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# Possibilities for Anaerobic Digestion of Slaughter Waste and Flotates for Biomethane Production

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**Abstract:** Anaerobic digestion for biomethane production is an important tool regarding sustainable energy production. The objective of this study was to investigate the effects of the substrate composition and operating parameters on biomethane production during anaerobic digestion, focusing on the use of flotates and slaughterhouse waste as substrates with a high organic content. A novelty here was the use of a moving bed biofilm reactor (MBBR) with circulation pump for the anaerobic treatment of flotates, slaughter waste (SW), and their mixture. Flotates and waste from slaughterhouses offer a substrate with a high organic content. In this work, it was shown that both substrates provide a high biochemical methane potential (BMP). The highest methane yield was achieved by mixing both substrates. In continuous operation, special challenges arose, due to the high nitrogen and fat content of the substrates. These could be overcome by mixing the substrates and using a circulation pump in the reactor for improved back-mixing. As a result, the highest average methane yield of 0.65 $NL_{CH4} \cdot g_{TS\ eli}{}^{-1}$ was achieved in mesophilic operation at an organic loading rate (OLR) of 4.2 $g_{TS} \cdot L^{-1} \cdot d^{-1}$.

**Keywords:** anaerobic digestion; slaughter wastewater; slaughter waste; bio gas; methane potential

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## 1. Introduction

The demand for food and energy is increasing worldwide, due to the growing population and rising standards of living [1]. Meat is a popular source of nutrients for people of all income levels, which has led to an increase in global meat consumption, particularly of chicken and pork [2]. In comparison to plant-based foods, meat causes more emissions and consumes more energy per unit of calorific value, since energy is lost at each trophic level [2]. The meat industry alone contributes approximately 200 MWh per year to global energy consumption, with an annual production of 340 million tons and an average energy demand of 570 kWh per ton of carcass weight [1,3]. In order to reduce the environmental impact of the meat industry, the production of biogas from SW and slaughter wastewater (SWW) has been proposed as a potential solution. Both waste streams contain large amounts of readily biodegradable organics, which results in a high biogas potential [4,5]. In addition, these materials also contain a comparatively high proportion of fats and nitrogen, which can lead to inhibition during anaerobic degradation [6–8].

Many slaughterhouses operate flotation plants for wastewater treatment, producing an organic-rich stream and a pretreated wastewater stream that can often be released into municipal wastewater treatment plants [3]. However, due to concerns about bovine spongiform encephalitic and other diseases, the disposal of slaughter waste is heavily regulated in many countries [9]. To explore the feasibility of using anaerobic digestion for methane production from slaughterhouse flotates and waste, batch tests were conducted using both substrates. A two-stage anaerobic MBBR was then used to investigate the effect of certain operating parameters on the BMP in a continuous long-term experimental set up.

Several researchers have conducted studies related to anaerobic digestion of slaughterhouse waste, identifying challenges and opportunities regarding this process. Aklilu and Waday [10] investigated the effect of certain variables on biogas yield via anaerobic co-digestion of poultry manure and alkali-treated corn stover. A response surface methodology and artificial neural network were used to optimize and predict biogas production. The results suggested that co-digestion is a promising way to increase biogas production. Tsegaye et al. [11] optimized the operating parameters of a hydrolytic–acidogenic reactor in a two-phase anaerobic digestion process treating slaughterhouse wastewater. Lower hydraulic retention time (HRT) and higher OLR resulted in a higher hydrolysis and acidification. An HRT of 3 days and an OLR of 1.7 $g_{COD} \cdot L^{-1} \cdot d^{-1}$ were found to be optimal for high performance and stability. Kumar et al. [12] presented an experimental investigation of the use of anaerobic batch reactors for the treatment of oily sludge for methane production. The findings suggested that anaerobic digestion can effectively treat oily sludge and produce biogas and methane gas with a relatively high yield, making this a promising method for the effective treatment of this challenging waste. Overall, anaerobic digestion of SW and SWW has the potential to provide a sustainable and cost-effective solution to waste management, while also generating renewable energy and a digestate with a high fertilizing potential [13].

The novelty of this study lies in the demonstration that a mixture of high-fat SW and nitrogen-rich flotate sludge can be anaerobically digested through utilization of a laboratory scale MBBR. In this process, the use of a circulation pump could suppress fat accumulation at the top of the reactor and achieve increased degassing.

## 2. Materials and Methods

### *2.1. Substrate for the Digestion Experiments*

The substrate for the digestion experiments was flotate from the dissolved air flotation (DAF) plant of a large slaughterhouse, as well as SWW. Additionally, SWW and SW from a small rural slaughterhouse were used. The large slaughterhouse, which produces about 600 $m^3$ SWW and 25 $m^3$ flotate per day, operates a main stream DAF, which follows a mixing and equalization tank. Afterwards, the SWW is discharged to the municipal wastewater treatment plant. The flotate sludge is then collected by an external operator for further processing. The flotate samples used came from the flotate tank. Representative flotate samples were collected from the flotate tank. They were analyzed and then stored at −20 °C until use.

The small rural slaughterhouse mainly slaughters pigs and cattle and produces on average 1 $m^3$ SWW and 0.2 $m^3$ SW per day. Mixed SWW samples were taken from the fat separators, in which the SWW remains for 3 to 7 days, depending on the quantity that arises. The SW samples were taken immediately after slaughter and were composite samples of soft offal from the animals slaughtered that day. Afterwards, the samples were ground to a grain size of 5 mm, hygienized for 1 h at 80 °C,analyzed, and stored at −20 °C until use. The rumen contents of a Demeter cow slaughtered at the small slaughterhouse served as the inoculum for the starting phase of the first continuous experiment. The cow had been raised according to the guidelines of Demeter organic meat production, without the use of prophylactic antibiotics.

### *2.2. Setup Continuous Experiment*

The experimental plant consisted of a pre-acidification unit and an anaerobic digestor. The pre-acidification unit is a cylindrical 1 L container made of plastic with a volume of 400 mL. The digester is a cylindrical 3 L container made of PMMA. In the pre-acidification unit, 50 mL and in the digester, 500 mL of biocarriers was added, which consisted of bamboo pieces about 1 cm long. These biocarriers came from a bamboo shoot about 3 m high from the Berlin area. The bamboo was harvested, had all leaves and branches removed, and then cut. Before use, these pieces were dried. The digester has a double outer wall and was connected to a continuous flow heater that ensured a constant water temperature in the outer walls. The digester was mixed by a built-in stirrer. In addition, a peristaltic pump was integrated via two inlets, by means of which substrate could be fed, samples taken, and a circulation generated. A sketch of the experimental setup is shown in Figure 1.

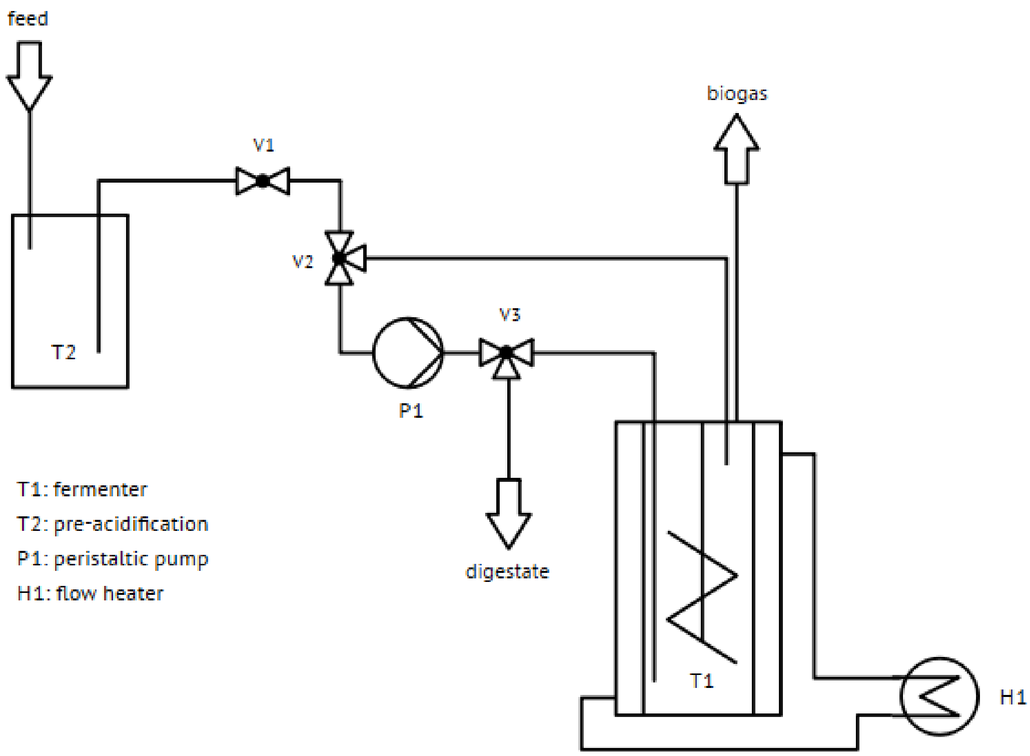


**Figure 1.** Experimental plant.

Three continuous experiments were performed, and an overview is given in Table 1. The operating conditions of the pre-acidification unit were the same for all three experiments. The pre-acidification tank was not heated, it was left at room temperature (20 °C). The HRT in the reactor was 4 days. The pH was monitored and kept at 6.

The first experiment included the startup phase of the reactor. Prefermented rumen content from the small rural slaughterhouse was used for inoculation. After an adaptation phase, the flotate sludge from the large poultry slaughterhouse was used as substrate in this experiment. The experiment was conducted over a period of 170 days. The OLR was increased step by step from 1 to 4 $g_{TS} \cdot L^{-1} \cdot d^{-1}$. Due to the strong inhomogeneity of the substrate, deviations occasionally occurred. Increasing the OLR also reduced the HRT, from an initial 25 days to 12 days. A constant temperature of 35 °C was maintained in the reactor to enhance the growth of mesophilic methane-producing microorganisms.

In the second experiment, SW was mixed with flotate in the ratio at which it occurs in the slaughterhouse and then diluted with SWW, until the mixture had a TS of 17%. An OLR of 4.2 $g_{TS} \cdot L^{-1} \cdot d^{-1}$ with a HRT of 40 days was set. The organic content in the SWW was so low in relation to the SW and flotate that it was neglected for balance. A constant temperature of 35 °C was also maintained in the reactor for this experiment. In the third experiment, the same substrate was used as in the second experiment. The OLR was also set at 4.2 $g_{TS} \cdot L^{-1} \cdot d^{-1}$, with the HRT at 40 days. In this case, however, a temperature of 60 °C was set in the reactor, to enrich the content of thermophilic methane-producing microorganisms. After the temperature increase and before the start of the third experiment, the reactor was left to rest for 60 days to give the microbiology time to adapt. During this time, no substrate was added initially, and from day 30 the substrate addition was slowly increased. Only after this adaptation phase was experiment 3 started.

**Table 1.** Performed fermentation experiments.

| | Substrate | Temperature | Duration | Organic Loading Rate |
|---|---|---|---|---|
| 1 | flotate | 35 °C | 170 days | 1–4.2 $g_{TS} \cdot L^{-1} \cdot d^{-1}$ |
| 2 | slaughter waste + flotate | 35 °C | 110 days | 4.2 $g_{TS} \cdot L^{-1} \cdot d^{-1}$ |
| 3 | slaughter waste + flotate | 60 °C | 120 days | 4.2 $g_{TS} \cdot L^{-1} \cdot d^{-1}$ |

### *2.3. Biochemical Methane Potential Measurements*

Four measurements with three replicates each were performed to determine the BMP of the two substrates. The measurements were carried out according to the recommendations of Holliger et al. [14]. The substrates used were flotate and SW, as well as a mixture of both. Additionally, three replicates without substrate were performed with only an inoculum as reference. The inoculum was digested sludge from the post-digestion of an anaerobic reactor. For all measurements, the ratio of total solids (TS) inoculum to substrate was 5:1 or, in relation to the volatile solids (VS) 5:1.7. 15 g TS of inoculum was added to each 500 mL vial followed by the substrate. The BMP measurements are summarized in Table 2.

**Table 2.** Biochemical methane potential measurements.

| Series | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| substrate | - | flotate | slaughter waste | mix (1:1) |
| duration | 90 days | 90 days | 90 days | 90 days |
| V inoculum | 100 mL | 100 mL | 100 mL | 100 mL |
| V flotate | 0 mL | 75 mL | 0 mL | 37.5 mL |
| V slaughter waste | 0 mL | 0 mL | 5.5 mL | 2.7 mL |
| V water | 400 mL | 325 | 395 mL | 360 mL |
| total TS | 15 g | 18 g | 18 g | 18 g |

### *2.4. Analytical Methods*

To characterize the used substrates, consisting of SW and flotate from SWW, and to monitor the digestion experiments, the following parameters were analyzed: pH, chemical oxygen demand (COD), fat oil and grease (FOG), total organic carbon (TOC), total carbon (TC), dissolved organic carbon (DOC), total nitrogen (TN), $NH_4$-N, dissolved ions, and TS. The pH value was measured with a METTLER TOLEDO pH meter. The TS were determined in accordance with DIN 12880, by drying the sample for 6 h and then weighing it. The VS were determined following the TS measurement, by incinerating the dried sample for 24 h at 550 °C and then weighing. The COD was analyzed with a $QuickCOD_{lab}$-03D0318 from the company LAR Process Analysers AG via a thermal disintegration process. The measurement of TOC, DOC and TN was performed with an Analytik Jena TOC analyser multi N/C 3100 (Jena, Germany), whereby the DOC samples were prepared by filtration through Whatman 0.45 µm membrane filters (Kent, UK). For the determination of FOG, first an acid disintegration according to Weibull–Stoldt was carried out. Subsequently, a Soxhlet extraction was carried out for the FOG determination. The solved ions were measured by means of ion chromatography from Metrohm, using a Metrosep A Supp 17–150/4.0 column for anions and a Metrosep C 4–150/4.0 column for cations. The biogas was collected and then the methane, carbon dioxide, hydrogen sulfide, and oxygen content were determined using an infrared method with a Pronova SSM 6000. The BMP measurements with the method described by Holliger et al. [14] were performed with an Automatic Methane Potential Test System (AMPTS®) from BPC InstrumentsAB produced in Haining, Zhejiang, China.

## 3. Results and Discussions

### 3.1. Characterization of the Substrates

The different flotate and SW samples were analyzed for relevant parameters. Table 3 shows the mean values of the parameters analyzed. In the repeat measurements of the different flotate and SW samples, a deviation in the following parameters was observed: TOC, TC, TS, VS, and TN. Due to the high inhomogeneity of the samples, the variances of these parameters are shown as a box plot in Figure 2. The total carbon and total solids content of SW was 35- and 15-times higher, respectively, compared to the flotate.

**Table 3.** Substrate characterization.

| Parameter | Unit | Flotate | SW |
|---|---|---|---|
| COD | [g·L$^{-1}$] | 40 ± 7 | n.d. |
| TOC | [g·L$^{-1}$] | 20 ± 5 | n.d. |
| TC | [g·L$^{-1}$] | n.d. | 670 ± 20 |
| TN | [g·L$^{-1}$] | 3.8 ± 1 | 27 ± 2 |
| TS | [g·L$^{-1}$] | 4 ± 2 | 550 ± 10 |
| FOG | [g·L$^{-1}$] | 28 ± 4 | 300 ± 20 |
| pH | | 8.3 ± 0.2 | 6.3 ± 0.2 |
| $NH_4^+$ | [mg·L$^{-1}$] | <0.5 | n.d. |
| $F^-$ | [mg·L$^{-1}$] | <0.5 | n.d. |
| $Cl^-$ | [mg·L$^{-1}$] | 540 ± 30 | n.d. |
| $NO_3^-$ | [mg·L$^{-1}$] | 13 ± 2 | n.d. |
| $SO_4^{2-}$ | [mg·L$^{-1}$] | 55 ± 3 | n.d. |
| $PO_4^{3-}$ | [mg·L$^{-1}$] | 75 ± 5 | n.d. |

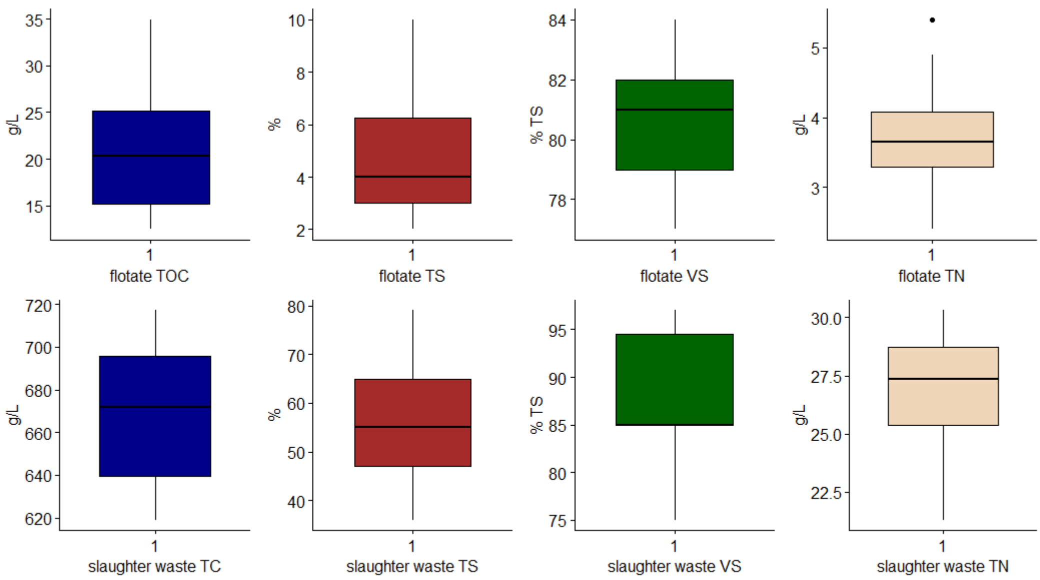


**Figure 2.** Characterization of the flotate and slaughter waste.

### 3.2. Biochemical Methane Potential

The progression of the methane yield in the BMP measurements is shown in Figure 3. The long measurement period of 90 days was compared to similar works by Renggaman et al. [15] and Ware and Power [5]. This can be explained by the comparatively low fermentation temperature of 32 °C. This was chosen to shift the ammonia to ammonium ratio towards ammonium to reduce inhibition of the biology. The highest methane yield was measured for the substrate mixture, followed by the flotate, and the lowest methane yield was measured for the SW sample. The measured maximum methane yields were comparable to previous works and were close to the theoretical maximum methane yield, for the substrate mixture in particular [5,8,15]. However, it is noticeable that an increased methane formation was only observed in all samples after 30 days. On the one hand, this could indicate a slowed hydrolysis of the fatty substrates; on the other hand, it could also indicate inhibition and slow adaptation of the microorganisms to the high ammonia and organic acid concentrations in the batch reactor.

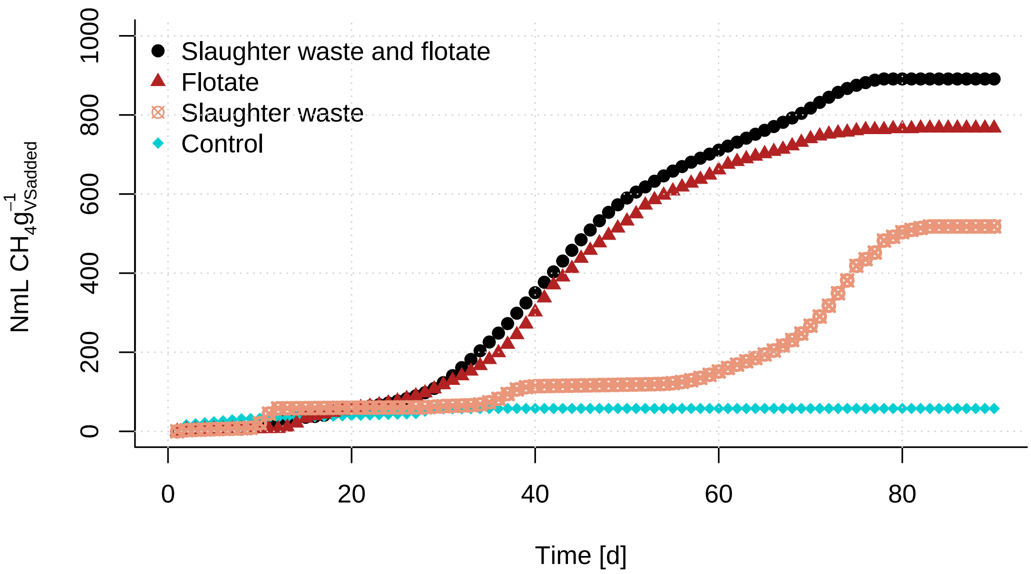


**Figure 3.** Trend of the methane yield for the biochemical methane potential measurements of the different substrates. The values represent the mean values of the three repetitions.

*3.3. Continuous Mesophilic Digestion of Flotate Sludge*

The first part of the mesophilic continuous digestion experiment included the startup phase of the reactor. The reactor was filled with rumen contents mixed with water and set at a constant temperature of 35 °C. Subsequently, the dosage of flotate as substrate was gradually increased. Figure 4 shows the degradation rate, OLR, $NH_4$-N concentration, and methane yield for the experimental period. Experiment 1 started with the adjustment of the substrate dosage to 1 $g_{TS}{\cdot}L^{-1}{\cdot}d^{-1}$, which was gradually increased to 2 $g_{TS}{\cdot}L^{-1}{\cdot}d^{-1}$. The reactor stabilized after 50 days. This can also be seen in Figure 5, which shows the pH value in the reactor over the experimental period. After this startup phase, the pH-value stabilized at about 8. In addition, the reduction of the parameters TS and TOC stabilized at 90 and 95 %, respectively. In contrast, the $NH_4$-N concentration in the reactor increased steadily during the first 120 days, to reach its first peak at 4 $g{\cdot}L^{-1}$. Despite the reduced OLR, the large drop in the degradation rate of TS and TOC could not be prevented. After about 20 days, the $NH_4$-N concentration in the reactor decreased again and the degradation rates stabilized. Towards the end of the experiment, the $NH_4$-N concentration in the reactor increased again and reached its highest peak at 5 $g{\cdot}L^{-1}$. Again a decrease of the degradation rate could be observed. Similar observations were made with respect to the methane yield. With high $NH_4$-N concentrations, the methane yield was reduced. A decrease in methane yield was observed at $NH_4$-N concentrations above 4 $g{\cdot}L^{-1}$. On average, a methane yield of 0.7 $NL_{CH4}{\cdot}{g_{TS\ eli}}^{-1}$ was obtained for the flotate as substrate. This is comparable to other research results and is below the result of Harris et al. [16] of 0.9 $NL_{CH4}{\cdot}{g_{TS\ eli}}^{-1}$, but for whose work a lower OLR of 1 $g_{TS}{\cdot}L^{-1}{\cdot}d^{-1}$ was applied. Damaceno et al. [17], however, were able to achieve a maximum methane output of 0.38 $NL_{CH4}{\cdot}{g_{TS\ eli}}^{-1}$ from a sweet potato flotate sludge mixture at an OLR of 1.8 $g_{TS}{\cdot}L^{-1}{\cdot}d^{-1}$, but they also observed ammonia concentrations of a maximum of 3.3 $g{\cdot}L^{-1}$ in the reactor.

This observation leads to the hypothesis that the anaerobic biocenosis can tolerate $NH_4$-N concentrations of up to 4 $g{\cdot}L^{-1}$, but experiences inhibition at higher concentrations.

However, both this inhibition and the $NH_4$-N concentrations can be regulated by reducing the nitrogen load and increasing the HRT. Similar observations were made in previous works [6,18].

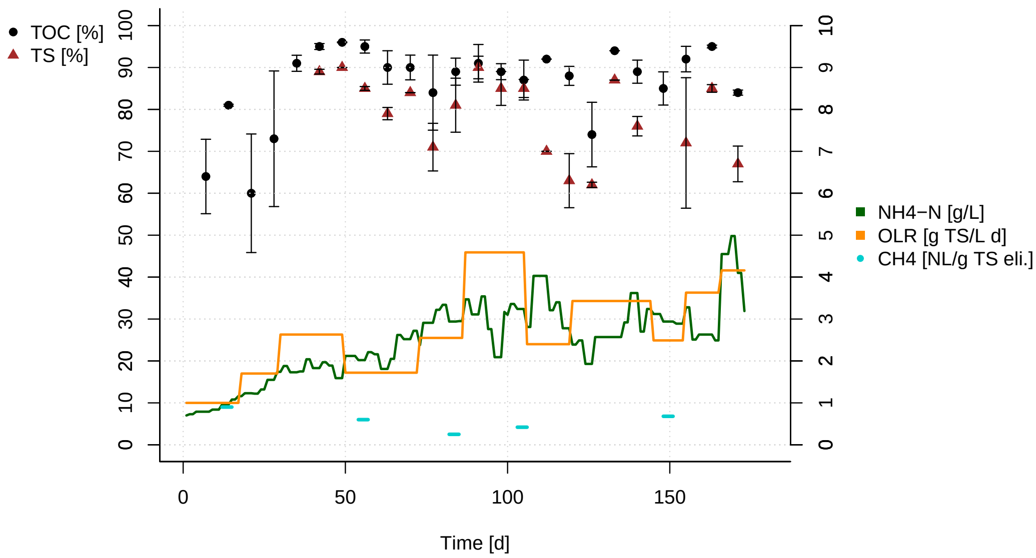


**Figure 4.** Mesophilic anaerobic digestion of slaughterhouse flotates at different organic loads.

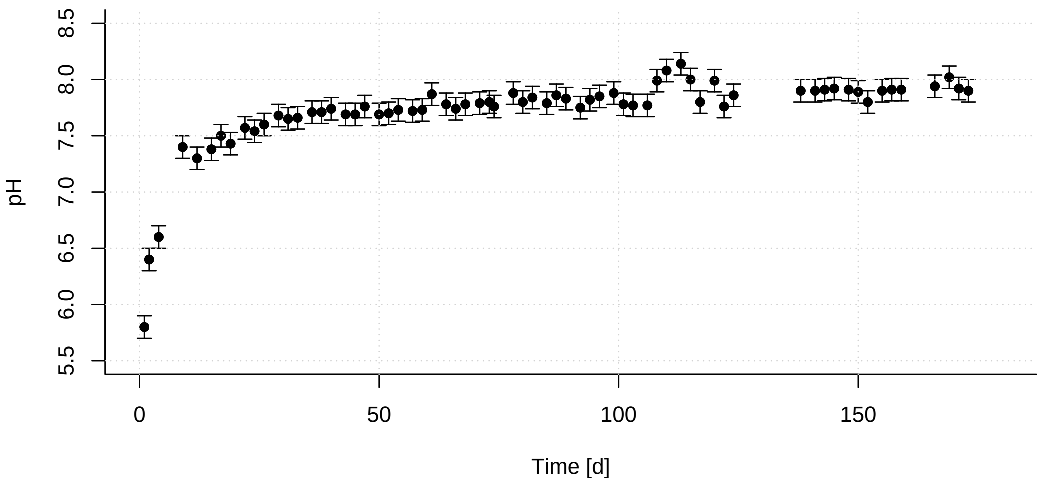


**Figure 5.** pH values for anaerobic digestion of slaughterhouse flotates.

*3.4. Continuous Mesophilic Digestion of Slaughter Waste*

In the second part of the mesophilic continuous digestion experiment, a mixture of SWW and SW was used as substrate with the anaerobic sludge from the first experiment. The OLR was kept constant at 4.2 $g_{TS} \cdot L^{-1} \cdot d^{-1}$ in this experiment. This was achieved by mixing the SW used with flotate in a ratio reflecting the occurrence of these waste streams in the sampled slaughterhouse, and finally diluting until the mixture had a TS of 17%, and establishing a HRT of 40 days. Due to the high solid content, only the solid TC and not the TOC could be determined in the experiments, but the inorganic carbon contained in the samples was assumed to be negligible compared to the TOC. Thus, the TOC and the solid TC was considered comparable. Figure 6 shows the TS and TC degradation, the concentration of $NH_4$-N, and organic acids, as well as the methane yield over 110 days.

As in the experiment using the flotate, a high degradation rate for TC and TS of 98 and 93% on average, respectively, was observed. However, a decrease in the degradation rate, and thus an inhibition of the anaerobic microorganisms, could also be observed at a high $NH_4$-N concentration in the reactor. Due to the high FOG content in the substrate, the concentration of organic acids in the reactor was additionally determined in this experiment, but the concentration did not correlate with the degradation rate. Regarding the methane yield, a strong decrease was observed after day 20, following the highest $NH_4$-N concentration of 3 $g \cdot L^{-1}$ in the reactor, which may have indicated inhibition of the microorganisms. However, during this experiment, an accumulation of fat on the surface of the reactor fluid level was observed, which suppressed the degassing of the biogas, as depicted in Figure 7. This layer increased with time until day 35, when the circulation pump was started. Sludge from the bottom of the reactor was pumped up and trickled onto the fat layer, as well as being degassed. This resulted in contact between the anaerobic sludge and the floating fat, resulting in its degradation. This resulted in an increase in methane yield from day 50. The highest methane yield was determined in the period from day 80 to 100 with 0.76 $NL_{CH4} \cdot g_{TS\ eli}{}^{-1}$.

In a comparable study by Rodríguez-Méndez et al. [19], the highest methane yield of 0.66 $NL_{CH4} \cdot g_{TS\ eli}{}^{-1}$ was achieved in a stirred anaerobic reactor at a OLR of 0.83 $g_{TS} \cdot L^{-1} \cdot d^{-1}$; whereby, clear inhibition of the methane yield could be observed from a OLR above 1.5 $g_{TS} \cdot L^{-1} \cdot d^{-1}$. In the study by Dalantai et al. [20], an investigation was carried out of counteracting the inhibition with ammonium and volatile fatty acid in SW digestion, by slowly adding degradable lignocellulosic carbon. Using liquid-state digestion, a methane yield of 0.65 $NL_{CH4} \cdot g_{TS\ eli}{}^{-1}$ with an OLR of 1.8 $g_{TS} \cdot L^{-1} \cdot d^{-1}$ was achieved, although ammonium concentrations of 4.7 $g \cdot L^{-1}$ and volatile fatty acid concentrations of 1.9 $g \cdot L^{-1}$ were measured in the reactor. In comparison, the methane yields measured in this experimental setup appear to be slightly higher, especially taking into account the higher OLR.

Figure 8 shows the variation of the pH value over the duration of the experiment. This value oscillated between 7 and 8 and was within the optimal range for the entire duration of the experiment.

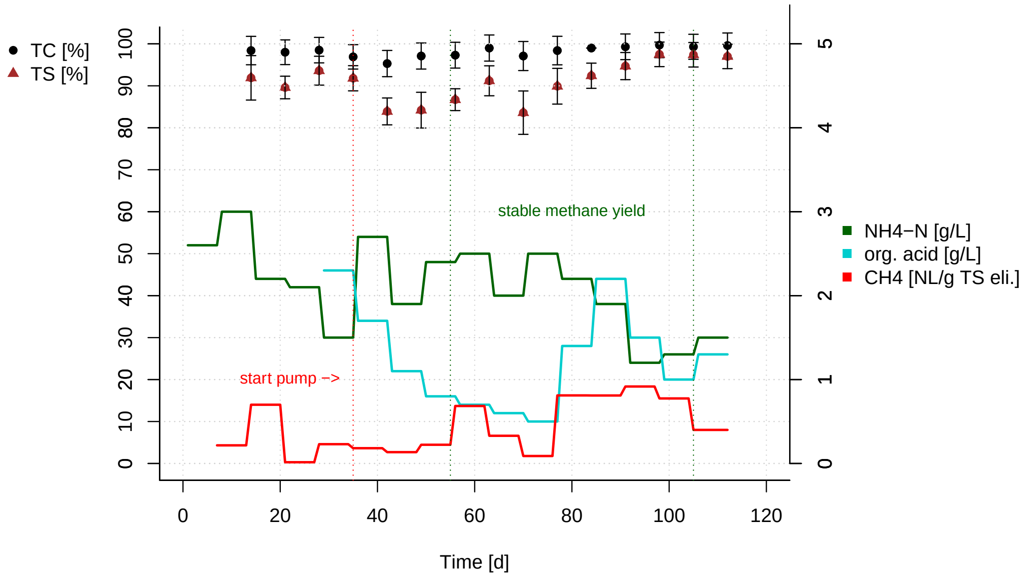


**Figure 6.** Mesophilic anaerobic digestion of slaughter waste and wastewater at an organic load of 4.2 $g_{TS} \cdot L^{-1} \cdot d^{-1}$.

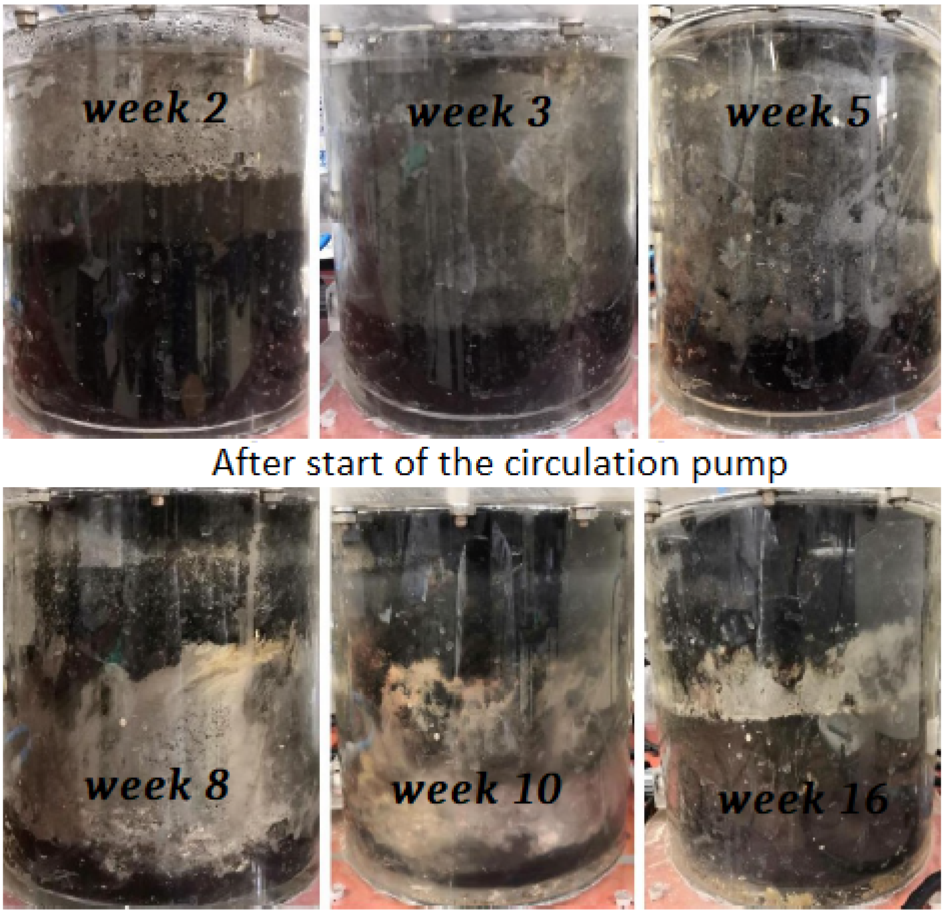


**Figure 7.** Grease layer built up in reactor over the experimental period with and without recirculation.

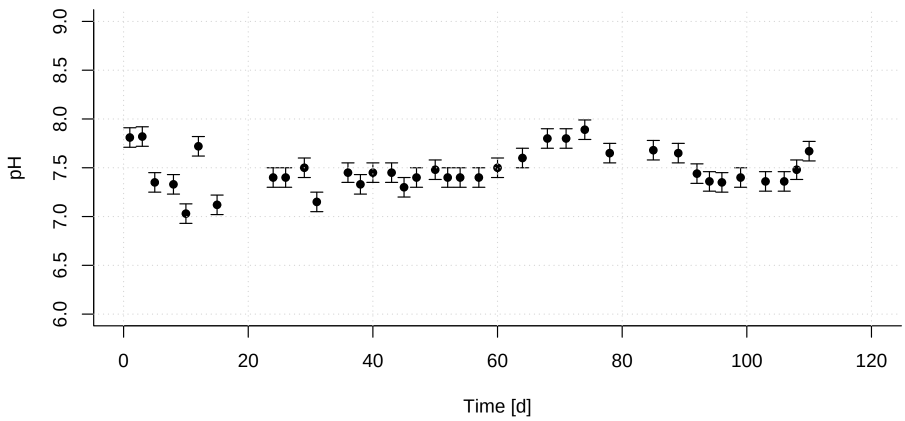


**Figure 8.** pH values for the mesophilic anaerobic digestion of slaughter waste and wastewater.

*3.5. Continuous Thermophilic Digestion of Slaughter Waste*

In the third part of the continuous experiment, the thermophilic anaerobic treatment of SW mixed with flotate was investigated. For this purpose, the reactor was heated to 60 °C. Subsequently, thermophilic anaerobic sludge was built up over 4 weeks, with a very low substrate addition. After this initial phase, an OLR of 4.2 $g_{TS}{\cdot}L^{-1}{\cdot}d^{-1}$ and a HRT of 40 days were set, to ensure comparability with the previous experiment. Figure 9 shows the progression of the degradation rates of TC and TS, the concentrations of $NH_4$-N and organic acids in the reactor, and the methane yield during the experiment. The increased temperature allowed the introduced fats to liquefy in the reactor. Therefore, in this experiment, no accumulation of fat on the reactor surface or associated hindered degassing was observed. In addition, the increase in temperature led to reduced gas solubility in the reactor liquid, which also improved the degassing. Furthermore, the mixability in the reactor could be regained, which made use of the circulation pump no longer necessary. During the experiment, an average high degradation performance for TC and TS of 95 and 85%, respectively, was recorded. However, the methane yield was lower than in the mesophilic experiment, with an average of 0.52 $NL_{CH4} \cdot g_{TS\,eli}{}^{-1}$ and a maximum of 0.59 $NL_{CH4} \cdot g_{TS\,eli}{}^{-1}$. The $NH_4$-N concentration in the reactor during the experiment fluctuated between 1 and 3 $g \cdot L^{-1}$, as in experiment 2, and did not increase to the critical level. This can be attributed to the higher C/N ratio in the substrate compared to the flotate. However, the concentration of organic acids in the reactor with an average value of 6.3 $g \cdot L^{-1}$ was significantly higher throughout the entire experiment than in the mesophilic experiment. This was also reflected in the pH, which is shown in Figure 10. This was lower in the reactor during the entire experiment than in the other two experiments, although the pH value of the substrate was increased to 6.9 after pre-acidification in this experiment using caustic soda.

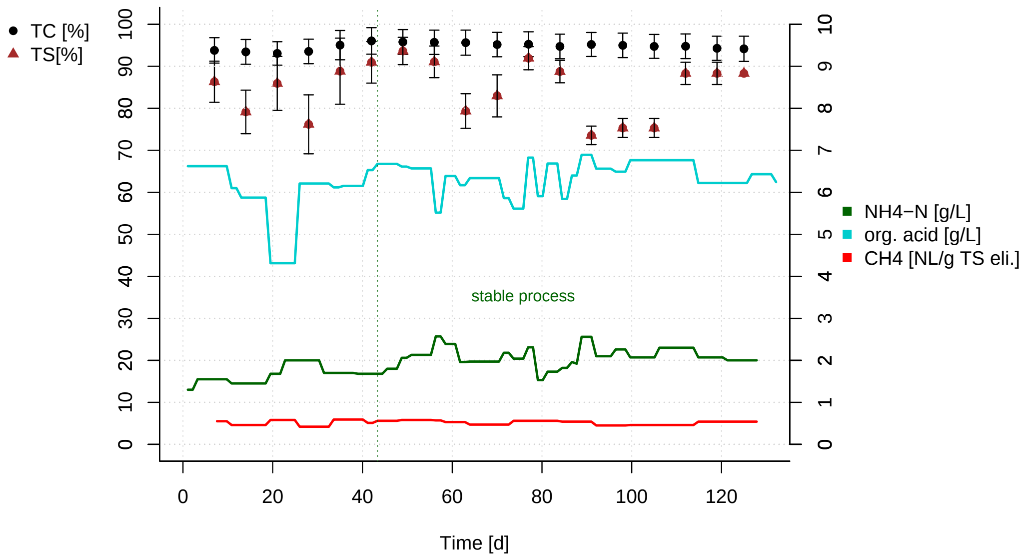


**Figure 9.** Thermophilic anaerobic digestion of slaughter waste mixed with wastewater at an organic load of 4.2 $g_{TS} \cdot L^{-1} \cdot d^{-1}$.

Since the concentration of organic acids remained constant above 5 and below 7 $g \cdot L^{-1}$ from day 30, and as the TC degradation and methane yield were constant from day 40, the reactor was considered stable from this point on. The reduced methane yield compared to the mesophilic experiment allowed the following conclusions to be made: The thermophilic operation of the reactor liquefied the fat contained in the substrate. This led to an increased bioavailability of the fats and improved degassing. However, due to the accumulation of organic acids in the reactor, it can be assumed that the organic acids formed faster than they could be converted into methane, which again led to inhibition of the methanising microoganisms. Thermophilic operation can thus accelerate hydrolysis and improve degassing; however, at the same time, the potential for inhibition by organic acids increases [7,19].

In a study by Bayr et al. [21], SW mixed with rendering plant waste was treated anaerobically and thermophilically in a constantly mixed reactor, achieving a methane yield of 0.8 $NL_{CH4} \cdot g_{TS\,add}{}^{-1}$ at an OLR of 1.6 $g_{TS} \cdot L^{-1} \cdot d^{-1}$. However, the fermentation became unstable after 100 days, which the authors attributed to the high ammonium and volatile fat concentrations of 4 $g \cdot L^{-1}$ and 2 $g \cdot L^{-1}$, respectively. In a comparable study by Wu et al. [22], waste from restaurant grease traps was treated anaerobically with a constantly stirred reactor. In thermophilic operation, a maximum methane yield of 0.6 $NL_{CH4} \cdot g_{TS\,add}{}^{-1}$ was achieved at a OLR of 1.8 $g_{TS} \cdot L^{-1} \cdot d^{-1}$. In addition, a recommendation for a maximum lipid concentration of 3.6 $g_{lipid} \cdot L^{-1}$ for the thermophilic digesters was made. Since this value was exceeded in the experiment carried out in this work, this may also explain the comparatively low methane yield.

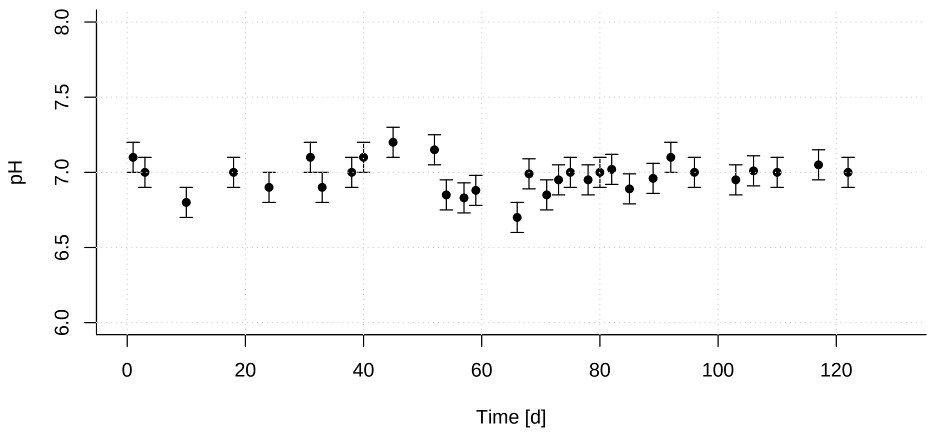


**Figure 10.** pH values of thermophilic anaerobic digestion of slaughter waste mixed with wastewater.

### 3.6. Comparison of Methane Yields

Figure 11 shows the methane yields of the continuous fermentation experiments as a box plot. The experiment with the mesophilic fermentation of the SW and flotate mixture resulted in the highest mean methane yield, but also the highest variance. The lowest averaged methane yield was observed in the thermophilic fermentation experiment. In comparison, it can be concluded that the mixture of flotate and SW is a potential substrate for anaerobic methane production, and compared to the flotate-only fermentation, the risk of $NH_4$-N accumulation and the associated inhibition of microoganisms through ammonia formation can be controlled. Although the C/N ratio can be increased by mixing the flotates with the SW, new challenges arise, due to the high fat content in the substrate. Therefore, it can be assumed that obtaining the highest methane yield is not only dependent on the substrate but also on the specific reactor design [7].

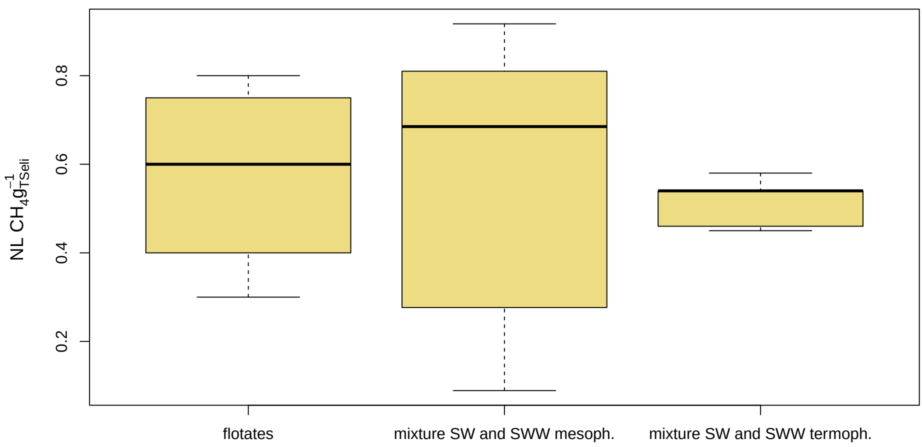


**Figure 11.** Comparison of the methane yields of the continuous experiments, without the first 40 days.

## 4. Conclusions

The BMP measurements showed that the methane yield potential of the mixture of flotate and SW was 0.75 $NL_{CH4} \cdot g_{TS\ added}{}^{-1}$ higher than that of the flotate alone. This led to the conclusion that mixing the substrates can counteract both the ammonia inhibition due to the high nitrogen content in the flotate and the inhibition due to the formation of organic acids because of the high fat content in the SW.

The continuous mesophilic fermentation experiments showed similar results. Inhibition at a $NH_4$-N concentration in the reactor higher than 4 $g \cdot L^{-1}$ was observed for the flotate digestion. The highest averaged methane yield of the continuous experiments resulted from the mesophilic fermentation of the flotate-SW mixture. However, problems occurred in connection with the accumulation of fat on the reactor liquid surface. This was solved by using a circulation pump. The pump regularly pumped anaerobic sludge from the reactor bottom and reintroduced it at the reactor surface. The thermophilic reactor operation was also able to counteract the fat accumulation, but this experiment led to an increased accumulation of organic acids in the reactor and thus also to the lowest average methane yield. In summary, anaerobic digestion of flotates and SW together appears to be feasible, as long as a OLR of 4.2 $g_{TS} \cdot L^{-1} \cdot d^{-1}$ is not exceeded and the mixing of anaerobic sludge with the fatty substrates at the liquid surface is ensured in the reactor design.

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## Abbreviations

| | |
|---|---|
| BMP | biochemical methane potential |
| COD | chemical oxygen demand |
| DAF | dissolved air flotation |
| DOC | dissolved organic carbon |
| FOG | fat oil and grease |
| HRT | hydraulic retention time |
| MBBR | moving bed biofilm reactor |
| OLR | organic loading rate |
| SW | slaughter waste |
| SWW | slaughter wastewater |
| TC | total carbon |
| TN | total nitrogen |
| TOC | total organic carbon |
| TS | total solids |
| VS | volatile solids |

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