INFLUENCE OF PHOTOPERIOD ON BIOMASS PRODUCTION AND REMOVAL OF NUTRIENTS FROM TANNERY EFFLUENTS WITH MICROALGAE CONSORTIUM

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Abstract. Wastewater from tanneries besides having toxic compounds also contain nutrients such as carbon, phosphorus, and nitrogen, which facilitate the rapid multiplication of microalgae. Currently, several researches search microalgae capable of growing in industrial effluents, exploiting the advantages of removing the nutrients present in these waters and producing biomass with high value added. The liquid effluents produced in tanneries for finished leather have essential nutrients for the growth of microalgae, but also some compounds that may restrict or hinder the growth of microalgae in this medium. Therefore, the present work has the objective to evaluate the growth of a microalgae consortium for the removal of phosphorus and ammonia from wastewater streams of a tannery processing wet-blue to finished leather with different photoperiods. Microalgae consortium was cultivated at two different compositions of mixtures of raw wastewater (R) and wastewater after secondary biological treatment (B): 50% of R + 50% of B, (50R50B) and 75% of R + 25% of B, (75R25B), in photoperiod of 24 hours and 12 hours of light, temperature of 25 °C and constant aeration. The growth of microalgae in the effluent and the removal of phosphorus, nitrogen and ammonia were monitored throughout the cultivation. The highest growth was achieved in the 24-hours condition with maximum biomass concentrations in the 75R25B effluent (1.40 g L^{-1}) and phosphorus removal (97.94% for the 50R50B), nitrogen removal (71.53% for the 50R50B) and ammonia removal (100% for both effluent).

1 Introduction

In most stages of leather production, clean water is utilised as transport liquid to diffuse the chemicals and for the extraction of undesirable materials from the hide. In this way, the liquid effluents generated in the beamhouse stage, as well in the tanning and finishing stages, have high impact potential to the environment due to high concentrations of nitrogen, phosphorus, toxic metals, sulphides, biological oxygen demand (BOD), oxygen chemical demand (COD) and suspended solids. Thus, because of the large volumes generated with high chemical and organic loads, the effluents need adequate treatments before being discarded in the water bodies GUTTERRES et al., 2015; SHARMA & MALAVIYA, 2016, DE AQUIM; HANSEN; GUTTERRES, 2019). The characteristics of tannery wastewater vary widely, depending on the nature and preservation of the hide, the tanning and leather processing technology, the amount of water used, and the procedure adopted by the industry to reduce pollution.

In the finishing stage, wet-blue leather receives the desired final characteristics, such as physical-mechanical strength, softness, color, durability, stamping and surface coating. The leather finishing consists of wet end (deacidulation, retanning, dyeing and fatliquoring), drying, pre-finishing and finishing. In these steps various chemical is used in the processing, such as deacidulants, dyes, oils, surfactants, polymers, pigments, solvents, resins and other chemical products, as well as remainings of organic matter inherent to the process, result in contamination of the effluents that require treatment (PICCIN et al., 2016).

Several researches have investigated in the last years treatment techniques of effluents generated in the leather industry, involving biotechnology, as it is a sustainable and economical way to treat pollutants. These studies have used biological agents as microalgae, bacteria, fungi and

their bioproducts to treat effluents (FONTOURA *et al*., 2017, ORTIZ-MONSALVE *et al*., 2017; SINGH, VYAS & MALAVIYA 2016;).

Microalgae represent a versatile possibility for treatment of effluents since they have high capacity of fixation of carbon dioxide of the air and phosphorus and nitrogen dissolved in the water, adapt easily to the changes in the environment (temperature, pH, salinity, and availability of nutrients) making possible their cultivation in effluents (WHITTON, 2012). These microorganisms can also be used for the removal of metals that are present in the effluents since their surfaces contain negative charges and adsorb the metal ions of the liquid effluent (SUNDARAMOORTHY *et al*., 2016). In addition, they can achieve high rates of cell growth in these media and present cleaner solutions when compared to other alternatives of effluent treatment (ANGELIS *et al*., 2012; HU *et al*., 2017). However, raw effluents from the leather industry, that is, without previous treatment, are a challenge for the growth of microalgae due to the high chemical load and turbidity, which can often be toxic to these microorganisms, inhibiting their growth (AJAYAN *et al*., 2015). Fontoura *et al*. (2017) used raw wastewater from the beamhouse stage in different concentrations to grow the *Scenedesmus* sp. microalgae. Results were obtained with 88.4% effluent concentration, reaching a maximum biomass concentration of 0.90 g L^{-1}, maximum removal of ammoniacal nitrogen, phosphorus and COD of 85.63%, 96.78% and 80.33%, respectively.

Pena *et al*. (2018) carried studies with the microalgae *Tetraselmis* sp. in the effluent from the finishing phase with continuous light regime. Removal of 96.59% and 99.81% for phosphorus, 99.90% and 89.2% for ammoniacal nitrogen, 89.06% and 54.78% for total nitrogen, 40, 46% and 43.54% for COD, 59.24% and 57.90% for total organic carbon, 32.70% and 44.73% for biological oxygen dissolved, were achieved at the 50R50T concentrations (50% raw/50% treated effluent), and 75R25T (75% raw/25% treated effluent), respectively.

Microalgae become an attractive alternative for wastewater treatment, since these microorganisms present many benefits, as they also remove unwanted substances from the effluent, they have high storage capacity of reserve substances in their biomass, which can be transformed into bioproducts (JAHAN *et al*., 2014). Some studies have reported higher efficiency when using a microalgae consortium in the removal of pollutants and nutrients, such as nitrogen, phosphorus and ammonium from wastewater, when compared to individual microorganisms (KOREIVIENĖ *et al*., 2014; HENA *et al*., 2015).

In this way, the present study was carried out with the purpose of analysing the growth of microalgae consortium and the efficiency for removal of total Nitrogen, phosphorus, and ammonia from effluent of wet end to leather finishing processing at different effluent concentrations and photoperiods.

2 Material and methods

2.1 Cultivation of microalgae

A sample of microalgae was collected in a deactivated effluent treatment pond from a tannery located in Montenegro / RS, Brazil and throughout microscopic analysis, it was possible to see that there was a consortium of microalgae (**Fig. 1**). When analysing the microalgae present in this consortium Pena *et al*., (2018) identified the predominance of the microalgae *Tetraselmis* sp.. The culture of microalgae consortium was cultured under constant aeration with 1 L min^{-1} of compressed air at room temperature under continuous light. 20 mL of the microalgae consortium was maintained using 180 mL of the Tris-Acetate-Phosphate (TAP) in 250 mL Erlenmeyer every 10 days. All culture inoculation and maintenance procedures were performed with glassware and sterile culture medium inside a vertical laminar flow hood with air filtration system and ultraviolet (UV) lamps.

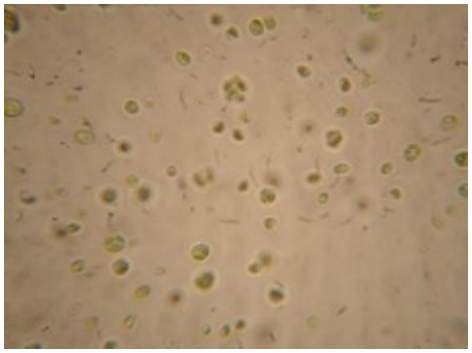

Fig. 1. Optical microscopy of the microalgae consortium (10x) (PENA *et al*., 2018).

2.2 Tannery Wastewater

The wastewater was collected from a tannery that processes leather from wet-blue to finished leather, located in the city of Novo Hamburgo/RS, Brazil. Two kinds of effluents were collected in the treatment effluent plant: raw effluent without treatment (R) and effluent after primary physicochemical treatment (coagulation-flocculation-sedimentation) followed by biological secondary treatment with sludge-sedimentation (B). Nevertheless, this treated effluent does not meet environment standards for discharge to water bodies, requiring advanced treatment realized in the wastewater treatment plant (WWTP).

The microalgae consortium was cultivated with continuous light and at room temperature in 5000 mL bottles for 19 days, in the two following compositions:

- 50% raw/50% wastewater after secondary biological treatment (50R50B): (i) 1800 mL of raw effluent; (ii) 1800 mL of treated effluent; and (iii) 400mL of the microalgae consortium pre inoculum, totalling 4000mL.
- 75% raw/ 25% wastewater after secondary biological treatment (75R25B): (i) 2700 mL of the raw effluent; (ii) 900 mL of treated effluent; and (iii) 400 mL of the microalgae consortium pre inoculum, totalling 4000 mL.

The culture of microalgae consortium was cultured under constant aeration with 1 L min^{-1} of compressed air at room temperature, under two photoperiod conditions: continuous fluorescent light (24-hours) and fluorescent light in 12 h light/12 h dark cycles (12-hours).

2.3 Analytical methodology

The quantification of ammonia (N-NH_3) present in the culture was analysed on the Ion Chromatograph (Metrohm) using the Metrosep C4-150 column, eluent HNO_3 2.5 g L^{-1} and dipicolinic acid 1.5 g L^{-1} with a flow of 0.9 ml min^{-1}.

Phosphorus (P-PO_4) was analysed according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The effluent analysis followed the colourimetric method with quantification using UV / VIS spectrophotometer (880 nm).

Total nitrogen (TN) analyse was performed on the Shimadzu TOC-L analyzer equipped with a total nitrogen measurement unit (TNM-L Shimadzu) and 8-port sampler (OCT-L Shimadzu).

3 Results and Discussion

The maximum biomass concentrations of the cultures of 24-hours light and 12-hours light for composition 50B50S and 75B25S are presented in Table 1. The highest biomass concentration of the microalgae consortium was 1.40 g L^{-1} on day 11, for 75B25S in 24-hours light. The condition

75B25S favored the growth of the microalgae consortium explained by the higher concentrations of nutrients in this effluent mixture.

However, 12-hours did not favor the growth of the microalgae consortium, mainly in the 75R25B, which has a lower incidence of light because it is a more concentrated and turbid effluent, making the passage of light difficult. It is necessary a balance between the luminosity and the absence of light, since under low illumination the available energy is insufficient, whereas the opposite, that is, the excess of light causes photoinhibition (YAN *et al*., 2011). The light in the cultures varies both in space (depth and latitude) and in time (daily), making it a determinant factor for microalgae growth. This explains the lower growth of the consortium in the 12-hours culture when compared to the 24-hours cultivation since there was no light every 12 hours and as it is a turbid effluent the entry of light is hindered into the culture medium due to the time reduction.

Table 1. Maximum biomass concentration of the microalgae consortium during of cultivation 24-hours light and 12-hours light in tannery wastewater.

	24-hours light	12-hours light
50R50B (g L^{-1})	1.04±0.03	1.26±0.005
75R25B (g L^{-1})	1.40±0.02	0.79±0.01

Removal of TN, N-NH_3 and P-PO_4 over the 19 days of culture for 50R50B and 75R25B concentrations is shown in **Fig. 2** for 24-hours. The removals were 100% of ammoniacal nitrogen for both, 71.54% and 58.84% total nitrogen and 97.94% and 95.54% phosphorus for 50R50B and 75R25B, respectively.

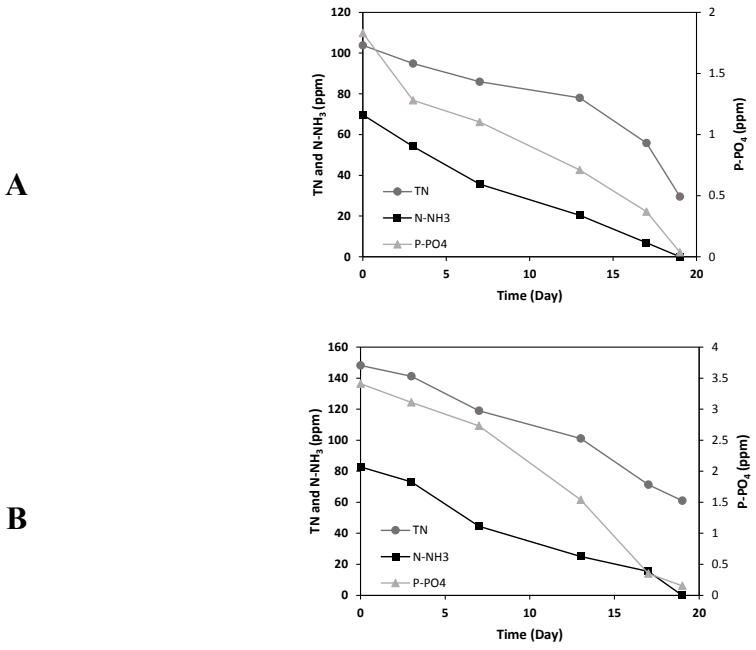

Fig. 2. Removal of TN, N-NH_3 and P-PO_4 in composition 50R50B (A) and 75R25B (B) in 24-hour culture with light.

Removal of TN, N-NH_3 and P-PO_4 over the 19 days of culture at 50R50B and 75R25B concentrations are shown in **Fig. 3** in 12-hours. The removal of 70.16% and 56.36% for ammoniacal nitrogen, 53.28% and 41.67% for total nitrogen and 97.37% and 97.39% for phosphorus were observed in assays with tannery wastewater concentration 50R50B and 75R25B, respectively.

The metabolism of phosphorus and nitrogen present in wastewater is directly linked to the production of biomass and metabolic activities. Ammonia is a form of nitrogen that is more easily assimilated by microalgae, and usually, nitrite and nitrate are assimilated after the complete removal of ammonia (Maestrini et al., 1986). Phosphorus is an essential element for the growth of microalgae and plays many roles in the cells. The initial amounts of phosphorus are low and decay rapidly in the cultivation, it becomes a growth-limiting factor since it is directly linked to some functions of the cell.

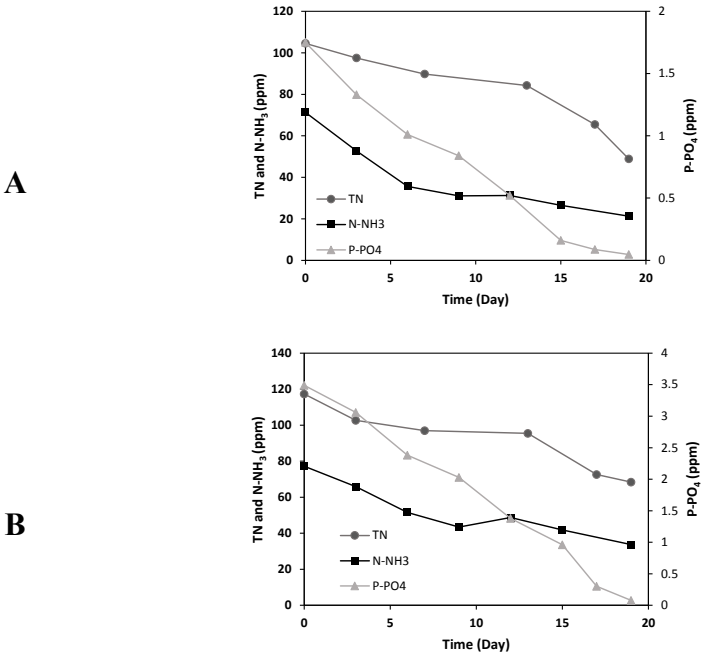

Fig. 3. Removal of TN, N-NH_3 and P-PO_4 in composition 50R50B (A) and 75R25B (B) in 12-hour culture with light.

Conclusion

This study showed that the microalgae consortium growed in raw tannery wastewater being efficient in the removal of nitrogen, ammonia and phosphorus. The highest growth was achieved in the 24-hours condition with maximum biomass concentrations in the 75R25B effluent (1.40 g L^{-1}) and phosphorus removal (97.94% for the 50R50B), nitrogen removal (71.53% for the 50R50B) and ammonia removal (100% for both effluent). Thus, the study showed that tannery wastewater can be used as an alternative source of nutrients for the production of biomass microalgae.

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