ISSN 2175-6813
Revista Engenharia na Agricultura
V.25, n.06, p.491-499, 2017
Viçosa, MG, DEA/UFV - DOI: https://doi.org/10.13083/reveng.v25i6.687

EFFECTIVE MICROORGANISMS (EM) AS BIOFEEDERS FOR ANAEROBIC DIGESTION

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Keywords:
animal production
biogas
cattle
digesters
waste management

ABSTRACT

Soil micro-organisms called Effective Microorganisms (EM) were first cultivated and used in the 1970s. Researches about these cultures have since then demonstrated their effectiveness in improving soil characteristics and as an alternative for accelerating organic matter decomposition in waste treatment systems. The objective of this study was to test whether the addition of EM to substrates incubated in anaerobic digesters would increase the efficiency of waste treatment and biogas production. EM cultures were obtained from bacterial colonies captured within the A-horizon of a Brazilian forest soil. They were left to grow during 15 days on cooked rice contact with the soil; afterwards, the established colonies were separated according to their colors, discarding all shades of black, gray and white, according to recommendations from related literature. Remaining colonies were further grown in sugarcane broth medium for 18 days, being this the final EM culture. Twelve bench digesters were used, each with a total capacity for three liters. The experiment was composed by four treatments consisting of different concentrations of EM inoculum [15% (T1), 10% (T2), 1% (T3) and 0% (T4)] applied to dairy cattle manure, with three replications per treatment. Anaerobic digestion was carried out under controlled temperature (35°C) over 99 days. Data collected included concentrations of total, fixed and volatile solids (TS, FS and VS), pH and Chemical Oxygen Demand (COD). The pH of the EM inoculum was 3.34 and COD was 24.25 mg L^{-1}. The best reduction efficiencies for COD and TS removal were 79.44% and 42.50%, respectively, in T4. Among the treatments with EM addition, 1% (T3) resulted in better COD reduction. The maximum accumulated biogas production was 20.60 L biogas L substrate^{-1}, also for T3. In conclusion, EM as an inoculum in low concentrations may be advantageous to anaerobic digestion.

Palavras-chave:
produção animal
biogás
gado
digestores
manejo de resíduos

MICRORGANISMOS EFICIENTES (ME) COMO BIOALIMENTADORES PARA DIGESTÃO ANAERÓBIA

RESUMO

Microrganismos benéficos ao solo, nomeados "Microrganismos Eficientes" (ME) foram cultivados e utilizados nos anos 70. Pesquisas sobre tal cultura tem demonstrado sua eficiência na melhoria das características do solo e também como forma alternativa de acelerar a decomposição da matéria orgânica em sistemas de tratamento de resíduos. O objetivo desse estudo foi testar se a adição da cultura ME como inóculo a substratos incubados em digestores anaeróbios aumentaria a eficiência do tratamento de resíduos. A cultura ME foi obtida de colônias capturadas no horizonte A do solo em uma floresta brasileira. Elas foram desenvolvidas em arroz cozido posto em contato com o solo por um período de 15 dias, após o qual as colônias estabelecidas foram separadas de acordo com padrões de cores, sendo descartadas as de tons cinza, preto e branco, conforme recomendado. As colônias remanescentes foram transferidas para o meio de caldo de cana, onde se desenvolveram por mais 18 dias, sendo esta a cultura ME. Foram utilizados 12 biodigestores de bancada, com capacidade total de três litros cada. Foram aplicados quatro tratamentos, referentes às diferentes adições de ME como inóculo [15% (T1), 10% (T2), 1% (T3) e 0% (T4)] a esterco de bovino de leite, com três repetições por tratamento. A digestão anaeróbia foi conduzida sob condição de temperatura controlada (35ºC), durante 99 dias. Os dados coletados incluíram concentrações de sólidos totais, fixos e voláteis (ST, SF e SV), pH e Demanda Química de Oxigênio (DQO). O pH do inóculo ME foi de 3,34 e sua DQO foi de 24,25 mg L^{-1}. As maiores eficiências de redução de DQO e de ST foram de 79,44% e 42,50%, respectivamente para T4. Dentre os tratamentos com adição de ME, 1% (T3) resultou em melhor eficiência de redução de DQO. A produção máxima acumulada foi de 20,60 L de biogás por L de substrato, também para T3. Concluiu-se que o uso do ME como inóculo, em baixa concentração, pode ser vantajoso para a digestão anaeróbia.

Recebido para publicação em 13/06/2016 • Aprovado em 10/10/2017 • Publicado em 21/12/2017

INTRODUCTION

Anaerobic digestion is a microbial treatment process which occurs under sequential reactions and produces a biogas mixture of approximately 50-80% methane, 20-50 % carbon dioxide and traces of other gases (MOGAMI, 2005). This is a more efficient process when the residue is readily biodegradable. Anaerobic digesters are widely applied for waste treatment, including agricultural crops residue, animal manure, sludge from sewage treatment stations and solid urban wastes. They are also used for wastewater treatment in agricultural, food and beverage industries. Benefits of anaerobic digestion include solid and organic load reduction, relatively low power consumption, tolerance to high organic loads and the possibility of operation with high solids retention times and low hydraulic retention times (CHERNICHARO, 2007).

To reduce required digestion start time and to improve efficiency of biogas production, various additives or inoculants may be added to the substrates in a process known as bioaugmentation, which is an improvement of the hydrolysis and acetogenesis phases in largely cellulosic materials (MARTIN-RYALS et al., 2015) and in fish wastes with moderately high organic load (LI et al., 2011, 2012). In addition, bioaugmentation may be used as a one-time inoculant or as a routine additive (MARTIN-RYALS, 2012), and the additional population of microorganisms is useful for biodigestion (XAVIER & LUCAS JUNIOR, 2010; LI et al., 2011; MARTIN-RYALS, 2012; MARTIN-RYALS et al., 2015). The amount of inocula added to the substrate can positively influence biogas production and result in significant reduction of hydraulic retention time (QUEIROZ, 2003). Distinct inoculants have different nutrient conditions, pH and viable methanogenic organisms, providing distinct effects during anaerobic digestion.

In the 1970s, the Japanese horticulturist and researcher Dr. Teruo Higa initiated the use of a culture of soil beneficial microorganisms (HIGA & PARR, 1994), which he named "Effective Microorganisms" (EM) due its reviving capacity. The main species involved in EM culture include lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus casei*, *Streptoccus lactis*), photosynthetic bacteria (*Rhodopseudomonas palustrus*, *Rhodobacter spaeroides*), yeasts (*Saccharomyces cerevisiae*, *Candida utilis*), and actinomycetes (*Streptomyces albus*, *Streptomyces griseus*). These EM culture are useful because they contain various organic acids due to the presence of lactic acid bacteria, which secretes organic acids, enzymes, antioxidants, and metallic chelates (SHALABY, 2011).

Research about EM has demonstrated their effectiveness in improving soil characteristics and promoting organic matter degradation in wastewater treatment systems. In addition, literature has reported advantageous results of EM culture use, such as biological control for diseases (BEEVI & QUADRI, 2010), better nutrient absorption by plants (HU & QI, 2013), enhanced association between plants and mycorrhizae (BAJAWA et al., 1999), higher crop production (KHALIQ et al., 2006), and efficient domestic sewage treatment (NAMSIVAYAM et al., 2011; SHALABY, 2011). Brazilian agriculturists who have used EM culture have noticed an increase of organic matter degradation in the soil (ANDRADE, 2011).

The objective of this work was to evaluate whether an EM culture applied as inoculum on cattle manure subjected to anaerobic digestion could enhance biogas production and improve the efficiency of organic load removal.

MATERIALS AND METHODS

The experiment was carried out in twelve bench digesters, in the Laboratory of Anaerobic Digestion (LAD), which belongs to the Agricultural Engineering Department, Federal University of Viçosa, Minas Gerais state, Brazil.

The EM used as inoculum were obtained according to the "Homemade Method for Microorganism Capture and EM/soil Preparation" described by Andrade (2011). We cooked 750 g of raw dry rice and placed it in a wooden box with perforated lid. This box was then taken to a forest and the lid was placed in contact with the soil A-horizon at 2.5 cm below the surface. After 15 days, the box was collected and taken back to the laboratory so microorganisms could be selected for the EM culture preparation. Microorganism colonies with colors in shades of black, gray and white were rejected. The remainder colonies were grown in 1.5 L bottles containing 1.0 L of sugar cane juice under environmental temperature. Every two days, the gas produced by these microorganisms during their growth was released, and at the end of

18 days the culture did not produce more gas and, therefore, was ready to be used.

The digesters, as well as the digestion chambers, were glass flasks with plastic lids, each with a total volume of 3.1 L. Each digester had an independent gasholder, made of PVC cylinder (4 L volume), immersed in oil in order to avoid gas leakage. Silicone tubes, with an internal diameter of 8mm, were used to link the chambers to the gasholders (Figure 1). Alongside the tubes, in the gas circuit, Mohr clamps were placed on specific points to allow gas flow control. A digital manometer (Instrutemp, ITMP 120) was used to check the pressure inside the gasholders. The digestion chambers were wrapped in black plastic to avoid light interference and were immersed in a heated water bath that controlled temperature range from 34.1 to 35.1ºC (SOUZA et al., 2005). Graduated scales were attached to the external surfaces of the gasholders to measure its displacement and quantify gas production.

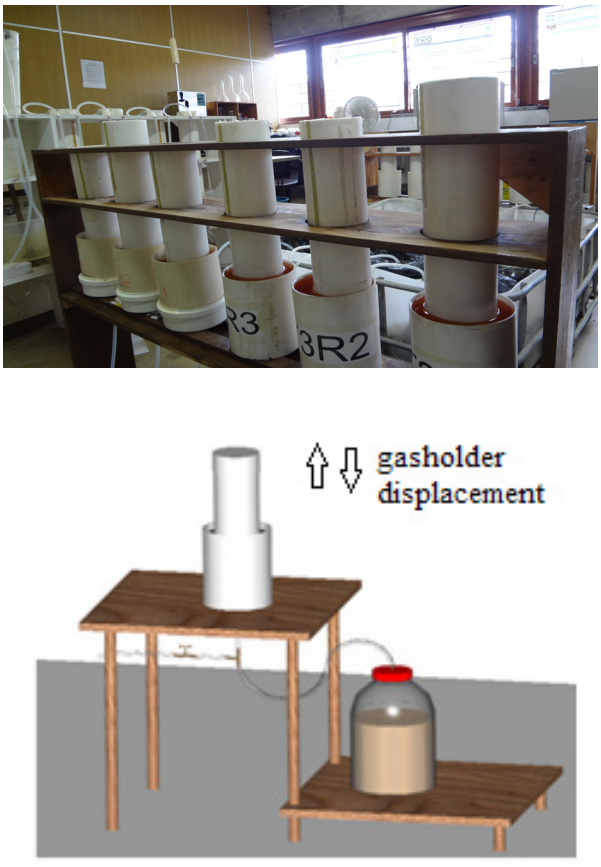

Figure 1. Bench digesters used in this experiment and a scheme of the set detailing the gasholder displacement [From: Inoue (2008)].

The substrate was made from fresh dairy cattle manure collected from the "Research Center for Dairy Production", Animal Science Department, Federal University of Viçosa. Considering the recommendation from Xavier & Lucas Junior (2010) as for the best substrate consistency to facilitate fluid flow along the tubes and to improve anaerobic digestion performance, the cattle manure was diluted in water to reach 8% of total solids (TS). Following the same authors, who mentioned the maximum inoculum concentration in the substrate must be 15%, four different percentages of inoculum were added to the substrate, composing the treatments designated as: T1 (15% EM), T2 (10% EM), T3 (1% EM) and T4 (0% EM), with three replications each.

The biogas production was corrected for standard conditions of temperature and pressure at 20º C and 10332.3 mm H_2O, respectively, based on the combination of Boyle's Law and Lussac's Law:

$$V_2 = \frac{P_1 V_1 T_2}{T_1 P_2} \quad (1)$$

where,
P1 = biogas pressure, measured in the gasholder at the moment of displacement reading;
P2 = atmospheric pressure, reference to calculate corrected biogas volume (10332.72 mmH_2O);
T1 = air temperature (K);
T2 = temperature, reference to calculate corrected biogas volume (293.15 K);
V1 = biogas volume, based on the gasholder displacement; e
V2 = corrected biogas volume.

Before feeding the digesters, the fresh cattle manure was analyzed to determine the total solids (TS) content, as well as the volatile and fixed solids (VS and FS). The diluted material used as the feedstock was also analyzed. Chemical Oxygen Demand (COD) was also determined by means of the closed reflux method, i.e., chemical digestion using potassium dichromate. COD and solids were also determined for digestate at the end of the experiment. In order to correct alkalinity, a specific acid was added to the samples until the pH reached 4.2. Afterwards, measurement was done by using a potentiometer. All these analytical processes were done according to Apha (2005). Evaluation of anaerobic digestion efficiency for

removing organic material was accomplished based on reduction of both solids and COD, which were verified according to the data for the initial substrates and the final digestates.

The experiment was set up under completely randomized design in a Split-Plot system and was carried out for 99 days, with the plots as treatments with three replications. Each treatment was applied at the beginning and the digestion was carried out for a complete cycle, which means it was carried out in a batch process. In order to evaluate the effect of the EM inoculum, as mentioned before, analysis of variance was performed as well as the Dunnett average tests for data related to substrates and digestates. The software used for such analysis was ASSISTAT. In addition, regression equations were developed based on daily records to estimate the accumulated biogas production during the experimental period. From these equations, an estimate of accumulated biogas production was obtained using SigmaPlot software.

RESULTS AND DISCUSSION

Results from the sample analysis are presented in the Table 1.

For fresh manure, 89.13% of TS were volatile, suggesting, according to Chernicharo (2007), a highly biodegradable feedstock amenable to digestion. EM inoculum in its natural state presented a pH of 3.34 and COD of 24,250 mg L^{-1}. Thus, this inoculum can be characterized as acidic and rich in organic matter; furthermore, almost all of its total solids were volatile (94%).

The average value for pH of the EM inoculum differs from that reported by Shalaby et al. (2011), who applied a commercial culture of EM, pH 7.6, for industrial wastewater treatment. Sigstad et al. (2013) used another commercial EM culture, with pH 3.5, for soil improvement. Based on such references, these differences in pH may be related to the phase of development of the culture, as well as the varied species found in the culture medium. These differences may be also related to characteristics of the medium in which the culture grows.

The high value found for COD of the EM inoculum, compared to the one reported by Shalaby et al. (2011), may be explained by the type of culture medium used, composed by sugarcane juice, and subsequent specific microbial growth which occurred in that medium. Despite the liquid aspect, the amount of organic matter that could be chemically oxidized was high. The is also true for the TS values. Around 94.26% of these solids were volatile, therefore most of the medium components could be oxidized. Again, the characteristics of both the culture medium and microorganisms living in it may explain such tendency.

In Table 2, results are shown for alkalinity and pH of the substrate used in the experiment, as well as of the digestate generated after the anaerobic digestion.

Table 1. Characterization of fresh dairy cattle waste and EM inoculum before being added to the anaerobic digestion substrate

	pH	COD (mgL^{-1})	TS (%)	FS (%)	VS (%)
Fresh dairy cattle manure	-	-	15.18	1.65	13.53 (89.13% of TS)
EM	3.34	24,250	12.20	0.70	11.50(94.26% of TS)

Table 2. Alkalinity and pH of the substrate and of the digestate generated after the anaerobic digestion process

	Alkalinity ($mg\ L^{-1}\ CaCO_3$)*		pH*	
	Substrate	Digestate	Substrate	Digestate
T1 (15%EM)	0.03203 b	0.01130 b	5.99 b	4.31 b
T2 (10%EM)	0.03595 b	0.01080 b	6.09 b	4.31 b
T3 (1%EM)	0.04875 a	0.05441 a	6.57 a	7.31 a
T4 (0%EM	0.05128 a	0.05476 a	6.58 a	6.73 a

*Averages in a column with same superscript are not different from those for the treatment with no addition of inoculum, by the Dunnett test, at

In high concentration, EM influenced negatively the alkalinity by decreasing it, hence the buffering power was lower and the pH could change easily. Namsivayam et al. (2011) also observed this when they worked with domestic sewage, in which the alkalinity diminished along the digestion period when EM was added. With the acid characteristic of the EM inoculum in this experiment, the buffering capacity of the system was reduced, causing pH to be unstable when EM additions in the substrate were 15% and 10%. This was not strongly observed for the 1% addition, and, therefore, for low EM concentrations, the decrease in alkalinity is not considerate when compared to the substrate with no inoculum addition, many times being equal to it. At the end of the digestion, the alkalinity of the treatments with 1% and 0% EM additions was higher than the one observed at the beginning, which probably is related to higher stability among organic compounds and enzymes present in the medium. Wan et al. (2011), working on anaerobic digestion of activated sludge also verified this tendency of decreased pH and alkalinity along the digestion time, also with higher values for these variables when the added inoculum amounts were low.

Due to a low alkalinity verified at the beginning and at the end of the digestion, the process was not completed in treatments T1(15% EM) and T2(10% EM) because. during the acidogenic phase, when short chain organic acids are produced and the environment is not favorable to the microorganisms, pH drop disrupts the anaerobic digestion steps. The application of the digestate is, consequently, more difficult due the verified acidity, and pH must be corrected before use. Biological post-treatment of the digestate must also be considered to analyze procedures for discarding in a water stream. For this, the pH of the digestate must be increased. In continuous digesters, a basic solution is added at the beginning of the process when feeding the digester, aiming to increase the pH in the digestate. This procedure generates a change in the medium and may enhance the methane generation phase, however the addition of a basic solution is not recommended for a batch system, because the sealing of the digester could be interfered.

In Table 3, results are listed of the analysis for average content of Total Solids of substrates with the distinct percentages of EM addition and respective digestate. Also, the percentages of reduction observed in such solids are showed.

Treatments with higher additions of inoculum, T1(15% EM) and T2 (10%EM), generated digestates with ST concentrations, respectively, lower and statistically equal to the control, T4 (0%EM). These treatments also resulted in better efficiency in solid reduction at the end of the digestion process among the treatments that received the inocula. Steil et al. (2002), evaluating the use of different percentages of inocula on manures of laying hens, poultry and swine during an anaerobic digestion process, also found the same results, or, in other words, the higher additions presented better solid content reduction at the end of the process.

Table 4 presents the averages for the Chemical Oxygen Demand (COD), in mg L^{-1}, in the substrates with the distinct percentages of EM addition and respective generated digestate, as well as the percentages of reduction observed in such COD.

Table 3. Results of the analysis for average content of Total Solids (TS), in %, in the substrates with the distinct percentages of EM (effective microrganisms) addition and respective generated digestate, as well as the percentages for the reduction observed in such solids

Treatments	Total Solids (TS), in %*		Percentages of Reduction
	Substrate	Digestate	
T1 (15%EM)	8.00 a	4.82 a	39.75
T2 (10%EM)	8.00 a	5.02 a	37.25
T3 (1%EM)	8.00 a	5.72 b	28.50
T4 (0%EM)	8.00 a	4.60 a	42.50

* Averages followed by the same lower case, in the column, are not different from those for the treatment with no addition of inoculum, by the Dunnett test, at 5% of probability.

Table 4. Average values for the Chemical Oxygen Demand (COD), in mg L^{-1}, in substrates with distinct percentages of EM (effective microrganisms) addition and respective generated digestate, as well as the percentages of reduction observed in such COD

Treatments	$COD_{substrate}^{*}$ (mg L^{-1})	$COD_{digestate}^{*}$ (mgL^{-1})	reduction in COD* (%)
T1 (15%EM)	133,472 a	103,020 b	22.73
T2 (10%EM)	116,917 a	85,850 b	26.17
T3 (1%EM)	106,571 a	48,278 a	53.62
T4 (0%EM)	137,956 a	28,549 a	79.44

*Averages followed by the same lower case, in the column, are not different from those for the treatment with no addition of inoculum, by the Dunnett test, at 5% of probability.

The best performance in COD reduction (53.62%) was found in the treatment with the lowest concentration of inoculum, T3 (1%EM). Therefore, COD concentration in the digestate for T3 (1%EM) was lower than those with higher percentages of inoculum in the substrate, T1 (15% EM) and T2 (10% EM), but statistically equal to the control, T4 (0%EM), by means of the Dunnett test, 5% probability. Such value of COD reduction is in accordance with Chernicharo (2007), which mentions theoretical ranges of 60 to 80% for anaerobic digestion. Higher EM concentrations may have generated incomplete organic matter conversion, probably due the acidification identified in the medium, which is also mentioned by Chernicharo (2007).

In Table 5, the analysis of variance are shown for the regression referring to the accumulated biogas production during the 99 days of anaerobic digestion of dairy cattle manure, according to distinct percentages of EM addition (treatments).

In Table 6, regression equations are shown for the estimation of the accumulated biogas volume, based on daily values, and its respective determination coefficients (R^2), as well as the maximum accumulated volume, in liters, according to the distinct percentages of EM addition (treatments).

The maximum biogas accumulation volume for all the treatments with EM addition was inversely proportional to the concentration of EM in the substrate (Table 6). The addition of 1% EM inoculum resulted in highest biogas accumulated volume, followed by the treatment with 10% EM. The treatment with no inoculum addition presented the lowest accumulated biogas production, supposing a positive interference of the EM application on cattle manure only in low concentrations of the inoculum. However, the accumulated biogas production for the treatment based on pure dairy cattle manure was lower than expected. The low values for R^2 are due to the biological characteristics imposed on the system, which may have disturbed the correlation between the studied variables, i.e., accumulated biogas production and the distinct percentage of EM addition to the substrate.

The accumulated biogas production along the experimental period for each treatment is shown in figure 2.

Substrates with the lowest concentrations of inoculum presented better results for the anaerobic digestion (figure 2). For the treatments with 15% and 10% inoculum, an initial peak of biogas production was reached fast and remained constant. In T3, with 1% of EM inoculum added to the substrate, the first peak of biogas production happened around the eighth day of digestion, being constant up to the fortieth day, when an increase occurred. This treatment obtained superior values when compared to the other ones, emphasizing the use of low concentrations of inoculum to maximize biogas production.

The maximum biogas production results for the treatment with no EM addition and for ones with the highest concentrations of inoculum was not in accordance to the literature. Galbiatti et al. (2010) obtained 900.4 m^3 of biogas at the end of the digestion of 70 liters substrate prepared from cattle manure diluted in water. In such case, the accumulated production can be considered of 12.86 liters of biogas per each liter of substrate put

Table 5. Analysis of variance of the regression referring to the accumulated biogas production during the 99 days of anaerobic digestion of dairy cattle manure, according to distinct percentages of EM (effective microrganisms) addition, as treatments

	T1(15%EM)		T2(10%EM)		T3(1%EM)		T4(0%EM)	
	DF	MS	DF	MS	DF	MS	DF	MS
Regression	2	58.197*	3	36.030*	3	29.252*	3	38.352*
Residual	261	0.049	260	0.072	260	2.416	260	2.439
Total	263	0.492	263	0.075	263	2.722	263	2.849

* Significant at 5% probability, according to the F test.

Table 6. Equations of regression and respective parameters for estimation of the accumulated volume of biogas and its respective determination coefficients (R^2) as well as the maximum accumulated volume (Max), in liters, according to the distinct percentages of EM (effective microorganisms) addition (treatments)

Treatments	f	Parameters				R^2	Max*
		a	b	x_0	y_0		
T1(15%EM)	$f=y_0+a/(1+\exp(-(x-x_0)/b))$	1.9231	0.3318	0.4823	0.0000	0.8999	1.9231
T2(10%EM)	$f=y_0+a/(1+\exp(-(x-x_0)/b))$	1.7519	-0.0153	-0.0379	1.6894	0.0548	3.4413
T3(1%EM)	$f=y_0+a/(1+\exp(-(x-x_0)/b))$	40.0575	-0.0530	-0.1832	1.1427	0.1226	41.2002
T4(0%EM)	$f=y_0+a/(1+\exp(-(x-x_0)/b))$	2.0967	0.0452	0.0316	-0.1979	0.1536	1.8988

* Estimation according the equation of regression.

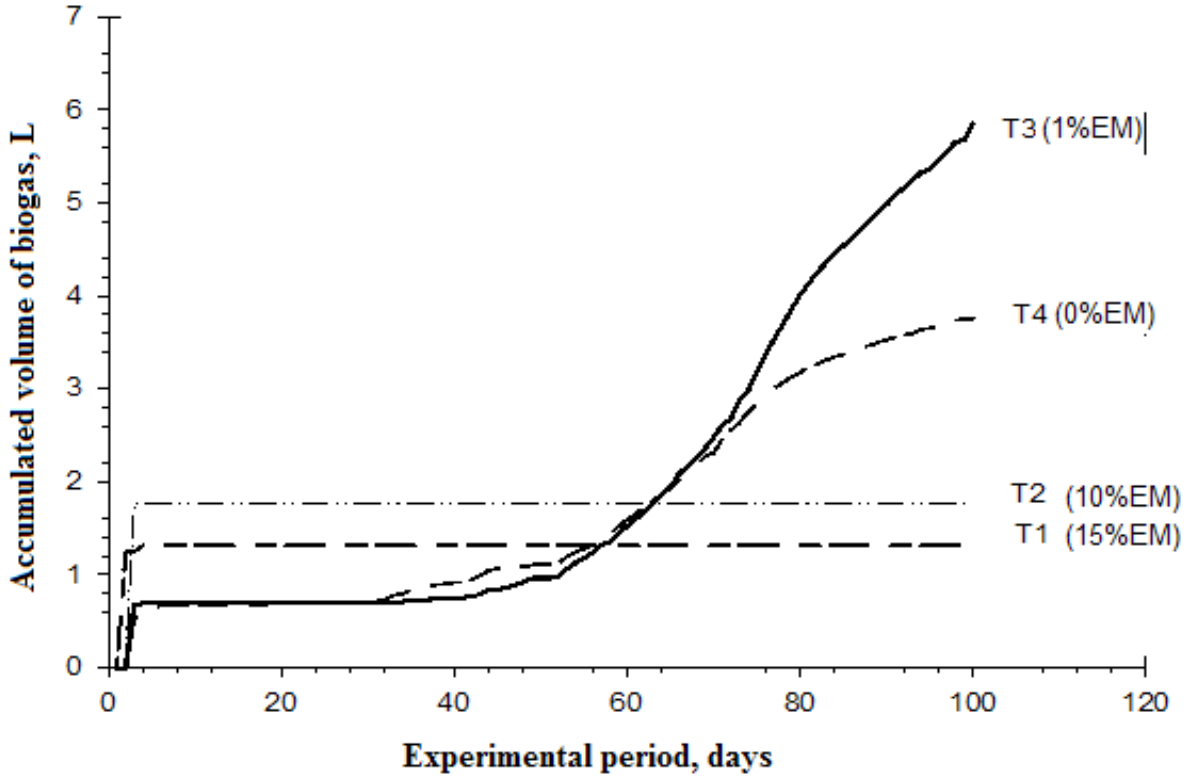

Figure 2. Estimates for accumulated biogas production along the experimental period, for each treatment [(T1 (15% EM), T2 (10% EM), T3 (1% EM) and T4 (0% EM)].

in the digestion chamber. For the present research, the result was 20.60 liters of biogas per each liter of substrate for the treatment with 1% of EM added to the substrate.

CONCLUSION

- The EM (Effective Microorganisms) inoculum used in the present work was too acidic, thus reducing alkalinity of the substrates from the cattle manure. Therefore, to use EM in higher concentrations, a rigid control in the pH is essential. In lower concentrations, EM showed advantage for biogas production, however they also presented less COD (chemical oxygen demand) reduction when compared to the substrate with no EM addition.
- The decision about the added EM percentage must be done according to the purpose of the anaerobic digestion - if the goal is energetic or treatment related. In both cases, to use EM as an inoculum may add beneficial characteristics to the generated digestate, depending on its final use.

ACKNOWLEDGEMENTS

To CAPES (Coordination for the Improvement of Higher Education Personnel) for providing financial support for this work.

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