Aquacultural Engineering 101 (2023) 102328

Contents lists available at ScienceDirect

Aquacultural Engineering

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

Simultaneous biomethane production and nutrient remineralization from aquaculture solids

Victor Lobanov [a], Jo De Vrieze [b], Alyssa Joyce [a,*]

[a] University of Gothenburg, Department of Marine Sciences, Sweden
[b] Center for Microbial Ecology and Technology (CMET), Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Gent B-9000, Belgium

ARTICLE INFO

Keywords:
Recirculating aquaculture systems
Biomethane potential
Waste-revalorization
Onsite treatment
Solids management
Circular economy

ABSTRACT

The rapid expansion of the aquaculture industry has brought about a heightened focus on the waste produced by high intensity fish farming. In closed-containment, recirculating aquaculture systems (RAS), fish solids are mechanically separated and/or coagulated before being disposed as waste. Subsequent revalorization is typically limited to the direct dispersal of aquaculture solids onto agricultural fields. Here, we developed a novel, continuous flow, low-cost solids waste treatment system for freshwater and saline RAS. Rotating drum filter backwash was collected as the primary feedstock for anaerobic digestion. A laboratory scale set up was used to monitor the conversion of the solids into a methane-rich (60–80% purity) biogas stream. Iron supplementation (ferric iron at 100 mg/L and 1000 mg/L) improved salt tolerance of the methanogenic community, leading to higher methane yields in a supplemented ($FeCl_3$ at 1000 mg/L) saline treatment than the saline control. The application of iron additionally improves pH stability and volatile fatty acid utilization. The methane yield ranged from 0.1 to 0.4 NL CH_4/ g VS across the three freshwater treatments and the iron-supplemented saline treatment, however, it was significantly lower for the saltwater control: ranging between 0.08 and 0.25 NL CH_4/ g VS. These values correspond to a percentage yield of 57–86% of the total biomethane potential. Overall, implementing anaerobic digestion for RAS waste valorization may generate significant amounts of biomethane to be used in electricity and heating for large-scale aquaculture facilities, while even for smaller facilities it may offset costs and mitigate environmental impacts of the waste streams.

1. Introduction

In 2018, world salmon aquaculture production reached 2.2 million tons, corresponding to an estimated nutrient loss of 889 kilotons of carbon, 1.13 million tons of nitrogen, and 20.6 kilotons of phosphorus into coastal waters (Wang et al., 2012; Fisheries, 2010). This discharge is related to metabolic processes (the excretion of carbon-rich mucous, exhaled ammonia, and urea), uneaten feed (partial digestion of the carbon source, nitrogen and phosphorus in other forms, such as proteins) as well as all microbially-mediated derivatives of the decomposition process (Moraes et al., 2015).

Aquaculture waste streams can be divided into two broad categories, i.e., dissolved and suspended fractions. Treatment of the dissolved fraction focuses on the simultaneous removal and neutralization of nitrogenous species, resulting in the formation of nitrate (Bartelme et al., 2017; Brown et al., 2013; Crab et al., 2007; Keuter et al., 2017; Holl et al., 2011), although the removal of dissolved organic carbon occurs simultaneously (Guerdat et al., 2011; Michaud et al., 2006). Other mineral nutrients (Tetreault et al., 2021) are also carried downstream to varying degrees, depending on their solubility at the neutral pH typical of the upstream water source and their complexation in the fish solids or feed (Hussain et al., 2014; Cerozi and Fitzsimmons, 2017; Gartmann et al., 2019; Nichols and Savidov, 2011; Yogev et al., 2016). Under pressure from both regulatory agencies and the public, solids waste management is an increasingly important issue for the further development of the global aquaculture industry (Miller and Semmens, 2002; Bergheim and Brinker, 2003; Patrice Takoukam KE, 2013). The development of closed containment systems for land and coastal

---

Abbreviations: Acronym, Description; BMP, Biomethane potential; COD, Chemical Oxygen Demand; mCHP, (micro) Combined Heat and Power; OLR, Organic loading rate; RAS, Recirculating Aquaculture System; SRT, Solids Retention Time; SVI, Sludge volume index; TN, Total Nitrogen; TS, Total Solids; VFA, Volatile fatty acid; VS, Volatile Solids.

* Corresponding author.
E-mail address: alyssa.joyce@gu.se (A. Joyce).

https://doi.org/10.1016/j.aquaeng.2023.102328
Received 21 November 2022; Received in revised form 3 March 2023; Accepted 9 March 2023

cultivation facilities, referred to as recirculating aquaculture systems (RAS), has been indicative of this paradigm shift towards increased water and nutrient-use sustainability (Fisheries, 2010; Chu et al., 2020). In RAS, suspended solids 60–200 μm are removed from the circulating loop through the use of a rotating drum filter (Dolan et al., 2013; Bao et al., 2019). These solids are recoverable – in contrast to the relatively more open net-pen or flow-through raceways.

Solids management in closed aquaculture systems is essential due to the deleterious direct and indirect effects of suspended solids on finfish health (Bao et al., 2019; Pedersen et al., 2017; Becke et al., 2018; Schumann and Brinker, 2020). There are many types of solids collection systems available for freshwater aquaculture facilities, each with unique advantages and disadvantages (Schumann and Brinker, 2020; van Rijn, 2013). Solids are taken out of the circulating system through diverse, and often facility-specific, collection designs (drum filter, swirl separator, or radial flow settler) at which point there are two terminal options for fish solids: neutralization (e.g., biological degradation and disposal of residual sludge where, if allowed, it is often redirected towards municipal waste treatment streams), or revalorization as part of other bioprocesses (Bao et al., 2019; Davidson and Summerfelt, 2005). The inherent financial costs associated with the collection and removal of fish solids is increasingly incentivizing aquaculturists to explore sludge revalorization, including reselling dewatered wastes as organic fertilizers (Badiola et al., 2018, 2017; Song et al., 2019). Anaerobic biorefineries have been shown to integrate well with aquaculture systems, although their current stage of development suggests that considerable time and innovation is still needed before they become economically viable at a commercial scale (Sawatdeenarunat et al., 2016; Van Den Hende et al., 2015; Chen et al., 2018; Goddek et al., 2018).

These refineries prioritize biogas production through anaerobic digestion as a crude mixture of methane and CO_2 from carbon-rich waste streams. When the methane fraction is purified to remove potential contaminants (nitrogenous species, oxygen, and H_2S), the resulting stream is referred to as biomethane. Varying in size and complexity, combined heat and power (CHP) systems combust biogas or biomethane with the aim of generating heat and electricity (Wang et al., 2019). Recent years have seen the biogas market grow considerably in scale to meet increasing energy demands, while also better achieving sustainability and climate goals (Deublein and Steinhauser, 2011; Ferella et al., 2019; Korberg et al., 2020; Zhu et al., 2019). The potential applications of anaerobic digestion in recirculating aquaculture has been recently reviewed (Choudhury et al., 2022), however, data is limited for aquaculture compared to other agricultural resources, such as livestock farming (Ferella et al., 2019; Korberg et al., 2020; Pellegrini et al., 2015).

The development of cost-effective waste treatment solutions in closed containment systems is critical for the aquaculture industry to reduce discharge, especially as nutrient pollution contributes to eutrophication of local water bodies, which is being increasingly regulated (Xiao et al., 2019). In this context we quantified the stability of biogas production from fish solids over an extended period of time (95 days) with the goal of assessing the capacity of this technology to alleviate waste treatment costs for recirculating aquaculture systems. We furthermore addressed the role of iron in maximizing the biomethane potential. Iron, known to be an essential nutrient for methanogenesis (De Vrieze, 2020; De Vrieze et al., 2013), was supplemented to the aquaculture solids as ferric chloride at a low and high concentration as part of an initial investigation into the iron requirements of the anaerobic community. The choice in concentrations allowed for the dichotomy between a control treatment where iron is a limiting reactant for biological and chemical processes, a situation where iron is sufficient for biological processes only (low iron concentration; 100 mg/L) and a situation where iron is not limiting for biological nor chemical reactions (high iron concentration, 1000 mg/L). Both freshwater and saline (12 g/L) environments were explored in this study to broaden the applicability of the technology to include a wide range of fish-production types. Iron supplementation under saline conditions was explored in a deficiency/excess duality (1000 mg/L addition). The multiplicity of treatments was then contextualized at scales relevant for aquaculture farms, creating a framework for the implications from this study for environmentally and economically sustainable aquaculture solids treatment.

2. Materials and methods

2.1. Inoculum and feedstock

Aquaculture solids were collected from a rainbow trout (*Oncorhynchus mykiss*) recirculating aquaculture system at the Brussel Integrated Greenhouse (BIGH), Belgium. Sludge was collected from the backwash coming from a 10 L rotating drum filter (0.85 μm mesh), corresponding to flow rate of 0.24 m^3/h. The sludge was allowed to settle in the collection containers for at least 24 h, resulting in ca. 2% w/v sludge. Only the settled solids were used in the experiment (Table 1) and were stored at 4 °C until use. An anaerobic inoculum was obtained from a full-scale mesophilic digester provided by Innolab (Belgium) and was used to jumpstart methanogenic activity (Table 1). The inoculum was diluted with tap water to a final concentration of 10 g COD/L.

2.2. Reactor set-up and operation

Anaerobic digestion was carried out in Schott bottles (1 L) filled to 80% with sludge. These Schott bottles were homogenized by gently stirring before sampling but were not otherwise mixed during the experiment. Biogas collection columns were set up for each Schott bottle (tubing connection) to allow for biogas capture and quantification. Thrice weekly, two 5 mL syringes were used to collect biogas for each treatment directly from the column, whereupon samples were immediately processed (see Section 2.3). In this way, sampling represents the average headspace composition produced between any two feeding points. An acid salt bath (HCl solution at pH ≈ 3) stained with methyl orange prevents CO_2 dissolution and escape from the column headspace (Supplementary Figure 1).

Treatments included three freshwater and two simulated saline water treatments consisting of 12 g/kg salt mix (Instant Ocean, USA) added to the aquaculture solids at each feeding. Each treatment was performed in triple biological replicates, and all treatments were kept in a temperature-controlled room (28 °C). The three freshwater treatments included a control (no iron addition), a low iron (100 mg/L) and a high iron (1000 mg/L) treatment, with ferrous chloride added from a stock solution during feeding. The two saline treatments were divided between a control (no iron addition) and high iron (1000 mg/L), likewise added at each feeding.

The anaerobic digesters were operated as a continuous stirred-tank

Table 1
Initial characterization of the settled aquaculture solids and anaerobic inoculum used in this study. FW = fresh weight.

Parameter	Unit	Settled aquaculture solids	Anaerobic inoculum
Chemical Oxygen Demand (COD)	g COD kg^{-1} FW	56.63 ± 1.14	85.31 ± 1.98
Total solids (TS)	g TS kg^{-1} FW	36.64 ± 1.34	59.69 ± 0.84
Volatile solids (VS)	g VS kg^{-1} FW	21.34 ± 1.23	42.37 ± 0.77
VS/TS	%	58.26 ± 1.05	70.98 ± 0.56
COD: VS ratio	-	2.65 ± 0.16	2.01 ± 0.06
Total Kjeldahl Nitrogen (TKN)	g N kg^{-1} FW	8.90 ± 0.38	1.52 ± 0.05
Volatile fatty acids (VFA)	mg COD kg^{-1} FW	1340 ± 131	281 ± 92

reactor with manual shaking in which hydraulics retention time is always the same as the solids retention time as there is no separation of the liquid from the solids. Here, we will refer only to the SRT. The SRT was slowly reduced from 80 days to 20 days over a two-week period with the effect of gradually increasing the organic loading rate (OLR) while allowing for the microbial community in the inoculum to adapt to the aquaculture solids feedstock (Table 2).

Feeding consisted of manually replacing digestate with new substrate (aquaculture solids) as per the volume exchange rate (SRT × interval of days between feeding). To do this, Schott bottles were shaken to homogenize the digestate, then briefly opened to remove digestate and add new feedstock. This was carried out thrice weekly at which time digester pH was measured and biogas potential was assessed (quantification of biogas volume and composition). Once weekly, samples were taken for total and volatile solids measurements, as well as nutrient and volatile fatty acid analysis.

2.3. Analytical techniques

Total and volatile solids were measured using a drying oven (100 °C) and a muffle oven (550 °C) using standard methods (Eaton et al., 2014). Kjeldahl nitrogen was likewise measured using standard methods (Eaton et al., 2014). The COD was measured using the Hach LCK 514 (Hach-Lange, Germany). Volatile fatty acid (VFA) composition was measured by 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) and a flame ionization detector (FID) calibrated for VFA concentration range of 30–1000 mg/L using a nitrogen gas carrier (Andersen et al., 2013). The COD-adjusted volatile fatty acid values were calculated by multiplying the measured acid concentration by the ratio of the required oxygen for combustion to acid molecular weight (e.g., 1.07 for acetic acid).

A 2 mL syringe was used for CH_4 and CO_2 analysis (two syringes per treatment), with sampling taken from a gas sampling tube (Lenz, Germany). The gas phase composition was analyzed with a Compact GC (Global Analyser Solutions, Breda, Netherlands), equipped with a Molsieve 5 A pre-column and Porabond column (CH_4, O_2, H_2, and N_2) as well as a Rt-Q-bond pre-column and column (CO_2, N_2O, and H_2S). Concentrations of gases were determined by means of a thermal conductivity detector, with detection limits for each gas range from 0.05% v/v to 100% v/v. Anion and cation concentrations were measured using ion chromatography (Metrohm, Switzerland) using a Metrosep A Supp 5–150/4.0 (61006520) column. Detection limits for ions ranged between 0.05 and 100 mg ion/L.

The sludge volume index was calculated based on the height of settled sludge inside the Schott bottle observed immediately prior to feeding. As 800 mL of sludge was present per liter digester, index values were adjusted for one liter of sludge.

Table 2
Description of the adjustment protocol to acclimate the inoculum to the aquaculture solids feedstock.

Period (d)	Target SRT (d)	Organic loading rate (OLR) (g COD/L digester * d)	Success criteria
0 – 7	80	0.57	No significant signs of instability.
8 – 14	40	1.13	The pH should be > 7 without adjustment.
15 – 25	20	2.27	The pH should stabilize to within 0.2 units.
25 – 95	20	2.27	The pH should be > 7, biogas production will determine which treatment is more successful.

2.4. Biogas estimation

From the GC results, the percentage CH_4 as part of the headspace gas composition was calculated by:

$$\%CH_4 = 100 * \frac{CH_4}{CO_2 + CH_4}$$

The volume of CH_4 produced per liter reactor at standard temperature and pressure was calculated by:

$$Volume_{CH_4} = \%CH_4 * Volume_{biogas,daily} \frac{273K}{301K}$$

The CH_4 yield was calculated from the volume of CH_4 produced per liter reactor divided by the volume of feed sludge added (L) multiplied by its VS or COD (g/L sludge) content. This results in the methane yield were related to the initial COD of the sludge (L CH_4 / g COD) or to volatile solids (L CH_4 / g VS).

$$CH_{4yield,VS} = \frac{Volume_{CH_4}}{\frac{g\ VS}{L\ sludge} * Volume_{feed}}\ CH_{4yield,COD} = \frac{Volume_{CH_4}}{\frac{g\ COD}{L\ sludge} * Volume_{feed}}.$$

Based on these yield products, the annual energy and electricity production was estimated assuming a CHP electricity conversion efficiency of 40% and a methane to electricity conversion of 1 m^3 $CH_4 = 10$ kWh and calculating the MJ energy produced as 3.6 $MJ = 1$ kWh (Deublein and Steinhauser, 2011; Szarka et al., 2013). The electricity production is then calculated by:

$$Electricityyield \frac{KWh}{Lsludge} = CH_{4_{yield,VS}} * \frac{10kWh}{1m^3 CH_4} * 40\%efficiency * \frac{gVS}{Lsludge}$$

The economic sustainability was worked out from the minimal biogas production volume as outlined by Cucchiella et al. (2019), and typical rainbow trout farm feed conversion yields of 1.1 – 1.36 kg Feed/kg biomass, plugged into a calculator derived from the above formulae.

2.5. Data analysis

Data analysis was performed in Excel and R version 4.0.3, with figures generated entirely in R. Relevant libraries used include: ggplot2 (Wickham et al., 2016), ggpubr (Kassambara and Kassambara, 2020), dplyr (Wickham et al., 2014), tidyverse (Wickham, 2017), tidyr (Wickham and Wickham, 2017), cowplot (Wilke et al., 2019), grid (Murrell, 2002), and gridExtra (Auguie et al., 2017). A paired t-test was used to confirm the significance of results wherever stated in the text, with normality and homoscedasticity determined through Shapiro–Wilk test and Bartlett's test, respectively. All significance tests were performed in Microsoft Excel.

3. Results

3.1. Yields and energy production rates

The primary focus of this study was to determine the capacity for long-term biomethane production from aquaculture solids under optimized conditions for methanogenesis. This included an inoculum from a working anaerobic digester provided the starter community, a solids retention time based on the influent carbon and nitrogen loading rates to align with literature values for maximal methanogen growth (Bischofsberger et al., 2005; Metcalf et al., 1991). The temperature set point of 28 °C provides a preferential environment for methanogenesis over aceto- and acidogenesis while minimizing heating costs. In this study, we evaluate the capacity of iron supplementation to additionally bolster methanogenesis of the aquaculture solids.

The yield of methane produced per volume of incoming solids is the primary indicator of this performance, here displayed in terms of volatile solids (Fig. 1A) and chemical oxygen demand (Fig. 1B). The saltwater treatments performed worse than freshwater treatments, with the

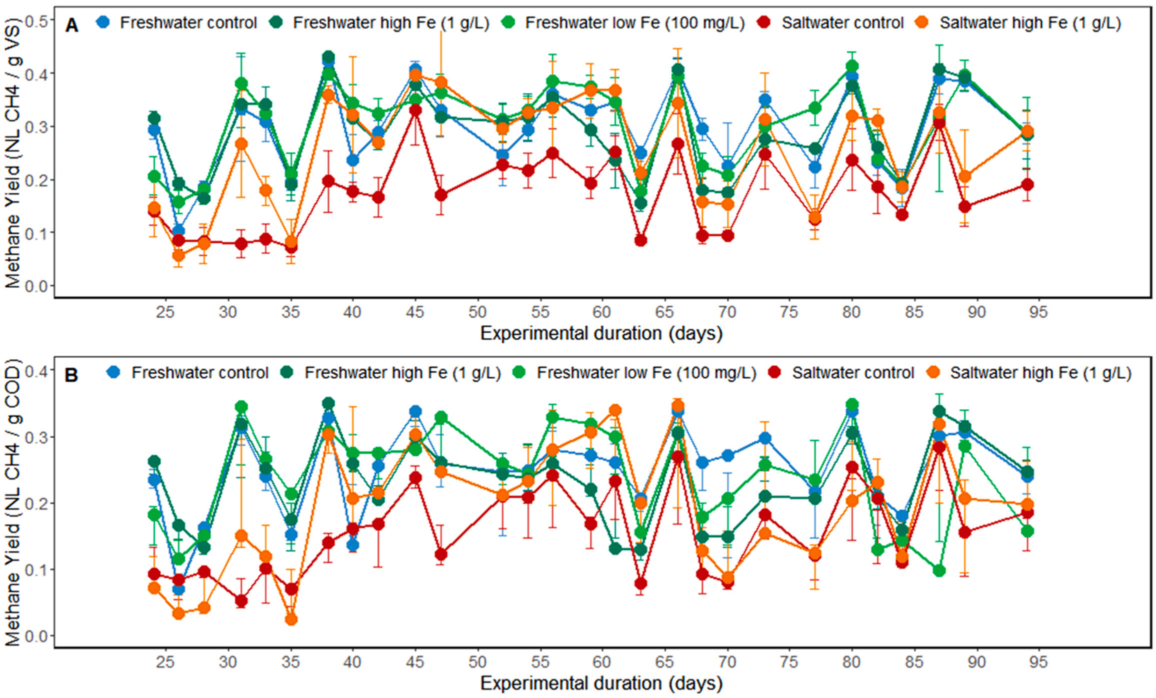

Fig. 1. Methane yields per liter sludge, based on volatile solids (VS) (A) and chemical oxygen demand (COD) (B).

saltwater control lagging furthest behind ($p < .001$). With the exception of a dip around day 70, a yield in the range of 0.3 – 0.4 NL CH_4 per g VS was typical for all other treatments over the experimental duration. Taken per gram COD the yield was less, around 0.2 – 0.3 NL CH_4/ g COD, corresponding to a percentage yield of 57–86% of the total biomethane potential (Fig. 2 A, B). The percentage yield was calculated as the realized BMP as a percentage of the theoretical BMP based on the volatile solids. In other words, it is a reflection on the efficiency with which the feedstock can be converted into methane.

The percentage of methane as a component of the biogas was consistent across all treatments. Gas chromatography analyses suggest that CH_4, and CO_2 are the main gas components, with no H_2S or N_2O detected. Most treatments fluctuated between 70% and 80% methane purity over the duration of the experiment (Fig. 2 A). The saltwater control achieved a lower methane production rate than other treatments ($p < .001$). Nonetheless, rate differences across treatments favored freshwater treatments and the iron supplemented saline treatment compared to the saline control treatment ($p < .001$ for each comparison, respectively) (Fig. 2B).

Methane production rates translate proportionally into electricity

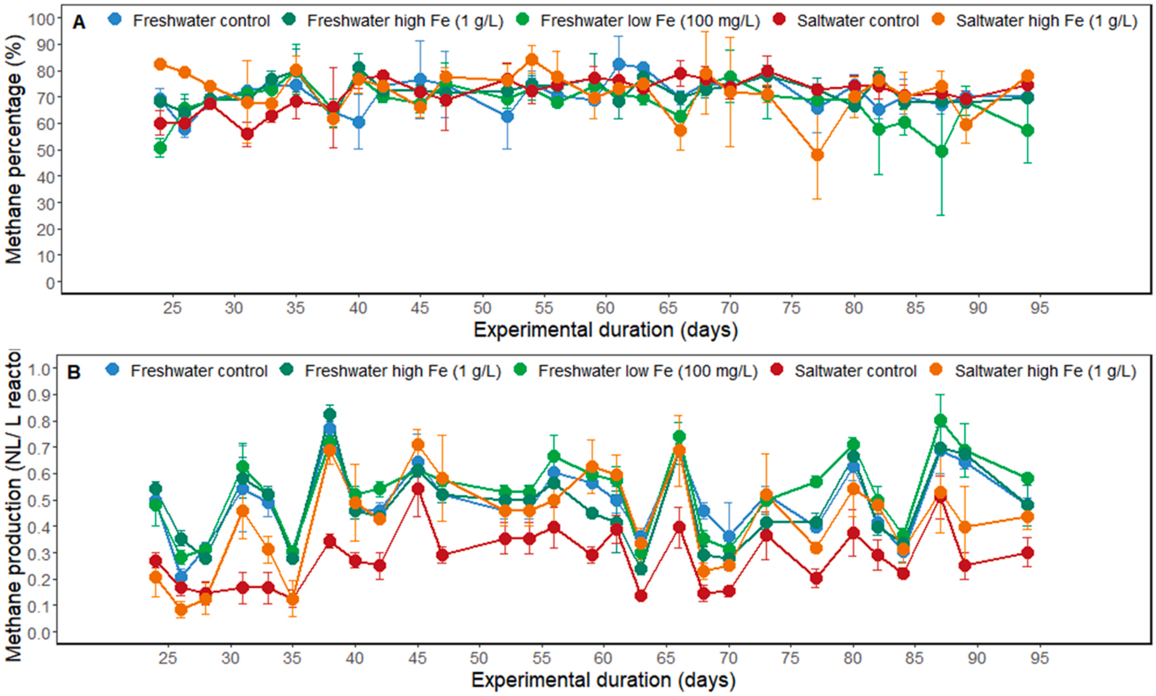

Fig. 2. (A) Methane purity in the biogas across treatments. (B) Volume of methane produced across treatments, normalized per liter reactor at STP.

and energy production rates (Fig. 3A, B). With the exception of the saltwater control performing worse than other treatments ($p < .001$), a range of 0.02–0.035 kWh/ L reactor of electricity and 0.05–0.12 MJ/ L reactor was typical for most of the experimental duration.

Backyard farms will likely not warrant investing in their biomethane potential through the purchase of a combined heat and power system. However, even small industrial scale aquaculture facilities (1 000 tons annually) could produce 88.14 kWh per day, assuming an electricity generation of 0.0275 kWh/ L reactor and a feed conversion ratio of 1.1 for rainbow trout weighing on average 3.5 kg and growing at 16 °C.

3.2. Long-term stability of the anaerobic digester

The pH fluctuated over the duration of the study (Fig. 4), however, each treatment remained within a range of ± 0.5. The freshwater treatments maintained significantly ($p < .001$) higher pH values (near pH = 7), compared to saline water treatments, suggesting the presence of an environment conducive to methanogenesis. Saltwater treatments regularly skirted along the lower tolerable range for methanogenesis (ca. pH 6.5), however, this did not result in a reduction in biogas production compared to other treatments.

The volatile fatty acid accumulation was highest in the saltwater control treatment, with longer chains ($\geq$C3) accumulating significantly ($p < .001$) more than in other treatments. The methanogenesis efficiency was similar across treatments with minimal accumulation of VFAs $>$ C5 (Fig. 5A). Iron supplementation apparently fortified pH under saline conditions, however, the effect likely requires only low ($\leq$ 100 mg/L) iron concentrations to meet methanogen demand, as no significant difference ($p = .104$) was observed between the two freshwater iron treatments. The ratio of acetate to total VFA ratio over time (Fig. 5B) indicates the acetate utilization efficiency by the microbial community. Despite a higher total VFA load compared to other treatments, the saline control did not deviate significantly ($p = .284$).

The IC anion and cation analyses revealed similar patterns across treatments, with the clearest distinctive factor being the presence of the sea salt mixture (Na^+, Cl^-, Mg^{2+}, Ca^{2+}). Of the nitrogenous compounds, ammonium was initially high, but decreased to a stable concentration at ca. 250–500 mg/L after 20 days of operation. This is likely due to a shift in the feedstock composition compared to the initial inoculum. Other ions were detected at stable concentrations in the digestate for the entire duration of the experiment: sulphate 3.3 ± 2.1 mg/L, phosphate 58.5 ± 13.2 mg/L, and potassium 227.0 ± 37.1 mg/L (Supplementary Figure 2).

Total solids were highest for saline treatments (Fig. 6A). Volatile solids remained similar across all treatments (Fig. 6B), as similarly reflected in the TS/VS ratio (Fig. 6C). Important to note is the high variability during the start-up period (days 0 – 25), which is typical in anaerobic digesters as the microbial community adapts to the increasing SRT. Total and volatile solids were taken from shaken digesters, meaning they comprised both settled and soluble particles. While a constant TS/VS ratio suggests the microbial activity was maintained at the same rate throughout the study, looking at the VS as a percentage of TS suggests that an accumulation of undigested solids occurred over time. While at the beginning of the study this value was greater than 50%, the average dropped below 50% towards the end of the study, however the high standard deviation limits our capacity to draw definitive conclusions from these data (Fig. 6D).

While the sludge volume index (SVI) was only measured during the last stage of experimental period, the divergences were consistent across 25 days of observation (Fig. 7). It was hypothesized that a higher SVI would be observed in saline treatments owing to the higher ionic stabilization of the solids and floc and decreased microbial activity, however this was not the case ($p = .132$ between freshwater and saline controls). While low iron supplementation significantly reduced the SVI ($p < .001$ between the 100 mg/L iron treatment and the control), it did not appear to reduce the SVI significantly at the higher concentration (1000 mg/L Fe addition) under freshwater ($p = .367$) nor saline conditions ($p = .063$).

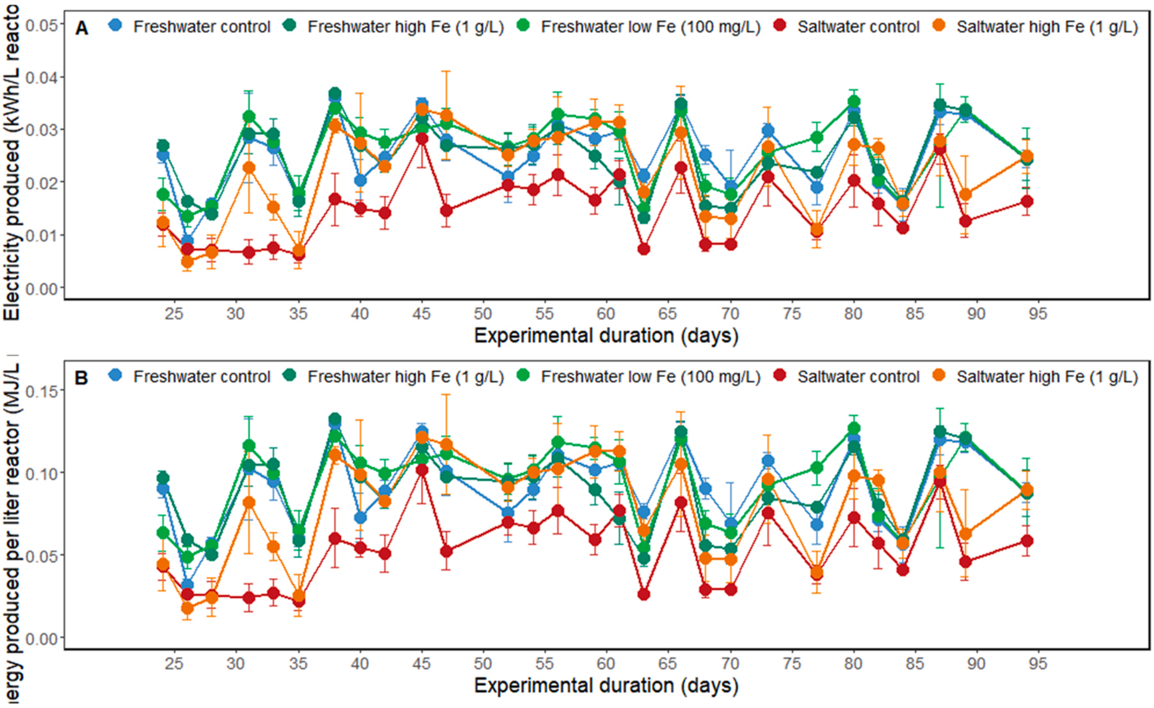

Fig. 3. Estimated electricity production per liter reactor (A) and energy yield per liter reactor (B).

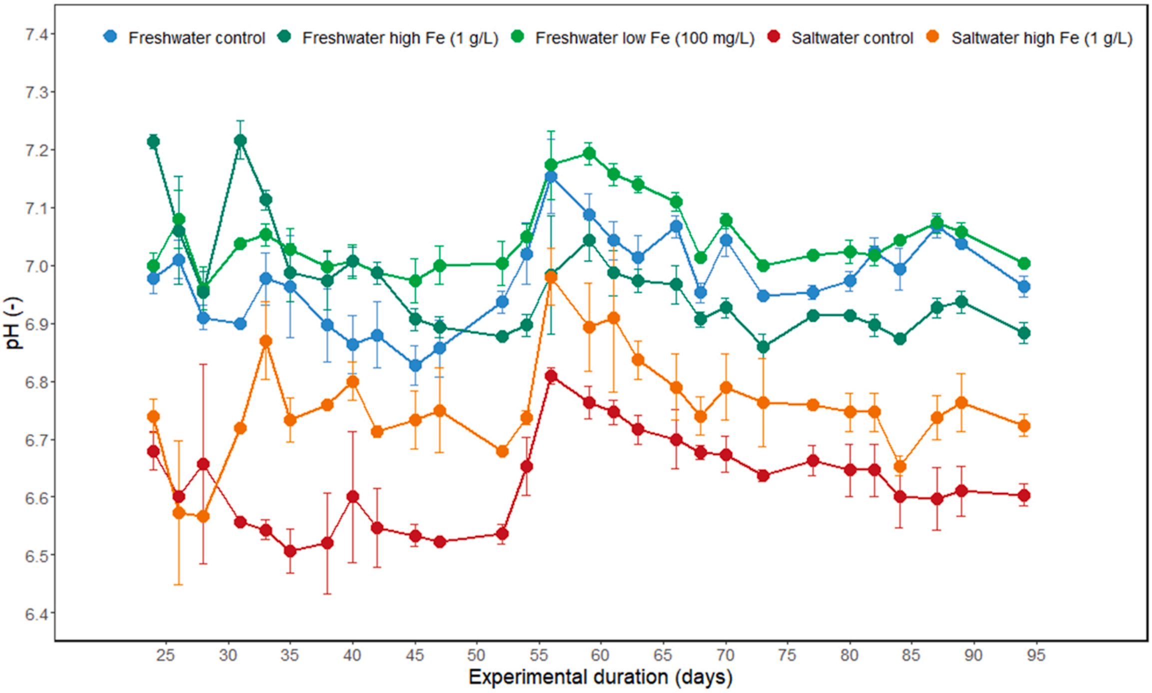

Fig. 4. Consistency of pH across treatments.

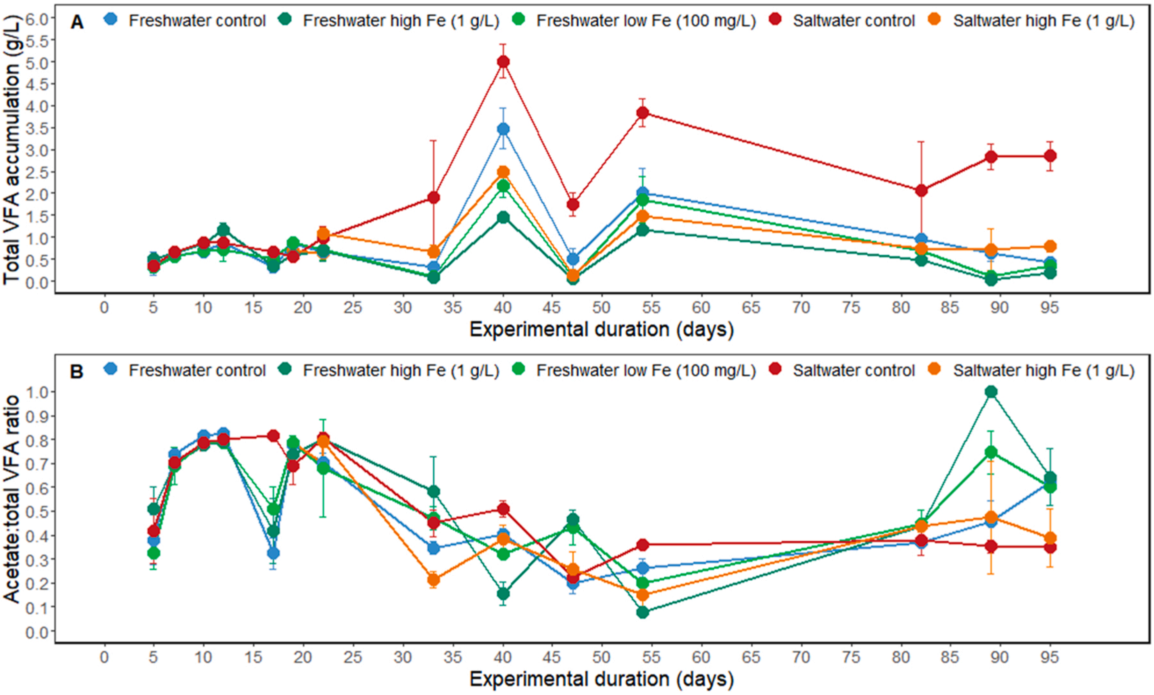

Fig. 5. Total COD-adjusted volatile fatty acid accumulation over the experimental duration (A). Ratio of acetate to total VFA (B).

4. Discussion

4.1. Both saline and freshwater anaerobic digestion of solid aquaculture waste results in stable biogas production

In this study, we demonstrated the feasibility for long-term biogas production in simulated freshwater and saltwater anaerobic digestion systems using rainbow trout solids as the feedstock. The novelty of the approach in this study is its ability to incentivize responsible solids management through the potential for electricity generation, applicable in any aquaculture farm where fish solids are selectively removed from the water column. Using pH as the most immediate signal for reactor health, it is evident that freshwater conditions were less stressful on the methanogenic community, which is further supported by the volatile fatty acid profile in which fewer C3 and longer VFAs are present. This suggests that under saline conditions, the methanogenic community was partially inhibited from converting acetate (C2) into methane. The consistency of volatile solids measurements indicated a similar amount

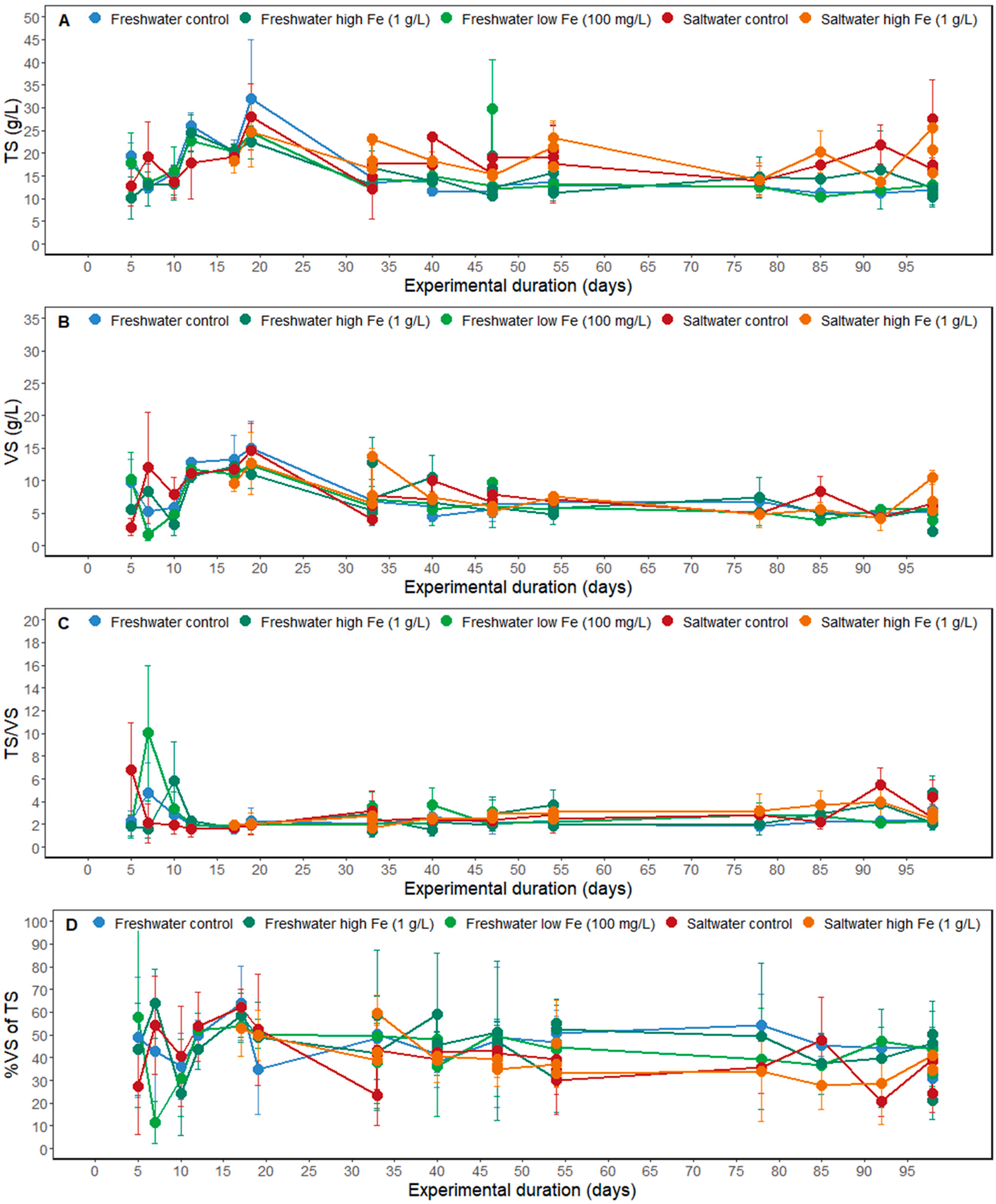

Fig. 6. Evolution of total solids (TS) (A), volatile solids (VS) (B), as well as their ratio (C) over time, and (D) the percentage of VS as a portion of TS.

of organic matter across treatments, indicative of the metabolically active fraction of the microbial community. With the exception of some outliers, the TS:VS ratio remained between 1.5 and 3, resulting in a %VS of TS between 40% and 50%. As the %VS relates to the degree of microbial activity in the digester, it is worth noting the consistency of these results with municipal wastewater treatment systems (Metcalf et al., 1991; Turovskiy and Mathai, 2006). Furthermore, while regular variations were noticeable across the experimental duration, these trends affected all treatments simultaneously. The fed-batch model used in this study can create a feast-famine alternation across the three days between each feeding, possibly explaining the observed fluctuations.

4.2. The contribution of biogas to the economic and sustainable picture depends on the scale of the aquaculture farm

One of the key goals of this work was to gauge the practicality of biogas collection for aquaculture farms. The lab-scale reactor experiments performed in this study enable the estimation of the electrical and heat potential from fish solids. While previous studies on the biomethane potential (BMP) from saline aquaculture solids achieved similar yields as observed here (0.279–0.3 NL/g VS compared to 0.2–0.4 NL/g VS in this study) (Chiumenti et al., 2021; da Borso et al., 2021), the novelty added through the current investigation is in determining the long-term stability of the anaerobic digestion microbial community and potential energy yield. Methane yields fluctuated for each treatment

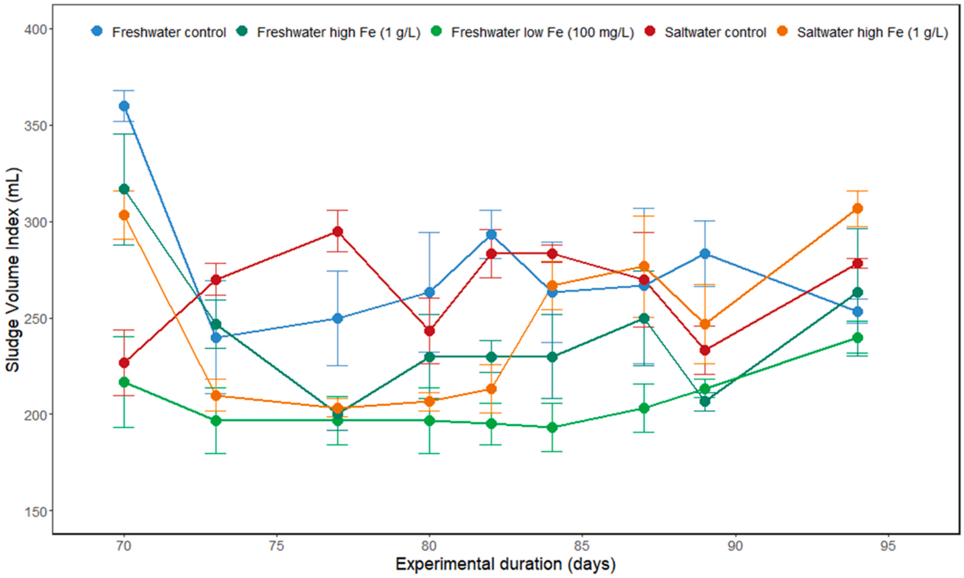

Fig. 7. Sludge volume index measurements across treatments.

ranged generally between 0.2 and 0.4 L CH_4/ kg VS with a variation of ≈ 0.2 NL CH_4 / g VS. These yields are comparable to other agricultural waste streams, such as cow or sheep manure (Eggeling et al., 1986). Importantly, methane purity was generally higher than literature values: 60–80% CH_4 in this study, compared to 65% for cow manure (Eggeling et al., 1986) and 60% previously reported for freshwater aquaculture solids (Ndiaye et al., 2020). We attribute this observation to a few factors: a relatively optimized anaerobic digestion design compared to previous studies on BMP generation from aquaculture solids (temperature, pH, iron addition, the use of the inoculum from a BMP anaerobic digester, ideal retention time and volumes based on feedstock characteristics) and as well a homogenous, nitrogen-rich feedstock lacking inhibitive products (as may occur in wastewater treatment). Considering that modern CHP systems run at around 40% electrical efficiency and around 45% heat efficiency (Deublein and Steinhauser, 2011; Szarka et al., 2013), these results suggest that establishing a biogas-generating waste treatment system could address operational and maintenance costs of an aquaculture facility (Scarlat et al., 2018).

The reduced biogas volume produced by the saline control treatment emphasizes the importance of iron supplementation, at least for saline aquaculture systems. Methanogens use iron as an electronic shuttle, allowing them to prevent interference from the high environmental ionic load created by the saline environment. Typically, iron-based coagulants result in a denser sludge than other common coagulants (e.g., aluminum) (Brandt, 2017), corroborated by the sludge volume index results observed in this study. One study investigating the use of inorganic coagulants ($FeCl_3$ and polymeric aluminum sulfate) for pretreatment prior to BMP from brackish aquaculture solids found an improved yield in the iron but not aluminum treatments (Zhang et al., 2014). Aluminum exposure has likewise been associated an increased risk of Alzheimer's disease, limiting the downstream applications for aluminum-treated solids (Ferreira et al., 2008; Jansson, 2001). As such, $FeCl_3$ is both a safer and more effective coagulant to augment sludge density and settling efficiency.

Investing in the CHP system represents a critical consideration vis à vis the return-on-investment for aquaculture facilities. For large facilities, solids disposal is as great a concern as is reducing operational costs such as electricity and heat. The process of collecting biomethane from organic waste has become widespread over the past two decades for large-scale agricultural and intensive animal husbandry facilities within the European Union (Zhu et al., 2019; Hamelin et al., 2021), with biogas deployment schemes elsewhere around the world growing at a slower rate (Outlook, 2020). A recent case study in Italy determined that for a biogas plant to be profitable, a minimum production level of 200 kWh is necessary (Cucchiella et al., 2019). The US energy market is significantly more privatized and as such, there are wider price fluctuations both geographically and temporarily. Recent federal incentives for biogas (US Federal Energy Regulatory Commission Order 2222 (FERC-2222)) make the operation more attractive, especially for large facilities (Erickson et al., 2023). The ability to accumulate biogas before combustion could allow facilities to time their electricity generation with peak demand hours, however the economics of this process will need to be worked out for a given operation. Naturally, further capital investments and technical innovation in the sector have the potential to make biogas production at lower volumes more profitable in the near future (Ferella et al., 2019). To produce 200 kWh daily, we estimate needing a rainbow trout farm size producing 42 T annually based on the electricity yields and methane production rates from this study. For smaller aquaculture farms (50–150 kWh; equivalently 10–32 T production), the advent of smaller scale electricity generation units, such as micro combined heat and power (mCHP) systems, may provide a more practical solutions (Maghanki et al., 2013; Hammond and Titley, 2022). The smallest of facilities may opt for makeshift options, such as the Mini Methane Generator Project (Howe, 2017). Ultimately, most energy in CHP goes towards heat generation, with possible outputs including pressurized steam, hot air, and hot water.

Aquaculture solids contain a majority fraction of mineral nutrients compared to those dissolved in the water column (Schumann and Brinker, 2020). While optimizing the remineralization of nutrients was not a priority in this study, several trends could be observed. Firstly, virtually all nitrogenous species were reduced to ammonia. In the reducing environment of the anaerobic system, proteinic nitrogen is liberated during the decomposition of organic matter. Ammonium toxicity would not be a concern as the pH never exceeded 8, however ammonia concentrations in the digester were low (stabilizing around 500 mg/L reactor) compared to other anaerobic digestion feedstocks (Yenigün and Demirel, 2013). Simultaneously, mineral nutrients are liberated from the colloidal matrices within the aquaculture solids. While this was outside the scope of this study, there is clearly a possibility for further treatment (i.e., aeration of the reactor digestate), which could allow the effluent stream to be applied to hydroponic plant cultivation as we have investigated previously (Lobanov et al., 2021).

One element of concern in anaerobic digestion is sulfur, due to its propensity to form a noxious gas. In our study, H_2S was not detected in the reactor headspace nor were sulfate concentrations in the digestate exceptionally high (remaining below 10 mg/L reactor). Sulfate levels were higher in the saline treatments owing to the contribution of the Instant Ocean salt mix. Previous studies claimed that the use of an inoculum derived from anaerobic processes improved methane yield while reducing H_2S production when added to aquaculture solids (Li et al., 2011), however, our results suggest that the H_2S is instead precipitated chemically by cations in the digestate. No significant difference was detected in the soluble sulfate concentrations between iron versus control treatments. Future work on the solids treatment system described in this study will need to review the potential for H_2S production, as there is evidence from the literature that it is likely produced as a byproduct during this process (Letelier-Gordo et al., 2020a, 2020b).

4.3. Iron addition stabilizes biogas production under saline conditions

High salinity typical to full strength seawater (35 g/L) has been previously claimed to be a cause of low methane yields from aquaculture solids (Zhang et al., 2013). While full-strength seawater was not investigated in this study, our results demonstrate that methane production from saline water at 12 g/L is similar to freshwater yields. Saline anaerobic digestion of the aquaculture solids remains stable well after the effect of the inoculum would have diminished, suggesting that the methanogenic community of the inoculum successfully colonized the new digester conditions. Crucially, the addition of iron to the saltwater sludge seems to have alleviated salt stress compared to the control, as evidenced by the pH stability. Under freshwater conditions, low iron supplementation (100 mg/L) as well as high (1000 mg/L) iron supplementation significantly improved methane yield compared to the freshwater control ($p < .001$ for each comparison, respectively). However, methane production rates were not higher in the iron supplemented freshwater sludge at low ($p = .195$) nor high ($p = .790$) concentrations.

Although it was hypothesized that the addition of iron would help coagulate the aquaculture solids under saline conditions, the total solids concentration was not significantly lower in the iron-supplemented saline treatment compared to the saltwater control ($p = .995$), nor was the sludge volume index significantly decreased ($p = .063$). However, the saline control treatment yielded significantly ($p < .001$) less methane than the iron supplemented saline treatment. These discrepancies are visible in the volume of biogas produced (production rate), although they are not reflected in the percentage of methane in the biogas ($p = .422$ between saltwater control and iron-supplemented saltwater treatments). Biogas purity was likewise similar between freshwater and saltwater control treatments ($p = .481$), suggesting that the methanogenic community was able to maintain its niche under the higher ionic conditions. These observations suggest that the salinity of the sludge does not exclude its use for biogas production. A stressed methanogenic community in the saline control treatment was likewise portrayed through the volatile fatty acid profile. While other treatments did not have a significant accumulation of VFAs longer than C3, the saltwater control had consistently higher VFA concentrations up to C8. The fact that this backlog was alleviated through iron supplementation further lends credence to the notion that iron may improve the resilience of the methanogen community to exogenous perturbations. A previous study investigating the use of $FeCl_3$ as a coagulant for a brackish aquaculture solids digestion system indicated an inhibitory effect when using 6 g/L $FeCl_3$ (Zhang et al., 2014) – a concentration much higher than those used in this study. This discrepancy might explain why our study did not observe any inhibitory effect. Rather, we encourage further research to explore lower $FeCl_3$ concentrations to determine the minimal effective concentration (i.e., whether enough iron should be present to satisfy the biological demand of methanogens or is a higher concentration needed to chemical precipitate inhibitory elements such as sulfur).

4.4. Limitations and future outlook

The use of biological replicates allows for a better investigation into the variability of the microbial communities as they adapt to the feedstock. The biogas yield and production calculations incorporate measurable inputs from all three biological replicates with the result that the standard deviations in this study were wide. Biological variation is always present; however, we believe that many of these variations will be resolved at larger scales as aquaculture solids entering in an active facility – solids entering the treatment system – will be consistently fresh, and an automated pumping system will regularize the handling process. The effect of variable temperature – even if the digester itself is maintained at 28 °C – may create seasonal variations in the microbial community as described elsewhere (Resende et al., 2016; Kandhro et al., 2022). Nonetheless, trends are visible for treatment groups (freshwater vs. saltwater) in a way consistent across all parameters. To simulate a saline sludge input, Instant Ocean was added to the incoming aquaculture solids to achieve a concentration of 12 g/L. However, this resulted in the digester reaching a sodium concentration of between 3.5 and 4 g/L (supplementary figure 2) over the course of the experiment due to the low volume exchange rate. While biogas yield was similar for the freshwater and brackish water treatments in this study, the effect of salinity will require further study. Namely, the digestate salinity should be raised to higher concentrations (e.g., 6, 12, and 35 g/L) to map out the influence in BMP. Section 4.2 describes the complex interplay between sulfur, biomethane production, and iron requiring further research - especially under saline conditions where sulfur concentrations are considerably higher.

Bringing this experiment to the next technology readiness level will require pilot and industrial scale studies, as well as measurements over a longer period. There are several tiers of aquaculture facilities as described in Section 4.2, and, likely, the profitability model differs tremendously based on size and usage (aquaculture vs. aquaponic farms).

5. Conclusion

The investigation into the biogas potential from aquaculture solids reveals promising results both in terms of biogas yields achieved and the long-term process stability. We also provide initial estimates for electricity and energy yields at an SRT of 20 days. Advantages of this approach are the low operational costs, the stability of the biogas production, and the possibility to recuperate investment/operational costs through electricity generation. Globally this study indicates a high consistency in biomethane composition (%CH_4) across treatments, suggesting that while the methanogenic community may be suppressed under saline conditions or in the absence of sufficient iron, it is not outcompeted. Iron supplementation was found to be useful under saline but not freshwater conditions. However, the effect appears to improve the rate of methane production but not the yield. Hence, it is possible to change the paradigm of waste treatment from a costly burden into a cost-alleviating activity with direct implications for industrial stakeholders in aquaculture.

Funding sources

Support for this project was generously provided by a Swedish Research Council FORMAS grant (Joyce 2020/0003), AquaExcel (AE1600012) and the Adlerbertska Foundation (travel scholarship for Victor Lobanov).

CRediT authorship contribution statement

Victor Lobanov: Conceptualization. Victor Lobanov and Jo de Vrieze: developement of experimental design and analysis of data. Victor Lobanov: Writing- Original draft preparation, investigation. Alyssa

Joyce, Jo de Vrieze: Supervision. Joe Pate, Victor Lobanov, Alyssa Joyce: Writing- Reviewing and Editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgements

We thank the support staff, Tim Lacoere and Greet van de Velde, at the University of Gent (Belgium) for their assistance in performing GC and IC measurements during this study. We thank the Brussels Integrated Greenhouse (BIGH), specifically François Latrille, for their provision of the aquaculture solids used in this study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquaeng.2023.102328.

References

Andersen S., Gildemyn S., Hennebel T., Coma M., Rabaey K., editors. Electrolytic membrane extraction enables fine chemical production from biorefinery side streams. Francqui symposium on Recent Advances in Microbial and Enzymatic Electrocatalysis; 2013.

Auguie B., Antonov A., Auguie M.B. Package 'gridExtra'. Miscellaneous Functions for "Grid" Graphics. 2017.

Badiola, M., Basurko, O.C., Gabiña, G., Mendiola, D., 2017. Integration of energy audits in the Life Cycle Assessment methodology to improve the environmental performance assessment of Recirculating Aquaculture Systems. J. Clean. Prod. 157, 155–166.

Badiola, M., Basurko, O., Piedrahita, R., Hundley, P., Mendiola, D., 2018. Energy use in recirculating aquaculture systems (RAS): a review. Aquac. Eng. 81, 57–70.

Bao, W., Zhu, S., Jin, G., Ye, Z., 2019. Generation, characterization, perniciousness, removal and reutilization of solids in aquaculture water: A review from the whole process perspective. Rev. Aquac. 11 (4), 1342–1366.

Bartelme, R.P., McLellan, S.L., Newton, R.J., 2017. Freshwater recirculating aquaculture system operations drive biofilter bacterial community shifts around a stable nitrifying consortium of ammonia-oxidizing Archaea and Comammox Nitrospira. Front Microbiol 8, 101.

Becke, C., Schumann, M., Steinhagen, D., Geist, J., Brinker, A., 2018. Physiological consequences of chronic exposure of rainbow trout (Oncorhynchus mykiss) to suspended solid load in recirculating aquaculture systems. Aquaculture 484, 228–241.

Bergheim, A., Brinker, A., 2003. Effluent treatment for flow through systems and European environmental regulations. Aquac. Eng. 27 (1), 61–77.

Bischofsberger W., Dichtl N., Rosenwinkel K.-H., Seyfried C.-F., Böhnke B. Anaerobtechnik: Springer-Verlag; 2005.

Brandt, M., 2017. Chapter 8–Storage, Clarification and Chemical. In: Treatment, M.J., Brandt, K.M., Johnson, A.J., Elphinston, D.D., Ratnayaka (Eds.), Twort's Water Supply. Butterworth-Heinemann, Boston, USA.

Brown, M.N., Briones, A., Diana, J., Raskin, L., 2013. Ammonia-oxidizing archaea and nitrite-oxidizing nitrospiras in the biofilter of a shrimp recirculating aquaculture system. FEMS Microbiol Ecol. 83 (1), 17–25.

Cerozi, B.S., Fitzsimmons, K., 2017. Phosphorus dynamics modeling and mass balance in an aquaponics system. Agric. Syst. 153, 94–100.

Chen, P., Anderson, E., Addy, M., Zhang, R., Cheng, Y., Peng, P., et al., 2018. Breakthrough technologies for the biorefining of organic solid and liquid wastes. Engineering 4 (4), 574–580.

Chiumenti A., Owono B.O., Fait G., Mainardis M., Goi D., Stella E., et al., editors. Anaerobic Digestion of Brackish and High Salinity Aquaculture Sludges. 2021 ASABE Annual International Virtual Meeting; 2021: American Society of Agricultural and Biological Engineers.

Choudhury, A., Lepine, C., Witarsa, F., Good, C., 2022. Anaerobic digestion challenges and resource recovery opportunities from land-based aquaculture waste and seafood processing byproducts: A review. Bioresour. Technol., 127144

Chu, Y., Wang, C., Park, J., Lader, P., 2020. Review of cage and containment tank designs for offshore fish farming. Aquaculture, 734928.

Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W., 2007. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270 (1–4), 1–14.

Cucchiella, F., D'Adamo, I., Gastaldi, M., 2019. An economic analysis of biogas-biomethane chain from animal residues in Italy. J. Clean. Prod. 230, 888–897.

da Borso, F., Chiumenti, A., Fait, G., Mainardis, M., Goi, D., 2021. Biomethane potential of sludges from a brackish water fish hatchery. Appl. Sci. 11 (2), 552.

Davidson, J., Summerfelt, S.T., 2005. Solids removal from a coldwater recirculating system—comparison of a swirl separator and a radial-flow settler. Aquac. Eng. 33 (1), 47–61.

De Vrieze, J., 2020. The next frontier of the anaerobic digestion microbiome: from ecology to process control. Environ. Sci. Ecotechnol., 100032

De Vrieze, J., De Lathouwer, L., Verstraete, W., Boon, N., 2013. High-rate iron-rich activated sludge as stabilizing agent for the anaerobic digestion of kitchen waste. Water Res. 47 (11), 3732–3741.

Deublein, D., Steinhauser, A., 2011. Biogas from Waste and Renewable Resources: an Introduction. John Wiley & Sons.

Dolan, E., Murphy, N., O'Hehir, M., 2013. Factors influencing optimal micro-screen drum filter selection for recirculating aquaculture systems. Aquac. Eng. 56, 42–50.

Eaton A.D., Clesceri L.S., Greenberg A.E. Standard methods: for the examination of water and wastewater2014.

Eggeling, G., Mackensen, G., Sasse, L., 1986. Production and utilization of biogas in rural areas of industrialized and developing countries. Rev.

Erickson, E.D., Tominac, P.A., Zavala, V.M., 2023. Biogas production in United States dairy farms incentivized by electricity policy changes. Nat. Sustain. 1–9.

Ferella, F., Cucchiella, F., D'Adamo, I., Gallucci, K., 2019. A techno-economic assessment of biogas upgrading in a developed market. J. Clean. Prod. 210, 945–957.

Ferreira, P.C., Piai, Kd.A., Takayanagui, A.M.M., Segura-Muñoz, S.I., 2008. Aluminum as a risk factor for Alzheimer's disease. Rev. Lat. Am. De. Enferm. 16, 151–157.

Fisheries, F., 2010. Aquaculture Department. The State of World Fisheries and Aquaculture. FAO, Rome.

Gartmann F., Schmautz Z., Junge R., editors. Influence of pH change on the phosphorus cycle in aquaponics. 9th International Phosphorus Workshop (IPW9), Zurich, 8–12 July 2019; 2019: ZHAW Zürcher Hochschule für Angewandte Wissenschaften.

Goddek, S., Delaide, B.P.L., Joyce, A., Wuertz, S., Jijakli, M.H., Gross, A., et al., 2018. Nutrient mineralization and organic matter reduction performance of RAS-based sludge in sequential UASB-EGSB reactors. Aquacult. Eng. 83, 10–19.

Guerdat, T.C., Losordo, T.M., Classen, J.J., Osborne, J.A., DeLong, D., 2011. Evaluating the effects of organic carbon on biological filtration performance in a large scale recirculating aquaculture system. Aquac. Eng. 44 (1), 10–18.

Hamelin, L., Møller, H.B., Jørgensen, U., 2021. Harnessing the full potential of biomethane towards tomorrow's bioeconomy: A national case study coupling sustainable agricultural intensification, emerging biogas technologies and energy system analysis. Renew. Sustain. Energy Rev. 138, 110506.

Hammond, G.P., Titley, A.A., 2022. Small-scale combined heat and power systems: the prospects for a distributed micro-generator in the 'net-zero'transition within the UK. Energies 15 (16), 6049.

Holl, C.M., Glazer, C.T., Moss, S.M., 2011. Nitrogen stable isotopes in recirculating aquaculture for super-intensive shrimp production: tracing the effects of water filtration on microbial nitrogen cycling. Aquaculture 311 (1–4), 146–154.

Howe M. DIY Methane Generator: Fantastic Farms; 2017 [Available from: https://small-farm-permaculture-and-sustainable-living.com/methane_generator/.

Hussain, T., Verma, A., Tiwari, V., Prakash, C., Rathore, G., Shete, A., et al., 2014. Optimizing koi carp, Cyprinus carpio var. koi (Linnaeus, 1758), stocking density and nutrient recycling with spinach in an aquaponic system. J. World Aquac. Soc. 45 (6), 652–661.

Jansson, E.T., 2001. Aluminum exposure and Alzheimer's disease. J. Alzheimer'S. Dis. 3 (6), 541–549.

Kandhro, B., Sahito, A.R., Nixon, J.D., Uqaili, M.A., Mirjat, N.H., Harijan, K., et al., 2022. Seasonal variation in biogas production in reinforced concrete dome biogas plants with buffalo dung in Pakistan. Biomass. Convers. Biorefinery 1–15.

Kassambara, A., Kassambara, M.A., 2020. Package 'ggpubr'. R. Package Version 01, 6.

Keuter, S., Beth, S., Quantz, G., Schulz, C., Spieck, E., 2017. Longterm monitoring of nitrification and nitrifying communities during biofilter activation of two marine recirculation aquaculture systems (RAS). Int J. Aquac. Fish. Sci. 3 (3), 051–061.

Korberg, A.D., Skov, I.R., Mathiesen, B.V., 2020. The role of biogas and biogas-derived fuels in a 100% renewable energy system in Denmark. Energy 199, 117426.

Letelier-Gordo, C.O., Aalto, S.L., Suurnäkki, S., Pedersen, P.B., 2020a. Increased sulfate availability in saline water promotes hydrogen sulfide production in fish organic waste. Aquac. Eng. 89, 102062.

Letelier-Gordo, C.O., Mancini, E., Pedersen, P.B., Angelidaki, I., Fotidis, I.A., 2020b. Saline fish wastewater in biogas plants-biomethanation toxicity and safe use. J. Environ. Manag. 275, 111233.

Li P., Schideman L., Wilkinson H., editors. Improving Anaerobic Digestion Processes with Bioaugmentation: Case Study for Sustainable Bioenergy Production from Aquaculture Wastes. Energy Conference 2011; 2011: Water Environment Federation.

Lobanov, V.P., Combot, D., Pelissier, P., Labbé, L., Joyce, A., 2021. Improving plant health through nutrient remineralization in aquaponic systems. Front. Plant Sci. 12, 1064.

Maghanki, M.M., Ghobadian, B., Najafi, G., Galogah, R.J., 2013. Micro combined heat and power (MCHP) technologies and applications. Renew. Sustain. Energy Rev. 28, 510–524.

Metcalf, L., Eddy, H.P., Tchobanoglous, G., 1991. Wastewater Engineering: Treatment, Disposal, and Reuse. McGraw-Hill, New York.

Michaud, L., Blancheton, J.-P., Bruni, V., Piedrahita, R., 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Aquac. Eng. 34 (3), 224–233.

Miller D., Semmens K. Waste management in aquaculture. West Virginia University Extension Service Publication No AQ02–1 USA. 2002;8.

Moraes, Md.A.B., Carmo, C.Fd, Ishikawa, C.M., Tabata, Y.A., Mercante, C.T.J., 2015. Daily mass balance of phosphorus and nitrogen in effluents of production sectors of trout farming system. Acta Limnol. Bras. 27 (3), 330–340.

Murrell, P., 2002. The grid graphics package. R. N. 2 (2), 14–19.

Ndiaye, N.A., Maiguizo-Diagne, H., Diadhiou, H.D., Ndiaye, W.N., Diedhiou, F., Cournac, L., et al., 2020. Methanogenic and fertilizing potential of aquaculture waste: towards freshwater farms energy self-sufficiency in the framework of blue growth. Rev. Aquac. 12 (3), 1435–1444.

Nichols M., Savidov N., editors. Aquaponics: A nutrient and water efficient production system. II International Symposium on Soilless Culture and Hydroponics 947; 2011.

Outlook, I., 2020. For biogas and biomethane: prospects for organic growth. World Energy Outlook Spec. Rep. 93.

Patrice Takoukam KE. Aquaculture Regulatory Framework. FAO Legal Papers Online. 2013(No. 91).

Pedersen, P.B., von Ahnen, M., Fernandes, P., Naas, C., Pedersen, L.-F., Dalsgaard, J., 2017. Particle surface area and bacterial activity in recirculating aquaculture systems. Aquac. Eng. 78, 18–23.

Pellegrini L.A., De Guido G., Consonni S., Bortoluzzib G., Gatti M., editors. From biogas to biomethane: how the biogas source influences the purification costs. ICheaP12 International Conference on Chemical & Process Engineering; 2015: Italian Association of Chemical Engineering-AIDIC.

Resende, J.A., Godon, J.-J., Bonnafous, A., Arcuri, P.B., Silva, V.L., Otenio, M.H., et al., 2016. Seasonal variation on microbial community and methane production during anaerobic digestion of cattle manure in Brazil. Microb. Ecol. 71, 735–746.

Sawatdeenarunat, C., Nguyen, D., Surendra, K., Shrestha, S., Rajendran, K., Oechsner, H., et al., 2016. Anaerobic biorefinery: current status, challenges, and opportunities. Bioresour. Technol. 215, 304–313.

Scarlat, N., Dallemand, J.-F., Fahl, F., 2018. Biogas: developments and perspectives in Europe. Renew. Energy 129, 457–472.

Schumann, M., Brinker, A., 2020. Understanding and managing suspended solids in intensive salmonid aquaculture: a review. Rev. Aquac. 12 (4), 2109–2139.

Song, X., Liu, Y., Pettersen, J.B., Brandão, M., Ma, X., Røberg, S., et al., 2019. Life cycle assessment of recirculating aquaculture systems: A case of Atlantic salmon farming in China. J. Ind. Ecol. 23 (5), 1077–1086.

Szarka, N., Scholwin, F., Trommler, M., Jacobi, H.F., Eichhorn, M., Ortwein, A., et al., 2013. A novel role for bioenergy: a flexible, demand-oriented power supply. Energy 61, 18–26.

Tetreault, J., Fogle, R., Guerdat, T., 2021. Towards a capture and reuse model for aquaculture effluent as a hydroponic nutrient solution using aerobic microbial reactors. Horticulturae 7 (10), 334.

Turovskiy, I.S., Mathai, P., 2006. Wastewater Sludge Processing. John Wiley & Sons.

Van Den Hende, S., Laurent, C., Bégué, M., 2015. Anaerobic digestion of microalgal bacterial flocs from a raceway pond treating aquaculture wastewater: need for a biorefinery. Bioresour. Technol. 196, 184–193.

van Rijn, J., 2013. Waste treatment in recirculating aquaculture systems. Aquac. Eng. 53, 49–56.

Wang, J., You, S., Zong, Y., Træholt, C., Dong, Z.Y., Zhou, Y., 2019. Flexibility of combined heat and power plants: A review of technologies and operation strategies. Appl. Energy 252, 113445.

Wang, X.X., Olsen, L.M., Reitan, K.I., Olsen, Y., 2012. Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquac. Environ. Interact. 2 (3), 267–283.

Wickham H. The tidyverse. R package ver. 2017;1(1):1.

Wickham H., Wickham M.H. Package 'tidyr'. Easily Tidy Data with'spread'and'gather (.)'Functions. 2017.

Wickham H., Francois R., Henry L., Müller K., editors. dplyr. useR! Conference; 2014.

Wickham H., Chang W., Wickham M.H. Package 'ggplot2'. Create elegant data visualisations using the grammar of graphics Version. 2016;2(1):1–189.

Wilke C.O., Wickham H., Wilke M.C.O. Package 'cowplot'. Streamlined Plot Theme and Plot Annotations for 'ggplot2. 2019.

Xiao, R., Wei, Y., An, D., Li, D., Ta, X., Wu, Y., et al., 2019. A review on the research status and development trend of equipment in water treatment processes of recirculating aquaculture systems. Rev. Aquac. 11 (3), 863–895.

Yenigün, O., Demirel, B., 2013. Ammonia inhibition in anaerobic digestion: a review. Process Biochem. 48 (5–6), 901–911.

Yogev, U., Barnes, A., Gross, A., 2016. Nutrients and energy balance analysis for a conceptual model of a three loops off grid, aquaponics. Water 8 (12), 589.

Zhang, X., Spanjers, H., van Lier, J.B., 2013. Potentials and limitations of biomethane and phosphorus recovery from sludges of brackish/marine aquaculture recirculation systems: A review. J. Environ. Manag. 131, 44–54.

Zhang, X., Hu, J., Spanjers, H., van Lier, J.B., 2014. Performance of inorganic coagulants in treatment of backwash waters from a brackish aquaculture recirculation system and digestibility of salty sludge. Aquac. Eng. 61, 9–16.

Zhu, T., Curtis, J., Clancy, M., 2019. Promoting agricultural biogas and biomethane production: Lessons from cross-country studies. Renew. Sustain. Energy Rev. 114, 109332.