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Research article

Anaerobic co-digestion of swine manure and crude glycerol derived from animal fat—Effect of hydraulic retention time

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Abstract: Crude glycerol (CG), an abundant by-product of bio-diesel production, has been identified as a suitable co-substrate for improving the biogas production of livestock manure through anaerobic digestion (AD). In this study, the potential of utilizing CG generated from the esterification of animal fats for biogas production was studied in both batch and continuous AD experiments, with emphasis on the importance of the hydraulic retention time (HRT). Batch experiments showed that the limiting step in the methane production rate during CG mono-digestion was the 1,3-propanediol uptake. Additionally, biochemical methane potential tests indicated that the addition of 1% w/w CG to swine manure-AD is more efficient in terms of percent of theoretical amount of methane obtained than the addition of 3% w/w. However, in continuous experiments, co-digestion of manure with 3% w/w CG did not exhibit any sign of inhibition within the HRTs tested (17–22 days). Moreover, a 222% increase of biogas productivity was observed with 3% CG supplementation at an HRT of 17 days, in contrast to a 146% increase at an HRT of 22 days. Based on this, and on the similar efficiency of soluble COD removal among the processes (ca. 93%), it was shown that it is possible to reduce the HRT without affecting negatively the efficiency of conversion of manure. Moreover, it was shown that CG from 2nd generation biodiesel based on animal fat, is a suitable feedstock for boosting the methane production of manure-based biogas plants.

Keywords: anaerobic digestion; manure; crude glycerol; co-digestion; biogas; methane

1. Introduction

Anaerobic digestion (AD) is a widely applied waste management process for stabilizing the nutrient content of livestock manure, and ensuring a safer disposal and application to agricultural soils. An additional benefit of AD is that during the process, biogas is produced which is a renewable energy source. However, as a result of the low C/N ratio and the high dilution of manure, mono-digestion results into poor biogas production. In the last decades, the co-digestion of manure with other organic substrates has been successfully applied and undoubtedly improved the economic performance of biogas plants [1].

In recent years, a significant increase of biodiesel production through the esterification process of vegetable oils and animal fats has been evidenced [2,3], resulting in the generation of large amounts of crude glycerol (CG), the main by-product of biodiesel production. Crude glycerol has been identified as a suitable co-substrate for improving manure-based AD. Depending on the origin of the initial feedstock, CG is composed of glycerol (also called glycerin) along with impurities such as methanol, oils, salts, heavy metals, volatile fatty acids (VFAs) and water, that make its purification and commercialization a complex and expensive process [4]. Consequently, this substrate is highly available and not easily absorbed by the current glycerol market. On the other hand, it has a high carbon content and low nitrogen concentration that could balance the AD of manure and boost the biogas production.

Earlier studies have shown that an increase of 150–400% of biogas productivity is possible when adding CG to livestock manure [5,6]. On the other hand, at high CG concentrations, inhibitive effects such as accumulation of VFAs, overproduction of H_2S and foaming phenomena have been reported [5,7], resulting in failure of the AD processes. These effects are a result of the impurities of CG, which are highly dependent on the origin of the substrate and any further processing. The majority of studies up to now have tested the co-digestion of manure with CG derived from the transesterification process of vegetable and cooking oils. However, CG originating from biodiesel production of animal fats and meat processing industry residues that often present high content of impurities [8] could be more inhibitory to the microbial community, and to our knowledge its co-digestion with swine manure has not been tested yet.

The aim of this study is to investigate the performance of co-digestion of swine manure with CG originating from the transesterification of animal fats. Initially, biochemical methane potential (BMP) tests were carried out for assessing the biodegradability of the substrates used and of their different co-digestion mixtures. Subsequently, continuous anaerobic digesters were operated with increasing supplementation of CG to swine manure AD at different hydraulic retention times (HRTs) for evaluating the importance of this factor on the stability and performance of the process.

2. Materials and methods

2.1. Substrates and inocula

The swine manure used in this study was collected from the Hashøj biogas plant in Sjealand (Denmark) and stored in sealed containers at −18 °C. Prior to use, each container was thawed and maintained at 4 °C. The Total (TS) and Volatile solid (VS) content of the manure used were 23.34 ± 0.24 g TS/L and 15.49 ± 0.43 g VS/L respectively, and the pH was 7.5 ± 0.1. The CG used was a by-product of the biodiesel production from butchery waste (based on animal fat

categories 1 and 2 according to the EU regulation number 1069/2009 [9] and 142/2011 [10]) and was collected from Daka Biodiesel (Denmark). The specific characteristics of CG are shown in Table 1. The pH of the CG was equal to 1.5. The inoculum used for both the BMP tests and the continuous AD experiments originated from two bench-scale digesters operated with swine manure at mesophilic conditions (37 °C).

Table 1. Characteristics of CG *.

Component	Content
Moisture	10%
Ash	5%
Glycerol	75%
Fat	10%
Methanol	<1%
Sulphur	1–2%

*adapted from [11].

2.2. Biochemical methane potential tests

Two sets of BMPs were run. The first set comprised of BMP tests of CG at three different organic loadings, in order to test if any inhibitory effect occurred. The CG A, CG B, and CG C loadings corresponded to the following inoculum-to-substrate ratios (I:S): 4:1, 2:1 and 1:1, respectively (VS basis). The second set of BMPs was carried out for determining the ultimate CH_4 yield of the swine manure alone, the mixture of 99% w/w swine manure and 1% w/w CG (1% CG), and 97% w/w swine manure plus 3% w/w CG (3% CG).

All BMP tests were set in 320 mL infusion bottles and were run in triplicates. In each set of experiments, BMP tests were also set only with inoculum and were used as blanks. The bottles were flushed with a mixture of 80% N_2 and 20% CO_2, sealed with rubber stoppers, secured with aluminum crimps and placed in an incubator at 37 °C. The CH_4 production was monitored periodically until the end of the experiments. The CH_4 production of the BMP tests of substrates was corrected for the residual production of the inoculum by subtracting the CH_4 production of the blank tests. Liquid samples from the CG A, B, and C BMP tests were withdrawn periodically for monitoring the concentration of glycerol, 1,3 propanediol (1,3-PDO) and VFAs. Concentrations of soluble compounds were corrected for residual concentrations of the blank tests (containing only inoculum).

2.3. Continuous mode experiments

Two continuous stirred tank reactors (CSTR)-type digesters (3 L active volume) were used in the present study, both running under mesophilic conditions (37 °C). In order to ensure comparability of the two digesters, the initial inoculum was well mixed and distributed simultaneously to the CSTRs. Initially, the digesters were inoculated and left to acclimate for 2 days without any feeding. In continuation, both digesters were fed with swine manure (phase I), one with a HRT of 17 days (digester A) and the other one with a HRT of 22 days (digester B). After 30 days of operation, the feed of the digesters was changed to a mixture of 99% w/w manure and 1% w/w CG on a wet mass basis (Phase II, 30–78 days). Following phase II a mixture of 97% w/w manure

and 3% w/w CG was initiated and lasted until the end of the experiments (Phase III, 79–120 days). In order to reduce errors associated to inhomogeneity of the mixture and ensure that no degradation of glycerol would occur prior to digestion, the mixture was prepared in a large container and subsequently divided into 2-L plastic containers and stored at −20 °C. The 2-L containers were thawed twice per week and added to the influent flasks of the digesters that were kept at 4 °C.

Both digesters were fed once per day by means of peristaltic pumps after rigorous mixing of the feed for 10 min. The stirring of the digesters was intermittent and took place every 3 hours for 10 min by means of overhead stirrers. The heating of the digesters was achieved by water jackets using a recirculating water bath. Biogas production was measured continuously with Ritter MilliGas counters (Ritter, Germany).

The digesters were monitored twice per week on the biogas composition in CH_4, and weekly on the concentration of volatile fatty acids (VFAs), glycerol, 1,3-PDO, soluble COD, NH_4^+-N content, and pH. Samples for TS and VS determination were taken weekly from the influent, inside the digesters and effluents. The biogas productivities and the CH_4 yields of the two digesters at different phases were subjected to Student's t-test at a 95% significance level.

2.4. Analytical methods

The TS and VS content of the samples was determined following Standard Methods [12]. Soluble COD and NH_4^+-N were determined by means of HACH Lange kits LCK 514 and LCK 305 respectively after centrifugation at 10,000 rpm for 10 min and filtration through Whatman filters with a pore size of 0.45 μm. The total COD of the substrates was determined by HACH Lange LCK 914. VFAs, 1,3-PDO and glycerol quantification was done by means of an HPLC (Shimadzu, USA) equipped with a refractive index detector and an AMINEX HPX-87H (Bio-Rad) column at 63 °C. A solution of H_2SO_4 12 mM was used as eluent at a flow rate of 0.6 mL/min. The samples for the HPLC were centrifuged at 10,000 rpm for 10 min, filtered through 0.45 μm, acidified with H_2SO_4 (10% w/w), centrifuged at 10,000 rpm for 10 min and filtered through 0.20 μm. CH_4 determination was done by a GC (Mikrolab Aarhus, Denmark) equipped with a packed column (6 ft. and I.D. 3 mm) and a thermal conductivity detector. N_2 was used as a carrier gas and the injector, oven and detector were all set at 70 °C. All gas volumes are given at 20 °C, unless otherwise stated. All CH_4 yields reported are expressed per g VS of substrate added.

3. Results and discussion

3.1. Theoretical CH_4 yields of substrates

The theoretical CH_4 yields of the substrates and their mixtures were calculated based on the COD measurements and assuming 0.35 m^3/kg O_2 at STP. The total COD of the swine manure was measured to be 29.00 ± 1.15 g/L, and of the CG it was 1255 ± 21 g/L. Thus, the ratios of g COD/g VS of manure and CG were 1.89 g COD/g VS and 1.52 g COD/g VS respectively. Finally, the theoretical maximum CH_4 yield of manure was calculated to be 658 ± 50 mL/g VS and the maximum CH_4 yield of CG was 573 ± 9 mL/g VS.

3.2. Ultimate CH_4 yield of CG

The cumulative CH_4 yields of the BMP tests of CG at different organic loadings are shown in Figure 1. As observed in the graph, all BMP tests presented a lag phase as almost no CH_4 production was detected after 2 days of incubation. However a sharp increase of the CH_4 production was detected after 5 days of digestion. Thereafter, only the lowest CG loading continued to produce CH_4 at a high rate, while the higher loadings presented signs of partial inhibition (reduced rates compared to CG A). The latter could be either due to overloading of easily degradable organic matter, or due to a higher concentration of inhibitory compounds such as salts [13].

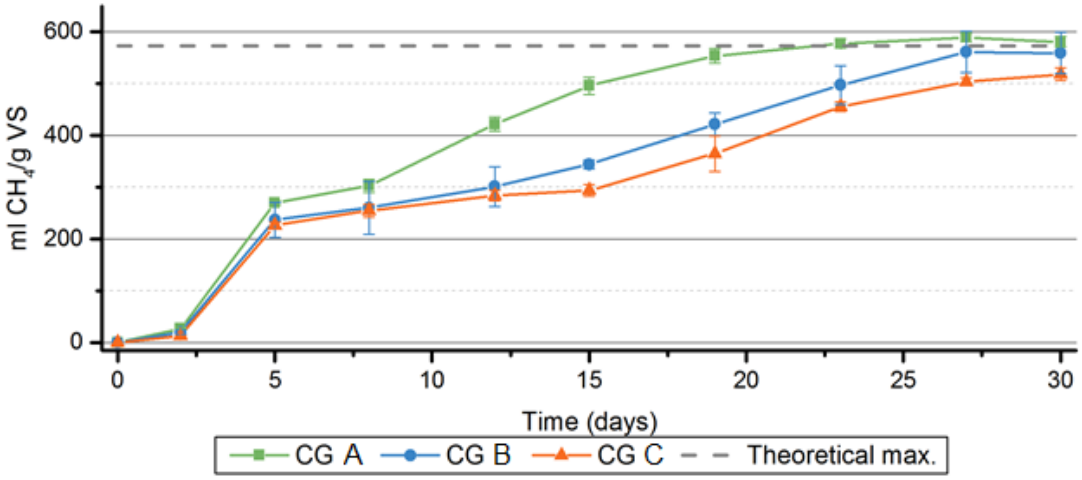

Figure 1. Cumulative CH_4 yields of BMPs of CG at different organic loadings. CG A, B and C correspond to I:S ratio on a VS basis of 4:1, 2:1 and 1:1 respectively.

The evolution of the glycerol concentration in BMPs (Figure 2a) shows that glycerol was broken down to intermediate products after 5 days of digestion. The main intermediate was 1,3-PDO as it can be seen in Figure 2b, along with VFAs (Figure 2c), comprised mainly of caproic and acetic acid. The further degradation of 1,3-PDO has been suggested to be towards acetyl CoA [14]. The uptake of 1,3-PDO appeared to be the limiting step due to the slow consumption rate evidenced in all BMP tests (Figure 2b). Moreover, a minor peak of VFAs was detected in the BMPs with the lower organic loading (CG A) after 12 days compared to peaks after 19 and 23 days in the case of CG B and CG C, respectively (Figure 2c). These findings are in agreement to [15] who reported an increased inhibitory effect of 1,3-PDO in comparison to other intermediate products. Finally, after 30 days of digestion all BMP tests have reached an end producing on average 544 ± 29 mL CH_4/g VS, corresponding to 94.9% of the theoretical maximum (section 3.1). This indicates that the entire substrate was consumed, as a 5% COD could be attributed to the fraction utilized by microbes for growing.

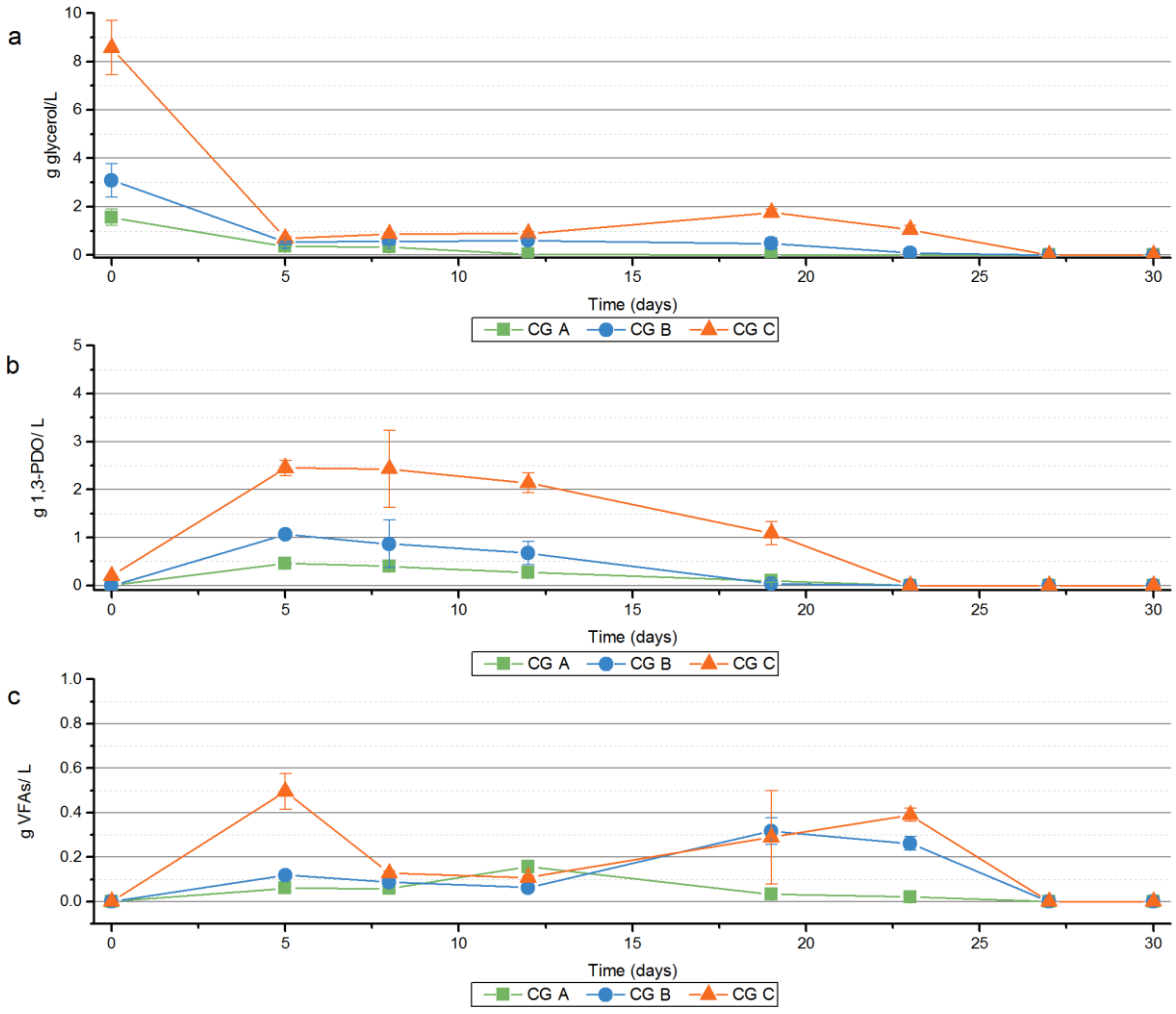

Figure 2. Evolution of (a) glycerol concentration, (b) 1,3-PDO concentration and (c) VFA concentration in BMP tests of CG at different organic loadings. CG A, B and C correspond to I:S ratio on a VS basis of 4:1, 2:1 and 1:1 respectively.

3.3. Ultimate CH_4 yield of manure and CG mixtures

The second set of BMPs was carried out for determining the ultimate CH_4 yield of swine manure and the mixtures of 1% CG and 3% CG that were subsequently fed to the continuous anaerobic digesters. The cumulative CH_4 yield curves are presented in Figure 3, where it can be seen that all substrates were consumed rapidly. The ultimate CH_4 yield of the swine manure reached 605 ± 15 mL CH_4/g VS on average, while the 1% CG and 3% CG produced 639 ± 10 mL/g VS and 530 ± 11 mL/g VS of CH_4 respectively. Thus the addition of 1% CG to manure resulted in a 6% increase of the CH_4 yield. However, with the addition of 3% CG, a decrease of 12% of the ultimate CH_4 yield was observed. Interestingly, the efficiency of the co-digestion of 99% manure with 1% CG was the highest in comparison to the other mixture and to manure alone, even though this mixture did not have the highest theoretical CH_4 yield. More specifically, manure reached approximately the 91.9% of the theoretical CH_4 yield (see section 3.1), while the 1% CG and 3% CG reached 99.7% (641 ± 36 mL CH_4/g VS) and 93.5% (567 ± 30 mL CH_4/g VS) respectively. This indicates that the co-digestion of 97% manure with 3% CG was probably partially constrained either

from nitrogen limitation, or due to the presence of impurities that produced inhibition. N deficiency has been mentioned by [16] to be the reason why a supplementation of CG to sewage sludge, higher than 1%, did not result in improved efficiency. However, in contrast to sewage sludge, swine manure has a significantly higher N concentration that can permit the glycerol consumption. Thus, the lower efficiency of the 3% CG supplementation is probably a result of the impurities present in CG generated from the processing of animal fats for biodiesel production.

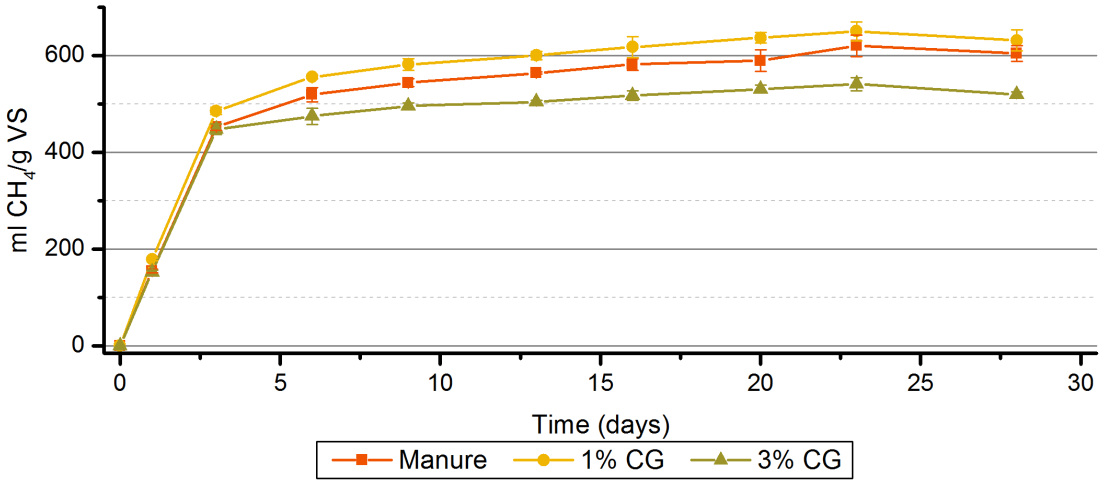

Figure 3. Cumulative CH_4 yields of second set of BMPs of manure, 99% of manure and 1% CG (1% CG), and 97% manure and 3% CG (3% CG).

3.4. Continuous AD experiments

BMP tests are very useful for providing information on the ultimate CH_4 yield of different substrates and constitute the common approach for obtaining preliminary information regarding the incorporation of new feedstocks in industrial AD processes. However, continuous AD experiments can offer information on both the productivity and stability of co-digestion, which are more relevant to an industrial process where the HRT can be decisive on how well the process performs [17]. Additionally, inhibition effects that have been evidenced in batch tests might change, as accumulation of inhibiting factors is not favored in continuous experiments. Based on this, it was of interest to compare two co-digestion processes at different HRTs, for evaluating whether it was possible to reduce the HRT without affecting significantly the process stability and efficiency. The approach followed in this study was based on increasing the share of CG in consecutive steps, in order to permit adaptation of microorganisms to CG. During phase I, the two digesters were fed only with swine manure and the HRT of digester A and digester B was set at 17 and 22 days respectively. This permitted to compare the process performance of the co-digestion to the mono-digestion of swine manure at different HRTs.

During phase I, digester A was operated at an organic loading rate (OLR) of 0.97 g VS/L/day in comparison to digester B that ran with 0.75 g VS/L/d. As expected, digester B presented lower biogas productivity and higher CH_4 yield compared to digester A (Table 2), as more time was allowed for the microbes to biodegrade the organic matter of manure. This resulted also in a slight increase of the NH_4^+-N concentration in digester B, probably due to an improved digestion of nitrogenous compounds such as proteins. The VFA concentration was slightly higher in digester A,

though in both processes the VFA concentration was low enough (<0.5 g/L) to avoid a drop of pH. Overall, both processes were operated without any signs of instability and the reduction of soluble COD was similar among the digesters, corresponding to approximately 84%. The biogas productivity of digester A was 0.46 ± 0.02 L/L/d, similar to 0.41 ± 0.02 L/L/d observed from digester B. The CH_4 content of the biogas produced in both digesters was the same, reaching 64.5%.

Following the addition of 1% CG after day 30, both digesters responded with an increase in the biogas productivity and no process instability occurred as indicated by the low VFA concentration and the stable pH values (Table 2). However, the biogas productivity of digester B was stabilized faster as it can be observed in Figure 4 and the glycerol concentration in digester A was 0.12 g/L after 3 days of mixture-based digestion in comparison to 0.04 g/L in digester B. After 1 week of co-digestion the glycerol concentration in both digesters was less than 0.03 g/L. Similarly, the 1,3-PDO concentration in digester A was 0.06 g/L and in digester B 0.04 g/L during the first week and thereafter no 1,3-PDO was detected. Consequently, it can be assumed that during the continuous process, microbes were adapted faster to 1,3-PDO than in the batch experiments. The CH_4 content of biogas was significantly increased reaching 68.0% and 70.9% from digester A and B respectively. The biogas productivity from digester A in phase II was 0.81 ± 0.06 L/L/d, corresponding to a 76% increase as compared to phase I where the digester was fed only with swine manure. On the other hand, the increase of biogas productivity of digester B at phase II corresponded to 46% in comparison to phase I. These increments were of the same magnitude to the increase observed by Astals et al. [5] when adding 1% w/w of vegetable-oils-derived CG to swine manure at a digester with similar HRT (20 d), indicating thus that even when using CG generated from processing animal fats for biodiesel production (as used in this study) and when the supplementation is as low as 1% w/w, similar improvements can be obtained in manure-based AD processes.

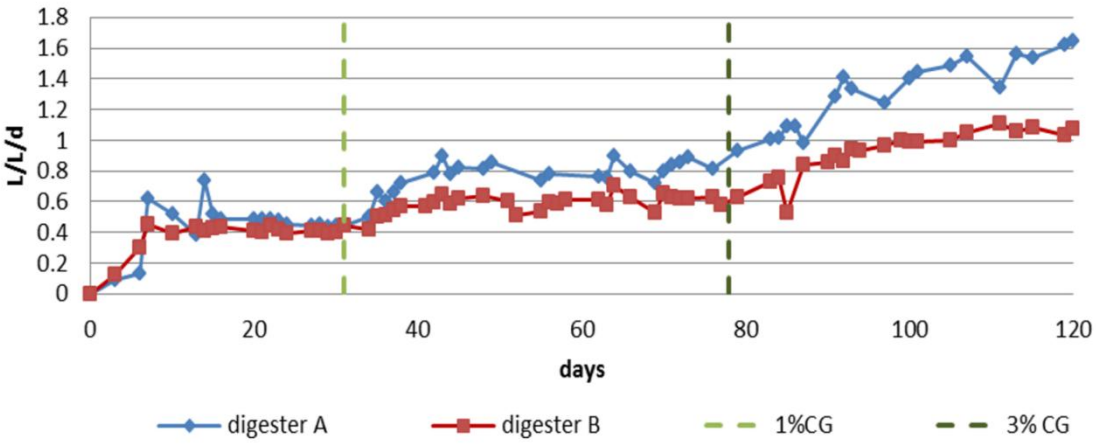

Figure 4. Biogas productivity of digester A and B (with HRT of 17 and 22 days respectively) fed with swine manure and an increased CG supplement.

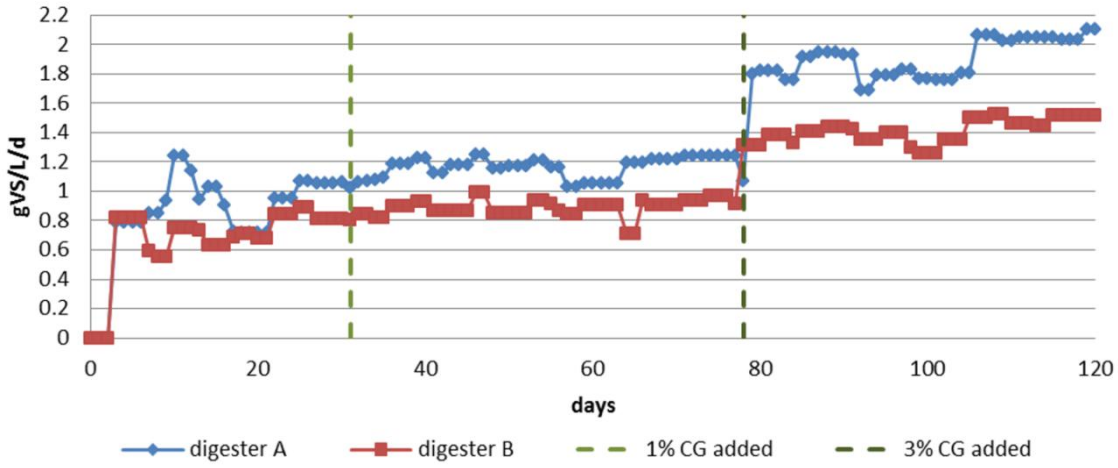

Figure 5. Organic Loading Rate of digesters A and B during the entire course of experiments.

On day 78, feeding of 3% CG mixture was initiated (phase III), resulting to a 2-fold increase of the OLR on both digesters as compared to phase I (Figure 5). Both digesters responded relatively fast. The biogas production started to increase sharply in both processes, and no signs of inhibition were observed as implied also by the very low levels of intermediate products, 1,3-PDO and acids (Table 2). The concentrations of both glycerol and 1,3-PDO remained lower than 0.001 g/L and no accumulation of VFAs was detected. However, the biogas productivity of digester B was more stable than the productivity of digester A that continued to increase for more than 20 days after the increase of the OLR (Figure 4). Overall, during phase III, digester A and digester B presented a 222% and 146% increase of biogas productivity.

Table 2. Average characteristics of digesters A and B during Phase I (manure only), II (1% CG added) and III (3% CG added)*.

Characteristic	Phase I (days 14–30)		Phase II (Days 43–76)		Phase III (Days 100–120)	
	Digester A	Digester B	Digester A	Digester B	Digester A	Digester B
g VS manure: g VS CG	100:0	100:0	65:35	65:35	38:62	38:62
Organic Loading Rate (g VS/L/d)	0.94 ± 0.16	0.75 ± 0.10	1.17 ± 0.07	0.91 ± 0.09	1.91 ± 0.13	1.42 ± 0.08
pH	8.3 ± 0.1	8.3 ± 0.1	8.3 ± 0.1	8.3 ± 0.1	8.2 ± 0.0	8.2 ± 0.1
NH_4^+-N (g/L)	2.24 ± 0.8	2.54 ± 0.3	1.96 ± 0.44	2.34 ± 0.06	2.16 ± 0.17	2.21 ± 0.21
VFAs (g/L)	0.42 ± 0.15	0.33 ± 0.15	0.43 ± 0.09	0.39 ± 0.15	0.33 ± 0.04	0.23 ± 0.02
Biogas productivity (L/L/d)	0.46 ± 0.02a	0.41 ± 0.02b	0.81 ± 0.06c	0.60 ± 0.04d	1.48 ± 0.13e	1.01 ± 0.07f
CH_4 yield (L/g VS)	0.33 ± 0.08a	0.35 ± 0.07a	0.48 ± 0.04b	0.47 ± 0.04b	0.48 ± 0.04b	0.48 ± 0.02b
Sol COD reduction (%initial)	83.6 ± 2.1	84.3 ± 2.0	90.2 ± 2.4	91.0 ± 2.9	92.7 ± 2.7	93.5 ± 2.4

*different letters next to the values indicate a significant statistical difference based on t-test with $p < 0.05$ (with same letters indicating a non- significant difference).

The CH_4 yield of both processes reached 0.48 L/g VS at a 3% CG supplementation, which is similar to values reported in previous studies on co-digestion of manure with CG derived from vegetable oils. Usack and Angenent [18] ran a long-term co-digestion of cattle manure and CG and reported a CH_4 yield of 0.55 L/g VS. However, in that study the share of glycerol in the mixture was lower as the feed ratio was equal to 62 g VS manure: 38 g VS glycerol, in contrast to this study where a ratio of 38 g VS manure: 62 g VS glycerol (Table 2) was achieved without process instability issues. Generally, the limits of CG concentration before having signs of process failure or reduced yields vary among studies. Lobato et al. incremented the CG content up to 8% v/v and evidenced an increase of the CH_4 yield from 11 L/g COD to 17 L/g COD [19]. Amon et al. [20] found a 170% increase of the CH_4 yield of manure mono-digestion by adding 6% CG. Astals et al. found a biogas yield equal to 0.78 L/g VS under mesophilic co-digestion with 4% w/w CG, corresponding to a 400% increase of biogas productivity and reported process instability at 5% w/w CG [5]. The same group increased the supplementation of CG up to 3% w/w CG and found a biogas yield equal to 0.47 L/g VS under thermophilic conditions [21]. In this sense, it is clear that not only the origin of the CG used may affect the efficiency of the co-digestion process but the process conditions (temperature, HRT, etc.) as well.

The effect of the HRT on the performance of the co-digestion of manure with CG was evaluated by comparing the data of the two digesters. The biogas productivity of digester A was found to be 35% higher than the productivity of digester B during phase II. Interestingly though, the CH_4 yields of the two processes were similar during phase II, being these 0.48 L CH_4/g VS for digester A, and 0.47 L CH_4/g VS for digester B. A possible explanation for this could be that the CG added was quickly degraded by the microorganisms in both digesters as it is a more readily available carbon source, while a larger part of the organic content of manure was permitted to degrade in digester B due to its higher HRT. Thus, the high yield originating from CG covered the difference in the CH_4 yields originating from the degradation of manure, making the difference in the CH_4 yield among digesters less significant. Similarly, in phase III, only the CG was probably converted to CH_4 in both digesters, resulting into the same yields. This hypothesis is also supported by the soluble COD reductions found (Table 2). Based on the soluble COD reductions of phases I and II, the expected CH_4 yields were calculated to be 0.30 L and 0.39 L respectively in both digesters. However, in phase III, the expected CH_4 yield based on the reductions of soluble COD observed were calculated to be 0.48 L. This means that during phase I and II, part of the particulate matter was hydrolyzed and contributed to the CH_4 yield. On the other hand, at phase III, the share of CG was high and the expected CH_4 yields coincide with the observed yields indicating that only the soluble matter was converted to CH_4. This is in agreement with a previous study reporting that the addition of CG to manure may reduce the digestion efficiency of manure due to the presence of more favorable compounds for microbes [7]. This could also explain why the NH_4^+-N content in digester B was higher than in digester A (especially during phase II), as the NH_4^+-N probably originated mainly from manure components. Additionally, the removal of soluble COD achieved by digester B was found to be slightly higher than in digester A (Table 2). However, the difference among the two processes is not that significant. Based on these observations, it appears that a low HRT of 17 days can be applied without significantly reducing the extent of AD of manure.

4. Conclusions

The co-digestion of crude glycerol, CG, derived from the transesterification of animal fats with swine manure was shown to be an efficient way to improve the methane production of manure-based biogas plants. Batch experiments of CG mono-digestion indicated that the limiting step for CH_4 generation was the uptake of the intermediate product 1,3-PDO. However, this did not appear to limit the continuous co-digestion of manure with CG. Continuous experiments showed no inhibition effects with concentrations up to 3% CG and reached a 222% increase of biogas productivity compared to manure mono-digestion. More importantly, in this study it was shown that decreasing the HRT from 22 days to 17 days allowed for a significant increase of the biogas productivity while not reducing the soluble COD removal efficiency importantly.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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