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EXPERIMENTAL INVESTIGATION OF BIOGAS PRODUCTION FROM CITRUS WASTE: CO-DIGESTION WITH CATTLE MANURE

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ABSTRACT

Biogas production through anaerobic co-digestion of a mixture of cattle manure and citrus waste using an experimental facility for testing the biochemical methane potential (BMP) was investigated. No buffer solution is added to the mixture in order to use the buffer capacity from cattle manure. Regular measurements of pH, alkalinity, chemical oxygen demand, methane and biogas net production were performed. Three substrate inoculum ratios (SIR) 1:1, 2:1 and 3:1 (g COD/g VSS) were evaluated. Maximum COD removals of 56.4%, 51.3% and 48.0% for the SIRs 1:1, 2:1 and 3:1 were obtained. For all SIR, pH was on the range of 6.5 to 7.5, while the maximum VFA concentration was 4250 mg CH_3COOH l^{-1}. Alkalinity ranged between 2250 to 4500 mg $CaCO_3$ l^{-1}. Both maximum methane production rate (MMPR) and percentage of anaerobic biodegradability were established. BMP of 94.3 to 146.6 $ml_{STP-CH4}$/gVSS were calculated for the ratios 1:1 and 3:1, respectively. The highest feasibility for biogas production and methane was established for SIR 3:1.

Keywords: Anaerobic digestion, biodegradability, cattle manure, citrus waste, biogas, methane

1. Introduction

Energy production from renewable agro-industrial waste represents an important role in the global energy context. Its use as energy source benefits the sector´s productivity and the environmental impact caused by improper disposal is eliminated. Anaerobic co-digestion involves the simultaneous digestion of two substrates from different origins, allowing an efficient process of degradation due to a synergistic action between the available nutrient content of both organic materials that strengthens the development of the microbial populations responsible for the process (Norsberg and Edstrom, 2005; Callaghan *et al.*, 1999; Neves *et al.*, 2004; Gunaseelan, 2007). The anaerobic co-digestion of cattle manure and biodegradable wastes has presented excellent results in both mesophilic and thermophilic temperature range (Alvarez and Lidén, 2009; Brinkman, 1999). That is often attributed to an increase in the concentration of organic solids in the reactor or the satisfaction of specific nutrient requirements necessary to the effective development of microbial populations (Callaghan *et al.*, 1999; Jae and Soon, 1995).

Orange juice world production in 2014 amounted to 1.84 Million ton. Brazil is the main orange juice producer with 55% of the total production followed by USA (26%), México (7%) and the European Union (5.5%) (USDA, 2015). During manufacture of juices and nectars derivatives from citrus fruits, especially orange, a large volume of waste composed of shells, seeds, fibres and membranes is generated. This waste causes many environmental problems due to its high water and organic material content, so it cannot be easily dried or disposed (Tripodo *et al.*, 2004). From the total volume of citrus waste generated, a small fraction is used in the production of concentrated feed for animals, due to the high amount of carbohydrates and protein; another portion is disposed for the extraction of food preservatives such as pectin (Waheed *et al.*, 2008; Moser *et al.*, 1991; Nguyen, 2012). In recent decades the most economically viable process for treatment is its incineration after drying (Mamma *et al.*, 2008). The high water content of this residue makes the drying process expensive, thus a large volume of wastewater, together with particulate material, which requires further treatment is generated (Aslanzadeh and Özmen, 2009; Srilatha *et al.*, 1995).

Anaerobic digestion is emerging as an alternative post-processing of citrus waste, as it enables an adequate reduction of the organic load of the waste with a simultaneous generation of biogas and fertilizers. Anaerobic digestion of citrus waste has been analysed by several authors (Mizuki *et al.*, 1990; Lane, 1984; Tekippe, 1972; Martín *et al.*, 2013; Martín *et al.*, 2010a; Doughterty *et al.*, 1956). In these investigations, an inhibition of the digestion process has been observed, thus the addition of buffer solutions in order to control pH and alkalinity during long periods of operation was necessary. Inhibition is developed mainly by a cyclic terpene called Limonene that is present in the exocarp of citrus fruits. The reduction of the inhibitory effect of Limonene during anaerobic digestion can be accomplished by addition of buffer solutions as well as through thermal and mechanic pre-treatments, such as steam extraction and grinding. Martín *et al.*, (2010a) concluded that anaerobic digestion using this waste required an inoculum previously adapted to the substrate and the addition of a $CaCO_3$ solution that neutralize VFA generated during the initial stages of the process establishing a pH value near neutrality. Kaparaju *et al.*, (2006) investigated the behaviour of the anaerobic digestion process of citrus waste at laboratory scale in batch and semi-continuous reactors. These researchers conclude that anaerobic co-digestion of citrus waste and other biodegradable wastes as cattle manure overcomes inhibitory process conditions such as acidification of the substrate evident during the anaerobic digestion of citrus waste only.

The objective of the present research is to evaluate the production and composition of biogas generated during anaerobic co-digestion of the homogeneous mixture of citrus waste and cattle manure. As the alkaline systems present in cattle manure can act as buffer solutions for the systems, the process is studied without the addition of any buffer solution to control pH or alkalinity. The evaluation is based on the determination of the biochemical methane potential (BMP) and the periodically measure of pH, VFA and Alkalinity using an experimental facility implemented according to standardized methodologies.

2. Experimental methods

2.1. Substrate and the inoculum

The substrate used in this study is a homogeneous mixture of cattle manure and citrus waste; the latter consisted of a mixture of orange peels, hulls, inner fibres and seeds obtained in the extraction of orange juice (*Citrus Sinensis-Valencia variety*). Equal mass fractions for each residue were used. Citrus waste was selected and subjected to a mechanical pre-treatment which consisted in cutting and grounding it to a particle size of approximately 0.5 mm; the citrus waste was then left outdoors for several hours. This pre-treatment was performed to allow evaporation of the greatest part of the essential oils, mainly composed of limonene, contained in the outer shell of the orange (*exocarp*). Citrus waste samples were then mixed with fresh manure obtained from a farm in a town near Bogotá and diluted with tap water. As inoculum sludge from the wastewater treatment plant “El Salitre” in Bogotá was used. Substrate and inoculum were stored at 4 °C for 2 days before the beginning of the BMP tests.

2.2. BMP tests

The experimental procedure used for the determination of BMP was accomplished based on the techniques proposed by several authors (Owen *et al.*, 1978; Owens and Chynoweth, 1993; Battersby and Wilson, 1988; Shelton and Tiedje, 1984) as well as on ISO 11734:1995 and ASTM E2170-01:2008. The experimental facility comprises a set of batch reactors in which different mixtures of substrate and inoculum together with mineral medium type Balch (Balch *et al.*, 1979) are incubated. Balch solution is required to allow an efficient development of the bacterial populations. Additionally, reactors containing a mixture of inoculum and Balch solutions were mounted in order to compare the measurements performed on these reactors, against the reactors with the mixture substrate/inoculum. Hence, an assessment of the net values of the parameters tested is possible. Incubation was carried out at a mesophilic temperature of 30 °C +/- 2 °C for a period of 40 days. Sealed bottles with a total volume of 600 ml, from which 20 % corresponds to the gas phase, were used as reactors. Methane and total biogas volume generated were measured through a volumetric displacement technique using bottles containing a strong base aqueous solution of NaOH 15 % (m/v) and distilling water, respectively. Each bottle was connected to the anaerobic reactor and placed upside down hanging from the same structure which supports the thermostatic equipment in which the reactors are placed. This configuration originates a slight vacuum in the bottle used for gas measurement so that the volume of soda and distilled water that is getting out equals the gas volume from the anaerobic reactor at room temperature and pressure conditions.

Experimental design was implemented through the use of three substrate inoculum ratios (gCOD/gVSS) corresponding to the values 1:1, 2:1 and 3:1. Table 1 shows the content and number of reactors used in the experimental setup together with the parameters measured from each one. Four reactors containing ratios 1:1 and 3:1, two reactors containing ratio 2:1 and one reactor containing inoculum/mineral solutions were installed. Additionally, three reactors containing all ratios (one for each ratio) were used in order to measure the total biogas production. A total of 14 reactors were used. Their number was chosen according to the available space in the thermostatic equipment.

Table 1. Content and number of reactors used in the experimental setup

Content	Substrate/Inoculum + Balch solution			Inoculum/ Balch solution	Substrate/Inoculum + Balch solution		
gCOD/gVSS	1:1	2:1	3:1	---	1:1	2:1	3:1
Parameter measured	Methane production, process control parameters			Reference	Total biogas production		
Number of Reactors	4	2	4	1	1	1	1

Experimental design included daily methane and total biogas volume determination and five periodic measurements of the control process parameters (pH, alkalinity and volatile fatty acids) for all SIR (every ten days). Process parameters for SIR 1:1 and 3:1 were measured on samples taken at the time of installation and samples from one of the four installed reactors that was periodically discarded. As for SIR 2:1 only two reactors were installed, the measurements performed after 10 and 30 days were done on samples obtained from the extraction of a small sample of substrate from the reactors using an especial syringe The reported methane net production volume corresponds to the mean value of the measurements performed on the reactors that were available at the moment of the determination.

2.3. Analytical methods

Table 2 summarizes the parameters and the methods used for the characterization of the samples obtained during the anaerobic biodegradability tests. Measurements were done in triplicate. The reported results include the mean calculated value together with its standard deviation.

Table 2. Methods used in the determination of physicochemical parameters

Parameter	Method
Total Solids (TS)	APHA/SM 2540-B
Total Volatile Solids (VTS)	APHA/SM 2540-B
Total Suspended Solids (TSS)	APHA/SM 2540-D
Volatile Suspended Solids (VSS)	APHA/SM 2540-D
Alkalinity	APHA/SM 2330-B
$COD_{total}/COD_{soluble}$	APHA/SM 5220-C
Volatile fatty acids	Method proposed by Montgomery *et al.,* (1962)
pH	APHA/SM 4500 H^+-B
Methane Volume	NaOH 5% (Collazos and Díaz, 2010)
Biogas Volume	Distilled Water (Collazos and Díaz, 2010)

3. Results and discussion

3.1. Characterization of the substrate and the inoculum

Table 3 presents the results of the characterization of the inoculum and the homogeneous mixture of cattle manure and citrus waste used in this research.

Table 3. Physicochemical parameters of the inoculum obtained from WWTP "El Salitre" and the homogeneous biodegradable organic mixture

Parameter	Inoculum	Organic mixture
Total Solids (TS)(mg l^{-1})	37 640±308	84 865±693
Total Fixed Solids (TFS) (mg l^{-1})	14 900±121	18 475±151
Total Volatile Solids (VTS) (mg l^{-1})	22 740±183	66 390±542
Total Suspended Solids (TSS) (mg l^{-1})	32 550±266	20 300±165
Fixed Suspended Solids (FSS)(mg l^{-1})	14 450±118	5200±43
Volatile Suspended Solids (VSS) (mg l^{-1})	18 100±147	15 100±124
Alkalinity (mg $CaCO_3$ l^{-1})	1290±10	28 400±232
pH (pH Value)	6.30±0.10	6.13±0.39

3.2. pH, alkalinity and VFA variation during anaerobic co-digestion

Fig. 1(a) shows pH values of the substrate in reactors measured during the process for each SIR. The values obtained show that pH remained in the range between 6.25 and 7.5, having a minimum value half way through the experiment. This range is reported as optimal for the efficient development of the anaerobic digestion process (pH between 6.5 y 7.5) (Khanal, 2008; Madigan *et al.*, 1999).

Fig. 1(b) shows the evolution of the concentration of volatile fatty acids calculated as the concentration of acetic acid in the substrate. Maximal standard deviation amounted to ± 1 mg CH_3COOH l^{-1}. In this figure, a slight increase during the first ten days of the tests for all SIR, followed by a continuous decrease can be observed. These results evidence the efficient development of the anaerobic process on the initial stages of hydrolysis and fermentation. SIR 3:1 and 1:1 showed higher and lower fatty acid concentrations, respectively. SIR 3:1 shows a rapid decrease of the acid concentration after it reaches a maximum; this behaviour can be attributed to a high availability of these fatty acids for acetogenic and metanogenic bacteria that use them directly as a substrate. SIR 1:1 has the lowest rate of decrease in the concentration of organic acids after reaching the maximum, which is associated to the lack of the required nutrient for the optimal development of the microbial biomass, responsible for consumption of these acids. The initial decrease of pH is consistent with the gradual increase of volatile fatty acids during this period. The obtained results are consistent with the fact that pH and volatile fatty acids are indicators of the presence of easily biodegradable constituents in the substrate, which are readily used by fermentative microorganisms present in the anaerobic substrate (Cun-Fan *et al.*, 2008). The increase in pH after twenty days of process can be explained by a continuous decrease of VFA observed in Fig. 1(b).

The behaviour of alkalinity is shown in Fig. 1(c). Maximal standard deviation amounted to ± 0.97 mg $CaCO_3$ l^{-1}. SIR 3:1 has a considerable buffer capacity which is reflected in the initial value of alkalinity and in its progressive increase during the first days of the anaerobic digestion process. SIR 1:1 shows a slight decrease in alkalinity since the beginning of the test until almost half of the process and a gradual increase towards the end of the test period. This is consistent with the increase in pH and the decrease in the content of volatile fatty acids in the same period. On the other hand, SIR 2:1 presents intermediate values for alkalinity, but at the end of the test this SIR has the lowest value of all SIR tested. The values of pH are lower than the results obtained by Koppar *et al.,* (2013) who performed BMP test for anaerobic digestion of peel waste and wastewater.

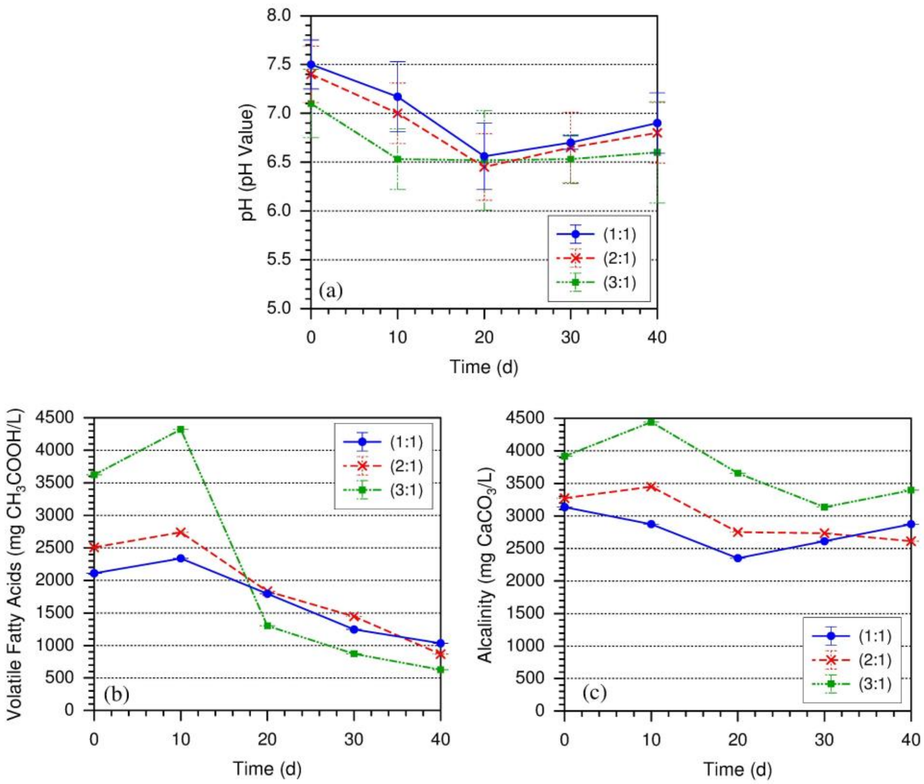

Figure 1. Variation of (a) pH, (b) volatile fatty acids and (c) alkalinity during the anaerobic co-digestion

3.3. Soluble chemical oxygen demand

Table 4 presents the measured values of soluble chemical oxygen demand (COD_S) for each of the reactors that were discarded with a periodicity of ten days. From the differences in the values of soluble chemical oxygen demand for each periodical measurement and the initial soluble chemical oxygen demand the percentage of removed chemical oxygen demand (% $COD_{S\text{-}Rem}$) is calculated using Eq. 1. The results are also presented in Table 4. The maximum value of % $COD_{S\text{-}Rem}$ is established as an indicator of the effectiveness of the anaerobic treatment for the stabilization of the organic biodegradable waste. Maximum values of

56.40±0.02, 51.33±0.01 and 48.07±0.01 % for SIR 1:1, 2:1 and 3:1 were obtained. These percentages are lower than the results obtained by Cun-Fang *et al.,* (2008) and Alkaya *et al.,* (2011) who reported values between 63.7 and 87.3 % when BMP tests were performed using OFMSW and beet-pulp with wastewater respectively. Martín *et al.* (2010a, 2013) reported values of % $COD_{S\text{-}Rem}$ near to 80 and 85 % (VS), in anaerobic digestion of wastewater derived from the pressing of orange peel and semi-continuous anaerobic co-digestion of orange peel waste and residual glycerol with addition of buffer solutions.

$$\%COD_{S\text{-}Rem}=\frac{COD_{S\text{-}t=0}-COD_{S\text{-}t}}{COD_{S\text{-}t=0}}\cdot 100 \quad (1)$$

Table 4. COD measured values and percentage of removed COD for all SIRs tested

	SIR 1:1		**SIR 2:1**		**SIR 3:1**	
Time (t)	**COD_S**	**$\%COD_{S\text{-}Rem}$**	**COD_S**	**$\%COD_{S\text{-}Rem}$**	**COD_S**	**$\%COD_{S\text{-}Rem}$**
d	**$mg_{O_2}\ l^{-1}$**	**%**	**$mg_{O_2}\ l^{-1}$**	**%**	**$mg_{O_2}\ l^{-1}$**	**%**
0	2786.16±0.35	0.00±0.02	3751.23±0.41	0.00±0.02	5244.12±0.27	0.00±0.01
10	2446.35±0.92	12.20±0.03	3370.92±1.23	10.13±0.03	4832.13±0.51	7.86±0.01
20	2372.65±0.46	14.84±0.02	2354.59±0.54	37.23±0.02	4114.95±1.06	21.53±0.02
30	2012.43±0.63	27.77±0.02	1976.96±0.75	47.30±0.02	3031.54±1.16	42.19±0.02
40	1214.80±0.66	56.40±0.02	1825.48±0.49	51.33±0.01	2723.46±0.34	48.07±0.01

Initial rate of removal of COD_S (IRR-COD_S) is a parameter that describes the rate at which the microorganisms break down the organic substrate at the beginning of the test. This parameter is calculated according to Eq. 2, where $rCOD_{S\text{-}Rem}$ represents the rate of COD_S remotion at the beginning of the process (Eq. 3), and $VSS_{t=0}$ is the initial concentration of volatile suspended solids.

$$IRR\text{-}COD_S=\frac{rCOD_{S\text{-}Rem}}{VSS_{t=0}} \quad (2)$$

$$rCOD_{S\text{-}Rem}=\frac{COD_{S\text{-}t=0}-COD_{S\text{-}t=10}}{t_{10}-t_0} \quad (3)$$

Table 5 lists the values calculated for $rCOD_{S\text{-}Rem}$ and the initial rate of removal of COD_S (IRR-COD_S) for each of the SIR tested. Comparing the values presented in Tables 4 and 5, an inverse relationship between the percentage of chemical oxygen demand ($\%COD_{S\text{-}Rem}$) and initial rate of removal of COD_S (IRR-COD_S) with SIR can be observed. This means that the treatment performed with by the lower SIR 1:1 is more effective for the reduction of soluble chemical oxygen demand while the treatment performed by SIR 3:1 reduces this parameter faster.

3.4. Biogas and methane yield

Fig. 2 presents the net cumulative production of methane and biogas during the anaerobic biodegradability tests for each SIR. Since the measurement of the process parameters presented requires the discarding of a reactor of each SIR every 10 days, the methane yield corresponds to the daily average of the values obtained in the reactors available at the time of the measurement. The maximal standard deviation of methane production amounted to ±1.2 ml.

The production of both biogas and methane (Fig. 2(a) and 2(b)) shows the same trend. First, an initial adaptation period during approximately seven to ten days (depending on each SIR) can be observed. Next, there is an increase in the production of biogas and methane for all SIR indicating an effective development of the process, prior to a final phase in which the rate of production considerably decrease due to the end of the anaerobic digestion process. SIR 1:1 has the lowest production of both methane and biogas, and also maintains the same rate of generation during the majority of the time after the initial adaptation period. The longest adaptation time for this SIR can be attributed to the fact that it has the lowest concentration of volatile fatty acids, as shown in Fig. 1(b). This implies that the production of methane is delayed due to the requirement of production of these organic acids in the early stages until the metanogenesis phase is reached. Consequently, SIR 2:1 presents a shorter adaptation time followed by a similar behaviour, as shown by SIR 1:1.

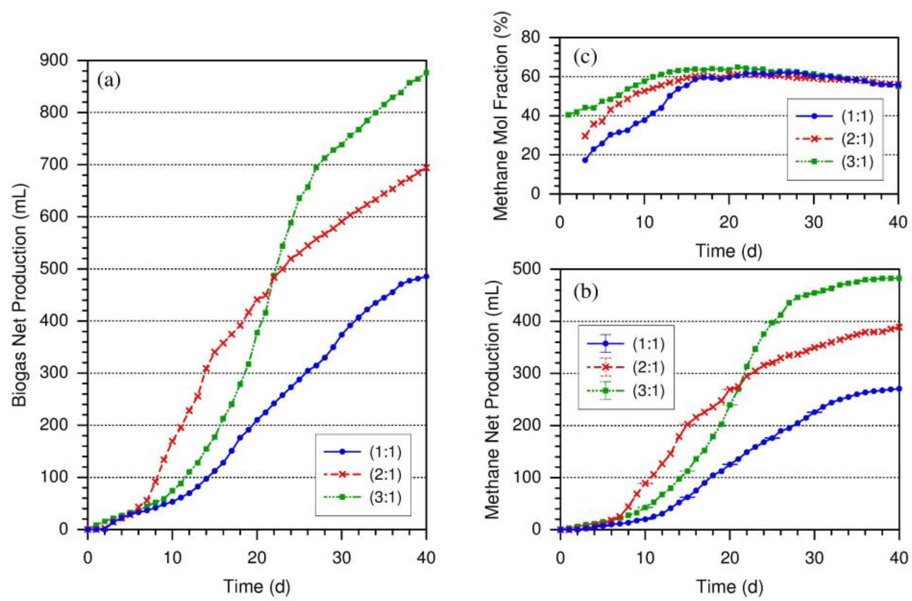

Figure 2. Net cumulative production of methane and biogas and mole fraction of methane in biogas during the anaerobic biodegradation tests for each SIR

SIR 3:1 shows a behaviour which differs from the other SIRs tested. This SIR presents a longer adaptation time than SIR 2:1, resulting in a lower production of both biogas and methane at the beginning of the next stage of the process. This behaviour can be attributed to the high accumulation of VFA, as shown in Fig. 1(b), which implies a slight inhibition of the biochemical sub-processes of transformation of VFA to methane. After overcoming this initial inhibition, this SIR presents the highest methane and biogas generation rate possibly caused by the high VFA content, resulting in the highest methane and biogas production at the end of the test. This behaviour was observed by Alkaya *et al.* (2011), who made BMP test using a sugar beet and beet pulp.

Fig. 2(c) presents the development of the methane mole fraction in biogas. It was calculated assuming that the biogas corresponds to a gas mixture of only methane and carbon dioxide. The figure show similar trends for all SIR: a low methane composition at the beginning of the test followed by a continuous increase until reaching a concentration of approximately 60 %. Next, a slight decrease in the methane concentration until the end of the test is observed. For all SIR the same final biogas composition was determined. These results agree with the values reported by other researches (Montañés *et al.*, 2014; Zhang and Noike, 1994; Qiao *et al.*, 2011) who used different biomass wastes.

3.5. Maximum methane production rate and biochemical methane potential

Maximum methane production rate (MMPR) is an indicator of the maximum generation rate of methane as a function of the content of cellular biomass in the substrate. It is calculated as the maximum slope of the linear part of the curve of methane production vs. time per initial content of volatile suspended solids and is expressed in terms of methane chemical oxygen demand ($COD_{CH4}/(VSS_{t=0}\ d)$) (Neves *et al.*, 2004). COD_{CH4} is obtained from the values presented in Fig. 2(b) using a conversion factor that considers the ambient pressure and temperature during the test (for P = 752 mbar and T=30 °C this value was calculated as 0.00192 $gCOD_{CH4}/(l\ ml_{CH4})$). The biochemical methane potential (BMP) is obtained as the maximum methane production (Fig. 2(b)) per initial $VSS_{t=0}$. Table 4 presents the calculated values of COD_{CH4}, MMPR and BMP for each SIR. The standard deviations of the calculated values are less than ± 0.0002.

Table 5. Characteristic values of the anaerobic biodegradability tests

Ratio / gCOD/gVSS	1:1	2:1	3:1
VSSt=0 / mg l^{-1}	1943±18	2120±16	2230±19
rCODS-Rem / mg_{O_2}/ (l d)	33.98±0.1	38.01±0.13	41.2±0.06
IRR-CODS / gCODRem/(gVSS d)	0.0175±0.0002	0.0179±0.0002	0.0185±0.0002
CODCH4 / mgCOD/ (l d)	23.1	42.2	67.5
MMPR / gCODCH4 / (gVSS d)	0.011	0.019	0.030
BMP / mlSTP-CH4 / gVSS	94.3	124.3	146.6

Fig. 3 presents BMP values vs. SIR reported by different authors together with the results of the present work. A short description of the experimental conditions is included in the legend of the figure in the form: type of substrate/origin of the inoculum/process temperature/type of operation.

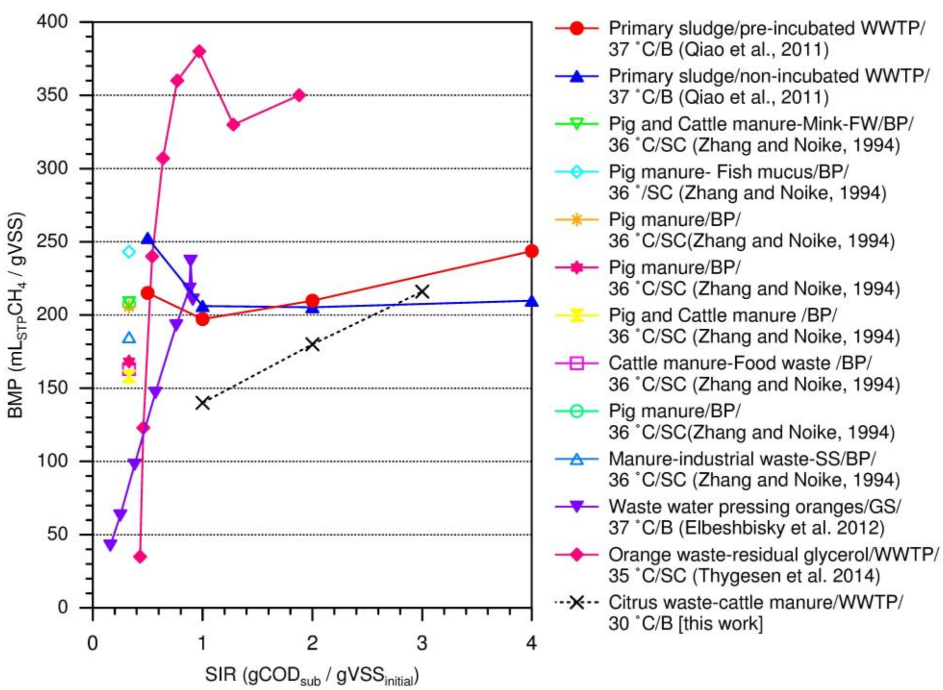

Figure 3. BMP vs. SIR values reported by other researchers. B= Batch operation, SC = Semi-continuous operation, BP = Biogas plant, IFW = Industrial food waste, GS = Granular sludge, OFMSW = Organic fraction municipal solid waste, OPF = Organic plant facility, SSO = source separated organics

For the same SIR, BMP values present a great variation depending on the kind of substrate, inoculum and experimental conditions used. All experiments were done at higher temperatures (35 to 37 °C) than the ones used in the present work (30 °C). Elbeshbisky *et al.,* (2012) obtained BMP values for primary sludge similar to the ones obtained in the present work for SIR 2:1 and 3:1. They performed tests using non- and pre-incubated inoculum finding no influence in the obtained results. In the same work this authors reported SIR values for the anaerobic digestion of the organic fraction of municipal solid waste between 590 and 1250 $ml_{STP-CH4}$/gVSS. Martín *et al.* (2010b, 2013) evaluate the anaerobic digestion of sub-products of the orange juice industry. In order to increase the buffer capacity in the reactors, the authors added 1 g $KHCO_3$ l^{-1} during the initial phases of the process obtaining values up to 380 $ml_{STP-CH4}$/gVSS. Thygesen *et al.,* (2014) evaluated different substrates using a constant SIR of 1:3, the calculated BMP values lie in the range of 150 to 250 $ml_{STP-CH4}$/gVSS. Qiao *et al.*, (2011) (not indicated in the figure) evaluated the biodegradability of different wastes (cow manure, pig manure and fish mucus, sludge and food waste) with an hydrothermal pre-treatment at 170 °C for 6 h using an initial VSS of 4 g l^{-1}. BMP values for untreated fruit/vegetable waste and food waste were 250 and 475 $ml_{STP-CH4}$/gVSS respectively, for the corresponding treated samples BMP values were 290 and 438 $ml_{STP-CH4}$/gVSS, so a clear dependence of the process on the pre-treatment could not be determined.

4. Conclusions

The feasibility of biogas production though anaerobic co-digestion of an homogeneous mixture of citrus waste and cattle manure without the use of buffer solutions is established. BMP values that are among the values reported by other authors using similar substrates are obtained. SIR 3:1 is established as the most suitable ratio for an energetic application as it produces the highest methane volume.

The implementation of a system for biogas production using low process temperatures, simple pre-treatment conditions and high available raw materials, as used in this work, results in lower production costs; making it accessible for areas away urban centres in developing regions. Nevertheless, further work must be concentrated in the optimization of the process parameters and the study of the continuous operation of the system.

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