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Biogas and Humic Biofertilizer Production from Biphasic Anaerobic Digestion of Fruit and Vegetable Waste with Lignocellulosic Packing

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ABSTRACT :*A biphasic anaerobic process for fruit and vegetable wastes (FVW) with pig manure as co substrate in the acidogenic step and lignocellulosic packing in the methanogenic step was tested. A process starting up with three days of a thermophilic acidogenic digestion, followed by a twelve days methanogenic mesophilic digestion packed with wood chips, was implemented, and assessed in terms of efficiency in methane generation and content in biogas. Additionally, alkaline extraction of humic compounds from the residual waste sludge was characterized as liquid humus fertilizer. Average results for 15 days of total hydraulic retention time (HRT), showed a production of 270.3 ml of methane per gram of volatile solids (VS) with 63.5 % of them removed, this is 14 % higher than without lignocellulosic packing, while biogas content of 65.9 % of methane, 9 % higher also. On the other hand, the liquid humus obtained qualified as carbonated organic fertilizer. Even though the conversion efficiency of volatile solids achieved was moderate, the combination of the hydraulic retention time (HRT) and the yield in methane generation, permits a reduction in the size of digestion reactors and the possibility to obtain a high quality liquid biofertilizer.*

KEYWORDS:*biogas, humic, biofertilizer, anaerobic digestion, biphasic.*

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I. INTRODUCTION

Fruit and vegetable wastes (VWF) are generated at different stages of harvesting, transportation, storage, marketing, and processing of the agro-industrial activity. In urban markets, where they are usually commercialized, large volumes of this type of substrate are generated, which in some cases is deposited in landfills and other times is used for composting [26]. These wastes, due to their high moisture content, close to 90 % and due to its high degradability, constitute a substrate that is likely to be treated by anaerobic digestion to produce bioenergy and bio-fertilizers. The most limiting fact for anaerobic digestion is its rapid acidification due to the low pH of waste and high production of volatile fatty acids (VFA), which reduces the methanogenic activity in digestion [3]. A possible anaerobic reactor configuration is the two-phase system, consisting of two reactors in series: a hydrolytic-acidogenic stage in the first reactor and a second stage, predominantly methanogenic in the second reactor. The two-phase process allows the development of an optimum for both types of bacterial environment which allows greater stability of the process, an increased production of biogas, a higher content of methane and a smaller volume of reactors [20]. There is some experimental evidence concerning anaerobic treatment of waste of fruit and vegetables which include the work done by Bouallagui et al. (2003) [2], who used waste from fruit and vegetables (FVW), in a tubular digester, varying the hydraulic retention time (HRT) between 12 and 20 days and the concentration of total solids (TS) between 4 and 8 %. For 4 % of TS, they obtained an efficiency of degradation of volatile solids (VS) that ranged between 61.8 and 74.45 %, with a production of biogas which varied between 0.582 and 0.695 l/gVS for HRT between 12 and 20 days, respectively. A maximum degradation efficiency of 75.91 % of VS was achieved when using 6 % of TS, and a maximum production of biogas from 0.707 l/gVS in 20 days of HRT. For 8 % of TS, the maximum degradation efficiency of 64.58 % VS was achieved and a maximum production of biogas of 0.638 l/gVS in 20 days of HRT. Bolzonella et al. (2003) [4] studied the anaerobic digestion of the organic fraction of municipal waste in thermophilic conditions with a rate of organic load (OLR) of 9.2 gVS/l d at a concentration of 20 % of TS and attained a specific production of biogas of 0.23 l/gVS fed. Mata-Alvarez et al. (2003) [16] studied the anaerobic digestion of waste fruit and vegetables, in a completely stirred tank reactor (CSTR) with a 1.6 OLR g VS/l d and HRT of 20 days. In this case, the removal of VS was 88 % and the production of methane was 0.47 l/g VS fed. Callaghan et al. (2002) [5] worked in co-digestion of cattle slurry (CS), chicken manure (CM) and FVW. In a CSTR type reactor at mesophilic temperature of 35°C they studied the effect of adding FVW and CM to a system of digestion of CM with HRT of 21 days and an OLR of 3.19-5.01 kgVS $m^{-3}d^{-1}$. They found that, by increasing the proportion of FVW from 20 to 50% increased the production of methane from 0.23 to 0.45 m^3 of methane per kg of VS added. Nallathambi (2004) [18] studied the performance of methane from a set of 54 different FVW's from fresh fruits and vegetables. The maximum observed methane yield varied between 0.19 and 0.40 l/gVS added, while most of the waste showed a yield greater than 0.3 l/g of VS added. Other experiences with vegetable substrate have been reported by Yu et al. (2002) [27] that used hay in a two-phase anaerobic digestion process, with a primary solid phase, and a second recirculation liquid phase using the grout of the first phase. The results showed a solubilization of 67% of the VS in the substrate with a production of methane of 0.15 m^3 per kg of hay and an average of 71 % of methane content. Also some results of FVW-two-phase anaerobic digestion have been reported, as it is the study by Kirtane et al. (2010) [14] that proved a semi-continuous process for digestion of mango puree, in a pilot plant using three acidogenic reactors operated aerobically at room temperature with a substrate of 8-12 % of TS. Hydrolysis was conducted in a methanogenic anaerobic reactor at mesophilic temperature of 35° C with 30 % of the substrate recirculation to the acidogenic phase reactor. The observed yield was 90% of VS conversion to biogas containing 78 % of methane once stabilized the process within 180 days. On the other hand, Shen et al. (2013)[23] worked on co-digestion of VWF and waste of food (FW), comparing the single stage with the two-stage process. The results showed that for OLR less than 2 gVS $L^{-1}d^{-1}$ the single phase process was 4.1 % better in terms of production of methane, whereas for OLR greater than 2 gVS $L^{-1}d^{-1}$, the two-phase process yielded a greater production of methane from 0.351-0.455 L g $VS^{-1}d^{-1}$, being between 7.0 and 15.8% higher than the result obtained in a phase, with a much more stable operation than in the single phase case. De La Rubia et al. (2009)[9] studied the influence of the HRT and OLR on the acidogenic stage of a biphasic digestion process for a cake of sunflower oil in a CSTR type of reactor at mesophilic temperature of 35 ° C. The process was tested for OLR between 4 and 9 g $VSL^{-1}d^{-1}$ and HRT between 8 and 15 days. It was concluded that at the ranges studied the solubility of organic matter yield varied between 20.5 and 30.1 % and the degree of acidification of the substrate was primarily influenced by the OLR only and not by the HRT.

Even though enough research has been reported with respect to the anaerobic digestion of FVW, scarce or nil research evidence is available concerning the extraction of carbonated or humic organic compounds of the solid waste originated from that digestion process. Carbonated or humic acid compounds could be defined as stabilized organic matter and can be fractioned into humic acid, fulvic acid and humin [8]. Humic acid is soluble in an alkaline solution, but precipitates when the extract is acidified. It has a dark brown color, a high molecular weight (5,000–300,000 Da), is highly polymerized, in the soil is closely linked to clays, it is resistant to degradation and its carbon content varies from 50–62 % [22]. Fulvic acid is the humic fraction that remains in solution when this is acidified; therefore it is soluble in acids and bases. It has a gray-yellowish color, lower molecular weight (900–5,000 Da), and a carbon content of 43–52 % [10]. Finally, humins constitute the non-soluble fraction, and therefore non extractable humic substances [13], [21], [24]. This fraction is the most polymerized and has the highest molecular weight. The functional carboxylic and hydroxyl groups present in humic material could play a major role in its activity. Thus, low molecular weight fractions would have a higher metal binding capability, which could account for their ability in improving nutrient assimilation [29]. Moreover, humus has numerous physical and chemical characteristics that cause positive effects on soil and plants. Some of these effects are: to improve soil structure [11], [25] and soil water retention capacity [1], facilitate nutrient absorption by plants [7], [15] and stimulate plant development [12]. Traditionally, the organic matter has been applied to the soil raw, in solid or suspension form, or composted. More recently, “liquid humus” has been available as a mixture of humic and fulvic acids, in a liquid form, that can be applied through the irrigation system [28]. They would have the same benefits as traditionally applied humus; however, applied rates are much smaller. There are clear specifications for these products, particularly in terms of their composition, like the ones set by the European Community; they specify that liquid humus products must have at least a 15 % of total humic extract and maximum levels of heavy metals such as cadmium (Cd), copper (Cu), lead (Pb),zinc (Zn), mercury (Hg), nickel (Ni), and chromium (Cr). Most “liquid humus” products commercialized in the world are made from leonardite, which is an oxidized form of carbon of lignite origin, formed for thousands of years, but eventually, liquid humus can be made from any organic material, including soil, compost, and plant residues [19].

II. METHODOLOGY

Anaerobic Digestion

The methodology used in this research is in accordance with the well-known empirical evidence, which indicates that the optimal pH for the operation of acidogenic bacteria is close to 6, while for methanogenic bacteria is between 7 and 8, and additionally in accordance with the fact that the recommended range of a substrate carbon to nitrogen ratio C/N is between 20 and 30, which allows to minimize the risk of inhibiting the digestion conditions. On the other hand, accepting that the kinetics of the methanogenic stage is slower than the kinetics of the acidogenic stage [6], the method proposed in this approach, incorporate lignocellulosic wood chips as a supporting packing, to amplify and sustain the proliferation of methanogenic bacterial mass in the second stage of the process where the main product is methane. To run the anaerobic digestion tests, a combined sample of substrate of fruit and vegetable waste (FVW) with pig manure as co-substrate (PM) was prepared, in proportion of mass needed to generate a composite substrate with C/N ratio of 25, reference value that was used for all experiments. After completing the acidogenic stage, rumen of sheep (SR) was injected as inoculums along with the addition of the lignocellulosic co-substrate. The compositions of each of the organic substrates are shown in Table 1. The trialswere conductedin three cylindrical 6.5 L reactors made of acrylic material implemented with a mechanical blade stirring system rotating at 10 rpm, and instrumentation for measuring pH and temperature. The mass percentage of each substrate initially loaded into reactors was determined according to a calculated mass balance for C/N = 25, whose data is displayed in Table 2. The experimental methodology utilized 4.16 L of substrate of fruit and vegetable waste previously crushed by means of a food waste disposer brand Terminator, model Commander SLC-1500, added to each of the three reactors, followed by adjustment of the pHto a value of 6.0 using a concentrated solution of NaOH 10 N. Subsequently 0.84 L of pig manure was incorporated to the reactors and the temperature control set point adjusted to 50° C, to initiate the stage of hydrolysis of volatile solids to generate volatile fatty acids and finally acetate and propionate compounds. The acidogenic process then was held at pH between 5.5 and 6.0 and maintaining pH and temperature control for 72 hours. After 72 hours of primary processing, a mass of 10% of pretreated wood chips was added to each reactor, the pH was adjusted to a value within the range of 7.0-7.5 using NaOH 10 N, and sheep rumen inoculums was added in mass proportion of 5 %, at process temperature setting of 35 °C. The wood chips were pretreated at 120 °C for 30 minutes, to ensure the absence of additional bacterial input to the process. The second anaerobic stage was maintained in conditions of temperature and pH control for a period of twelve days, as shown in the experimental diagram of Figure 1.

Table 1. Composition of substrates.

Component	Fruit & Vegetable Waste (FVW)	Pig Manure (PM)	Sheep Rumen (SR)
Water content (%)	94.01	94.29	90,49
TotalSolids (TS) (%)	5.99	5.71	9,51
Volatile Solids (SV) (%)	36.83	79.70	80,45
C/N Ratio	5340.0	8.0	18.0

Table 2. Mass balance of substrates.

Component	Total Solids (g/L)	Volatile Solids (g/L)	Total Carbon (g/L)	Total Nitrogen (g/L)	C/N Ratio	Volume (%)	Volume (L)
FVW	59.9	22.06	12.79	0.0024	5340	83.18	4.16
PM	57.1	45.51	29.6	3.7	8.0	16.82	0.84
Mixture	59.41	26.01	15.617	0.624	25.0	100.00	5.0

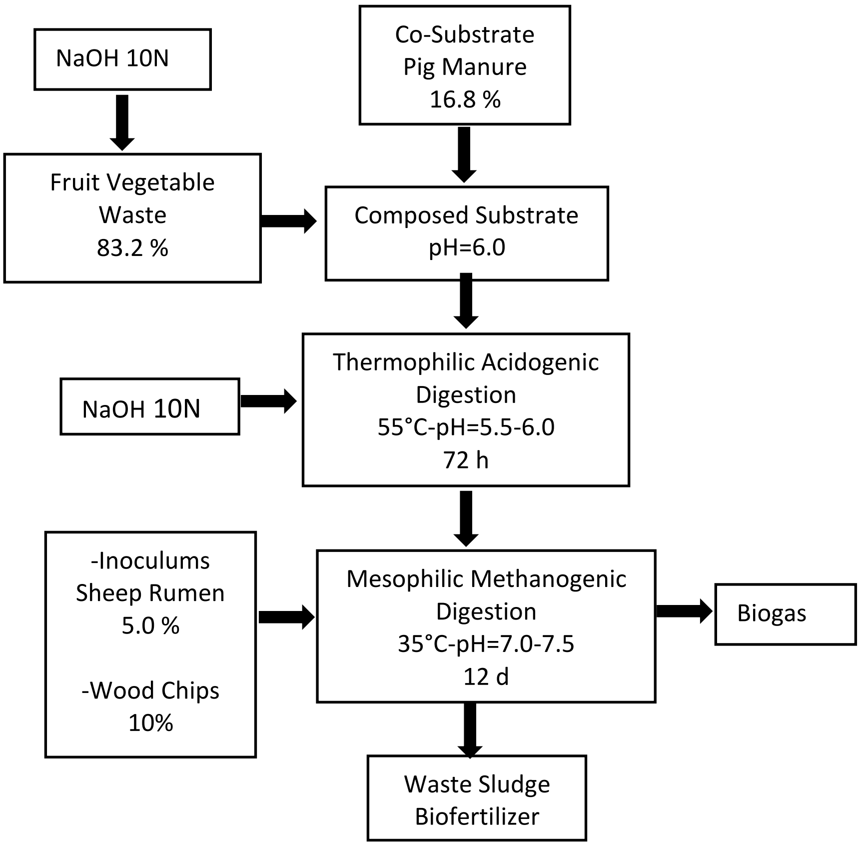


Fig. 1. Flowsheet of laboratory tests.

During the methanogenic stage, the volume of the biogas generated, was measured through an automated system consisting of a 1.0 L volume flexible and transparent lung of polyvinyl chloride, attached to a cylinder of suction. This system allowed to measure the biogas volume generated and the composition of the gas in % of methane, by means of a methane sensor system brand Wuhan Cubic, model Gasboard - 3200L, in connection with a suction cylinder. Twelve experiences were carried out under the protocol described above, but only six outcomes were considered in the analysis since in half of them there were operational failures that affected the control of process variables, especially the loss of calibration of pH sensors. To assess the actual influence of the lignocellulosic material in the methanogenic step, additional experimental tests were executed following the same methodology but without adding the packing material in the methanogenic phase. Finally, a comparison of biogas generation results, with respect to other similar anaerobic digestion tests using the same co-substrate profile as in the present research, was explored. In that sense, according to an experimental design of biogas production with the same substrate mixture (Molinuevo, 2010), a reliable model of the digestion of fruit and vegetable waste with co-substrate of pig manure, to assess the upper limit of volatile solids conversion to methane without HRT limitation can be expressed as:

$$C_{VS} = 71.9 + 6.7 \times VS + 6.7 \times FV - 5.7 \times VS^2 - 0.7 \times FV^2 + 4.7 \times VS \times FV \quad (1)$$

where C_{VS} is the mass percentage of conversion from volatile solids to methane, VS is the concentration of volatile solids in the substrate mixture expressed as (gVS/L) and FV is the percentage in volume of fruit and vegetable waste in the substrate mixture. Likewise, the following expression is proposed to assess the maximum yield of methane production Y_M in milliliters of methane per gram of initial volatile solids ($mLCH_4/gVS$):

$$Y_M = 286.2 + 49.9 \times VS + 59.3 \times FV - 55.3 \times VS^2 - 21.4 \times FV^2 + 35.6 \times VS \times FV \qquad (2)$$

Carbonated Biofertilizer

In order to assess the feasibility to generate a liquid humic biofertilizer, using the waste sludge discarded from the anaerobic digestion, a set of three samples of dried sludge coming from the three methanogenic laboratory reactors, were treated using a solution of KOH with different molar concentrations of 0.1, 0.3, 0.5 and 1 M, maintaining the ratio between material and extractant of 1: 10. The alkaline extraction was performed at ambient temperature and constant stirring conditions of 120 rpm for 12 hours. After extraction, the final solution was analyzed to measure its pH, EC, and to determine the percentage of carbon in total humic extract (THE), carbon percentage in humic acids (HA) and fulvic acids (FA) of the liquid biofertilizer produced.

III. RESULTS AND DISCUSsION

Twelve trials were carried out using three parallel experimental reactors whose characteristics were individualized before, according to the already described methodology, but only six of these experimental trials showed a consistent behavior, with respect to methane generation. The rest of the experiments delivered results of low methane productivity, due to a series of troublesome operational factors such as failure in the pH control system, excessive foam formation in the acidogenic stage, discontinuous digestate stirring, poor inoculums quality and unexpected breakdowns. The results of cumulative productivity of methane are shown in Figure 2, which considers the two stages of the process where the methanogenic stage starts on day four. As shown in Figure 2, methane production begins during the first day of the methanogenic stage (day four), and methane production rate grows until the sixth day, at that point a decremented rate tendency is observed, probably in coincidence with the beginning of the consumption of chemical compounds convertible to biogas, generated in the first stage. It is interesting to notice that the biogas production always begins within twenty-four hours after starting the methanogenic stage, which could be attributed to the presence of wood chips as a microbiological supporting media. Daily production speed of biogas throughout the process can be best appreciated in the plot of Figure 3, which shows that methane production speed is maximum, on the third day of the methanogenic stage, probably in coincidence with the beginning of the exhaustion of the compounds produced in the previous acetogenic stage, as is has been mentioned before. In essence, the biogas generation occurs between the first and tenth day of methanogenesis, suggesting an exhausting of acetate and intermediate compounds generated in the acidogenic step that should be digested by methanogenic bacteria. Conversion parameters of the anaerobic digestion process in terms of overall energetic efficiency in removing of volatile solids, volume of methane produced per unit of volatile solids and content of methane in the biogas generated are showed in Table 3. As this table indicates, average soluble solids removed were around 63.5 % and the biogas produced contained an average value close to 66 % of methane. Also, this Table includes a comparison of the actual conversion efficiency, with the maximum experimental conversion values, according to empirical equations (1) and (2), in the last column. When comparing the conversion values of the last two columns in Table 4 in terms of % of conversion of volatile solids to biogas and biogas productivity expressed as ml CH_4/g VS, it can be seen that the actual results are approximately 25 % behind the maximum attainable figures with the specific mixture of FVW and PM used in this research. However, these rates seem to be satisfactory if we consider that the whole process was designed for a total hydraulic residence time of only 15 days, which is a clearly shorter time compared to a

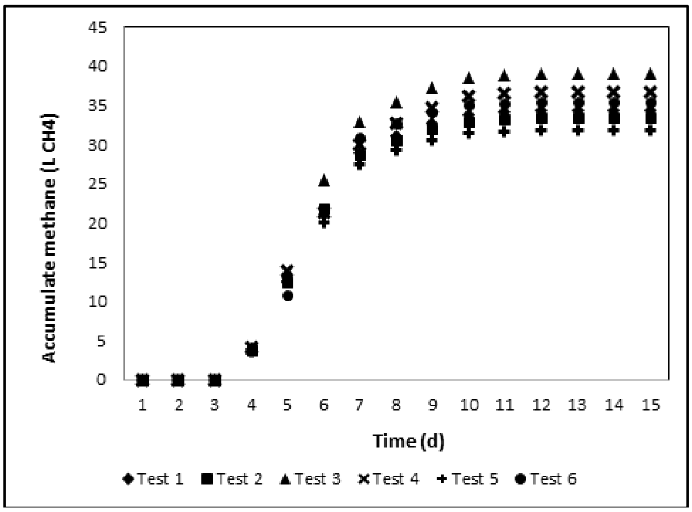


Fig. 2. Cumulative methane

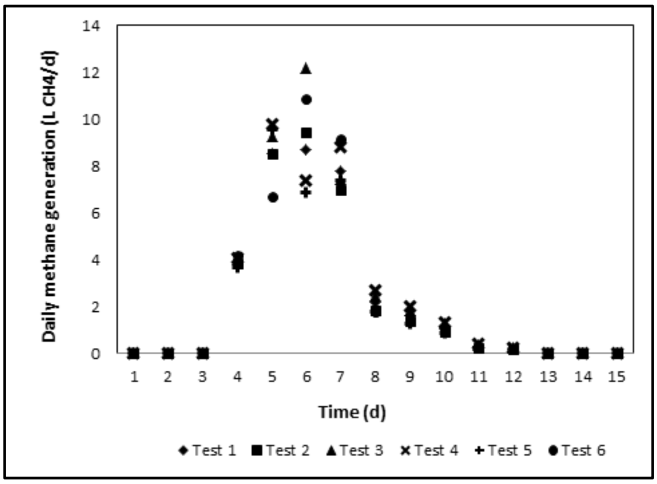


Fig. 3. Daily methane production

traditional anaerobic digestion of single stage, particularly the modeling of equations (1) and (2) which were developed for HRT greater than 30 days. This results however seem to suggest that it could be feasible to improve the percentage of volatile solids removal by increasing efficiency in the hydrolysis and acetogenic compounds generation in the first stage, since a rapid depletion of them in the methanogenic stage is observed. The comparison of cumulative methane generation with and without adding lignocellulosic packing in the methanogenic stage is depicted in Figure 4.

Table 3. Comparison of conversion figures with and without packing.

Test N°	ml CH_4/g VS	% VS Removed	% CH_4
With packing	270.2	63.4	65.9
Without packing	230.6	54.1	59.5
Increment (%)	14.7	14.7	9.7

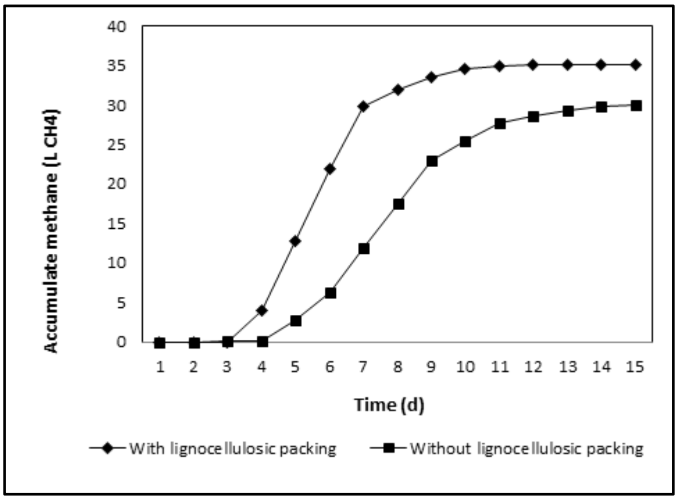


Fig. 4. Comparison of cumulative methane production with and without lignocellulosic packing.

The upper curve corresponds to the average of the six tests with wood chips packing, detailed previously in Figure 2. The lower curve is the average of three tests of methanogenic digestion without lignocellulosic packing. Is evident that the profile of methane generation is different insofar the upper curve indicates that methane production starts one day earlier and with a greater slope, but it reaches its maximum accumulate production approximately at day tenth of the methanogenic step, earlier than the process without packing, which in the twelfth day of the methanogenic phase is still generating some biogas even though at very small rate. The difference in the methanogenic generation kinetics between both cases can be better observed from Figure 5 that shows the daily methane rate generation. The improved process with lignocellulosic packing reaches its highest rate at the second day of the methanogenic phase, approximately two days before than in the process without packing. At the same time its maximum methane production rate is about a 50 % greater than the maximum corresponding to the treatment without packing. In global terms, the process that utilized lignocellulosic packing permits enhancing biogas production and better conversion indexes as indicated in Table 3. The percentage of VS removed and the volumetric methane production per unit of mass of VS is about 15 % higher while the average percentage of methane in the biogas is increased nearly to 10 %.

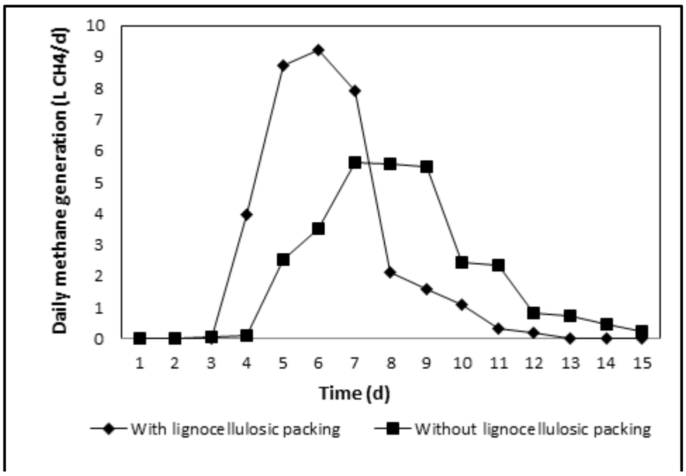


Fig. 5. Comparison of daily methane production with and without lignocellulosic packing

The characterization of the dried sludge samples in terms of carbon and organic matter content, pH, and electric conductivity (EC) is shown in Table 4. The results of the alkaline extraction of humic compounds, from the digested waste sludge, at different concentrations of KOH, can be seen in Fig. 6 and Fig. 7. Figure 6 indicates that the highest rate of carbon extraction occurs for a KOH concentration of 1.0 M , which allows to generate a solution of nearly 7% of total carbon in the humic extract, however Fig. 7 shows that when KOH 1 M is used, the EC of the liquid product is too high. The best combination of carbon extraction and EC can be obtained when using KOH 0.5 M, while the pH is maintained approximately unchanged around a value of 12. Under these extraction conditions, the total equivalent carbon extracted from the sludge was near 20 %.

Table 4. Characterization of sludge samples.

Sample	C Total	C Soluble	Organic Matter	pH	EC
	%	mg/L	%		dS/m
1	48.1	2.48	69	10.7	22.1
2	51.2	1.67	72	10.4	14.0
3	47.2	1.76	69	10.3	19.5
Average	48.8	2.0	70	10.5	18.5

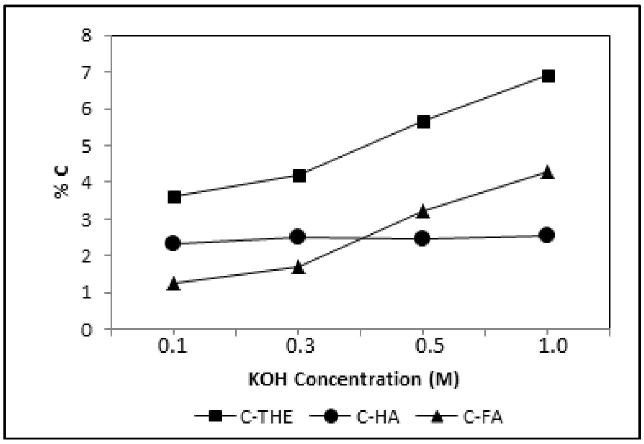


Fig. 6. Organic carbon distribution in humic extract. C-THE: Total Humic Extract, C-HA: HumicAcids, C-FA: Fulvic Acids.

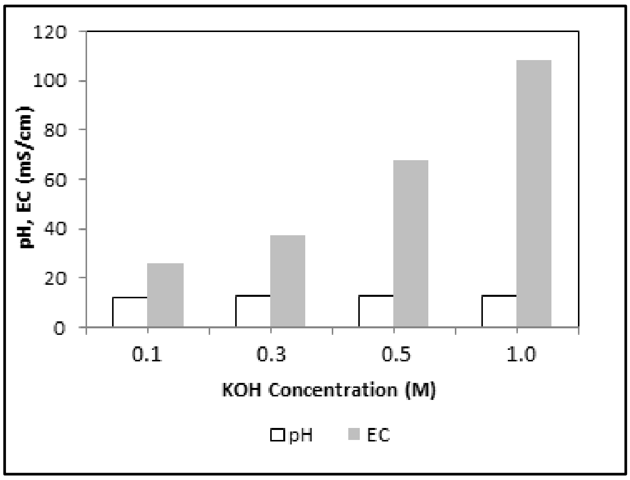
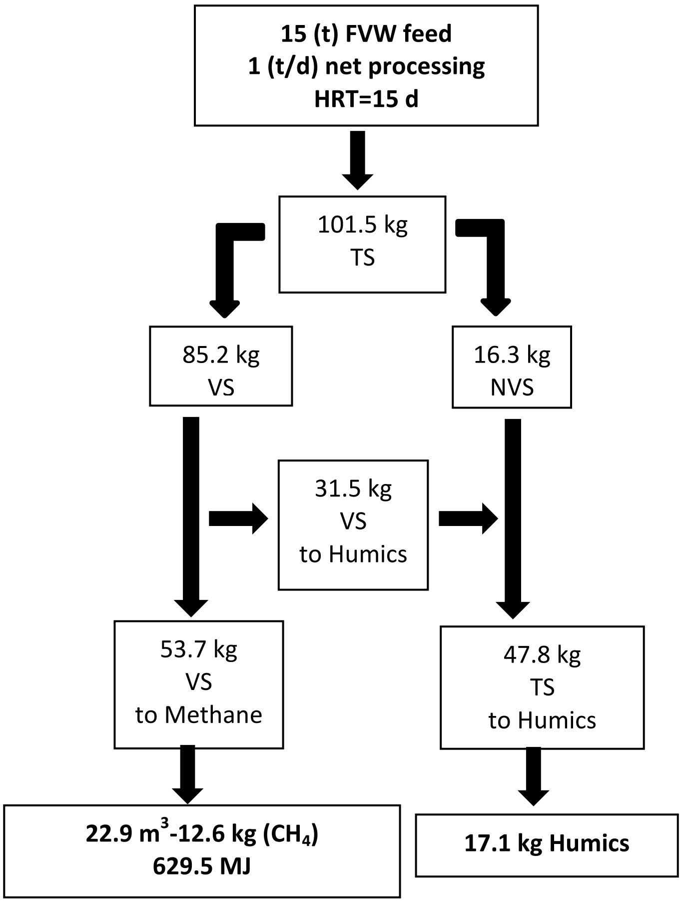


Fig. 7. Electrical conductivity and pH of humic extract

A mass and energy balance of the whole process to produce biogas and a liquid humic biofertilizer is depicted in Fig. 8. For a 1 ton of daily processing or 15 t/d of FVW fed to the process, with 15 days of HRT of the FVW substrate, in order to supply the production parameters, that has to be considered to carry out an economic evaluation, for a commercial escalation of the technology.



IV. CONCLUSION

The main innovations proposed in this research, were the incorporation of lignocellulosic packing into the methanogenic step to enhance the bacterial proliferation and the efficiency in methane production, and the post treatment of the waste sludge to generate a valuable carbonated biofertilizer. The results are promising in terms of the speed of methane production, but improvements need to be achieved in the step of hydrolysis and acetogenesis. The co-substrate of pig manure acts as bacterial inoculums in the acidogenic stage and allows to balance the substrate to a properly C/N relationship. The reactor packing of wood chips as bacterial supporting medium, allowed to reinforce the methanogenic activity which was confirmed when methane generation in the methanogenic stage was compared with the methanogenic activity in absence of lignocellulosic packing. The moderate efficiency in the conversion of volatile solid have a counterpart in the amount of humic compounds remaining in the waste sludge that allows recovering most of organic matter as a carbonated biofertilizer or liquid humus, of broad application in agricultural irrigation systems. However, the moderate HRT of the substrate should imply a certain degree of size reduction in anaerobic digestion reactors. The combined biogas and liquid biofertilizer process deployed, has to be economically evaluated, to determine its feasibility for commercial escalation.

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