Article

Methane and Hydrogen Sulfide Production from the Anaerobic Digestion of Fish Sludge from Recirculating Aquaculture Systems: Effect of Varying Initial Solid Concentrations

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Abstract: Recirculating aquaculture systems (RAS) are efficient at solid waste capture and collection but generate a concentrated waste stream. Anaerobic digestion (AD) could be one potential treatment option for RAS facilities. However, the concentration of organic matter in the sludge can significantly affect the biogas quality from AD. This study evaluated the effect of fish sludge (FS) solid concentration on biogas quality. Three FS treatments consisted of different initial total solid concentrations (1.5%, 2.5%, and 3.5%) from a mixture of sludge produced by Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss). Methane (CH$_4$) production was measured, quantified, and normalized on a volatile solids (VS) basis. The highest solid concentration treatment produced 23% more CH$_4$ than the lowest solid concentration (519 mL/g VS versus 422 mL/g VS, respectively). Peak CH$_4$ production occurred on Day 7 for the lowest FS concentration (78.2 mL/day), while the highest FS concentration peaked on Day 11 (96 mL/day). Peak hydrogen sulfide (H$_2$S) concentrations ranged from 1803–2074 ppm across treatments, signifying the requirement of downstream unit processes for H$_2$S removal from biogas. Overall, this study demonstrated that increasing the FS concentration can significantly enhance CH$_4$ production without affecting the stability of the digestion process.

Keywords: biogas; fish waste; biosolid; volatile fatty acids; total solids

1. Introduction

Aquaculture, the intentional production of aquatic organisms, plays a significant role in global food security by providing 49% of all seafood consumed. As the global population rises, aquatic food production is expected to increase an additional 15% by 2030, with aquaculture providing a substantial fraction of that growth [1]. The industry’s rapid expansion has resulted in the development of new intensive farming methods which improve productivity, environmental control, biosecurity, and environmental sustainability. One promising technology is land-based recirculating aquaculture systems (RAS), which can treat and reuse over 95% of the water from fish culture tanks through a series of mechanical and biological unit processes. However, the benefits of RAS are undermined by their challenges, such as high capital and operating expenses, intense energy use, and the disposal/treatment of a large volume of concentrated waste material comprised of fish fecal matter and uneaten feed [2,3].

Waste particulates generated by RAS are typically collected and concentrated into sludge using mechanical filtration and gravitational systems, respectively [4]. Once removed from fish culture tanks, disposal of the resulting sludge often presents challenges related to dewatering, storing, and relocating. Additionally, disposal is generally costly in areas with stringent discharge regulations, which limits the amount of concentrated organic matter and nutrients into local water bodies or wastewater treatment facilities [5]. Fish sludge, biologically rich in organic matter and nutrients such as nitrogen and phosphorus, presents a unique waste-to-value opportunity. Incorporating RAS technology in the circular economy by diverting and valorizing waste sludge for other uses offers an economic
incentive for RAS farmers. One possibility for the valorization of the organic matter may be anaerobic digestion (AD), a method that provides a sustainable and productive solution to organic matter degradation and could assist facilities in reducing energy costs [6].

Anaerobic digestion is a biological process occurring in environments sans oxygen, where the degradation of organic matter by specialized microorganisms produces energy-rich biogas [5]. Biogas contains 50–75% methane (CH$_4$), 25–50% CO$_2$, and small amounts of water vapor, ammonia (NH$_3$), hydrogen sulfide (H$_2$S), and other trace gases [7]. In particular, the CH$_4$-rich biogas can then be used to generate heat and electricity. The AD process also reduces and stabilizes the total mass of solids and solubilizes nutrients, creating a fertilizer byproduct. Anaerobic digesters have been utilized for the remediation of animal wastes and municipal wastewater sludge with high success due to their treatment capabilities and subsequent production of renewable energy [5,8–10].

However, different feedstocks (i.e., waste materials to be digested) produce varying quantities and qualities of biogas from the AD process. If the biogas contains high H$_2$S concentrations, then using it for electricity becomes more complex, as the H$_2$S may cause significant corrosion in energy generation equipment [11]. Additional expenses are incurred because the biogas will need to be scrubbed of H$_2$S before utilization. Naturally produced during the AD process, the H$_2$S concentration varies depending on the amount of sulfur-rich components in the waste. Concentrations generally range from 50–10,000 ppm, but as high as 15,000 ppm may be observed when treating sulfur-rich material [12,13]. The presence of organic matter and sulfate in the AD feedstock promotes the growth of sulfate-reducing bacteria (SRB). Under the anaerobic condition, the SRBs can reduce sulfur and sulfate to produce H$_2$S in the presence of a carbon source such as volatile fatty acids (VFAs) (an intermediary product during the AD process), pyruvates, and succinates [12]. It is highly recommended that new AD feedstocks are evaluated for their H$_2$S potential to determine if the desired feedstock is economically viable.

While the AD treatment of sewage sludge, animal waste, and food waste as feedstocks have been well documented, the treatment of RAS-produced sludge is still considered novel [5]. Previous studies have suggested that the solids content in mechanically filtered freshwater RAS sludge may be too low and require further dewatering processes to increase AD efficiency [3]. However, excess organic loading in the AD reactor after dewatering sludge can also lead to process instability due to the production of excess VFAs. To the best of the authors’ knowledge, no studies have specifically focused on the optimal solid concentration for freshwater RAS sludge mono-digestion. Additionally, previous research has not quantified the H$_2$S production potential of freshwater RAS sludge. The purpose of this study was to investigate the change in CH$_4$ and H$_2$S production from freshwater RAS sludge due to a change in the total solids (TS) content. It is expected that the results from this study will help inform RAS farms on the degree of dewatering and desulfurization required for successful AD of fish sludge.

2. Materials and Methods

2.1. Sample Collection

The effluent from an AD system on a dairy farm was selected as the inoculum for the study (PA, USA). Samples were stored on ice during transit and refrigerated at 4 °C until use. The inoculum from this digester was selected to gauge its effectiveness for mono-digestion as it treats a single substrate (i.e., only dairy manure, no co-digestion of other materials), albeit for a different type of substrate (i.e., fish sludge). The substrate consisted of fish sludge (FS) samples collected from a gravity-thickening settler used for dewatering at The Conservation Fund Freshwater Institute (TCFFI), Shepherdstown, WV, USA. At the time of collection, salmon (Salmo salar) and trout (Oncorhynchus mykiss) on pelleted feed diets were cultured at the facility. Inoculum and substrate composition are shown in Table 1.
Table 1. Total and volatile solids content, total ammonia, nitrogen, phosphorus, volatile fatty acid (VFA), pH of the substrate (fish sludge), and inoculum used for the experiment (expressed as mean ± std. error).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fish Sludge (FS)</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (mg/L)</td>
<td>35,700 ± 794</td>
<td>53,700 ± 587</td>
</tr>
<tr>
<td>Volatile solids (mg/L)</td>
<td>31,133 ± 1471</td>
<td>42,367 ± 936</td>
</tr>
<tr>
<td>Chemical oxygen demand (mg/L)</td>
<td>54,875 ± 1151</td>
<td>64,650 ± 2406</td>
</tr>
<tr>
<td>Total ammonia (mg/L)</td>
<td>422 ± 2</td>
<td>1072 ± 16</td>
</tr>
<tr>
<td>Total nitrogen (mg/L)</td>
<td>2265 ± 33</td>
<td>2535 ± 64</td>
</tr>
<tr>
<td>Total phosphorus (mg/L)</td>
<td>750 ± 6</td>
<td>440 ± 6</td>
</tr>
<tr>
<td>Total VFA (mg/L)</td>
<td>6540 ± 232</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>pH</td>
<td>5.5 ± 0.0</td>
<td>7.7 ± 0.0</td>
</tr>
</tbody>
</table>

2.2. Experimental Design and Setup

For this bench-top batch study, FS was mixed with de-ionized water to create three different TS concentration treatments (labeled as 1.5% FS, 2.5% FS, and 3.5% FS) on a w/v basis. The three FS treatments were mixed with inoculum, each conducted in triplicate, plus an inoculum control. An inoculum-to-substrate ratio (ISR) of 2:1 (volatile solids basis) was used for the experiment, which meant that there was a gram of substrate volatile solids (VS) for every 2 grams of inoculum VS [14]. The total mass of organic matter or VS was kept constant for each treatment. Experimental design and treatment levels are shown in Table 2.

Table 2. Experimental design using a 2:1 inoculum-to-substrate ratio (by VS). All treatments were conducted in triplicate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inoculum (mL)</th>
<th>Fish Sludge (mL)</th>
<th>Substrate TS (g)</th>
<th>Substrate VS (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoculum Control</td>
<td>75</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.5% FS</td>
<td>75</td>
<td>122.5</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>2.5% FS</td>
<td>75</td>
<td>73.5</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>3.5% FS</td>
<td>75</td>
<td>52.5</td>
<td>1.8</td>
<td>1.6</td>
</tr>
</tbody>
</table>

The study followed the biochemical methane potential protocol to characterize CH₄ production potential [14]. After substrate and inoculum were added to 300 mL glass serum bottles, the headspace was purged of oxygen using nitrogen gas. Bottles were capped using self-healing rubber septa (Sigma-Aldrich, St. Louis, MO, USA) and incubated at 35 °C (MaxQ 6000; Thermo Fisher Scientific, Waltham, MA, USA). Biogas volumes and CH₄ and H₂S concentrations were monitored regularly until daily biogas production was less than 1% of the total biogas production for all treatments (day 39).

2.3. Analytical Methods

Biogas volume was measured using a graduated, gas-tight 50 mL borosilicate glass syringe (Air-Tite, Virginia Beach, VA, USA) inserted through the septa covering the serum bottles and equilibrated to atmospheric pressure. Biogas samples were collected in 2 mL Luer-lock A-2 gas sampling syringes (VICI; Houston, TX, USA) and tested on a micro-GC Fusion (Inficon; Bad Ragaz, Switzerland) with a thermal conductivity detector (µ-TCD), using Helium as the carrier gas. The injector and detector jacket temperatures were set at 90 °C and 72 °C, respectively. Column temperatures for CH₄ (Column A) and H₂S (Column B) were 100 °C and 65 °C, respectively. Gas production from the inoculum control was subtracted from the daily and cumulative CH₄ and H₂S from the three treatments to account for the production of these gases from the AD of the fish sludge only.

Pre- and post-digestion treatment mixtures were tested for pH (Thermo Fisher Scientific; Waltham, MA, USA). Additionally, pre- and post-digestion treatments sampled
for chemical oxygen demand (COD), total ammonia (TAN), total nitrogen (TN), and total phosphorus (TP) measurements were conducted using standardized methods (DR6000 User Manual, 7th ed. Hach Company, USA), as well as triplicate samples for TS and VS, according to Standard Methods for the Examination of Water and Wastewater 23rd Ed. (2017) within 24 h of collection. Volatile fatty acid concentrations in the pre- and post-digested samples were analyzed at Cumberland Valley Analytical Services (Waynesboro, PA, USA) using a gas chromatograph (Perkin Elmer; Shelton, CT, USA) with a flame ionization detector.

2.4. Statistical Analysis

Collected data were analyzed for significant differences in CH₄, H₂S, TS, VS, COD, TN, TP, TAN, and VFA concentrations using one-way ANOVA (α = 0.05) with post hoc Tukey–Kramer multiple mean comparison tests (SigmaPlot 14.0; Systat Software, Inc., Palo Alto, CA, USA). Data are reported as mean ± standard error.

3. Results and Discussion

3.1. Methane Production

There were no significant differences between the treatments’ percent CH₄ values by the end of the study. All treatments peaked at >70% CH₄ between Days 9 and 12, although peak CH₄ concentrations were achieved faster for 1.5% FS than 3.5% FS. Consequently, the initial rate of CH₄ production was higher for 1.5% FS, but after Day 12, the rate of CH₄ production from 3.5% FS was the highest (Figure 1). It is well known that increasing the organic loading in batch studies can reduce initial CH₄ production rates due to the rapid production and accumulation of VFAs from the hydrolysis and fermentation of complex polymers [15]. Once the methanogens start consuming the accumulated VFAs and proliferating, CH₄ production rates may increase, as observed in this study (Figure 1). However, it should be noted that excess loading of substrates should be avoided since elevated VFA concentrations (typically above 3500 mg/L) may result in an unstable process with partial or complete inhibition of methanogenesis, eventually leading to the complete failure of the AD system [16,17]. No such inhibition was observed in this study at higher organic loading treatments, i.e., 2.5% FS and 3.5% FS. Additionally, all treatments had produced >90% of the cumulative CH₄ production observed at the end of the study period by Day 22, indicating that a 22-day hydraulic retention time (HRT) should be sufficient for practical applications.

Figure 1. Daily methane production rate using fish sludge as the anaerobic digestion substrate at three different initial solid concentrations.
Increasing the solids concentration from 1.5% to 3.5% in the treatment units resulted in a significant increase in the cumulative CH$_4$ production ($p$-value < 0.0001; Table 3). Theoretically, the amount of VS in each treatment was kept constant for this study, which should have resulted in similar values for the normalized cumulative CH$_4$ production. However, studies have shown that microbial activity may be reduced at low solid concentrations (reduced VFA production, lowered methanogenesis), which could have resulted in the 19% decrease in CH$_4$ production for the 1.5% FS treatment, as compared to the 3.5% FS treatment [18]. The logarithmic increase in the CH$_4$ production with respect to the increase in solids concentration ($R^2 = 0.9991$) also suggests that CH$_4$ production may not increase indefinitely with the increase in solids content.

The cumulative CH$_4$ production for all treatments (422–519 mL CH$_4$/g VS; Figure 2) was also comparatively higher than dairy manure, swine manure, and poultry manure seen in the literature [14]. The increase may be due to undigested fats in the sludge, as the peak CH$_4$ concentration for all treatments was >70%, typically seen in waste feeds containing fatty substrates [19,20]. Fish feed for salmonids is typically rich in lipids to provide energy to the fish. While trout feed can contain around 20–24% fat, salmon feed could have fat content up to 35% [21,22]. Lanari and Franci (1998) showed that 400–460 mL CH$_4$/g VS could be obtained from the digestion of rainbow trout manure in freshwater, similar to the values obtained in this study [23].

![Cumulative methane production using fish sludge as the anaerobic digestion substrate at three different initial solid concentrations.](image)

Figure 2. Cumulative methane production using fish sludge as the anaerobic digestion substrate at three different initial solid concentrations.

However, some studies have shown that the CH$_4$ potential from freshwater FS is comparable to or significantly lower than manure sources from terrestrial animal farms [24,25]. The research on AD of freshwater FS is limited, which makes it difficult to compare results comprehensively. Furthermore, the fish species cultured, feed characteristics, and diet digestibility affect waste characteristics and, therefore, the CH$_4$ potential [3,26]. Inoculum may also affect the CH$_4$ potential from the substrate [6]. Using a microorganism source that is unacclimated to a particular substrate may result in extended lag phases, reduced process stability, and reduced CH$_4$ production [27]. In this study, however, there was no visible lag phase as the percent CH$_4$ in the biogas for all treatments was >50% by Day 5 and >70% by Day 9, indicating that the inoculum was suitable for FS as the substrate.

On a per gram (wet) basis, increasing the concentration by 1% and 2% resulted in a 100% and 200% increase in CH$_4$ production, respectively, thereby reinforcing the need for sludge concentration at RAS facilities [3,5]. The higher solid concentration also allows for more sludge to be digested within a smaller digester volume. In the typical ‘Cornell-type’ dual drain fish culture tank, standard in RAS, the bottom drain discharge’s total
suspended solid concentrations are <20 mg/L [28]. Most RAS facilities use mechanical filtration systems, such as microscreen drum filters, to rapidly remove and concentrate the waste generated in the fish culture tanks. However, this process only increases the TS concentration in the backwash to 1000–2000 mg/L, which is considerably lower than the recommended TS for manure digesters [4]. According to the EPA, complete mix digesters function optimally at TS concentrations of 30,000–100,000 mg/L (3–10% solids) [29]. Dewatering and thickening of the dilute backwash using equipment such as gravity thickening settlers are necessary to increase the TS content to 5–10% [4].

Table 3. Methane (CH$_4$) and hydrogen sulfide (H$_2$S) production data from the batch digestion testing.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CH$_4$ (%)</th>
<th>mL CH$_4$/g VS</th>
<th>mL CH$_4$/g COD</th>
<th>mL CH$_4$/g wet sludge</th>
<th>mL CH$_4$/g feed*</th>
<th>mL H$_2$S/g VS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5% FS</td>
<td>59.4 ± 0.3</td>
<td>422 ± 11</td>
<td>240 ± 6</td>
<td>5.5 ± 0.1</td>
<td>92 ± 2.4</td>
<td>0.90 ± 0.04</td>
</tr>
<tr>
<td>2.5% FS</td>
<td>58.8 ± 0.2</td>
<td>483 ± 6</td>
<td>274 ± 3</td>
<td>10.5 ± 0.1</td>
<td>105 ± 1.3</td>
<td>0.99 ± 0.00</td>
</tr>
<tr>
<td>3.5% FS</td>
<td>58.9 ± 0.3</td>
<td>519 ± 5</td>
<td>295 ± 3</td>
<td>15.9 ± 0.1</td>
<td>113 ± 1.1</td>
<td>1.10 ± 0.03</td>
</tr>
</tbody>
</table>

* The % CH$_4$ shown is the average value of the last week of the 39-day experiment. * Assuming 0.25 g of dry solid waste is generated per gram of dry feed [30].

The viability of AD as a waste treatment technique for RAS farms will depend on the energy that can be recovered using this process. In this study, using salmon and trout waste sludge as the feedstock, the maximum CH$_4$ production achieved was 0.415 m$^3$/kg dry sludge. For a farm producing 1000 metric tons (MT) of fish annually with a feed conversion ratio of 1.2, 1200 MT of feed would be required. Assuming fish feed contains 20% moisture, it is estimated that the fish would consume 960 metric tons of dry feed, which would result in an estimated 240 metric tons of dry sludge per year (assuming 0.25 g of dry sludge is generated per gram of dry feed) [30]. An anaerobic digester on the 1000 MT RAS farm would theoretically be able to produce 99,600 m$^3$/year or 273 m$^3$/CH$_4$/day. Assuming 36 MJ/m$^3$ CH$_4$, a combined heat and power (CHP) unit (80% efficiency) can produce 7900 MJ/day (2200 kWh/day) or 2.9 million MJ/year (0.8 million kWh/year). Energy use on RAS farms growing market-size fish can range between 2.9 kWh/kg fish and 81.48 kWh/kg fish due to differences in farm size and type (full recirculation or partial reuse), rearing stage of the fish, water temperatures and recirculation rates, aeration technologies, solids removal, and waste treatment [2]. From the results of this study, it can be estimated that a 1000 MT farm raising salmonids could meet up to 28% of its energy needs using a CHP. Considering only electrical energy, up to 10% of a RAS farm’s energy needs may be met using CH$_4$ from an AD system [5].

3.2. Hydrogen Sulfide Production

The three FS treatments produced biogas with peak H$_2$S concentrations ranging from 1803–2074 ppm by Day 3 (Figure 3). This trend is typically seen in most methane potential studies, as SRBs tend to outcompete the slow-growing methanogens for the same carbonaceous substrates within the first few days of the study [31,32]. Once the sulfur source begins to dwindle, the methanogens start outcompeting the SRBs, which increases CH$_4$ production. After Day 3, the H$_2$S concentrations experienced a sharp drop to under 1000 ppm by Day 8 and finally stabilized between 600 and 1000 ppm after Day 20. The sharp decline could be correlated to the methanogenic archaea outcompeting the SRB after Day 3, as the average CH$_4$ concentration for all treatments increased rapidly to >50% by Day 5 of the study. The H$_2$S concentrations observed in this study are comparable to values typically seen during the digestion of animal manures [33]. However, the peak concentrations were higher than the maximum recommended limits for biogas boilers and generators for heat and electricity generation, respectively [34]. As such, it is recommended that the H$_2$S in the biogas from fish sludge be scrubbed before its utilization for energy
The concentration of H$_2$S would likely vary based on the feed input rate and its composition [35].

![Figure 3. Variation of hydrogen sulfide concentrations in the biogas with fish sludge as the anaerobic digestion substrate at three different initial solid concentrations.](image)

The normalized cumulative H$_2$S volumes from the treatments ranged from 0.9–1.1 mL H$_2$S/g VS (Table 3). These values are similar to cumulative H$_2$S productions observed during the AD of dairy manure but may depend on the sulfur content of the fish sludge [34]. Sulfur is mainly present in proteins, forming a substantial fraction of organic material in aquaculture sludge [36]. The protein content in the fish feed and its digestibility (dependent on the fish species and culture conditions, among other factors) would likely determine its concentration in the sludge. Letelier-Gordo et al., 2020 noted that most commercial feeds currently used in the industry have a high protein digestibility (>90%) [13]. As such, the protein content of the sludge (dry basis) would most likely be between 20% and 25%. However, the proportion of wasted feed pellets in the sludge could contribute to an overall increase in the protein content of the sludge. Concentrated salmonid sludge samples collected from radial flow settlers (connected directly to the fish culture tanks) at TCFFI (within 24 h of accumulation) have shown that the crude protein in the sludge may be as high as 47% (dry basis; unpublished data), which may significantly impact the H$_2$S concentration in the biogas. Other authors have also observed high protein concentrations in the sludge (up to 52% on a dry matter basis), which may be attributed to a higher fraction of wasted feed in the sludge [13,37]. It is also important to note that TCFFI tests experimental feeds from different manufacturers, which could have impacted the residual protein content in the sludge.

Additionally, sludge salinity is another factor that could impact the H$_2$S concentration in the biogas. A recent study suggested that dissolved sulfide concentrations under anaerobic conditions tend to increase significantly as the salinity increases from 0 parts per thousand (ppt) to 35 ppt due to the increase in saline sludge sulfate content [38]. While in this study with freshwater sludge (0 ppt salinity), H$_2$S in the biogas peaked at 2074 ppm, it is expected that more concentrated saline sludge may result in H$_2$S concentrations peaking at over 15,000 ppm [13].

3.3. Volatile Fatty Acids

The concentration of VFAs in the raw sludge sample collected from the gravity thickening settler was 6540 ± 232 mg/L. Acetic acid (36%) and propionic acid (28%) were the primary constituents of the VFAs present in the sample. After dilution, the total initial VFA
concentrations were 2748 mg/L, 4580 mg/L, and 6412 mg/L in the 1.5%, 2.5%, and 3.5% raw sludge samples, respectively. After mixing the sludge with the inoculum, the initial VFA concentrations were 1704 mg/L, 2267 mg/L, and 2640 mg/L for the treatments 1.5% FS, 2.5% FS, and 3.5% FS, respectively. However, the total mass of VFA in each treatment was the same, i.e., 337 mg each. At the end of the study, 99.5–100% of the VFAs were consumed in all the treatments, which suggests that the digestion process was complete. The remaining VS in the treatments were likely more recalcitrant and could not be easily broken down into VFAs after the 39-day study period [39]. The change in VS in the substrate ranged between 27% and 30% (Table 4), which provides further evidence that the remaining organic matter in the substrate was more challenging to degrade without any pretreatment. Future studies should investigate the VFA profile throughout the study, which would help correlate the VFA concentrations to biogas production [40].

Table 4. Percent reduction in fish sludge mass of total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) post digestion.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>%TS Reduction</th>
<th>%VS Reduction</th>
<th>%COD Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5% FS</td>
<td>44%</td>
<td>30%</td>
<td>57%</td>
</tr>
<tr>
<td>2.5% FS</td>
<td>42%</td>
<td>27%</td>
<td>62%</td>
</tr>
<tr>
<td>3.5% FS</td>
<td>50%</td>
<td>29%</td>
<td>69%</td>
</tr>
</tbody>
</table>

The initial concentration of VFAs in FS was 117–225% higher than the sludge collected from a radial flow settler (VFA concentration around 2–3 g/L; unpublished data) within 24 h of capture from the fish culture tanks. It is important to note that the FS collected from the bottom of the gravity-thickening settler for this study had a retention time of two weeks, which may have been the reason for the high initial VFA concentration. The higher VFA concentrations, however, did not significantly impact the pH of FS, as both fresh sludges from a radial flow settler and FS had similar pH (5.4–5.5).

Kuruti et al. (2017) reported that a substantial fraction of the complex organic matter in waste streams could be degraded rapidly into VFAs in a storage system under acidic conditions before AD [41]. This setup essentially acts as a two-reactor system as the hydrolysis and fermentation steps to produce VFAs occurs in the storage system. At the same time, the methanogens in the digester consume the VFAs to produce biogas [42]. While this two-step process may be beneficial for AD (e.g., reduced HRT) in some instances, it is essential to ensure that the buffering capacity of the digester is sufficient to counteract the low pH of the VFA-rich feed sludge. Alkaline chemicals such as lime may need to be added to increase the pH to neutral for successful methanogenesis [41]. In this study, the buffering capacity of the inoculum was sufficient to maintain a neutral pH in the mixture throughout the study. Additionally, the breakdown of proteins in the substrate during the incubation period increased the TAN concentration in the treatments (up to 1100 mg/L, from 420 mg/L), which may also have fortified the buffering capacity of the AD system.

3.4. Solids and Organic Matter Reduction

The VS reduction was low but similar for all treatments (27–30%) (Table 4). The similarities in VS removal indicate that hydrolysis was not limiting for the treatments [43]. This is also supported by the fact that all treatments had similar ratios of readily biodegradable material to recalcitrant material, as the raw FS was diluted with water to obtain the three different TS concentrations. The reduction in substrate TS was also similar for all three treatments, ranging from 42–50%. High COD reductions were observed for all treatments (57–69%), and the percent removal exhibited an increasing trend with increasing TS content. From the COD mass balance, it was calculated that 1.6–1.9 g of substrate COD was removed from the initial mass of 2.8 g substrate COD in each treatment. The high COD removals can also be correlated to the normalized CH₄ production on a COD basis, as the treatments produced 69–84% (240–295 mL CH₄/g COD; Table 3) of the maximum theoretical CH₄
(350 mL CH$_4$/g COD). However, many authors have reported inaccuracies and challenges with measuring COD for high TS samples and recommend using VS to normalize CH$_4$ production [18].

4. Conclusions

Results from this study suggest that concentrating freshwater RAS sludge may help increase the normalized volume of CH$_4$ produced from anaerobic digestion. Increasing the TS concentration from 1.5% to 3.5% resulted in a 23% increase in the CH$_4$ production when normalized by VS. When normalized on a mass basis, the maximum increase in CH$_4$ production observed in this study was 200%. Maximum methane production was 519 mL CH$_4$/g VS (15.9 mL CH$_4$/g wet sludge), substantially higher than animal waste or sewage sludge as the digester substrate. However, increasing the TS concentration also increased the H$_2$S concentration in the biogas and total H$_2$S production. Peak H$_2$S concentration in the biogas was >2000 ppm, and the biogas from freshwater RAS sludge may need to be scrubbed before its utilization for heat and electricity production. Additionally, the storage of sludge in dewatering systems (such as gravitational settling columns) available in RAS facilities may aid in VFA production through hydrolytic breakdown and fermentation of the organic matter, thereby reducing the HRT of the digester.

Author Contributions: Conceptualization, A.C.; Methodology, A.C.; Software, A.C. and C.L.; Validation, A.C. and C.L.; Formal Analysis, A.C.; Investigation, A.C. and C.L.; Resources, C.G.; Data Curation, A.C.; Writing—Original Draft Preparation, A.C.; Writing—Review & Editing, A.C., C.L. and C.G.; Visualization, A.C.; Supervision, A.C. and C.G.; Project Administration, C.G.; Funding Acquisition, C.G. All authors have read and agreed to the published version of the manuscript.

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References


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