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Biochemical potential of methane of wastewater ultrafiltration in the processing of unriped green acerola (Malpighia emarginata)

Potencial bioquímico de metano de águas residuárias da ultrafiltração no processamento da acerola (Malpighia emarginata) verde

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Abstract

Ultrafiltration clarifies fruit juices, in the food industry, but generates retentive, recalcitrant wastewater, which, by its organic nature, may present a potential for biodegradation and methane production. This study aimed to evaluate the biochemical methane potential (BMP) in wastewater from the processing of unripe green acerola, obtaining the mass balance and the speed of organic load removal in COD terms. The BMP assays followed the German Guidelines VDI 4630, by applying three COD loads per liter of reactor vial (0.86 g $COD_{applied}L_R^{-1}$, 1.5 g $COD_{applied}L_R^{-1}$, and 2.0 g $COD_{applied}L_R^{-1}$), in batches, inoculated with the anaerobic sludge from reactors treating domestic sewage, at 30 °C. The pH, COD, and methane production were evaluated every 48 hours. The biodegradability and the decay rate constant of the COD (K_d) were determined, thus obtaining the methanized COD, the COD for the formation of new cells, and the COD present in the wastewater, in the form of volatile acids. The best BMP was 0.100 L CH_4 g^{-1} $COD_{removed}$, the percentages of methanization were above 62 %, and the highest K_d occurred for the lowest load applied. The anaerobic digestion of the wastewater proved viable for in full-scale, with its application being suggested at a pilot scale.

Keywords: Agro-industry. Organic load. Malpighia emarginata. Methane. Ultrafiltration.

Resumo

A ultrafiltração clarifica sucos de frutas, na agroindústria alimentícia, mas gera o retentado, água residuária recalcitrante, que por sua natureza orgânica, pode apresentar potencial para biodegradação e geração de metano. Objetivou-se nesse estudo avaliar o potencial bioquímico de metano (PBM) da água residuária do processamento da acerola verde, obtendo o balanço de massa e a velocidade de remoção da carga orgânica em termos de DQO. Os ensaios do PBM seguiram a Norma Alemã VDI 4630, aplicando-se três cargas de DQO por litro de frasco reator (0,86 g $DQO_{aplicada}L_R^{-1}$, 1,5 g $DQO_{aplicada}L_R^{-1}$ e 2,0 g $DQO_{aplicada}L_R^{-1}$), em batelada, inoculados com lodo anaeróbio de reatores tratando esgotos domésticos, a 30 °C. Avaliou-se pH, DQO e produção de metano a cada 48 horas. Determinou-se a biodegradabilidade e a constante de velocidade de decaimento da DQO (K_d), obtendo-se a DQO metanizada, DQO para formação de novas células e DQO presente na água residuária em forma de ácidos voláteis. O melhor PBM foi 0,100L L CH_4 g^{-1} $DQO_{removida}$, os percentuais de metanização foram acima de 62 % e o maior K_d ocorreu para a menor carga aplicada. A digestão anaeróbia da água residuária mostrou-se viável, com possibilidade de uso em escala real, sugerindo-se para isso, sua aplicação em escala piloto.

Palavras-chave: Agroindústria. Carga orgânica. *Malpighia emarginata*. Metano. Ultrafiltração.

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Introduction

The fruit processing agroindustry generates millions of tons of residues with varied characteristics as a function of the different processes employed. If not properly treated, such residues generate environmental problems (CHENG *et al*. 2018; RAJAGOPAL; MASSÉ; SINGH, 2013), with biological treatments being recommended for the treatment of organic and agro-industrial wastewaters (BASTOS *et al*., 2019; CORREIA; DEL BIANCH, 2008).

Among the fruit species produced in the Northeast region of Brazil, the West Indian Cherry (*Malpighia emarginata D.C*) is highlighted for its production and volume of residues generated (PEREIRA; CAMPOS; MOTERANI, 2010). According to the 2017 Agricultural Census (IBGE, 2017), 6,646 producers cultivate West Indian Cherry in Brazil in an area of 5,753 hectares, with an annual production of 60,966 tons, of which the state of Pernambuco is the largest producer, with 21,351 tons.

West Indian Cherry processing is still in expansion due to the high content of ascorbic acid present in the fruits, which, for being climacteric, present two maturation stages for harvest and industrialization: unripe and ripe. This characteristic generates two processing cycles and diversified residues (CORRÊA *et al*., 2017).

As one of the stages of fruit processing, ultrafiltration is a process that clarifies the fruit juice using semipermeable membranes. The fraction that does not permeate the membrane is called retentate or residue of the ultrafiltration process, which, due to the large amount generated and its recalcitrant chemical composition, may constitute an obstacle for the application of this technology on an industrial scale, given the environmental impact of its disposal (PENHA *et al*., 2001) since the compounds present in this wastewater do not exist naturally in the environment and, therefore, microorganisms would hardly metabolize them. In the West Indian Cherry agro-industry, this stage of the process is responsible for the residue with the highest organic matter content in terms of chemical oxygen demand (COD), with lower contents of salts and sugars, conferring it a characteristic of hard biological degradation, especially due to the presence of recalcitrant polymers, such as pectins and lignin complexes.

Shen *et al*. (2019) classify fruit industry wastewaters as recalcitrant or of low biodegradation, thus presenting complex characteristics that complicate their treatment.

In this perspective, studies on new use potentials of these residues are necessary, and Nascimento Filho and Franco (2015) cite biological treatments as alternatives to be studied. It is known that the application of the anaerobic digestion process constitutes a viable and efficient alternative that promotes energy reuse through methane production (GONZÁLEZ-SÁNCHEZ *et al*., 2015; SOUZA *et al*., 2019), in addition to the transformation of residues into a stable matter of lower polluting potential (LEIVA *et al*., 2014).

The importance of anaerobic digestion can also be observed in the results obtained by Lima *et al*. (2018), when, based on indicators of economic, environmental, and social sustainability, the authors affirmed that anaerobic digestion in upflow anaerobic sludge blanket reactors is the adequate method for wastewater treatment. Anaerobic digestion is known as a methane-generating method that can convert organic compounds into a sustainable energy source (SIDDIQUE; WAHID, 2018).

According to Dechrugsa, kantachote and Chaiprapat (2013) and Hagos *et al*. (2017), different types of residues provide different methane productions, which can be evaluated by biochemical methane potential (BMP) assays and by the assessment of the waste degradation kinetics (SAMADI; MIRBAGHERI, 2019; VAN *et al*., 2018).

The BMP assays provide a method for comparing methane productions (ESPOSITO *et al*., 2012; LESTEUR *et al*., 2010) and optimizing anaerobic digestion, whose advantages are the simplicity in performing the method and its low cost and quick response (SANTOS FILHO *et al*., 2018). Therefore, it is the most used methodology by academics and technicians to previously determine the maximum methane production of a specific residue (GONZÁLEZ-SÁNCHEZ *et al*., 2015; RODRÍGUEZ *et al*., 2017), when subjected to anaerobic digestion.

The kinetics of degradation is one of the most used parameters for verifying the ability of microorganisms to metabolize the organic matter of residues. First-order models represent most of the biological degradations that occur in the metabolization of residues, such as domestic wastewaters (BERTOLINO; CARVALHO; AQUINO, 2008; LEW *et al*., 2009) and those from hog and cattle raising (MORAES; PAULA JUNIOR, 2004).

According to Lebon *et al*. (2019), the rate at which the process of organic matter removal by the anaerobic digestion of juice processing wastewaters can also be represented by mathematic models, based on first-order equations. It is an important tool to estimate the efficiency of the process and reduce the number of experiments required to evaluate degradation (VELÁZQUEZ-MARTÍ *et al*., 2018) and to project full-scale anaerobic reactors (DEEPANRAJ; SIVASUBRAMANIAN; JAYARAJ, 2015).

Several studies have been performed aiming at optimizing the anaerobic degradation of agroindustry residues (KOUPAIE *et al*., 2014; MONTALVO *et al*., 2020; PENTEADO *et al*., 2018; ZERROUKI *et al*., 2015) and ultrafiltration residues in the processing of food products (KHALIGH *et al*., 2017). However, although Fan, Lee and Klemes (2017) have shown that Brazil was among the top 10 countries in the world that most researched anaerobic digestion, between 2016 and 2017, studies with ultrafiltration residues from West Indian Cherry processing, with either unripe or ripe fruits, are still scarce.

Therefore, this study aimed to evaluate the biochemical methane potential of the wastewater resulting from ultrafiltration the processing of unripe West Indian Cherry fruits, in laboratory-scale anaerobic reactors, and to obtain the mass balance and the organic load removal rate in terms of chemical oxygen demand. Such results will allow evaluating the degradation rate of residues considered as recalcitrant or of low biodegradation and to evaluate the viability of the application of the anaerobic technology for the treatment of fruit industry wastewaters.

Materials and methods

Substrate and inoculum of the BMP tests

The substrate used was the wastewater originated in the ultrafiltration stage of the processing of unripe West Indian Cherry fruits by NIAGRO (Nichirei do Brasil Agrícola LLC), a company located in the Industrial District of Petrolina-PE, being characterized by APHA procedures (2017) for the parameters of Table 1.

Table 1 – Characterization of the wastewater from the ultrafiltration process of unripe West Indian Cherry fruits

Parameter	Values
PH	3.5
COD	129 g O_2. L^{-1}
BOD	34 g O_2. L^{-1}
Total solids	144 g ST. L^{-1}
Nitrogen	0.29 g NH_3. L^{-1}
Phosphorus	1 g P-PO_4. L^{-1}
COD:BOD	3.79
COD:N	129:0.29

COD: chemical oxygen demand; BOD: biochemical oxygen demand; N: nitrogen.

Source: The authors.

The inoculum used was the anaerobic sludge of a UASB reactor treating domestic sewage in the State Sanitation Company of Pernambuco, Petrolina–PE, with a specific methanogenic activity of 0.125 NL CH_4.kg STV d^{-1}.

Experimental design of the BMP assays

Three COD loads of the wastewater were applied (T_1 = 0.85 g $COD_{applied}L_R^{-1}$, T_2 =1.5 g $COD_{applied}L_R^{-1}$, and T_3 = 2.0 g $COD_{applied}L_R^{-1}$) by adapting the VEREIN DEUTSCHER INGENIEURE-VDI 4630 German Guidelines (2016) to the bottle sacrifice methodology described by Amorim *et al*. (2013), which consists of the sacrifice of a triplicate set of reactor vials for wastewater analysis over the time of degradation, with each flask containing a substrate, an inoculum, and 20 % of a nutritive solution.

The experiment was conducted in a batch reactor for 288 h (12 days), by sacrificing, for the analyses of COD, pH, and VFA (Volatile Fatty Acids), one triplicate at every 48 h, totaling, with the control, 75 reactor flasks with a useful volume of 0.118 L and headspace of 0.026 L.

The VFAs were determined by the Kapp method, according to Ribas, Moraes and Foresti (2007), since, according to Aquino and Chernicharo (2005), acetic acid is the most important VFA for contributing with about 70 % of the biologically-produced methane.

The inoculum concentration was 5g of volatile solids per liter (BERTOLINO; CARVALHO; AQUINO, 2008) in the mixed liquor of each vial, and nutritional supplementation was made according to Aquino *et al*. (2007).

The pH was adjusted to neutrality with NaOH 1M when the reactor vials were set up (day zero), without providing neither alkalinity nor nitrogen supplementation, aiming at obtaining the BMP of the raw wastewater.

Afterward, the vials were sealed with nitrile rubber and aluminum seals, using a vial crimper, connecting 10 mL syringe septa in the rubbers for the collection and measurement of the biogas, for later composition analysis.

After the set-up, the vials were incubated at 30 ± 2 °C and agitated at every 24 hours. The initial conditions of the assays (day zero) are exhibited in Table 2.

Biogas composition, methane volume, and BMP calculation

The composition of the biogas was obtained with a 7890A gas chromatograph with an FID-type detector equipped with a methanator. The Agilent Hayesep Q80/100 column was used, with N_2 as a carrier gas, at

Table 2 – Experimental conditions of the BMP assays in the reactor vials, in day zero

Treatments	Applied load ($g\ COD_{applied} L_R^{-1}$)	Inoculum ($g.\ L^{-1}$)	T (°C)	pH	VFA ($g\ HAc\ L^{-1}$)	Alkalinity ($g.\ L^{-1}$)
T_1	0.86	5	30	6.98	0.646	1.84
T_2	1.50	5	30	7.13	0.614	2.36
T_3	2.00	5	30	6.94	0.678	1.88

HAc: Acetic acid; VFA: volatile fatty acids.

Source: The authors.

a 25 mL min^{-1} flow rate. The running time was 11 minutes, and the temperature of the detector and the oven was 300 °C and 60 °C, respectively.

The methane volume was obtained based on the composition of the biogas in terms of CH_4 percentages, applying this percentage to the biogas volume measured daily using the syringes connected to the vials. Afterward, the BMP expressed in NL CH_4 $g^{-1}COD_{removed}$ was calculated.

Biodegradation rate (K_d)

The organic matter removal in terms of COD applied per liter of the reactor ($COD_{applied}L_R^{-1}$) was used as a parameter to evaluate the degradation kinetics. The K_d constant was determined by considering that, in a batch reactor, the rate of change in the concentration of the reagent is proportional to the concentration of this reagent at a given moment, admitting, therefore, a first-order reaction (METCALF *et al*., 2016), given by

$$r = \frac{dS}{dt} = -K_d S, \tag{1}$$

where r is the reaction speed (g L^{-1} days), S is the concentration of the limiting reagent (COD) (g), t is the hydraulic retention time (days), and K_d is the constant speed for the firs-order reaction (d^{-1}).

Biodegradation mass balance

For the mass balance, the percentage of anaerobic biodegradability (% BD) and the methanized COD percentage (% COD_{CH_4}) were calculated according to Elbeshbishy and Nakhla (2012), using equations (2) and (3), respectively

$$\%\ BD = \left(\frac{COD_{applied} - COD_{final}}{COD_{applied}} + \frac{COD_{AGV}}{COD_{applied}}\right) * 100, \tag{2}$$

$$\%\ COD_{CH_4} = \left(\frac{COD_{CH_4}}{COD_{applied}}\right) * 100. \tag{3}$$

The COD transformed into methane (COD_{CH_4}) and the COD used for the formation of new cells (COD_{Cel}) were calculated according to Metcalf *et al*. (2016), in which COD_{CH_4} is the COD of the accumulated methane production based on 0.395 L CH_4/g COD.

For the calculation of the COD_{Biom}, the solids production coefficient of 0.15 $gCOD_{Iodine}$/$gCOD_{applied}$ was adopted. The COD present in the wastewater in the form of volatile fatty acids (COD_{AGV}) not converted into methane was calculated by the difference between the $COD_{applied}$ and the sum of the last two values (COD_{CH_4} and COD_{Cel}).

Statistical analysis

The results were statistically evaluated using the software Sisvar@ (version 5.6), through descriptive statistics and analysis of variance (ANOVA), applying Tukey's test at 5 % of significance.

Results and discussion

The highest values of biodegradability (% BD) and methanized COD (% COD_{CH_4}) occurred in T_2 and T_1, in which T_1 presented the best BMP (0.100 NL CH_4 $g^{-1}COD_{removed}$), Table 3. Although these treatments presented practically the same percentages of COD converted into methane and new cells, the BMP (NL CH_4 g^{-1} $COD_{removed}$) differed, with the treatment of lower $COD_{applied}$ load (T_1) presenting the highest potential. This fact may be associated with non-soluble substances measured by the COD, which, for not being biodegradable, are not converted into methane. The treatment with the highest $COD_{applied}$ load presented the lowest percentage of biodegradation (% BD), the lowest value of final pH (4.89), and, consequently, the highest % COD_{AGV}, demonstrating the accumulation of volatile fatty acids.

Table 3 – Experimental conditions of the BMP assays in the reactor vials, in day zero

Treatments	[1]% BD	[2]% COD_{CH_4}	[3]% COD_{Cel}	[4]% COD_{AGV}	NL CH_4 g^{-1} $COD_{removed}$
T_1	88a	72	16	12	0.100 ± 0.001
T_2	91a	73	17	10	0.070 ± 0.001
T_3	76b	62	13	25	0.045 ± 0.002

Means followed by the same letter do not differ from each other by Tukey's test at 5% of significance. [1]BD: Biodegradability; [2]COD_{CH_4}: Methanized Chemical Oxygen Demand; [3]COD_{Cel}: COD used for the formation of new cells; [4]COD_{AGV}: COD present in the wastewater as volatile fatty acids not converted into methane.

Source: The authors.

By observing the BMP results, Table 3, T_1 presented the highest rate among the treatments. Cremonez *et al*. (2016) obtained 0.161 L CH_4 g^{-1} $COD_{removed}$ when treating starch wastewater, which possesses similar organic matter and acidity features to the West Indian Cherry wastewater due to the high contents of initial VFAs (Table 2). This methane production rate demonstrates that the wastewater studied is a potential source of bioenergy, as affirmed by Khan *et al*. (2015) when citing that citrus fruit wastes are promising for energy generation. On the other hand, the differences between biodegradability and methanization (% BD - % COD_{CH_4}) of 16, 18, and 14 % for T_1, T_2, and T_3, respectively, evidence an inhibition of methanogenesis for the three treatments when based on the affirmation by Elbeshbishy and Nakhla (2012), by which differences higher than 5 % between biodegradability and methanization indicate that there is inhibition in methane production.

The lowest BMP value of T_3 is related to the higher accumulation of VFAs (4.641 g HAc L^{-1}). The potential for the accumulation of fatty acids in the system is limited by the concentration of polluting agents in the wastewater and their liability or not to degradation; in fact, T_3 presents the highest organic load and is recalcitrant. VFAs are toxic to methanogenic microorganisms only in their non-ionized form, which occurs when the pH is below 6.0.

Thus, the acidification of the wastewater in the reactor results in growth inhibition for the Archaeans, consequently reducing the methane production rate. This behavior of the West Indian Cherry wastewater is similar to that observed by Zerrouki *et al*. (2015) when treating fruit juice wastewater in batch reactors, observing a reduction in methane production and the inhibition of microorganisms when the wastewater was acidified.

This acidification comes from the hydrolysis of carbohydrates, which results in glucose, fructose, and galactose. These, in turn, are quickly fermented, leading to a pH reduction and an increase in the concentration of VFAs. The absence of alkalinity, which is characteristic of these wastewaters, contributes to the pH decrease. In fact, except for T_1, which presented a 6.3 value for the pH, there was a 35 % decrease in the alkalinity values (T_2 reduced from 2.361 to 1.546 g L^{-1}; T_3 reduced from 1.880 to 1.226 g L^{-1}), resulting in pH values of 5.1 for T_2 and 4.8 for T_3. T_1 maintained the alkalinity of the system (1.947 g L^{-1}), showing a slight increase, with this factor justifying its better performance regarding the BMP.

The nitrogen contents also contribute to the accumulation of VFAs (VRIEZE *et al*., 2012). In conditions of limitation of nitrogen availability (high C/N ratio), microorganisms are unable to metabolize carbon, which leads to the inefficiency of the process and an intense accumulation of organic acids, reducing the quality of the biogas (GUERI *et al*., 2018; SGORLON *et al*., 2011). Although the ultrafiltration wastewater presents a value of 0.29 g NH_3 L^{-1}, a concentration considered beneficial to the system (CHEN; CHENG; CREAMER, 2008; RAJAGOPAL; MASSÉ; SINGH, 2013), it does not present a balanced ratio between organic matter and nitroge, Table 1. The ratio was calculated for 350:0.78, which is six times lower than the recommended by Metcalf *et al*. (2016) (350:5).

According to the data of the adjustments of the first-order model, Figure 1, and the data exhibited in Table 4, it is seen that the substrate degradation rates for the loads applied in T_1 and T_2 were similar, with both treatments presenting a progressive COD reduction up to com 192 h (eight days).

At the end of the degradation period, the COD mass removal for T_1 was 89%, keeping the pH at 6.0. In the three conditions evaluated, there were COD mass removals at 48 h, presenting satisfactory results with values above 73 %.

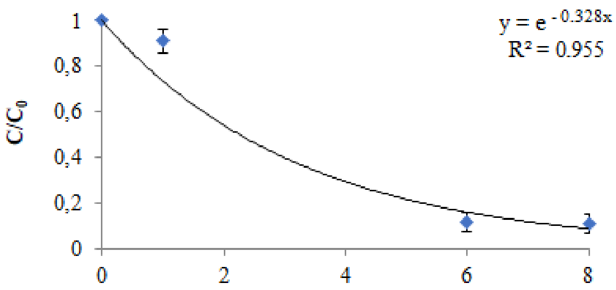
The treatment with the lowest $COD_{applied}$ (T_1) presented the highest reaction speed, as well as the best adjustment to the curve (R^2 = 0.955), demonstrating fitness to the model, which did not occur with T_3, which had higher

Table 4 – Loads removed and kinetic parameters

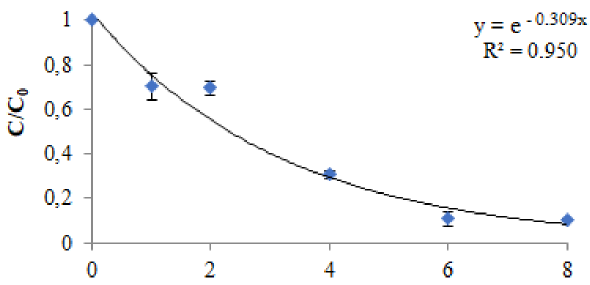
	$g\ COD_{removed}L_R^{-1}$	K_d (h^{-1})	K_d (d^{-1})	R^2
T_1	0.78 ± 0.06	0.0113	0.328	0.955
T_2	1.24 ± 0.22	0.0124	0.309	0.949
T_3	1.45 ± 0.13	0.0090	0.209	0.677

Source: The authors.

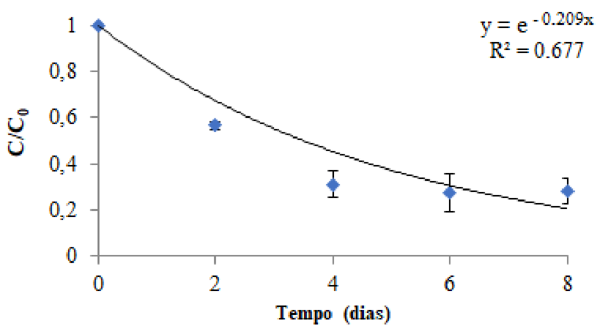
Figure 1 – COD depletion



(a) T_1 adjusted to the first-order model.



(b) T_2 adjusted to the first-order model.



(c) T_3 adjusted to the first-order model.

Source: The authors.

$COD_{applied}$ ($R^2 = 0.677$). This behavior is attributed to the lower availability of biodegradable organic matter or the higher load of particulate material in the wastewater with the highest load applied. This is also associated with higher acidity since this treatment showed to be acid at the end of the 12 day, equivalent to 4.7, as well as with a higher accumulation percentage of VFAs.

By comparing the biodegradation of wastewater from wet coffee processing (K_d 0.01 d^{-1} and K_d 0.0075 d^{-1}) performed by Matangue and Campos (2011) and Campos, Prado and Pereira (2014) with the constant obtained for West Indian Cherry wastewater, the results revealed that the organic compounds from the later residue are more rapidly degraded. These results resemble those obtained with the wastewater from palm oil production (K_d 0.0658 d^{-1} and 0.106 d^{-1}), verified by Wun *et al.* (2019), and are higher than the values obtained by Amorim *et al.* (2019) (K_d 0.169 d^{-1}) when biodegrading starch wastewater.

The organic matter decay coefficients obtained for the three loads applied are low compared to the values obtained for the degradation of carbohydrates and proteins (0.780 and 0.650 d^{-1}) (ELBESHBISHY; NAKHLA, 2012), for textile wastewaters (kd 0.615 d^{-1}) (ISIK; SPONZA, 2005), for hog raising wastewater (K_d 1.52 d^{-1}) (MATOS; FREITAS; BORGES, 2010), and for domestic sewage (K_d 1.62 d^{-1}) (BRASIL; MATOS; SOARES, 2007).

However, these results are in agreement with Zerrouk *et al*. (2015), who modeled the anaerobic digestion of fruit processing wastewaters, concluding that they presented a lower rate of biodegradation and conversion of methane compared to other wastewaters in studies present in the literature, although with potential for anaerobic biodegradation.

Conclusions

The results of the BMP assays demonstrate that the anaerobic digestion of the ultrafiltration wastewater from the processing of unripe West Indian Cherry fruits is viable, with the possibility of full-scale use, requiring, for that, the application in pilot reactors, with alkalinity correction.

The mass balance evidenced the inhibition of methanogenesis for the three loads applied, being associated with the accumulation of VFAs in the process, and the later to nitrogen deficiency in the wastewater.

Although the mass balance has indicated the inhibition of methanogenesis, the wastewater showed to be anaerobically biodegradable at a pH close to neutrality with organic loads of 1g $COD.L_R^{-1}$, with organic fraction removals of 90% and faster degradation rates than those found in the literature for the biodegradation of other agro-industrial wastewaters.

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