Impact of substrate to inoculum ratio in anaerobic digestion of swine slurry

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Abstract

Batch tests were conducted to elucidate the impact on the anaerobic digestion of different ratio of substrate/inoculum using swine slurry as substrate. In order to completely understand possible inhibitions, soluble COD, VFAs concentration, and pH were periodically analysed.

The results showed that the same methane yield was achieved with the three ratios (COD/VS of 1, 2 and 3). Methane production rates were clearly different. A faster degradation was observed with a ratio of 1, meanwhile a decline in methane productivity was remarked for ratio of 2 and even more severe with the ratio of 3. This behaviour was explained by the accumulation of VFA (predominantly HAc and HPr), reflected on the soluble COD profile as well.

Introduction

The final goal of anaerobic digestion (AD) is the reduction of organic matter and even total mineralization of the waste. This technique is considered as an environmentally friendly process since in turn of degrading organic matter, it produces energy in form of methane. The use of swine waste as sole substrate for AD resulted in a poor methane production; hence it is usually combined with sewage sludge (Hansen et al., 1998). AD is a biological treatment that entails a correct concentration of substrate (swine waste) and inoculum (sludge). The ideal balance to overcome the limitation of biomass and to avoid the overloading of organic matter has to be found. A limitation of anaerobes produces a slow methane production, meanwhile an excess of organic matter results in total inhibition of biomass activity or at least a lag phase for acclimation. In that manner, both situations result in a longer digestion time and consequently larger digesters.

Only a few works have been found in order to set a correct substrate/inoculum ratio elsewhere (Raposo et al., 2006 with maize waste, Neves et al., 2004 with kitchen waste or Hashimoto (1989) with straw). However, the study of methanogenic productivity employing different substrate/inoculum ratio on livestock effluents has not been reported. The purpose of this research was to elucidate the impact on methanogenesis by using different substrate (COD)/inoculum (VS) ratio. To fulfil this goal, VFA, soluble COD and pH were periodically analysed.

Materials and methods

Substrate and Inoculum collection

The mixture of nursery, sow and feeder-to finish swine waste was the substrate employed for this experiment. The slurry samples were collected from a farm located in Avila (Spain). Samples were analysed for total solids (TS), volatile solids (VS), total COD, and pH according to the Standard Methods for the Examination of Water and Wastewater (2005). The mean concentrations calculated for the substrate were 8.5 and 4.4 g/L for TS and VS respectively, and 12000 mg/L of COD.

The anaerobic sludge used as inoculum was obtained from the anaerobic digester situated at the wastewater treatment plant of Valladolid (Spain). The mean concentration for TS and VS was 31.4 and 14.0 g/L respectively.

Biodegradability Method

Serum glass bottles with chlorobutyl septum were used as reactors. The tests were conducted in a constant temperature room at 35ºC. The incubation time was approximately two months. The swine slurry was mixed with anaerobic sludge and water to get a ratio substrate (COD)/inoculum (VS) of 1 (R1), 2 (R2) and 3 (R3). The quantities were calculated to get a final volume of 50 mL of liquid mixture, allowing a headspace for the gas of approximately 70 mL. The mediums for the biodegradability tests were prepared according to González-Fernández et al. (2008).

For the determination of endogenous methane production, blanks containing only sewage sludge from anaerobic digester were run. The experiment involved two sets of samples. One of the series included duplicate samples for each ratio and sampling time. This group of samples was sacrificed periodically for VFA, soluble COD, and pH analysis in the liquid phase, as well as biogas quality in the headspace. Meanwhile, the second set of samples (triplicates per ratio) was monitored for biogas pressure in the headspace over the digestion period.

Gas chromatography (HP 5890) equipped with a TCD detector was used to determine the content of methane and carbon dioxide and a FID detector was employed in order to discard the presence of volatile fatty acids (VFA) at the end of the assay, meaning that the conversion to methane was complete.

Results and discussion

Parameters controlled with digestion time

The first set of samples (duplicates per COD/VS ratio and blank) were periodically sacrificed to get the profile degradation of soluble COD and VFAs concentration.

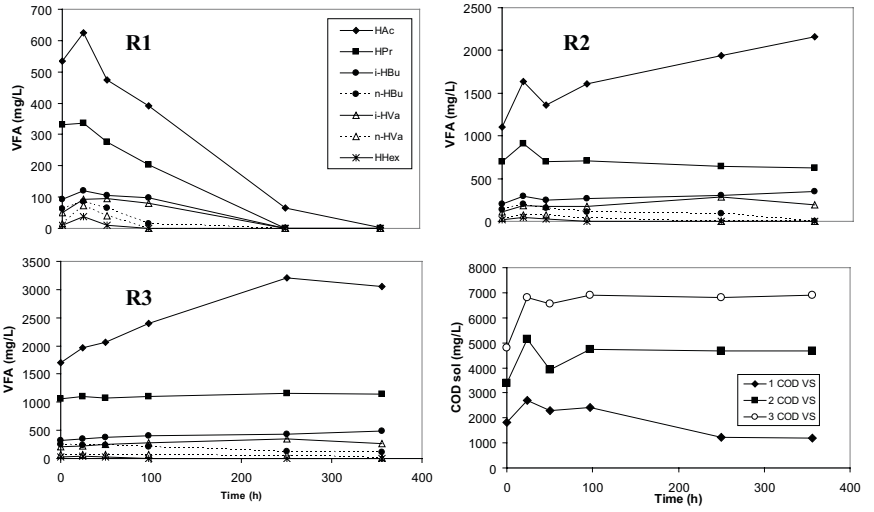
The fate of VFAs was followed in order to investigate the possibility of methanogens inhibition. Albeit in the first sampling a slight accumulation of VFA occurred, as it can be seen in Figure 1, the general tendency for R1 was to decrease along the time. An opposite behaviour took place for the other two ratios, being accumulated constantly during the sampling period (356 h) reaching values of 2160 and 3200 mg HAc/L for R2 and R3 respectively. It has to be pointed out the fact that for all the ratios the iso-forms of butyric and valeric acids were more persistent to anaerobic digestion. The increase of the i-form was attributed to isomerization of the n-form by rearrangement of the carboxyl group. In the case of i-HVa a longer period was needed for complete degradation, while the n-form was degraded faster due to β-oxidation producing other intermediates products (HAc and HPr) as suggested by Wang et al. (1999). This phenomenon was in accordance as well with the accumulation of HAc for R2 and R3.

Even tough Raposo et al. (2006) indicated the inhibition of HPr degradation when HAc concentration was greater than 1400 mg/L, this study revealed that degradation was still taking place at values of 2160 mg HAc/L (plot correspondent to R2). Meanwhile in the R3 graph, HPr was not degraded during the last three sampling times (97, 250 and 356 h) when HAc concentration was over 2400 mg/L. Even more, at the end of the assay (1587 h) HAc was still present in small amounts, 47 mg/L for R2 and 102 mg/L for R3. It should be noted that in the case of R1, total removal of VFAs were accomplished.

Soluble COD during the first six sampling points is shown in Figure 1. The initial COD were 1830, 3380 and 4789 mg/L for R1, R2 and R3 respectively. As expected, during the first step of digestion the soluble COD increased due to hydrolysis of complex molecules and acidogenesis. In the case of R1, the soluble COD was then degraded continuously. For

R2 a slight decrease was remarked in the third point (50 h) that coincided with a decrease of VFAs and afterwards a release of soluble matter was observed concominantly with an increase of HAc and HPr concentration. Regarding R3, the soluble COD experienced an increase during the second sampling point (24 h) and remained constant at approximately 7000 mg/L during the following sampling times, probably related to the continuos increase of HAc.

Figure 1. VFA and soluble COD profile with time for the different ratios assayed (R1, R2 and R3)



Finally, the pH was set at 7.5 at the beginning of the experiment and after increasing to 8 it was constant along the digestion time. This slight variation may be explained by the high buffering capacity of swine manure (Campos et al., 1999).

Biogas and methane production

In the second set of samples (triplicates per COD/VS ratio and blank) the headspace pressure was continuously monitored. The headspace biogas was released seven times in order to avoid a high increment in pressure.

Biogas production rate observed was considerably different for each ratio indicating that some delay occurred for R2 and R3. Figure 2-A shows the cumulative biogas along digestion time calculated at standard temperature and pressure (STP).

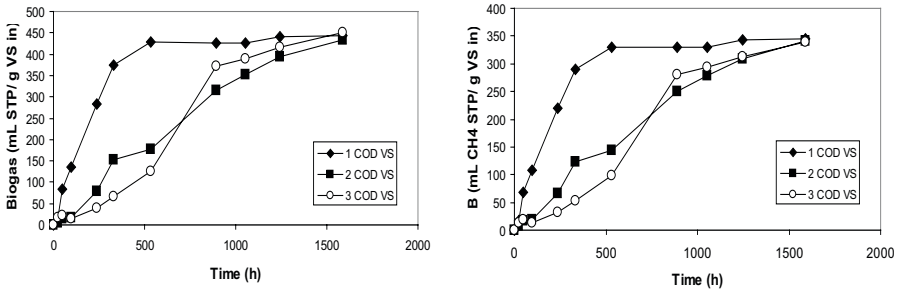
The biogas quality (methane percentage) was monitored weekly (Table 1). The values were in the range of 75 to 78% regardless the ratio. This percentage was in the same order of biogas quality obtained in previous experiments of swine manure (González-Fernández et al., 2008) and quite high when compared with Hill and Bolte (2000) that indicated the methane percentage of 60-65%.

Table 1. Overview over different measured and calculated parameters: accumulated methane per gram of VS, per gram of COD added, and biogas quality

Sample	1 COD/VS	2 COD/VS	3 COD/VS
B (L CH_4/g VS in)	0.345	0.341	0.340
mL CH_4/g COD added	0.137	0.135	0.134
Gas Quality (%CH_4)	75.3	78.1	77.9

Methane yield (B, mL CH_4 STP/ g $VS_{initial}$) was calculated for each ratio (Figure 2-B). A sharp production of methane was observed for R1, the plateau phase was reached at approximately 500 hours of digestion. Meanwhile R2 showed a delay of methane productivity from the beginning and R3 presented a longer lag phase than R2. From 500 h onwards a more stressed increase in methane production was obtained, probably due to the fact that at 365 hours the VFA concentration started to decline. In that manner, it can be suggested that some kind of inhibition influenced by VFA was occuring for R2 and R3. After a period of acclimation (approx. 500 h) the microorganisms were capable of degrading the remaining soluble organic matter.

Figure 2. Evolution of biogas (A) and methane production (B) with time for the different ratios assayed



Even more, when focusing in the production rate 90 % of total methane productivity was accomplished within the first 22 days for R1, while R2 and R3 needed 52 days to reach the same percentage. The final of the experiment was set at 66 days when the three ratios had achieved the same methane productivity.

With regard to methane productivity as a function of initial COD, the values were in the range of 0.137-0.134 mL CH_4 (STP)/ g CODadded. This value was low when compared with the ones obtained by Hill and Bolte (2000) 0.270 mL CH_4/g CODadded or 0.249-0.277 L CH_4/g CODadded achieved by González-Fernández et al. (2008).

In addition, methane productivity (B) obtained in the present work was in the range of 0.340-0.345 L CH_4 (STP)/g $VS_{initial}$ for the three ratios (Table 1). These results were higher than the ones obtained by Hartmann et al. (2000) who indicated a methane production of 0.200-0.250 L CH_4/g VS. On the other hand, the methane yield estimated in this study was similar compared with ultimate methane yield (time approaches to infinity) calculated by Moller et al. (2004), 0.356 L CH_4/g VS but low when compared with 0.450 L CH_4/g VS suggested by IPPC (1997). This fact was expected since up to 25 % of organic matter is unused by microorganisms due to the unsuccessful hydrolysis step (Hartmann et al., 2000). Thus, ultimate methane yield may be 25 % overestimated with respect to the experimental methane yield.

Conclusions

The results from this study help to understand the importance of using a correct substrate/ inoculum ratio in order to obtain a better control of the process. Soluble COD variation entailed the byproducts formed during hydrolysis and production of VFA. Indeed, soluble COD increased concominantly with increasing VFAs concentration. Even tough a fast VFAs degradation was accomplished with R1, the other two experimental ratios (R2 and R3) showed an accumulation of HAc and HPr. This fact resulted in a delay in methane production for R2, even more stressed for R3. The presence of HAc at the end of the assay for R2 and R3 indicated that the system could not consume all the generated VFAs.

The final methane yield was in the range of 0.342 L CH_4 (STP)/g $VS_{initial}$, regardless the used ratio. The main difference was the methane production rate, 90 % of the total was achieved on day 22 for R1, while R2 and R3 reached the same point on day 52. On the other hand, the methane percentage on the biogas was averaged to 77%.

Thereby, it may be suggested for anaerobic digestion of swine slurry to work in the range substrate/inoculum of around 1. Longer hydraulic retention time is needed to degrade organic matter when using higher ratios, hence resulting on larger digester volumes.

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References

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21th ed. American Public Health Association, Washington, DC

Campos, E., Palatsi, J., and Flotats, X., 1999. Codigestion of pig slurry and organic wastes from food industry. Proceedings of the II International Symposium on Anaerobic Digestion of Solid Waste. Barcelona. 192-195.

González-Fernández, C., Nieto-Diez, P.P., León-Cofreces, C., and García-Encina, P.A., 2008. Solids and nutrients removals from the liquid fraction of swine slurry through screening and flocculation treatment and influence of these processes on anaerobic biodegradability. Bioresource Technology, In Press, Corrected Proof.

Hansen, K.H., Angelidaki, I., and Ahring, B.K., 1998. Anaerobic digestion of swine manure. Inhibition by ammonia. Water Research, 32(1), 5-12

Hartmann, H., Angelidaki, I., and Ahring, B.K., 2000. Increase of anaerobic degradation of particulate organic matter in full-scale biogas plants by mechanical maceration. Water Science and Technology, 41(3), 145-153.

Hashimoto, AG., 1989. Effect of inoculum/substrate ratio on methane yield and production rate from straw. Biological Wastes, 28 (4), 247–55.

Hill, D.T., Bolte, J.P., 2000. Methane production from low solid concentration liquid swine waste using conventional anaerobic fermentation. Bioresour. Technol. 74, 241-247.

IPPC Guidelines, 1997. Guidelines for National Greenhouse Gas Inventories: Reference Manual. Revised 1996.

Moller, H.B., Sommer, S.G., and Ahring, B.K., 2004. Methane productivity of manure, straw and solid fractions of manure. Biomass and Bioenergy 26, 485-495.

Neves, L., Oliveira, R., and Alves, MM., 2004. Influence of inoculum activity on the biomethanization of a kitchen waste under different waste/inoculum ratios. Process Biochemistry, 39, 2019–24.

Raposo, F., Banks, C.J., Siegert, I., Heaven, S. and Borja, R., 2006. Influence of inoculum to substrate ratio on the biochemical methane potential of maize in batch tests. Process Biochemistry, 41, 1444-1450.

Wang, Q., Kuninobu, M., Ogawa, H., and Kato, Y., 1999. Degradation of volatile fatty acids in highly efficient anaerobic digestion. Biomass Bioenergy, 16, 407–16.