Anaerobic Co-digestion of Sewage Sludge and *Typha Latifolia* and the Impact of Cattle Rumen Fluid on Biogas Production

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Abstract This study investigated the potential for anaerobic co-digestion of sewage sludge and the Typha latifolia plant in biogas production. The impact of cattle rumen fluid added to reaction mixtures was also investigated regarding biogas production. Four different combinations of these substrates were tested. The anaerobic digestion studies were performed in 1 L batch reactors under mesophilic conditions, with a hydraulic retention time of 55 days. The results showed that co-digestion of sewage sludge and the Typha latifolia plant significantly enhanced biogas production in comparison with mono-digestion of sewage sludge, since the total amount of biogas produced increased by almost 50 %, from 385 up to 578 mL of biogas/g of dry matter. The highest amount of biogas (614 mL/g of dry matter) was produced when cattle rumen fluid was added to the mixture, suggesting that rumen microorganisms exerted a positive effect on substrate degradation.

Keywords: anaerobic co-digestion, biogas production, sewage sludge, *typha latifolia*, cattle rumen fluid

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1 Introduction

Sewage sludge (SS), a by-product of wastewater treatment, could, if not properly treated, create huge environmental risks, given the presence of pollutants such as pathogens, heavy metals and dioxins. On the other hand, it is rich in nutrients (nitrogen, phosphorus, potassium, etc.), and thus offers great potential for recovering these nutrients to become valuable products (Kacprzak *et al.*, 2017). In Europe, about $10 \cdot 10^6$ t/y dry matter (DM) of sewage sludge is produced (Milieu Ltd, 2008). As a result of more stringent requirements for water treatment, its production is increasing in all European countries. Thus, there is increasing pressure and challenge to find better treatment and disposal methods for this waste (Bianchini *et al.*, 2016).

Commonly used technologies for energy and/or resource recovery from sewage sludge include anaerobic digestion, incineration, pyrolysis and gasification (Raheem *et al.*, 2018). Anaerobic digestion is among the more widely employed processes for sludge stabilization, owing to its relatively high methane production capacity, lower energy consumption, and the ability to deactivate pathogens in biomass (Montalvo *et al.*, 2017). In order to balance the C/N ratio, which significantly impacts biogas production, sewage sludge could be co-digested with various organic substrates such as a range of food wastes (Mehariya *et al.*, 2018), animal wastes (Borowski *et al.*, 2013) and other organic wastes (Elalami *et al.*, 2019). The l*ignocellulosic* plant *Typha latifolia*, which grows in or near water and is widely used for wetlands to remove nitrate, ammonia and heavy metals from (waste)water, represents one of the renewable substrates that could be applied in anaerobic co-digestion (Hu *et al.*, 2006).

Anaerobic digestion performance depends on many factors, especially since methanogens, the microorganisms mainly responsible for the methane production during anaerobic digestion, are sensitive to contaminants and can easily be inhibited (Paulo *et al.*, 2015). A similar microbial consortium can be found in the stomachs of ruminant animals (Neshat *et al.*, 2017). Various authors have claimed that rumen microbial cultures have shown good prospects for anaerobic digestion of lignocellulosic materials because of their ability to increase the hydrolysis of lignocellulosic substrates (Čater *et al.*, 2014), such as cattail (Hu & Yu, 2006) and corn straw (Jin *et al.,* 2018).

The aim of this study was to examine the potential of sewage sludge and *Typha latifolia* grass (cattail) for anaerobic co-digestion and to investigate the impact of cattle rumen fluid (microbial consortium) on biogas production.

2 Material and methods

2.1 Substrate characteristics

Sewage sludge containing 18.47 % total dry solids was taken from a local municipal wastewater treatment plant, while the *Typha latifolia* grass was gathered near the Dravinja riverbank. The inoculum was obtained from a local biogas plant that produces biogas from poultry manure, and rumen fluid was acquired from a nearby slaughterhouse. Before being used in the experiments, the rumen fluid was filtered through fabric to remove larger particles. The *Typha latifolia* grass was cut into pieces (with a size of approximately 0.8 cm x 0.4 cm).

The basic characteristics of sewage sludge, *Typha latifolia* grass, inoculum and cattle rumen fluid were determined before performing anaerobic digestion, such as total dry solids content (TS), water content, total nitrogen (TN) content, total phosphorus (TP) content and total carbon (TC) content (Table 1). The following analytical methods were used: SIST EN 16168:2013 for determining total nitrogen content, SIST EN ISO 11885:2009 for the total phosphorus content, SIST EN 13137:2002 for total carbon content and SIST EN 14346:2007 for the dry matter content.

Table 1: Basic characteristics of the substrates.

Parameter	Sewage sludge	*Typha latifolia* grass	Inoculum	Cattle rumen fluid
Total dry solids (TS, %)	18.47	11.11	8.02	2.42
Water content (%)	81.53	88.89	91.98	97.58
Volatile solids (% TS)	80.06	/	/	/
TN (% TS)	7.83	3.48	3.65	3.67
TP (% TS)	0.94	0.30	0.33	0.06
TC (% TS)	45.71	46.65	28.61	31.27

2.2 Anaerobic digestion studies

The anaerobic digestion studies were performed in 1 L batch reactors with a working volume of 600 mL and a retention time of 55 days. Four different reaction mixtures were prepared in two parallel batches (Table 2):

- Mixture 1 containing inoculum and sewage sludge (samples S1, S2) in a ratio of 1:1;
- Mixture 2 consisting of inoculum, sewage sludge and the *Typha latifolia* grass (samples SG1, SG2) in a ratio of 2:1:1;
- Mixture 3 containing a mixture 1 + 50 mL of cattle rumen fluid (samples SR1, SR2);
- Mixture 4 containing a mixture 2 + 50 mL of cattle rumen fluid (samples SGR1, SGR2).

Table 2: The composition of reaction mixtures.

Reaction mixture	**Inoculum (g TS)**	**Sewage sludge (g TS)**	***Typha latifolia* grass (g TS)**	**Cattle rumen fluid (mL)**
S1, S2 (Mixture 1)	15	15	/	/
SG1, SG2 (Mixture 2)	15	7.5	7.5	/
SR1, SR2 (Mixture 3)	15	15	/	50
SGR1, SGR2 (Mixture 4)	15	7.5	7.5	50

30 g of total solids per 500 g of liquid were added to each reactor. Thus, the dry matter content of each reaction mixture was 6 wt.%. For all mixtures 1-4, the ratio between inoculum and substrates for anaerobic digestion was 1:1. (15 g: 15 g). To achieve a dry matter concentration of 6 wt.%, a buffer solution (Angelidaki *et al.*, 2009) was added to each reaction mixture. After preparing the mixtures, the reactors (filter flasks for vacuum use, sealed with PTFE septa) were placed into a heating bath and flushed with inert argon gas for about 30 s to achieve anaerobic conditions. A constant temperature of 42 °C (mesophilic conditions) was maintained by using an immersion circulator (Thermo Scientific™ SC100). The flasks were hand-mixed daily for about 30 s.

Biogas production was measured daily by a water displacement method, while pH and conductivity were measured periodically. Wireless pH and conductivity sensors (Pasco) were used for this purpose, whereas around 4 mL of the samples were taken for analysis from the reactors using a 5 mL syringe with a needle. After the analysis, the samples were returned to the flasks. The composition of biogas produced was tested with an Optima 7 Biogas analyser.

3 Results and discussion

Anaerobic digestion depends on various operating parameters such as temperature, pH, system configuration, substrate composition and hydraulic retention time (Fang *et al.*, 2020). In the following, the results obtained in mono- and co-digestion of sewage sludge (SS) and *Typha latifolia* grass are presented in regard to some of these operating parameters.

3.1 Biogas production and the effect of hydraulic retention time (HRT)

Hydraulic retention time significantly affects biogas production, digestion rate, microbial flux and the C/N ratio of the digestate (Mehariya *et al.*, 2018). The volume of biogas produced in regard to hydraulic retention time in mono-digestion of sewage sludge is shown in Figure 1a) (samples S1 and S2), while results from the co-digestion experiments are shown in Figures 1b), c) and d).

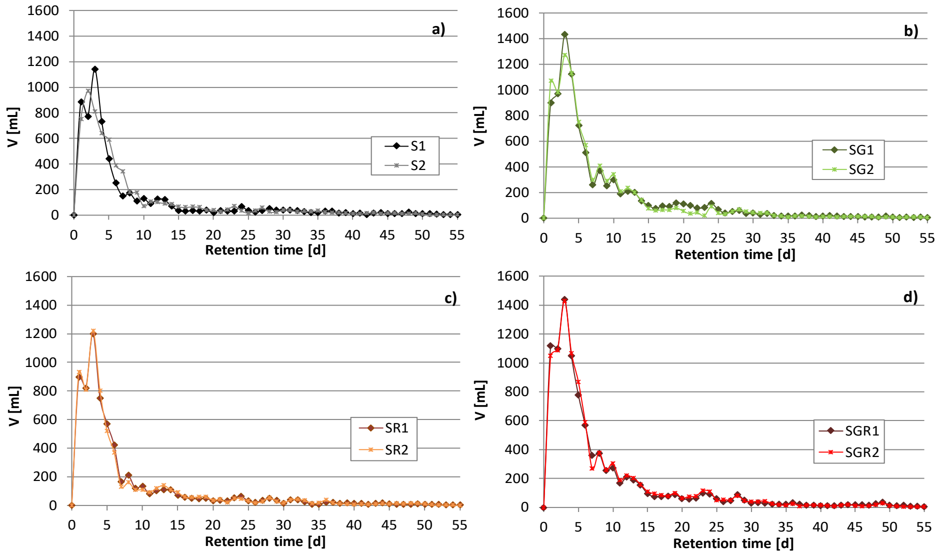

Figure 1: Volume of biogas produced regarding HRT in the case of: a) mono-digestion of SS; b) co-digestion of SS and *Typha latifolia* grass; c) co-digestion of SS and rumen fluid; and d) co-digestion of SS, grass and rumen fluid.

The majority of the biogas was produced in the first 15 days of the experiment; between days 16 and 35, significantly smaller amounts of biogas were measured, while after 35 days, the values were almost negligible. In the co-digestion of SS and rumen fluid (samples SR1, SR2; Fig. 1c), characteristics similar to those in the mono-digestion of SS were observed, except that between days 10 and 20, slightly more biogas was produced. This suggests that rumen fluid microorganisms and enzymes did contribute significantly to sludge decomposition.

When SS was co-digested with the *Typha latifolia* grass (samples SG1, SG2; Fig. 1b), up to 50 % higher biogas yield was noted, as compared to the SR1 and SR2 samples or to the S1 and S2 samples. The reason for improved biogas yield could be the improved C/N ratio of the substrate with the addition of grass. Co-digestion with organic substrates like *Typha latifolia* in comparison with mono-digestion offers better digestibility, better nutrient availability (Grosser *et al.*, 2018), increased pH buffering capacity and dilution of toxic substances such as ammonia (Fang *et al.*, 2020).

The highest biogas yield was achieved with the mixture of SS, grass and rumen fluid (samples SGR1, SGR2; Fig. 1d). For this sample, even after 25 days of the experiment, biogas yield was still relatively high. The microbial communities in rumen fluid increase the hydrolysis of lignocellulosic substrates and the production of fatty acids (Čater *et al.*, 2014). Rumen microorganisms comprise ~10^8-10^{10} methanogens/g, with great potential in biogas production (Jin *et al.*, 2018). The systems containing rumen microorganisms showed improved degradation efficiency and conversion rate (Hu & Yu, 2006).

Average cumulative volume of biogas produced for all the samples (in mL/g of dry matter) is shown in Figure 2. The lowest biogas production was obtained in mono-digestion of SS (samples S), 385 mL/g, while in the case of co-digestion with grass, it was 578 mL/g, an improvement of almost 50 %. With the addition of cattle rumen fluid to the mixture of SS and grass, total biogas production increased even more (6 % more), achieving the highest value among all tested reaction mixtures (614 mL/g of dry matter). Similarly, the addition of rumen fluid slightly improved biogas yield during mono-digestion of SS (7 %).

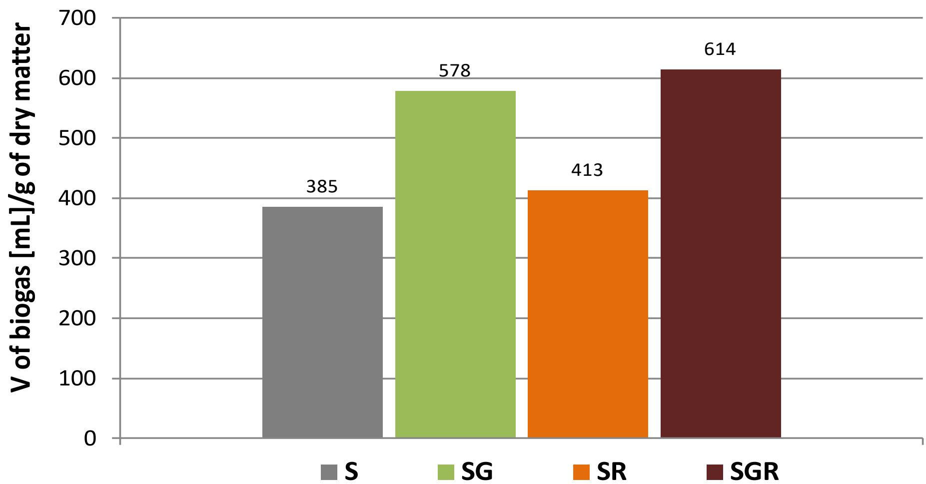

Figure 2: Total volume of biogas produced (mL)/g of dry matter.

3.2 Impact of C/N ratio

The optimal C/N ratio for anaerobic co-digestion is around 20 and might differ according to the operating temperature and type of substrate used (Ma *et al.*, 2018). SS typically has a lower C/N ratio (6–9), which can be improved by mixing with substrates with a higher C/N ratio (18-22), such as food wastes (Awe *et al.*, 2018). The C/N ratio for SS in this study was 5.8, and the C/N ratio of *Typha latifolia* grass was 13.4 (see Table 1), which means that mixing these two substrates improved the C/N ratio of SS and increased biogas yield. The C/N ratio of rumen fluid amounted to 8.5 (see Table 1), but since the rumen fluid was added only in small quantities, its impact was not significant to the overall C/N ratio.

3.3 pH impact

The majority of studies show that temperature (Obulisamy *et al.*, 2016) and pH value are among the key parameters in the anaerobic digestion process (Fang *et al.*, 2020). The system's performance is highly dependent on its buffering capacity, which can be attained through the addition of a buffering agent (Mehariya *et al.*, 2018). pH values for all the studied reaction mixtures for each parallel batch separately are shown in Figure 3.

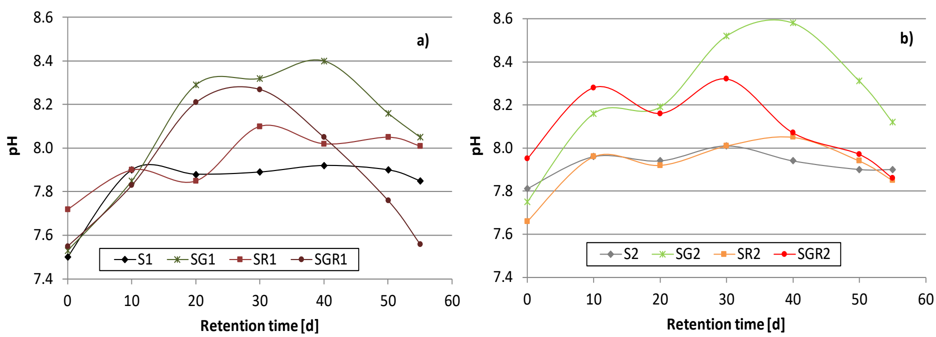

Figure 3: pH values for reaction mixtures with retention time for: a) first parallels and b) second parallels.

pH values in all reaction mixtures increased in the first 10 days of the experiment. In the case of mono-digestion of SS (samples S1, S2), after 10 days the values were relatively stable. These stable values could be due to the buffering agent that was added to each reaction mixture. Similar trends were observed for samples SR1 and

SR2, where rumen fluid was added to the SS. For both, the pH values were mainly below 8.0. For co-digestions of SS and *Typha latifolia* grass (samples SG and SGR), changes in pH value were more significant (a higher increase initially, and a greater decrease at the end of the experiment). The highest pH values were measured in samples SG1 and SG2 and were 8.4 and 8.58. The pH values are just within the optimal pH range, which is between 7 and 8.5 (Fernández-Cegrí *et al.*, 2012); thus, no greater inhibition effect was observed during these experiments.

4 Conclusions

In this study the potential of anaerobic co-digestion of sewage sludge and the *Typha latifolia* plant with/without the addition of rumen fluid was investigated. Mono-digestion of SS (samples S) produced the lowest biogas yield, 385 mL/g of dry matter, which is compared to previous studies in the literature, higher than the average amount reported, which is 350 mL/g of dry matter (Grosser & Neczaj, 2018). Thus, the results of this study are comparable with those from similar systems and show good prospects for further investigation.

Co-digestion of SS with *Typha latifolia* grass enhanced biogas production compared to mono-digestion of SS by almost 50 %. The addition of cattle rumen fluid slightly improved the process, since it increased biogas production by 6-7 %. However, greater improvements were expected. Thus, it would be interesting to further study the influence of the quantity of cattle rumen fluid added to the samples on biogas and digestate production.

Acknowledgments

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