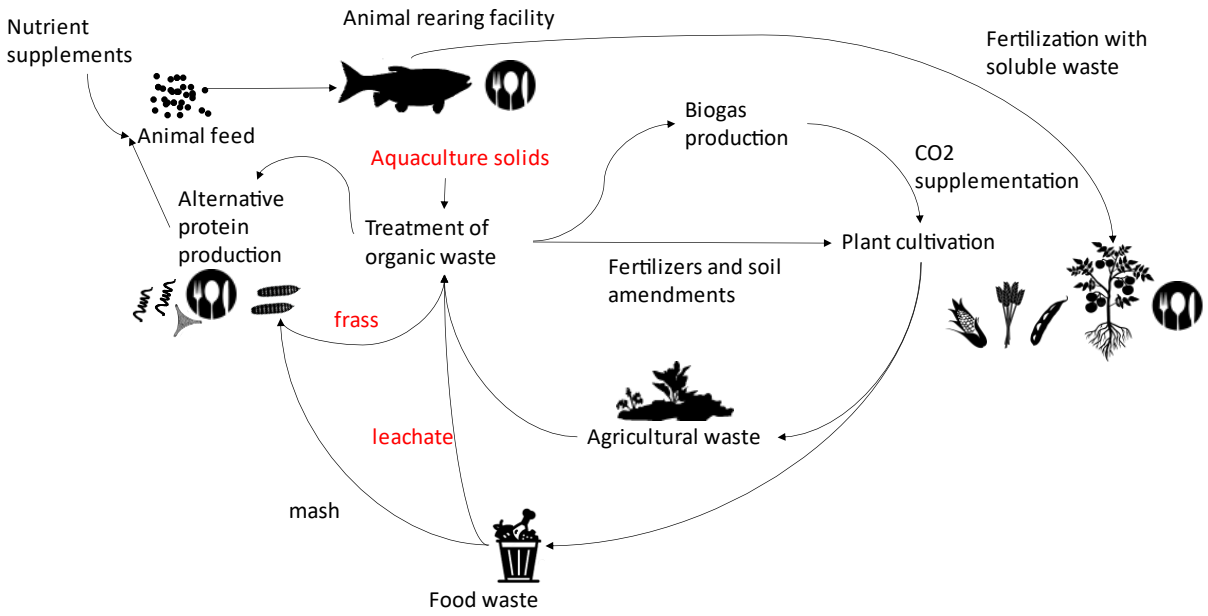

Figure 6: Overview of the circular integration of aquaponics and insect cultivation around a solids treatment system for nutrient remineralization. The feedstocks used in the experiments in this thesis are highlighted in red.

2 OBJECTIVES

In this thesis, the potential of anaerobic digestion to treat waste streams within a circular food production system will be investigated. The objective is to investigate the potential to integrate aquaponics with insect rearing, thereby, enhancing the circularity of the process. This research aims to address the need for sustainable waste management solutions in modern food production systems.

The first objective in this thesis involves assessing the suitability of black soldier fly larvae (BSFL) frass for methanogenesis through co-digestion with food waste leachate. This investigation serves as a crucial step towards optimizing the utilization of organic waste within the system.

The second part of the study focuses on evaluating the anaerobic digestion performance of aquaculture solids derived from recirculating aquaculture systems. A substantial proportion of high-value fish in European aquaculture originates from saltwater systems, highlighting the importance of investigating the digestibility of saltwater aquaculture sludge. In a first phase, the long-term stability of the anaerobic digestion microbial community and potential energy yield of freshwater and saltwater (12 g/L) aquaculture solids will be investigated. Additionally, this study aims to determine the maximum salinity tolerance of methanogens for optimal biogas production from an economic perspective, investigating the effects of gradual versus abrupt increases in salinity. In the last phase, the salinity will be decreased again to look at the recovery capacity of the methanogens.

The following research questions will be addressed in this master's thesis:

- Is BSFL frass suitable for anaerobic digestion, by co-digesting it with food waste leachate?
- Is there a difference in biogas yield, resulting from the anaerobic digestion of freshwater aquaculture solids and saltwater aquaculture solids at a salinity of 12 g/L?
- What is the maximum salinity tolerance in the anaerobic digestion of aquaculture solids?
- What’s the recovery capacity of the microbial community after salinity toxicity?

3 MATERIALS AND METHODS

3.1 Experimental set-up

3.1.1 Inoculum and feedstock

Anaerobic digestion was conducted using three distinct feedstocks: saltwater aquaculture solids, freshwater aquaculture solids, and a mixture comprising food waste leachate and black soldier fly larvae frass.

Aquaculture solids were collected from BIGH (Brussels Integrated Green Houses), an aquaponics farm in Brussels, annually producing 20,000 kg of salmon trout, 12,000 kg of fruits and vegetables, and 180,000 pots of herbs (BIGH, 2022). The sludge was collected from the backwash of a 10L rotating drum filter (0.85 µm mesh). Initially, aquaculture solids with a rather low COD content (4.36 g COD/L) were used for the start-up of the experiment, resulting in a very low organic loading rate (0.05 g COD/L/day). A setup was constructed to facilitate the settling of the aquaculture solids within a collection container resulting in ± 2% w/v sludge. The settled solids were stored at 4°C before use. The inoculum used was a mesophilic anaerobic sludge obtained from the sludge digester in the wastewater treatment plant in Ghent (RWZI Gent), and was also stored at 4°C until use. The black soldier fly larvae and food-processing leachate were obtained from Wastech, a company rearing black soldier fly larvae which feed on food-processing waste. They have partnerships with, amongst others, Delhaize and the food market Abbatoir in Anderlecht, Brussels, enabling the utilisation of food-processing waste as feed for the cultivation of black soldier fly larvae.

3.1.2 Reactor construction and operation

Anaerobic digestion experiments were conducted in fed-batch mode using three different feedstocks: aquaculture solids derived from both freshwater and saltwater aquaculture and a treatment in which BSFL frass and leachate from food-processing waste were combined in a 1:10 ratio respectively as leachate, on its own, exhibits a low percentage of total solids, which is not favourable for anaerobic digestion in a fed-batch reactor. The saltwater aquaculture solids were obtained by adding a salt mix (Instance Ocean, Aquarium Systems, France), to the freshwater aquaculture solids, achieving a sea salt concentration of 12 g/L. The experiment was operated under mesophilic conditions in a temperature-controlled room of 28°C.

The anaerobic digestion was performed in 1L Schott-bottles with a working volume of 800 mL. The bottles were closed with a rubber through which a syringe was inserted, connected with gas-tight tubing. These tubes led to the biogas collection system. The biogas was gathered in 5L biogas collection columns immersed in an acid water bath with a pH of approximately 3, stained with methyl orange. The low pH prevented the dissolution of CO_2. The amount of biogas accumulated in the upper part of the column could be read. The acid water level was reset to zero after each feeding and gas sampling cycle. Biogas was collected three times a week using 3 mL syringes, preceded by rinsing twice with a 50 mL syringe. The collected biogas samples were immediately analysed and represented the average headspace composition between any two feeding points. The set-up is depicted in Figure 7.

Inoculum was diluted with tap water, reaching a volatile solids (VS) content of 10 g/L. Biological triplicates were performed for each of the three treatments.

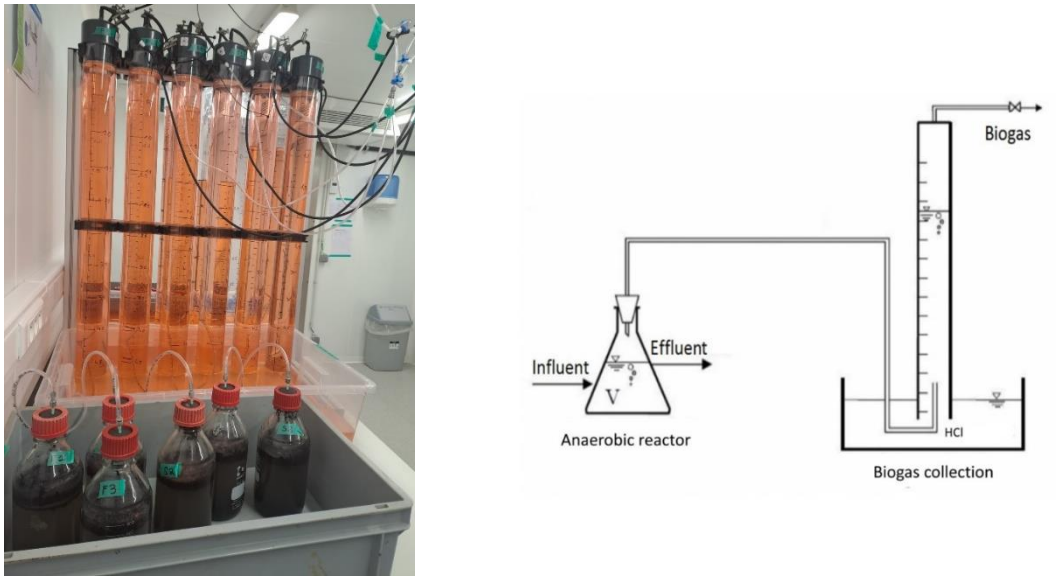

Figure 7: Left: Nine fed-batch reactors, with six reactors visible in the forefront and three additional reactors positioned behind the gas collection tubes. Three different treatments were performed, each in biological triplicates (aquaculture solids from freshwater aquaculture, aquaculture solids from saltwater aquaculture at concentrations of 12 g/L and a mixture of leachate and BSFL frass). Right: Schematic overview of the experimental set-up of one reactor.

A fed-batch reactor approach was used for the anaerobic digestion experiments. In these reactor systems, the sludge retention time (SRT) equals the hydraulic retention time (HRT), since there is no separation of liquid and solids in these reactors. The experiment consisted of a start-up phase of 50 days in which the SRT was slowly reduced from 80 days to 20 days by increasing the organic loading rate (OLR) so that the microorganisms in the inoculum could adapt to the feedstocks (Table 1). After the start-up, the reactors were operated for a duration equivalent to three times the SRT, totalling 60 days, while maintaining a constant SRT of 20 days.

Table 1: Description of the adjustment protocol to acclimate the inoculum to the feedstocks.

Period (d)	Target SRT (d)	OLR aquaculture sludge (g COD/L digester/d)	OLR BSFL frass and leachate (g COD/L digester/d)
0 - 12	80	0.05	1.38
12 - 42	40	0.109	-
42 - 142	20	0.865	-
142 - 170	20	1.995	-

The Freshwater treatment initially involved the anaerobic digestion of freshwater aquaculture solids whereas the Saltwater treatment involved the anaerobic digestion of aquaculture solids with a sea salt concentration of 12 g/L. Food waste leachate and BSFL frass were co-digested in Treatment 3. Subsequently, the Freshwater and Saltwater treatment progressed to their second phase, wherein the reactors were fed with aquaculture sludge at a salinity of 20 g/L, continuing for 35 days. In the final phase, the reactors of both treatments were again fed with freshwater aquaculture solids, lasting for a period of approximately 30 days. The various phases are detailed in Table 2. The reactors were fed thrice weekly on Monday, Wednesday, and Friday, which involved removing the desired volume of the reactor content (digestate) and replacing this with the same amount of fresh feed.

The pH was determined thrice weekly and the biogas production and the percentage of methane in the biogas were also measured three times a week. Samples of the digestate were taken once a week for the determination of total solids, volatile solids, anions concentration, cations concentration, and volatile fatty acids (VFA). Samples for microbial analysis were taken weekly and stored at – 20°C.

Table 2: sea salt concentrations in the Treatment 1 and Treatment 2, digesting aquaculture sludge.

Period (d)	Salinity level aquaculture solids fed to the Freshwater treatment (g/L)	Salinity level aquaculture solids fed to the Saltwater treatment (g/L)
0 - 110	0	12
110 - 144	20	20
145 - 170	0	0

3.1.3 Biochemical methane potential tests

Biochemical methane potential (BMP) tests were performed to determine the feedstock biodegradability and methane potential at anaerobic conditions. They were performed according to standard protocols from the literature (Angelidaki et al., 2009; Chynoweth et al., 1993; Owen et al., 1979). Biogas volumes and composition were reported under standard temperature (273 K) and pressure (101325 Pa) conditions (STP).

Experiments were conducted on six distinct feedstocks, namely aquaculture solids from freshwater aquaculture, aquaculture solids at sea salt concentrations of 12 g/L, 20 g/L, and 35 g/L, as well as leachate from food processing waste in both total and soluble forms. Both a negative (only inoculum) and positive control (inoculum and cellulose) were included. The negative control quantified the residual methane production by the inoculum itself, whereas the positive control was used as a quality check as its BMP should be within an expected range, based on the theoretical BMP. Biological triplicates were performed for all the treatments, and were placed in a warm water bath of 28°C in a temperature-controlled room of 28°C.

The tests were carried out in penicillin bottles with a volume of 120 mL, and were closed with a rubber stopper sealed with a metal cap. A needle inserted through the rubber was attached to gas-tight tubing, directing towards the gas columns. The gas columns were standing in an acid water bath with a pH lower than 4.3 to avoid the dissolution of CO_2. Methyl orange was added to visually monitor the pH. The liquid level was pulled up at the beginning of the experiment. The amount of biogas generated could be read on the columns as the water level decreased due to the accumulation of biogas in the upper part of the column. The set-up is shown in Figure 8.

Inoculum was added in such an amount so that a VS-concentration of 10 g/L was reached whereas substrate was added to each bottle to reach a COD load of 0.5 g COD/g VS inoculum. Tap water was added to a total volume of 80 mL to ensure consistency across all tests and eliminate any variations due to volume differences.

The pH was measured right before the start of the BMP-test. The BMP-test was left to run until biogas production went below 1-3% of total biogas production in all treatments for 3 consecutive days. The biogas volumes were written down on regular basis. At the end of the experiment, the pH and gas composition were measured.

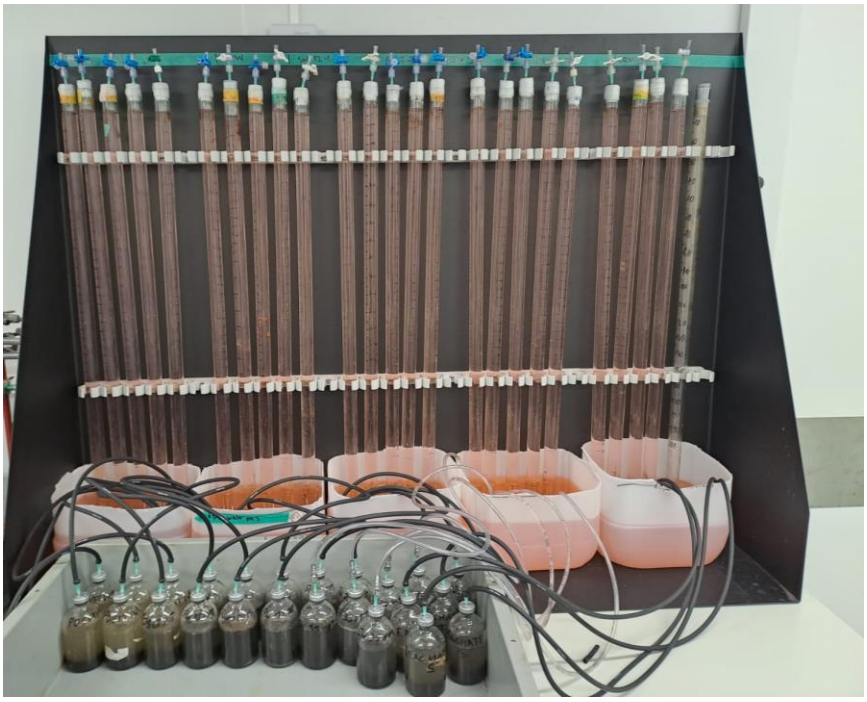

Figure 8: Set-up for conducting biochemical methane potential tests for six treatments (aquaculture solids from freshwater aquaculture, aquaculture solids at sea salt concentrations of 12 g/L, 20 g/L, and 35 g/L, and leachate in total and soluble forms), each executed in biological triplicates.

3.2 Analytical techniques

3.2.1 Total solids (TS) and volatile solids (VS)

Total solids and volatile solids were determined based on the standard methods (Eaton et al., 1999). Approximately three grams of the sample was placed into small aluminium trays. Total solids were determined by drying the samples in a 105°C oven for about two days. The total solids were calculated by determining the weight before and after oven drying, using equation 1. Here, *A* represents the final weight of the dried residue and tray, while B denotes the weight of the tray. The sample volume assumes a density of 1000 g/L.

$$TS = \frac{A-B}{sample\ volume} \quad (1)$$

The volatile solids were subsequently determined by igniting the sample in the muffle oven at 450°C for 3 hours after which the sample was weighted again. The solids lost to ignition are considered to be volatile solids and could be calculated by using equation 2 where C represents the final weight of the residue and tray after ignition.

$$VS = \frac{A-C}{sample\ volume} \quad (2)$$

The determination of both TS and VS were performed in technical triplicates.

3.2.2 Volatile fatty acids

The volatile fatty acids from C2 to C8 were measured by performing an extraction in diethyl ether. A sample of 2.000 mL was transferred into a plastic test tube. In the test-tube, 0.5 mL of H_2SO_4 was added to convert the acids to their undissociated form, which is more water insoluble, after which approximately 0.4 g natrium chloride was added to enhance the separation process. Next, 400.0 µl internal standard was added. Lastly, 2.00 mL diethyl ether was added for the extraction of the VFA to the ether phase. The test tubes were closed and mixed by turning it for two minutes in the tube rotator (L26 Small rotary mixer, Labinco, The Netherlands) after which the ether phase and the water phase were separated by centrifuging the test-tube for three minutes at 3000 rpm (Mega Star 600/600R, VWR, USA). The organic ether layer was then transferred into a GC vial.

Quantitative analysis of the VFA was done by means of capillary gas chromatography (GC-2014, Shimadzu®, The Netherlands) with a DB-FFAP 123–3232 column (30 m x 0.32 mm × 0.25 µm; Agilent, Belgium), which was coupled with a flame ionisation detector (FID). The carrier gas was nitrogen. The detection limit was 30 mg/L for acetate and 10 mg/L for the other VFA. The COD-adjusted concentrations of volatile fatty acids were determined by multiplying the measured VFA concentration with the ratio of the necessary oxygen for combustion to the acid's molecular weight.

3.2.3 Chemical oxygen demand

The chemical oxygen demand (COD) is the amount of oxygen required to oxidize organic carbon completely to CO_2 by chemical means. It was measured by using a test kit (Tube test Nanocolor COD 1500, Macherey-Nagel, Germany). It was based on the oxidation of organic compounds by potassium dichromate in an acid medium. Silver sulphate (Ag_2SO_4) acted as a catalyst, enhancing the oxidization of aliphatic substances whereas mercuric sulphate (Hg_2SO_4) was added in order to precipitate the chlorides thereby minimizing their interference. Potassium dichromate formed reactive oxygen species in acid medium as shown in equation 3 (Macherey-Nagel, 2021).

$$K_2Cr_2O_7 + 8\,H^+ \rightarrow 3\,O\ + 2\,Cr^{3+} + 2\ K^+ + 4\,H_2O \quad (3)$$

These oxygen compounds were able to oxidize organic compounds to carbon dioxide. The decomposition was carried out for 30 minutes at 160°C after which the decrease in concentration of the yellow potassium dichromate was determined photometrically.

3.2.4 Total nitrogen (TN)

The total nitrogen was determined by using a test kit (Tube test NANOCOLOR total-nitrogen TNb 22, Macherey-Nagel, Germany). The TNb stands for total bound nitrogen and includes both organic and inorganic or mineral nitrogen. All organic and inorganic nitrogen-containing substances were oxidized to nitrate in an acidic medium. Nitrate reacted in acidic solution with 2,6-dimethylphenol to form 4-nitro-2,6-dimethylphenol, which could be determined photometrically.

3.2.5 Total phosphate

The overall phosphate content was assessed using a test kit (Tube test NANOCOLOR ortho- and total phosphate 15, Macherey-Nagel, Germany). This measurement encompassed both ortho-phosphates and poly- and organo-phosphates present within the sample. The assessment relied on the reaction between ortho-phosphate and ammonium molybdate, resulting in the formation of phosphomolybdic acid. Subsequently, this compound was reduced to phosphorus molybdenum blue with the aid of a reducing agent. To account for poly- and organophosphates, an acidic oxidation process was conducted at temperatures between 100 and 120°C during the determination of total phosphate.

3.2.6 Alkalinity

The alkalinity was determined by using a test kit (Titrimetric test kit Viscocolor HE alkalinity, Macherey-Nagel, Germany). The method relied on a titration, using methyl red as a pH indicator. A titration solution, containing a strong acid, was added dropwise to the sample. The acid reacted with the alkaline compounds present in the samples, resulting in a decrease in pH. The alkalinity could be determined based on the quantity of titration solution added to the sample when it turned red.

3.2.7 Ion chromatography (IC)

The cations and anions were determined by using ion exchange chromatography (930 Compact IC Flex, Metrohm, Belgium). Separation of the ions is allowed due to the difference in retention time of each ion. The samples were first centrifuged for 3 minutes in the centrifuge (Centrifuge 5430/5430 R, Eppendorf™, Germany) at 20817 rfc, after which the supernatant was filtered using a 0.20 µm filter. When dilutions were necessary, milli-Q water was utilized. After separation, the ions were quantified based on conductivity by means of a calibration curve. Cations were analysed with a metrosep C6-150/4.0 column with P-polybutadienemaleic acid on a silica gel base as carrier material whereas anions were analysed with a metrosep A Supp 5- 150/4.0 column with polyvinyl alcohol with quaternary ammonium groups as carrier material. Cations in the range of 1 to 100 mg ion/L could be determined whereas anions in the range of 0.05-100 mg/L could be determined.

3.2.8 Biogas composition via gas chromatography

The composition of the produced biogas was measured thrice weekly for the fed-batch reactor and once at the end of the BMP-experiment. The biogas composition was analysed with a compact GC (Global Analyser Solutions, The Netherlands) equipped with a Molsieve 5A pre-column and Porabond column (CH_4, O_2, H_2 and N_2) and a Rt-Q-bond pre-column and column (CO_2, N_2O and H_2S). Helium was used as carrier gas. Biogas samples were taken with a 3 mL syringe, after rinsing with 50 mL syringes. Two syringes were used per treatment in order to analyse both CH_4 and CO_2. The concentrations of the gases were determined by a thermal conductivity detector which had a Limit Of Quantification (LOQ) of around 500 ppm_v for each gas.

The proportion of methane within the headspace gas composition was determined through:

$$\%CH_4 = 100 \cdot \frac{CH_4}{CH_4 + CO_2} \quad (4)$$

The amount of methane generated per litre of reactor volume under standard temperature and pressure conditions (STP) was calculated by:

$$Volume_{CH_4} = \%CH_4 \cdot volume_{biogas,daily} \cdot \frac{273K}{301K} \quad (5)$$

The normalized methane yield was calculated by dividing the volume of methane produced at STP by the volume of feed sludge added (L) multiplied by its VS (g/L sludge) content:

$$CH_{4_{yield,VS}} = \frac{volume\ CH_4}{\frac{g\ VS}{L\ sludge} \cdot Volume_{feed}} \quad (6)$$

The normalized methane percentage yield could be calculated by dividing the normalized methane yield by the theoretical amount of methane that could be maximum produced:

$$CH_4\ percentage\ yield = \frac{normalized\ methane\ yield}{0.35} \quad (7)$$

An estimation of the annual energy and electricity production could be calculated based on a CHP electricity conversion efficiency of 41% and a methane to electricity conversion rate of 1 m³ CH_4 = 10.55 kWh. The calculation involved converting MJ energy produced to kWh using the conversion factor of 3.6 MJ = 1 kWh:

$$elecetricity\ yield\ \left[\frac{kWh}{L\ sludge}\right] = \frac{Normalised\ CH_4\ yield\ [\frac{L\ CH_4}{g\ VS}]}{1000\ L/m^3} \cdot 10.55 \frac{kWh}{m^3 CH_4} \cdot 0.41 \cdot \frac{g\ VS}{L\ sludge} \quad (8)$$

3.2.9 pH

The pH of both the digestate and the feedstocks was measured using the C3010 series with an SP10 pH electrode (Consort, Belgium). The pH probe was calibrated weekly using three buffer solutions with pH 4.0, 7.0, 9.0.

3.2.10 Conductivity (EC)

The conductivity was measured using the C3010 series with an SK10 conductivity electrode (Consort, Belgium). The probe was calibrated weekly using three solutions with concentrations of 1, 0.1 and 0.01 M KCl. The measured conductivity is expressed in mS/cm.

3.3 Data analysis

Chat gpt 3.5 was used during this thesis to rephrase sentences.

3.3.1 Statistical tests

Independent sample t-tests were carried out to assess whether differences occurred between the two treatments. Before conducting a parametric test, several conditions needed to be satisfied. These conditions include independence of observations, normal distribution, and homoscedasticity (homogeneity of variance). The first condition was already met, while the second and third condition were assessed using the Shapiro-Wilk test and Levene's test, respectively. If these conditions were met, *i.e.*, the null hypothesis of both tests was not rejected at the significance level of α=0.05, then the parametric unpaired t-test was conducted. The Mann-Whitney U-test was used if one of the required conditions was not fulfilled. A p-value <0.05 was considered significant.

4 RESULTS

4.1 Fed-batch anaerobic digestion

4.1.1 Characterisation of the feedstocks and inoculum

The physicochemical characteristics of the anaerobic inoculum and the feedstocks used in the experiments are depicted in Table 3 and Table 4, respectively.

Table 3: Initial characterisation of anaerobic inoculum used in this study with standard deviations from technical replicates (n=3).

Parameter	Unit	Anaerobic inoculum
Chemical oxygen demand (COD)	g COD/L	51.2 ± 2.0
Total Kjeldahl nitrogen (TKN)	g TKN/L	5.2 ± 0.6
Total solids (TS)	g/L	57.4 ± 3.3
Volatile solids (VS)	g/L	23.6 ± 1.2
TS/VS	-	2.43 ± 0.02
Volatile fatty acids (VFA)	mg COD/L	376 ± 46

Table 4: Initial characterisation of the different batches of aquaculture solids, processing food waste leachate, and BSFL used in this study with standard deviations from technical replicates (n=3).

Parameter	Unit	Aquaculture solids	Processed-food waste leachate	Black soldier fly larvae
Chemical oxygen demand (COD)	g COD/L	40 ± 11	113 ± 7	84 ± 3
Total Kjeldahl nitrogen (TKN)	mg TKN/L	167 ± 25	1044 ± 114	15 ± 2
Total solids (TS)	g/L	32.0 ± 0.2	102.9 ± 1.9	466.5 ± 1.8
Volatile solids (VS)	g/L	12.6 ± 0.1	43.6 ± 2.7	145 ± 4.5
TS/VS	-	2.5 ± 0.0	2.4 ± 0.1	3.2 ± 0.1
Volatile fatty acids (VFA)	g COD/L	2 ± 0	3 ± 0	-

Table 4 shows that the chemical oxygen demand (COD) of the processed-food waste leachate was notably the highest, whereas the COD of the black soldier fly larvae was also quite high, compared to the aquaculture solids. In terms of nitrogen content, the food waste leachate also surpassed both the leachate and the BSFL frass. The total solids and volatile solids were the highest for the BSFL frass, whereas the ratio of TS to VS was in the same order of magnitude for all the feedstocks.

4.1.2 Anaerobic digestion of aquaculture solids

4.1.2.1 Electrical conductivity (EC)

The aim of this study was to look at the impact of salinity on the anaerobic digestion of aquaculture solids and consequently on biogas and energy production.

Figure 9 illustrates the electrical conductivity (EC) of the digestate from the reactors. The EC is a measure for the salinity of the digestate, and, therefore, reflects the salt concentration in the digestate. In the first phase, in which the reactors of the Freshwater treatment were fed with freshwater aquaculture solids and the reactors of the Saltwater treatment with aquaculture solids at a salinity of 12 g/L, the electrical conductivity (EC) of the digestate in the Saltwater treatment was consistently higher than in the Freshwater treatment. Specifically, the EC of the Saltwater treatment was almost three times higher.

The salinity of both treatments was increased to 20 g/L after 110 days to assess the capacity for biogas production under even higher salinity concentrations. This adjustment aimed to investigate whether it is more effective to allow methanogens to gradually acclimate to high salinity levels or to subject them to a sudden shock. Although there was no significant difference ($p = 0.35$) in the EC of the digestate between both treatments during this period, there was a discernible rise in EC for both treatments. The rise in electrical conductivity (EC) occurred gradually and continued to increase throughout the entire phase. The Freshwater treatment exhibited a more rapid increase in electrical conductivity, reaching levels comparable to those of the saltwater treatment within 20 days of introducing saltwater aquaculture solids at a concentration of 20 g/L.

The salinity of both treatments was in the last phase (Figure 9 from day 145) reduced to 0 g/L to look at the ability of the microorganisms to recover from the high salinity. The EC decreased in this phase across all replicates. There was no significant difference between the EC of the digestate of both treatments in this phase ($p=0.46$). Furthermore, it was observed that the decrease in EC occurred gradually and continued to decrease throughout the entire phase.

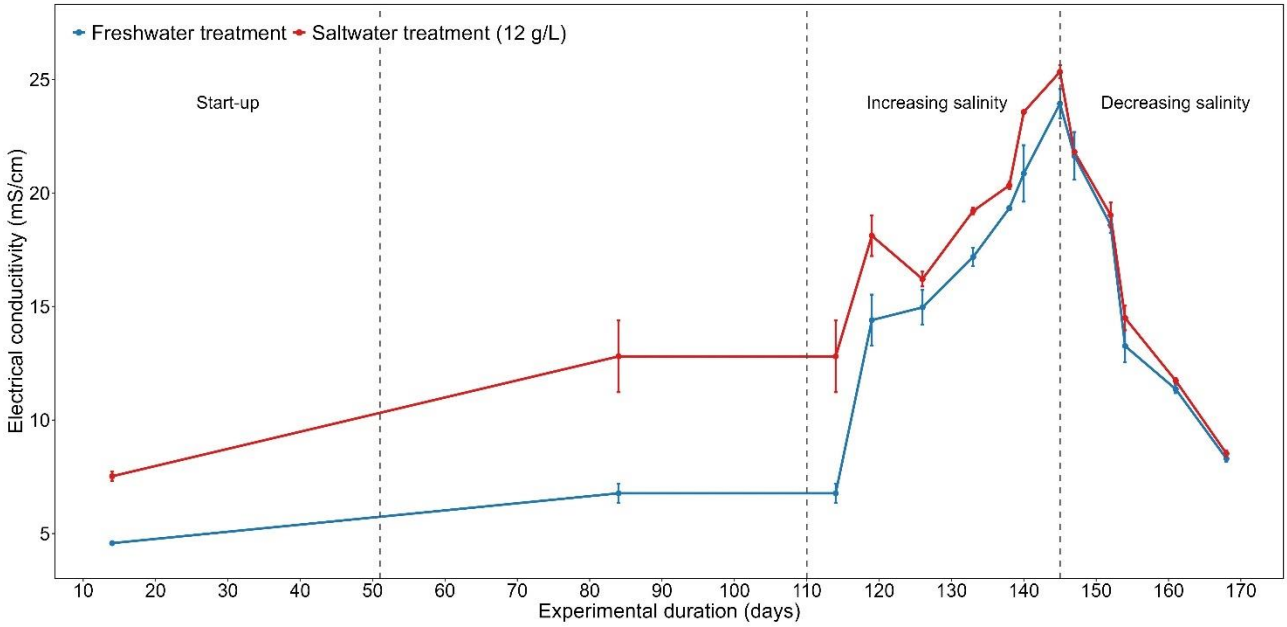

Figure 9: Electrical conductivity (EC) of the digestate, with standard deviations from biological triplicates. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

4.1.2.2 Biogas production rates

The yield of methane produced per gram of volatile solids in the feedstock is depicted in Figure 10.

In the first phase, when the reactors in the Freshwater treatment were fed with the freshwater aquaculture solids and the reactors in the Saltwater treatment with the saltwater aquaculture solids at a salinity of 12 g/L, the methane yield globally increased, reaching values around 0.3 NL CH_4/g VS. However, there were no significant differences between the methane yield of the Freshwater treatment and the Saltwater treatment ($p = 0.74$). Some values from the first replicate of the Freshwater treatment were removed, due to inaccuracies in the measurements by the compact GC leading to statistical outliers.

The yield decreased to approximately 0.1 NL CH_4/g VS when the salinity of the aquaculture solids was elevated to 20 g/L for all reactors, with the yield of the initially Saltwater treatment significantly higher than the yield of the initially Freshwater treatment ($p = 0.047$). In the final phase, when all reactors were again fed with freshwater aquaculture solids, the methane yield increased again to methane yields similar to those observed prior to the salinity increase of the aquaculture solids to 20 g/L. No significant difference was observed in methane yield in this phase between both treatments ($p=0.44$).

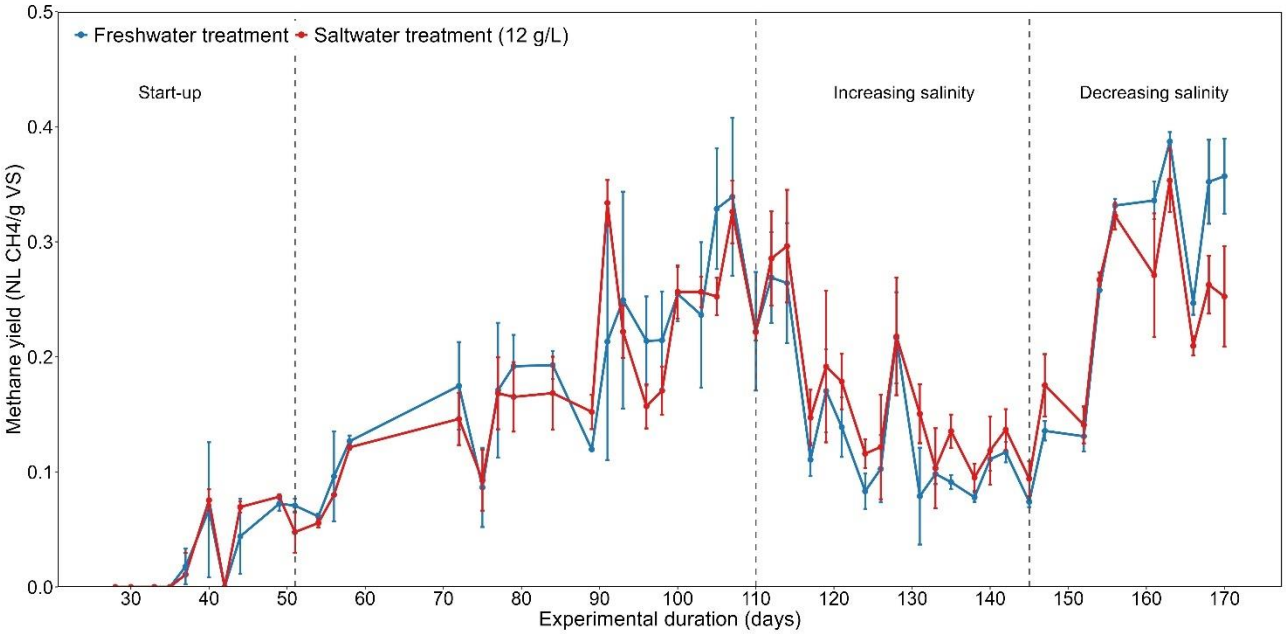

Figure 10: Methane yield per litre reactor, based on volatile solids (VS), with standard deviations from biological triplicates. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

Methane production rates directly correspond to rates of electricity and energy generation. Figure 11 shows the potential electricity production from a 1L-reactor, calculated based on the equations in section 3.2.8. The trend observed in Figure 11 closely resembles that of Figure 10, indicating the direct connection between the two figures. There were no significant differences in electricity production, neither in the steady-state period (Freshwater treatment and Saltwater treatment at 12 g/L) ($p=0.70$), nor in the period where the salinity of both treatments was raised to 20 g/L ($p=0.17$) or in the phase where salinity was decreased again ($p=0.39$).

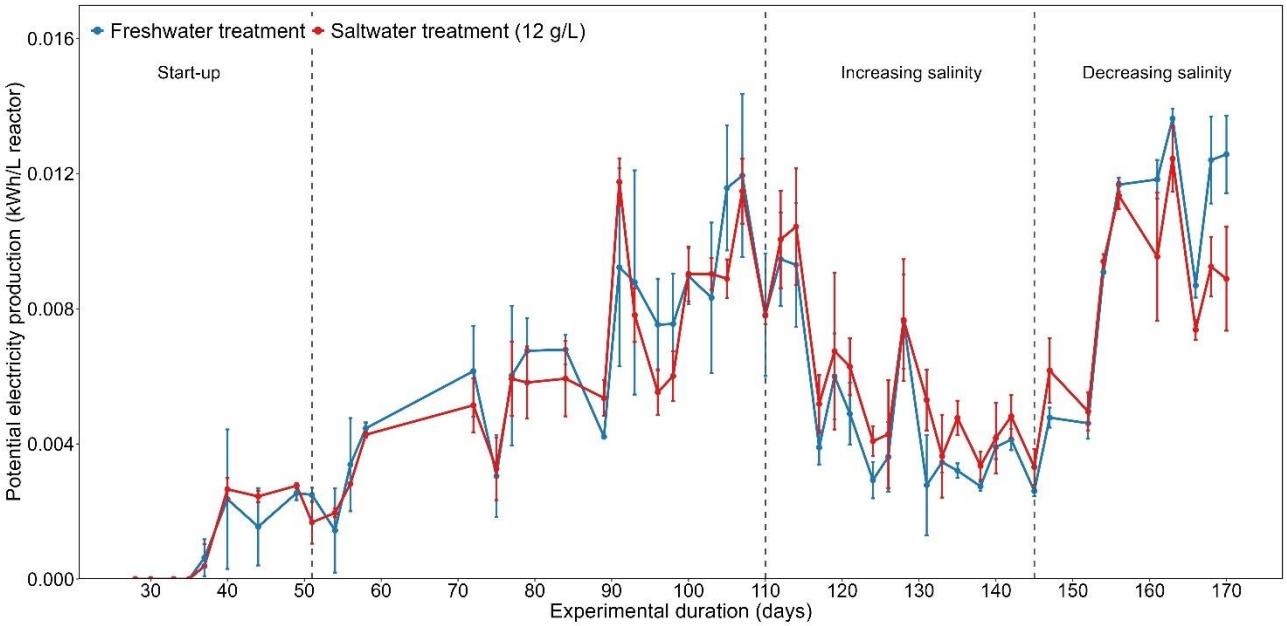

Figure 11: Potential electricity production (kWh/L reactor) with standard deviations from biological triplicates. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

4.1.2.3 Biogas composition

Figure 12 illustrates the relative proportions of methane and carbon dioxide in the biogas, collectively comprising 100%. Initially, there was no methane production at the outset of the experiment, but this started approximately one month later. The methane fraction of replicate 1 in Freshwater treatment exhibited considerable variability during the first phase of the experiment (Freshwater and Saltwater (12 g/L) aquaculture solids) attributable to inaccuracies in the measurements taken by the compact gas chromatograph (GC). Values that were considered as outliers were removed from the data. The methane fraction in the biogas during the first phase of the experiment was significantly higher in the Saltwater treatment (12 g/L) compared to the Freshwater treatment ($p = 0.0024$). When the salinity of both treatments was increased to 20 g/L, the methane fraction in the initially Saltwater Treatment remained significantly higher than in the initially Freshwater treatment ($p < 0.001$). However, when the salinity of the aquaculture solids supplied to all reactors is decreased to 0 g/L (phase d), there was no significant difference in the methane percentage between the two treatments ($p=0.42$).

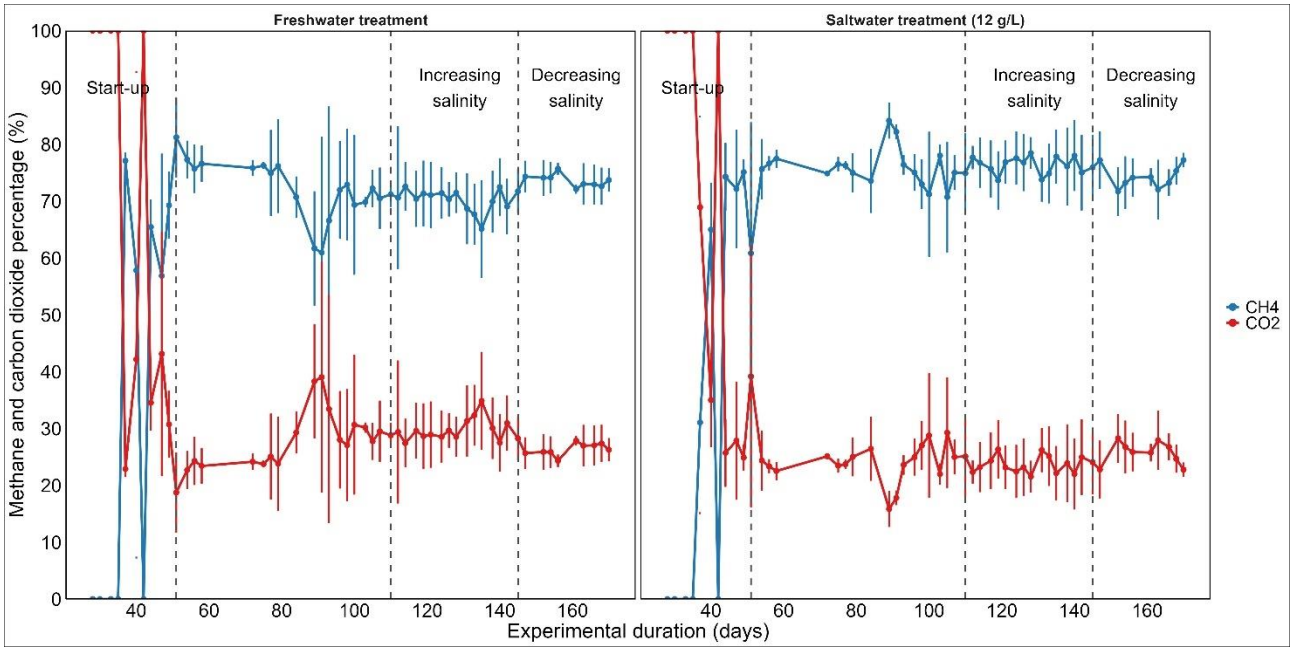

Figure 12: Percentage of methane and carbon dioxide in the biogas, collectively comprising 100% with standard deviations from biological triplicates. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

4.1.2.4 Process performance

During anaerobic digestion, process parameters, such as pH (see 4.1.2.4.1), volatile fatty acids (VFA) (see 0), total solids, and volatile solids (see 4.1.2.4.3) are good indicators of the performance of the anaerobic digestion process.

4.1.2.4.1 pH

The pH fluctuated between 6.32 and 7.33 throughout the experiment (Figure 13) with its highest levels observed at the beginning. Around day 50, the pH declined to its lowest values for the entirety of the experiment. Subsequently, the pH began to rise again, although it never reached the same levels as before this decrease. When excluding the data from the start-up and the strong decrease around day 50, the pH remained within a range of ± 0.50 for all the separate replicates. There was no significant difference in pH in the first phase between the Freshwater treatment and the Saltwater treatment at 12 g/L ($p=0.52$). However, the pH of the Freshwater treatment was significant lower when the salinity of both treatments was raised to 20 g/L ($p=0.023$). The pH decreased when both reactors were subsequently fed with freshwater aquaculture solids; but started to increase again after 10 days until the end of the experiment.

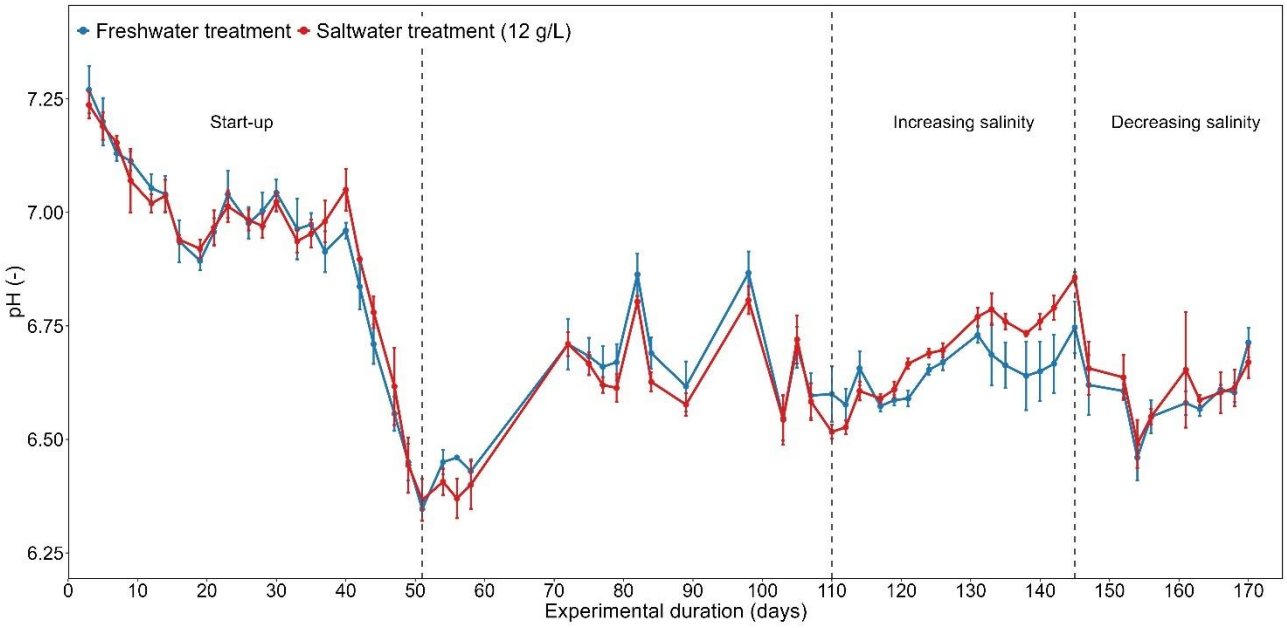

Figure 13: pH trends in the digestate. The dashed lines show the change in treatment with standard deviations from biological triplicates. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

4.1.2.4.2 Volatile fatty acids

The accumulation profile of volatile fatty acids (VFA) is illustrated in Figure 14, expressed in grams of chemical oxygen demand per litre (g COD/L). This encompasses the presence of volatile fatty acids ranging from 2 to 8 carbon atoms. A peak in VFA accumulation was observed around day 50, followed by a subsequent decrease. Despite some fluctuations, the VFA accumulation in the digestate started to diminish globally, for both treatments. Initially, following the elevation of salinity levels in both treatments to 20 g/L, VFA accumulation continued to decrease. However, one to two weeks after this salinity increase, a reversal occurred, marked by an increase in VFA accumulation, which continued during the start of the last phase. Approximately 20 days after the commencement of the last phase, the VFA accumulation began to decline again.

In the first phase, the volatile fatty acids accumulation was the highest in the Saltwater treatment, fed with aquaculture solids at a salinity of 12 g/L ($p=0.016$). There was no significant difference in VFA accumulation in the two subsequent phases ($p = 0.11$ and $p=0.89$ for respectively the increased salinity to 20 g/L and the decreased salinity).

Over time, the ratio of acetate to total VFA serves as an indicator of the microbial community's efficiency in utilizing acetate (Figure 15). None of the different regions exhibited a significant difference between the two treatments. In the steady-state period, the ratio of acetate to total VFA fluctuated for all the replicates. Notably, an increase in the ratio occurred when both treatments experienced a rise in salinity to 20 g/L Subsequently, upon reducing the salinity of the feedstock back to 0 g/L, the ratio decreased again

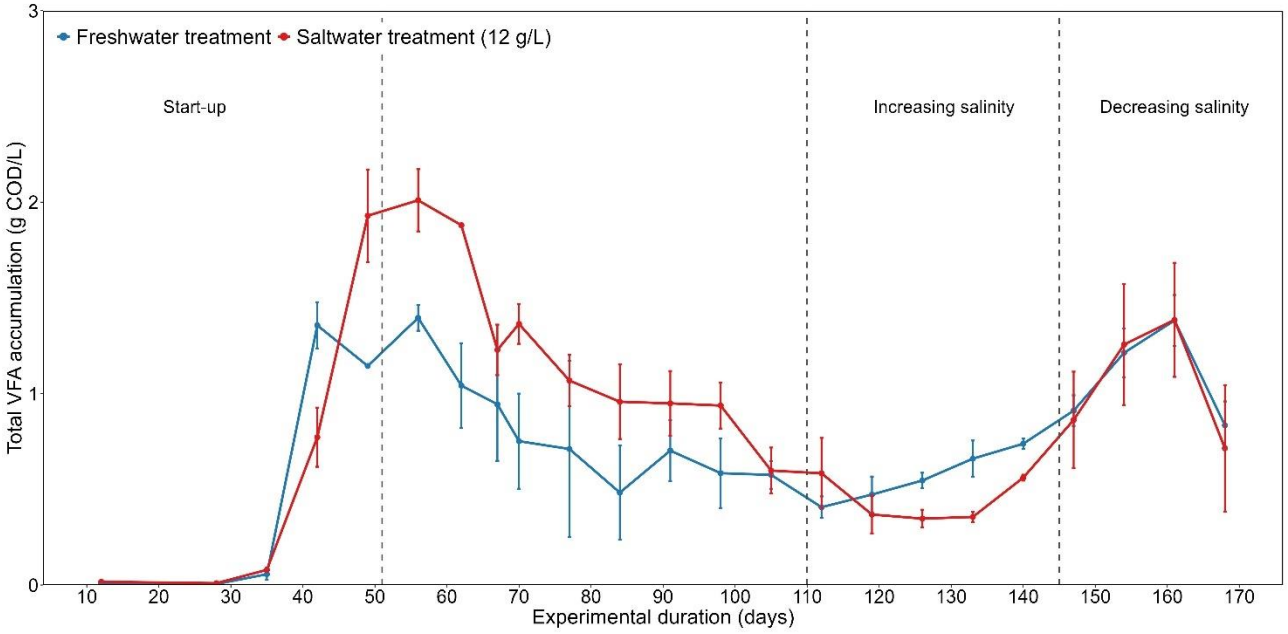

Figure 14: Total COD-adjusted volatile fatty acid accumulation over the experimental duration with standard deviations from biological triplicates. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

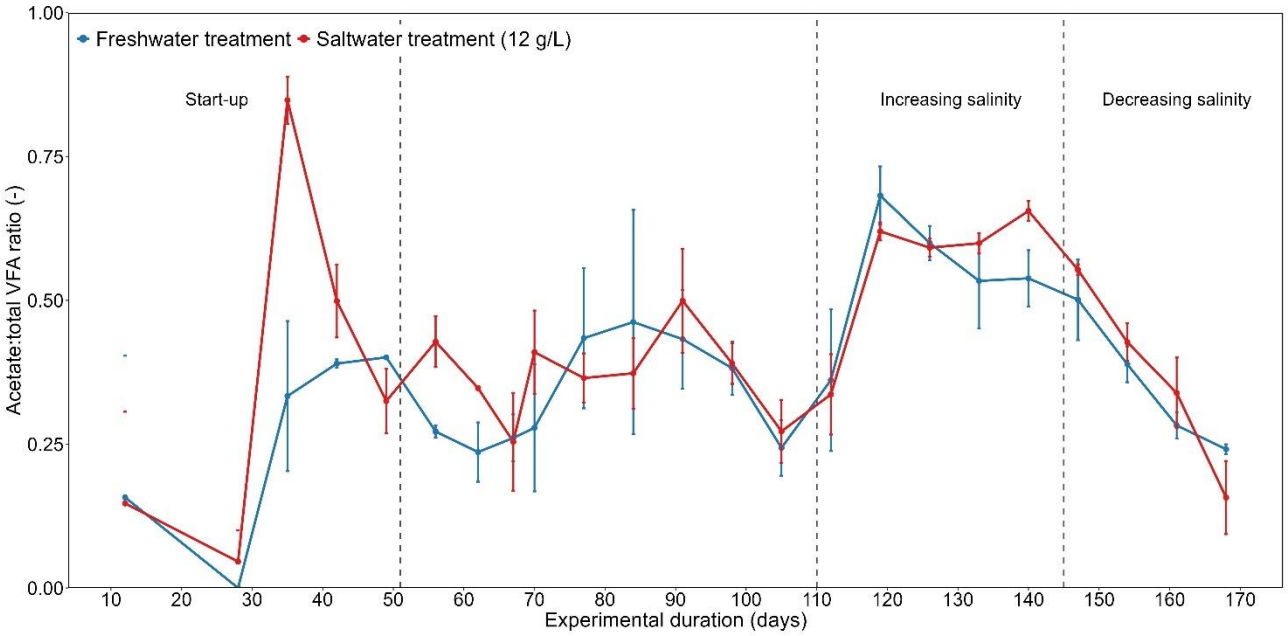

Figure 15: Ratio of acetate to total VFA with standard deviations from biological triplicates. The dashed lines show the change in treatment. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

4.1.2.4.3 Total solids and volatile solids

Figure 16 and Figure 17 illustrate the variations of total solids (TS) and volatile solids (VS) within the digestate over time. There was an increase in both TS and VS during the start-up period. The rise in both TS and VS was more pronounced for the Saltwater treatment. Following the elevation of the salinity of the fed aquaculture solids to 20 g/L, an increase in the TS and VS from the Freshwater treatment was observed in the plots, whereas no remarkable increase was observed for the Saltwater treatment. However, this period of increased salinity was also marked by a high variability in both TS and VS.

The digestate from reactors supplied with saltwater aquaculture solids (12 g/L) exhibited significantly higher levels of TS compared to those supplied with freshwater aquaculture solids ($p<0.001$) in the initial phase (day 51-110). This pattern was also observed in the case of VS ($p<0.001$). Following the introduction of aquaculture solids with a salinity of 20 g/L to the reactors, there was no longer a statistically significant difference between the two treatments for TS ($p=0.56$) and VS ($p=0.50$).

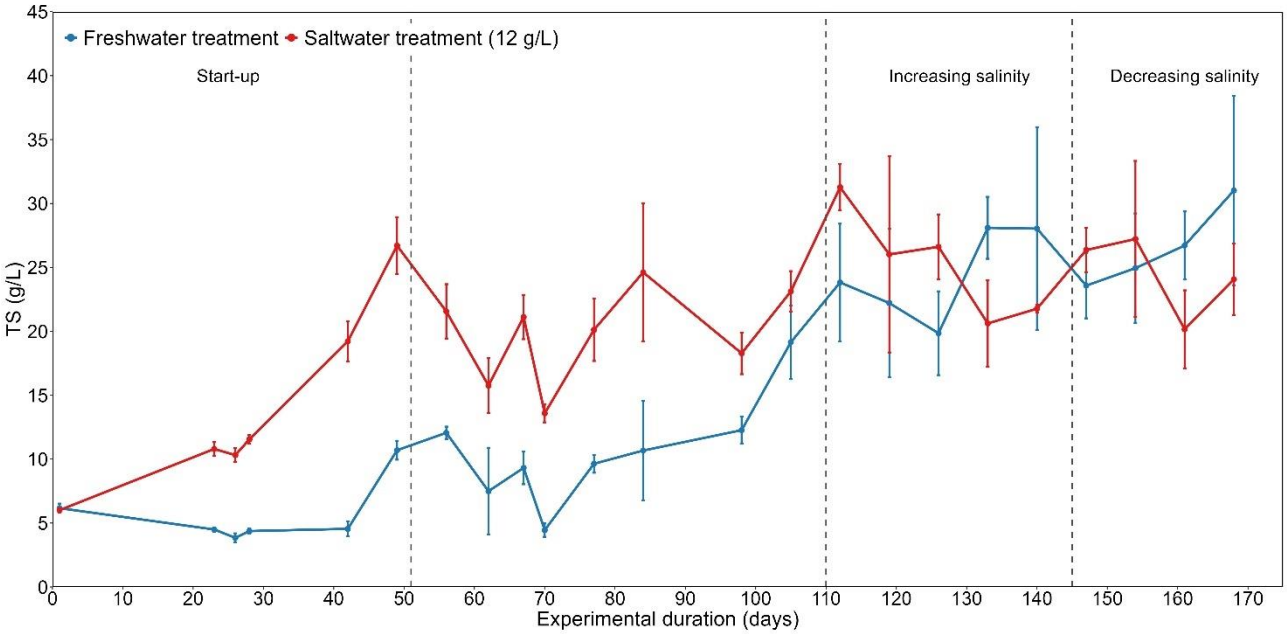

Figure 16: Evolution of total solids (TS) over time with standard deviations from biological and technical triplicates. The dashed lines show the change in treatment. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

Figure 18 shows the ratio of TS to VS over time, which represents the ratio of the organic to the inorganic fraction. A constant TS/VS ratio suggests the microbial activity was maintained at the same rate throughout the experiment. The ratio of TS to VS was significantly higher for the Saltwater treatment compared to the Freshwater treatment in the initial phase (day 51-111), which can also be seen in the figures ($p=0.0007$). However, this difference vanished when the salinity of the aquaculture solids was raised to 20 g/L, with no significant difference observed anymore ($p=0.44$). The figure illustrating the VS:TS ratio can be found in Appendix A.

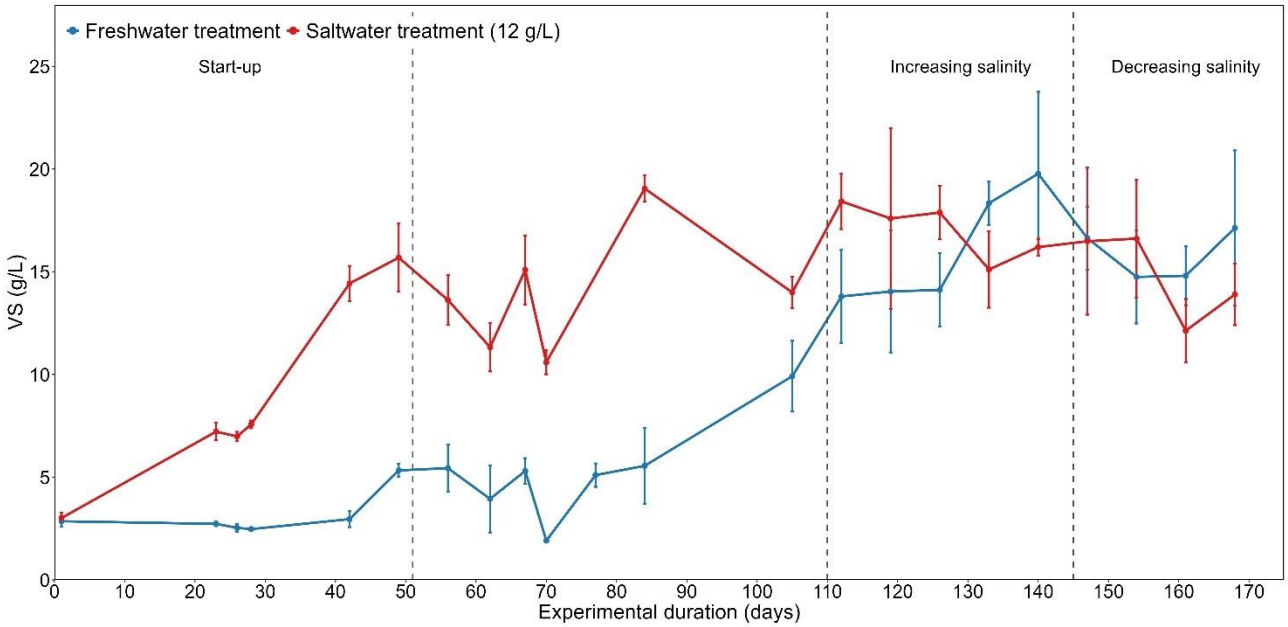

Figure 17: Evolution of volatile solids over time with standard deviations from biological and technical triplicates. The dashed lines show the change in treatment. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

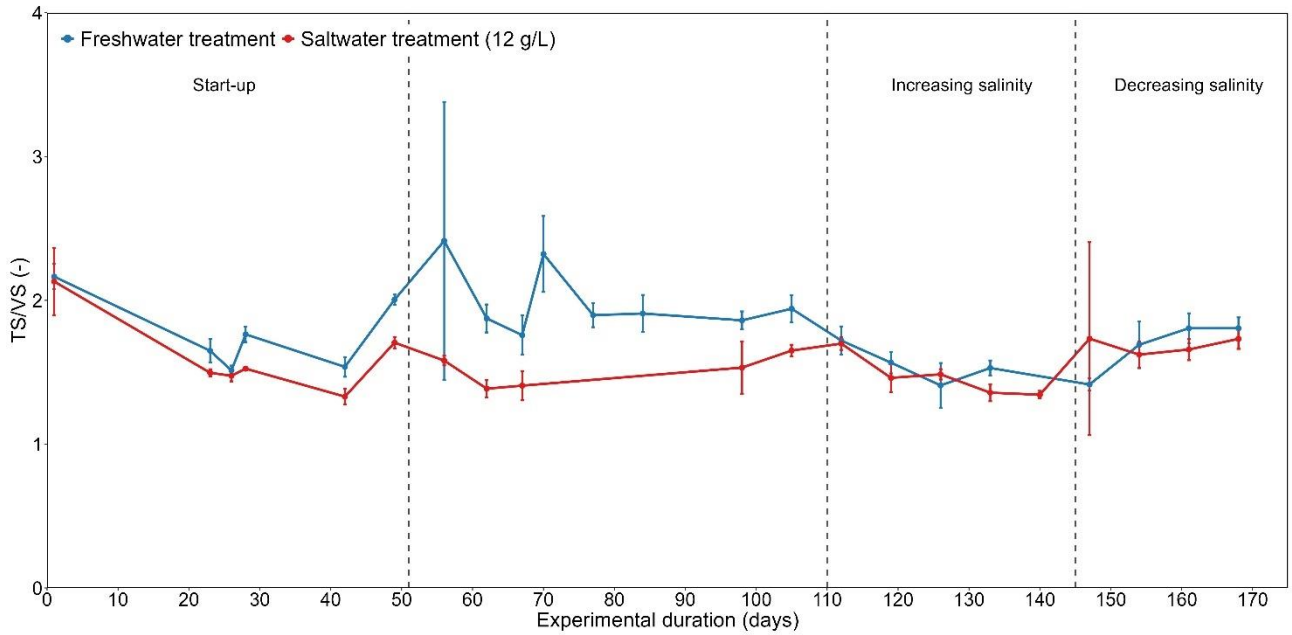

Figure 18: Evolution of the ratio of total solids to volatile solids (VS) over time with standard from biological and technical triplicates. The dashed lines show the change in treatment. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

4.1.2.4.4 Anions and cations

The IC anion and cation analyses measured across the treatments are depicted in Figure 19. The presence of sodium, magnesium, calcium, potassium, and chloride are linked to the sea salt mixture. In the first phase, when the reactors in the Freshwater treatment were fed with the freshwater aquaculture solids and the reactors in the Saltwater treatment with the saltwater aquaculture solids at a salinity of 12 g/L, the concentrations of these ions remained relatively constant with higher concentrations observed for the Saltwater treatment. Increasing the salinity of the aquaculture solids to 20 g/L in the next phase resulted in a rise in salt concentrations within the Freshwater treatment. It took approximately two weeks for these ion concentrations in the digestate of both treatments to become comparable.

Among the nitrogenous compounds, total ammonia nitrogen (TAN) predominated in the digestate. The TAN-concentration was 285.6 ± 2.6 mg/L in the freshwater aquaculture solids, accounting for 49% of the total Kjeldahl nitrogen. The TAN-concentrations measured in the digestate were higher than those in the freshwater aquaculture solids. During the first phase of the experiment, TAN-concentrations were slightly increasing to values around 400-500 mg/L after 100 days. Initially, TAN-concentrations were higher in the Saltwater treatment, yet this discrepancy diminished over time. After increasing the salinity of the aquaculture solids, the concentrations of TAN initially decreased across all the reactors, before stabilizing between 400 and 500 mg/L.

Phosphate concentration trends over time, as shown in Figure 19-H revealed higher levels in the Saltwater treatment during the first phase, except for a dip around day 50. Elevating fish solid salinity to 20 g/L didn't result in considerable changes in phosphate concentration. Reducing the salinity of the aquaculture solids to 0 g/L has led to an increase in phosphate concentration, peaking 15 days after the introduction of the freshwater aquaculture solids. Next to the phosphate concentration, the total phosphate (TP, encompassing both ortho-phosphates and poly- and organo-phosphates) was measured at day 126 of the experiment, two weeks after the introduction of aquaculture solids at a salinity of 20 g/L. An average TP concentration of 3.4 ± 1.1 g/L and 3.0 ± 1.2 g/L was measured in the digestate of respectively the Freshwater and the Saltwater treatment. In addition, the TP concentration was measured for the aquaculture solids at different salinity levels, which were 1.26, 2.75, 0.8, and 0.58 g TP/L for respectively aquaculture solids at a salinity of 0 g/L, 10 g/L, 20 g/L, and 35 g/L. The highest TP concentration was measured at a salinity of 10 g/L whereas the concentration decreased at higher salinities. In addition, the fraction of ortho-phosphates and poly- and organo-phosphates was higher in the digestate than in the influent.

The sulphate concentrations are depicted in Figure 19-F. Following the elevation of salinity in the aquaculture solids to 20 g/L, sulphate concentrations in both treatment groups increased, peaking approximately 18 days after the introduction of these high-salinity aquaculture solids. Subsequently, sulphate concentrations decreased again.

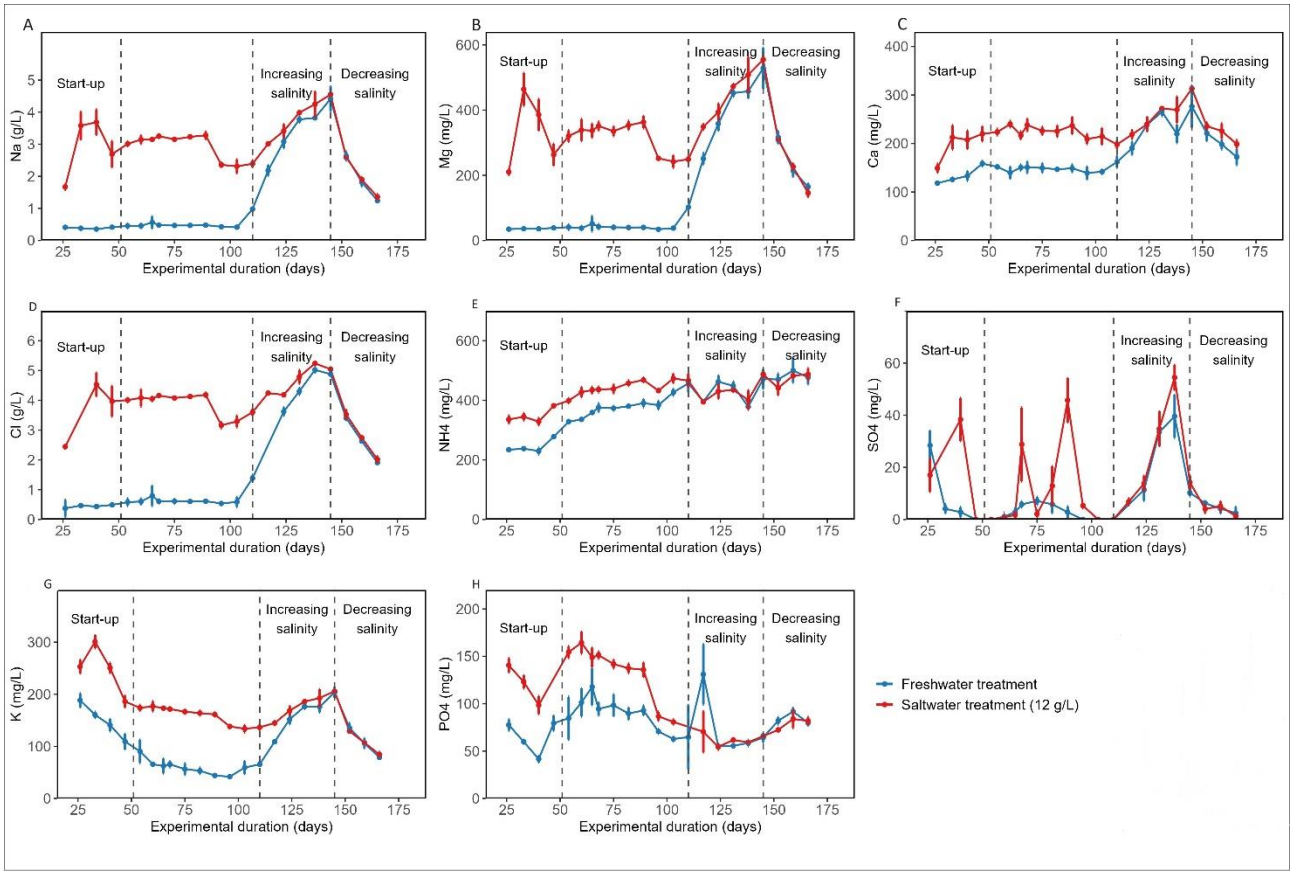

Figure 19: IC results for anions and cations measured across treatments in this study with standard deviations from biological triplicates. The dashed lines show the change in treatment The dashed lines show the change in treatment. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the Freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the Saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.

4.1.3 Anaerobic co-digestion of food waste leachate and black soldier fly larvae frass

Figure 20 illustrates the pH levels of the digestate resulting from the co-digestion process involving leachate from processed-food waste and black soldier fly larvae frass over a period of twelve days. The pH at the beginning of the experiment was approximately 6, which was lower than the initial pH recorded during the anaerobic digestion of aquaculture solids, standing at about 7.25. Over the course of the twelve-day period, the pH decreased to approximately 5. This indicates a daily decrease exceeding 0.1 pH units, in contrast to a decrease of roughly 0.02 pH units observed during the initial twelve days of the anaerobic digestion of aquaculture solids.

In addition, VFA concentrations in the digestate were measured at 7.4 ± 0.4 g/L with the highest fraction being acetic acid and butyric acid with respectively 31.1 ± 0.4% and 43.3 ± 0.7%. These concentrations were almost 1000 times higher than the VFA concentrations measured in the digestate of the aquaculture solids in the beginning of the experiment. The food waste leachate was also marked by a very low alkalinity, close to zero, as no alkalinity was detected by the alkalinity test.

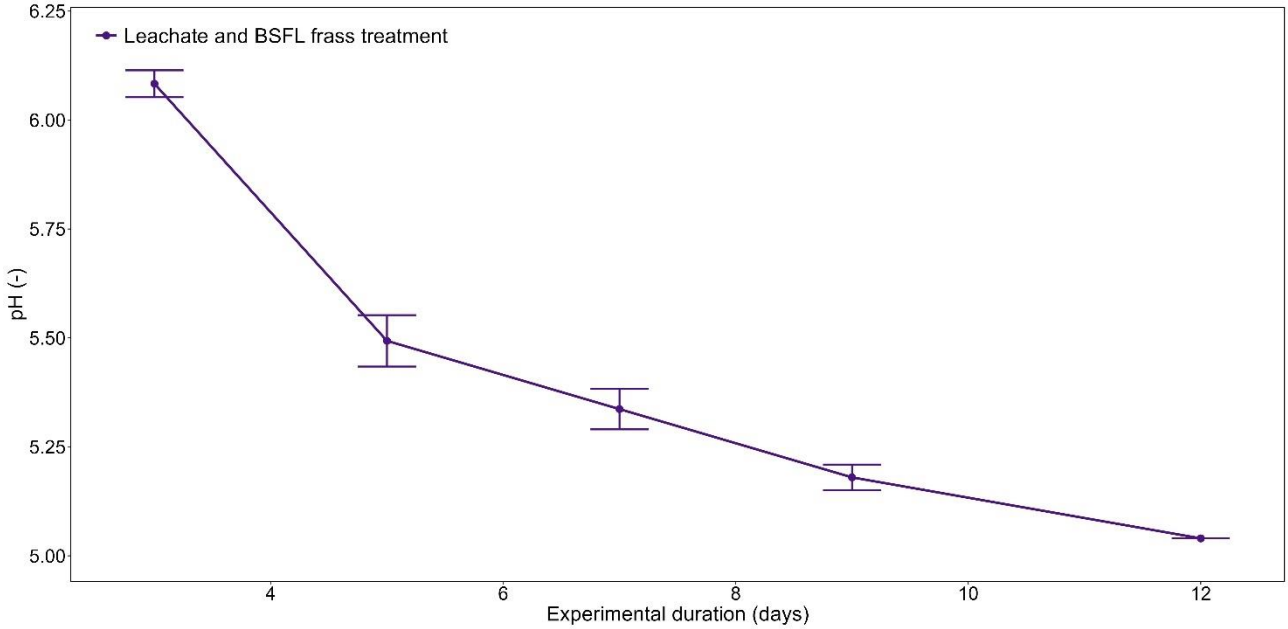

Figure 20: pH trends in the digestate resulting from the anaerobic co-digestion of food waste leachate and black soldier fly larvae frass with standard deviations from three biological triplicates.

4.2 Biochemical Methane Potential tests

A Biochemical Methane potential (BMP) test was performed to quantify the differences in biodegradability and conversion efficiencies to biogas from aquaculture solids at different salinity levels (0 g/L, 12 g/L, 20 g/L, 35 g/L), as well as for both total and soluble leachate. As depicted in Figure 21, Figure 22, and Figure 23, each of the BMP samples, along with the positive and negative controls, was conducted in biological triplicates. The biochemical methane potential in normal litres per gram of volatile solids over time is shown in Figure 21. Corrections were made for the residual biogas production by the digestate (negative control) by which the negative methane potentials for some feedstocks can be declared.

An immediate increase was observed in all samples, except for the saltwater aquaculture solids at 35 g/L, after which a plateau was reached. The duration until reaching the plateau varies among the different feedstocks. For both freshwater aquaculture solids and aquaculture solids at 12 g/L and 20 g/L, the plateau was already reached after 12 days.

The methane yield was the highest for the total leachate from food-processing waste with values of 0.64 ± 0.22 NL CH_4/gVS after 44 days, whereas the methane yield from the soluble leachate was much lower, namely 0.04 ± 0.03 L CH_4/gVS.

The biochemical methane potential from anaerobic digestion of aquaculture solids exhibited the highest yield at a salinity of 12 g/L, with an average of 0.39 ± 0.06 L CH_4/gVS during the steady-state period. The methane yield from freshwater aquaculture solids was only slightly lower at 0.32 ± 0.02 L CH_4/gVS. Notably, the methane yield from aquaculture solids at a salinity of 35 g/L was the lowest, with an average value of 0.007 ± 0.05 L CH_4/gVS.

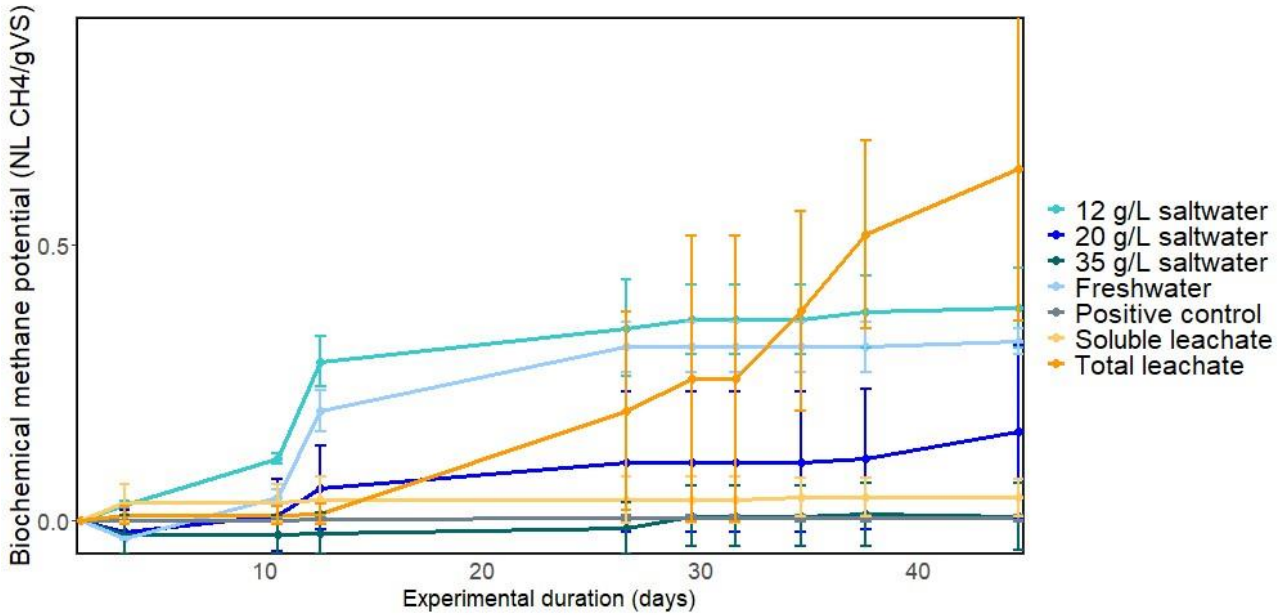

Figure 21: cumulative methane production in function of time for six different feedstocks with standard deviations from biological triplicates (aquaculture solids at a salinity of 0 g/L, 12 g/L, 20 g/L, and 35 g/L, total and soluble leachate from food-processing waste). The volumes are expressed in mL at STP per gram volatile solids.

The fractions of methane and carbon dioxide, collectively comprising 100%, are depicted in Figure 22. Methane fractions in both the positive and negative controls were generally comparable, although one replicate of the negative control showed no methane production. Methane fractions in the anaerobic digestion of freshwater aquaculture solids, as well as those at salinities of 12 g/L and 20 g/L, exhibited comparable values of respectively 52.3 ± 2.3%, 45.7 ± 7.1%, and 44.4 ± 6.5%. However, the methane percentage in the biogas produced by aquaculture solids at a salinity of 35 g/L was notably lower, albeit with one exception showing a high methane fraction of 72%, causing the high standard deviation. Furthermore, the methane fraction in the biogas was higher for the total leachate compared to the soluble leachate, with values of 74.8 ± 6.6% and 63.3 ± 4.2%, respectively.

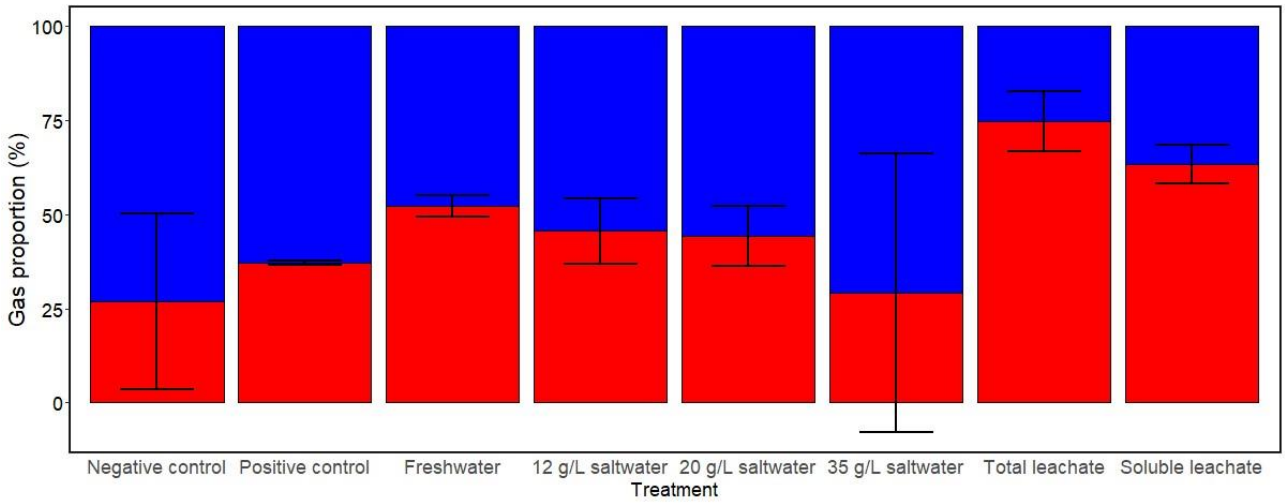

Figure 22: fraction of methane and carbon dioxide in the biogas, collectively comprising 100% for six different feedstocks with standard deviations from biological triplicates (aquaculture solids at a salinity of 0 g/L, 12 g/L, 20 g/L, and 35 g/L, total and soluble leachate from food-processing waste).

The pH of the digestate of the different treatments at the end of the BMP-test is shown in Figure 23. The pH of the digestate of the aquaculture solids at different salinities were comparable with a slight decrease observed with increasing salinity. The average pH of the digestate from the soluble leachate was almost 2 pH-units lower than the average pH of the total leachate.

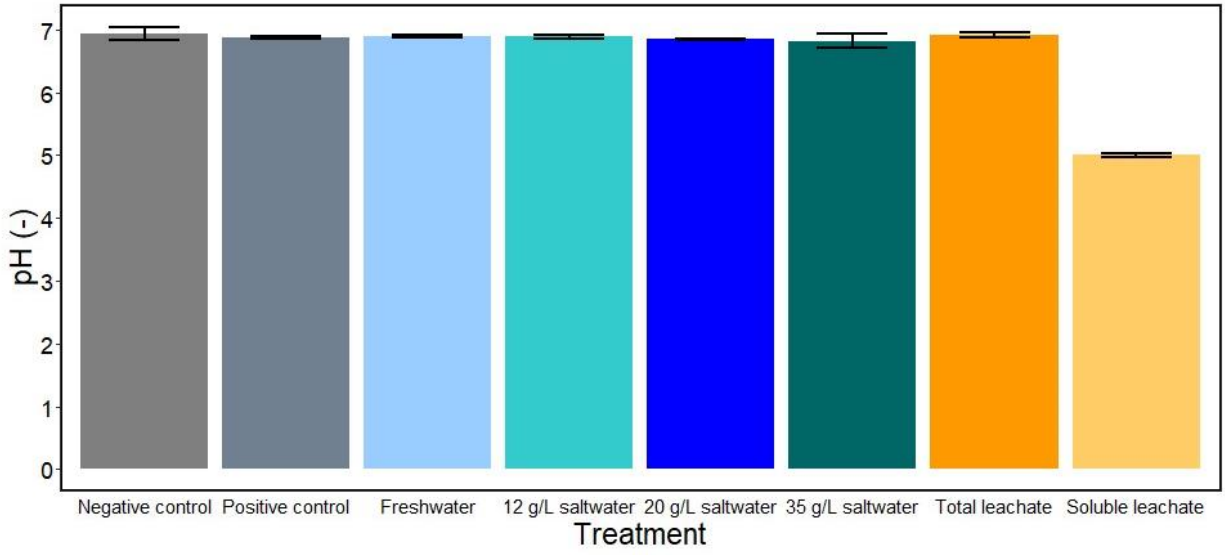

Figure 23: pH of the digestate at the end of the BMP-test for six different feedstocks with standard deviations from biological triplicates (aquaculture solids at a salinity of 0 g/L, 12 g/L, 20 g/L, and 35 g/L, total and soluble leachate from food-processing waste).

5 DISCUSSION

5.1 Fed-batch reactor not suitable for the co-digestion of food waste leachate and BSFL frass

In this research, the decision was made to co-digest food waste leachate and BSFL frass, which are both components of the circular integration of aquaponics and insect cultivation (Figure 6). Food waste leachate typically exhibits a low total suspended solids content and a high proportion of volatile soluble solids, which could be rapidly degraded during anaerobic digestion. This high amount of easily biodegradable organic matter can cause rapid volatile fatty acid generation, which can lead to the inhibition of the methanogens (Ghanimeh et al., 2018; Shen et al., 2013). Black soldier fly larvae frass, on the other side, had a high total solids content (466 ± 2 g/L of TS). The biogas yield of BSFL frass is high with values obtained by Elissen et al. (2019) of 0.506 NL/gVS and a methane purity of 58%. By adding BSFL frass to the anaerobic digestion of food waste leachate, the total suspended solids content can be increased as well as the alkalinity, which can act as a buffer, stabilizing pH-values. The co-digestion of both waste-streams can also slow down the fast degradation of organic substances in the food waste leachate. Adding a slowly biodegradable feedstock, such as BSFL frass, can prevent acidification, caused by the fast degradation of the easily biodegradable organic matter of the food waste leachate.

From the BMP-test, methane yields were determined for both the soluble fraction of the food waste leachate and the total food waste leachate. The highest methane yield was recorded for the total leachate, with a yield of 0.64 ± 0.2 NL CH_4/gVS, and pH levels reaching approximately 7. In contrast, the methane yield of the soluble fraction of the leachate was much lower, at 0.04 ± 0.03 NL CH_4/gVS. Centrifugation of the total leachate removed most solid particles from the solution. Consequently, the soluble leachate primarily comprised dissolved organic matter, which is more readily accessible to microorganisms. This readily available substrate was broken down rapidly by the microorganisms, leading to the accumulation of VFA and a subsequent decrease in pH. The pH of the soluble fraction of the leachate was measured at 5, whereas the optimal pH range of methanogens is between 6.5 and 7.8. So the high content of easily biodegradable chemical oxygen demand in the soluble fraction caused an imbalance between acidogenic and methanogenic processes. This led to the accumulation of VFA and a subsequent drop in pH, which inhibited the methanogenesis, resulting in low methane yields. Furthermore, a lower methane purity was observed for the soluble leachate, which was 63.3%, compared to 74.8% for the total leachate.

At the outset of the fed-batch experiment, there were some indicators during the start-up of the experiment suggesting that this anaerobic co-digestion won't succeed. First, VFA accumulation occurred in the digestate with an average concentration of 7.4 ± 0.4 g COD/L. Acetic acid and butyric acid represented the highest fractions with respectively 31.1 ± 0.4% and 43.3 ± 0.7%. The high fraction of butyrate was due to the low pH, as butyrate-producing bacteria are acid-tolerant bacteria and subsequently thrive in these acid conditions (Kong et al., 2016). The VFA accumulation can diminish the activity of microorganisms, and may cause the imbalance between the acid stage and the methanogenic stage, leading to acidification (K. He et al., 2024). Long-chain fatty acids (LCFA) have the capacity to adhere to microorganism surfaces, impeding the mass transfer process. This can also lead to reduced microorganism activity and instability (K. He et al., 2024).

Furthermore, acidification resulted in a decrease in the pH of the digestate to approximately 5 within a span of 12 days. This low pH was caused by the rapid acid generation of the easily biodegradable matter of the leachate. Despite the addition of the of BSFL frass, the pH couldn't be kept above 6.5, due to the low alkalinity of the leachate. Fermentative bacteria can survive these low pH-values, as they remain active within the pH-range of 4.0 to 8.5 (Appels et al., 2008). Methanogens, on the other hand, have an optimal pH range between 6.5 and 7.8 and inhibition risk occurs when the pH is below 6.5 (Atelge et al., 2018b; Mara & Horan, 2003). Qiu et al. (2023) showed that a 100%, and 71.7% suppression on methanogenesis was triggered at

respectively pH 4.0 and 5.5, compared to that at pH 7.0. Gene abundance and/or activity of most enzymes involved in methanogenesis, such as acetate kinase, was reduced. Moreover, the presence of crucial enzymes involved in the electron transport process, like CO hydrogenase, is reduced under acidic conditions (Qiu et al., 2023). Consequently, pH values around 5 indicate that methane production by methanogens may be hindered. Considering these indications pointing to the system's imbalance, the choice was made to close down the reactors.

These problems related with the anaerobic digestion of food waste leachate are already encountered in literature (K. He et al., 2024). The acidification problem could be temporarily solved by adding a base, such as NaOH, however this only treats the symptom instead of addressing the cause. The problem of acidification of the reactor and the VFA accumulation could be solved by introducing a two-stage digestion system. In this kind of system, the anaerobic digestion process is physically split in an acid-generating phase and an acid-consuming phase. The environmental conditions can be optimized for hydrolysis/acidogenesis phases in one reactor and for the methanogenesis phase in the second. It's already proven that these two-phase systems solve the pH inhibition issues of one-stage systems (Dinsdale et al., 2000; Shen et al., 2013).

5.2 The critical role of salinity on anaerobic digestion of aquaculture solids

5.2.1 Chemical composition of the aquaculture solids

The total solids content in the first batch of aquaculture solids was quite diluted leading to a low total solids content (1.43 g/L), consequently leading to a low initial organic loading rate. However, the TS-content was higher in the next two batches, due to a better settling. The organic fraction in the batches of aquaculture solids used in this experiment was comparable (40-50%), which is in line with the aquaculture sludge used in the experiment of Gebauer (2004). However, a lot of variation is found in literature, depending on the type of fish feed, feeding frequency, stocking density, etc. The organic fraction in fish sludge is typically lower compared to organic fractions found in agricultural waste, black soldier fly larvae (BSFL) frass, or manure, which often have organic fractions exceeding 80% (Elissen et al., 2019; Kafle & Chen, 2016; Lalander et al., 2018; Martínez et al., 2017; Szilá gyi et al., 2021).

5.2.2 Stability of the aquaculture solids as feedstock

The BMP test was performed to see if anaerobic digestion could deal with saltiness when processing fish waste. The methane yields were the highest for the aquaculture solids at a salinity of 12 g/L, with an average of 0.39 ± 0.07 NL CH_4/gVS. However, the difference in yield compared to the anaerobic digestion of freshwater solids, which obtained an average yield of 0.33 ± 0.02 NL CH_4/gVS, was low. The biochemical methane potential for the freshwater aquaculture sludge is comparable with data found in literature: Lanari & Franci (1998) obtained a yield of 0.43 ± 0.03 L CH_4/gVS. The yield obtained for the fish sludge at a salinity of 12 g/L was in line with the methane yield of 0.32 obtained by da Borso et al. (2021) for sludge from a brackish water fish hatchery. However, a higher yield was obtained than in the study of (Zhang et al., (2014), whereas in that case anaerobic digestion was performed on sludge from brackish water (15.2 g/L salinity), using glycine betaine and trehalose to improve biogas production rates, resulting in a yield ranging from 0.27 to 0.33 NL CH_4/g VS.

The methane yield in this thesis gradually decreased when the salinity was further increased to 20 g/L and 35 g/L. The biomethane potential for aquaculture solids at a salinity of 20 g/L obtained yields of 0.21 ± 0.13 NL CH_4/gVS. Methane yields observed from the digestion of aquaculture solids at 35 g/L was almost the same as the residual methane production by the negative control, suggesting that almost no methane was produced from the conversion of the substrate due to inhibition of the methanogens. This inhibition could also be caused by the elevated concentrations of sulphate in the aquaculture solids. Sulphate can be reduced to hydrogen sulphide (H_2S) by sulphate reducing bacteria (SRB). The H_2S has the potential to inhibit methane

production, with an IC_{50} (half-maximal inhibitory concentration) of 160 mg H_2S/L for acetoclastic methanogens and 220 mg H_2S/L for hydrogenotrophic methanogens (Yamaguchi et al., 1999). In this study, only sulphide seemed to contribute to the methane inhibition. The concentrations of sodium in the penicillin bottles were only 0.51, 0.72, and 1.11 g/L for respectively freshwater aquaculture solids, and for aquaculture solids at salinities of 12 g/L, and 20 g/L, due to the dilution of the feedstocks. Elevated salinity levels resembling full-strength seawater (35 g/L) have previously been identified as a contributing factor to diminished methane yields from aquaculture solids (Zhang et al., 2013). Zhang et al. (2014) showed that methanogens experienced sodium toxicity at salinity levels exceeding 25 g/L, despite the long-term adaptation of the inoculum to high salinity levels.

The BMP-test results showed a decline in biochemical methane potential starting from salinity levels of 20 g/L in aquaculture sludge. This indicates that at high salinity, anaerobic digestion of aquaculture sludge may face some challenges and may not work effectively. However, it is crucial to find proper management options for this saline waste stream to prevent environmental harm. Despite the decrease in conversion efficiency, saline aquaculture waste still possesses substantial methane potential, suggesting that it's worth investigating the anaerobic digestion of saltwater aquaculture solids in longer term experiments to determine if substantial methane yields can still be achieved. Conducting fed-batch experiments over an extended period will provide valuable insights into the viability of anaerobic digestion for high-salinity aquaculture waste.

5.2.3 Start-up phase

During the start-up phase, there was a gradual decline in pH, primarily caused by the production and buildup of volatile fatty acids (VFA). These VFA originated from water-insoluble macromolecules, and were converted by hydrolytic and acidogenic microorganisms. Thus, before the VFA were utilized by methanogens, a pH decrease can be observed (Zhai et al., 2015). Following this initial decrease, the pH stabilized due to the consumption of the VFA by the methanogens, and the production of alkalinity (Yulisa et al., 2022). The increase in alkalinity is caused by the conversion of CO_2 to carbonic acid, bicarbonate, and carbonate, together with the generation of NH_3 by the degradation of proteins (Yulisa et al., 2022).

During the initial phase of biogas production (day 51-110), only carbon dioxide (CO_2) was observed, with no methane (CH_4) being produced yet. This could be explained by the high sensitivity and low growth rates of methanogens (Olafadehan & Alabi, 2009). It took around 35 days to produce any methane. The sudden rise in VFA after 40 days can be attributed to the decrease of the SRT to the target SRT of 20 days as the organic loading rate was increased. This increase in VFA accumulation caused subsequently a decrease in pH, which in turn gave rise to the increase in phosphate concentration as the solubility of phosphorus increases with decreasing pH-values (Latif et al., 2015). In addition, the TS, VS, and ratio of TS to VS also reached peak levels during this period due to the decrease in SRT. The reduced SRT implies that organic matter spends less time in the digester, undergoing degradation and conversion to biogas, resulting in a higher proportion of the organic material remaining in the digestate as volatile and total solids. In addition, more organic material was added to the reactors as the organic loading rate is increased. The increase in TS and VS was more pronounced in the saltwater treatment, like the accumulation of VFA. The higher amount of salts introduced in the reactors resulted in higher TS concentrations in the digestate as salts are part of the TS-content. The combined effect of heightened organic loading rate and increased salinity led to a more substantial surge in VFA in the saltwater treatment, which subsequently led to an increase in VS.

5.2.4 Both saline (12 g/L) and freshwater anaerobic digestion of aquaculture solids resulted in stable biogas production

Instant ocean was added to the aquaculture solids to achieve a sea salt concentration of 12 g/L, corresponding to brackish water salinity, was obtained. The instant ocean mix consisted out of sodium, magnesium, calcium, potassium, and chloride. It can be seen in Figure 19 that the concentration of these salts show similar trends, with higher concentrations observed for the saltwater treatment. At very low salt concentrations, stimulation of the activity of microorganisms can be achieved, whereas inhibitory effects can occur at higher concentrations (McCarty, 1964). The concentrations at which these inhibitory effects occur, is dependent on the type of cation: 3500 mg/L for sodium, 2500 mg/L for both potassium and calcium, and 1000 mg/L for magnesium (McCarty, 1964).

The methane yields observed for both treatments were comparable with no significant differences observed ($p=0.74$), reaching values around 0.3 NL CH_4/gVS at the end of the phase. This methane yield is comparable to other experiments performing anaerobic digestion of aquaculture solids. For instance, Swedish Gas Technology Centre (2012) reported methane yields of 0.36 NL CH_4/gVS, and Ahsan et al. (2019) recorded methane yields of 0.32 NL CH_4/gVS. Gebauer (2004) reported methane yields of 0.22 g CH_4/gVS for the anaerobic digestion of saltwater aquaculture solids, with the sodium concentration in the digestate measured at 5.3 g/L. In contrast, in the review of Miranda et al. (2016), a median of 0.124 L CH_4/gVS was reported for the anaerobic digestion of dairy cattle manure. This suggests that the methane yield obtained from aquaculture solids can be higher than from manure, highlighting the potential for the anaerobic digestion of aquaculture solids. The higher methane yield observed from aquaculture solids can be attributed to their higher lipid content compared to manure. Higher lipid fractions are associated with a higher methane fraction in the biogas, as lipids are more reduced compared to carbohydrates, making them more conducive to methane production during anaerobic digestion. Therefore, the presence of a greater proportion of lipids in aquaculture solids results in an increased methane production compared to manure, which typically contains lower lipid content (Anglade et al., 2024; Atelge et al., 2018; Møller et al., 2004).

Even though the yields of both treatments were comparable, there were significantly more VFA (>C2) in the Saltwater treatment ($p=0.016$). Additionally, the accumulation of acetate was significantly higher for the Saltwater treatment ($p=0.024$). This can be caused by two factors: (1) less acetate consumption by the acetoclastic methanogens and syntrophic acetate-oxidizing bacteria or (2) more acetate production by the fermenting bacteria or propionate-oxidising bacteria (Yue et al., 2021). Research of Zhao et al. (2017) showed that higher salinity levels can improve the solubilization of organic substrates, leading to higher concentrations of soluble carbohydrates and proteins. In addition, the presence of NaCl may also enhance the degradation rates of proteins and carbohydrates. However, the degradation rate of acetate can decrease if the salinity is increased due to inhibition of the methanogenic community (Zhao et al., 2017). The VFA accumulation in both the Saltwater treatment and the Freshwater treatment decreased after the sudden peak, due to the decrease in SRT. The decrease in VFA concentration was achieved by consumption of the VFA by the methanogens, leading to increasing methane yields.

A reactor's stability can be determined using VFA-criteria, wherein stability is assumed when VFA-accumulation remains below 1000 mg/L (Gebauer, 2004). Based on this criterium, the Freshwater treatment could be considered stable approximately 70 days after the initiation of the experiment. However, it took longer for the Saltwater treatment to reach VFA values below 1000 mg/L, roughly around 95 days. Despite the extended time required for the saltwater digester to achieve stability, it suggests that the methanogenic community had successfully adapted to the new, saline digester conditions.

The concentration of both total solids and volatile solids was significantly higher for the Saltwater treatment ($p<0.001$ for both). The total solids represent the residue remaining after evaporation of the water content, including both organic and inorganic matter, whereas volatile solids are the solids that volatilize during ignition of the dry solids, representing the organic solids in the sample. The higher concentrations of total solids can be attributed to the higher salt concentrations. The higher portion of volatile solids in the digestate indicates the higher amount of biodegradable matter that was still present in the digestate, which consequently remained unconverted into biogas. This is in line with the higher levels of VFA detected in the digestate of the Saltwater treatment. The ratio of volatile solids to total solids (VS:TS) remained relatively stable across the treatments, although higher values were observed for the Saltwater treatment ($p<0.001$), averaging around 65%, compared to approximately 50% for the saltwater treatment. The consistency of these values alongside the experiment indicates the maintenance of the microbial activity; the microorganisms continued to degrade organic matter efficiently without substantial fluctuations in their activity levels (Leite et al., 2017).

Despite the higher VFA accumulation and VS concentrations in the Saltwater treatment, the methane fraction was significant higher for the Saltwater treatment ($p=0.0024$). Changes in salinity could have influenced the composition of the microbial community. Typically, 70% of the methane is produced from acetate (acetoclastic methanogenesis). The other 30% of methane is produced from the conversion of H_2 and CO_2 (hydrogenotrophic methanogenesis) (Mara & Horan, 2003). The higher methane fraction in the saltwater treatment can suggest that a higher fraction of methane was produced by the hydrogenotrophic methanogenesis. This is in line with the findings of X. He et al. (2024), as they discovered that there was a significant increase in the relative abundance of the hydrogenotrophic methanogenic archaea, *Methanobacterium* and *Methanomassiliicoccus*, under high salinity conditions, leading them to become the dominant genera. On the other side, a strong inhibitory effect on the acetoclastic *Methanothrix* was observed, indicating that the methanogenic pathways were shifting from acetoclastic to hydrogenotrophic methanogenic pathways with increasing salt concentration. These findings support that the higher methane fractions in the Saltwater treatment could be caused by the increased relative abundance of the hydrogenotrophic methanogenic archaea. Despite the higher methane fraction, no higher methane yields were obtained, due to the lower biogas volume for the Saltwater treatment. The VFA accumulation and the higher concentration of volatile solids still present in the digestate of the saltwater treatment suggest that less acids were converted to biogas, causing these lower volumes of biogas. The methane purity, which was 71.9% ± 5.0 for the freshwater treatment and 76.4 ± 3.7% for the saltwater treatment, was generally higher than literature values: A methane content of 60% was reported for previous freshwater aquaculture experiments and a 65% methane content was observed for cow manure (Eggeling et al., 1986; Ndiaye et al., 2020). This can be attributed to a several factors: an anaerobic digestion design that is optimized compared to previous studies on generating biogas from aquaculture solids (including factors such as temperature, utilizing inoculum from a BMP anaerobic digester, ideal retention time, and volumes based on feedstock characteristics) and also a homogenous, nitrogen-rich feedstock free from inhibitory products (Lobanov et al., 2023).

5.2.5 Maximum Salinity Tolerance in Anaerobic Digestion of Fish Sludge

Increasing the salinity in the aquaculture solids, supplied to the fed-batch reactors, to 20 g/L resulted in a gradual decrease in yield, with methane yields reaching slightly above 0.1 NL CH_4/gVS, corresponding with an electricity yield of around 0.004 kWh/L incoming sludge. A big aquaculture company, producing around 17,000 tonnes sludge per year can consequently produce around 68 MWh per year (Čekanavičius, 2023). This amount of energy is sufficient to meet the annual energy needs of roughly 10 people. However, this low energy output does not justify the installation of a biogas plant. The decline in yield aligns with the gradual rise in salinity within the reactor, attributable to limited volume exchange during each feeding cycle as the SRT of 20 days involved only adding 40 mL of fresh feed daily. The salt concentrations in the digestate of both

treatments, one initially fed with freshwater aquaculture sludge and the other with saltwater aquaculture sludge at a salinity of 12 g/L, were comparable at the end of this period. The reduction in yield is attributed to limitations on the capacity of methanogens to adapt to the higher osmotic pressure caused by the increasing salinity.

It can be seen in Figure 19A that the sodium-concentrations in the digestate increased to 5 g/L, due the salinity increase in the aquaculture solids to 20 g/L. Sodium concentrations at such high levels are considered to be moderately inhibitors to anaerobic treatment, meaning that they could be tolerated, but acclimation of the microorganisms is required and the process can be retarded significantly (McCarty, 1964). The toxicity may be caused by an increase of the osmotic pressure between the microbial cells and the surrounding environment, resulting in cell dehydration, and the reduction of the activity of enzymes and microorganisms (Zhang et al., 2014). The inhibiting impact of sodium is considered to be lower when sea water is used instead of NaCl, due to the antagonistic effect of the other nutrients present in the seawater (Feijoo et al., 1995). Cations such as magnesium, potassium, and calcium can act as antagonists reducing the toxicity of sodium. Kugelman & Chin (1971) stated that the antagonist might stimulate certain mechanisms or processes within the system that mitigate the impact of the toxin, so it doesn't directly counteract the toxin, but rather triggers responses within the system that diminish its effects.

A reduction in methane yield could also be caused by high concentrations of sulphate. Sulphate concentrations in the digestate increased to around 60 mg/L after the increased salinity. Next to methanogens, sulphate reducing bacteria are present in anaerobic digestion reactors. These microorganisms reduce sulphate to hydrogen sulphide, which can inhibit methanogenesis. However, Choi & Rim (1991) state that methane producers and sulphate reducers are competitive at a COD:SO_4^{2-} ratio lower than 2.7. However, the ratio of COD:SO_4^{2-}, equalling 1188 ± 20, remained well above 2.7 in this experiment, suggesting that limited competition occurred between the methanogens and the sulphate reducing bacteria. So, the inhibition of the microorganisms is primarily caused by the increased osmotic pressure.

The higher salinity levels were stressful for the methanogens, which is supported by the increase in VFA. The delay in the impact of heightened salinity on VFA levels is attributed to the gradual rise in salinity within the digestate, as only a limited volume of new feed was introduced to the reactors during each feeding cycle. The VFA concentration continued to rise until the end of this phase. Despite the increase in VFA, no decrease in pH was observed, due to high alkalinities related to aquaculture solids (Gebauer, 2004; Luo et al., 2013). Next to that, an increase in TS and VS and the ratio of VS:TS was observed, due to the lower amount of organics converted to biogas. The fraction of acetate to total VFA also increased, suggesting that the methanogenic community was partially inhibited from converting acetate to methane in these high salinity conditions. Despite the decrease in biogas volume production and methane yield when the salinity was increased to 20 g/L, this is not reflected in the methane purity in the biogas as the average methane percentage was similar to the percentage before the salinity increase. As discussed in section 5.2.4, X. He et al. (2024) found that the relative abundance of hydrogenotrophic methanogens increased, indicating that the methanogenic pathways shifted from acetoclastic to hydrogenotrophic methanogenic pathways with increasing salt concentration. However, the consistency in methane purity suggests that further increases in salinity don’t lead to additional enrichment of hydrogenotrophic methanogens. Conversely, feeding the initially freshwater reactors with aquaculture solids at a salinity of 20 g/L, didn’t result in increased methane purity (initially 71.4 ± 4.8% vs 70.3 ± 2.0% when the salinity was increased to 20 g/L). This is in line with the observations of Wang et al. (2017). They noted that the shift from acetoclastic methanogens to hydrogenotrophic methanogens was clearly inhibited at high salinity levels, particularly at sodium concentrations of 20 g/L. This suggests that even though salinity can cause a shift to more hydrogenotrophic methanogens, high salinity levels can hinder this transition.

As the sodium concentrations in the digestate are not considered as strongly inhibitory, microbial adaptation to saline conditions may lead to an increase in methane yield over time. This is also supported by the fact that the methane purity was significantly higher for the reactors that were initially fed with the aquaculture solids at a salinity of 12 g/L ($p < 0.001$). Methanogens had the possibility to slowly adapt to increased salinity levels by first adapting to salinity levels of 12 g/L. Non-saline inocula can adapt to saline conditions by gradually introducing sodium ions, driving the microbial community to a halotolerant state (Buenaño-Vargas et al., 2024). Microorganisms change their physiological and morphological properties over time, which is generally reversible. By accumulating Cl^- and K^+, while maintaining low Na^+ concentrations, microorganisms can counteract the high osmotic pressure imposed by high salinity. This can also be done by accumulating organic solutes, such as glycine betaine, ectoine, and other amino acids and sugar derivatives (Yoo et al., 2023). These adjustments serve to adapt metabolism, enhancing the organism's survival in response to a change in the environment. So the acclimation of non-halophilic biomass to saline conditions can be improved by adding osmolytes such as potassium and organic osmolytes in order to reduce the osmotic stress whereas iron and calcium can be added to enhance microbial aggregate stability and prevent biomass wash-out (Buenaño-Vargas et al., 2024). However, it is unlikely that the methane yield will reach pre-salinity increase levels, as biological activity may remain lower at elevated sodium concentrations.

The yield from the anaerobic digestion of aquaculture solids at a salinity level of 20 g/L is lower than the yield obtained from the BMP-test. This is attributed to the shorter solids retention time (SRT) of 20 days in the fed-batch limiting the extent to which organic matter can be converted to biogas. This shorter retention time allows only partial conversion of the feedstock to biogas. In contrast, the BMP test allows sufficient time for complete conversion of the aquaculture solids to biogas.

5.2.6 Recovery from high salinity levels

By feeding the reactors with freshwater aquaculture sludge (without sea salt addition), after the salinity increase, the performance of the anaerobic digesters was again improved, due to the gradual decrease of salinity in the reactors to sodium concentrations of around 2 g/L, however, the sodium level was still decreasing. The concentration of volatile solids in the digestate of both treatments was subsequently reduced, suggesting that more organic material was again converted into biogas. This was also supported by the decrease of the VS:TS ratio. This is in line with the observed decrease of VFA that were still present in the digestate and the lower fraction of acetate to total VFA, indicating the higher acetate utilization efficiency by the microbial community.

This highlights the ability of the microbial community to recover from high salinity levels, suggesting that they were only temporarily inhibited by the high salinity levels. The sodium concentrations decreased gradually to values slightly above 1 g/L, which is below the range of concentrations causing moderate inhibitory effects among McCarty (1964). The microorganisms were able to survive the increased salinity levels of 20 g/L, indicating they were only temporarily inhibited. This is in line with McCarty (1964), who stated that at the sodium concentrations, observed in the reactors when they were fed with aquaculture solids at salinity levels of 20 g/L, only moderate inhibition occurred, which involves the decreased activity of the microbial activity. By decreasing the salinity of the aquaculture sludge again, the microbial activity increased again, leading to the faster rate of organic conversion to biogas. Strong inhibition only occurs at sodium concentrations of 8 g/L, involving the biological activity approaching zero (McCarty, 1964).

The higher conversion rate was mirrored in the increase in methane yield. As the methane fraction remained comparable to before the salinity increase, the increase in methane yield was attributed to the increased rate of biogas production, due to the increased activity of the methanogenic activity. No significant differences were observed in methane yield between both treatments ($p=0.86$). This means that the microbial community in both treatments were equally efficient in converting organic material into biogas

under the decreased salinity conditions. Therefore, the microbial communities in both treatments demonstrated adaptability, ultimately leading to comparable methane production rates despite the initial salinity variations.

5.3 Biogas production from aquaculture solids at full-scale

The biogas produced by anaerobic digestion can be used by a Combined Heat and Power (CHP) system to produce electricity and heat. However, it's important to look if it is economically feasible to invest in such an installation. The interest in producing biogas from organic waste becomes more widespread in the world, which is reflected in the accelerated growth in biogas production worldwide with Europe as world leader, responsible for more than half of global production. However, a lot of improvement is still possible and will be necessary to increase circularity in our food production systems. Cucchiella et al. (2019) performed an economic analysis of biogas plants, and stated that a minimum production level of 200 kWh is needed for a biogas plant to be profitable. However, further technical innovations or subsidies can reduce this minimum required production level. By using the maximum electricity yields obtained in the experiment, it's estimated that a trout farm yearly producing 6000-ton sludge is needed to make the biogas installation economically feasible. Large-scale fish farms, yearly producing 50,000 tonnes of fish, can produce up to 17,000 tonnes of sludges on a yearly basis, making the installation of a biogas plant feasible (Čekanavičius, 2023).

However, for smaller aquaculture farms, other options are also possible for smaller scale electricity production, such as micro combined heat and power systems (mCHP). In these kinds of systems, biogas can be used as fuel to generate both electricity and heat simultaneously, typically on a smaller scale suitable for residential or small commercial applications (Maghanki et al., 2013).

Sulphur poses a concern in anaerobic digestion due to its tendency to produce a noxious gas. Sulphate (SO_4^{2-}) and elemental sulphur (S^0) can be converted to hydrogen sulphide in anaerobic conditions by sulphate reducing bacteria. Thus, after the anaerobic digestion process, the sulphur compounds present in the inlet stream leave the reactor via the biogas in the form of H_2S. This H_2S is an unwanted gas, due to its potential to cause corrosion (Zulkefli et al., 2016). Therefore, its removal is essential to enhance the quality of the raw biogas. The sulphate concentrations in the digestate of the Freshwater treatment weren't extremely high, exceeding 10 mg/L. However, higher concentrations were observed in the digestate of the saltwater treatment and after the salinity increase to 20 g/L, due to the higher amount of sulphur present by adding the Instant Ocean salt.

Another component of concern is ammonia. Even though ammonium is an essential nutrient for bacterial growth, elevated concentrations of total ammonia nitrogen (TAN) can impede methanogenesis. During anaerobic digestion, nitrogen in proteins is liberated as ammonia during the decomposition of organic matter. Ammonia toxicity was not a concern since, after the start-up period, the pH never exceeded 7. At pH-values lower than 7, TAN is almost entirely present as ammonium, which is less toxic than free ammonia. Therefore, complete inhibition of anaerobic digestion by free ammonia, which occurs at concentrations of 150 mg/L, was not a concern in this study (Yenigün & Demirel, 2013). The TAN always remained below 500 mg/L, which is considered low compared to other feedstocks, whereas reactor failure only occurs at TAN-concentrations of above 1700 mg/L (Yenigün & Demirel, 2013).

Beside the use of the biogas, there is also the possibility for recovering the minerals present in the digestate. Although prioritizing the optimization of nutrient remineralization was not the main focus of this study, several trends were noticeable. Considering the pollution mitigation needed within the rapidly growing aquaculture industry and the potential of supernatant, rich in nutrients, for aquaponic system, it's crucial to consider the phosphate concentration in the sludge as a critical parameter to monitor. Suzuki et al. (2003)

found that 69% of the phosphorus fed to the fish, ends up in the sludge. The total phosphate content in aquaculture sludge, consisting of orthophosphate, poly- and organophosphates, was 1.26 g/L, accounting for 3.9% of total solids. The total phosphate in the digestate of both treatments was higher than the concentration in the influent. This elevation occurred as organic matter, broken down during anaerobic digestion, released various forms of phosphorus, including orthophosphates, polyphosphates, and organophosphates. However, the phosphate concentrations were lower in the digestate than in the aquaculture solids, fed to the digesters. The decreased phosphate concentrations in the digestate compared to the feedstock was also observed in the research of Güngör & Karthikeyan (2008). They suggest that the orthophosphate may not remain in the dissolved phase. Instead, they can become associated with particulate solids or undergo re-precipitation as inorganic phosphate solid phases. The orthophosphate could be also used by the microbial community for their metabolic processes.

Differences were observed in phosphate concentrations in the digestate at the different treatments applied. Figure 19-H illustrates that the phosphate concentration in the first phase was notably higher in the digestate of the Saltwater treatment (12 g/L). However, during the period of elevated salinity (20 g/L), the phosphate concentrations in the digestate decreased. Remmen et al. (2017) investigated the impact of salinity on the phosphorus concentrations in the supernatant of sewage sludge. They observed a peak in dissolved phosphorus concentration at 12% salinity, followed by a subsequent decrease. This phenomenon can be attributed to the salting-out principle, where increased ionic strength promotes precipitation. This precipitation likely involves calcium phosphate. So the higher phosphate concentration observed in the digestate of the Saltwater treatment (12 g/L) compared to the freshwater treatment could be attributed to increased solubility, whereas the decrease in phosphate concentrations at higher salinities suggests precipitation induced by the salting-out effect. However, further research is needed to clarify these underlying mechanisms further.

6 CONCLUSION

This research on the biogas potential from aquaculture solids showed promising results in terms of biogas yield and long-term process stability from the anaerobic digestion of both freshwater aquaculture solids and aquaculture solids at a sea salt salinity of 12 g/L. Methane yields were approximately 0.3 NL CH_4/g VS showing that biogas production can remain efficient under saline conditions, making anaerobic digestion a viable solution for waste streams produced in saltwater aquaculture farms. Despite significantly higher VFA, TS, and VS accumulation in the digestate of the Saltwater treatment, methane yields were not significantly lower compared to the Freshwater treatment. However, it took longer for the Saltwater treatment to reach stability, reflecting the extended adaptation period needed for microorganisms to adapt to higher salinity levels. Furthermore, higher methane purity was obtained for the Saltwater treatment, suggesting an increased relative abundance of the hydrogenotrophic methanogenic archaea.

When investigating the maximum salinity tolerance from an economic perspective, increasing the salinity to 20 g/L in the aquaculture solids resulted in elevated VFA, TS, and VS levels in the digestate, indicating that more biodegradable organic matter remained unconverted to biogas. This highlights the limitations and potential inefficiencies of the anaerobic digestion process at higher salinity levels. The higher acetate:VFA ratio suggested partial inhibition of the methanogenic community from converting acetate to methane under these conditions, reducing methane yield to around 0.1 NL CH_4/gVS. Economically, biogas production from anaerobic digestion of aquaculture solids at a salinity of 12 g/L would be feasible, but at 20 g/L, it would not be profitable anymore, yielding only 68 MWh annually at large-scale land-based aquaculture farms, enough for the annual energy needs of roughly ten people. Therefore, further research is needed to explore strategies to manage these high salinity levels, such as the addition of osmolytes. Developing such strategies could enhance process efficiency and expand the applicability of anaerobic digestion under higher salinity conditions.

Microorganisms showed the ability to recover from high salinity conditions when the salinity was reduced to 0 g/L, with methane yield eventually returning to pre-salinity increase levels. This indicated that the inhibition by salinity toxicity was only temporary and highlights the resilience of the microbial community in anaerobic digestion.

Lastly, this research explored the suitability of co-digesting BSFL frass and food waste leachate. However, the co-digestion using a fed-batch reactor failed, due to multiple factors, primarily VFA accumulation in the digestate, with an average concentration of 7.4 ± 0.4 g COD/L. This led to the imbalance between the acid stage and the methanogenic stage, causing acidification, which can lead to the inhibition of methanogens. This experiment highlighted the challenges accompanied with the high fraction of easily biodegradable organic matter in the processing food waste leachate. These challenges could be addressed by using a two-stage reactor system to cope with the acidification issue, but further research is required.

Future perspectives

The co-digestion of food waste leachate and Black Soldier Fly Larvae (BSFL) frass in a fed-batch reactor encountered challenges, due to acidification. To address this issue, it would be valuable to investigate the use of a two-stage reactor system. This approach could potentially mitigate acidification, thereby, improving the overall stability and efficiency of the anaerobic digestion process.

The anaerobic digestion of both aquaculture sludge at freshwater conditions and at 12 g/L salinity has shown promising results with feasible methane yields. However, it’s essential to conduct studies on pilot and industrial scale to evaluate the process at larger scale.

Given the significant decrease in methane yield during anaerobic digestion of aquaculture solids at a salinity of 20 g/L, it's crucial to explore strategies to manage these high salinity levels. One approach is to add osmolytes, such as potassium and organic compounds, to reduce osmotic stress. Additionally, employing halotolerant microorganisms can be beneficial. Further research is needed to evaluate the effectiveness of these strategies.

Biosafety is a crucial aspect of the solids treatment system. Anaerobic digesters have the potential to reduce pathogenic bacteria, which is beneficial for both waste management and nutrient remineralization in aquaponics. Future investigations should include monitoring the survivability of pathogenic strains in anaerobic digesters. Advanced techniques like flow cytometry could be used to determine the presence and reduction of these pathogens, ensuring the safety and sustainability of the process.

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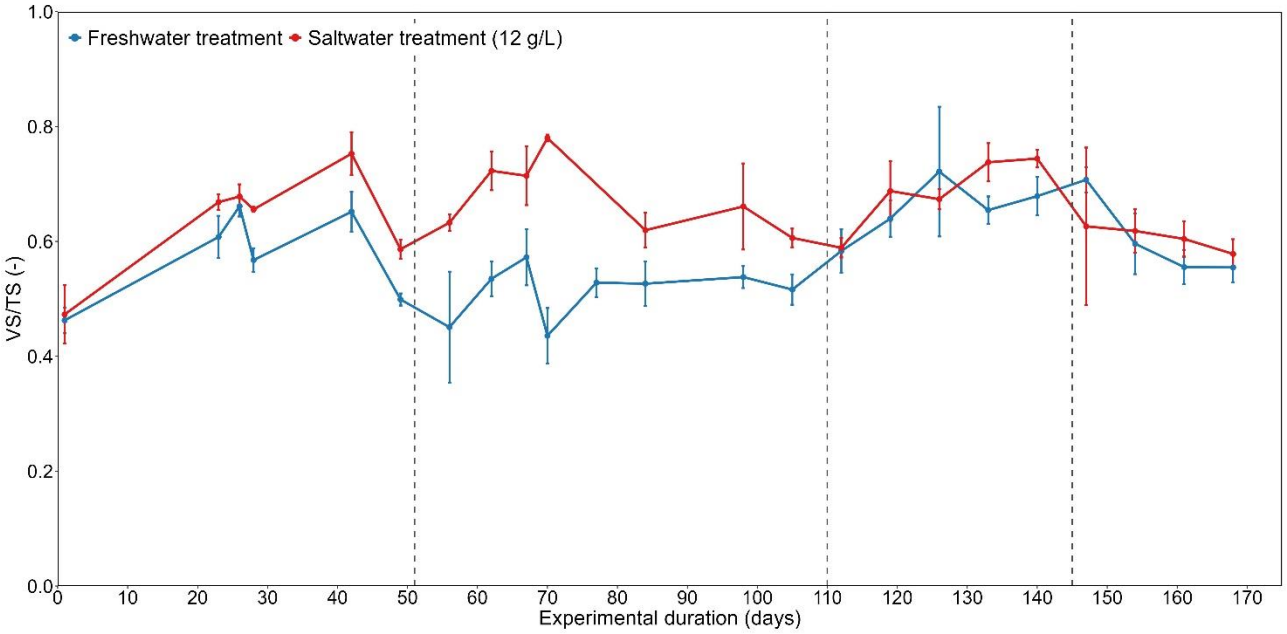
Appendix

Appendix A



Evolution of the ratio of total solids to volatile solids (VS) over time with standard deviations from biological and technical triplicates. The dashed lines show the change in treatment. The dashed lines show the change in treatment. Day 1 to 50 was the start-up period, whereas the SRT was kept constant from day 51 to 110. During these periods, the freshwater treatment involved the anaerobic digestion of freshwater aquaculture solids and the saltwater treatment (12 g/L) involved the anaerobic digestion of saltwater aquaculture solids (12 g/L). From day 110 to 145 the salinity of both treatments was increased to 20 g/L whereas the salinity of both treatments was decreased again to 0 g/L after day 145.