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Anaerobic digestion of fruit and vegetable waste in semi-continuous reactors from a municipal market in Tumbaco, Ecuador

Digestión anaeróbica de desechos de frutas y hortalizas en reactores semicontinuos de un mercado municipal en Tumbaco, Ecuador

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Abstract

Organic solid wastes are generated in large quantities in Ecuador. In 2010, the whole production of municipal solid waste in Quito was estimated to be 1500 tons per day and the organic fraction represented about 60% of the solid waste. Landfills which are the most common disposal way of municipal solid waste in the country pose a threat to the environment and public health. Anaerobic digestion of municipal organic waste has shown to be a suitable option for stabilizing the organic matter and preserving the ecosystem and well-being of the population. In addition, the conversion of the solid waste into biogas and bio-fertilizer provides an alternative energy source and organic fertilizer with great potential for agricultural usage, respectively. In this research, the anaerobic treatment of fruit and vegetable waste (FVW) coming from a municipal market in Tumbaco was studied in laboratory scale semi-continuous bioreactors (4 L capacity). First, FVW was characterized to be used as a feedstock for AD process. Subsequently, the bioreactors were incubated with 10% weight of prepared anaerobic inoculum and fed with different concentrations of FVW to identify the optimal organic load. Prepared microbial inoculum was composed of 40% granular sludge obtained from an anaerobic reactor of a wastewater treatment plant in Quito and 90% (v/v) cow dung slurry (CDS). The mesophilic mixed digesters were fed with raw FVW in a semi-continuous mode with organic loads of total solids (TS) of 5, 7.5, 10 and 12.5% during 90 days in three different periods of FVW loading. The biogas production as well as the performance of the bioreactors was periodically monitored. The highest cummulative methane generation was achieved by the bioreactor fed with 5% FVW with a value of 2401.92 L CH_4 kg VS^{-1}. Finally, an effective technology based on AD was successfully developed as an alternative to landfills for the treatment of municipal organic waste in Ecuador.

Keywords. Fruit and vegetable wastes (FVW), anaerobic digestion, granular sludge, cow dung slurry (CDS), methanogenic activity and cumulative methane production.

Resumen

En Ecuador se generan una gran cantidad de residuos orgánicos. En el año 2010, el promedio de recolección de desechos en el Distrito Metropolitano de Quito fue de aproximadamente 1500 t por día, de los cuales más del 60% fueron identificados como residuos orgánicos. Los rellenos sanitarios que son comúnmente empleados en el país para el tratamiento de desechos generan grandes problemas al medio ambiente y a la salud pública. El tratamiento biológico de residuos orgánicos bajo condiciones anaerobias constituye una alternativa viable para estabilizar la materia orgánica, proteger el medio ambiente y reducir el riesgo de afección a la salud pública; adicionalmente, se generan productos con valor agregado como son el biogás y el fertilizante orgánico que pueden ser empleados como fuente de energía alternativa y en las actividades agrícolas, respectivamente. En el presente trabajo de investigación, el tratamiento anaeróbico de residuos sólidos de frutas y vegetales (RFV) del mercado central de Tumbaco fue estudiado en cuatro biorreactores de 4L en modo de operación semi-continuo escala laboratorio. En primer lugar los RFV fueron caracterizados para ser utilizados como materia prima en el proceso de digestión anaeróbica. A continuación, los biorreactores fueron incubados con 10% de inóculo microbiano preparado y diferentes porcentajes en peso de residuos de frutas y vegetales con el fin de identificar la concentración optima de carga orgánica. La composición del inoculo microbiano preparado fue 40% de lodos granulares provenientes del biodigestor de una planta de tratamiento de aguas de la ciudad de Quito y 90% (v/v) de estiércol vacuno disuelto. Los biodigestores mesófilos de mezcla completa fueron alimentaron con diferentes porcentajes en peso de RFV equivalentes a 5, 7.5, 10 and 12.5% sólidos totales. Los bioreactores fueron operados durante 90 días en tres períodos correspondientes a las diferentes alimentaciones. La mayor produccion acumulada de metano se alcanzó con el biorreactor alimentado con 5% RFV registrando un valor de 2401.90 L CH_4 kg SV^{-1}. Finalmente, una tecnología efectiva de digestión anaerobia fue exitosamente desarrollada como alternativa a los rellenos sanitarios para el tratamiento de residuos orgánicos municipales en Ecuador.

Palabras Clave. Residuos sólidos de frutas y vegetales (RFV), digestión anaeróbica, lodos granulares, estiércol vacuno disuelto, actividad metanogénica y producción acumulada de metano.

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Introduction

Solid wastes are organic and inorganic residues produced during domestic, commercial and industrial activities. Organic wastes are of biological origin and mostly characterized as biodegradable waste. This type of waste is able to disintegrate rapidly under anaerobic conditions in order to transform into biogas and organic fertilizer [1, 2]. Municipal waste, depending on their nature and/or their physical characteristics are classified as food wastes, paper and cardboard, textiles, plastics, metals, glass and others [3]. According to Ministry of Environment in Ecuador, in 2002 the country generated close to 7423 tons of solid waste per day which was classified as organic origin (58%), paper (9%), plastic (11%), glass and aluminum (2%) and other components (8%) [4].

In Latin America, in average 70% of urban solid wastes are collected by the municipalities and the rest ends up as contaminants in rivers or lakes [4]. Sanitary landfills are the most common way employed to eliminate municipal solid waste (MSW); however, the generation of toxic leachates, unpleasant odors, wild fires caused by generation of methane from organic matter, proliferation of pets and diseases cause a negative impact in the environment and public health [5]. Anaerobic digestion (AD) of municipal solid waste MSW is currently used in Europe, Japan and USA to reduce the amount of material being landfilled, stabilize organic material before disposal to reduce impacts to the ecosystem due to air and water emissions and produce renewable energy [6]. This technology has been successfully employed in Europe for more than 15 years and the interest is growing worldwide. Agricultural and industrial wastes are good candidates for anaerobic digestion because they contain high levels of easily biodegradable materials. Low methane yield and process instability are often encountered in anaerobic digestion, preventing this technique from being widely applied [7]. Nevertheless, anaerobic digestion (AD) of municipal organic waste is today one of the best alternatives for the comprehensive treatment of solid waste because it allows the recovery of energy and the generation of a bio-based fertilizer [8]. In addition, the concentration of nutrients is appropriate and the presence of contaminants is low [9].

There are several factors that affect the anaerobic digestion process to be performed. Some of the factors that need to be considered are the characteristic of the feedstock, the reactor design and the operation mode [10]. The physical and chemical characteristics of the organic waste are important information for designing and operating anaerobic bioreactors, because they are related to biogas production and the stability of the AD process [10]. The characterization parameters include but are not limited to moisture content, pH, solids, chemical oxygen demand (COD), among others. Fruit and vegetable wastes are good feedstock for anaerobic digestion due to their easily biodegradable nature and high moisture content (75 – 90%) [11]. However, the rapid acidification of FVW decreasing the pH in the reactor and the larger production of volatile fatty acids (VFA), inhibit the activity of methanogens and affect significantly the stability of the reactor [7]. Microbial inoculum plays an important role in the anaerobic digestion process because it is the source of microorganisms responsible for organic matter stabilization and biogas production. The health of the microbial community can be estimated based on the methanogenic activity [12]. Maximum specific acetoclastic methanogenic activity of granular sludge of 0.5 kg COD-CH_4 kg VSS^{-1} d^{-1} suffices to economically operate a UASB-reactor [13]. Several reactor designs and operation modes can be employed in the AD process. The treatment of FVW has been studied in different types of reactors such as batch reactors, continuous one-stage reactors, continuous two stage reactors [11], continuously stir tank reactor (CSTR), sequencing batch reactors [8], tubular anaerobic digester [14], upflow anaerobic sludge blanket (UASB) [12] and anaerobic filters [2], among others with conversion of 70–95% of organic matter to methane [2, 8].

Anaerobic digestion has the potential to minimize the environmental impact of waste disposal by reducing the amount of biodegradable materials in landfills. The objective of this research is to study the anaerobic digestion of a mixture of fruit and vegetable waste (FVW) coming from a municipal market in Tumbaco in semi-continuous bioreactors at laboratory scale. First, FVW will be characterized to be used as a feedstock for AD process. Bioreactors will be then incubated with anaerobic inoculum and fed with different concentrations of FVW to identify the optimal organic load. Information on solid waste management adapted to the national situation will be generated to address one of the most important environmental issues in Ecuador.

Materials and Methods

Reagents

Sodium acetate (100%, purity), potassium dichromate (99.4%), sodium hydroxide (≥99%) and silver nitrate (≥99%) were obtained from Laboratorio de Reactivos H.V.O (Quito, Ecuador). Sulfuric acid (95-97%) was bought in MERCK. Zinc chloride (97.1%) was obtained from J.T. Baker. N_2 gas was delivered by AGA Ecuador (Guayaquil, Ecuador). All the chemicals were used in the condition they were received.

Microbial inoculum

Three inocula from different sources were evaluated in this study. Sediments of an artificial lagoon in the campus of Universidad San Francisco de Quito (USFQ), granular sludge from a wastewater treatment plant (WWTP) at "Cervecería Nacional" Cumbayá (GS), and the effluent from the digester installed at the Botanical Garden located at "Parque La Carolina" (BG digester).

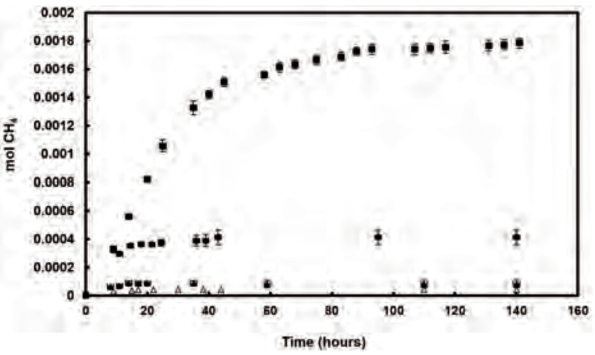

Figure 1: Cumulative methane production over time for different anaerobic sludge and sediments. Treatment bioassays were supplied with basal mineral medium, 10% v/v of the artificial lagoon sediments, or 20% v/v of granular sludge and BG digester sludge. Legend: (■) granular sludge (GS), (●) BG digester sludge, (▲) sediments of artificial lagoon and (△) abiotic control. Error bars represent the standard deviations of the bioassays performed in triplicates.

Cattle manure was used as part of the microbial inoculum in order to complement the selected anaerobic sludge. Manure was collected in a stable around Cumbayá, and it was diluted with tap water in a 1:1 ratio in order to produce the cow dung slurry (CDS). The inocula and CDS were stored in refrigeration at 4°C in plastic containers.

Culture media

The basal mineral medium used in the methanogenic activity and anaerobic degradation of FVW bioassays contained (in mg L^{-1}): NH_4Cl (280), KH_2PO_4 (250); $MgSO_4 \cdot 7H_20$ (100), $CaCl_2$ [10], $NaHCO_3$ (3000) yeast extract (50) and 1 mL L^{-1} trace element solution. The trace element solution contained (in mg L^{-1}): H_3BO_3 (50), $FeCl_2 \cdot 4H_20$ (2000), $ZnCl_2$ (50), $MnCl_2$ (32), $(NH_4)_6Mo_7O_{24}*4H_20$ (50), $AlCl_3$ (50), $CoCl_2*6H_20$ (2000), $NiCl_2*6H_20$ (50), $CuSO_4*5H_20$ (44), $NaSeO_3*5H_20$ (100), EDTA (1000), resazurin (200) and 1 mL L^{-1} of HCl 36% [15]. The pH of the basal mineral medium was adjusted to 7.1-7.3 with HCl and NaOH, as required.

Fruit and vegetable waste (FVW)

The fruit and vegetable waste used as substrate in batch bioassays and semi-batch bioreactors were obtained from the municipal market in Tumbaco. FVW was collected during fair days (Wednesday and Sunday) from 81 stores; then, the waste was selected to be used as a substrate based on its abundance as residues in the ground and around the stalls. Once collected, FVW was characterized based on physical-chemical parameters and then crushed with the shredder Proctor Silex (PS HB Group, SA, Polanco, Mexico), until particles were less than 5 mm in diameter. The FVW mixture was composed of apple (15% wt), lettuce (25%), bell pepper (20%), tomato (25%) and cabbage leaves (15%).

Microbial bioassays

In all bioassays the headspace was flushed with N_2 gas to assure anaerobic conditions. All bioassays were incubated in a home-made climate-controlled chamber at 30±2°C. The maximum specific methanogenic activity expressed as mg CH_4-COD g VSS-1 d-1 was calculated from the slope of the cumulative methane production and biomass concentration; respectively, versus time (d). The cumulative methane production in the presence of FVW was expressed in liters of methane generated per kilogram of volatile solids added (L CH_4 kg VS^{-1}).

Methanogenic activity: Batch bioassays were conducted in duplicates using glass serum flasks (160 mL) with butyl rubbers stoppers and aluminum crimp seals. Each flask was supplemented with basal mineral medium, 10% v/v of the artificial lagoon sediments, or 20% v/v of GS and BG digester sludge. The substrate in the methanogenic activity bioassays was 2.5 g COD-acetate L^{-1}. Abiotic controls (absence of microorganisms) were run in parallel.

Batch: The optimal composition of a prepared microbial inoculum composed of granular sludge (GS), cow dung slurry (CDS) and water was determined in batch bioassays in triplicates using 160 mL glass serum flasks with butyl rubbers stoppers and aluminum crimp seals. GS concentrations of 10, 20, 30 and 40 % wt and CDS concentrations of 25, 50 and 90 % (v/v) as well as physical conditions of operation and the effect of agitation were evaluated in this study. Each flask was supplemented with basal medium (75% v/v), prepared inoculum GS-CDS (20% v/v) and shredded FVW (5% v/v). Abiotic controls (absence of microorganisms), granular sludge controls (absence GS) and cow dung slurry control (absence of manure) were run in parallel.

Semi – continuous: Anaerobic digestion of different organic loads of FVW was evaluated in semi-continuous bioreactors. The bioreactors were plastic containers with a volumetric capacity of 4 L tightly sealed with Teflon tape and silicone to prevent leakage of biogas and to maintain anaerobic conditions. Each bioreactor contained prepared inoculum GS-CDS (20 % v/v), basal mineral medium, water and different mixture of fruit and vegetable wastes (FVW). The percentage of solid waste evaluated in each reactor varied from 5, 7.5, 10 and 12.5 % wt corresponding to bioreactors R1, R2, R3 and R4; respectively. The bioreactors were fed three times with FVW and 500 mL CDS. All reactors were agitated using magnetic stirring plates. The cumulative methane production rate, soluble COD removal rate, pH, nitrate, VS and TS were periodically monitored in each bioreactor.

Analytic methods

Total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) and chemical oxygen demand (COD) were determined according to Standard Methods for Examination of Water and Wastewater [16]. Methane production during the anaerobic bioassays was determined by the liquid displacement method with serum flasks as described by Field [17]. pH, temperature, dissolved oxygen and conductivity were measured by a Thermo Scientific Orion 5-Star portable multiparameter meter (Thermo Scientific, Beverly, MA, USA).Nitrate was measured using an Orion ion-selective chloride electrode (Thermo Scientific, Beverly, MA, USA).

Results and Discussion

Characterization of microbial inoculum

Table 1 presents the physical and chemical characterization of the different sludge and sediments evaluated in this study based on pH, TSS, VSS, ash, and total and soluble COD. The pH values were within the optimal pH of untreated primary inoculum ranging from 5.0 to 8.0 [15] and consistent with those found by Gomez - Lahoz et al. [18]. The VSS/TSS ratios of the two anaerobic sludges evaluated were within the range of healthy inoculum reported in the literature (60-80%) [19]. As expected, the sediments of the lagoon registered a low VSS/TSS ratio of 10.8% due to the significant presence of inert solids. The soluble COD values of BG digester sludge and GS were 685.9 and 360.6 mg O_2 L^{-1}, respectively. The BG digester registered a total COD value five times greater than that of GS. In the case of the sediments, the soluble and total COD values were very similar 186.5 and 228.6 mg O_2 L^{-1}; respectively, and these values are comparable to those obtained by Yang [20].

Figure 1 presents an illustrative example of the time course of cumulative methane production in the presence of 2.5 g COD-acetate L^{-1} in an abiotic control (absence of microorganisms) and in the treatment bioassay with different anaerobic sludge and sediments as microbial inoculum. There is no methane generation in the abiotic control while the methane production increased over time in the treatment bioassays conducted with granular sludge, BG digester sludge and sediments of the artificial lagoon as microbial inoculum. These results demonstrate that the anaerobic microorganism present in the sludge and sediments are responsible for the production of methane from acetate as electron donor. Table 2 presents the maximum specific methanogenic activity of the sludge and sediments evaluated in this study. The BG digester sludge registered the highest maximum specific methanogenic activity, 1216.38 mg COD-CH_4 g $VSS^{-1}d^{-1}$ while the values for GS and sediments were two orders of magnitude lower. A possible explanation for this difference is that the amount of horse manure present in the BG digester is 1.6 times greater than the amount of vegetal material, and it is known that the ratio of VSS/TSS of horse manure is 83.7% [21]; however, the high COD concentration makes this sludge not suitable for anaerobic biodegradation processes. The methanogenic activity of the sediments was 67.15 COD-CH_4 g VSS^{-1} d^{-1}. Yang and co-workers

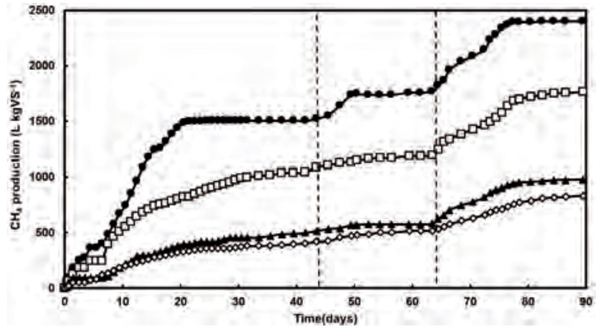

Figure 2: Cumulative methane production (L kg VS^{-1}) over time for the four bioreactors (R) operating in semi-continuous mode with 10% GS-CDS inoculum during anaerobic degradation of different amounts (weight) of FVW. Legend: (●) R 5%, (□) R 7.5%, (▲) R 10%, and (◇) R 12.5% of FVW. Period I corresponds to 0 - 42 days, period II corresponds to 43 - 63 days, and period III corresponds to 64 - 90 days.

studied the methanogenic activity in river and lake sediments from Taiwan and reported values very similar to the one found in this study, 54.54 mg COD-CH_4 g VSS^{-1} d^{-1} [20]. The maximum specific methanogenic activity of the microorganisms present in the GS inoculum was 89.03 mg COD-CH_4 g VSS^{-1} d^{-1} which is significantly lower that the values reported in the literature for granular sludge. For instance, Schmidt and Ahring reported a methanogenic activity for granular sludge in the presence of acetate of 500 mg COD-CH_4 g VSS^{-1} d^{-1} [22]. The low maximum specific methanogenic activity registered for the GS in the present study could be attributed to the concentration and health of the microbial community present in the sludge, the GS was acquired when the digester had been functioning just for 10 months and the sludge was kept refrigerated (4 °C) for several months; so presumably, the microorganisms were not healthy to reach typical values of methanogenic activity like the ones achieved by bioreactors operating for several years. Another explanation for obtaining low methanogenic activity for GS could be attributed to diffusion limitations that could occur during mass transfer from the substrate to the granular bead, or in the diffusion of methane from the bead to the liquid and gas phase [12]. In order to overcome these problems, an inoculum based on cow dung slurry (CDS) and granular sludge (GS) was prepared by adding different volumes of CDS to 10% GS. CDS had a positive effect on the process of anaerobic degradation of FVW, as the volume of CDS in the prepared inoculum (GS-CDS) increased, the maximum methanogenic activity also increased (refer to Table 2). Based on these results, it was established that the best composition of the prepared inoculum (GS-CDS) for the anaerobic degradation (AD) of FVW from Central Market in Tumbaco was 10% GS and 90% CDS.

Anaerobic digestion of FVW

A mixture of FVW from Mercado central in Tumbaco was characterized and prepared (by weight) with 15 % apple, 25% tomato, 25% lettuce, 20% bell pepper and 15% cabbage leaf as described in Table 3. The percentage of each compound in the mixture was established

Inoculum	**pH**	**TSS (gL^{-1})**	**VSS (gL^{-1})**	**Ash (gL^{-1})**	**VSS/TSS %**	**COD (mgL^{-1})**	**COD_T (mgL^{-1})**
Granular sludge (GS)	7.27	7.90±0.06	7.32±0.11	0.05±0.01	92.7	360.55±15.42	962.90±58.71
BG digester sludge (BG)	6.96	3.42±1.50	2.71±1.28	0.01±0.00	79.4	1685.91±22.03	5037.67±421.98
Sediments artificial lagoon USFQ	7.83	10.01±0.14	1.08±0.61	0.89±0.06	10.8	186.51±115.6	228.60±1.70

Table 1: Physical and chemical characterization of microbial inoculum based on pH, total suspended solids (TSS), volatile suspended solids (VSS), ash, soluble and total chemical oxygen demand (COD and COD_T).

Assay	**Treatments**	**Maximum specific methanogenic activity ($mg\ COD\text{-}CH_4\ g\ VSS^{-1}\ d^{-1}$)**
Microbial inoculum	GS (20%)	89.03
	BG digester (20% v/v)	1216.38
	Sediments (10%)	67.15
Prepared inoculum GS-CDS*	Treatment 1: 10% GS + 25% CDS	36.54
	Treatment 2: 10% GS + 50% CDS	101.33
	Treatment 3:10% GS + 90% CDS	119.12

*Maximum specific methanogenic activity in the bioassays conducted with prepared inoculum was calculated based on the VSS of the granular sludge.

Table 2: Maximum specific methanogenic activity with 2.5 g acetate-COD L^{-1} for the different anaerobic sludge and sediments.

Substrate	**pH**	**TS ($g\ L^{-1}$)**	**VS ($g\ L^{-1}$)**	**Ashes ($g\ L^{-1}$)**	**Humidity (%)**	**VS/TS (%)**	**COD ($mg\ O_2\ L^{-1}$)**	**COD_T ($mg\ O_2\ L^{-1}$)**	**Mixture composition (%)**
Tomato	4.31	4.70±0.14	3.86±0.10	0.08±0.003	95.29	82.02	-	-	25
Bell pepper	5.54	5.81±0.04	4.90±0.04	0.09±0.0002	94.18	84.25	-	-	20
Lettuce	6.19	6.33±0.04	5.63±0.04	0.07±0.0001	93.66	88.90	-	-	25
Cabbage leaves	6.53	6.90±0.02	6.51±0.02	0.03±0.0002	93.09	94.35	-	-	15
Apple	4.23	12.74±0.15	12.13±0.17	0.06±0.002	87.26	95.26	-	-	15
FVW Mixture	4.97	7.95±0.21	7.27±0.18	0.06±0.003	92.05	91.4	93.70±23.84	210.19±84	100

Table 3: Physical and chemical characterization of the components of the fruit and vegetable waste (FVW) and the mixture based on pH, total solids (TS), volatile solids (VS), ash, humidity, soluble and total chemical oxygen demand (COD and COD_T).

according to literature studies on anaerobic digestion processes of FVW [18, 23]. The pH of the municipal FVW components ranged from 4.23 to 6.53, while the pH of the mixture was 4.97. The humidity content of the FVW mixture was 92.1% with a total volatile solids (VS) content of 91.4%; these values are within the optimal ranges and are consistent with values reported in the literature [2, 8]. COD values of the mixture were quite low (120.19 mg O_2 L^{-1}), which shows that the amount of organic matter is relatively low suggesting that the FVW mixture might be anaerobically degraded in short periods of time.

Batch biodegradation bioassays were conducted to evaluate the impact of increasing concentration of granular sludge in the composition of the prepared inoculum GS-CDS and the effect of agitation during the anaerobic degradation of 5% weight of FVW. Table 4 presents the methane production rate for different bioassays. The rate of methane production achieved in the bioassays conducted with the prepared sludge was significantly higher than the ones recorded in assays conducted with manure or granular sludge only. Taking into consideration that granular sludge is a good candidate for anaerobic degradation processes because it is an excellent source of microorganisms [19], it is expected that the higher the GS concentration the higher the methane production. Surprisingly, the increasing concentration of granular sludge in the prepared inoculum GS-CDS did not have a significant impact in the rate of methane production. In fact, the rate of methane production in treatment 2 was practically the same as the rate obtained in treatment 4 with twice as many as granular sludge (T4: 40% GS + 90% CDS). These results suggest that the low methane production reported in the treatment with the highest concentration of GS can be attributed to mass transfer limitations inside the flask. In this reaction flask, the space needed for reaction was limited resulting in poor contact between the microorganisms and the substrate and affecting the degradation process.

Literature studies on the anaerobic degradation of organic residues have reported mass transfer limitations. For instance, in the research conducted by Rizk et al., FVW municipal waste was digested in a batch bioreactor [24]. The authors reported that low residue degradation and low methane production could be attributed to mass transfer limitations between the microorganisms and the substrate. In other study, Harms and Bosma concluded that low biodegradation rates of organic pollutants are often a result of limited accessibility of the pollutants [25]. Mechanical mixing can be applied to

Bioassay	Treatments	CH_4 production rate (L kg $VS^{-1}d^{-1}$)	Operation time (days)
Granular sludge concentration	T1: 10% GS + 90% CDS	39.56	
	T2: 20% GS + 90% CDS	44.18	
	T3: 30% GS + 90% CDS	42.07	25
	T4: 40% GS + 90% CDS	42.27	
	CDS Control	15.50	
	GS Control	17.44	24
	Abiotic Control	1.94	8
Agitation	T20% GS + 90% CDS Agitation	58.59	7
	T40% GS + 90% CDS Agitación	97.02	7

Table 4: Methane production rates during the anaerobic degradation of 5% FVW with increasing concentration of granular sludge in the prepared inoculum GS-CDS and the effect of agitation.

Period	Bioreactor	pH (average)	Cumulative methane production rate (L CH_4 kg $SV^{-1}d^{-1}$)	COD removal rate (% removal d^{-1})	VS/TS* (%)	Operation time (days)
I	R5%	7.14	62.25	1.56	-	20
	R7.5%	7.06	34.00	1.38	-	20
	R10%	7.06	20.17	1.02	-	20
	R12.5%	7.01	16.49	1.08	-	20
II	R5%	7.59	37.06	1.16	-	6
	R7.5%	7.43	6.14	0.66	-	6
	R10%	7.68	8.20	0.36	-	6
	R12.5%	7.52	9.72	0.13	-	6
III	R5%	7.41	44.28	1.20	84.62	13
	R7.5%	7.45	29.44	1.15	82.56	13
	R10%	7.63	23.93	0.21	80.25	13
	R12.5%	7.45	16.52	0.16	81.35	13

*VS/TS values measured in day 76.

*Soluble COD removal rate was calculated using the slope of the curve of COD removal related to time in each period.

Table 5: Operational parameters of semi-continuous bioreactors performance based on cumulative methane production rate, effluent pH, total and soluble COD removal rate, nitrate concentration, and VS/TS concentration during the three operation periods of AD of FVW.

overcome the limitations resulting from mass transport mechanisms, the main idea is to reduce the distance between cells and substrate molecules in order to obtain a faster flow of the substrate, retaining a greater number of microorganisms [25]. Taking into consideration this information, additional bioassays were conducted using a stirring plate. The cumulative methane production rate in treatment 4 under stirring conditions was 2.3 times higher than the one recorded in the same treatment without agitation in 7 and 25 days of incubation, respectively (Table 4). These results are consistent with data obtained by Rizk et al. and Adhikari et al., where they concluded that the lack of agitation during AD reduces the amount of methane produced and increases the residence time [24, 26]. The optimal composition of the prepared inoculum GS-CDS (40% GS and 90% CDS) and agitation were employed in the subsequent semi-continuous bioassays during the treatment of FVW.

The anaerobic digestion of fruit and vegetable waste (FVW) coming from a municipal market in Tumbaco was studied in semi-continuous bioreactors on laboratory scale applying the optimal conditions obtained in batch bioassays. 4 bioreactors (R) of 4 L capacity were set-up with the prepared inoculum (40% GS + 90% CDS) and different concentration of FVW under agitation conditions. The bioreactors R1, R2, R3 and R4 were fed with 5, 7.5, 10 and 12% of total solids of FVW, respectively. Figure 2 illustrates the time curse of the cumulative methane production expressed in L kg VS^{-1} in the semi-continuous bioreactors (R) incubated with the prepared inoculum GS-CDS during the AD of different percentages of FVW. The bioreactors were operated in three periods directly identified by the addition of fresh mixture of FVW. Period I lasted for 42 days, period II lasted for 20 days, and period III lasted for 26 days. The bioreactors were incubated for 90 days and the methane production increased with time in all periods. In period I, R1 reached stationary phase in 20 days while R4 reached the stationary phase in 42 days and a second load of fresh substrate was added initiating period II. In this period, the only bioreactor that showed an exponential methane production was R1 with 5 % FVW. The other reactors recorded low values of methane production. In period III, the third FVW load was added and all reactors produced methane.

During the three periods of operation, the R1 fed with

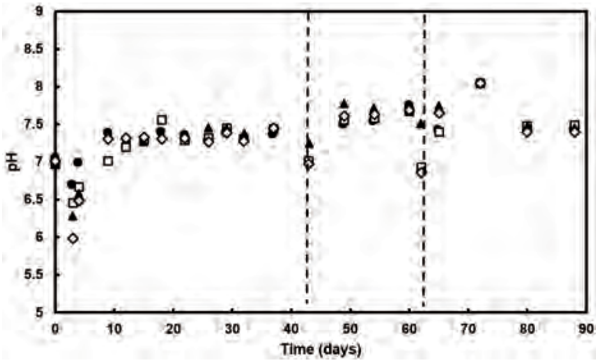

Figure 3: pH variation over time of the effluent of each reactor (R) in semi-continuous operation mode, using GS - CDS inoculum to degrade different amounts of FVW(•) R 5%, (□) R 7.5%, (▲) R 10%, and (◇) R 12.5% of FVW. Period I corresponds to 0 - 42 days, period II corresponds to 43 - 63 days, and period III corresponds to 64 - 90 days.

5% FVW showed the highest methane production, followed by the bioreactors R2, R3 and R4 fed with 7.5, 10 and 12.5 % FVW, respectively. The data indicates that AD is limited to substrate degradation capacity of microorganisms. Therefore the cumulative biogas production is related to the amount of organic matter added, so concentrations higher than the optimal organic load result in a decrease in the methane production. These results are comparable with previous publications. Bouallagui et al. employed tubular digesters to study the AD of FVW and demonstrated that biogas production increased as the organic load augmented from 4 to 6 % total solids [14]. However, the biogas production decreased dramatically when the organic matter content increased to 8%, and the process even stopped when the load reached 10% of total solids. This behavior could be attributed to the rapid conversion of the substrate into volatile fatty acids (VFA), and their accumulation inhibits the activity of the methanogenic microorganisms [7, 27]. As the amount of loaded substrate increases, the degradation of VFA into acetate, hydrogen and carbon dioxide is significantly augmented, while the degrading activity of the methanogenic microorganisms that use acetate and hydrogen does not increase at the same rate [7, 27, 28]. In fact, if hydrogen accumulates, acidogenesis gets partially inhibited causing disproportion in the methanogenic reactions which results in cessation of methane production [7, 27].

Table 5 presents the operational parameters of semi-continuous bioreactors performance based on cumulative methane production rate, effluent pH, total and soluble COD removal rate, nitrate concentration, and VS/TS concentration during the three operation periods of AD of FVW. The highest cumulative methane generation was achieved by the bioreactor with the best performance (5% FVW) and reached values of 2401.92 L CH_4 kg VS^{-1} after 90 days of operation. Methane production in this bioreactor is comparable to the values recorded in the study of Bouallagui et al. where they produced 907.18 L CH_4 kg SV^{-1} feeding an amount of FVW of 6% total solids in 20 days of operation [14].

Figure 3 illustrates the pH variation in the four bioreactors during 90 days of operation. The pH values in the effluent of the semi-continuous bioreactors dropped during the first three days of the experiment from 7 to 6. According to studies published in the literature, in the early days of AD process the concentration of VFA increases, probably causing acidification in the bioreactor [2, 7, 27]. This happens because the microorganisms are not able to remove organic acids and hydrogen at the same rate they are being produced. In addition, in the first days of reaction the pH drop could also be attributed to the low pH of the FVW mixture [14]. The pH of the FVW mixture fed into the bioreactors evaluated in this study was 4.97, which could have caused a slight acidification in the systems. In general terms, it can be concluded that pH values of the bioreactors remained stable during the 90 days of operation, except for few occasions near the organic matter loading where an increase in the substrate could have caused a disturbance in the system.

Regarding COD removal, it can be observed in Table 5 that the removal rate in the four bioreactors was higher during period I. In the case of R1 and R2, the COD removal rates were comparable in the following 2 periods while the rates for bioreactors R3 and R4 decreased with operation time. The low COD removal rates in these two reactors are consistent with the low cumulative methane production discussed previously. Finally, the semi-continuous bioreactors achieved similar percentages of VS removal, varying between 80.3 and 84.6 %, the highest removal values were reached by R1 treating 5% FVW. The VS removal values obtained in this research are quite high, indicating good levels of organic matter degradation in comparison with literature studies. For instance, Bouallagui et al., reported a 75.9% VS removal during AD in a tubular bioreactor fed with 6% total solids and 20 days retention time [14].

Conclusions

Anaerobic digestion of fruit and vegetable waste from municipal market in Tumbaco, Ecuador was successfully studied in semi-continuous bioreactors. The use of prepared inoculum resulted in a higher methane production probably due to synergism among the involved microorganisms. Agitation was identified as an important operational parameter that needs to be incorporated to avoid mass transfer limitations. pH value demonstrated to play a significant role during AD and needs to be controlled since it could inhibit the activity of methanogens in the reactor. The optimal operational conditions as well as the organic load to be treated in the AD process of FVW were established based on the cumulative methane production rate. Finally, anaerobic digestion of FVW was shown to be an effective alternative to landfills for stabilizing the organic matter and preserving the ecosystem and well-being of the population. This study contributes to the development of an integrated hazardous waste management of municipal organic solid wastes that could be successfully implemented in Ecuador.

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References

[1] Kato, K.; Miura, N.; Tabuchi, H.; Nioh, I. 2005. "Evaluation of maturity of poultry manure compost by phospholipid fatty acids analysis". *Biology and Fertility of Soils*, 41:399 – 410.

[2] Bouallagui, H.; Touhami, Y.; Cheikh, R.; Hamdi, M. 2005. "Bioreactor performance in anaerobic digestion of fruit and vegetable wastes". *Process Biochemistry*, 40 (3-4):989 – 995.

[3] Tchobanoglous, G.; Theisen, H.; Vigil, S. 1998. "Integrated Solid Waste Management". *McGraw-Hill*.

[4] Rubio, M. 2011. "Manejo de Residuos Sólidos". *EMASEO. Quito*.

[5] Renou, S.; Givaudan, J.; Poulain, S.; Dirassouyan, F.; Moulin, P. 2007. "Landfill leachate treatment: review and opportunity". *Journal of Hazardous Materials*, 150 (3):468 – 493.

[6] Rapport, J.; Zhang, R.; Jenkins, B.; Williams, R. 2008. "Current anaerobic digestion technologies used for treatment of municipal organic solid waste". *California Integrated Waste Management Board. Davis, California*: 1 – 90.

[7] Chen, Y.; Cheng, J.; Creamer, K. 2010. "Inhibition of anaerobic digestion process: A review". *Bioresource Technology*, 99(10):78 – 90.

[8] Bouallagui, H.; Lahdheb, H.; Romdan, B.; Rachdi, B.; Hamdi, M. 2009. "Improvement of fruit and vegetable waste anaerobic digestion performance and stability with co-substrates addition". *Journal of Enviomental Management*, 90(5):1844 – 1849.

[9] Elango, D.; Pulikesi, M.; Baskaralingam, P.; Ramamuthi, V.; Sivanesan, S. 2007. "Production of biogas from municipal solid waste with domestic sewage". *Journal of Hazarodus Waste Materials*, 141:301 – 304.

[10] Zhang, R.; El-Mashad, H.; Hartman, K.; Wang, F.; Liu, G.; Choate, C.; Gambie, P. 2007. "Characterization of food waste as feedstock for anaerobic digestion". *Bioresource Technology*, 98:929 – 935.

[11] Velmurugan, B.; Ramanujam, A. 2011. "Anaerobic digestion of vegetable wastes for biogas production in a fed-batch reactor". *International Journal of Emeging Sciences*, 1(3):455 – 486.

[12] Van Leeuwenhoek, A. 1995. "Anaerobic digestion and wastewater treatment systems". *Lettinga*, 67(1):3 – 28.

[13] Rinzema, A.; van Lier, J.; Lettinga, G. 1988. "Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor". *Enzyme and Microbial Technology*, 10(1):24 – 32.

[14] Bouallagui, H.; Cheikh, R.; Marouani, L.; Hamdi, M. 2003. "Mesophilic biogas production from fruit and vegetable waste in a tubular digester". *Bioresource Technology*, 86(1):85 – 89.

[15] Ochoa - Herrera, V.; Banihani, Q.; Leon, G.; Khatri, C.; Field, J.; Sierra - Alvarez, R. 2009. "Toxicity of fluoride to microorganisms in biological wastewater treatment". *Water Research*, 43:3177 – 3186.

[16] APHA. 1998. "Standard Methods for the Examination of Water and Wastewater". *20th. Washington, DC: American Public Health Association*.

[17] Field, J. 1987. "Parameters measurements". *Wageningen Agricultural University*, 13:22 – 34.

[18] Gomez - Lahoz, C.; Fernandez Gimenez, B.; Garcia - Herruzo, F.; Rodriguez - Maroto, J.; Vereda, A. 2007. "Biomethanization of mixtures of fruits and vegetalbes solid wastes and sludge from a municipal wastewater treatment plant". *Journal of Environmental Science and Health Part A*, 42(4):481 – 487.

[19] Metcalf & Eddy. 2007. "Wastewater Engineering Treatment and Reuse". *5th Edition AECOM. New York*.

[20] Yang, S. 1998. 'Methane production in rever and lake sediments in Taiwan". *Environmental Geochemistry and Health*, 20:245 – 249.

[21] Wartell, B. 2009. "Anaerobic digestion of Equine Waste". *The State University of New York, Tesis de Maestria: New Jersey*.

[22] Schmidt, J.; Ahring, B. 1996. "Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors". *Biotechnology and Bioengineering*, 49(5):229 – 246.

[23] Virtutia, A.; Mata - Alvarez, J.; Cecchi, F.; Fazzini, G. 1989. "Two-phase anaerobic digestion of a mixture of fruit and vegetable wastes". *Biological Wastes*, 29(3): 189 – 199.

[24] Rizk, M.; Bergamasco, R.; T., G.; R., C. 2007. "Anaerobic co-digestion of fruit and vegetable waste and sewage sludge". *International Journal of Chemical Reactor Engineering*, 5(1):1 – 10.

[25] Harms, H.; Bosma, T. 1997. "Mass transfer lilmitation of microbial growth and pollutant degradat". *Journal of Industrial Microbiology and Biotechnology*, 18(2-3):97 – 105.

[26] Adhikari, R. 2006. "Sequential batch and continuous anaerobic digestion of municipal solid waste in pilot scale digesters". *Asian Institute of Technology. Thailand*: 1 – 184.

[27] Dogan, E.; Demirer, G. 2009. "Volatile fatty acid production from organic fraction of municipal soild waste through anaerobic acidogenic digestion". *Environmental Science and Engineering*, 26(9).

[28] Liu, H.; Walter, H.; Vogt, G.; Holbein, B. 2002. "Steam pressure disruption of municipal solid waste enhances anaerobic digestion kinetics and biogas yield". *Biotechnology and Bioengineering*, 77(2):121 – 130.