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BEHAVIOR OF THE MESOPHILIC AND THERMOPHILIC ANAEROBIC DIGESTION IN THE STABILIZATION OF MUNICIPAL WASTEWATER SLUDGE (PART 1)

COMPORTAMIENTO DE LA DIGESTIÓN ANAEROBIA MESOFÍLICA Y TERMOFÍLICA EN LA ESTABILIZACIÓN DE LODOS RESIDUALES MUNICIPALES (PARTE 1)

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Abstract

The objective of this study was to evaluate the performance of the mesophilic and thermophilic anaerobic digestion process as an alternative for the biosolids production. Biological sludge from a municipal and industrial (brewery and pulp and paper) wastewater treatment plant using a UASB reactor was used. During the operation of the mesophilic batch system, the volatile solids (VS) were reduced from 60.3% (15.67 g/L) up to 37.5% (9.67 g/L) with 31 d of treatment time. The mesophilic process presented a stable operation reducing 38% of VS with 1.76 Kg VS m^{-3} d^{-1} of organic load feed fulfilling with the criterion of vectors attraction reduction. However, in mesophilic conditions, the digested sludge must be post-treated with a process of pathogenic microorganism inactivation because only remove low concentrations of pathogenic microorganism. The obtained results demonstrated that the process in thermophilic conditions (55°C) is capable to stabilize 3.9 Kg VS m^{-3} d^{-1} and to inactive the totality of fecal coliforms, *Salmonella* spp. and helminth ova fulfilling with the permissible limits for class A biosolids.

Keywords: mesophilic anaerobic digestion, thermophilic anaerobic digestion, municipal wastewater sludge.

Resumen

El objetivo de este estudio fue evaluar el desempeño del proceso de digestión anaerobia mesofílica y termofílica como una alternativa para la producción de biosólidos. Se utilizó lodo biológico de un reactor UASB de una planta que trata aguas industriales (industria cervecera y papelera) y municipales. Para el sistema mesofílico por lote, la materia orgánica (SV) fue reducida de 60.3% (15.67 g/L) hasta 37.5% (9.67 g/L) con un tiempo de tratamiento de 31 días. El proceso mesofilico presentó una estabilidad en la operación reduciendo el 38% SV con 1.76 Kg SV m^3d^{-1} de la carga orgánica suministrada cumpliendo con el criterio de la reducción de atracción de vectores. Sin embargo, en condiciones mesofílicas, el lodo digerido debe ser post-tratado con un proceso de inactivación de microorganismos patógenos. Los resultados obtenidos demostraron que el proceso en condiciones termofílicas (55º C) es capaz de estabilizar una carga orgánica de 3.9 Kg SV m^3d^{-1} inactivando el total de coliformes fecales, *Salmonella* spp. y huevos de helmintos cumpliendo con los límites máximos permisibles para biosólidos clase A.

Palabras clave: digestión anaerobia mesofílica, digestión anaerobia termofílica, lodos residuales municipales.

1. Introduction

The sludge produced in wastewater treatment plants should be treated for its disposal or beneficial reuse. Unfortunately, in Mexico they are not properly treated and even in some cases, they do not receive treatment at all before being disposed, causing several negative effects in public health and to the environment. According to several authors (Méndez *et al.*, 2009; 2008 and Jiménez *et al.*, 2004) the sludge generated in Mexico contains high concentrations of pathogenic microorganisms which exceed the maximum limits of the official regulation. The concentration of pathogens in

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Mexico (faecal coliforms 6.9x10^9 MPN g^{-1} TS and *Salmonella* spp. 2.1x10^6 MPN g^{-1} TS) are more elevated than those reported in countries like USA (faecal coliforms 2x10^7 MPN g^{-1} TS and *Salmonella* spp. 2.1x10^2 MPN g^{-1} TS) and in the United Kingdom who reported faecal coliforms concentration from 3.6x10^4 up to 1.4x10^6 MPN g^{-1} TS and *Salmonella* spp. from 1.4x10^2 up to 4.2x10^4 MPN g^{-1} TS. In Mexico, sludge contains up to 90 helminth ova (HO) g^{-1} TS. Additionally, in Argentina concentrations of 20 HO g^{-1} TS has been reported (Sanguinetti *et al.,* 2005) while in France, ranged from 1 to 7 helminth ova g^{-1} TS (Schwartzbrod and Banas, 2003).

A wastewater treatment plant using a biological anaerobic treatment in a UASB reactor located in the central region of Veracruz State produces approximately 110 ton day^{-1} of wastewater sludge which, because of their source, requires a stabilization treatment before being deposited. Currently, the sludge is incinerated, however this is a high-cost process and the organic matter and nutrients (N and P) are totally eliminated. Biological sludge treatments as the mesophilic process is the most utilized for the reduction of organic matter in sludge; although a better treatment in the reduction of volatile solids and pathogen microorganisms inactivation can be obtained by the digestion at 55°C (Young-Chae *et al*., 2004). Moreover, because during the anaerobic digestion methane is produced, these biological processes can be considered as attractive options to produce a bio-combustible, (Nidal *et al.,* 2004). The thermophilic anaerobic digestion process is faster than the mesophilic (Ahring *et al*., 2001) since higher hydrolysis of particles is obtained. However, in some cases, the thermophilic process can be more unstable, because of the bigger toxicity of certain compounds at high temperatures, as the ammonia ($N\text{-}NH_4$) or the long chained fatty acids (Gallert and Winter, 1997). In an attempt to demonstrate the applicability of alternating stabilization technologies in Mexico, the aim of this research was to evaluate the anaerobic digestion process, comparing the mesophilic process (35°C) against the thermophilic (55°C) in the pathogen microorganisms inactivation and to biosolids obtained.

2. Materials and methods

2.1. Substrate

The sludge under consideration was obtained from a wastewater treatment plant (WWTP), which operates with UASB reactors and it feeds with municipal and industrial wastewater from Orizaba's valley. The physicochemical characterization of the substrate was carried out by determining the contents of total solids (TS) using method 2540B from Standard Methods (American Public Health Association),

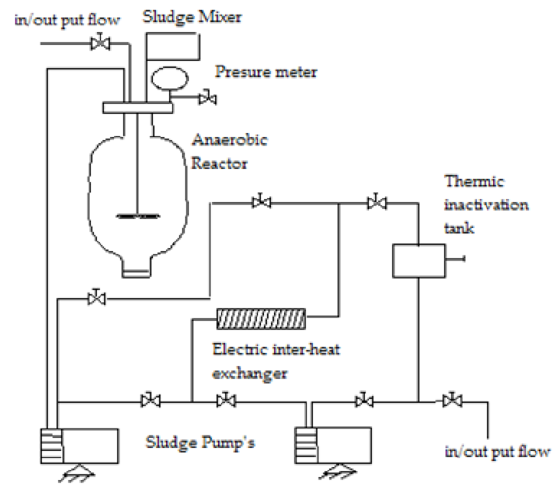

Fig. 1. Schematic diagram of the anaerobic digester.

volatile solids (VS) with the 2540E SM method. The microbiological characterization was determined quantifying faecal coliforms, *Salmonella* spp. and helminth ova, with the methods established by the NOM-004-SEMARNAT-(2002). Alkalinity ratio was determined with partial alkalinity (pH end point 5.75) and total alkalinity (pH end point 4.3).

2.2. Experimental setup

The experimental setup consisted of two ovoid shaped reactors constructed in fiberglass with 4 liters capacity used for the mesophilic anaerobic digestion (MAD) and 8.5 liters for the thermophilic anaerobic digestion (TAD) operated at 35°C and at 55°C, respectively. The temperature in the reactors was controlled by an external heating system based in a system of dry heating using an electronic inter-heat exchanger; the system is auto-adjustable to temperature variations and it is equipped with alarms to indicate temperature variations. Both reactors were mixed with an agitator to 200 rpm and were operated previously in batch and after continuously.

To start up process, the mesophilic reactor was fed with 3500 ml of sludge containing TS of 2.6% and 1.57% of VS, which corresponds to 54.56 Kg VS m^{-3}. The TAD process was start up feeding the reactor with 7000 mL of sludge with TS contents of 2.6% and 1.61% of VS corresponding to 109.2 Kg VS m^{-3} of organic load. The schematic diagrams of the anaerobic digestion system used for the experiments are shown in Fig. 1.

2.3. Experimental methods

To monitor the performance of the anaerobic processes, digested sludge samples were daily taken from the MAD and TAD in triplicate. Due to the anaerobic nature of the substrate, the incorporation

Table 1. Characteristics of feed sludge and initial experimental conditions.

Parameter	MAD	TAD	Units
pH	7.04 ± 0.04	7.02 ± 0.03	
Temperature	35 ± 2.0	55 ± 1.0	°C
Total Solids (TS)	2.6 ± 0.2	2.6 ± 0.2	% ww^{-1}
VS	1.57 ± 0.1	1.61 ± 0.1	% ww^{-1}
Alkalinity ratio	0.59 ± 0.03	0.43 ± 0.02	Alk 5.75 Alk^{-1} 4.3^{-1}
$N\text{-}NH_4$	14.54 ± 0.30	14.54 ± 0.15	g $N\text{-}NH_4.Kg^{-1}$ TS
N-Total	19.92± 0.15	19.92 ± 0.2	g N-Kjel.Kg^{-1} TS
Phosphorus	29.34 ± 0.04	29.34 ± 0.04	mg P.Kg^{-1} TS
Faecal coliforms	$3x10^6$	$3x10^6$	MPN g^{-1} TS
Salmonella spp.	4.17 $x10^5$	4.17 $x10^5$	MPN g^{-1} TS
Helminth ova	13	13	HO g^{-1} TS
Initial Organic load	54.56	109.20	Kg VS m^{-3}

Note: MAD: Mesophilic anaerobic digester; TAD: Thermophilic anaerobic digester.

Table 2. Characterization of the stabilized sludge in MAD and TAD processes.

Parameter	MAD	TAD	Units
pH	6.8 ± 0.03	6.9 ± 0.20	
Temperature	35 ± 2.0	55 ± 1.0	°C
Treatment time	31	28	days
Total solids (TS)	2.01 ± 0.20	2.03 ± 0.30	% ww^{-1}
$N\text{-}NH_4$	11.85 ± 0.30	10.50 ± 0.20	g $N\text{-}NH_4.Kg^{-1}$ TS
N-Total	17.23 ± 0.03	15.62 ± 0.09	g N-Kjel.Kg^{-1} TS
Phosphorus	19.99 ± 0.09	18.96 ± 0.06	mg P.Kg^{-1} TS
Organic matter (VS)	37.2 ± 1.04	35 ± 1.02	% ww^{-1}
Alkalinity ratio	0.48 ± 0.04	0.49 ± 0.03	Alk 5.75 Alk^{-1} 4.3^{-1}
Reduction of VS	37.90 ± 0.5	37.00 ± 0.42	%

of inoculum for the starting up of the reactors was not required. During the four batch tests four MAD and TAD batch tests, the microbiological parameters were determined at the beginning and at the end of the sludge treatments. At the end of the batch tests, the continuous-flow operation of both mesophilic and thermophilic systems was carried out during seven months taking in account the experimental conditions obtained in the batch stage. The biogas production was daily evaluated by using a volume displacement method. The biogas production and the methane content were daily monitored by a gas chromatography using a thermal conductivity detector; again, VS reduction, biogas production and pathogen inactivation were used as output variables.

3. Results and discussion

The average characteristics of fed sludge and the experimental conditions for the batch stage anaerobic digestion systems are shown in Table 1. The sludge contains high concentrations of organic matter (60.3%ww^{-1} and 61.84%ww^{-1} of TS, respectively) and high content of pathogenic microorganisms; 3 x 10^6 and 4.17 x 10^5 MPN g^{-1} TS for faecal coliforms and *Salmonella* spp., for MAD and TAD, respectively, these concentrations of pathogenic microorganisms are slightly lower than those reported by Mendez *et al.*, (2008) for poultry industry sludge but very similar to the reported in biological sludge from Mexico City by Cabirol *et al.*, (2002) (1.4 x 10^6 to faecal coliforms). The values of pH (7.04 and 7.02) are appropriate for the sludge treatment in anaerobic conditions, contrasting with those produced in physicochemical treatments, which partially are acidified (pH 5.7) by the coagulant presence and slow down the adaptation of biological processes (Jiménez *et al*, 2004; Méndez *et al.*, 2004).

As seen in Fig. 2 the batch processes started with a 60.3% (15.67 g VS /L) and 61.84% (16.07 g VS /L) of organic matter for the MAD and TAD, respectively. During 31 days of batch operation the VS concentration was reduced down to 37.9% (9.96 g/L SV) in the MAD and reduced up to 37% (9.62 g VS /L) in only a treatment time of 28 days in the thermophilic digester (TAD), reaching with the Mexican environmental rules (NOM-004-SEMARNAT-2002) that considers a reduction of 38% in the initial concentration of volatile solids in the treated sludge to reduce the vector attraction and to be considerate as biosolids. Additionally, it is observed a higher removal rate in the TAD due to the higher temperature, agree with other authors (Cabirol *et al.*, 2002), who have reported that the process at 55°C presents higher removal efficiency (from 58 up

Table 3. Effect of the mesophilic and thermophilic anaerobic digestion in the pathogenic microorganisms inactivation

Microorganism	Biological Sludge (UASB)	Digested sludge in MAD at 35° C with 31 days of treatment time	Digested sludge in TAD at 55° C with 28 days of treatment time	Maximum Permissible Limit NOM-004-SEMARNAT-2002		
				Class A	Class B	Class C
Faecal coliforms (Log MPN g^{-1} TS)	6	4	0	< 3	<3	< 6.3
Salmonella spp. (Log MPN.g^{-1} TS)	5	3	0	<0.47	<0.47	<2.47
Helminth ova (HO.g^{-1} TS)	13	13	0.25	<1 [(a)]	< 10	< 35

Note: [(a)] Viable helminth ova; HO: helminth ova; MPN: most probable number

Table 4. Genera distribution of helminth ova in raw and digested sludge

Genera	Biological sludge (UASB)		Digested sludge in MAD (35°C)		Digested sludge in TAD (55°C)	
	HO g^{-1} TS	%	HO g^{-1} TS	%	HO g^{-1} TS	%
Necator americanus / Ancylostoma duodenale	4.5	34.6	4.5	37.5	0.0	0.0
Ascaris spp.	4.0	30.8	4.0	33.3	0.0	0.0
Trichuris spp.	3.5	26.9	2.5	20.8	0.5	100.0
Enterobius vermicularis	1.0	7.7	1.0	8.3	0.0	0.0
Total	13.0	100.0	13.0	100.0	0.5	100.0

Note: HO: helminth ova

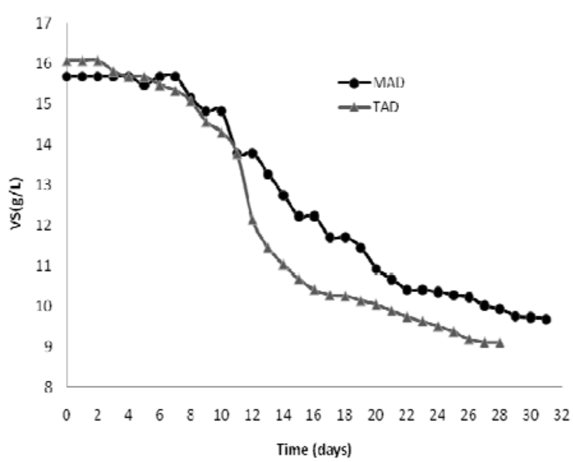

Fig. 2. Degradation of volatile solids in mesophilic and thermophilic anaerobic conditions.

to 74% SSV) due to the temperature increases the velocity of the biochemical reactions.

Significant removal of volatile solids was attained within 31 d of treatment time with an organic load of 54.56 Kg VS m^{-3} for the MAD and 28 d treatment time and an organic load of 109.2 Kg VS m^{-3} for the TAD. Table 2 shows the average sludge characteristics at the end of the stabilization period for the mesophilic and thermophilic systems. In this study, the initial ammonia concentrations in the sludge, before stabilization, was of 14.54 g N-NH_4 Kg^{-1} TS, equivalent at 0.378 N-NH_4 L^{-1}, lower concentration to those reported in other studies that cause inhibiting in the thermophilic digestion process (Zeeman *et al.*, 1985; Hashimoto *et al.*, 1986; Krylova *et al.*, 1997).

Since the content of pathogen microorganisms is one of the main sludge pollution problems in Mexico; in this study, the inactivation efficiencies of faecal coliforms, *Salmonella* spp., and helminth ova were evaluated in both of the anaerobic digestion systems. According with the results presented in Table 3, most indicator organisms and pathogenic bacteria were not significantly reduced in the first anaerobic reactor (MAD), inactivating only two logs of the raw sludge concentration. From the results it was also evident that helminth ova (HO) were the more resistant group of microorganisms at the end of the mesophilic digestion, which in turn explains the mesophilic treatment inefficiency in the elimination of HO. A similar effect was previously reported by some authors (Kunte, *et al.*, 2000; Rojas-Oropeza *et al.*, 2001). The process did not eliminate the indicators microorganisms, pathogenic bacteria or HO; but the final values for faecal coliforms and HO met the Mexican regulation limits for biosolids class C. This kind of biosolids can be use in agricultural

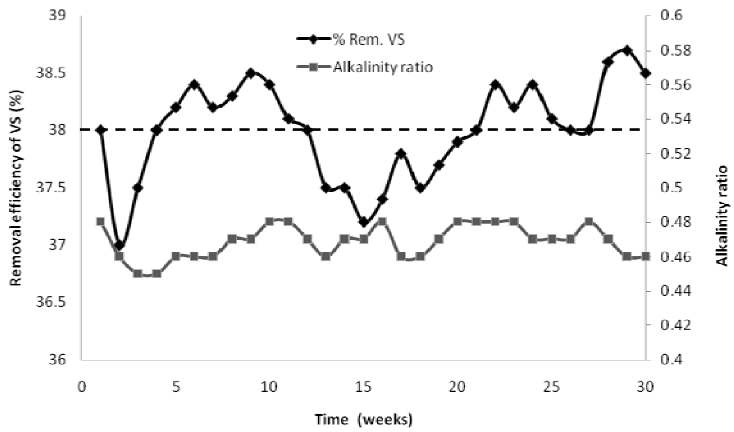

Fig. 3. Volatile solids behaviors in the continuous-flow mesophilic anaerobic digester.

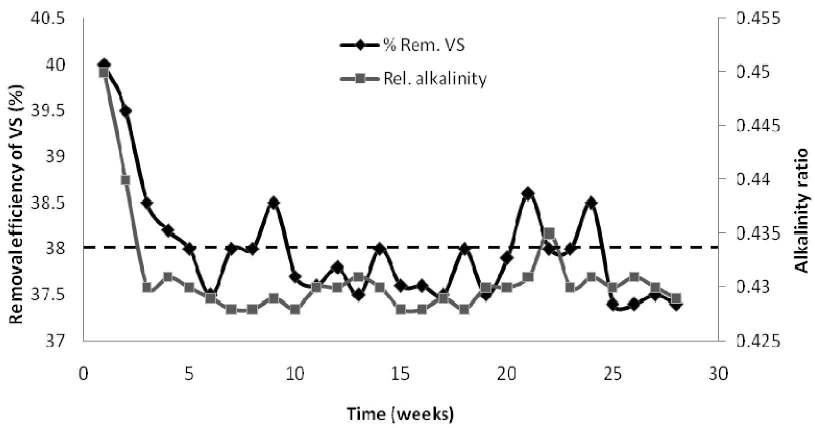

Fig. 4. Volatile solids and alkalinity ratio behaviors through the time in the continuous-flow thermophilic anaerobic digester.

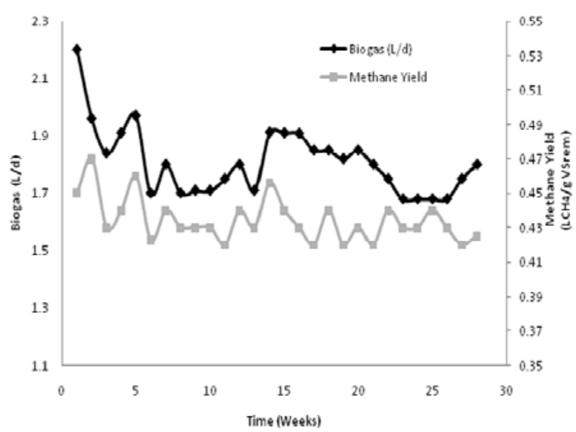

Fig. 5. Biogas Production during the continuous-flow operation of the mesophilic anaerobic digester.

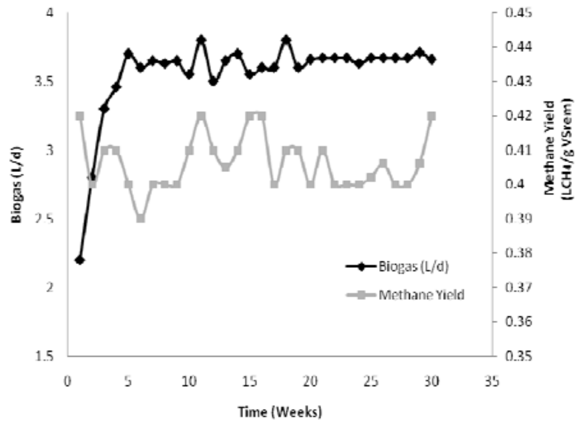

Fig. 6. Production of biogas during the continuous-flow operation of the thermophilic anaerobic digester.

lands or disposed in reclamation soils but the concentration of *Salmonella* spp. was not significantly reduced at 35°C and surpassed the Class C limit. This group of pathogens does not permit the beneficial reuse of this kind of treated sludge. On the other hand, the faecal coliforms and *Salmonella* spp., were totally eliminated and the helminth ova groups counted were significantly removed in the thermophilic anaerobic digester. The TAD produced biosolids by thermophilic conditions comply with the limits of this kind of microorganism in both, the Mexican environmental regulation for biosolids class A and rule Part 503 (1996) of the US Environmental Protection Agency.

Table 4 shows the distribution of different genera of helminth ova found in the biological and treated sludge. The initial helminth ova concentration was 13 HO g^{-1} TS. *Necator americanus/Ancylostoma doudenale* and *Ascaris* spp. were the predominant genera (34.6% and 30.8%, respectively). The eggs of *Necator americanus and Ancylostoma doudenale* are similar when these are observed at microscope and it is almost impossible to differentiate them with the used method. Since helminth ova are not inactivated in the mesophilic conditions, the concentration of the biological sludge was the same after the mesophilic treatment at 35°C (13 HO g^{-1} TS) in comparison with the thermophilic treatment the increase of the temperature to 55°C resulted in a significant decrease of the level, up to 0.5 HO g^{-1} TS (*Trichuris* spp.) of helminth ova. The inactivation the of pathogen bacteria and the helminth ova can be related to long thermal treatment (55°C) applied during the batch tests (28 days), which is an excessive thermal treatment in comparison with the three hours at 60°C of pasteurization treatment applied for sludge disinfection, to obtain zero viability and infectivity (Morris *et al.*, 1986) that demonstrated the efficiency of the increase in temperature and the need for a time exposure of just a few hours.

To evaluate the performances of both processes, mesophilic and thermophilic, in continuous flow, tests were performed with an organic load of 1.76 Kg VS m^{-3} d^{-1} in a period of 30 weeks for MAD and 3.9 Kg VS m^{-3} d^{-1} and 28 weeks for TAD. In Fig. 3 it is observed the behavior of the total volatile solids in the continuous-flow mesophilic system; in the first three weeks, the substrate consumption presents instability because of the addition of 112 ml d^{-1} (1.76 Kg VS m^{-3} d^{-1}) of biological sludge. The process continues on adaptation but it is capable to consume an average of 38 ± 0.46% of the volatile solids per day complying permanently with the Mexican regulations for the biosolids production.

Fig. 4 shows the behavior of volatile solids in the thermophilic continuous-flow system; when an organic load of 3.9 Kg VS m^{-3} d^{-1} was fed, an average reduction of 37 ± 0.45% of the volatile solids was attained, this value can be seen low by comparing with the reached by Cabirol *et al.*,(2002) who reported an efficiency of 58% volatile solids reduction; however in the cited work a organic load of 1.5 Kg VS m^{-3} d^{-1} was used. The alkalinity ratio, relation between the alkalinity due to the volatile fatty acids (VFA) and the one due to bicarbonate, ranged from 0.46 to 0.48 in mesophilic temperature and from 0.43 to 0.44, in thermophilic conditions. This interval is considered itself stable, in according to some authors (Iza, 1995; Noyola and Tinajero, 2005), who established values for the relation of alkalinity between 0.42- 0.48 as acceptable for the stability of the anaerobic digestion process.

The biogas production behavior in the continuous-flow system is shown in the Fig. 5, where the daily removal of total volatile solids generates an average production of 1.8 L ± 0.12 L d^{-1} of biogas with a concentration of 82 ± 5% of methane in volume. In Fig. 6 the permanent volatile total solid removal generates a very high production of biogas with an average of 3.6 ± 0.25 L d^{-1} with a concentration of 80 ± 3% of methane in volume. The average specific methane yield of the mesophilic process was 0.435 m^3 CH_4/Kg $VS_{removed}$ which is higher than 0.407 m^3 CH_4/Kg $VS_{removed}$ of the thermophilic digester. These obtained results are slightly lower to those reported by Tapana *et al.,* (2000) for a sludge mesophilic anaerobic digestion process (0.51 m^3 CH_4/Kg $VS_{removed}$) and Rotcher *et al.*, (2008) in a thermophilic anaerobic sludge digestion (0.47 m^3 CH_4/Kg $VS_{removed}$) and can be explained because the higher maintenance energy of the anaerobic thermophilic microorganisms (Kim *et al.*, 2002), as well as, the lower methane content of the biogas.

Conclusions

Based on the obtained results, the anaerobic digestion is an alternative for the stabilization of residual sludge produced not only in the central region of Veracruz State but for all the sludge produced in México. The applied process must take into account the organic compound removal, as well as, the inactivation of pathogens microorganisms. The operation of the process at 55°C is recommended.

The mesophilic anaerobic digestion process (at 35°C) can be successfully used for the stabilization of agro-industrial or food industries sludge (>55% VS), which, generally contains lower concentrations of bacteria and helminth ova. The presence of helminth ova in the sludge suggests faecal contamination in origin wastewaters. Its evaluation in all stages of the treatment process (WWTP) is recommended. The inactivation of helminth ova in sludge is attained in processes that use the increase of temperature (TAD) or chemical treatments for the sludge stabilization. The anaerobic digestion in thermophilic conditions (55°C) may replace stabilization processes as the incineration, because its operation requires lower cost and it produces a source of energy (CH_4). But, the results suggest that the mesophilic anaerobic digestion may be improved including a thermal stage to inactivate the pathogenic bacteria densities. Finally, the product of the thermophilic anaerobic digestion (biosolids) may be applied without restrictions for the soil improvement or agricultural lands in according with the current regulation in Mexico.

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