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TREATMENT OF WASTEWATER FROM BIODIESEL GENERATION AND ITS TOXICITY EVALUATION BY *Raphidocelis subcapitata*

Nádia Hortense Torres[1,2]*, Álvaro Silva Lima[1,2], Luiz Fernando Romanholo Ferreira[1,2], Joyce de Andrade Oliveira[1,2] and Eliane Bezerra Cavalcanti[1,2]

[1] Post-Graduated Program on Process Engineering, Tiradentes University. Av. Murilo Dantas, 300, Farolândia, 49032-490. Aracaju-Sergipe, Brazil.
[2] Institute of Technology and Research. Av. Murilo Dantas, 300, Farolândia, 49032-490. Aracaju-Sergipe, Brazil.

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Abstract - Biodiesel was developed to solve vast economic and environmental problems related to the use of petroleum as fuel. However, large quantities of water are necessary for biodiesel production, generating a high volume of effluent waste. Therefore, it is important to treat this wastewater in order to meet the environmental legislations according to the Brazilian Resolution Number 430/2011 (CONAMA). This work aimed to treat this effluent by coagulation/flocculation (CF) and an electrochemical advanced oxidation process. The combination of CF techniques (pH = 6.5) and electrochemical oxidation (boron doped diamond - BDD - electrode with NaCl) led to 78.5% removal of total organic carbon. The evaluation of the toxicity with *Raphidocelis subcapitata* indicated that the treated samples ($IC50_{72h}$ = 1.66%) had lower chronic toxicity when compared to raw effluent samples ($IC50_{72h}$ = 0.17%). This indicates that the proposed treatment was effective to decrease the organic load and reduce the environmental impact of these effluents in water bodies.

Keywords: Waste treatment, advanced oxidative process, ecotoxicology, biodiesel residue, toxicity.

INTRODUCTION

Biofuel is considered a renewable fuel (Karatzos et al., 2016; Zhu and Hiltunen, 2016) because it is produced from biomass and it may be used to replace fossil derived fuels. A type of biofuel is biodiesel - a methyl-ester compound obtained primarily from vegetable oils (inedible and edible) from soybean, cottonseed, coconut, nuts, canola, sunflower, safflower, castor oil plant, or from animal fats and used frying oil (Widayata et al., 2013). The production of biodiesel has a higher cost when compared to petroleum due to the cost of raw material and processing (Ferrero et al., 2014). However, in Brazil, due to its dynamic ecosystem, an interest has emerged in finding an alternative way to solve the current oil crisis utilizing agricultural sources (Brito et al., 2012). Biodiesel has been effectively part of the Brazilian energy matrix since 2005 (BRASIL, 2005)

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* e-mail: nadiahortense@gmail.com

and, since it is incorporated to mineral diesel, there is a continuous increase in its production.

In the process for obtaining biodiesel, the selected feedstock is subjected to a chemical reaction known as transesterification (Low et al., 2011; Sebastiana et al., 2016; Yellapu et al., 2017). This process generates significant quantities of waste (glycerin and purification washing water) (Leung et al., 2010), with an estimated ratio of 20 to 120 liters per 100 liters of biodiesel (Chavalparit and Ongwandee, 2009). The biggest environmental concern is to develop an effective methodology for the treatment and reutilization of the washing water (Veljković et al., 2015), since this residue has inadequate physical and chemical characteristics for *in natura* release into bodies of water under the Brazilian Legislation CONAMA 430/2011. The existing technologies in refineries are not able to fully retrieve the compounds found in the effluent. Therefore, there is a need to find an alternative and effective treatment method to remove the carbonaceous compounds, which are the most undesirable compounds in the environment (Rocha et al., 2012).

A very important step in wastewater treatment is the coagulation/flocculation process, which is widely used due to its simplicity and cost-effectiveness. Depending on the nature of the treated sample and the overall applied treatment scheme, coagulation-flocculation is usually included, either as a pre- or post-treatment step (Tzoupanos and Zouboulis, 2014).

In this context, the treatment of wastewater containing recalcitrant organic pollutants by an electrochemical advanced oxidation process (EAOP) has been shown to be a promising alternative. EAOP has the main feature of generating hydroxyl radicals (HO•), which react rapidly and indiscriminately with many organic compounds, producing innocuous species, typically inorganic anions, CO_2 and H_2O (Munter, 2001). There are many studies of other treatment processes involving the production of hydroxyl radicals. These use primarily ozone derivatives, hydrogen peroxide, Fenton's reagent or employ electrochemical technologies (Safarzadeh-Amiri et al., 1997; Munter, 2001).

Wastewater treatment techniques such as electroflocculation, electroflotation and electrocoagulation have shown promising results in the treatment of various types of waste water residues (Brito et al., 2012). Treating recalcitrant residues, such as the effluent from biodiesel production, the most appropriate techniques are electrochemical oxidation and electrocoagulation (Chavalparit and Ongwandee, 2009; Jaruwat et al., 2010), and the use of a synthetic effluent allows control of the composition of the solution to facilitate the optimization of the treatment method to be applied to the raw effluent.

To better assess the removal of wastewater pollutants from biodiesel production, chronic toxicity tests are often applied. Employing the Chlorophyceae unicellular alga *Raphidocelis subcapitata*, considered to be an important component in the aquatic ecosystem, is a common technique to measure chronic toxicity (Yan et al., 2015). Due to the lack of scientific work that describes effluent toxicity tests in biodiesel wash waters with *R. subcapitata*, the development of bioassays with this test organism for such effluent is extremely important.

The purpose of this work was to combine the physical-chemical processes of coagulation/flocculation with an electrochemical advanced oxidation process to treat effluent samples (raw and synthetic) resulting from the biodiesel purification step. The ecotoxicological assessment employed the alga *Raphidocelis subcapitata* as a model organism to estimate the quality of the treated effluent.

MATERIALS AND METHODS

Materials

The wastewater used in the treatment and toxicity assays was raw biodiesel wash water. In order to observe and compare the behavior of the electrodes, electrolyses were conducted for the synthetic effluent. Two liters of synthetic effluent were produced with the approximate characteristics of a normal biodiesel refinery effluent. The synthetic effluent was composed of a methanol:glycerol ratio of 10: 1 (v/v), diluted in water, and adjusted according to the components described by Ahmad et al. (2010) and Oliveira et al. (2010). Methanol (purity 99.8%) and glycerol (purity 99.5%) were both purchased from Sigma-Aldrich.

The biodiesel washing water sample was obtained from the Fuel Plant Texas of Brazil/UNICOOPES, located in the city of Itabaiana, Brazil. This micro fuel plant produces biodiesel from vegetable oil used in food frying collected from local homes. This fuel plant delivers 20,000 L/day of biodiesel for the Central Transport Cooperatives of the State of Sergipe (Unicoopes). During the purification step of biodiesel, washing effluent was collected for this study. 50 liters of wash effluent sample was collected in February, 2015, and stored in a polyethylene container, then transferred to the Waste and Effluent Treatment

Laboratory at the Institute of Technology and Research, in Sergipe, Brazil. They were preserved in a dark, refrigerated environment (± 5 ºC) until the treatments. All experiments were performed in duplicate.

Experimental Procedure

The experimental sequence is described in Figure 1.

The procedures included effluent characterization, coagulation/flocculation (CF) tests, electrochemical advanced oxidative treatment assays and toxicity bioassays. The pH values tested in CF experiments (5.0, 5.5, 6.0, 6.5, 7.0 and 7.5) were chosen according to the appropriate disposal range established by Brazilian legislation (CONAMA Resolution No. 430/2011). The resolution indicates a pH between 5.0 to 9.0 to reduce further expense with subsequent pH corrections.

Physico-chemical characterization of biodiesel waste

Physicochemical characterization of samples was carried out according to the methodologies described in *20thStandard Methods for Examination of Water and Wastewater* (2012), as follows: pH (potentiometric Method, 4500 H, pH meter PG1800, GEHAKA); total oils and grease content (TOG) (gravimetric method of extraction by n-hexane, 5220 B/F); the electrical conductivity (method 2510 B) was measured with a conductivity meter (Hanna, HI 2300 model); the turbidity was measured with a turbidimeter (MS Tecnopon, TB100P model); to analyze the total organic carbon (TOC), a Shimadzu TOC-LCSN was used; the color of raw and synthetic effluent was determined by UV-Vis absorption spectroscopy (transmission) (Hach, DR5000 model); alkalinity (method 2320 B); and the hardness (method 2340 C).

Coagulation/flocculation (CF)

Coagulation/flocculation assays and decantation were carried out in a "jar test" type equipment (Floc Control II, PoliControl). This step only optimized the initial pH of the solution from the treatability study parameters for the effluent. The coagulant used was aluminum sulfate (purity 99.5%, Synth). This coagulant has a low cost compared to ferric chloride and is considered to be effective in reducing pollutants in wastewaters with pH 6-8 (Jaruwat et al., 2010). It is also widely used for exhibiting high clotting efficiency, low cost, availability and ease of handling (Ahmad et al., 2006). CA35 (Engeclean) was used as the flocculant at concentrations of 20 and 76.7 mg/L. A proportional amount of 0.45 mg/Lofthe alkalizing agent calcium carbonate ($CaCO_3$, Vetec) was utilized with the goal to obtain no alteration in pH after the treatments.

The CF test was conducted using 300 mL samples of raw and synthetic effluent separately. The initial pH-values were adjusted and varied between the range of 5.0 to 7.5 (Meneses et al., 2012; Cristóvão et al., 2014) using 5 M sulfuric acid (Ngamlerdpokin et al., 2011) and 0.1 M sodium hydroxide. As a coagulant, 5% aluminum sulfate and the polyelectrolyte CA 35 (an emulsified polyacrylamide based polymer) at 25% concentration as a flocculant. The rapid mixture time (100 rpm) was 1 minute and 20 minutes for the slow mixing (40 rpm) (Rattanapan et al., 2011).

After the settling of the flocs formed, 100 mL samples of the treated effluent's supernatant phase were collected for further TOC analysis to determine the removal rate. After identifying the optimum clotting pH for highest TOC removal, samples were run to the next treatment step to evaluate the DSA (Dimensionally Stable Anodes) and BDD (Boron Doped Diamond) electrodes (Jaruwat et al., 2010; Ngamlerdpokin et al., 2011; Ramírez et al., 2012), and the physicochemical parameters (conductivity, pH, TOC and turbidity) for each sample before and after the combined treatment.

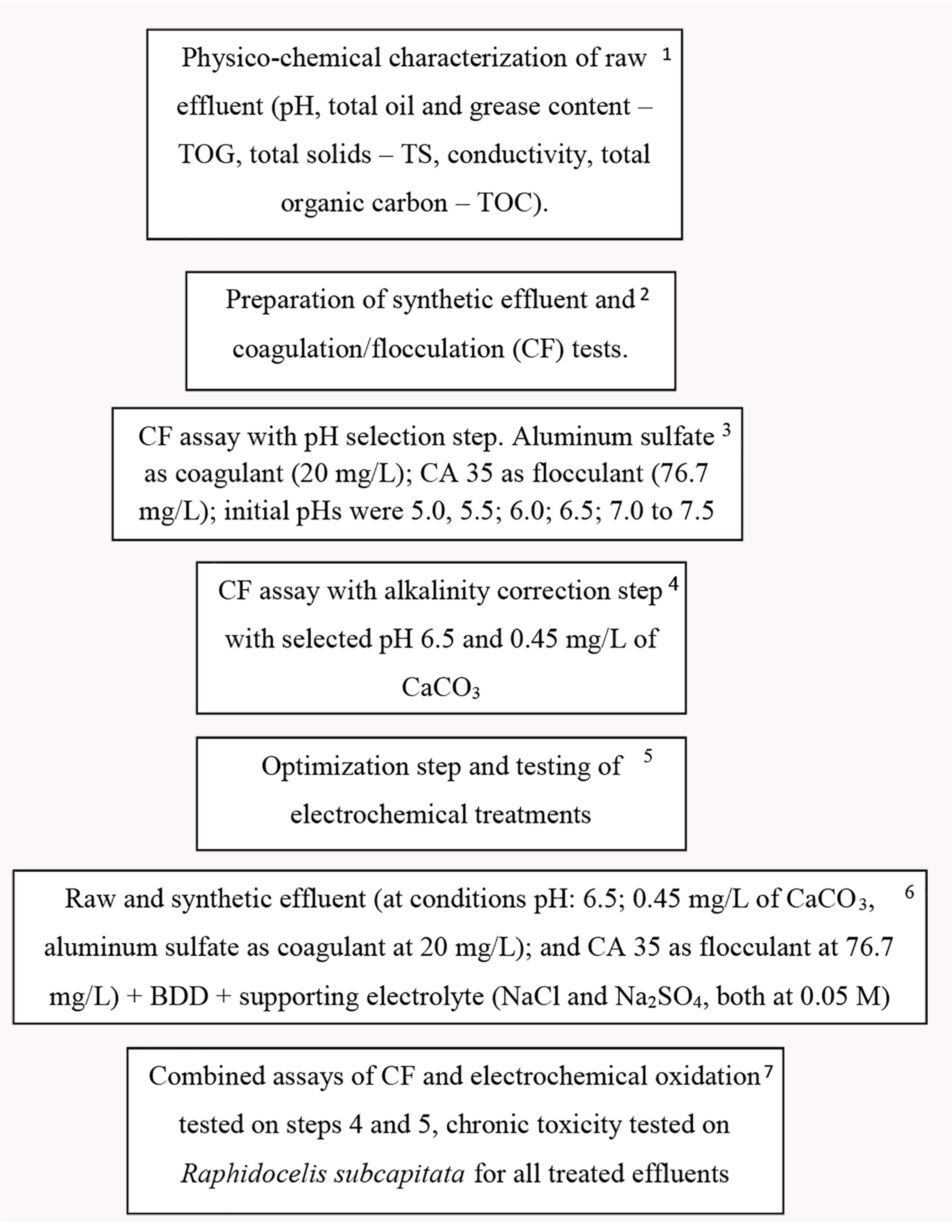
Electrochemical Treatment

100 mL of the homogenized samples were stirred at room temperature (±23 ºC). The electrodes used were an expanded DSA® anode (DeNora, Brazil) and another anode with an area of 8 cm^2 of Si/BDD ($Ti/Ru_{0,36}Ti_{0,64}O_2$, El-Tech, Denmark). A 316 stainless steel perforated cathode plate with an area of 8 cm^2 and 30 $mA.cm^2$ density (Iniesta et al., 2001; Martínez-Huitle and Ferro, 2006) was used together with a supporting electrolyte of NaCl and Na_2SO_4, both at 0.05 M concentration.

The current intensity of the BIOLOGIC VMP3 potentiostat/galvanostat, was set to 300 mA and the distance between the cathode and anode was 2 cm. The electrolytes tested were: NaCl and Na_2SO_4 (Jaruwat et al., 2010), both at 0.05 M. The reaction time was 180 minutes, in which 1 mL aliquots were collected at determined time points to monitor TOC as a function of time.

Toxicology Assay

The seaweed model organism *R. subcapitata* used in the toxicity tests was obtained from the Aquatic Ecosystems Study Group (GEEA), Department



1 Physico-chemical characterization of raw effluent (pH, total oil and grease content – TOG, total solids – TS, conductivity, total organic carbon – TOC).

2 Preparation of synthetic effluent and coagulation/flocculation (CF) tests.

3 CF assay with pH selection step. Aluminum sulfate as coagulant (20 mg/L); CA 35 as flocculant (76.7 mg/L); initial pHs were 5.0, 5.5; 6.0; 6.5; 7.0 to 7.5

4 CF assay with alkalinity correction step with selected pH 6.5 and 0.45 mg/L of $CaCO_3$

5 Optimization step and testing of electrochemical treatments

6 Raw and synthetic effluent (at conditions pH: 6.5; 0.45 mg/L of $CaCO_3$, aluminum sulfate as coagulant at 20 mg/L); and CA 35 as flocculant at 76.7 mg/L) + BDD + supporting electrolyte (NaCl and Na_2SO_4, both at 0.05 M)

7 Combined assays of CF and electrochemical oxidation tested on steps 4 and 5, chronic toxicity tested on *Raphidocelis subcapitata* for all treated effluents

Figure 1. Experimental procedure for effluent treatment optimization.

of Environmental Engineering, Federal University of Sergipe (Brazil). The inoculate was maintained according to Blaise et al. (2000). The assays were adapted from a previous methodology that assesses which concentration effectively inhibited 50% of algal growth after 72 hours ($IC50_{72h}$) compared to the negative control and using serial dilutions ranging from 3.125 to 75% (Blaise et al., 2000; Calomeni et al., 2015).

The negative control was prepared using seven diluted stock solutions in distilled water. The bioassay was performed with algae incubated in continuous illumination of 1,000 lux ± 10%, at a temperature of 24 ± 1ºC, pH 7.0 ± 0.1, and shaken manually three times a day. Assays were performed with a volume of 10 ml and in triplicate in glass test tubes, shaken manually three times a day during the experiments. The cell count was performed before and after the tests, using a Neubauer chamber.

For the statistical analysis of the effluent toxicity studies Microsoft Office Excel (2010) was used for the Tukey test with a significance level of $p<0.01$ (1%) and the software ASSISTAT, 7.5 beta version, 2008 (Ferreira et al., 2011) for the $IC50_{72h}$ values. Table 1 identifies the detailed toxicity tests using *R. subcapitata* with raw, synthetic and treated effluents (after 180 minutes).

RESULTS AND DISCUSSION

Effluent characterization and the Coagulation/Flocculation process

The characterization data of the effluent (Table 2) reflect the operating conditions in the purification process used in the biodiesel plant. The high pH of the raw effluent suggests that the plant applies alkaline catalysis in the transesterification process. The biodiesel differs both in input and in operation of its production. The synthetic effluent of this study was designed to obtain a relatively low pH, simulating the result of an effluent generated after a transesterification process by acid catalysis mixed with the effluent from raw material. This variation in the pH value of biodiesel effluent is common; other studies report values in both the acid and in the alkaline range (Chavalparit and Ongwandee, 2009; Jaruwat et al., 2010; Siles et al., 2010; Suehara et al., 2005). These variations are justified by the processes used for obtaining the biofuel.

The effluent was treated by a physicochemical process employing aluminum sulfate as a coagulating agent (Daud et al., 2015a). It is important to note that the concentration of coagulant used in this study is two times lower than that reported by Ngamlerdpokin et al. (2011) and the authors also used the same coagulant for treating an effluent from biodiesel production.

When using a smaller reagent volume in an effluent treatment process, such as a coagulant, it means that the method is optimized, therefore consuming less

Table 1. Effluents and their identification in chronic toxicity tests for *R. subcapitata*.

Effluent	Identification of effluent
Synthetic	1
Synthetic + CF	2
Synthetic + CF + BDD + Na_2SO_4(0.05M)	3
Synthetic + CF + BDD + NaCl (0.05M)	4
Synthetic + CF + DSA + Na_2SO_4(0.05M)	5
Synthetic + CF + DSA + NaCl (0.05M)	6
Raw	7
Raw + CF	8
Raw + CF + BDD + Na_2SO_4(0.05M)	9
Raw + CF + BDD + NaCl (0.05M)	10
Raw + CF + DSA + Na_2SO_4(0.05M)	11
Raw + CF + DSA + NaCl (0.05M)	12

Table 2. Physicochemical parameters of raw and synthetic effluent.

Parameter	Effluent	
	Raw	**Synthetic**
Wavelength (λ) (nm)	294	281
pH	8.3	4.9
Total oil and grease content (TOG) (mg/L)	650	600
Total Solids (TS) (mg/L)	3126	1560
Conductivity (µS/cm)	1140	5.35
Total Organic Carbon (TOC) (mg/L)	6004	41910
Turbidity (NTU)	107	1.18
Hardness (mg/L)	84.6	-
Alkalinity (mg/L)	152	-

reagents, reducing costs and minimizing the disposal impacts of these products in the environment.

The initial TOC value of raw effluent after the removal of the fatty layer was equal to 6.004 mg/L. Values close to this were reported in previous studies (Siles et al., 2010; Patiño et al., 2012; Daud et al., 2015b). Figure 2 shows results for conductivity, turbidity and TOC for the raw and synthetic effluent before and after treatment by coagulation/flocculation as a function of the different initial pH values.

The different physical and chemical characteristics of the effluent are a concern regarding the compliance with the environmental legislation for industrial wastewater under the CONAMA Resolution 430/2011.

The pH of the residue from the biodiesel purification process increases with time (Brito et al., 2012), hence the correction with the alkalizing calcium carbonate ($CaCO_3$) to avoid variation. Chen et al. (2000) found that, in the pH range of 6.87 to 9.10, pH values in the range of 7.47 to 8.95 were attained. These values did

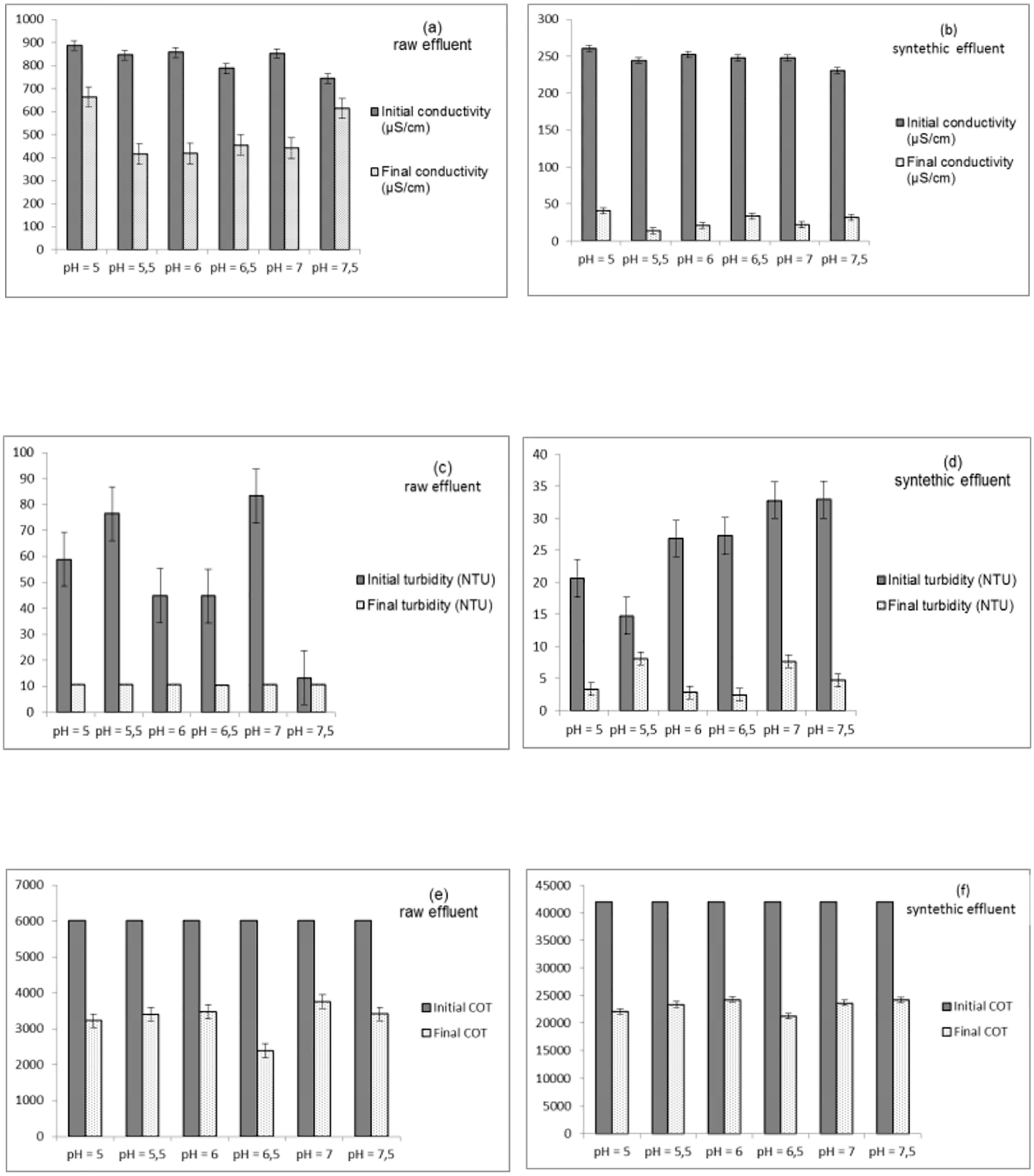


Figure 2. Parameter values of conductivity (a) and (b), turbidity (c) and (d) and TOC (e) and (f) in relation to the pH values evaluated for the samples of raw and synthetic effluent before and after CF treatment.

not occur in the samples analyzed due to the addition of alkalizing agent (Figure 2).

The data for electrical conductivity, turbidity and TOC, initial and final, for the two types of waste (Figure 2) show that the physicochemical treatment employed in the studied conditions was able to promote a reduction in the values of these parameters. With respect to conductivity, the synthetic effluent was more susceptible to the CF treatment, with reductions between 85% (pH 5) and 95% (pH 5). In general, this reduction is very significant for all pH values evaluated. For the raw wastewater samples, the reduction in this parameter was not so significant, ranging from 17% (pH 7.5) to 55% (pH 5).

Mohouedhen et al. (2008) studied the electrocoagulation process using aluminum electrodes and verified that, when the initial pH is acid, the final pH value increases; when the initial pH is alkaline, the final pH decreases, as occurred in this study where the initial pH decreased from 6.5 to 5.9.

This difference in the results for conductivity can be associated with the complexity of the aqueous matrix. However, regardless of the type of effluent, the pH 5 sample was the one with the greater reduction.

According to CONAMA 430/2011, conductivity indicates water salinity where values close to 1000 µS/cm are for industrially contaminated water, which is the case of the samples analyzed in this study.

Turbidity, which indirectly indicates the presence of colloidal substances in the effluent (Kirk, 1985), after physicochemical treatment and regardless of sample type or studied pH, is below the value set in the current class 2 wastewater release legislation, which sets the maximum of 100 uT (CONAMA, 430/2011). The minimum and maximum percentages of reduction achieved with treated raw effluent were 5% (pH 7.5) and 87% (pH 7.0), respectively, and for the synthetic effluent 43% (pH 5.5) and 91% (pH 7.5).

Employing the treatments by coagulation/flocculation and decantation, the values of the physicochemical parameters for the treated effluents are in compliance with the current legislation and lower than those found in the literature (Gomes et al., 2015). Also, the turbidity of the raw and synthetic effluent was reduced to 87 and 91% compared to the effluent before treatment (Figure 3).

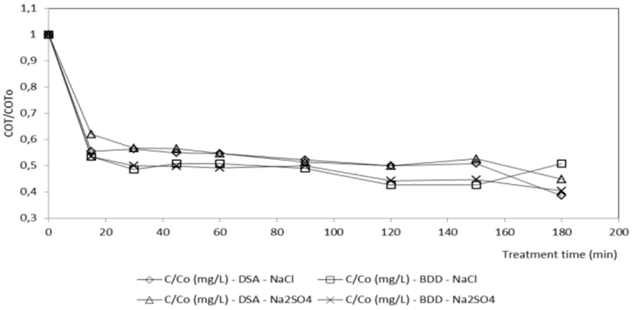


Figure 3. TOC removal rate as a function of treatment time, using the combinations: DSA electrode and NaCl (—□—); DSA electrode and Na_2SO_4 (—△—); BDD electrode and NaCl (—◇—); and BDD electrode and Na_2SO_4 (—×—), tested in synthetic effluent samples (T = 23 °C and $pH_i = 6.5$).

The percentage of mineralization of components of the raw and synthetic effluent samples was obtained from the equation: TOC = $[(TOC_{initial} - TOC_{final})/TOC_{intiial}] \times 100$ (Nellier et al., 2015). From the organic matter standpoint (Fig. 1-e and 1-f), it is observed that the TOC parameter was significantly affected by the physicochemical treatment. Greater removal efficiencies were achieved at pH 6.5, obtaining a reduction in TOC equal to 78.5% and 61.5% for raw samples and synthetic effluent, respectively. In another study the removal only reached 42% (Cotillas et al., 2016), meaning that the proposed treatment was efficient.

After analyzing the data for the physicochemical parameters of the treated effluent by the coagulation/flocculation and decantation process, it was possible to suggest that the best working pH is 6.5. It is important to note that, although the treated effluent has some suitable parameters for the release into the environment, it still requires further treatment in order to reduce the remaining organic load, considered high. Table 3 shows data for the physicochemical characterization obtained for the effluent (raw and synthetic) treated by the process of coagulation/flocculation and decantation, optimized for an initial pH of 6.5.

In the work reported by Yang et al. (2010), the optimal pH of 6.0 for both coagulants was determined in a study of the effect of the coagulant dosage and pH on the efficiency of aluminum sulfate coagulating performance in surface water in China. Other authors reported that efficient removal is accomplished when the pH is maintained near neutral (Siles et al., 2010).

Table 3. Physicochemical characterization of raw and synthetic effluent after treatment by optimized CF, both with pH = 6.5.

Parameter	Raw effluent		Synthetic effluent	
	Before CF	**After CF**	**Before CF**	**After CF**
Conductivity (µS.cm^{-1})	787	455	247.2	33.5
Turbidity (NTU)	44.7	10.5	27.2	2.45
TOC (mg/L)	6004	2390	41910	21259

Electrochemical Treatment

To conduct the electrochemical reactions, the pH was set to 6.5 as the initial pH for the raw and synthetic effluents, optimized and pre-defined in the section "Experimental Procedure". Previous studies have reported that the variables that influence the electrochemical wastewater treatment can be taken as an initial baseline for the development of this process (Chen et al., 2000; Mollah et al., 2001; Emamjomeh and Sivakumar, 2009). Therefore, some variables were tested here, such as the supporting electrolytes (NaCl and Na_2SO_4), treatment time and the DSA and BDD electrodes.

The results from the various electrochemical treatments show that synthetic wastewater samples treated by the CF process at pH 6.5, subjected to electrochemical treatment employing a DSA electrode and perforated 316® stainless steel plate, and NaCl as the supporting electrolyte were the experimental conditions that showed the highest removal rate of 61.5% (Figure 4).

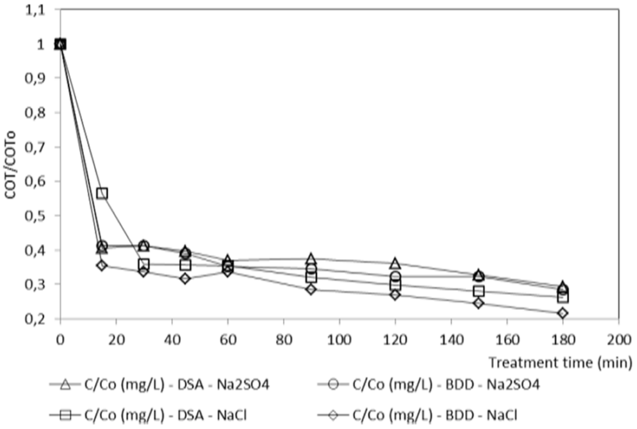


Figure 4. TOC removal rate as a function of treatment time, using the combinations: DSA electrode and Na_2SO_4 (—△—); DSA electrode and NaCl (—□—); BDD electrode and Na_2SO_4 (—○—); and BDD electrode and NaCl (—◇—), tested in raw effluent samples (T = 23 ºC and $pH_i = 6.5$).

The tests with treated raw effluent using a Si/BDD electrode, a perforated 316® stainless steel plate and NaCl as a supporting electrolyte were the experimental conditions that showed the highest removal rate of 78.5% (Fig. 4). Other studies have reported the removal of organic matter from sanitary effluent up to 89% by using the Fenton process. Other authors (Srirangsan et al., 2009) have achieved a removal rate of 55.4% using the electrocoagulation process, which was also used by Romero et al. (2013). Treatment times used by other authors, such as Cotillas et al. (2016), established 10 hours for the treatment of sewage and Brito et al. (2012) electrochemically treated the water wash effluent for 24 hours. These treatment times differ from this current study of 1 hour and 30 minutes (Fig. 4). This is because the BDD electrode has a large oxidation capacity for organic pollutants, requiring a smaller electrolysis time to achieve complete mineralization (Martínez-Huitle et al., 2012).

The electrochemical processes require acidification pretreatment, high electricity and process costs, although it offers efficiency in contaminants removal and low sludge production (Jaruwat et al., 2010). There are previous studies stating that the pH values of 2 and 12, in electrocoagulation processes, remain unchanged (Meneses et al., 2012).

Chavalparit et al. (2009) reported a 55.43% COD removal from biodiesel wastewater, a value lower than that observed for electrochemical treatments proposed for raw and synthetic effluents, which were 78.5% and 61.5%, respectively.

Emamjomeh and Sivakumar (2009) investigated the electrocoagulation process for treatment of oily wastewater and showed an electrocoagulation reactor that could successfully destabilized oil-in-water emulsions. Dispersed gas bubbles formed from electrolysis are extremely fine and uniform, and it justifies the use of electrochemical treatment in this study. A more efficient COD removal for raw and synthetic effluents, using BDD with NaCl as an electrolyte support, was due to the higher efficiency electrode for the reaction of chlorine release. This means that the ternary electrode has a greater potential for producing active oxygen species, such as hydroxyl radical. These radicals aid in the process of effluent degradation from used vegetable oil, which justifies the slightly higher performance of the electrode Si/BDD (Luu et al., 2015).

The choice of CF treatment followed by electrochemical oxidation is due to limitations of the electrochemical coagulation as follows (Khandegar and Saroha, 2013): the sacrificial anodes need to be replaced periodically; and electrocoagulation requires a minimum solution conductivity depending on reactor design, limiting its use with effluent containing low dissolved solids. And there is a lack of literature describing experiments using CF treatments followed by electrochemical oxidation, and this is why this combination was tested for biodiesel washing wastewater.

Chronic toxicityusing *R. subcapitata*

Toxicity tests using the algae *R. subcapitata* were based on the methodology proposed by Blaise et al. (2000). In this study 10 mL of sample was used in each test according to Nogueira et al. (2007), who also used the same assay volume with the algae *T. weissflogii*, and an initial inoculum of 1x104 cells/mL. Other authors also used *R. subcapitata* (Radix et al., 2000; Melo et al., 2013) for toxicity testing and an initial inoculum of 5x10^5 cells/mL of *R. subcapitata* in a volume sample of 50 mL. The same was observed in another study using 25 mL for the assays (Debenest et al., 2012).

The results of $IC50_{72h}$ values for *R. subcapitata* exposed to the various effluents are shown in Fig. 5. According to the data, the addition of new oxidative methods to treat the effluent in question promoted a reduction in the toxicity for *R. subcapitata*. This indicates that the treatments were effective and reduced growth inhibition for the tested organism (Figure 5).

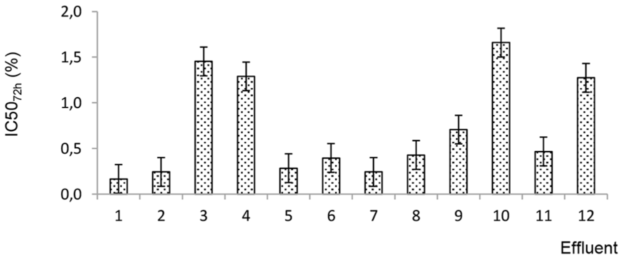


Figure 5. Percent $IC50_{72h}$ for *R. subcapitata* exposed to the effluents 1 to 12, untreated synthetic and raw, as well as treated by the coagulation/ flocculation, BDD, DSA, Na_2SO_4, NaCl for chronic toxicity tests.

Regarding the inhibition values, the untreated effluents 1 (synthetic) and 7 (raw) can be considered more toxic because the $IC50_{72h}$ values were 0.17% and 0.24%, respectively. Only the use of physicochemical treatment of coagulation/flocculation and sedimentation was not sufficient to improve the toxic response in the effluent samples 2 (synthetic + CF) and 8 (raw + CF). This shows that the isolated use of this technology it is not capable of reducing the toxic effects of biodiesel effluent against *R. subcapitata*. In relation to the various treatments proposed, the combined treatment (CF + electrochemical oxidation) decreased the toxic potential of the effluent because the higher the $IC50_{72h}$ value, the less toxic the effluent will be. It means that a larger volume of the effluent must be used in order to inhibit the growth of the algae.

Effluent No. 10 (raw + CF + BDD + 0.05M NaCl) showed better results for the electrochemical treatment with greater TOC reduction (78.5%), and the highest value of $IC50_{72h}$ (0.17%), indicating less toxic potential in relation to other tested effluents and treatments.

Toxicity tests carried out in Recife (Brazil), using the alga *T. weissflogii,* reported a toxic sensitivity after 72 h (Araújo and Souza-Santos, 2013). The proposed test period of 72 hours is recommended by OECD (2006) and it was also the time tested by Debenest et al. (2012).

Growth rates were significantly lower in cultures exposed to the three highest effluent concentrations (12.5, 25, 50 and 75% v/v). Through the coagulation/ flocculation, BDD, DSA electrodes and Na_2SO_4, inhibition ranged from 60.9 to 100% at these concentrations. The effluent tested in other publications (Debenest et al., 2012) showed a more toxic effect due to greater growth inhibition of *R. subcapitata* (from 20 to 40%) at concentrations of 12.25, 25 and 37.5%.

Tests with algae have been widely used to evaluate the inhibitory effects of natural waters, effluents and chemical substances (Stauber, 1995). Therefore, in the tests with raw and synthetic effluents without any treatment, no increase in algae concentration was observed. It is important to note that both inhibition and growth stimulation of organisms are equally undesirable, because any change in algae productivity or in the community composition, compared to the normal levels for that particular system, may threaten the entire balance of the ecosystem (Blanck et al., 1984).

CONCLUSIONS

This study concludes that the best treatment of biodiesel washing wastewater is by CF followed by electrochemical oxidation in combination with DSA electrodes and NaCl and BDD and NaCl, with samples at pH 6.5 and COD removals of 61.5% and 78.5%, respectively. The proposed combined treatment demonstrates effectiveness by toxicity tests with *R. subcapitata*. A significant decrease in toxicity was obtained following the described treatment, indicating that it can minimize the environmental impact caused by these effluents. Through this study, various physical and chemical treatments can be used in combination to reduce the pollutant parameters and CF treatment followed by electrochemical oxidation is a promising technique.

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