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The effect of different inoculums on anaerobic digestion of swine wastewater

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ABSTRACT

Methane production from swine wastewater was evaluated by using three inoculums: rumen (I1), stabilized swine wastewater (I2) and sewage sludge (I3). Experimental design was based on four treatments by duplicate: T0: swine wastewater as substrate (S) without inoculum (I), T1: S + I1, T2: S + I2 and T3: S + I3 all with 90 (S)–10 (I) % vol with a ratio S/I approximately constant (1:0.05). ANOVA test was applied to evaluate the significance of treatments at 95% confidence. After a batch experiment of 140 days, results indicated that the addition of any inoculum improved methane production rate and shortened the start-up of methane exponential growth stage. I2 and I3 promoted the highest percentage of organic matter removal (close to 50% in terms of VS and COD) and, in relation to the control test, a higher methane production achieving 0.25 L CH_4/g VS. The use of rumen (I1) did not improve methane production to the same extent as the other inoculums while organic matter removal only achieved 15%. The evolution of VFA and alkalinity show that methanogenic phase could be considered as the rate-limiting step of the global methane production rate.

1. Introduction

One of the most important and current challenges is to find a solution to the pollution caused by waste and wastewater of industrial and agricultural activities and to satisfy the growing demand for energy [1]. During the last decades in Argentina it has been a gradual process of concentration of the primary activity with the emergence of large companies and the disappearance of many traditional producers of mid and small scale. Accompanying this phenomenon there was a wide diffusion of technologies of high-capital investment based on a technological concept of industrialization of rural production. The increase in the number of animals per establishment and the regionalization of the production has generated strong pressure on livestock, poultry and dairy sectors because if production operations are not properly handled, the discharge of nutrients, organic matter, pathogens and emission gases cause significant pollution on water, air and soil [2,3]. Anaerobic digestion (AD) tend to solve the problem of wastewater with a high content of organic matter through the availability of renewable energy source based on the use of the generated methane [4–6]. However, the estimation of net energy to be produced through this process is a complex task due to the wide range of factors that affect methane production [7].

AD of organic matter is carried out by a consortium of microorganisms in sequential stage resulting a synergic action [8–10]. The first stage corresponds to the hydrolysis of the complex organic compounds that are not directly available for microorganisms. The result is the production of more simple organic compounds as fatty acids, alcohols, and sugars. The second stage is the acidogenic phase, which involves the conversion of volatile acids and alcohols into simple substrates such as acetic acid and hydrogen that can be used by methane-forming bacteria. The third and last stage is the methanogenesis phase where methane and carbon dioxide are produced [11,12]. In the final steps of AD process, the dominant species are acidogenic and methanogenic bacteria. The first is characterized by fast growing and less sensitivity to the environmental changes while methanogens are of slow growing (from a few days to some weeks depending on environmental conditions) and they usually are inhibited to low pH values [7,12]. Veeken and Hamelers [13] have determined that

Abbreviations: AD, anaerobic digestion; AR, alkalinity ratio; I, inoculum; IA, intermediate alkalinity; PA, partial alkalinity; S, swine wastewater; T, treatment; TS, total solids; VFA, volatile fatty acids; VS, volatile solids.

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the accumulation of volatile fatty acids (VFA) as intermediates is a signal that the rate of the hydrolytic step is greater than the methanogenic stage. Any perturbation in the system can cause changes in the type of dominant species and in the population of microorganisms that will be reflected in the performance of the bioreactor [7]. From that, the production and consumption of VFA must be balanced to avoid overloads of the system and low degradation of waste.

Kinetic studies of AD models can provide useful information for the analysis, design and operation of a fermentation process [14–16]. Kinetics description of the complex organic matter is accomplished through the so-called rate-limiting step approach, which could be defined as the step that will cause failure under imposed conditions of kinetic stress [16]. The rate-limiting step is related to the nature of the substrate, process configuration, temperature, and loading rate. The type of waste digested dictates which step need to be considered [17]. According to Seghezzo [17] hydrolytic step is usually regarded as the controlling step for wastewater with a high content of particulate matter. Other authors such as Pavlostathis and Gossett [18], Pavlostathis and Giraldo-Gómez [16] pointed out that the methanogenesis or acidogenesis have been indicated as controlling stages even when the hydrolysis may affect the overall process kinetics.

Between livestock waste, swine wastewater has characteristics that make it able to be a favorable substrate for AD due to its higher levels of water content and buffer capacity. Furthermore, swine wastewater is a complex substrate that contains undissolved and dissolved organic matter such as polysaccharides, lipids, proteins and VFA in addition to a wide variety of nutrients which are favorable for the growing of anaerobic microorganisms [14,19]. All these compounds interact during the anaerobic process in a complex system that should be analyzed to assure its stability when the use of energy is a goal. Previous studies [20] demonstrated that the use of fresh liquid swine manure a substrate in AD did not achieve a good and steady performance for methane production without the application of inoculums (I). Quality and quantity of inoculums are key factors that determine the length of the start-up and operation in a steady state of the reactor [12].

Forster-Carneiro et al. [21] reported that inoculum source is a very important operational parameter. Different types of inoculums have been used in mesophilic anaerobic digestion, such as swine wastewater, rumen, and sewage sludge that have demonstrated good quality due to its content in methanogenic bacteria [1,7]. Furthermore, these inoculums are waste from several sectors such as meat and other food productions (slaughterhouses, pig and cattle breeding, dairy sector) that highly impact the environment. Liquid swine manure have been used as inoculums in AD of urban solid waste [21], and in poultry manure [22]. Meanwhile Lopes et al. [23] and Budiyono et al. [1] analyzed the influence of bovine rumen fluid as inoculum during anaerobic treatment of the organic fraction of municipal solid waste and cattle manure respectively. González-Fernández and García-Encina [24] analyzed the influence of sewage sludge on AD of liquid swine manure. Neves et al. [25] used two types of sewage sludge as inoculum (granular and suspended) in the AD of kitchen waste finding that the first one (granular) was better in terms of methane production than the second one. Digested sludge resulted the best inoculums between six types (corn silage, restaurant waste digested mixed with rice hulls, cattle excrement, swine excrement, digested sludge, and swine mixed with sludge) in anaerobic thermophilic digestion of organic fraction of municipal solid waste [21]. Taken into account that not all substrate can be utilized by all methane-forming bacteria [12], the performances of the process is strongly dependent on the characteristics of the substrate as well as the inoculum and the whole mixed system. In this sense, no comparative studies were found relative to the influence of different inoculum on the DA of swine wastewater.

The objective of this study was to analyze the effect of three different inoculums-highly available in the region of this study- on the AD of fresh swine wastewater and evaluate the performance of the process regarding the methane production and identifying those factors that affect the start-up and stability of the process.

2. Materials and methods

2.1. Experimental design

Four treatments in duplicate were carried out in a batch laboratory-scale experiment. Reactors of 1 L capacity were filled with mixtures of substrate and inoculums. They were hermetically closed in order to assure anaerobic conditions and were manually agitated at least once a day to avoid stratification. Bioreactors were maintained in a water bath a constant temperature ($35 \pm 1\,^{\circ}C$) corresponding to mesophilic conditions (Fig. 1). Experiment were conducted for 140 days until, methane production declined in all treatments.

2.2. Substrates and inoculums

The substrate used was liquid fresh swine wastewater collected from a piggery the same day that was produced and previous to the discharge in a covered lagoon.

Three inoculums (I) were used: rumen (I1), swine liquid manure previously digested (I2) and sewage sludge (I3). I1 was provided by the local slaughterhouse the same day that was collected to avoid degradation and death of anaerobic bacteria. I2 was obtained from the Bioenergy Lab (College of Engineering, UNCPBA) from a previously conditioned material under mesophilic conditions and used when methane percentage achieved its higher and constant value ($67.61 \pm 3.39\%$) in order to assure the methanogenic activity [12]. I3 was obtained from the wastewater

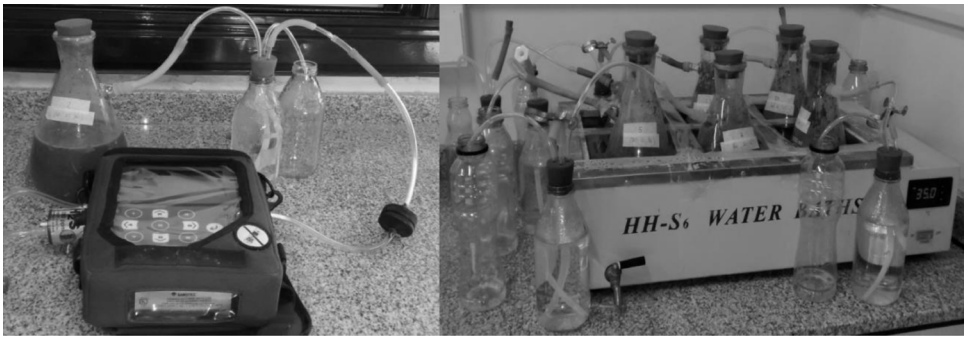

Fig. 1. Experimental arrangement; biogas and methane measurement.

Table 1
Experimental design.

Treatment	Inoculum	Percentage in vol.		S/I ratio
		Substrate	Inoculum*	g VS S/g VS I
T0		100	–	–
T1	I1	90	10	1:0.06[a]
T2	I2	90	10	1:0.05[a]
T3	I3	90	10	1:0.05[a]

*Base on several authors that evaluated the inoculums concentration and agreed that it should be applied between 5 and 10% in volume to assure an efficient process [9,19,20].
[a]Values with the same letter in the same column have no significant differences between treatment ($P > 0.05$) according to LSDs test.

treatment plant (WWTP) at Olavarría city and maintained in a batch reactor at mesophilic conditions at 35 °C ± 1 until achieved constant methane content of about 70%.

Samples were prepared by mixing S and I in the proportions showed in Table 1. Each treatment was carried out in duplicates, and no water was added to the digester due to the total solids content of the substrate resulted in less than 10% assuring wet anaerobic digestion [26]. The rate S/I resulted 1:0.06, 1:0.05 and 1:0.05 for T1, T2 and T3 respectively; differences between treatments resulted statistically non-significant at $P < 0.05$.

2.3. Analytical methods and procedure

The parameters analyzed for the characterization of S, I and mixtures were total solids (% TS), volatile solids (% VS), chemical oxygen demand (COD, mg/L), pH, and ammonia nitrogen (mg N-NH_4^+/L). All of them were performed according to Standard Methods [29]. These analyzes were carried out for each treatment before and after the AD.

The alkalinity of treatments along the experiment was determined according to the method suggested by Jenkins et al. [30] that involves titration of the centrifuged sample at two pH values (5.75 and 4.3). Three parameters were defined as a measure of alkalinity: total alkalinity (TA) measured by pH titration to 4.3; partial alkalinity (PA) associated with bicarbonate alkalinity and to the buffer capacity of the system measured at pH 5.75 and intermediate alkalinity (IA) associated with the VFA concentration and estimated as the difference between previous ones. Alkalinity ratio (AR) is an indicator of digester stability and is defined as the ratio between IA and PA. This parameter should not exceed the range 0.3–0.4 to avoid acidification of the reactor [12,19,31–33]. These parameters, as well as pH were measured once a week in all bioreactors.

The volume of biogas was evaluated in all the samples by volume displacement according to Córdoba et al. [20]. The quality of biogas was evaluated by the percentage (%) of methane. Measurements were done periodically (at least daily) using a portable Landgem GA2000, USA (Fig. 1) instrument with infrared cells to measure methane and carbon dioxide (maximum error ±0.5%), and electrochemical cell for oxygen content (maximum error ±1.0%). Calibration was done with certified standard type gas mixture 60–40 (CH_4–CO_2) from AGA (Certification Number 165342).

2.4. Statistical analyses

All treatments were conducted in duplicate. Methane and biogas production were expressed as mean value ± standard deviation. The values reported to each parameter analyzed in this work were the average of two samples. The analysis of variance (ANOVA) test was performed to determine the significance of mean values, Fisher's least significant difference (Fisher's LSD) was calculated at $\alpha = 0.05$. Statistical analysis of the data was carried out using Statgraphics Centurion XVI (v.16.2.04) program.

2.5. Waste characterization

Physic-chemical characterization of S and I utilized in the four treatments is detailed in Table 2. S showed a higher percentage of total solids (7.21%) than I. The high values of VS (86.39%) and COD (63724 mg/L) are indicators of abundance in organic matter. I2 and I3 showed higher matter content than I1. The value of alkalinity gives an indication of the buffer capacity of the system. Several authors [21,34–36] reported that values of TA higher than 3000 mg/L assure the stability of the process. All the inoculums used showed higher alkalinity value than the substrate, where showed the highest value (11374 mg $CaCO_3$/L). The relative high ammonia nitrogen concentration (1021 mg NH_4^+-N/L) should not be a risk regarding the range between 3000 and 5000 mg/L suggested by Drosg [37] that could because of inhibition.

Table 2
Physic-chemical characterization of S and I.

Parameter	S	I1	I2	I3
TS, %	7.21 ± 0.39	4.51 ± 0.38	3.53 ± 0.45	4.57 ± 0.07
VS, % d.b.	86.39 ± 0.24	73.96 ± 1.37	96.47 ± 0.45	56.70 ± 0.43
pH	6.18	6.85	7.88	8.27
Total alkalinity, mg $CaCO_3$/L	4776 ± 28	5903 ± 82	11374 ± 262	5063 ± 65
COD, mg/L	63724 ± 6061	28479 ± 3756	38766 ± 1834	38034 ± 8206
Ammonia nitrogen, mg N-NH_4^+/L	1021.0 ± 37.9	163.9 ± 33.1	2528.5 ± 175.1	882.2 ± 83.8

Table 3
Physic-chemical characterization of treatments.

Parameter	T0	T1	T2	T3
TS%	7.21 ± 039[a]	6.94 ± 0.31[a]	6.84 ± 0.30[a]	6.95 ± 0.34[a]
VS% d.b.	86.39 ± 0.24[c]	85.15 ± 0.08[b]	85.26 ± 0.00[b]	83.42 ± 0.17[a]
pH	6.18	6.40	6.49	6.45
Total alkalinity, mg $CaCO_3$/L	4776 ± 28[a]	4996 ± 129[b]	5264 ± 36[c]	5010 ± 155[b]
COD, mg/L	84576 ± 8045[b]	65890 ± 6007[a]	66747 ± 5189[a]	67243 ± 4694[a]
Ammonia nitrogen, mg N-NH_4^+/L	1021.0 ± 37.9[a]	922.1 ± 76.5[a]	1198 ± 124.0[a]	1016.5 ± 176.1[a]

The values are means of replicates ± standard deviation. Values with the same letter in the same row have no significant differences ($P > 0.05$) according to LSDs test.

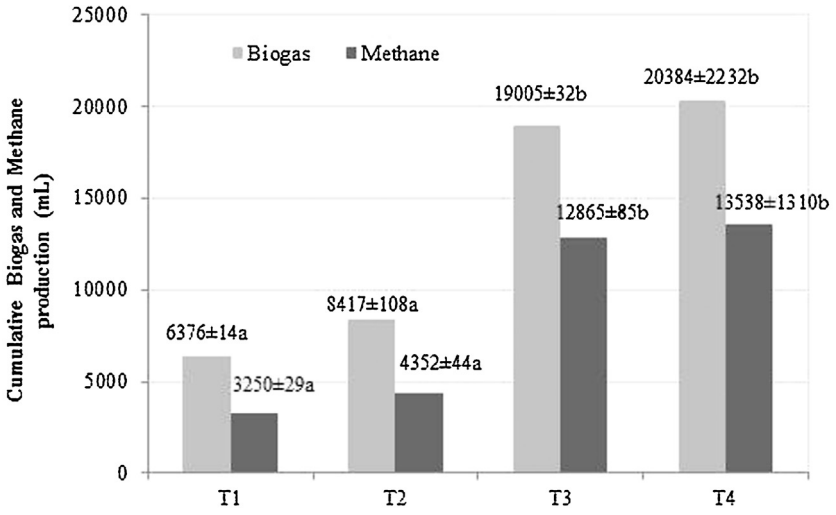

Fig. 2. Cumulative biogas and methane production for each treatment. Values obtained are means of replicates ± standard deviation (SD). Values with the same letter in the same type bar have no significant differences according to LSDs test. *P* values resulted 0.0005 and 0.0002 for biogas and methane production respectively in the ANOVA table.

3. Results and discussion

Physical and chemical characterization of the initial mixtures is shown in Table 3. The initial pH varied between 6.18 (T0) to 6.49 (T2), and alkalinity resulted in all the treatments higher than the value suggested to assure stability in the digester. Based on that, no pH adjustment was carried out on the mixtures. Regarding ammonia nitrogen concentration, T2 showed the higher value (1198 NH_4^+-N/L) due to the nature of the inoculums (I2) that provides a high concentration of this compound. However, the differences between the four treatments did not result statistically significant at $P > 0.05$ (Table 3).

3.1. Methane production and process efficiency in terms of organic matter removal

After 140 days of anaerobic treatment, the cumulative biogas production obtained achieved 6376, 8417, 19005 and 20384 mL for T0, T1, T2 and T3 respectively (Fig. 2). Higher methane productivity was achieved in T3 reaching a total production of 13538 mL followed by T2 with 12865 mL, T1 with 4352 mL and T0 with 3250 mL. Fig. 2 shows that differences between treatments resulted statistically different ($P < 0.05$) between T0 and T2 and between T0 and T3 while between T0 and T1 differences resulted non-significant ($P > 0.05$).

Biogas production in T2 and T3 resulted 198% and 219% respectively higher than T0 while in terms of methane generation the increase resulted in 296% and 317%. The application of rumen (T1) only improved 32.0% and 33.9% biogas and methane productions respectively compared to T0.

The fraction of methane in the biogas resulted 51.0, 51.7, 67.7and 66.4% in T0–T3 respectively indicating that inoculums improved not only biogas production but also enhanced its quality in terms of energy content.

Organic matter removal is a measure of the degradability of complex substrates [21,38]. Biogas and methane productions are related to the available organic matter fed to the bioreactor measured in terms of VS and COD. For that reason, the productivity of AD is usually indicated in units of L CH_4/g VS or L CH_4/g COD. T2 and T3 achieved higher organic matter removal (46.0 and 49.8% VS removal and 53.0 and 52.0% COD removal respectively) compared to T0 (32.0 % of VS and 18.4% COD removal) and T1 (35.9% SV and 14.6% COD removal) (Table 4).

Taking into consideration the methane production in terms of VS added to the bioreactor, rumen (T1) produced only 34.5% more methane than the substrate T0 (differences non-significant at $P > 0.05$) while T2 and T3 produced 320.7% and 341.4% (differences non-significant at $P > 0.05$) higher than T0 respectively. Final values obtained for methane production (Table 4) represent the maximum potential for each mixture. The results obtained in T2 (0.244 ± 0.002 L CH_4/g VS) and T3 (0.256 ± 0.025 L CH_4/g VS) resulted slightly lower than those reported by Chynoweth et al. [38], that indicated a typical potential methane yield in the range of 0.32 to 0.48 L CH_4/g VS with a VS removal between 40 and 60%, demonstrating that these parameters are largely dependent upon the composition and the digestibility of the feed ration. By other side, the experimental values obtained resulted close to those suggested by IPCC Guidelines [39] for the estimation of methane emissions from swine wastewater (the indicated value for Latin America region was 0.29 m^3 CH_4/kg VS).

Chae et al. [40] determined the theoretical methane yield at standard temperature and pressure based on the elemental analysis of swine wastewater by using the Buswell's equation [41] founding a production of 0.724 L CH_4/g VS destroyed, value 26% higher than those obtained in the present study (0.534L CH_4/g VS destroyed). This difference could be explained through several factors such as the fraction of the organic matter that will be use to synthesize bacterial mass, the lignin content that is not anaerobically degraded and the limitation of macro and micro nutrients as highlighted by Angelidaki and Sanders [41].

Table 4
Summary of the performance of the four applied treatments.

Treatment	Organic matter removal		Methane production	
	COD, %	VS, %	L CH_4/g VS_i	L CH_4/g VS_f
T0	18.41 ± 6.0^a	32.01 ± 2.0^a	0.058 ± 0.0005^a	0.181 ± 0.010^a
T1	14.61 ± 3.3^a	35.85 ± 1.6^a	0.078 ± 0.0008^a	0.227 ± 0.008^a
T2	53.06 ± 2.2^b	46.03 ± 6.4^a	0.244 ± 0.002^b	0.534 ± 0.077^b
T3	52.05 ± 9.2^b	49.85 ± 7.6^a	0.256 ± 0.025^b	0.517 ± 0.029^b
P-value	0.0031	0.0687	0.0002	0.002

i:initial, f: removed. These values are means of replicates ± standard deviation. Values with the same letter in the same column have no significant differences ($P > 0.05$) according to LSDs test.

3.2. Analysis of the methane production kinetics

Methane concentration in biogas resulted about 50% (average) from the startup of the process, and then decreased to about 22% in T0 (60th day) and among 40–50% in T1, T2 and T3 (between days 20th and 40th of the experiment). The action of the inoculums allowed a high recuperation of methane concentration, achieving percentages around 70–80% in T2 and T3 that remained from day 60th to the end of the experiment. Methane percentage in T0 started to growth from day 60th achieving values close to 70% at day 120th although methane production resulted lower than T2 and T3.

For all treatments, it was observed two phases of methane generation separated by an intermediate lag stage of low methane production (Fig. 3). The first stage stayed about 20 days and the second one resulted in longer depending on the treatment. During the first stage it was observed an initial fast increase in methane production rate (during the first 48 h approximately) showing a peak for all treatments (highest value observed in T1 was 325 mL CH_4/day). This behavior could be explained through the action of the bacteria consortium provided by the substrate itself, as can be observed from T0 that evidenced a peak of methane production of 281 mL CH_4/day in spite it does not contain any inoculums. Then, methane production rate decreased from the peak value achieving the minimum rates for each treatment: almost null for T0, 5.4 mL/day for T1, 10.6 mL/day for T2 and 5.7 mL/day for T3. This stage could be considered as the start up of an induced lag phase. The decrease of methane production observed could not be explained on the basis of a lack of a specific source of feed for methanogenic bacteria because VFA concentration steadily increased during this stage (as can be observed from Fig. 4). According to Swinnen et al. [42] the lag phase of a microbial population is typically observed as a delay in the growth of a microbial population caused by a sudden change in some environmental factor such as temperature. In this case, when the substrate was placed in the batch digester, biomass temperature suddenly changes from ambient temperature to 35 °C. These changes induced a lag phase in the process causing a methane production decrease achieving a null production for T0 (until day 75). This lag phase could also be detected in T1–T3 however, it resulted in shorter comparing to treatment T0 explained on the microbial population supplied by inoculum itself (Fig. 2).

After the induced lag phase, the second stage of methane production started approximately from day 40th to treatment T1, T2, and T3. During this stage, T0 achieved a maximum of 54.97 mL/day on day 104th from when average production was of 34.7 ± 9.1 mL/day. For T1–T3, the peak rates resulted in 56.09, 256.50 and 298.61 mL CH_4/day achieved on 62nd, 100 and 105th days respectively. These results evidenced the action of inoculums shorting the lag phase and increasing the methane production rate. In spite T1 started its exponential phase of methane growing at the same time than T2 y T3, from day 53rd T1 stayed approximately at constant rate (average 26.4 ± 13.7 mL/day) while T2 and T3 continued increasing the methane production achieving averages values of 120.7 ± 68.1 mL/day and 124.4 ± 71.6 mL/day respectively for the whole methane exponential growing stage.

These results evidenced that during the second phase, the bacteria responsible for the methane generation were provided by the inoculums which, required an adaptation time (lag-phase) to the new environment (substrate). Besides, the nature of inoculums influences the methane productivity according to the type of substrate. From results obtained, digested swine manure (I2) and wastewater sludge (I3) seems the inoculums that best suit to the substrate that is being studied due to they not only shortened the lag phase but increased methane production as well as methane generation rate.

3.3. Alkalinity and VFA analysis

High biogas and methane production are both associated with the consumption of VFA [21]. VFA are by-products of digestion and according to Shao Pin [43] wastewater from pig farms contains a high proportion of these compounds. The methane production observed during the initial stage of the AD was explained through the high values of VFA (3500–3800 mg $CaCO_3$/L, Fig. 4) that served as a substrate for methanogenic bacteria. This methane production caused an increase in alkalinity to values close to 2000 mg $CaCO_3$/L indicating a decomposition of the organic compounds (proteins and amino acids) that released ammonia and carbon dioxide allowing the alkalinity growth, favoring the increase of the buffer capacity of the system [9,12,33]. After this initial stage, the VFA continued increasing for all the treatments to approximately 4900 mg $CaCO_3$/L consuming alkalinity and decreasing the buffer capacity of the system. As a result, methane production decreased until values almost negligible. During the second stage, methanogenic bacteria mainly provided by the inoculums and already adapted to the new environment, starting to produce methane at the expense of the available VFA consumption. This behavior could be clearly observed in T2 and T3 where VFA consumption along the second stage resulted in 70^{b}% and 67.3^{b}% respectively while for T0 and T1 there were only consumed 36.6^{a}% and 37.5^{a}% respectively from the available VFA (Fig. 4). Different letters indicated significant differences at 95% confidence level. These results showed that bacteria provided by I2 were not able to consume the

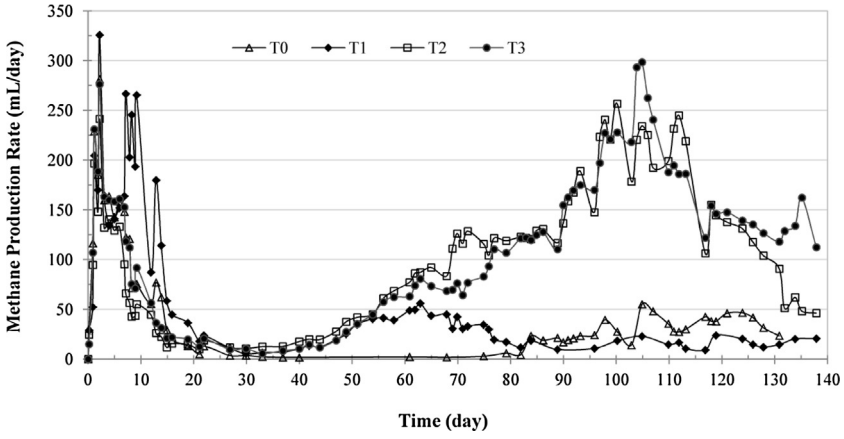

Fig. 3. Methane production rate along 140 days of AD for the four applied treatments.

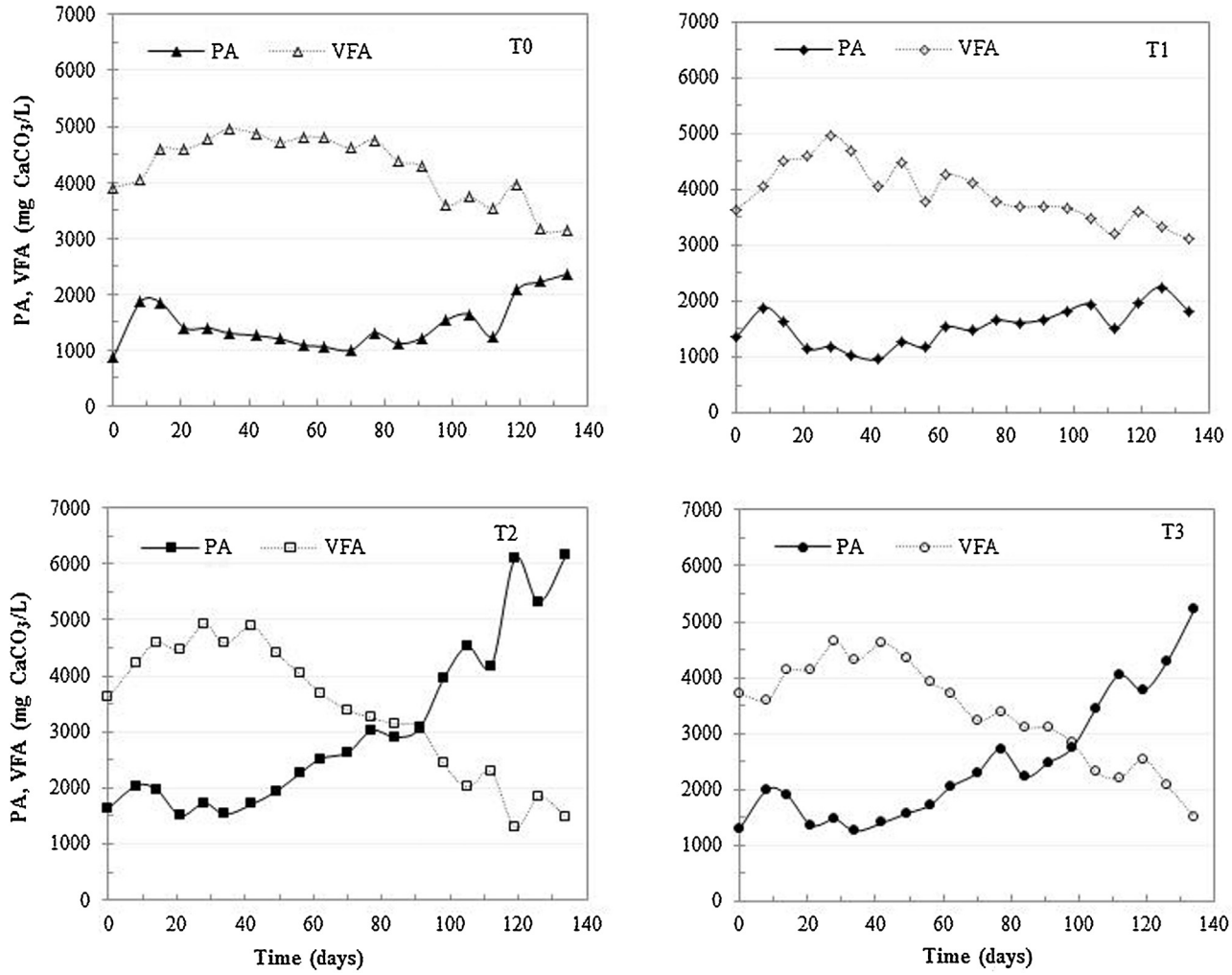

Fig. 4. Evolution of alkalinity (PA) and VFA concentrations along the experiment.

available VFA and, therefore showed lower potential methane production. The final values obtained of VFA at the end of the process for T2 (1476 mg $CaCO_3$/L) and T3 (1509 mg $CaCO_3$/L) resulted lower than those obtained for T0 (3178 mg $CaCO_3$/L) and for T1 (3112 mg $CaCO_3$/L) indicating that adequate inoculums promote VFA consumption and therefore methane production. The remainder non-consumed VFA in T0 and T1 are indicators of an excess of accumulation that could destabilize the system and inhibit the methanogenic action. This in accordance with Gerardi [12] that indicated that successful fermentation of substrates in an

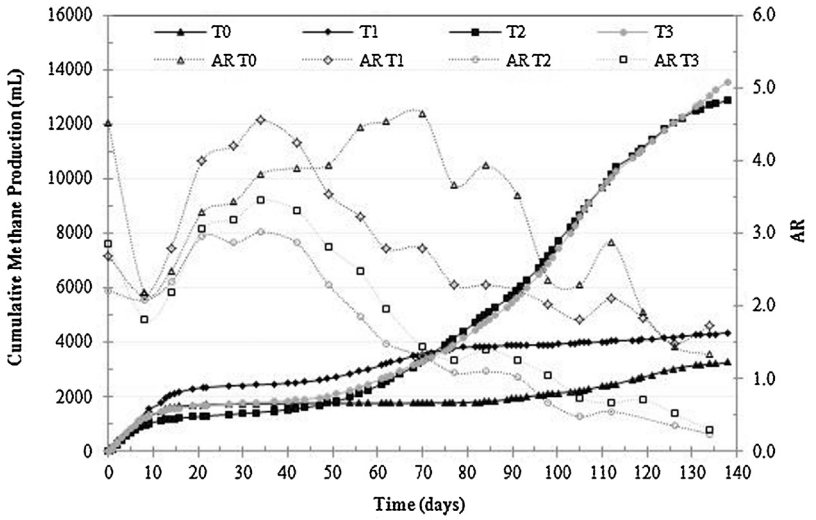

Fig. 5. Cumulative methane production and AR for the four applied treatments.

anaerobic digester requires the presence of a large diversity of methane-forming bacteria.

The analysis of the second stage in terms of the alkalinity showed for T0 a slow increase from day 70th to achieve a final value of 2358 mg $CaCO_3$/L; T1 exhibited similar behavior achieving a final value of 1820 mg $CaCO_3$/L; in T2 and T3, alkalinity showed a sustained growth from approximately day 20thachieving values at the end of the experiment of 6168 and 5215 mg $CaCO_3$/L respectively (Fig. 4). pH values followed a similar pattern to alkalinity.

Fig. 5 shows that the increase in methane production was associated with a decrease of AR and the consumption of VFA due to a higher methanogenic activity. In T0 and T1 a lower AR decrease reflected lower methane production while in T2 and T3the increase in the VFA consumption rate caused higher decrease of AR. In T2, only after the 90th day, AR decreased to less than 1.00, achieving an alkalinity value of 3058 mg $CaCO_3$/L. T3 achieved AR lower than 1.00 on 98th day, recording a value of alkalinity of 2741 mg $CaCO_3$/L. However, T0 and T1 were not able to reach AR lower than 1.At the end of the process, for T2 and T3, the buffering capacity of the system in terms of total alkalinity resulted higher than 3000 mg $CaCO_3$/L that ensures the stability of the process, in spite of AR resulted higher than the value suggested for a "stable system" [12,19,31–33,37].

Fig. 5 reflected the cumulative methane production explained through two stages with an intermediate induced lag-phase for the four treatments. The first stage produced 2318.4^{c} mL CH_4 (T1) followed by 1692^{b} mL (T0), 1668^{b} mL (T3) and 1271^{a} mL (T2) (different letters indicated significant differences at 95% confidence level). These values represented 52.1, 53.3, 9.9 and 3.7% of the whole accumulated methane production for T0–T3 respectively, so in this stage, methane-forming bacteria provided by I1 are able to produce more methane than the other treatments. After the lag phase, the second stage produced 1557 mL CH_4 (T0), 2033 mL (T1), 11593 mL (T2) and 11870 mL (T3) demonstrating that I2 and I3 allowed the best performance for the substrate studied obtaining higher methane production.

By comparing the kinetics of the four treatments, T0 showed the longer induced lag phase, the lower VFA consumption and, therefore the lower methane production. This behavior could also be explained through the high S/I ratio used in the experiment compared with values previously reported. High S/I ratio was selected to improve the performance of the system in terms of provide higher capacity of the digesters, however as could be observed it caused longer times of process and VFA accumulation and, therefore lower methane production [24]. The action of the inoculums contributed to reduce the lag phase and increase the methane production rate during the exponential phase allowing longer methane production stage as could be observed from the differences in these parameters when T1–T2 and T1–T3 were compared. This behavior is in accordance with those reported by Neves et al. [25].

For the experimental design applied and considering the high S/I ratio used, it was observed that methanogenic stage resulted the limiting of the process rate based on the analysis of the low methane production and low VFA consumption obtained for T0 compared with T2 and T3 during the second stage where can be observed the effect of inoculums.

4. Conclusions

Methane production from AD of swine wastewater was evaluated by using three inoculums (rumen, digested swine wastewater and sewage sludge) in low concentration. It was evaluated the performance of the process in terms of the methane production and the identification of the factors that affect the start-up and stability of the process.

Results showed that inoculation of fresh swine wastewater improved the productivity in the AD process in terms of biogas and methane production. Sewage sludge and stabilized liquid swine manure showed better capacity that rumen to act as inoculums when fresh liquid swine wastewater is used as the substrate. Both inoculums achieved a higher percentage of organic matter removal (close to 50% in terms of VS and COD) and the highest methane productivity attaining the value of 0.25 L CH_4/g VS, close to previous reported. Rumen did not produce significant differences regarding methane produced by the substrate itself, and the organic matter removal did not exceed 15%.

The nature of the substrate as carrier of a microbial consortium that provides enough methanogenic bacteria allowed to identify two stages in the methane production: the first one provided by the substrate itself, an intermediate lag phase that depends on the inoculums and a second one of exponential growth methane production where it was achieved the maximum methane potential production. The inoculation caused a decrease of the lag phase almost to the half. The evolution of VFA and alkalinity explained the observed methane production kinetics. For the substrate studied, the fast growing of VFA concentration as well as the long time that this concentration remains indicated that methanogenic stage is the rate limiting step of the global methane production rate.

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