



*Original Research Article*

# Addition of lipolytic enzyme in anaerobic co-digestion of swine manure and inclusion levels of waste vegetable oil

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Studies that aim the reusing of agro-industrial wastes have become very important due to the high production of this sector and environmental preservation. The objective of this work was to evaluate the performance of digesters loaded with swine manure, increasing inclusion levels of waste oil and lipolytic enzyme, through the potential of biogas and methane production, as well as reductions in levels of total solids (TS) and volatile solids (VS). Batch digesters were loaded with substrates containing 4% TS, composed of pig manure, waste oil (ratios of 8, 10 and 12% in relation to levels of TS in the substrate) and lipolytic enzyme (0.05, 0.10, 0.15, 0.20 and 0.25% in relation to levels of TS in the substrate), water and inoculum. Maximum reduction of TS and VS were 56.13 and 64.49% respectively and occurred in inclusion levels of 0.15 and 0.13% lipase and 12% oil. The greatest potential for methane for the amounts of TS and VS added (0.23 and 0.29L) were achieved by inclusion of 12% oil and levels of 0.12 and 0.11% lipase, and these values were up to 29.7% higher than yields observed for the lowest inclusion level of enzyme. The inclusion of 12% oil and 0.15% lipase in the composition of substrates containing pig manure improves the yields of methane and reductions in pollutant constituents.

**Key words:** biodigestion, lipase, lipid, methane, swine.

## INTRODUCTION

Consequently, as a result of the largest animal and agroindustrial productions there is also the generation of large amounts of waste, which in turn may represent an issue due to the high concentrations of organic matter and the lack of space to properly dispose such material. This situation is a reality in Brazil that has these characteristics within pork industry which is one of the most developed livestock production in the country with very significant indices. According to a survey of the Brazilian Association of Animal Protein (ABPA, 2014) Brazil produced and exported 3.37 million and 600 thousand tons of pork carcass-weight-equivalent, respectively, in 2013. These

numbers led the country to 4th position of the largest producer and exporter.

The possibility of generating power using such waste hence reducing the amount of material to be disposed into the environment has been studied. The anaerobic digestion is an efficient technology that may be used for the treatment of this material, rendering a stable and sanitized final product, and further adding values due to the production of biogas and biofertilizer (Zhang et al. 2013).

The yields of methane from anaerobic digestion of pig manure may vary mainly depending on the quality of the residue added to the digester (Li et al., 2011). Lansing et al.

(2010) working with the co-digestion of pig manure with cooking waste oil using continuous digesters, obtained a production of 0.29 m<sup>3</sup> / kg VS / day with substrates containing only manure.

Co-digestion is the simultaneous addition of two or more substrates with the aim of improving the economic feasibility of anaerobic digestion process due to increased methane production by their interaction through the balance that is established between the compounds (Mata-Alvarez et al., 2014). Fats, oils and greases have been mentioned as substrates that can increase the biogas production by 30% or more when added directly in an anaerobic digester (Hunter Long et al., 2012).

Still, according to Mata-Alvarez et al. (2014), only 4% of the work performed about this issue has used lipid residue added to wastes and therefore, the process of co-digestion using these substrates should be further studied.

As stated by the aforementioned authors, co-digestion is a technique that has been extensively explored thanks to the individual characteristics of each residue and the improvement that occurs when they are combined. In this study the authors attribute the limitations of the waste to its low organic content and high levels of ammonia nitrogen, in addition to the high concentration of long chain fatty acids and low levels of N for lipid residues. Among the benefits of co-digestion of pig manure and lipid residues there are the adequacy of buffering capacity and adjustment the concentrations of ammonia nitrogen, which would be sufficient to meet microbial growth without compromising the formation of biogas (Chenxi et al., 2011).

Thus the addition of lipid residues to animal waste improves anaerobic digestion process, however there are few studies related to this topic.

Lansing et al. (2010) evaluated the additions of 0; 2.5; 5.0 to 10% cooking waste oil in relation to the digesters volume capacity during co-digestion of pig manure and verified the benefits of the combination of these residues, once the largest inclusion of oil doubled the biogas productions and the increment of methane in the biogas composition in comparison to the lowest addition level.

Nonetheless, high amounts of lipids in the substrates may have toxic effects on microorganisms, slowing the degradation process of the material in digestion. According to Mata-Alvarez et al. (2014) proportions of long chain fatty acids and polyphenols inhibit the degradation of the substrate in fermentation by the microorganisms and also have inhibitory action on certain microbial groups. Zhang et al. (2013) reported that the process of toxicity is related to adsorption of long chain fatty acids (oleic and stearic acid) into the membrane of microbial cells hence reducing the transport of nutrients.

Valladão et al. (2011) observed improvement on the production of methane (0.393 L CH<sub>4</sub> / g COD reduced) from digesters supplied with effluent from poultry slaughtering and inclusion of lipolytic enzyme - lipase (1.0% volume).

Thus, the usage of certain inclusion levels of lipase

allowed the decomposition of compounds and enhanced the process of co-digestion; however, its excess can cause increased production of long chain fatty acids which are harmful to microorganisms, limiting the degradation process (Mata-Alvarez et al., 2014).

Based on this assumptions, this work aimed to evaluate the performance of digesters supplied with pig manure and increasing levels of waste oil (8, 10 and 12% in relation to the contents of the TS in the substrate) in the presence of lipase enzyme (lipase) through the productions and potential productions of methane and as well as the reductions in the levels of total (TS) and volatile (VS) solids.

## MATERIAL AND METHOD

Swine manure was collected from pig farming in Jaboticabal - SP / Brazil. Prior to the collections, the pig pens were cleaned the day before and the removal of feces and urine excreted by the animals until the next day, followed by scraping the floor without the addition of water. The animals were in the finishing phase and fed diets formulated to meet the requirements of this phase. The oil was obtained by donation from commercial pastry at the point of being discarded (after several frying).

The loads of the digesters were prepared with substrates of pig manure, cooking waste oil (in ratios of 8, 10 and 12% in relation to the levels of TS in the substrate), lipase (0.05, 0.10, 0.15, 0.20 and 0.25% in relation to the levels of TS in the substrate), water for dilution of this waste and inoculum with the initial concentration of 4% ST. The inoculum was considered ready after approximately 90 days of fermentation when reached a maximum concentration of methane.

The co-digestion was arranged in 30 bench-scale batch digesters, which were housed in a barn with roof and masonry walls, protected from the sun and rain.

The digesters used in this study were built basically with two straight PVC cylinders with diameters of 150 and 100 mm and a plastic bottle with 65 mm diameter for storage of material to be fermented. The digesters were characterized as bench-scale digesters with an average capacity of 1.3 liters of substrate in fermentation in each one. The cylinders with 100 and 150 mm in diameter were inserted into each other, so that the space between the outer wall of the inner cylinder and the inner wall of the outer cylinder could hold a volume of water ("water seal"). The cylinder of 100 mm diameter had one end closed and one opening for the release of biogas and was also submerged onto the water seal to provide anaerobic condition and store the gas produced (Figure 1).

Concentrations of total solids (TS), volatile solids (VS) and the most probable number (MPN) of total and fecal coliforms were determined from the influents and effluents, according to the methodology described by APHA (2005).

The manure used to feed the digesters had the following

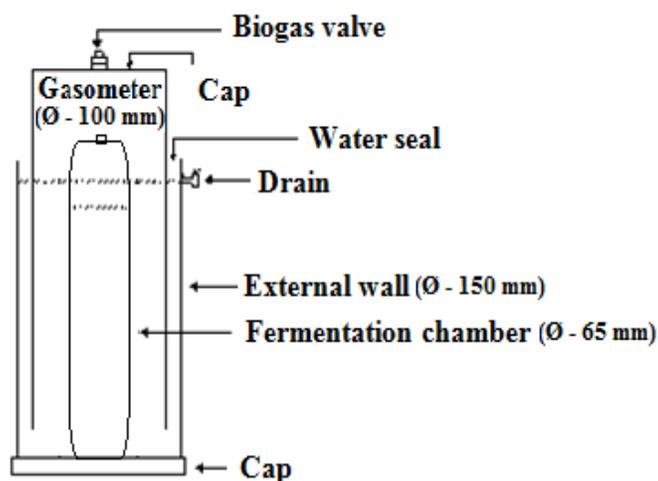


Figure 1: Schematic representation of bench-scale batch digester.

**Table 1.** Levels (%) of TS and quantity (g) of manure, inoculum, water and oil for composition of one liter of effluent, and levels of TS, VS and COD (g O<sub>2</sub>/L effluent) and pH of effluents prepared with pig manure, increasing doses of waster oil and lipase.

Variables	Experimental Treatments (% of oil in TS)														
	8					10					12				
	0.05	0.1	0.15	0.2	0.25	0.05	0.1	0.15	0.2	0.25	0.05	0.1	0.15	0.2	0.25
Levels of TS%															
	<b>Quantity (g) for composition of one milliliter of effluent</b>														
Inoculum	217	217	217	217	217	217	217	217	217	217	217	217	217	217	217
Oil	3.27	3.27	3.27	3.27	3.27	4.08	4.08	4.08	4.08	4.08	4.9	4.9	4.9	4.9	4.9
Manure	131	131	131	131	131	128	128	128	128	128	124	124	124	124	124
Enzyme	0.02	0.04	0.06	0.08	0.1	0.02	0.04	0.06	0.08	0.1	0.02	0.04	0.06	0.08	0.1
Water	649	649	649	649	649	651	651	651	651	651	654	654	654	654	654
	<b>Composition of Experimental Substrates</b>														
TS (%)	4.29	4.55	4.27	4.41	4.18	4.48	4.72	4.28	4.38	4.31	4.23	4.29	4.30	4.10	4.27
VS (%)	3.48	3.43	3.43	3.48	3.42	3.52	3.45	3.35	3.48	3.42	3.32	3.30	3.37	3.27	3.25
pH	7.36	7.41	7.40	7.37	7.35	7.33	7.30	7.32	7.37	7.31	7.27	7.26	7.27	7.31	7.24
COD (g O <sub>2</sub> /L effluent)	94.80	94.76	94.72	94.68	94.65	95.46	95.42	95.38	95.35	95.31	96.12	96.08	96.05	96.01	95.97

composition: 28.9% of TS, of which 78.3% were volatile. The inoculum contained 2.77% TS, with 67.4% volatile. Waste oil presented 98% TS. MPN of total and fecal coliforms were  $15 \times 10^{15}$  per 100 grams of manure, however no coliforms were detected in the inoculum.

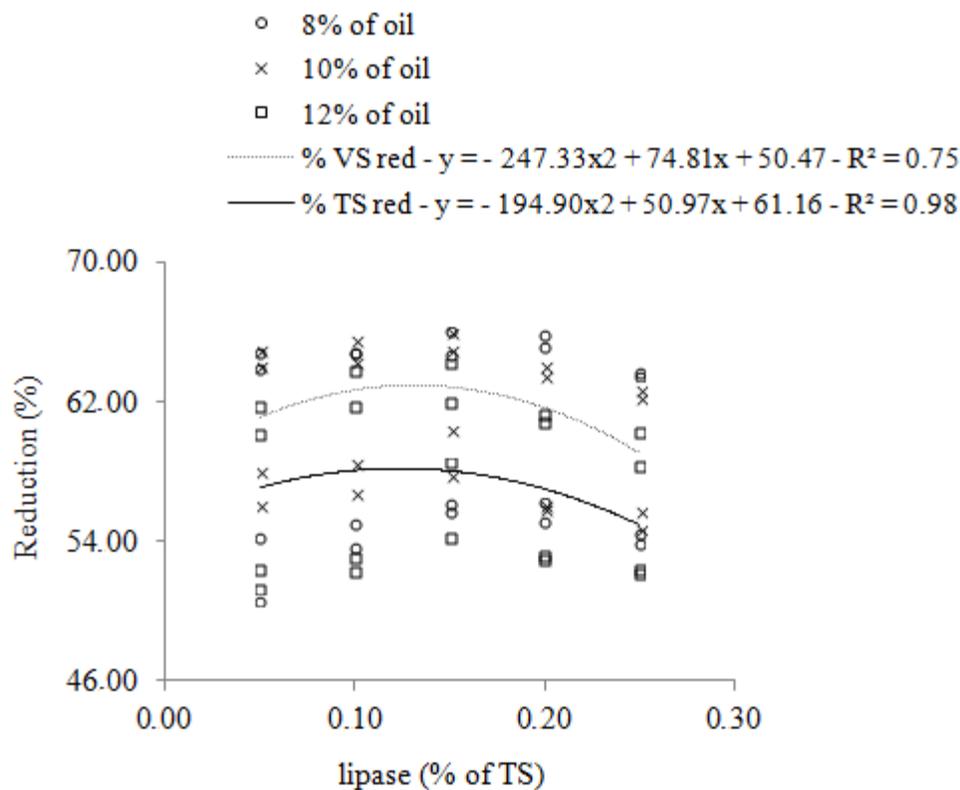
The composition of the substrates as well as the contents of TS present in each component is shown in Table 1 which presents the results of the composition of the substrates subjected to anaerobic co-digestion. These results indicate the similar composition of the substrate in concentration of TS and VS, as well as pH and are used to calculate the reductions obtained during the process and the potential for production of methane.

The volume of biogas produced per day was determined by measuring the vertical displacement of gasometers, and

multiplying by the area of the inner cross section thereof. After each reading the gasometers were zeroed using the record of release of the biogas. The gas volume was corrected according to conditions of 1 atm and 20 ° C. The biogas production was measured and subsequently performed the calculations of potential production, dividing the production values by the amount of TS and VS added and reduced on the digesters.

The composition of biogas was assessed using a gas analyzer, GA-21 Plus, Madur Electronics, equipped with sensors for determining CO, CO<sub>2</sub> and CH<sub>4</sub>. The potentials of methane production were calculated by the production of methane, dividing the production values by the amount of TS and VS added and reduced in the digesters.

The results were analyzed from a completely randomized



**Figure 2:** Reductions of TS (%) and VS (%) in substrates prepared with swine manure, doses of waste oil and lipase.

factorial design 5 x 3 (five inclusion levels of lipase and three inclusion levels of waste oil) with two replications. The results were subjected to analysis of variance considering as sources of variation the levels of oil and levels of lipase. Orthogonal contrasts were used to determine linear, quadratic and cubic effects of oil and lipase levels. These analyses were performed using the statistical computer package R (version 3.1.0 for Windows).

## RESULTS AND DISCUSSION

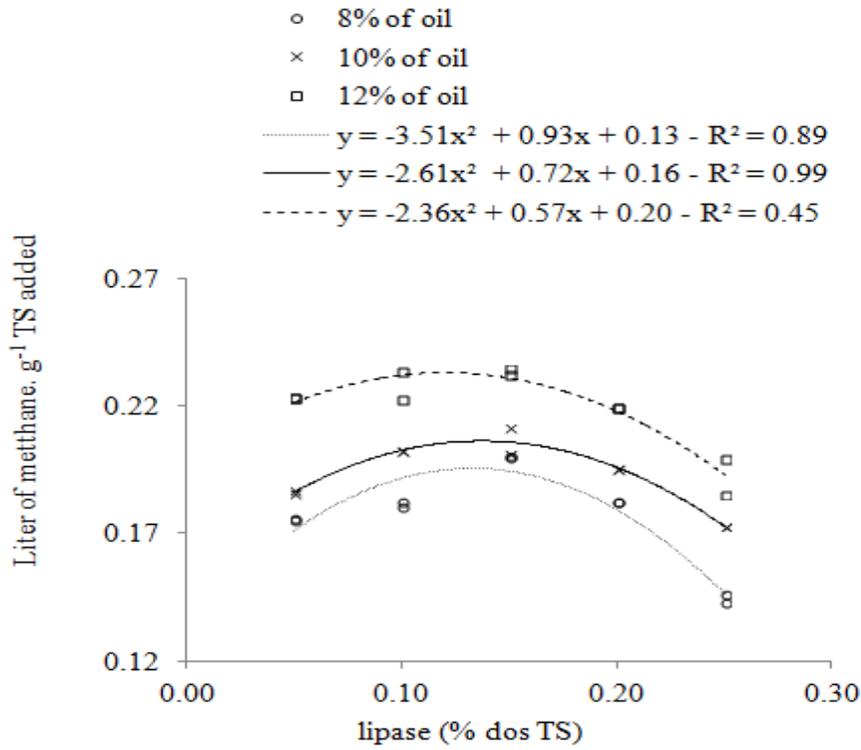
Reductions in MPN of total and fecal coliforms were not influenced by addition of oil and / or lipase in the substrates. The results allowed obtaining biofertilizers with maximum values of  $0.91 \times 10^3$  per mL of material and were superior to those found by Junior Orrico (2010) working with the anaerobic digestion of pig manure with and without separation of the solid fraction. This result may corroborate a safe value for the use of this material, which is recommended for maximum of 1,000 fecal coliforms per 100 mL of effluent (CONAMA, 2005).

As shown graphically in Figure 2, independent of the inclusion levels of waste oil, the optimal dose of lipase inclusion was 0.15% for reductions of TS levels which were

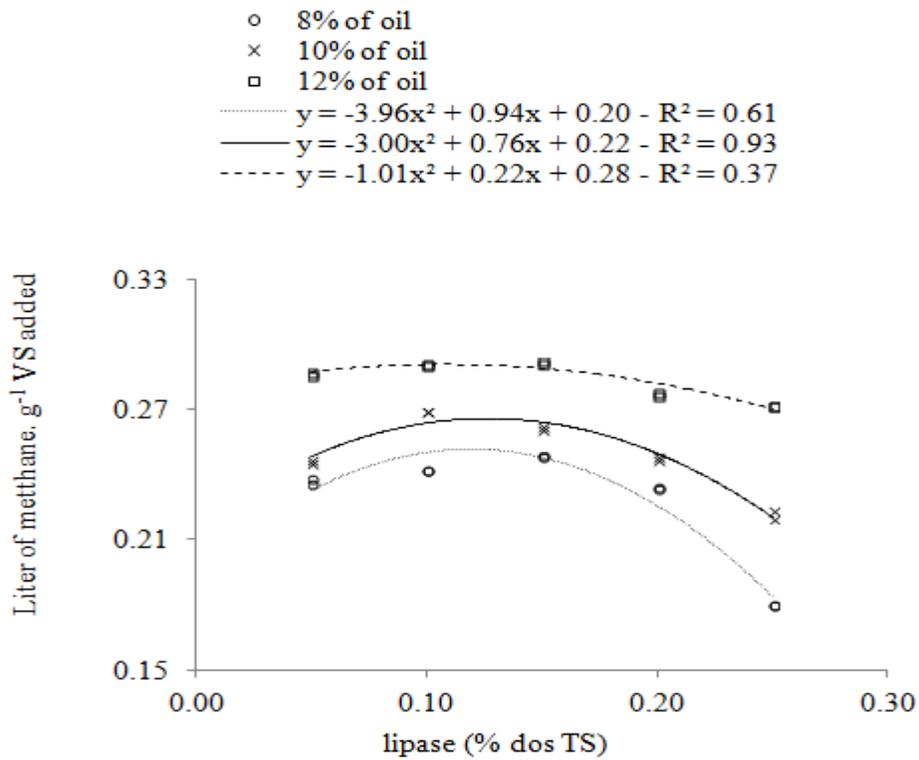
7.41% greater than the reductions in the lowest level of inclusion of lipase (0.05%). For VS reductions, also independent of the inclusion levels of waste oil, the optimal inclusion level (0.13%) had reduction 7.27% greater than the inclusion level of lipase at 0.05%.

The values of reductions in TS and VS (56.13 and 64.49%) at the optimum levels of inclusion of lipase were superior to results carried out with high lipid content, such as in Luste and Luostarinen (2010), who used by-products of the meat processing industry added to manure and observed VS reductions of 38%. In research conducted by Pastor et al. (2013) using the waste oil in co-digestion of waste from landfill, found VS reductions of  $41 \pm 5.5\%$ , which reflects the improvement in the rate of degradation of organic material due to the inclusion of lipase as a lipolytic agent that enhances the process, mainly by the presence of oil in the substrates.

The potential of methane production, which is the gas of greatest interest (Figure 3), was increased per amount of TS added and the inclusions of enzyme that showed the largest increase for the inclusion levels of 8, 10 and 12% waste oil were 0.13; 0.14 and 0.12% lipase, respectively. The most favorable inclusions of enzyme for VS (Figure 4) were 0.12; 0.13 and 0.11% lipase for 8, 10 and 12% waste oil, respectively.



**Figure 3:** Potential of methane production (L methane.g<sup>-1</sup> TS added) from substrates prepared with swine manure, doses of waste oil and lipase.



**Figure 4:** Potential of methane production (L methane.g<sup>-1</sup> VS added) from substrates prepared with swine manure, doses of waste oil and lipase.

**Table 2.** Regression models, followed by CV (%), P (probability) and R<sup>2</sup> for potentials of biogas and methane production per g<sup>-1</sup> of TS and VS reduced, obtained during co-digestion of substrates prepared with swine manure, increasing doses of waste oil and lipase.

Variables	Level of oil	Regression Model	CV	P	R <sup>2</sup>
l of methane per g of TS reduced	8	$y = -9.14x^2 + 2.71x + 0.16$	2.5	< 0.001	0.67
	10	$y = -4.71x^2 + 1.24x + 0.29$		< 0.001	0.90
	12	$y = -10.40x^2 + 2.60x + 0.31$		< 0.001	0.98
l of methane per g of VS reduced	8	$y = -7.53x^2 + 1.94x + 0.27$	1.4	< 0.001	0.91
	10	$y = -5.13x^2 + 1.41x + 0.32$		< 0.001	0.87
	12	$y = -3.96x^2 + 1.12x + 0.41$		< 0.001	0.75

CV: coefficient of variation; P: probability; R<sup>2</sup>: correlation coefficient; TS: total solids; VS: volatile solids

A similar behavior between the two solid constituents was expected since VS is part of the TS, and they reflect even better the quality of digesters, once the integrant part of these constituents is the corresponding fraction degraded by microorganisms, generating biogas production and consequently methane. Thus, the highest production of methane (0.29 L per gram of SV added) might be achieved during co-digestion with addition of 0.11% lipase and 12% waste oil, providing productions of methane 73% higher than with enzyme inclusion of 0.05% with the same level of oil.

A decrease in the potential of methane production depicted in Figures 3 and 4 indicates a possible toxic effect of the long chain fatty acids that occurred with an average inclusion of lipase about 0.15%. According to Pastor et al. (2013), this behavior can be explained by the adsorption of long chain fatty acids in the microbial cell membrane, which will interfere with the mass transfer and consequently affect the methanogenesis.

The analysis of potential of methane production considering the reduction in the amounts of TS and VS (Table 2) presented a similar result to those generated by reductions of these constituents during co-digestion, since the inclusion level of lipase up to 0.15% produced higher values of potential of methane production due to the most suitable conditions generated by the degradation of the lipids in the substrate.

It was observed that the optimal values for the inclusion of lipase were achieved between values of 0.13 and 0.15% for the production of methane per kilogram of ST reduced and 0.14% for methane production per kilogram of SV reduced. These values indicate that the inclusion of lipase enhanced the degradation of the constituents present in the substrate, probably due to increased proportion of lipids, resulting in higher concentration of the nutrients in the medium and possibly favoring the action of microorganisms.

Rodrigues et al. (2014) conducted a study using co-digestion of pig manure, increasing inclusion levels of oil and increasing inclusion levels of lipase with the objective

of evaluating reductions and potential of biogas production and methane and reported that the reduction of the constituents solids, especially the volatile ones, was related to the production of biogas and hence, methane.

Thus, once the co-digestion with lipid residues on the substrates were improved by inclusions levels of lipase, there were higher production of biogas and methane, which means that probably undesirable conditions, as accumulation of long chain fatty acids did not occur in the substrate during digestion process.

## CONCLUSION

The inclusion of lipase at levels up to 0.15% (relative to levels of TS in the substrate) in the composition of substrates in co-digestion with oil at levels up to 12% (relative to levels of TS in the substrate), and swine manure improves the yields of methane and reductions in pollutant constituents.

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