Aerobic, anaerobic treatability and biogas production potential of a wastewater from a biodiesel industry

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Abstract: The purpose was to investigate the treatability of a wastewater from a biodiesel production industry under (i) aerobic conditions using domestic activated sludge as inoculum (ii) anaerobic conditions using sludge from an anaerobic domestic wastewater treatment digester (iii) utilization of wastewater for biogas production. Methods: The process was evaluated in terms of primary degradation, i.e. ultimate degradation of pollutants as total dissolved chemical oxygen demand (COD) removal. The aerobic biodegradation batch tests were conducted in reactors with a working volume of 1.0L according to *Zahn Wellens* methodology proposed by the Organization for Economic Co-operation and Development (OECD). The anaerobic treatability was determined by the methodology proposed by Field *et al.* [9]. Based on the results of anaerobic biodegradation, four new reactors with a working volume of 1.0 liter were inoculated to evaluate the biogas production potential. Results: The experiments showed that wastewater can be degraded under aerobic conditions with no lag-phase. COD maximum concentration of 780 mg.L^{-1} could be metabolized aerobically. The anaerobic biodegradation only started after the adaptation phase (3 days). After 28 days, it was possible to achieve removal efficiencies above 90% for the conditions applied in anaerobic tests. It was possible to obtain 114 mL of biogas for the highest influent COD concentration of 800 mg.L^{-1} and F/M ratio = 0.25.
Keywords: aerobic, anaerobic, biodegradation, biodiesel, wastewater

1. INTRODUCTION

The world energy matrix is mainly composed of non-renewable resources, especially fossil fuels. Due to the escalating costs associated with the increase of crude oil prices and also to the concern with problems related to the emission of greenhouse gases, alternative energy sources are sought, in an attempt to gradually allow an overcome, regarding the dependency on fossil fuels at a global scale.

In 2004, the Brazilian government launched the National Program for Biodiesel Production (NPBP). Biodiesel can replace, partially or completely, mineral diesel. The addition of biodiesel in Brazil was legally established in 2008. Initially, the mandatory blend was 2% and, year by year, it was increased to 5% in 2010, which corresponds to a production of 2.4 billion liters per year. The NPBP gives special value to the participation of small producers of castor and palm oil, from the poorest regions of the country. This means social inclusion and consideration of the ecological reality found, although the program also requires quality assurance and the ability to compete.

The amount of effluent generated in biodiesel production plants is strongly influenced by the production method used to obtain the fuel. According to Suehara *et al.* [1] when using the transesterification process, for each 100L of biodiesel, 20L of effluent are generated. Kolesárová *et al.* [2] (2011) state that, depending on the washing method utilized, up to 300L of effluent can be generated for each 100L of biodiesel produced. As for the qualitative aspects, the wastewater, which comes from the biodiesel production process, has a high organic load, pH value (when used alkaline catalysis), content of oils, fats and suspended solids, and low nitrogen and phosphorus concentrations [3-4].

Part of the success of wastewater treatment processes is determined by the physicochemical characterization of the effluent. Generally, the characterization studies are limited to the examination of non-specific organic material in the form of BOD or COD, total or suspended solids, nitrogen compounds, phosphorus, and chloride, among others. Although COD is the most appropriate parameter to characterize and quantify the organic matter present in a wastewater, this statement is only of practical manner, since the concentration of COD provides no information about the biodegradability or the identity of the pollutants present in a determined wastewater. In this case, one can apply sophisticated analytical techniques such as gas or liquid chromatography and mass spectrometry, or even perform biodegradability tests.

The biodegradability of the effluent is affected by many factors. Among the most relevant are: source and amount of microorganisms, abiotic and physicochemical conditions of the medium (dissolved oxygen concentration, temperature, pH, alkalinity and availability of macro and micro-nutrients).

There are a variety of methods to assess the biodegradability of wastewater. Typically, the methods are based on an indirect measurement of the pollutants degradation by means of oxygen consumption or generation of biogas and carbon dioxide production. The anaerobic biodegradation can be evaluated by monitoring the methane gas production or also by monitoring the depletion of the substrate concentration during the degradation of organic matter by microorganisms in batch reactors under controlled temperature [5-6].

The purpose of this study was to evaluate aerobic and anaerobic treatability and potential biogas production from a wastewater generated in a biodiesel production industry, located in the city of *Candeias, Bahia,* Brazil.

2. METHODS

Specifically during biodiesel production, the raw material used in the transesterification, considerably affects the characteristics of the waste generated. The physicochemical characterization (Table 1) indicated that the industrial effluent collected downstream of the oil and grease removal system (flotation unit) in the Wastewater Treatment Plant of Biodiesel of *Candeias* had high concentrations of chemical oxygen demand (COD), total organic carbon (TOC), solids and chlorides, which are likely to seriously compromise the efficiency of the biological treatment. All analyzes were performed according to Standard Methods for the Examination of the recommendations of Water and Wastewater [7].

Table 1: Pollutant concentrations in the biodiesel production wastewater

Parameters	**Samples**	**Minimum**	**Maximum**	**Mean ± SD**
pH value	15	5.4	7.8	6.7 ± 0.7
Alkalinity (mg $CaCO_3.L^{-1}$)	15	24.1	208.4	117.2 ± 55.5
COD ($g.L^{-1}$)	15	230	910	561.4 ± 224.8
Dissolved COD ($g.L^{-1}$)	15	60	350	175 ± 62.2
Chloride ($mg.L^{-1}$)	15	749.8	1 916.1	1 344.8 ± 337.8
Total Nitrogen ($mg.L^{-1}$)	15	5.5	15.2	11.7 ± 3.2
Total suspended solids ($mg.L^{-1}$)	15	20	112	57.4 ± 29.7

The aerobic sludge used as inoculum in biodegradability batch tests was collected in an activated sludge system that treats domestic wastewater. It is noteworthy that the original proposal would collect the anaerobic and aerobic sludge of the wastewater treatment plant installed in the industry, but it was not possible, since the aerobic reactor was with the air supply pipe ruptured, therefore the sludge from the reactor would not be suitable for aerobic biodegradation tests.

The aerobic biodegradation batch tests were performed in reactors with a working volume of 1.0L according to *Zahn Wellens* methodology proposed by the Organization for Economic Co-operation and Development (OECD) and were monitored by COD analyzes performed on samples filtered through membrane filter (porosity of 0, 45µm). Ten reactors were used, being that two of these were used for a test control experiment. The working volume (1.0 L) was filled with sludge and substrate, respecting the Food/Microorganisms (F/M) ratios, described in Table 2. The reactors were involved with dark plastic to prevent direct incidence of light. Within each reactor was placed a ceramic air stone diffuser connected to a small air pump similar those used in domestic aquariums, in an attempt to spread the air and ensure the mixing of the solution. The pH value was controlled and maintained between 6.5 and 8.0 throughout the addition of stock solutions of NaOH and H_2SO_4. The process was monitored until the end of the depletion of the substrate concentration. The volume removed for analysis did not exceed 10% of the working volume of the reactor in any of the tests. The degradation at a given time was calculated using Equation 1.

$$\%COD_{removed} = \left[1 - \left(\frac{C_t - C_B}{C_A - C_{BA}}\right)\right] \times 100 \quad equation\ (1)$$

C_A = COD concentration after (3 h ± 30 min) incubation;

C_t = COD concentration at time t;

C_{BA} = COD concentration of the control experiment after (± 3h 30min) incubation;

C_B = COD concentration of the control experiment at time t

The anaerobic sludge was coming from an upflow anaerobic sludge blanket reactor (UASB) that treats domestic wastewater. The anaerobic biodegradability was determined using the methodology proposed by Field *et al.* [9] by monitoring the consumption of the substrate. Ten reactors were monitored with a working volume of 1.0 L, two of those were used as control experiment and therefore no substrate addition was made (Table 2). To eliminate the production of methane which originated from endogenous

respiration, the vials were kept at rest for seven days at room temperature, with only the sludge, thoroughly washed, and 100 mL of nutrient solution.
After the incubation period, the produced gas was purged, then, a solution comprising deionized water and industrial effluent was added, in order to meet the set F/M ratio. All flasks were stanched with rubber septa and sealed. All of them were stored in the dark at rest, in a container with a mercury bulb thermometer to monitor the temperature (28 ± 1°C). It is noteworthy that, the studies found in the literature report that anaerobic biodegradability testing should be performed within the range of 30-35°C, so to allow the methanogenic microorganisms to have the best growing conditions. However, the anaerobic reactors in full scale wastewater treatment plants are submitted to different environmental conditions and rarely operate in the ideal temperature range for methanogenic activity. Therefore, to make the process as close as possible to the reality found in the wastewater treatment plant of the biodiesel industry, the tests were performed at room temperature. Immediately before collection of samples for quantification of COD, a manual agitation of the reactors was held. The COD removal was monitored for 28 days.

Table 2: Conditions applied during the aerobic and anaerobic biodegradability tests

Aerobic Test	**COD ($g.L^{-1}$)**	**VSS ($g.L^{-1}$)**	**F/M Ratio**	**Organic Loading Rate kg COD.($m^{-3}.day^{-1}$)**
1 and 5	1.0	3.2	0.31	0.58
2 and 6	1.0	2.5	0.40	0.58
3 and 7	0.8	3.2	0.25	0.47
4 and 8	0.8	2.5	0.32	0.47
Anaerobic Test	**COD ($g.L^{-1}$)**	**VSS ($g.L^{-1}$)**	**F/M Ratio**	**Organic Loading Rate kg COD.($m^{-3}.day^{-1}$)**
Control 1	-	2.5	-	-
Control 2	-	3.2	-	-
1 and 5	1.0	3.2	0.31	0.036
2 and 6	1.0	2.5	0.40	0.036
3 and 7	0.8	3.2	0.25	0.028
4 and 8	0.8	2.5	0.32	0.028

To ensure the presence of essential nutrients to the biodegradation process, two stock solutions were prepared, one of micronutrients and the other of macronutrients (Table 3). The nutrient solution that was added to the aerobic and anaerobic reactors was made by addition of 2 mL of the micronutrient solution and 200 mL of the macronutrient solution.

Table 3: Stock solutions used in the aerobic and anaerobic biodegradability tests

Stock Solution of Micronutrient ($g.L^{-1}$)		Stock Solution of Macronutrient ($g.L^{-1}$)	
$FeCl_3.H_2O$	2.0	KH_2PO_4	1.5
$ZnCl_2$	0.05	K_2HPO_4	6.5
$CuCl_2.2H_2O$	0.03	NH_4Cl	5.0
$MnCl_2.4H_2O$	0.50	$NaS.9H_2O$	0.5
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.05	$CaCl_2.2H_2O$	1.0
$NiCl_2.6H_20$	0.05	$MgCl_2.6H_2O$	1.0
$AlCl_3$	0.05		
$CaCl_2.6H_20$	2.00		
H_3BO_4	0.01		
HCl	1.00		

The tests conducted to determine the biogas production potential were performed only after obtaining the results of the anaerobic biodegradability. Therefore, three new reactors with a working volume of 1.0 liter were inoculated to evaluate the biogas production potential. It was performed a control experiment that allowed evaluate the production of biogas derived from the degradation and cell lysis of microorganisms (endogenous respiration). The test response was obtained by monitoring the internal pressure of the reactors, which provided the volume of the accumulated biogas over time. The tests lasted for 28 days. The equipment used was: pressure transducer coupled to a digital device for reading the biogas pressure, borosilicate glass vial (digester) of 1L with plastic lid, sealed with rubber septum, gas portable monitor, model GEM™ 2000 with accuracy of 0.5 to ± 0.1 mbar. The net cumulative methane production was expressed as a percentage of the theoretical methane production.

3. RESULTS AND DISCUSSION

3.1 Aerobic biodegradation batch tests

Figures 1a and 1b show the COD concentration of filtered aliquots during the aerobic biodegradability tests. It is important to highlight that the wastewater was collected after the flotation unit, responsible for removing oil and grease. The experiments showed that biodiesel production wastewater can be degraded under aerobic conditions using activated sludge of a domestic wastewater treatment plant as inoculum with no lag-phase. A maximum COD concentration of 780 $mg.L^{-1}$ could be metabolized aerobically under the experimental conditions applied. The results show that the COD decreases until reaching the minimum point of degradation. After 40 hours of tests, there was an increase of COD, probably due to the production of microbial soluble compounds that are associated with the decay mechanism and cell lysis. This phenomenon begins when there is a low F/M ratio and substrate limitation, in association with the increase in active biomass degradation.

Fig. 1 Time evolution of the COD concentration in the aerobic biodegradation tests (a) Tests 1 to 4 (b) Tests 5 to 8 - Here

It is noteworthy that over 70% of the organic matter present in the biodiesel production wastewater was degraded in the first fifteen minutes of the tests with no lag-phase. In fact, in most tests, it was possible to obtain removal of COD concentration greater than 90% after fifteen minutes of aeration (Figure 2). After 40 hours, all the tests presented COD removal values above 97%. The substrate utilization rate (SUR) tests ranged between 0.11 and 0.19 mg COD/(gVSS·day). The F/M ratio is a widely adopted parameter, but it is also often set by experience during operation. The procedure to control the F/M ratio is based on the adjustment of the influent load of the substrate in function of the concentration of biomass (VSS) present in the reactor. In this case, monitoring process is essential for its proper performance. Nonetheless, the results permit to conclude that the choice of an aerobic route can provide stability to the wastewater treatment process. This is particularly important along the production of biodiesel, since the raw material used in the transesterification can be altered by environmental or economic reasons and that decision affects the production process and consequently the qualitative and quantitative characteristics of the wastewater generated.

Fig 2. Temporal evolution of the COD removal efficiency in the aerobic biodegradation tests (a) Tests 1 to 4 (b) Tests 5 to 8 - Here

3.2 Anaerobic biodegradability tests

The Figures 3a and 3b show the COD concentration variation along the anaerobic biodegradation tests of the biodiesel production wastewater. The results indicate that within the first 72h, the removal of organic matter has been limited in the majority of anaerobic tests (Figure 4). This fact is probably related to the nature of the inoculum that comes from a domestic wastewater treatment plant and therefore is not adapted to the pollutants present in the wastewater from biodiesel production, as the major presence of methanol. After this adaptation phase, such inhibition of the anaerobic degradation caused by methanol was not expected. In fact, previous studies indicate that methanol has been added as a co-substrate to improve the anaerobic biodegradation of various different complex substrates, such as phenolic wastewater [10-11]. Wang *et al.* [12] stated that the presence of methanol can reduce the toxicity of wastewater from coke production. Confirming this statement, after the period of adaptation (10 days), the soluble COD concentration began to reduce and it was possible to observe the production of biogas in the reactors. From the twenty-second day of tests, it was observed that the concentration of COD reduced significantly. At the same time, the reactors had become less and less pressurized. Only after 27 days, near at the end of the tests, the COD values remained almost constant.

Fig. 3 Time evolution of the COD concentration in the anaerobic biodegradation tests (a) Tests 1 to 4 (b) Tests 5 to 8 - Here

However, it is important to state that chlorides are formed during the acid neutralization step with hydrochloric acid (HCl) as alkaline compounds are used as reaction catalysts and must be neutralized to prevent the formation of soaps. The chloride ion (Cl^-) binds to the cation of the catalyzer providing saline features to the wash water [2]. The characterization tests of the biodiesel production wastewater have shown chloride concentrations between 750 and 1 916 mg.L^{-1}. Some researchers have shown that the chloride concentrations required to cause inhibition of methanogenesis can range from 5 to 9 g.L^{-1} [13-15]. Although the chloride concentrations found in wastewater used in this study were smaller, they may have been responsible for the lag-phase observed in the tests.

Fig 4. Temporal evolution of the COD removal efficiency in the anaerobic biodegradation tests (a) Tests 1 to 4 (b) Tests 5 to 8 – Here

Figure 5 shows the net production of biogas in the three reactors inoculated after the anaerobic tests. The cultures exerted more than 90% of the total biogas production during the first 21 days. It is emphasized that we carried out a new series of tests to detect the biogas production, i.e. the sludge used had not been yet exposed to wastewater from biodiesel production. Therefore, the results seem to confirm a certain inhibition of the process in the first three days. It was possible to obtain 114 mL of biogas for the highest influent COD concentration of 800 mg.L^{-1} and F/M ratio = 0.25. The result of a series of determinations indicated that the CH_4 content of the biogas produced from a wastewater of a biodiesel industry was 84 ± 4%.

Fig 5. Temporal evolution of the Biogas Production experiments for the biodiesel wastewater – Here

4. CONCLUSIONS

Considering the results of anaerobic and aerobic biodegradation tests, using the industrial effluent generated in a biodiesel production plant located in *Candeias*, *Bahia*, Brazil, it can be concluded that:

- The effluent has high biodegradability in both aerobic and anaerobic environment, indicating that organic material present in industrial wastewater is subject to degradation in the biological treatment processes.
- In most aerobic tests, it was possible to obtain removal of COD concentration greater than 90% after fifteen minutes of aeration. After 40 hours, all the tests presented COD removal values above 97%. The substrate utilization rate (SUR) tests ranged between 0.11 and 0.19 mg COD/(gVSS.day) with no lag-phase.
- The degradation of the substrate by anaerobic sludge from a UASB reactor which treats domestic wastewater was only possible after a faster adaptation period of 3 days. After 28 days, it was possible to achieve removal efficiencies above 90% for the conditions applied in anaerobic tests.
- It can be stated that anaerobic treatment presents a viable alternative for the treatment of biodiesel production wastewaters yielding significant energy recovery in the form of methane gas.

ACKNOWLEDGMENTS

The authors would like to thank the Foundation for Research Support of the Bahia State and The Center for Research and Development *Leopoldo Américo Miguez de Mello* (PETROBRAS®) for financial and material support.

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