Int. J. Environ. Res., 8(2):483-492,Spring 2014
ISSN: 1735-6865

Anaerobic Digestion of Slaughterhouse Solid Waste for the Optimization of Biogas Production

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Received 24 July 2013; Revised 25 Dec. 2013; Accepted 11 Jan. 2014

ABSTRACT: Anaerobic digestion is a feasible technology to stabilize the solid waste generated in slaughterhouses obtaining significant quantities of biogas, considered as a clean and renewable fuel. This is why it is important to optimize the digestion process in order to eliminate organic matter and maximize the production of biogas. In this work, a system was developed for treating slaughterhouse solid waste while maximizing the production of biogas. This system is based on the separation of the acidogenic and the methanogenic phases of anaerobic digestion. The study was conducted in two phases. First, the effect of thermal pretreatment of the substrate and inoculation of the bioreactor with granular sludge were evaluated. In this phase, two variables were analyzed: the pretreatment temperature and whether or not inoculum was added. The results showed that the greatest decrease of total chemical oxygen demand (57%) and the highest biogas production (753 mL) were obtained from the inoculated sample pre-treated at 60 °C. In the second phase of the study, we analyzed the effect of running the anaerobic digestion stages, fermentation and digestion, in two separated steps. We found that the removal of organic matter is the same (56%) but more biogas (0.376 m^3/m^3 reactor*day) was produced in a two-step process.

Key words: Biogas, Slaughterhouse, Anaerobic digestion, Pretreatment, Inoculum

INTRODUCTION

La digestión anaerobia es un proceso biológico donde un grupo de diferentes microorganismos en ausencia de oxígeno son capaces de degradar la materia orgánica permitiendo la formación de una mezcla de gases principalmente dióxido de carbono (CO_2) y metano (CH_4) llamada biogás y sedimentos estabilizados que pueden ser utilizados como abono orgánico (Chen Y. *et al* ., 2008).Anaerobic digestion is a biological process by which microorganisms breakdown organic matter in the absence of oxygen, producing biogas (primarily methane (CH_4) and carbon dioxide (CO_2)), and a stabilized sediment that can be used as an organic fertilizer (Chen *et al.*, 2008). El proceso consiste en cuatro fases metabólicas hidrólisis, acidogénesis, acetogénesis y metanogénesis. This process consists of four metabolic phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. La hidrólisis es considerada como el paso limitante de la digestión anaerobia. La desintegración mecánica y los pretratamientos al sustrato tienen la finalidad de mejorarla, así como de solubilizar la materia ya que los microorganismos solo pueden degradar materia orgánica en fase soluble, entre los pretratamientos, están los del tipo químico mediante la adición de una base o ácido, tratamientos térmicos de 40 – 100 °C e hidrólisis biológica mediante la adición de enzimas (Eastman JA, Ferguson JF, 1981; Miah MS *et al* ., 2005).Hydrolysis is the limiting step of anaerobic digestion (Demirer and Chen, 2005). Since microorganisms can only transform solubilized organic matter, mechanical disintegration and pretreatment of the substrate are employed to improve the solubilization of the organic material. Chemical pretreatments include the addition of an acid or a base, heat treatment at temperatures between 40 and 100 °C and biological hydrolysis using specific enzymes (Eastman and Ferguson, 1981, Miah *et al.*, 2005). Heat treatment breaks down the chemical bonds of the complex compounds (denaturing proteins and solubilizing fats) making them more soluble and suitable for microorganism digestion. It also breaks down the chemical bonds of the cell walls and membranes, and solubilizes cell components.

Several authors have described the use of thermal pretreatments to improve anaerobic digestion. The

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effects of thermal pre-treatments on the biogas production for two types of solid slaughterhouse waste (poultry and swine by-products), were reported by Rodríguez *et al.*, (2011). They found that thermal pre-treatments produced a significant solubilization of particulate chemical oxygen demand (COD) in both types of slaughterhouse waste. However, there were different results related to protein decomposition, biogas, methane production potential and maximum methane production rates, which suggests the importance of the influence of composition on the anaerobic bioavailability of treated substrates. Strong and Gapes (2012) presented a study in which four solid waste substrates (coal, Kraft pulp solids, chicken feathers and chicken processing waste) were thermally pre-treated (70, 140 and 200 °C), under an inert (nitrogen) or oxidative (oxygen) atmosphere, and then anaerobically digested. They reported that thermal pre-treatments allows for more rapid conversion of waste carbon to methane during anaerobic digestion, while thermo-chemical pre-treatments can rapidly destroy waste solids and convert them to biologically amenable compounds such as acetic acid (which would be particularly useful for toxic or recalcitrant wastes). They also found that methane yield more than doubled for the Kraft pulp solids with the 200 °C pre-treatment under oxidative conditions. On the other hand, Liu *et al.*, (2012) investigated the effects of thermal pretreatment on the physical and chemical properties of three typical municipal biomass wastes, kitchen waste, vegetable/fruit residue, and waste activated sludge. They found that thermal pretreatment at 175 °C for 60 min significantly decreased viscosity, improved waste dewatering and increased soluble chemical oxygen demand, soluble sugar, soluble protein, and especially organic compounds. They concluded that thermal pretreatment improves settling velocity and dewatering of the waste and that thermal pretreatment not only disintegrates particulate organics, but also destroys cell flocs and releases organic matter inside cells.

The optimal conditions and the magnitude of such improvement vary considerably. This is consistent with studies of Gavala *et al.*, (2003), who concluded that the optimal temperature and duration of the pretreatment depend on the nature of the substrate. Substrates that are more difficult to hydrolyze require more intense pretreatment (temperature and time). In general, thermal pretreatment can significantly increase methane production for mesophilic anaerobic digestion, but not so much for the thermophilic stage, so preconditioning is more effective in low-speed (slow kinetics) systems such as mesophilic digestion.

On the other hand, the uncontrolled growth of undesirable microorganisms is a problem because from the beginning of the start-up process until stability is reached, the most important factor is retaining a viable biomass in the reactor. This highlights the importance of inoculating the digester, as the first step of anaerobic digestion (Giraldo, 1998). Initially there is no need for anaerobic conditions, as these conditions are met the first day. The inoculum should have some methanogenic activity, the higher this is, the lower the starting period. Inoculating with mature sludge from an operating anaerobic reactor is highly recommended, but beef or pork manure or even domestic sludge can be used.

Anaerobic digestion is extremely complicated because of different types of bacteria that may be present. Separating the stages provides better process control and higher productivity, as was shown by Jeyaseelan and Matsuo (1995). They investigated the treatment characteristics of two different synthetic substrates processed by two-phase anaerobic digestion at 20°C. The liquefaction-acidification and gasification phases of anaerobic digestion in a plug-flow reactor charged with feed slurries were evaluated by Liu and Ghosh (1997). Numerous studies have been conducted on the production biogas using two-stage anaerobic digestion, including: sludge from wastewater treatment (Ghosh *et al.*, 1995); the organic fraction of municipal solid waste (Chanakya *et al.*, 1992); sludge and industrial water (Ghosh *et al.*, 1985); solid wastes from olive ground (Rincón *et al.*, 2009); fat (Yu *et al.*, 2002); and food waste (Verrier *et al.*, 1987). Other studies have focused on reactor design improvements and the control and operating parameters (Zoetemeyer *et al.*, 1982; Vavilin *et al.*, 2001; Von Sachs *et al.*, 2003).

The reason for separating the stages is that the same types of microorganisms (fermentative bacteria) perform both the hydrolysis and acidogenesis phases. These metabolic phases are the first stage of anaerobic digestion process, known as fermentation. The second stage, known as methanation because it produces methane, consists of acetogenesis and methanogenesis phases. In this stage, two types of bacteria are used: acetogenic bacteria that transform the volatile fatty acids (VFA) to acetate, and methanogenic bacteria that produce methane from acetate. La formación de estos gránulos es una característica que distingue a los sistemas anaerobios de flujo ascendente, de los otros sistemas (Castro *et al* , 1999; López *et al* 2000). Another important factor in anaerobic systems is the type and quality of anaerobic sludge. Granular sludge is the key factor in the efficient operation of an upûow anaerobic sludge blanket (UASB) reactor. The formation of those granules is a characteristic that distinguishes the

UASB reactors (Castro *et al.*, 1999). Una de las causas más frecuentes del mal funcionamiento de los reactores anaerobios es el desequilibrio entre las bacterias productoras y consumidoras de ácidos. Based on these issues and earlier studies, the objectives of this work are:

a. To evaluate the effect of thermal pretreatment on biogas production and removal of organic matter, and to assess the need to inoculate the reactor with anaerobic granular sludge to maximize the production of biogas.

b. To evaluate separating the fermentation and methanation phases of anaerobic digestion on the production of biogas, and to determine the efficiency of the biological process in one and two stages for the removal of organic matter.

MATERIALS & METHODS

The objective of pretreatment is to solubilize a major fraction of organic material so that it becomes available for bacteria, since bacteria can only degrade soluble organic material (Navia *et al.*, 2002). There are various studies on low temperature thermal pretreatment of substrates with high protein and fat content. These compounds in these substrates are too complex to be broken down by microorganisms. However, proteins can be denatured by heating. In the case of fats, temperature directly affects their solubility, and the higher the temperature the greater the solubility (Hiraoka *et al.*, 1989).

Substrate: Los residuos de rastro fueron recolectados en fresco y en las siguientes proporciones: 9% Estiércol, 11% sangre con restos de carne y tripas y 80% de contenido ruminal.Fresh waste from slaughterhouses was collected in the following proportions: 9% manure, 11% blood, meat scraps and guts, and 80% of rumen contents (Alvarez, 2004). Posteriormente se trituraron, homogenizaron y caracterizaron con los siguientes parámetros de acuerdo con Standard Methods (1995): Demanda Química de Oxígeno Total y Soluble (DQOt y DQOs), Sólidos Totales y Volátiles (ST y SV), Sólidos Suspendidos Totales (SST), Sólidos Suspendidos Volátiles (SSV), pH y Ácidos Grasos Volátiles (AGV). The waste was crushed, homogenized and characterized with the following parameters from Standard Methods (1995): Total and Soluble Chemical Oxygen Demand (TCOD and SCOD), Total and Volatile Solids (TS and VS), Total Suspended Solids (TSS) Volatile Suspended Solids (VSS), pH and Volatile Fatty Acids (VFA). La mezcla obtenida fue diluida hasta alcanzar una concentración de 50g/Kg.

Pretreatment: In order to reduce the hydrolysis period for organic matter (Gonzalez *et al.*, 2008) and to increase the removal efficiencies for TCOD, SCOD, TS and VS, as well as maximize the production of biogas, the residue was thermally pretreated. The residue was diluted to an organic load of 50 g of ST/kg (as determined in a previous study by Flores (2008) for the treatment of slaughterhouse waste). The diluted samples were pretreated at temperatures of 50, 60, 70 and 80 °C. A sample without pretreatment was used as control (25 °C). These temperatures were selected based on the range found in the literature as optimal for agro industrial organic waste (Li et al, 1992). The residue was pretreated for one hour after it reached the pretreatment temperature.

The pretreatment was performed in Erlenmeyer flasks and glass beakers, both 500 mL, using a mercury thermometer and a laboratory electric hot plate. Before and after the pretreatment, the following parameters were measured: TCOD, SCOD, TS and VS. The ratio SCOD/TCOD was calculated to monitor the relationship of the substrate solubility to the pretreatment temperature.

Inoculum: The inoculum used in this work was mature anaerobic granular sludge from the wastewater treatment at a municipal slaughterhouse. This was assessed in the work of Flores (2008), and showed a high methanogenic activity (1.32 g COD/g VSS*d). This parameter was decisive in the sludge selection, since it is a measure of biological quality, its potential for the production of methane, as well as to fast adaptation to the substrate and/or environmental parameters. The inoculum was characterized using the physicochemical techniques of Standard Methods (1995). The inoculum was kept active by adding 300 mL of 1% sodium acetate once per week.

System inoculation: Immediately after pretreatment, the residue was placed in a 60 mL serological bottle. For each pretreated sample, anaerobic digestion was tested both with and without inoculum, according to the proposed experimental design. Each experiment was performed in triplicate. The residue-inoculum ratio was 5:1 (Forster-Carneiro *et al.*, 2008). The inoculum was the granular mature sludge described above. The process was performed in batch, the temperature was controlled at 35 °C and the mixture stirred at 90 rpm on an orbital shaker with incubator and monitored until biogas production stopped. In this phase of the study, two variables (temperature and inoculum) were evaluated, with five values for temperature (25, 50, 60, 70 and 80 °C) and two values for the inoculum (with and without inoculum). Each set of conditions was tested in triplicate.

Biogas production: Continuous biogas production was measured through a system based on volumetric

displacement using a 1 M NaOH every three days. This solution was replaced when the pH value fell below 12. TCOD, SCOD, TS and VS of the treated samples were also determined. In stage 1 of our study, we determined that the optimal pretreatment temperature for the substrate was 60 °C, and that using an inoculum improved the removal of organic matter and produced more methane. The goal of the second phase of this work was to evaluate and compare the effects of a one stage anaerobic process to a two-stage process on the treatment of slaughterhouse waste. Experiments of anaerobic digestion in one and two stages were performed, and the results were evaluated to identify the more efficient system.

For the one stage process, two 6 L anaerobic acrylic reactors were used. In the two-stage process, a 6 L reactor was also used for the fermentation stage. The methanation stage used two up-flow anaerobic sludge blanket reactors (UASB) also made of acrylic with a working volume of 28 L, maintaining a volume ratio of 1:4.7 between the fermentation and methanation stages. The reactor´s content was stirred by recirculating the biogas that was produced.

For the separated stage experiments, the VFA concentration was monitored, since they are produced in the fermentation phase and consumed in the methanation phase, i.e., when the VFA are increasing, the first phase fermentation is predominant. When VFA concentration begins to decline, then methanation is the predominant stage. Thus, by monitoring the VFA, we can determine when phase separation should be induced. It is important to note that complete separation of the stages is impossible to achieve due to the symbiotic relationship between the different microorganisms. For comparison purposes, both the one and two stages processes were carried out under the same operating conditions, shown in Table 1. The process was performed in parallel and in semi-continuous modes, maintaining a 5:1 inoculum ratio. Two experiments were conducted for each stage, one for the substrate without pretreatment and the other for the sample pretreated at 60 °C.

Table 1. Operating conditions for the single and double stage processes

Parameter	Process	
	One stage	Two stage
Useful volume, L	6	34
Volumetric flow, L/day	0.143	0.815
HRT, days	42	42
Organic loading rate (OLR), Kg TCOD/m^3*day	1.48	1.48
Solids load, Kg TS/m^3*day	1.19	1.19

RESULTAD & DISCUSSION

Table 2 shows tLa caracterización del sustrato (residuos de rastro) y del inoculo es presentada en la tabla 2.he characterization of the substrate (slaughterhouse solid waste) and the inoculum. Los valores fueron el promedio de tres determinaciones llevadas a cabo durante la experimentación y su correspondiente desviación estándar.The values are the average of three measurements and their corresponding standard deviation. The amount of TS in the residue was 174.60 g/kg of sample of which 74% was composed of organic matter (VS) and 26% was on fixed solids (FS) or ash. TCOD had a high value of 232.32 g/L sample, confirming a high content of organic matter in the substrate, but only 58% of this value corresponded to the SCOD. The sample also showed a high content (about 92%) of VSS, suggesting that the substrate contained a large amount of organic matter and microorganisms, mainly from the rumen contents. This represents 80% of the sample, and since the rumen microorganisms are anaerobic, it could be used as inoculum in the reactors.

For En el caso del Inoculo los SSV representan una medida indirecta del contenido de microorganismos presentes en este, alrededor del 77% de la muestra son microorganismos lo cual es importante para el proceso, ya que mientras mayor sea el contenido de microorganismos en nuestro medio mayor será la producción de biogás.the inoculum, the VSS is an indirect measure of its microorganism content. About 80% of the sample were microorganisms, which is important for the process since more biogas is produced when the microorganisms content is higher. The Methanogenic Activity (MA) is another major factor in the process because it enables the potential for methane production of microorganisms to be evaluated. In this case 1 g of microorganisms (1 g VSS) was able to transform 1.39 g of COD to methane in one day.

For the thermal pretreatment, Table 3 shows that, as expected, the SCOD increased with increasing pretreatment temperature. It was 62% and 57% higher for temperatures of 60 and 70 °C respectively (the reference temperature was 25 °C). For temperatures of 50 and 80 °C, it only increased by 19% and 23% respectively. The reason for this could be that fats and proteins are not soluble at 50 °C. At 80 °C on the other hand, the proteins in the system are precipitated to the bottom of the heating vessel so the available organic matter is decreased. This confirms that the residue was solubilized by thermal pretreatment.

The behavior of the TCOD was different from that of the SCOD. As the pretreatment temperature increased, the concentration of TCOD decreased. For

Table 2. Characterization of the substrate and the inoculum

Parameter	Substrate	Inoculum
TS, g/Kg	174.60 ± 3.65	76.31 ± 3.12
VS, g/Kg	128.60 ± 2.48	60.44 ± 2.39
TSS, g/Kg	134.21 ± 3.28	71.62 ± 0.93
VSS, g/Kg	123.92 ± 3.83	58.27 ± 0.19
TCOD, g/L	232.32 ± 5.73	NA
SCOD, g/L	135.89 ± 4.38	NA
VFA, g/L	15.33 ± 1.23	NA
pH	6.49 ± 0.27	NA
N-NH_3, mg/L	2134.60 ± 5.48	NA
Sludge Volumetric Index, SVI, mL/g.	NA	9.95 ± 0.09
MA, g COD/g VSS * day	NA	1.39 ± 0.12
NA - Not Applicable		

Table 3. Increase for TCOD and SCOD after thermal pretreatment

Pretreatment temperature, °C	TCOD, g/L	Decrease, %	SCOD, g/L	Increase, %
25	64.0 ± 1.60	-	35.3 ± 1.24	-
50	62.0 ± 2.58	3	42.2 ± 2.81	19
60	61.7 ± 1.40	4	57.2 ± 2.03	62
70	61.0 ± 2.34	5	55.6 ± 2.13	57
80	51.2 ± 2.32	20	43.6 ± 1.81	23

treatment at 80 °C, it decreased to 51.2 mg/L, starting from an initial value of 64 g/L for the substrate without pretreatment. For pretreatments at temperatures of 50, 60 and 70 °C, the decrease was minimal. From the above data, it can be concluded that substrate proteins are denatured at a temperature above 75 °C, which is why organic matter is decreased with protein precipitation.

Thus, the best pretreatment temperature is 60°C. Figs. 1 and 2 show the kinetics for the production of biogas for the tests with and without inoculum, respectively, for the substrate pretreated at 50, 60, 70 and 80 °C, as well as the same as for the sample without pretreatment (25 °C). Note that the inoculated samples, in addition to increased production of biogas compared to the non-inoculated, are characterized by an improvement in the boot process since bacteria do not need time to acclimatize because they are already active. Therefore, the biogas production starts from day 3. Non-inoculated samples on the other hand, do not begin to produce biogas until day 12, as expected. The highest biogas production was from the inoculated samples that were pretreated at 60 and 70 °C, since 90% of the organic matter present in these samples was solubilized.

Table 4 summarizes the removal of TCOD, SCOD, TS and VS, and Table 5 shows the biogas yield and productivity from the experiments. Yield is defined as the biogas produced per unit of organic material removed (mL biogas/g TCOD removed), and productivity is the amount of biogas produced from the total volume fed into the reactor over time (mL biogas/mL reactor*day). The highest yield was 398 mL biogas/g TCOD eliminated, which was from the inoculated sample pretreated at 80 °C, and the lowest was from the inoculated non-pretreated sample (25 °C), with a value of 271 mL biogas/g TCOD eliminated. The highest productivity was from the inoculated sample pretreated at 60 °C, with a value of 0.448 m^3 biogas/m^3 reactor*day, while the lowest value was from the non-inoculated sample pretreated at 80 °C, with only 0.169 m^3 biogas/m^3 reactor*day. For the yield and productivity, the results agree with those obtained for the removal of organic matter and net production of biogas.

After determining that the best operating conditions were to add inoculum to the reactor and pretreatment at 60 °C, the next phase of the experiment was to determine whether a one or two stages process

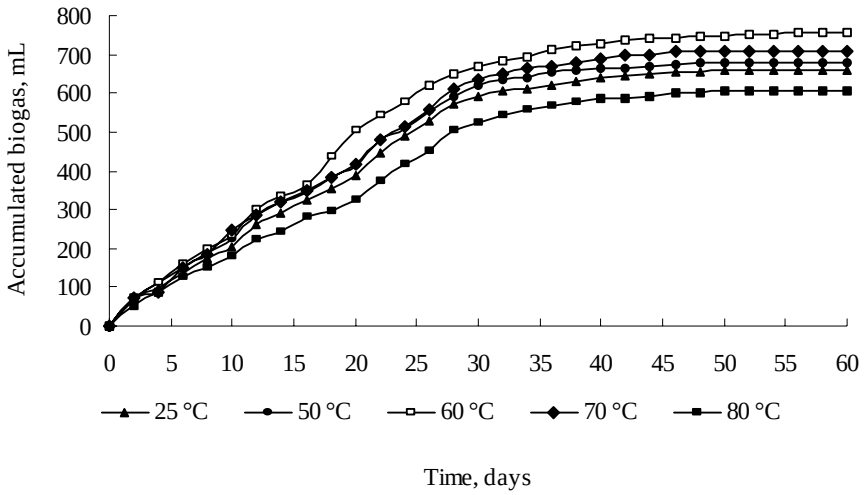

Fig.1. Biogas cumulative production, for the inoculated samples

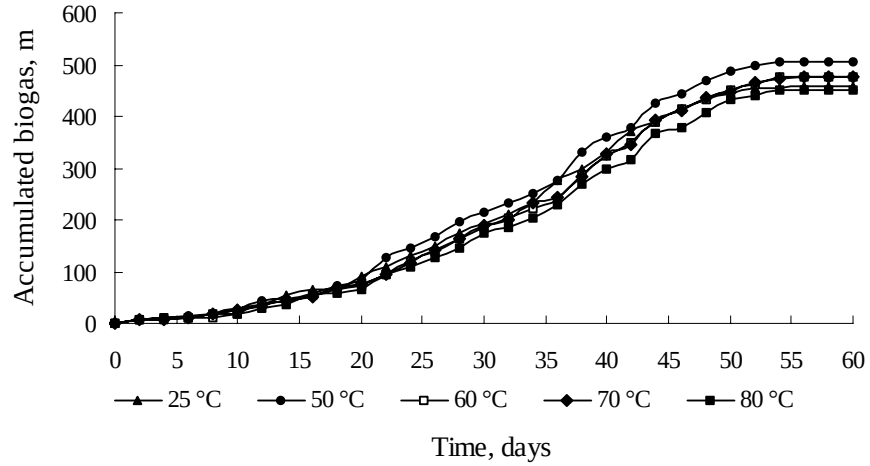

Fig. 2. Biogas cumulative production, for the non-inoculated samples

Table 4. Removal of TCOD, SCOD, TS and VS for the experiments

		% Removal			
	Pretreat. Temp. °C	**TCOD**	**SCOD**	**TS**	**VS**
Non-inoculated	25	50.51 ± 2.54	49.12 ± 0.83	48.92 ± 0.91	49.50 ± 2.81
	50	52.68 ± 2.15	50.24 ± 1.67	50.57 ± 0.86	51.63 ± 0.86
	60	57.64 ± 0.84	60.03 ± 1.46	55.99 ± 2.82	57.88 ± 1.26
	70	56.04 ± 3.67	56.52 ± 3.61	52.14 ± 1.20	53.57 ± 1.19
	80	54.32 ± 2.91	52.26 ± 1.59	47.69 ± 1.61	48.81 ± 2.00
inoculated	25	44.99 ± 0.98	39.20 ± 2.43	37.92 ± 2.26	36.98 ± 1.16
	50	43.61 ± 2.69	37.50 ± 0.58	39.52 ± 1.28	38.16 ± 2.26
	60	42.09 ± 1.43	38.24 ± 1.15	37.79 ± 0.63	37.74 ± 1.60
	70	38.70 ± 1.77	37.49 ± 2.17	39.99 ± 2.06	36.63 ± 0.58
	80	38.47 ± 1.07	36.71 ± 0.92	36.18 ± 0.54	32.24 ± 1.82

Table 5. Summary of results for the experiments

	Pretreat. Temp. °C	Yield (L biogas/g TCOD removed)	Productivity (m3 biogas/m^3 reactor* day)	Net production of biogas, mL
inoculated	25	378 ± 24.61	0.388 ± 0.008	659 ± 24.51
	50	384 ± 19.12	0.408 ± 0.014	685 ± 37.95
	60	387 ± 17.07	0.448 ± 0.025	753 ± 29.06
	70	380 ± 7.87	0.423 ± 0.021	710 ± 30.32
	80	398 ± 12.18	0.364 ± 0.022	612 ± 10.77
Non-inoculated	25	271 ± 10.87	0.173 ± 0.004	468 ± 31.02
	50	315 ± 15.40	0.190 ± 0.006	512 ± 33.07
	60	308 ± 12.75	0.178 ± 0.005	481 ± 7.26
	70	346 ± 15.99	0.181 ± 0.005	490 ± 8.04
	80	388 ± 25.10	0.169 ± 0.003	457 ± 14.12

maximized the production of biogas and removal of organic matter. This required comparing the efficiency of the two systems. Based on the previously obtained results, it was determined that it is necessary to inoculate the reactors for both the fermentation and methanation steps. In this section, we discuss the results for each process, which are summarized in Table 6.

The results show that the two-stage system maximized the production, yield and productivity of biogas, even though the removal of organic matter is the same in both systems. Thus, at two stages "fermentation and methanation" process is the most favorable.Fig. 3 shows the behavior of the VFA and pH during the fermentation step of the operation. The

Table 6. Summary of results for each treatment system

	System	
	One stage	Two stages
TCOD Removal, %	56.5 ± 1.08	56.1 ± 1.70
SCOD Removal, %	58.5 ± 1.04	55.8 ± 1.52
TS Removal, %	56.0 ± 2.23	61.2 ± 1.94
VS Removal, %	57.6 ± 1.33	60.3 ± 2.13
Net production of biogas (L)	69.7 ± 1.08	575.7 ± 19.52
Yield (L biogas/g TCOD removed)	0.310 ± 0.005	0.455 ± 0.040
Yield (L biogas/g TCOD fed)	0.175 ± 0.009	0.255 ± 0.007
Yield (L biogas/g VS removed)	0.296 ± 0.006	0.405 ± 0.017
Productivity (m^3 biogas/m^3 reactor*day)	0.258 ± 0.007	0.376 ± 0.011

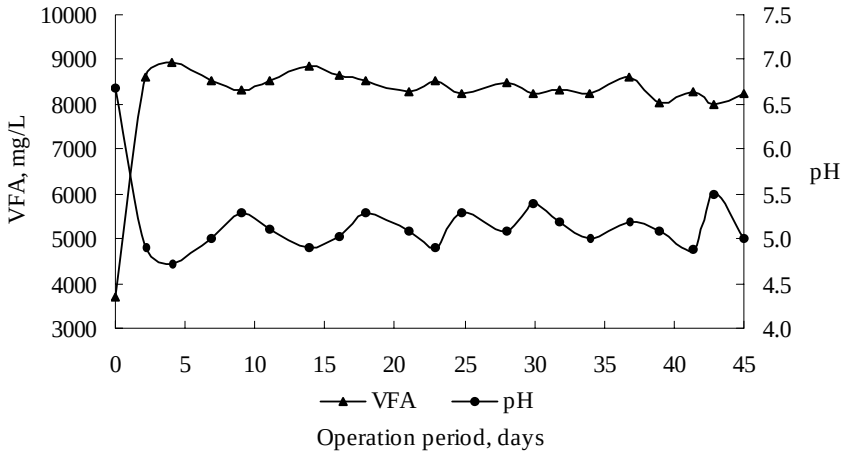

Fig. 3. Behavior of the VFA and pH during the fermentation phase

concentration of VFA increases early in the process and the acetogenic phase begins (days 3 and 4). After that, the concentration does not decrease significantly and becomes stable. This confirms that fermentation predominates over methanation, which was expected because VFA controls the process. The behavior of the pH was the opposite of VFA. Initially its value was close to neutral (6.7- 6.9), and then as the concentration of VFA increased, the pH became more acidic, due to the concentration of acids in the system. In the methanation stage, VFA concentration decreases rapidly as shown in Fig. 4, while the pH increases. The concentration of VFA decreases from 8400 to 4000 mg/L in less than 36 days. This occurred because acetogenic and methanogenic bacteria use VFA as food for growth and maintenance, then producing methane and other gases that compose biogas.

Ammonia nitrogen was measured once a week during the fermentation and methanation phases, as shown in Fig. 5. For the fermentation phase (a), from the beginning of the process, ammonia nitrogen levels were considered as inhibitory, consistent with Chamy *et al.*, (1998), Kroeker *et al.*, (1979); Soubes *et al.*, (1994); Sung and Liu (2003). However, the process was carried out without inhibition problems even when the concentration of NH_3-N increased from 2200 mg/L at the beginning of the process to values above 3500 mg/L. The absence of inhibition in the system may be attributed to the use of an inoculum already acclimated to this type of waste. In the methanation step (b), unlike the previous stage, NH_3-N has a passive evolution, i.e. neither increasing nor decreasing significantly. This suggests that proteins were primarily degraded during the fermentation stage.

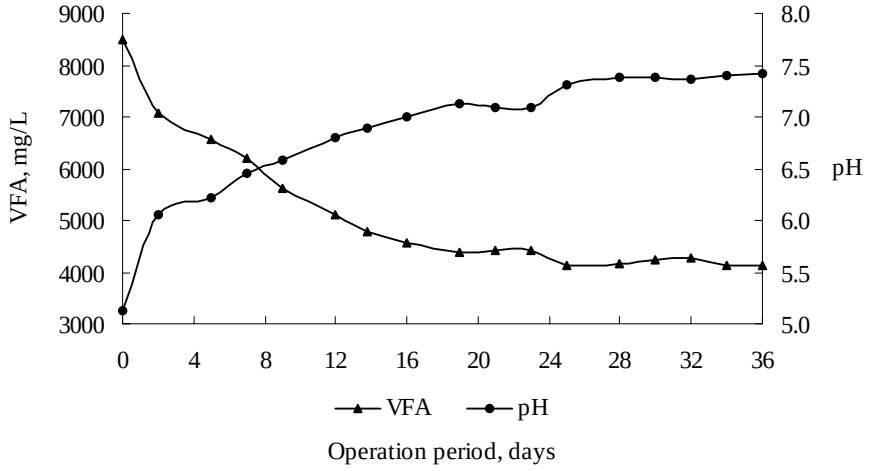

Fig. 4. Behavior of the VFA and pH during the methanation phase

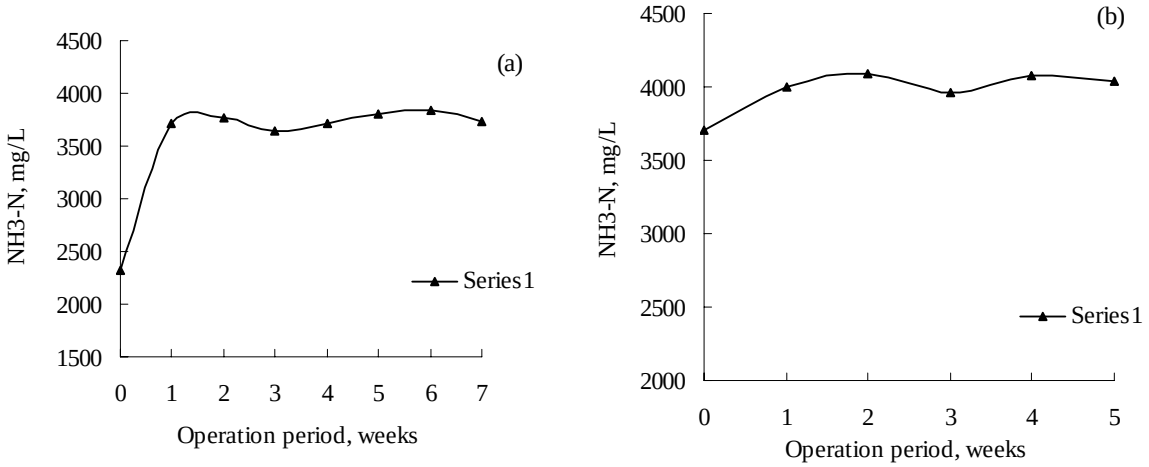

Fig. 5. Ammonia-N behavior of the steps of (a) fermentation and (b) methanation

CONCLUSION

It was determined that the solubility of the residue increases with respect to the temperature of pretreatment, showing a meaningful increase in solubility of COD for the pretreated samples. Inoculation increases the removal of organic matter, which was shown by the elimination of TCOD in comparison to the sample without inoculum. With the inoculum there was also production of methane. Without inoculum, there was less productivity and net production of biogas. The inoculated sample pretreated at 60 °C was the most efficient in both the material removal (58% elimination of TCOD) and productivity and net production of methane (753 mL biogas and 0.448 m^3 biogas/m^3 of reactor*day). Therefore, we concluded that thermal pretreatment improves the anaerobic digestion process and the addition of an inoculum increases the removal of organic matter and maximizes the production of methane. To induce the separation of the stages of the anaerobic digestion process we studied the behavior of VFA at one stage and found that they increased from the beginning of the process. The highest concentration was measured between the 3rd and 5th day of operation, and it was stable until day 9 and then fell to remain stable throughout the remaining time of operation. From these results, we conclude that from day 1 to 9 (or 9 days of operation) the fermentation stage dominates the process. In this stage, the organic matter hydrolyzes from complex organic compounds (carbohydrates, lipids and proteins) to more simple compounds (VFA), and then after day 10, methanization is the predominant stage. The removal of organic matter was the same for both one and two systems. However, the two-stage system produced more biogas. The two-step process also had higher yields with respect to the amount of organic matter supplied to the system. These results clearly show the superiority of the two-stage process for producing biogas, since the production increased by 45%. Thus, phase separation between the two groups of microorganisms involved in anaerobic digestion is an important way to improve the yield of total process.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Ibero-American Program for Science, Technology and Development (CYTED), which has facilitated this work.

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