Vol.62: e19170518, 2019
http://dx.doi.org/10.1590/1678-4324-2019170518
ISSN 1678-4324 Online Edition

Article - Environmental Sciences

Consortium of Microalgae for Tannery Effluent Treatment

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Received: 2017.08.15; Accepted: 2019.05.19.

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HIGHLIGHTS

- The microalgae consortium was able to grow in tannery effluents.
- The culture achieved higher growth in the diluted raw effluent.
- Treatment with a microalgae consortium led to a drastic reduction of pollutants.
- The use of the microalgae is an eco-friendly step in the treatment of effluents.

Abstract: Wastewater generated in tanneries have essential elements for microalgae growth, but it has also some toxic compounds that may hinder or restrain the growth of microalgae in this environment. This work tested microalgae consortium growth originating from a deactivated effluent treatment decanter of a complete tannery (beamhouse to finished leather) for the treatment of wastewater of a tannery processing wet-blue leather to finished leather. It was initially evaluated the growth of the microalgae consortium in the three effluents diluted in 50% distilled water: raw effluent (50RE50W), effluent after primary coagulation/flocculation (50PE50W), and effluent after primary and secondary biological treatment (50BE50W). After 16 days of cultivation, the 50PE50W presented the highest

biomass concentration (1.77 g L−1). The highest removal values for effluents 50RE50W, 50PE50W and 50BE50W were 51.02%, 99.90%, 82.88%, and 91.75% for chemical oxygen demand (COD), N-NH3, TKN, and P-PO4-, respectively. It was verified low levels of nutrient removal in the raw effluent (100RE), since the consortium was not able to grow in this medium. Finally, at concentrations of 25RE75BE (25% raw effluent diluted with 75% effluent after the biological treatment) and 50RE50B (50% raw effluent diluted with 50% effluent after the biological treatment), effective removal values were reached. Biomass growth concentration up to 1.3 g L-1 and removal values for N-NH3, TKN, P-PO4, COD, total organic carbon (TOC) and biological oxygen demand (BOD5), of 99.90%, 79.36%, 87.82%, 14.26%, 35.82%, and 42.86%, respectively, were reached in 50RE50B.
Keywords: Microalgae consortium, Tannery, Effluent treatment.

INTRODUCTION

Research in biotechnology has been very promising with numerous studies using microorganisms such as microalgae, bacteria, fungi, and their byproducts, for the treatment of effluents because they provide means of economical and eco-friendly alternatives[1-4]. Microalgae present a great potential for effluent treatment since they exhibit efficient removal of organic matter, nitrogen, phosphorus, and other nutrients present in the environment[5]. These substances, when released into the water sources, cause nutrient enrichment and consequently, the phenomenon of eutrophication, leading the decrease of dissolved oxygen, resulting in the death of some living species in the aquatic system[6].

The effluents generated in the leather industry present high pollutant loads due to the high content of organic nitrogen, toxic metals, sulfides, high biological oxygen demand (BOD), high chemical oxygen demand (COD) and high concentrations of suspended solids[7]. According to the Brazilian Leather Guide[8], in 2016 Brazil had the second largest herd of cattle in the world, exporting 152,864,109 m^2 of rawhide and leather (salted hide, wet-blue, crust and finished leather), being 60.5% finished material. The leather wet finishing and the final finishing are the end stages of leather processing when the material acquires the desired final characteristics such as physical-mechanical resistance, softness, color, durability, stamping and the protective coating[9]. The wet finishing steps of leather processing (deacidulation, retaining, fatliquoring and dyeing) generate effluents with several chemical compounds such retanning agents, neutralization salts, dyes, fatliquoring oils, surfactants polymers, pigments, solvents, resins, and other chemicals. These wastewater streams present high toxicity load that needs to be advanced treated before being discharged to the water sources[10].

Several studies reported the efficiency to employ the microalgae *Scenedesmus* sp. for the treatment of raw wastewater from effluents of the tannery industry for nutrients removal [11,12]. Furthermore, studies show that microalgae consortium is more efficient in the removal of pollutants and nutrients, like nitrogen, phosphorus, and ammonium, from wastewater when compared to individual microorganisms applied for the treatment local municipal wastewater[13] and dairy farm[14]. In this context, investigations about the isolation of the microalgae species present in the consortium in the environment are searched. There are several techniques for obtaining a single species of microorganism, described by some authors. However, these techniques require slow and detailed procedures[15-17].

In this context, the present study aims to investigate the use of a microalgae consortium collected from a deactivated tannery effluent decanter, for the treatment of three types of effluents collected in a treatment effluent plant of a tannery that processes leather from wet-blue to finished leather.

MATERIAL AND METHODS

Microalgae selection and growth

A collection of an effluent sample containing microalgae was performed in a deactivated effluent treatment decanter in an effluent treatment plant of a complete processing tannery (beamhouse stage to finished leather). The observed green color indicated the presence of some microorganism that could be probably already adapted to biodegrade this kind of effluent. The effluent sample was observed under the optical microscope, and several microalgae were visible (microalgae consortium).

Every 10 days 10% (v/v) of the microalgae culture was transferred, in their exponential growth phase, and supplemented with 90% Tris-Acetate-Phosphate (TAP) medium in 250 mL Erlenmeyer flasks[18]. The cultivation was kept at room temperature (24°C), under constant agitation by bubbling compressed atmospheric air (1.0 L min^{-1}) and continuous lighting (10,000 lux).

Isolation of the predominant species was made using the method of successive dilutions in the TAP medium, followed by plating in agar medium, as proposed by Lourenço[17]. This method is the most used when the desired species is abundant in the medium. It was performed under photoautotrophic growth conditions. The microalgae consortium is presented in Figure 1. The microalgae identification was based on the cell shape, ease of locomotion and adaptation in various medium[23] and it was done by the authors. Tetraselmis sp. was the dominant genus of microalgae present, as can be seen in the picture of the isolated microalgae already reported[19]. However, it was not possible to identify the strains at the species level, since no molecular structure studies have been carried out and there is little information on *Tetraselmis* imaging studies at species level[25].

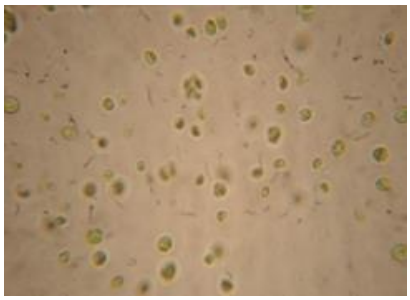

Figure 1. Optical microscopy of the microalgae consortium.

Effluents and Experimental setup

The same conditions of microalgae cultivation already described were used for experiments with tannery effluents (performed in duplicates). The effluents were collected from a tannery that processes leather from wet-blue to finished leather. The following effluents were collected: raw effluent without previous treatment (RE); effluent after physicochemical treatment (PE), that is, after primary coagulation/flocculation and sedimentation; and, effluent after the mentioned physicochemical treatment followed by secondary biological treatment and sedimentation (BE). Samples were stored in polyethylene bottles and kept refrigerated until used.

In the first run was tested the cultivation of the microalgae consortium for the treatment of the effluents RE, PE and BE diluted with 50% of distilled water, named respectively as 50RE50W, 50PE50W and 50BE50W. The cultivation was carried out in 500 mL photobioreactors filled with 300 mL of final volume (Figure 2).

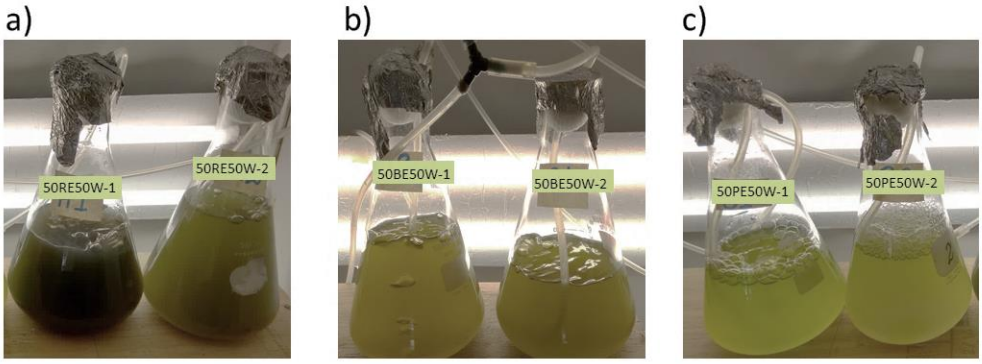

Figure 2. Image of microalgae in tannery effluents diluted (50% effluent + 50% water): a) 50RE50W, b) 50PE50W, and c) 50BE50W.

In order, to avoid spend clean water for the dilutions, in the second run was tested the cultivation of microalgae consortium using the biologically treated effluent (BE), for the dilutions, under the following conditions: 100% raw effluent (100RE); 50% RE diluted with 50% BE (50RE50BE); 25% RE diluted with 75% BE (25RE75BE). The cultures were grown for 19 days in photobioreactors of 5000 mL filled with 4.500 mL final volume.

Effluents Analysis and Biomass Growth

The microalgae growth in the effluent was daily monitored by optical density (OD) measurement at 570nm wavelength using a spectrophotometer (T80+UV/Vis, PG Instruments) after appropriate dilution. A calibration curve was set to obtain the biomass concentration through the absorbance as a function of the dry weight of the cells. In this way, samples of the microalgae consortium in TAP medium were analyzed in the spectrophotometer at different concentrations, and their respective dry cell weight was obtained by filtration of the samples in pre-weighed 0.7 µm membranes, followed by drying at 100 °C for 24 h.

Samples of effluents before treatment and at the end of the last day of cultivation were collected and filtered through membranes of 0.7 µm for analysis of the removal of Total Kjeldahl Nitrogen TNK, ammoniacal nitrogen (N-NH_3), phosphorus (P-PO_4), BOD_5 and Total Organic Carbon (TOC). The N-NH_3 quantification was analyzed on the Metrohm Basic IC Plus Package Ion Chromatograph (METROHM 20000). TKN and COD were quantified according to Standard Methods for the Examination of Water and Wastewater APHA (1998)[20], and phosphorus by ABNT NBR 1277221. The COD was carried out according to VELP Scientific BOD System 6, incubated (Eletrolab EL202/3 model) at 20°C for 5 days and TOC was performed using a Total Organic Carbon Analyzer (Model TOC-L TNM, Shimadzu).

The removal of pollutants R (%) was calculated by Equation (1):

$$R(\%) = \left(\frac{IC-FC}{IC}\right) * 100 \quad (1)$$

where IC is the initial concentration and FC is the final concentration on the last day of effluent treatment for the compounds: TKN, N-NH_3, P-PO_4, BOD, COD, and TOC.

The treatment of statistical data of parameters was performed with the software Statistica® 13, using analysis of variance (ANOVA) with a significance level of 95%.

RESULTS AND DISCUSSION

Figure 3 shows the average growth of the microalgae culture in the mediums composed by 50RE50W, 50PE50W and 50BE50W. Firstly, it was observed a lag phase until the 4th day of culture. Furthermore, the consortium was able to grow in all tested effluents. The best microalgae growth was observed for 50RE50W with highest growth rate. This can be explained because the 50RE50W effluent offers higher amounts of nutrients than 50BE50W and 50PE50W. After the 12th day of cultivation, the growth started to stabilize for the diluted effluents after the primary treatment (50PE50W) and after the biological treatment (50BE50W), this indicates that they were entering into stationary phase.

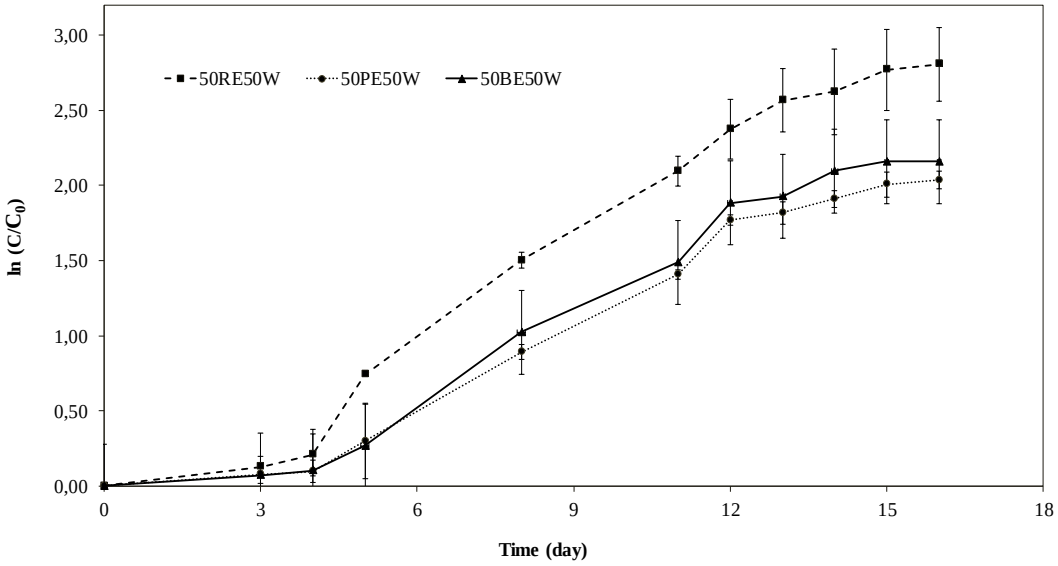

Figure 3. The average growth of microalgae in tannery effluents diluted (50% effluent + 50% water): raw effluent-RE, effluent after physical-chemical- treatment-PE, effluent after biological treatment-BE, and water-W.

The average results of analysis of the effluents with microalgae initial and final after 16 days of treatment are presented in Table 1. The results demonstrated significant differences between the removals of the COD, TKN, and P-PO_4^-, for the studied effluents. There was no significant difference between effluents for removal N-NH_3. The highest removal values for effluents 50RE50W, 50PE50W and 50BE50W diluted in water 50% were 51.02%, 99.90%, 82.88%, and 91.75% for COD, N-NH_3, TKN, and P-PO_4^-, respectively.

The concentrations of N-NH_3 at the end were below to the value detected by the method ion chromatography (0.05 mg L^{-1}), indicating that the removals were higher than 99.9%. This result is in agreement with the data found by Sun and Simsek[23] (99.0% of ammonia was removed from domestic wastewater at 21 days of incubation). It is reported that the ammonia is nitrified for nitrate or used to microalgae growth. The assimilation of nitrogen by microalgae is carried out in the form of nitrates, nitrites and mainly ammonia. Ammonia is easily absorbed by microalgae because it is the most reduced form of nitrogen

compounds. The phosphorus is consumed mainly in inorganic form and with the help of enzymes in its organic form[24].

Table 1. Average parameters of the diluted effluents before and after microalgae treatment.

Parameter		50RE50W[1]		50PE50W[1]		50BE50W[1]	
		Conc[2]. [mg l^{-1}]	Removal [%]	Conc[2]. [mg l^{-1}]	Removal [%]	Conc[2]. [mg l^{-1}]	Removal [%]
P-PO_4	IC[3]	2.16±0.03	84.72[b]	2.06±0.04	88.35[a]	2.06 ±0.03	91.75[a]
	FC[4]	0.33±0.02		0.24±0.03		0.17±0.02	
TNK	IC[3]	155.40±0.39	82.88[a]	140.00±0.21	76.00[b]	127.40±0.03	74.73[c]
	FC[4]	26.60±0.12		33.60±0.14		32.20±0.12	
N-NH_3	IC[3]	53.90±1.11	99.9[a]	58.54±0.49	99.9[a]	49.66±1.10	99.9[a]
	FC[4]	n.d.[5]		n.d.[5]		n.d.[5]	
COD	IC[3]	1721.72±2.37	51.02[a]	1150.20±1.25	43.64[b]	589.70±1.54	50.94[a]
	FC[4]	843.44±1.07		648.30±1.16		289.30±1.02	

[1]50RE50W, 50PE50W and 50BE50W were the effluents diluted with 50% in distilled water

[2]Concentrations [3]Initial concentration [4]Final concentration [5]Not determined.

Different letters a, b, c within a same line show significant differences according to honestly significant difference (HSD) test at a significance level of 95% and same letters indicate no significant difference.

The initial pH was not adjusted prior to inoculation. The pH values exhibited a discrete variation for all the tests throughout the treatment (Figure 4). The small elevation of pH (from 8.0 to 8.5) in the first days of cultivation suggests an increase in photosynthetic yield. The increase of the alkalinity of the culture medium is due to the consumption of the carbon dioxide by the microalgae and consequently increase the rate of growth and production of biomass[22].

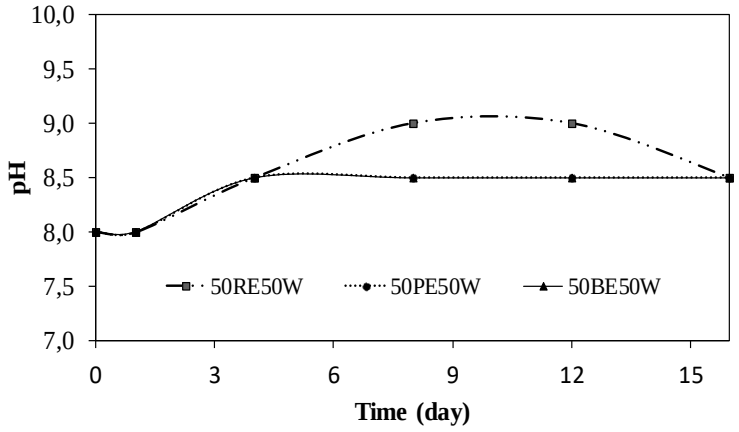

Figure 4. Variation of pH over the time by microalgae cultivation in tannery effluents diluted (50% effluent + 50% water): raw effluent-RE, effluent after physical-chemical- treatment-PE, effluent after biological treatment-BEW, and water-W.

The following experiments were carried out with the most polluted effluent (nutrients rich), that is, the raw effluent without treatment (RE), since similar nutrients removal were reached among the effluents. The dilutions were performed with the effluent after the secondary biological treatment (BE), as a sustainable way to reuse this water as this effluent (BE) had a lower COD than the (RE), leading to less toxicity. The objective was not to use the clean water for dilution. Figure 5 shows the average growth curve of the consortium in the effluents: the 50RE50BE and 25RE75BE obtained higher growth rates when compared to effluent 100RE. In the 50RE50BE, from the 11th day, the growth showed a small decline, differently to 25RE75BE which had a rapid decline after the 16th day, and the 100RE that on the 4th day began to enter the stage of cell death and presented smaller growth than the other 2 means.

When pure raw wastewater was applied (100 RE), it was possible to observe a reduction in the growth. In this case, the high concentration of pollutants leads to higher background turbidity that hinders the passage of light and reduces the growth of microalgae resulting in reduced nutrients removal, making it unviable for effluent treatment.

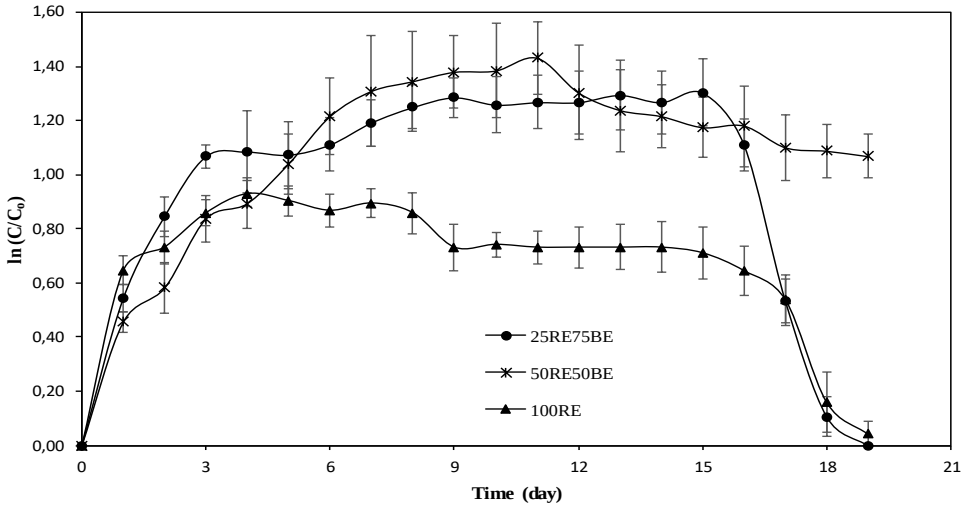

Figure 5. The growth of the microalgae consortium during 19 days of cultivation in tannery raw wastewater 100RE, 50RE50BE and 25RE75BE.

The P-PO_4, TKN, N-NH_3, COD, TOC and BOD initial and final average concentrations are shown in Table 2. The results showed high removals of ammoniacal nitrogen (approximately 100%) for the culture conditions 25RE75BE and 50RE75BE, and only 48.50% removal for 100RE. Higher removals of total nitrogen (86.56% and 79.36%) were also verified for the 25RE75BE, 50RE50BE conditions, respectively. However, a low total nitrogen removal (6.94%) was observed for the condition without dilution (100RE). It was also to observed the decrease in phosphorus concentration throughout the cultivation since it was achieved 87.82% of removal for the condition 50RE50BE followed by 75.86% in 25RE75BE effluents. According to the results, the removal of phosphorus, COD, TOC and total nitrogen were more efficient in the 50RE50BE effluent than in 25RE75BE effluent. As NTK is composed of free ammonia and organic nitrogen it can be stated that the remaining

concentrations are organic nitrogen since 99.9% ammonia was removed. All removal values presented significant differences for the distinct concentrations of effluent, except $N\text{-}NH_3$ that is not statistically different between effluents 25RE75BE and 50RE50BE.

Table 2. Average parameters of the raw effluents 25RE75BE, 50RE50BE and 100RE before and after microalgae treatment.

Parameter		25RE75BE[1]		50RE50BE[2]		100RE[3]	
		Conc[4]. [mg l^{-1}]	Removal %	Conc[4]. [mg l^{-1}]	Removal %	Conc[4]. [mg l^{-1}]	Removal %
P-PO_4	IC[5]	1.45±0.09	75.86[a]	2.38±0.12	87.82[a]	4.67±0.11	14.99[b]
	FC[6]	0.35±0.09		0.29±0.08		3.97±0.04	
TNK	IC[5]	69.20±0.53	86.54[a]	122.64± 1.02	79.36[b]	262.30± 0.64	6.94[c]
	FC[6]	09.30±0.25		25.31± 0.67		244.10±0.37	
N-NH_3	IC[5]	18.31±0.46	99.9[a]	39.80±0.97	99.9[a]	91.09± 1.27	48.50[b]
	FC[6]	n.d.[7]		n.d.[7]		26.91±1.10	
COD	IC[5]	525.00±1.03	21.14[b]	1340.00±1.89	35.82[a]	2450.00±1.90	15.14[c]
	FC[6]	414.00±0.83		860.00±0.67		2079.00±1.04	
TOC	IC[5]	49.80±0.76	11.04[a]	96.21±1.17	14.26[a]	202.20±0.98	0.15[b]
	FC[6]	44.30±0.56		82.49± 1.36		201.90±0.43	
BOD_5	IC[5]	690.00±1.86	49.35[a]	1540.00±2.12	42.82[b]	2790.00±1.95	8.14[c]
	FC[6]	349.50±1.53		880.00±2.31		2563.00±1.60	

[1]100% raw effluent (100RE); [2]50% RE diluted with 50% BE (50RE50BE); [3]25% RE diluted with 75% BE (25RE75BE) water; [4]Concentrations; [5]Initial concentration; [6]Final concentration, [7]No determined. Different letters a, b, c within a same line show significant differences according to honestly significant difference (HSD) test at a significance level of 95% and same letters indicate no significant difference.

CONCLUSION

The microalgae consortium showed high growth performance in tannery effluents treatment in diluted conditions. The highest removal values for effluents 50RE50W, 50PE50W and 50BE50W diluted in water 50% were 51.02%, 99.90%, 82.88%, and 91.75% for COD, N-NH3, TKN, and P-PO4-, respectively. When comparing the raw effluent at concentrations 25RE75BE (with 75% BE), 50RE50BE (with 50% BE) and 100RE, the best microalgae growth was reached in effluents with 50RE50BE dilution, being efficient in removal of nitrogen, nitrogenous ammonia, COD, TOC, and phosphorus. This can be justified by the fact that the dilution of 25RE75BE results in few nutrients necessary for the growth of microalgae. The dark color of the effluent (100RE) prevents light from entering the effluent, slowing the growth rate and resulting in low level of removal. In addition, the use of the microalgae consortium is an eco-friendly step in the treatment of effluents for the removal of nutrients, since it requires no additional treatment, chemicals are not used and biomass has the potential for various applications such as lipid and biodiesel production.

Acknowledgments: We would like to thank the scholarships provided by Brazilian Federal Agency for the Scholarship (CNPQ) National Council for Scientific and Technological Development, Agency Financier of Studies and Projects (FINEP) for the financial support by the public MCTI/FINEP CT-HIDRO 01/2013 and also we are grateful to Tannery CBC Leather Ltda for the samples of wastewater.

Conflicts of Interest: "The authors declare no conflict of interest."

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