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Influence of temperature on the anaerobic stabilization of organic solid residues

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This study was aimed at determining the effect of temperature on the stabilization of organic solid waste conjugated with sewage sludge in anaerobic batch reactors (ABR), and to estimate the efficiency of the process in producing biogas and methane. The substrate contained 36.2 g L^{-1} of total solids comprising residues from fruits and vegetables enriched with anaerobic sludge from sanitary sewage (4:1, w/w). The reactors were of 1.15 L capacity and were operated at 25.5, 40 and 50°C (ABR1, ABR2 and ABR3, respectively) for 160 days. The efficiencies of transformation of total volatile solids in ABR1, ABR2 and ABR3 were, respectively, 43.2, 34.2 and 32%, and the transformation of chemical oxygen demand showed a similar tendency with efficiencies of 39.5, 33.6 and 16.6%, respectively. The mean volumes of biogas accumulated by ABR1, ABR2 and ABR3 were 28.85, 21.24 and 20.54 L, respectively, while the respective mean volumes of methane were 9.04, 7.11 and 1.11 L. The results demonstrate that the activity of methane-producing microorganisms was inhibited at higher process temperatures. It is concluded that anaerobic digestion at ambient temperature represents an economical and environmentally viable strategy for the disposal of municipal solid wastes.

Key words: Plant residues, anaerobic digestion, biofuel, methane.

INTRODUCTION

Bordering on 260, 000 tons of municipal solid waste (MSW) are collected each day in Brazil and of this, 50.8% ends up in open dumping grounds, 22.5% in controlled landfills and 27.7% in sanitary landfills (Instituto Brasileiro de Geografia e Estatística, 2008). It is reported that some 50% (w/w) of MSW comprises organic matter (mainly food, kitchen and green waste) that is readily biodegradable and fermentable (Gossett et al., 1982).

This material represents an important resource that could be converted through anaerobic digestion into

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methane for use in the national energy matrix, or by aerobic processes into solid fertilizer for use in agriculture (Leite et al., 2009). However, the utilization of the biodegradable organic fraction of MSW, along with other recyclable fractions (such as paper, cardboard, plastics, glass, and ferrous and non-ferrous metals), requires implementation of integrated and sustainable waste management policies at least in the most heavily populated Brazilian cities (Maciel et al., 2011).

Anaerobic digestion is considered to be an efficient and environmentally friendly strategy for the disposal and conversion of biological waste into renewable energy in the form of biogas (Iglesias et al., 2000; Fdez-Guelfo et al., 2011). Moreover, anaerobic digestion of urban garbage is economically feasible since the biogas produced has the potential to replace fossil fuels, including natural gas, in heat generation and as a transportation fuel (Curry and Pillay, 2012). However, one of the main issues impeding the wider application of anaerobic digestion is the time required for the biostabilization of solid degradable wastes. The stability of the anaerobic process is crucial in maintaining the balance between the various microbial populations that are responsible for the conversion of complex organic compounds into simple substances; such as methane, carbon dioxide, nitrogen, ammonia, hydrogen sulfide and other products of low molecular weight (Lay et al., 1998; Raposo et al., 2011). By virtue of the greater proportion of cellulose and lignocellulosic polymers present in solid degradable materials compared with liquid wastes, the time required to attain stabilization during anaerobic digestion of the former is much longer (Schievano et al., 2010).

According to Zhang et al. (2006), temperature plays a key role in anaerobic processes and exerts a significant effect on conversion and process stability, kinetic parameters, the quality of the effluent and consequently, on the amount of methane produced. In the short term, waste decomposition generally increases with increasing temperatures up to a limiting value (Hartz et al., 1982). However, since the microbial population involved in anaerobic degradation may include psychrophilic, mesophilic and thermophilic organisms (El-Mashad et al., 2004), with respective optimal growth temperatures of < 25, 25 - 40 and > 45°C, alterations in temperature regimes will over the longer term influence the balance of the microbial population.

The optimal temperature ranges for various types of microorganisms that provide biostabilization process of organic matter present in liquid or solid wastes are psychrophilic (12 to 18°C), mesophilic (25 to 40°C) and thermophilic (55 at 60°C). In this work, three different levels of temperatures: room temperature (21.5°C), 40 and 50°C were studied. Considering the temperature range established by Metcalf and Eddy (2003), the three temperature levels adopted are conducive to the development of mesophilic microorganisms, given temperature of 21.5°C approaches the level set for mesophilic microorganisms and the temperature level 50°C, as is favorable for the development of thermophilic microorganisms. Therefore, the temperature range varied from 21.5 to 50°C and expressed theoretically in a medium largely to the development of mesophilic microorganisms, but their possibilities at room temperature prevailed in the presence of microorganisms and psychrophilic temperature of 50°C in the presence of thermophilic microorganisms.

The temperature is a parameter of fundamental importance to the process of anaerobic digestion of organic solid waste. There are numerous works about influence of temperature on the anaerobic digestion process, but with regard to the process of digesting anaerobic organic solid wastes with high solids concentration, there are still obvious gaps to be investigated; therefore, it was anchored in the existence of these gaps that aimed to study the three different temperature levels.

According to De La Rubia et al. (2005), the thermophilic microorganisms are more sensitive than mesophilic microorganisms for environmental changes. In terms of solid waste digestion, the yield and rate of biogas production depends mainly on operation temperature; but other factors such as setting the reactor and the chemical composition of the waste also influence it (Bouallagui et al., 2003).

Kim et al. (2006) indicated in their work that the temperature can influence the anaerobic biostabilization of organic waste, because it has direct influence on the growth rate of microorganisms, substrate utilization rate and biogas production rate. Low temperature may result in possible depletion of cellular energy or complete lysis, while high temperature may contribute to reducing the biogas production, due the production of volatile gases, such as ammonia, which limits the activity of methanogenic microorganism (Fezzani et al., 2010).

In general temperatures between 35 and 37°C are considered suitable for methane production and thermophilic temperatures may cause reduction in biogas production rate until there is complete adaptation and normal growth of thermophilic microorganisms.

Studies conducted by Ward et al. (2008) shown that growing optimal temperatures for some methanogenic microorganism were in the range 37to 45°C, with predominance for mesophilic Methanobacterium. Temperatures in the range 37 to 40°C for Methanobrevibacter and temperatures in the range 35 to 40°C showed the presence of Methanolobus, Methanococcus, Methanoculleus and Methanospirillum. Temperatures in the range of 30 to 40°C were predominant in Methanoplanus and Methanocorpusculum. Between 55 and 50°C predominance was of Methanohalobium and Methanosarcina.

Thus, this study is aimed at: (i) determining the effect of temperature on the stabilization of organic solid waste

Table 1. Physical and chemical characteristics of solid vegetable waste used in the preparation of the substrate.

Waste	Humidity (%)	Total solids (TS; $g.L^{-1}$)	Total volatile solids (TVS; $g.L^{-1}$)	Total fixed solids (TFS; $g.L^{-1}$)	Total organic carbon (TOC; $g.L^{-1}$)	Total Kjeldahl nitrogen (TKN; $g.L^{-1}$)	N - ammonium ($g.L^{-1}$)	Chemical oxygen demand (COD; $g.L^{-1}$)	pH	Total alkalinity (TA; g $CaCO_3.L^{-1}$)*	Volatile fatty acids (VFA; g $CH_3COOH.L^{-1}$)*
Chard	97.79	22.1	12.0	10.1	6.7	1.26	0.56	7.7	7.97	3.8	2.18
Potato	85.82	141.8	128.3	14.8	71.3	1.12	0.28	140.4	3.71	0	4.15
Banana	87.72	122.8	97.5	25.3	54.2	1.4	0.14	67.0	3.81	0	13.53
Eggplant	94.84	51.6	44.7	6.9	24.8	1.82	0.42	15.8	7.55	4.4	2.18
Carrot	87.78	122.2	76.9	45.3	42.7	1.82	0.28	111.1	4.26	0.8	6.76
Chayote	96.02	39.8	35.6	4.2	19.8	0.84	0.28	22.1	5.5	1.8	2.62
Coriander	92.24	77.6	39.1	38.5	21.7	1.96	0.84	26.2	8.49	15.6	3.93
Kale	93.28	67.2	51.1	16.1	28.4	1.26	0.70	11.3	8.01	4.4	1.53
Pumpkin	93.35	66.5	53.1	13.4	29.5	1.26	0.28	57.8	5.61	6	7.2
Watermelon	95.28	47.2	31.7	15.5	17.6	1.12	0.14	40.7	4.26	0.2	2.84
Melon	94.74	52.6	38.8	13.8	21.5	2.24	0.70	50.5	4.31	1.8	12.22
Papaya	93.87	61.3	51.9	9.4	28.8	2.10	0.14	44.5	3.67	0	11.78
Cucumber	97.59	24.1	16.0	8.1	8.9	1.12	0.28	23.5	5.39	3.2	3.27
Bell pepper	95.88	41.2	32.7	8.5	18.1	1.68	0.56	29.1	5.58	3.8	4.80
Gabagge	96.35	36.5	26.9	9.6	14.9	2.38	1.40	14.5	7.67	6	3.49
Tomato	97.21	27.9	17.2	10.7	9.6	1.26	0.84	24	5.58	4.2	5.45

conjugated with sewage sludge in anaerobic batch reactors (ABR), and (ii) estimating the efficiency of the process in producing biogas and methane.

MATERIALS AND METHODS

The experiments was conducted at the Estação Experimental de Tratamento Biológico de Esgoto (EXTRABES) located in the Universidade Estadual da Paraíba, Campina Grande, PB, Brazil (latitude 7°13'11''S; longitude 35°52'31''W; altitude 550 m). The substrate used as feedstock in the ABRs comprises of residues from fruits and vegetables, provided by the Empresa Paraibana de Abastecimento Agrícola (Campina Grande) and was used as inoculum for all reactors anaerobic sludge from sanitary sewage collected from an up-flow anaerobic sludge blanket (UASB) reactor located at the Experimental Station for the Biological Treatment of Sewage (Campina Grande, PB, Brazil). The UASB reactor was monitored at 25°C. The substrate was prepared by crushing the plant residues in a Trapp (Jaraguá do Sul, SC, Brazil) model TR 200 grinder and passing through a 6 mm mesh sieve until a viscous homogeneous mixture was obtained. Subsequently, 8 kg of sludge was added to 32 kg of the plant residue mixture, the concentration of total solids (TS) was adjusted to 36.2 $g.L^{-1}$ by addition of domestic wastewater collected from the eastern outfall pipe of the municipal sewer of Campina Grande, and the pH was increased from 4.3 to 6.0 or 6.5 with sodium carbonate.

Tables 1 and 2 presents data on chemical characterization of solid vegetable waste and anaerobic sludge used in the preparation of the substrate, respectively. In Table 3 the chemical characterization of the raw substrate are presented.

The domestic wastewater produced by the population of the city of Campina Grande has characteristics moderate with COD around 600 $mgO_2.L^{-1}$, BOD_5 300 $mgO_2.L^{-1}$, pH 7.5 and TN 60 $mgN.L^{-1}$. The vegetable solid waste used in the preparation of the substrate were acidic characteristics and the final pH was around 4.3, after the addition of anaerobic sludge the pH incresced to 5, valour below the recommended process of biological waste treatment.

Table 2. Physical and chemical characteristics of anaerobic sludge used in the preparation of the substrate.

Parameter	Raw substrate
Humidity (%)	93.61
Total solids (TS; $g.L^{-1}$)	63.9
Total volatile solids (TVS; $g.L^{-1}$)	29.62
Total fixed solids (TFS; $g.L^{-1}$)	34.29
Total organic carbon (TOC; $g.L^{-1}$)	16.46
Chemical oxygen demand (COD; $g.L^{-1}$)	48.48
pH	8.03
Total alkalinity (TA; g $CaCO_3.L^{-1}$)	7.05
Volatile fatty acids (VFA; g $CH_3COOH.L^{-1}$)	3.00
VFA/TA ratio	0.43
Total Kjeldahl nitrogen (TKN; $g.L^{-1}$)	0.70
N - ammonium ($g.L^{-1}$)	0.14
P - orthophosphate ($g.L^{-1}$)	0.61
Specific mass ($g.L^{-1}$)	1029.1
C/N ratio	23.5
C/P ratio	26.9

Then, it was corrected to 6.0 by addition of sodium carbonate. Each batch reactor (Figure 1) had a capacity of 1.15 L, of which 87% was employed for substrate storage and 13% was for biogas storage (headspace). Reactors were maintained at 25.5, 40 or 50°C (ABR1, ABR2 and ABR3, respectively) with constant monitoring of temperature, and each experiment was performed in triplicate to give a total of nine reactors. The raw substrate and the temperature stabilized substrates discharged from the reactors after digestion were characterized chemically. The parameters used in the analysis of raw and stabilized substrates were: Total solids (TS), total volatile solids (TVS) and total fixed solids(TFS) ($g.L^{-1}$); total organic carbon (TOC; $g.L^{-1}$); Total chemical oxygen demand (COD; $g.L^{-1}$); pH; Total alkalinity (TA; $g.L^{-1}$); Volatile fatty acids (VFA; $g.L^{-1}$); total Kjeldahl nitrogen (TKN; $g.L^{-1}$); N - ammonium ($g.L^{-1}$); P-orthophosphate ($g.L^{-1}$). The analyses followed the methods described by the American Public Health Association (2005). The biogas produced was measured daily and once a week was characterized by gas chromatography using thermic conductivity detector.

The processing period was 160 days during which quantitative and qualitative analysis of biogas was performed on a daily and weekly basis, respectively. The amount of biogas produced per day was quantified with the aid of a U-type manometer containing hydrated ethanol 46° INPM (*Instituto de Pesos e Medidas*; equivalent to 54% ethanol by volume) as the manometric fluid. The pressure exerted by the biogas accumulated in the headspace (P_x; atm), the number of moles of biogas produced (n_{biogas}; mol), the volume of biogas produced (V_{biogas}; cm^3), and the volume fraction of the component (methane) of the biogas ($V_{fraction}$; cm^3) were estimated according to Equations 1 to 3:

$$P_x = P_y + (\quad g \quad h) \tag{1}$$

$$n_{biogas} = (P_x \quad V) / (R \quad T) \tag{2}$$

$$V_{biogas} = (n_{biogas} \quad R \quad T) / (P) \tag{3}$$

Where, P_y is the atmospheric pressure (atm), ρ is the specific mass of the manometric fluid (g/cm^3); g the acceleration due to gravity, h is the height of the manometric column (cm), V is the volume of the head space (cm^3), R is the ideal gas constant ($cm.atm.. mol^{-1}. K^{-1}$), T is the temperature treatment (K), and $X_{fraction}$ is the proportion of the component (methane) in the biogas.

The biogas produced was analyzed qualitatively using a gas chromatograph coupled to a 250 mA thermal conductivity detector and equipped with a stainless steel column (3 m) packed with Porapak matrix (mesh size Q80-100). The carrier gas was helium supplied at a flow rate of 30 L min^{-1}, and the vaporizer, column and detector were maintained at 75, 75 and 100°C, respectively. Biogas samples were collected from the headspace by introducing a needle connected to a 0.5 mL syringe (equipped with a safety device to avoid gas loss) into the septum located on top of the reactor. The volume of methane was determined from the volume of biogas produced and the methane content of the biogas obtained in the chromatographic characterization.

The volumes of biogas and methane accumulated in the ABRs at the end of the experimental period were submitted to analysis of variance (ANOVA) and Tukey test in order to determine if the observed differences were statistically significant ($\rho < 0.05$; 95% confidence interval).

RESULTS AND DISCUSSION

Characterization of the wastes

The pH of the raw substrate used in the batch reactors was 6.52, a value that was within the pH range recommended for anaerobic stabilization processes. A slight reduction in the pH was observed in the stabilized substrates discharged from ABR1 and ABR2 after 160 days of digestion (Table 3). The total alkalinity (TA) of the raw substrate was equivalent to 8.95 $g.L^{-1}$ of $CaCO_3$, of

Table 3. Physical and chemical characteristics of the raw substrate and stabilized substrates discharged from the anaerobic batch reactors (ABR) operating at different temperatures.

Parameter	Raw substrate	Stabilized substrates		
		ABR1(25.5°C)	ABR2(40°C)	ABR3(50°C)
Humidity (%)	96.38	97.41	97.22	96.89
Total solids (TS; $g.L^{-1}$)	36.20	25.90	27.80	31.10
Total volatile solids (TVS; $g.L^{-1}$)	21.29	12.13	14.05	14.48
Total fixed solids (TFS; $g.L^{-1}$)	14.94	13.60	13.75	16.64
Total organic carbon (TOC; $g.L^{-1}$)	11.83	6.74	7.81	8.04
Chemical oxygen demand (COD; $g.L^{-1}$)	34.26	20.72	22.74	28.64
pH	6.52	6.27	6.03	6.48
Total alkalinity (TA; g $CaCO_3.L^{-1}$)	8.95	5.56	5.89	6.40
Volatile fatty acids (VFA; g $CH_3COOH.L^{-1}$)	8.45	4.49	4.99	5.36
VFA/TA ratio	0.94	0.81	0.85	0.84
Total Kjeldahl nitrogen (TKN; $g.L^{-1}$)	0.63	0.45	0.47	0.56
N - ammonium ($g.L^{-1}$)	0.17	0.34	0.36	0.35
P - orthophosphate ($g.L^{-1}$)	0.1661	0.1698	0.1666	0.1680
Specific mass ($g.L^{-1}$)	1009.6	-	-	-
C/N ratio	18.78	14.98	16.62	14.36
C/P ratio	71.22	39.69	46.88	47.86

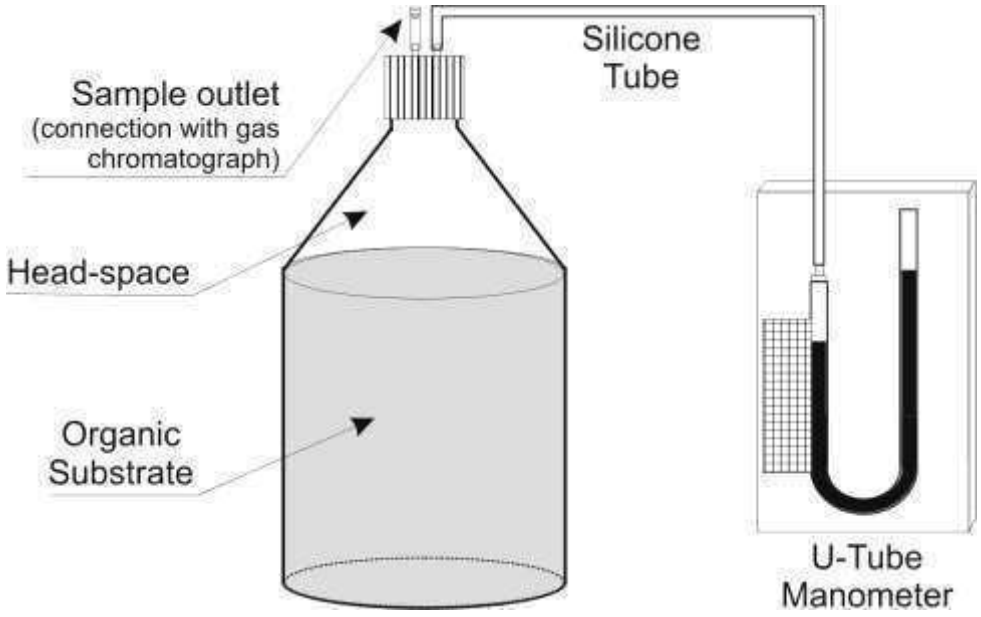

Figure 1. Schematic representation of the anaerobic batch reactors.

which a fraction of approximately 34% corresponded to bicarbonate alkalinity. The levels of TA in the stabilized substrates varied between 5.56 and 6.40, with the consumption of alkalinity being higher in ABR1 (43%) in comparison with ABR2 and ABR3 (37.7 and 28%, respectively). However, because of the mechanisms inherent in the anaerobic biostabilization of solid organic solid waste containing predominantly plant residues, the fractions of bicarbonate alkalinity in the stabilized structure ranged between 25.6 and 29%, and such small variations explain the insignificant differences in the pH values of the substrates.

The value of volatile fatty acids (VFA) decreased progressively from ABR1 to ABR3. The concentration of VFA present in the raw substrate reduced by 46.4, 41.6 and 35.7% in the substrates stabilized at 25.5, 40 or 50°C, respectively (Table 3). It is noteworthy that the ratio of VFA to TA in the raw substrate was 0.94, a value that is well above the theoretical recommended level for anaerobic processes. In the biostabilized substrates, this ratio was decreased to values that ranged from 0.81 (ABR1) to 0.84 (ABR3), thereby indicating the more efficient performance of anaerobic digestion at 25.5°C. Along with temperature, other factors may have influenced the performance of the process, and these include alterations in the particle size, heterogeneity and stationary mass of the substrate as well as acclimatization of the microorganisms.

The raw substrate fed to each reactor contained 36.2 $g.L^{-1}$ of total solids (TS), of which 21.29 $g.L^{-1}$ (~ 59%) represented total volatile solids (TVS) and 34.26 $g.L^{-1}$ (~ 95%) corresponded to chemical oxygen demand (COD). The efficiencies of transformation of TVS in ABR1, ABR2 and ABR3 were, respectively, 43.2, 34.2 and 32%, and a similar tendency was observed for COD transformation with efficiencies of 39.5, 33.6 and 16.6%, respectively (Table 3). These findings show that the efficiencies of the reactors were inversely proportional to the temperature.

The total Kjeldahl nitrogen (TKN) in the raw substrate was 0.63 $g.L^{-1}$ with the concentration of ammonia accounting for 16.6% of this value. In the stabilized substrates, TKN values were in the range 0.45 - 0.56 g L^{-1} and ammoniacal nitrogen was roughly 0.1 g L^{-1}. In the raw substrate, the ratio of total organic carbon (TOC) to TKN (the C/N ratio) was 18.78, a value that was within the range of approximately 20-30 recommended for anaerobic biostabilization. Owing to the insignificant conversion of TKN into other forms of nitrogen during anaerobic digestion, the reduction in the C/N ratio in the stabilized substrates was minimal and ranged from 11.5% in ABR2 to 23.5% in ABR3.

According to Weiland (2006) and Bouallagui et al. (2009), the C/N ratio between of 20/1 to 30/1 is good for performance of anaerobic digestion of the vegetable solid waste. On the other hand, works realized by Guermoud et al. (2009) and Lee et al. (2009) showed that the best C/N ratio in anaerobic digestion of organic waste was 20/1 to 30/1.

Biogas production

The accumulation of biogas as a function of process time in ABRs operating at different temperatures is presented in Figure 2 and the accumulation of methane as a function of its processing time in ABRs operating at different temperatures is presented in Figure 3, while the volumes of biogas and methane accumulated after 160 days of anaerobic digestion are shown in Table 4. Application of ANOVA and Tukey test revealed that the volumes of biogas produced in the three reactors were not significantly different. The accumulative biogas volumes in three reactors were quantitatively similar.

The volumes of methane accumulated after 160 days of anaerobic digestion are shown in Figure 3. While the volumes of methane produced in ABR1 and ABR2, did not differ significantly one from another; both were significantly higher ($\rho < 0.05$) than that generated in ABR3. According to Deublin and Steinhauser (2008), methane-producing microorganisms are more active at temperatures in the mesophilic range, and this explains why the volume of methane formed in ABR3 was much lower in comparison with ABR1 and ABR2. Considering that the three reactors were fed with the same substrate (similar particle size and concentration of TS), and received comparable organic loads, it is possible to state that the volume of biogas produced was negatively affected by a processing temperature of 50°C, probably because growth of the methanogenic Archeae was inhibited under such conditions.

The proportion of methane produced in ABR1 increased progressively throughout the experimental period and attained a value of 37% after 160 days of digestion. The percentage of methane formed in ABR2 exhibited a similar profile until day 140 (30%). In ABR3, no methane was detected in the generated biogas until day 100, after which the percentage increased steadily and attained 5% at day 153. These results demonstrate that the activities of methane-producing microorganisms were inhibited at the higher temperature, thereby reducing the efficiency of ABR3. In contrast, Lianhua et al. (2010) obtained biogas containing the equivalent of 65, 62.5 and 59.1% of methane by digestion of rice straw slurry containing 75 g L^{-1} of TS in reactors operating at 25, 35 and 55°C, respectively.

The reduced volumes of methane produced in ABR2 and ABR3 were likely associated with the lack of initial adaptation of the gas-producing organisms to high temperatures (40 and 50°C). Similar results have been reported by Komemoto et al. (2009) following experiments involving biogas production from food substrates at different temperatures. These authors found that,

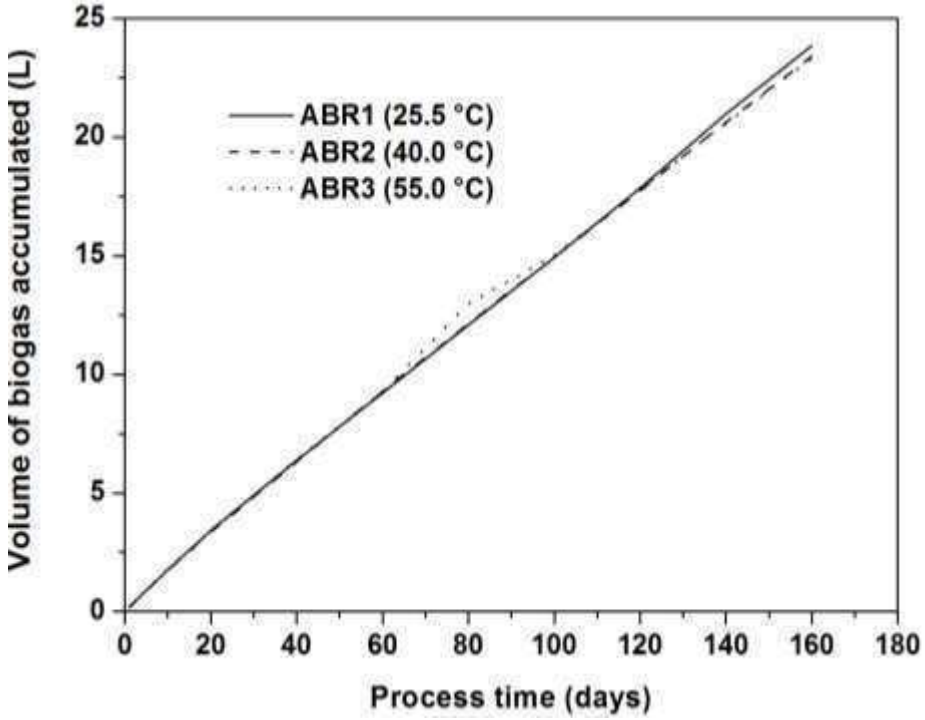

Figure 2. Accumulation of biogas as a function of process time in anaerobic batch reactors (ABR) operating at different temperatures.

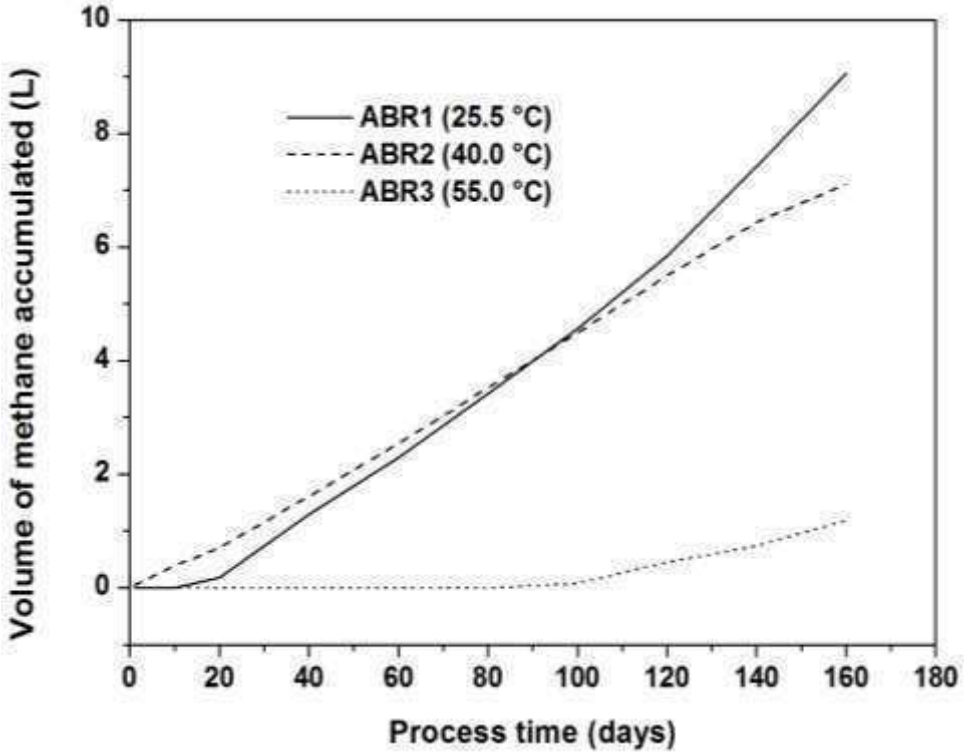

Figure 3. Accumulation of methane as a function of process time in anaerobic batch reactors (ABR) operating at different temperatures.

Table 4. Volume of biogas and methane accumulated in anaerobic batch reactors operating under different temperatures after 160 days of digestion.

Reactor	Mean accumulated volume (L)	
	Biogas	Methane
ABR1 (25.5°C)	23.85^{A}	9.04^{A}
ABR2 (40°C)	21.24^{A}	7.11^{A}
ABR3 (50°C)	20.54^{A}	1.11^{B}

In each column, values followed by dissimilar uppercase capital letters indicate significant differences according to Tukey test ($P < 0.05$).

Table 5. Theoretical and experimental values for methane production in anaerobic batch reactors (ABR) operating under different temperatures calculated in terms of total organic carbon (TOC) transformed.

Reactor	Volume of methane (L)		
	Theoretical value	Experimental value	Conversion efficiency (%)
ABR1 (25.5°C)	9.48	9.05	95.5
ABR2 (40°C)	7.52	7.11	94.6
ABR3 (50°C)	7.18	1.19	16.5

although temperatures in the thermophilic range promoted the solubilization of substrates, the production of biogas was reduced in comparison with other systems employing lower temperatures owing to the inhibition of gas-producing microorganisms.

As shown in Table 5, the experimental yields of methane generated in ABR1 and ABR2 (95.5 and 94.6%, respectively) were close to the theoretical values, while in ABR3, the experimental yield was less than 20% of the theoretical value.

Conclusions

Anaerobic digestion at ambient temperature (25.5°C) provided the most efficient conditions for the production of biogas and methane from a plant-derived organic sludge with a TS concentration of 36.2 g L^{-1}. Under these conditions, the total amounts of biogas and methane produced were 23.85 and 9.05 L, respectively, and these yields were significantly higher compared with those produced when a process temperature of 55°C was applied. Anaerobic digestion at ambient temperature represents an economical and environmentally viable strategy for the disposal of municipal solid wastes.

The best performance of the ABR1, monitored with temperature of 25.5°C, should be associated with the inoculum used. The reactor that was used to collect the inoculum was operated at the same temperature. In conclusion, the adaptation temperature of inoculum is a decisive factor for the performance of this type of process.

Conflict of interests

Authors did not declare any conflict of interest.

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