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Process performance of anaerobic co-digestion of raw and acidified pig slurry

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ABSTRACT

The effect of incorporating different ratios of acidified pig slurry on methane yield was evaluated in two scales of anaerobic digesters: Thermophilic (50 °C) pilot scale digester (120 l), operating with an average hydraulic retention time of 20 days and thermophilic (52 °C) full-scale digesters (10 and 30 m^3), operating with an average hydraulic retention time of 30 days. In the lab-scale digester, different inclusion levels of acidified slurry (0–60%) were tested each 15 days, to determine the maximum ratio of acidified to non-acidified slurry causing inhibition and to find process state indicators helping to prevent process failure. In the full-scale digesters, the level of inclusion of the acidified slurry was chosen from the ratio causing methane inhibition in the pilot scale experiment and was carried on in a long-term process of 100 days. The optimal inclusion level of acidified pig slurry in anaerobic co-digestion with conventional slurry was 10%, which promoted anaerobic methane yield by nearly 20%. Higher inclusion levels caused methane inhibition and volatile fatty acids accumulations in both experiments. In order to prevent process failure, the most important traits to monitor in the anaerobic digestion of acidified pig slurry were found to be: sulfate content of the slurry, alkalinity parameters (especially partial alkalinity and the ratio of alkalinity) and total volatile fatty acids (especially acetic and butyric acids).

1. Introduction

Slurry acidification by manipulating the balance between ammonia (NH_3) and ammonium is an effective measure to reduce NH_3 emissions from animal slurry (Berg et al., 2006; Kai et al., 2008). Different strategies to acidify slurry have been used in the last decades such as: the incorporation of additives in the slurry (McCrory and Hobbs, 2001), modifications in the formulation of feeds for animals (Canh et al., 1998) and changes in manure management and animal housing conditions at the farm level (Sommer and Hutchings, 1995). Nevertheless, most of these techniques have not a commercial application. Sulfuric acid (H_2SO_4) is currently used in Denmark to acidify slurry with success. Kai et al. (2008) reported reductions in NH_3 emission from pig houses by 70% when adding H_2SO_4 to the slurry, compared to untreated pig slurries.

Nevertheless, the full implementation of this technique is limited due to the increased activity and growth of sulfate reducing bacteria (SRB) and its negative effects on anaerobic digestion (O'Reilly and Colleran, 2006). Hydrogen sulfide (H_2S)

Abbreviations: SBR, Sulfate reducing bacteria; MPB, methane producing bacteria; ALK, Alkalinity, g $CaCO_3$ l^{-1}; PA, Partial alkalinity, g $CaCO_3$ l^{-1}; TA, Total alkalinity, g $CaCO_3$ l^{-1}; IA, Intermediate alkalinity, g $CaCO_3$ l^{-1}; TAN, Total ammonia, g NH_4 l^{-1}; TIDSS, Total inorganic dissolved sulphide, mg S^{2-} l^{-1}; VFA, Volatile fatty acids, g l^{-1}; TS, Total solids, %; VS, Volatile solids, %; RA, Ratio of alkalinity (IA/TA).

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produced by SRB during the degradation of organic matter can result in an inhibition of the anaerobic digestion process or even its total failure (Hulshoff Pol et al., 1998). In addition, SRB themselves can compete with methane producing bacteria (MPB) for electron sources such as acetate or hydrogen, and with the obligate hydrogen producing bacteria for propionate (O'Reilly and Colleran, 2006).

However, some research on bioreactors has shown that MPB can co-exist with SRB (Isa et al., 1986; McCartney and Oleszkiewicz, 1993; Yoda et al., 1987) under some specific conditions that depend on digester operational parameters like temperature, digester characteristics, the tolerance of anaerobic bacteria to sulfide, sulfate concentration and substrate composition (O'Flaherty et al., 1998; O'Reilly and Colleran, 2006; Visser et al., 1996).

Substrate composition is an important factor to take into account in the relationship between these two groups of bacteria and thus, in CH_4 production. The decrease of CH_4 production during anaerobic digestion of sulfate rich wastewaters using pure anaerobic cultures and synthetic appropriate substrates as volatile acids and ethanol has been widely studied in the literature (Isa et al., 1986; McCartney and Oleszkiewicz, 1993; O'Flaherty et al., 1998; Yoda et al., 1987). Concerning acidified animal slurry, solids from solid–liquid separation of acidified dairy cow manure has been tested in anaerobic co-digestion with non-acidified cow slurry with success (Sutaryo et al., 2012). However, to our knowledge, there are no studies concerning the effects of using the entire fraction of acidified animal slurries in anaerobic digestion. Eriksen et al. (2008) and Ottosen et al. (2009) reported that the activity of SRB in acidified pig slurries during storage is low, despite the presence of high amounts of acetate and sulfate, which are essential components for SRB development. Thus, the effects of high sulfate concentrations on the anaerobic digestion of slurry might differ from results achieved when digesting sulfate rich wastewaters.

The aim of this study was to investigate the effects of including acidified pig slurry in an anaerobic co-digestion process with conventional slurry used as the basic substrate, and to find the optimum ratio not having detrimental influence on the process performance. For this purpose two experiments were designed: a pilot scale experiment in which different sulfate concentration levels were added in short periods to find the range of sulfate concentration causing inhibition and the chemical process state indicators that could potentially be used for predicting process failure; and a full scale experiment in which we tested the results obtained in the pilot scale in a long term study to achieve the steady state conditions.

2. Materials and methods

2.1. *Substrates*

Conventional (raw) and acidified pig slurry were obtained from commercial pig farms in Denmark. In the farm where the acidified slurry was collected, acidification was performed by using the technology provided by Infarm® Company. Acidification was carried out in a process tank outside the fattening buildings in which concentrated H_2SO_4 was added at a ratio of approximately 7–8 kg per ton of slurry. The amount of H_2SO_4 added was controlled by a pH-sensor with the aim to reach a pH level of 5.5. Simultaneously to the acidification process, slurry was aerated by injecting compressed air to prevent the conversion of sulfate ion into hydrogen sulfide. The acidified pig slurry was used to flush the waste pits to ensure that pH under the slatted floor was always kept low.

Inoculum for starting up the digesters in this study was obtained from the thermophilic anaerobic digesters of the Research center in Foulum, Denmark. The digesters were completely filled with this inoculum before starting up the experiments.

2.2. *Pilot-scale digesters experiment*

This experiment was conducted using a continuously stirred tank with a working volume of 120 l. The digester was operated at 50 °C with an average hydraulic retention time of 20 days.

Mixing was performed by a central shaft with a propeller at the bottom, rotating at 60 rpm and programmed to work for 10 min every half hour. Therefore the sludge retention time can be considered equal to the hydraulic retention time under the steady-state conditions. The tank temperature was controlled by means of heating the tubes placed in the bottom of the digester and the body of the digester was provided with an insulating jacket for ensuring stable operational temperature.

In order to measure the volume of the biogas produced, the digester was continuously connected to a gas collection cylinder placed in an oil solution. Gas production was measured by the volume displacement method. Feeding and unloading the digester was performed automatically using electric pumps.

During one month the digester operated with normal pig slurry with the purpose of reaching steady state conditions. After the digester was stabilized, it was operated with 10% acidified pig slurry and 90% normal pig slurry. Every two weeks, the percentage of acidified pig slurry was increased. The experiment had a total duration of 90 days and the acidified slurry percentages tested were: 10%, 20%, 40% and 60%.

2.3. *Full-scale digesters experiment*

This experiment was conducted using two continuously stirred tanks with a working volume of 10 and 30 m^3. These two digesters were very similar in design, they were constructed in stainless steel and heated by an external water jacket; the two digesters were operated in thermophilic (52 °C) conditions with an average hydraulic retention time of 30 days. Continuous mixing in both digesters was performed by a central shaft with a propeller at the bottom, rotating at 60 rpm. Gas production was measured with a differential pressure transmitter device (EJX110A Yokogawa, Japan). Feeding and unloading the digesters was performed automatically using electric pumps and the exact amount of slurry feed and unload was measured with weighing cells.

The 30 m^3 digester was filled with inoculum and fed with a mixture of 30% acidified slurry + 70% raw slurry from the beginning of the experiment. The level of inclusion of the acidified slurry in this case was decided from the results obtained in the pilot scale experiment. In this experiment it was observed that the production of CH_4 was inhibited when acidified pig slurry was included in a ratio between 20 and 40%, thus, a 30% of acidified slurry inclusion level was tested in this experiment. The 10 m^3 digester was fed with raw pig slurry as control.

2.4. *Chemical analyses*

Samples for chemical analyses were taken from all digesters once a week in both experiments. After sample collection, pH, total solids (TS) and volatile solids (VS) were measured as described in APHA (2005) (methodology number: 2450-B and 2450-E). Alkalinity (ALK) was determined by titration method according with APHA (2005) (methodology number: 2320-B) with the titration equipment Mettler DL21 (Mettler Toledo). The method consists of a two steps titration, the first one at pH of 5.75 (Partial alkalinity, PA) and the second at 4.3 (total alkalinity, TA). The intermediate alkalinity (IA) was then estimated as the difference between TA and PA.

Total ammonia (TAN), sulfate (SO_4^{2+}) and total inorganic dissolved sulfide species (TIDSS: dissolved H_2S + HS^- + S^{2-}) were determined with photometric kits (Spectroquant® kit, Merk, USA). Volatile fatty acids (VFA) were analyzed using a gas chromatograph (5560-D of APHA, 2005) equipped with a flame ionization detector (HP 68050 series Hewelt Packard).

Biogas samples were taken twice a week in both experiments by flushing a 22 ml sample bottle with 300 ml of biogas. Methane (CH_4) and carbon dioxide (CO_2) concentration in the biogas were analyzed on a Perkin Elmer Clarus 500 gas chromatograph equipped with a thermal conductivity detector according with Sutaryo et al. (2012). The temperatures of injection port, oven, filament and detector were 110 °C, 40 °C, and 150 °C, respectively. The carrier gas was Helium with a flow rate of 30 ml min^{-1}.

Additionally, H_2S concentration in the biogas samples was determined colorimetrically using precision gas detector tubes (Kogyo K.K., Kitagawa, Japan). Hydrogen sulfide in the biogas was analyzed in the full-scale experiment from day 70 and onwards.

2.5. *Calculations and statistical analyses*

In anaerobic digestion, sulfate is reduced by SRB bacteria to sulfide which is distributed between H_2S in the gas phase and H_2S, HS^- and S^{2-} in the liquid phase (Isa et al., 1986). In this work the percentage of SO_4 reduced was calculated following Eq. (1):

$$SO_4\ \text{reduced}(\%) = \frac{S_{SO_4}\text{fed slurry} - S_{SO_4\ \text{sludge}}}{S_{SO_4}\text{fed slurry}} \times 100 \tag{1}$$

The percentage of VS degraded from the slurry during the anaerobic digestion process (removal efficiencies) was also calculated. During the drying process for VS determination, important losses of VFA can be expected depending of the sample's pH (Derikx et al., 1994; Rico et al., 2007). Derikx et al. (1994) estimated that more than 80% of the VFA present in the slurry were lost during the drying process in slurries with a pH around 7.5 and that the percentage of VFA lost during drying process increased as the pH decreased. Thus, in this study VFA content in the slurry were added to VS fraction when calculating the removal efficiency in the following manner: 100% of VFA was added to VS in slurries with a pH below 7, in slurries with pH between 7 and 8, 80% of VFA were added and in slurries with a pH higher than 8, 10% of VFA were added to VS.

Methane yield was expressed as the CH_4 production (l) per kg of VS introduced in the digesters. The inhibition of CH_4 yield in the pilot scale experiment was calculated following Eq. (2):

$$\text{Inhibition}(\%) = 100 - \left(\frac{\text{Average } CH_4 \text{ produced using the mix with acidified slurry}}{\text{Average } CH_4 \text{ produced using raw slurry}} \times 100\right) \tag{2}$$

The correlation between all the compositional variables and gas production determined in the pilot-scale experiment was assessed using SAS System® Software (Version 9.0, SAS Inst. Inc., Cary, NC) using PROC CORR procedure. In all cases, statistical significance level was set at 5% (P-value ≤ 0.05).

3. Results

3.1. *Chemical composition of acidified and raw slurries*

Table 1 shows the average chemical composition of acidified and raw slurries used in the two experiments. Sulfate concentration was nearly twenty times higher in the acidified slurries compared to raw slurries in both experiments. However SO_4 content was slightly higher in the acidified slurry used in the pilot-scale experiment compared to that used in the full-scale experiment.

Sulfide and VFA concentrations were lower in the acidified slurry than in the raw slurry in both experiments, especially in the full-scale experiment. As expected, pH was lower in the acidified slurry than in the raw slurry and TAN concentration was higher in the acidified slurry than in raw slurry. As shown in Table 1, ALK parameters were lower in the acidified slurry than in the raw slurry in both experiments.

3.2. *Pilot-scale digester experiment*

3.2.1. *Slurry and biogas composition*

Table 2 shows the chemical composition of the sludge used during anaerobic digestion with increased concentrations of acidified slurry. The pH remained almost constant until the period in which 60% of acidified slurry was added; the average pH value until then was 8.15. However, when 60% of acidified

Table 1 – Initial chemical composition of slurries used to feed the pilot-scale and the full-scale digesters.

Parameter	Units	Pilot-scale experiment		Full-scale experiment	
		Acidified slurry	Raw slurry	Acidified slurry	Raw slurry
Total solids	%	5.70	7.10	3.76	5.41
Volatile solids	%	4.12	5.29	2.59	4.29
Sulfate	g SO_4^{2-} l^{-1}	11.83	0.63	10.74	0.32
Sulfide	mg S^{2-} l^{-1}	32.85	64.22	37.71	56.33
Total volatile fatty acids	g l^{-1}	10.90	13.43	3.20	12.47
Acetic	g l^{-1}	3.94	6.69	2.00	6.15
Propionic	g l^{-1}	1.79	2.42	0.23	2.44
Butyric	g l^{-1}	3.02	2.42	0.56	1.99
pH		5.77	6.85	5.94	6.48
Total ammonia	g NH_4 l^{-1}	2.97	2.16	2.54	2.41
Total alkalinity	g $CaCO_3$ l^{-1}	11.92	19.19	5.11	13.58
Partial alkalinity	g $CaCO_3$ l^{-1}	5.74	9.33	2.42	5.99
Intermediate alkalinity	g $CaCO_3$ l^{-1}	6.17	9.86	2.69	7.59

slurry was added (7.50 g SO_4 l^{-1} of fed slurry) the pH value dropped until 7.53. Volatile fatty acids concentration in the sludge increased in the last two periods, when 40% and 60% of acidified slurry were added (Table 2). When adding 60% acidified slurry, acetic, butyric and total VFA were respectively 3.53, 21.6 and 3.15 times higher than at the beginning of the experimental period using no acidified slurry.

Partial alkalinity decreased and SO_4 increased proportionally with increases in the acidified slurry in the digester. The calculated percentage of SO_4 reduced was almost negligible at the beginning when no acidified slurry was added, and increased with the addition of acidified slurry to the digester.

The degradation of VS calculated as the removal efficiency is shown in Table 2. At the beginning of the experiment with up to 10% of acidified slurry in the mixed substrate, the removal efficiency was stable at around 52%. However the removal efficiency was reduced significantly when the digester was fed with a proportion of 20 and 60% of acidified slurry.

The methane yield increased by nearly 20% when the inclusion of acidified pig slurry increased from 0 to 10% of the total slurry. However, when the proportion of the acidified slurry was increased by 20%, 40% and 60%, methane yield was reduced by 18%, 70% and 96%, respectively. Methane concentration in the biogas was maintained constant (around 50–60%) until the addition of 40% of acidified slurry and decreased in the last period with addition of 60% of acidified slurry (7.50 g SO_4 l fed^{-1}); in this period CH_4 concentration reached levels below 30%. Fig. 1 shows the evolution of CH_4 yield and CH_4 content in the biogas with increasing concentrations of acidified slurry. Methane yield was linearly reduced with increasing sulfate concentrations in the slurry. Results show that the inhibition of CH_4 production started when the proportion of acidified slurry in the mixture was 20%. The drop in CH_4 yield was almost linear between 20% and 40% periods, showing values close to zero at the end of the period in which the percentage of acidified slurry reached 40% of the mixture. In the full-scale experiment we decided to test the effects of including a 30% of acidified slurry in co-digestion with raw slurry on CH_4 production for a long period of time. Both, the degree of CH_4 inhibition and a likely adaptation of methanogenic bacteria to these conditions with the time were evaluated.

3.2.2. *Relationship between slurry composition and CH_4 yield*

The correlation among process state indicators and CH_4 yield was studied in order to find the most important traits to be taken into account to detect a process failure in anaerobic digestion when acidified pig slurry is used as substrate. The most significant results of the correlation analysis performed are shown in Table 3. The trait most closely related to CH_4 yield was the SO_4 content in the slurry, explaining a 79% of the variation in CH_4 yield ($R = -0.79$, P-value < 0.05). The relationship between these two variables was negative, indicating that SO_4 was the main source of CH_4 inhibition in this study.

Alkalinity parameters were also well correlated with CH_4 yield, mainly PA ($R = 0.77$) and the ratio of alkalinity (RA = IA/TA) ($R = -0.73$). The relationship between CH_4 and PA in this study was positive indicating that CH_4 yield was reduced when PA decreased. Nevertheless RA was negatively correlated with CH_4 yield. Volatile fatty acids in the digester were negatively correlated with CH_4 yield in this study, especially total VFA, acetic and butyric acids.

Table 3 also shows the correlation among compositional variables. The results concerning the correlation between VFA and IA indicated that total VFA ($R = 0.98$) and acetic acid ($R = 0.98$) were highly and positively correlated with IA. Fig. 2 shows the positive and linear relationship between VFA and IA indicating that higher levels of VFA and acetic acid are correlated with higher IA values. Concerning RA (IA/TA) (Fig. 2), this parameter correlated well with total VFA ($R^2 = 0.98$) and butyric acid ($R^2 = 0.96$). The IA/PA ratio showed a low correlation with VFA (data not shown) being this only significantly correlated with butyric acid ($R^2 = 0.55$). The relationship between PA and VFA was also studied (Fig. 2). Partial alkalinity was highly correlated with total VFA ($R^2 = 0.86$) and acetic acid ($R^2 = 0.86$) showing a linear and negative relationship.

3.3. *Full-scale digesters experiment*

In the full-scale experiment, TS and VS remained stable over the 100 days with levels ranging between 4.0% and 2.5%. Ammonia was also stable and slightly higher in the acidified

Table 2 – Chemical composition and operational parameters during anaerobic digestion of increasing concentrations of acidified pig slurry in a pilot-scale experiment.

Parameter	Units	% Acidified slurry				
		0%, 0.4 g SO_4 l fed^{-1}	10%, 1.84 g SO_4 l fed^{-1}	20%, 2.82 g SO_4 l fed^{-1}	40%, 4.70 g SO_4 l fed^{-1}	60%, 7.50 g SO_4 l fed^{-1}
Total solids	%	5.31	5.69	4.14	3.22	4.09
Volatile Solids	%	3.74	3.93	2.79	2.04	2.71
pH		8.16	8.18	8.17	8.14	7.53
Ammonia	g NH_4 l^{-1}	2.00	2.77	2.55	2.16	3.23
Acetic	g l^{-1}	1.23	1.06	0.73	2.19	4.34
Propionic	g l^{-1}	1.46	2.95	3.12	2.83	2.47
Butyric	g l^{-1}	0.06	0.02	0.04	0.38	1.30
Total volatile fatty acids	g l^{-1}	3.19	4.60	4.20	6.60	10.06
Total alkalinity	g $CaCO_3$ l^{-1}	19.77	20.75	18.84	19.51	18.56
Partial alkalinity	g $CaCO_3$ l^{-1}	16.24	16.30	15.25	13.96	11.15
Intermediate alkalinity	g $CaCO_3$ l^{-1}	3.53	4.45	3.59	5.54	7.41
Sulfate	g SO_4^{2-} l^{-1}	0.39	0.68	0.73	1.74	2.51
Total dissolved sulfide	mg S^{2-} l^{-1}	45.92	44.62	46.13	32.59	34.95
Calculated sulfate $S{-}SO_4^{2-}$ reduced	%	1.61	64.42	74.26	63.47	62.33
H_2S biogas	%	0.27	0.29	1.01	0.91	<1.2
Calculated removal efficiency	%	51.99	52.28	25.87	64.98	39.06
Methane yield	l CH_4 kg VS^{-1}	220.24	262.87	179.92	62.91	6.96
Calculated methane inhibition	%		−19.36	18.91	71.43	96.84
Methane in the biogas	%	54.20	63.61	63.35	51.83	29.51

digester (1.89 g NH_4 l^{-1}) than in the non-acidified digester (1.57 g NH_4 l^{-1}). Table 4 shows the chemical composition (initial and final) in terms of pH, VFA, alkalinity traits, SO_4 and TIDSS content of the acidified and non-acidified digesters working in the full-scale experiment. The pH was stable in both digesters throughout the experiment. The concentration of VFA at the end of the study was higher than at the initial point in both digesters. This increment was more marked in the acidified digester compared to the non-acidified; in fact, total VFA in the non-acidified digester remained lower than 1.7 g l^{-1} during the experimental period. In the acidified digester the increase in total VFA started from day 40 of the experimental period (data not shown) and at the end of the study reached 5.48 g l^{-1}. This VFA concentration was almost four times higher than observed in the non-acidified digester. Regarding individual VFA, as expected from the pilot-scale

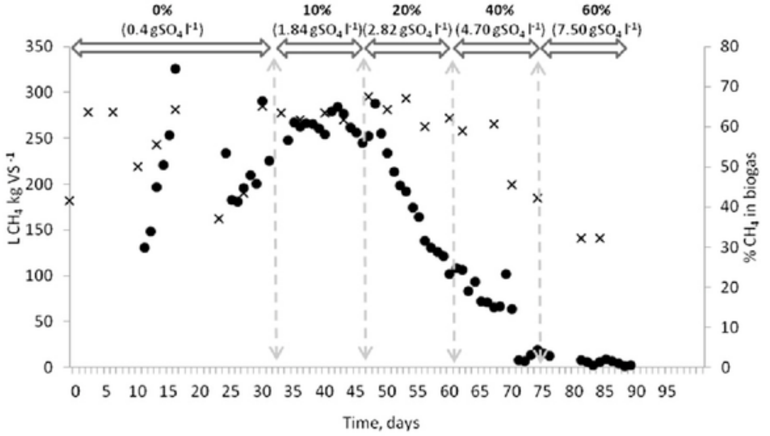

Fig. 1 – Methane yield per kg of volatile solids add to the digester from the digester of the pilot-scale experiment (●) and percentage of methane in the biogas (×). The concentrations of acidified pig slurry added were expressed as % of acidified slurry of the total slurry fed.

Table 3 – Sample coefficient of correlation (R) of the chemical parameters measured during anaerobic digestion of increasing concentrations of acidified pig slurry in a pilot-scale experiment ($n = 26$).

	Methane yield	Acetic	Propionic	Butyric	Total volatile fatty acids	Total alkalinity	Partial alkalinity	Intermediate alkalinity	Ratio of alkalinity
Acetic.	−0.50								
Propionic									
Butyric	−0.71	0.78							
Total volatile fatty acids	−0.46	0.86	0.52	0.88					
Total alkalinity									
Partial alkalinity	0.77	−0.93		−0.96	−0.93				
Intermediate alkalinity	−0.67	0.98		0.94	0.98		−0.93		
Ratio of alkalinity	−0.73	0.99		0.98	0.99		−0.85	0.99	
Sulfate concentration	−0.79	0.98		0.86	0.98		−0.97	0.92	0.97

experiment, all of them were higher in the digester treating acidified slurry compared to the digester treating non-acidified slurry at the end of the experiment.

Alkalinity parameters in the non-acidified digester were stable over the experimental period. Total and partial ALK remained lower in the acidified digester than in the non-acidified digester until day 60 (data not shown). From day 60, TA and PA in the acidified digester started to increase until they almost reached the values obtained in non-acidified digester. At the end of the experimental period, IA was higher in the acidified digester than in the non-acidified digester.

Regarding SO_4 and TIDSS content in the non-acidified digester, SO_4 concentration was stable and close to 0.40 g SO_4 l^{-1}. In the acidified digester, SO_4 concentration was stable and close to 0.55 g SO_4 l^{-1} until day 64; from this day, SO_4 content fell to reach non-acidified digester levels (0.40 g SO_4 l^{-1}) and remained at this concentration until the end of the experimental period. Total inorganic dissolved sulfide species in the non-acidified digester were stable and close to 43 mg S^{2-} l^{-1}. However, in the acidified digester, there was a slight increment in TIDSS concentration from day 40 until the end of the experimental period. The average H_2S concentration in the biogas of the acidified digester at the end of the experimental period was higher than that obtained in the non-acidified digester (1.85 vs. 0.05%).

Over 100 days of full-scale experiment, the average CH_4 yield in the digester operating with raw slurry was 221.80 ± 7.65 l CH_4 kg^{-1} (data not shown). The average CH_4 yield in the digester operating with 30% acidified slurry in this experiment was 154.65 ± 6.23 l CH_4 kg^{-1}.

Fig. 3 shows the CH_4 yield in the digesters over the experimental period. A varying CH_4 yield over time was observed in both digesters. However, it was observed that CH_4 yield from the digester fed with 30% of acidified slurry was lower at the beginning (days 30–50) of the experimental period, increasing thereafter to reach a similar CH_4 yield to that of the non-acidified digester. From day 75 of study, the acidified digester showed again a reduction in CH_4 yield. The methane concentration in the biogas produced by the acidified digester was stable (60.52 ± 0.91%) during the experimental period and close to that obtained in the non-acidified digester (60.80 ± 0.14%) and in the pilot-scale digester.

4. Discussion

Remarkable differences in acidified slurries between experiments were found in SO_4, TS and VS content, probably due to variability among seasons and sources. Important differences between acidified and non-acidified slurries were also found in the case of VFA, TAN and ALK parameters. In addition to natural variability on composition in slurries coming from different farms, VFA differences between acidified and non-acidified slurries could also be caused by a reduced biological activity in the acidified slurry during storage. Ottosen et al. (2009) observed a reduction greater than 98% in the biological activity of the acidified slurry

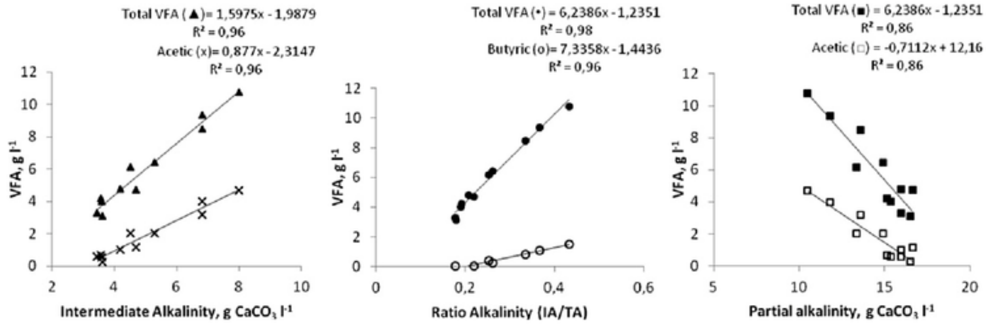

Fig. 2 – Correlation between the concentration of volatile fatty acids (acetic and butyric acids) with intermediate alkalinity (IA), the ratio of alkalinity (IA/total alkalinity (TA)) and partial alkalinity.

Table 4 – Initial and final composition of the digestate in the digesters used in the full-scale experiment after a 100-day working period.

Parameter	Units	Acidified digester		Non-acidified digester	
		Initial	Final	Initial	Final
pH		8.06	7.89	7.91	8.02
Acetic	g l^{-1}	0.25	7.87	0.32	0.88
Propionic	g l^{-1}	0.02	1.93	0.07	1.00
Butyric	g l^{-1}	0.00	0.24	0.00	0.05
Total volatile fatty acids	g l^{-1}	0.28	5.48	0.39	2.16
Total alkalinity	g $CaCO_3$ l^{-1}	12.17	17.47	16.51	17.53
Partial alkalinity	g $CaCO_3$ l^{-1}	9.86	13.42	14.01	14.56
Intermediate alkalinity	g $CaCO_3$ l^{-1}	2.31	4.05	2.00	2.98
Sulfate	g SO_4^{2-} l^{-1}	0.58	0.41	0.39	0.45
Total dissolved sulfide	mg S^{2-} l^{-1}	44.02	45.28	41.42	43.32
H_2S in biogas	%		1.85		0.05

compared to untreated slurries during storage. This diminution of the biological activity could decelerate the organic matter degradation process and thus VFA production and sulfate reduction. Therefore, as Berg et al. (2006) suggested, acidification might increase slurry potential for biogas production since it slows down the process of organic matter degradation, maintaining its initial chemical composition. Total ammonia nitrogen was also different between acidified and non-acidified slurries in both experiments, probably due to lower NH_3 losses to the environment in acidified slurries because the lower pH compared to raw slurries. Similar differences between acidified slurries and conventional pig slurries concerning pH and NH_3 were also observed by Kai et al. (2008).

Concerning differences in ALK parameters between acidified and non-acidified slurries, slurry acidification could have decreased ALK because the acid added consumes buffer carbonate capacity resulting in a reduction of PA. In addition, the lower VFA concentration found in acidified slurries can be the cause of the lower IA in these slurries. In fact, TA of a sample is the sum of PA, which is correlated with HCO_3^- components, and IA which is correlated with VFA and TAN components (Jantsch and Mattiasson, 2004; Ripley et al., 1986). Concerning digesters, the addition of H_2SO_4 to the digester may have consumed buffer carbonate capacity decreasing PA in the pilot-scale experiment. However IA increased with SO_4 addition to the digester, probably because of increments in VFA and TAN concentration in the digester. In this study, results from the lab-scale experiment (Fig. 2) indicated that PA was highly correlated with total VFA ($R^2 = 0.86$) and acetic acid ($R^2 = 0.86$) showing a linear and negative relationship and IA was linear and positively correlated with total VFA ($R^2 = 0.96$) and acetic acid ($R^2 = 0.96$). Similar correlations between VFA and ALK parameters were obtained by Ferrer et al. (2010). Total alkalinity in the pilot-scale experiment was stable probably because the reduction of PA might have compensated by increments in IA.

In addition to the good correlation between VFA and ALK, ALK parameters also resulted in high correlation with CH_4 yield in this study. The relationship between ALK and CH_4 yield has been widely reported in the literature (Ahring et al., 1995; Ferrer et al., 2010). A variation in ALK traits might indicate a failure in the anaerobic digestion process due to an overloading since, as observed in this study, IA was positively

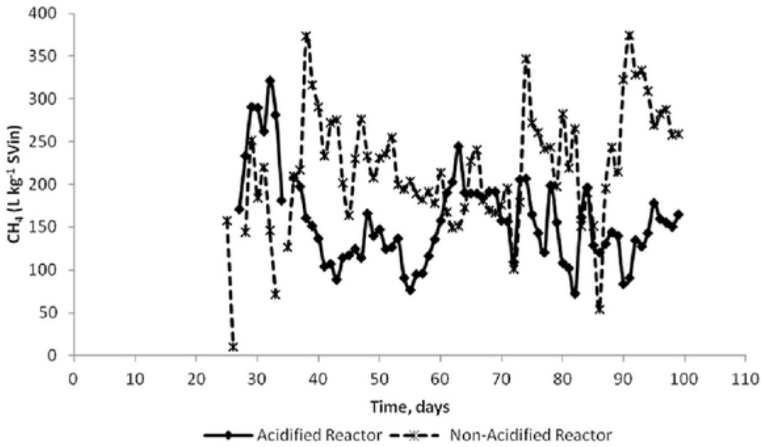

Fig. 3 – Methane yield per kg of volatile solids add to the two digesters, one operating with 30% of acidified slurry (3.45 gSO_4 l^{-1} in feed slurry) (Acidified Digester) and other operating with non-acidified slurry (Non-Acidified Digester).

correlated with total VFA and VFA were negatively correlated with CH_4 yield (Table 3), especially total VFA, acetic and butyric acids ($R > -0.4$, $P < 0.05$).

Volatile fatty acids accumulations are associated with anaerobic process imbalances. Increments in VFA concentration are related with a decoupling between the bacterial groups involved in anaerobic digestion (Ahring et al., 1995), mainly due to the higher growth velocity of the fermentative and acidogenic bacteria compared with methanogenic bacteria. Volatile fatty acids are one of the most important precursors of CH_4 production in anaerobic digesters and the accumulation of VFA indicates that they are not being converted to CH_4, and thus some of the potential production is lost with the effluent from the digester. In the present study, VFA concentration in the sludge was increased when adding acidified slurry in both experiments. This accumulation could have been caused by a higher fermentative and acidogenic bacteria tolerance to high sulfate and sulfite concentration than the methanogenic bacteria. Within individual VFA, butyric acid was more strongly correlated with CH_4 yield than other VFA ($R = -0.71$) and thus, butyric acid is the best predictor of instability in the anaerobic digestion process. Ahring et al. (1995) found increments in butyric acid concentration up to 875% within 2 days after a perturbation in anaerobic thermophilic digesters. In the present study, increments of butyric acid above 2000% were observed in the digester fed with 60% acidified slurry compared with the digester fed with 100% raw slurry.

The sulfate added to the digester in both experiments was reduced and transformed into H_2S. Total dissolved sulfide concentration in the digesters remained low and stable in both experiments, which means that almost all sulfur originated by SBR activity from SO_4 reduction must have been emitted to the gas phase as H_2S. In fact, the H_2S concentration in the biogas was higher when reactors treated acidified slurries in both experiments. Thus, a likely increase in biological activity of the SRB compared to other anaerobic bacteria populations might have occurred when acidified slurry is used in anaerobic digestion. This increase in the SRB activity could also explain the increase in ALK parameters found at the end of the full-scale experiment in the acidified digester. Sulfate reducing bacteria can use propionate and butyrate, among other organic compounds, as carbon source and electron donor producing CO_2 (Hirasawa et al., 2008; Sabumon, 2008). Carbon dioxide production and VFA consumption can promote a rise in PA. However the VFA accumulations at the end of the full-scale experimental period might indicate that the SRB were not well established. Accumulations of VFA, especially acetic (Damianovic and Foresti, 2009) and butyric (Briones et al., 2009) acids indicates an incomplete oxidation of organic matter by SRB that could probably be explained by the fact that H_2S is also toxic for SRB (O'Flaherty et al., 1998).

The increment in the SO_4 reduction in the digester fed with acidified slurry that took place from day 60 in the full-scale experiment, probably caused by higher SRB activity, could also explain the reduction in CH_4 yield that was observed from day 76 in this digester. McCartney and Oleszkiewicz (1993) also observed that SRB and MPB can compete for the substrate. These authors suggested that SRB are more efficient than MPB in utilizing organic matter. Additionally, in the present study CH_4 yield was not improved after 100 days of slurry anaerobic co-digestion with a 30% of acidified slurry, thus indicating that methanogenic bacteria could not be completely adapted to these conditions even after a long anaerobic digestion period. Probably, a new equilibrium between MPB and SRB has been reached.

Regarding CH_4 production, the average CH_4 yield in the digester operating with 30% acidified slurry in the full-scale experiment was approximately 30% lower than CH_4 yield in the non-acidified slurry. This result is in accordance with that obtained in the pilot scale digester where CH_4 inhibition increased from 18.91% to 71.43% when the percentage of acidified slurry included increased from 20% to 40%. Thus, as stated for sulfate rich wastewaters (McCartney and Oleszkiewicz, 1993; O'Flaherty et al., 1998) the inclusion of acidified slurry in an anaerobic digestion process reduce CH_4 production. However, in the pilot-scale experiment, CH_4 yield increased by nearly 20% when the inclusion of acidified pig slurry increased from 0 to 10% of the total slurry, probably due to the conservative effect of acid in terms of chemical composition of the slurry as suggested by Ottosen et al. (2009). In addition, it has been reported that sulfur is a nutrient for the anaerobic bacteria that can promote biological activity at low levels (Briones et al., 2009; O'Flaherty et al., 1998). However, this effect should be further tested in a long term anaerobic digestion.

5. Conclusion

From our two experiments concerning the inclusion of acidified pig slurry in an anaerobic co-digestion process with conventional slurry we can conclude that:

- Acidified slurry inclusion at a level higher than 40% in a pilot-scale experiment and 30% in a full-scale experiment, caused an increased in acetic, butyric, and total VFA contents.
- From our results, the most important traits to be taken into account to detect a process failure in the anaerobic digestion using acidified pig slurry were: SO_4 content of the slurry, alkalinity parameters (especially PA and the ratio of alkalinity), total VFA, acetic, and butyric acids.
- Alkalinity parameters like intermediate alkalinity and the ratio of alkalinity are good predictors of total VFA and acetic acid. Variations in ALK traits indicated VFA accumulations and these parameters can predict anaerobic digestion failure.
- The inclusion of a 30% of acidified slurry in co-digestion with raw pig slurry caused a reduction of around 30% in CH_4 yield. Nevertheless at a level lower than 10%, acidified slurry could promote CH_4 yield by nearly 20%.

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