Tetraselmis sp. Isolated from a Microalgae Consortium for Tannery Wastewater Treatment

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Abstract

Microalgae have been the subject of several studies in the wastewater treatment field due to their ability for the removal of various nutrients, organic load and to be a clean and economical way to treat pollutants. The effluents from leather finishing processing steps contain chemical pollutants due to the use of dyes, surfactants, toxic metals, emulsifying agents, retanning agents, oils, pigments, resins, among other chemicals added. In this work, the isolated microalgae *Tetraselmis sp.* was obtained from a microalgae consortium and evaluated for their ability for the treatment effluents collected from a tannery. The growth of microalgae biomass in these effluents in mixotrophic cultivation was analysed, as well as, the capacity of removal of total nitrogen (TN), total organic carbon (TOC), total carbon (TC), inorganic carbon (IC), ammonia ($N\text{-}NH_3$), phosphorus ($P\text{-}PO_4$), chemical oxygen demand (COD) and biological oxygen demand (BOD). The removal values observed for the 50R50T (50% raw/50% treated effluent) and 75R25T (75% raw/25% treated effluent) concentrations were 96.59% and 99.81% for phosphorus, 99.90% and 89.2% for ammoniacal nitrogen, 89.06% and 54.78% for TN, 40.46% and 43.54% for COD, 59.24% and 57.90% for TOC, 32.70% and 44.73% for BOD, respectively. The microalgae *Tetraselmis sp.* showed notable growth in mixotrophic cultivation, and the efficient removal of the controlled parameters indicate an enormous potential for application in tannery wastewater treatment.

1 INTRODUCTION

The biotreatment of effluents generated in the leather industry has increased in the last 5 years. These studies have applied microalgae, bacteria, fungi and their bioproducts for wastewater treatment.[3,12,14]

Microalgae represent a versatile possibility of wastewater treatment since they have a high fixation capacity of phosphorus and nitrogen dissolved in water and carbon dioxide of the air, besides adapting easily to environmental changes, such as temperature, pH, salinity, and availability of nutrients, making possible its cultivation in wastewaters.[16] These micro-organisms can reach high rates of cell growth in these media and present cleaner solutions when compared to other alternatives for wastewater treatment, such as conventional treatments methods.[2,6]

An essential step of the studies with microalgae is the pure culture isolation. Several techniques for obtaining single species are described by different authors,[4,1,9] even though they are slow and laborious processes. Studies on the use of mixed cultures of microalgae have been performed to obtain better results. Authors reported that the microalgae consortium containing *Chlorella sp.* and *Scenedesmus sp.*, showed itself to be more efficient in nitrogen and phosphorus removal from wastewater when compared to the individual culture of these micro-organisms.[8]

The objectives of this work are isolate and identify the microalgae present in a microalgae consortium and evaluate its performance when used to treat tannery wastewater.

2 MATERIALS AND METHODS

2.1 Isolation of microalgae from a consortium

A microalgae consortium was collected in a deactivated sedimentation tank in a wastewater treatment plant of a tannery located in the city of Montenegro/RS, Brazil. This tannery performs all the leather processing steps (beamhouse stage to finished leather) to obtain the final product.

To maintain the culture, a Tris-Acetate-Phosphate culture medium (TAP), was used in the proportion of 1:10, in 250mL Erlenmeyer.[5] Every 10 days the consortium was pealed to ensure a stock culture. The culture was maintained, on a full-time basis, under constant aeration with compressed air flow of $1Lmin^{-1}$ conducted for each vial. The experiments were held at room temperature under 3910 lux continuous light regime.

To analyse the consortium growth, an absorption scan in the visible light range of electromagnetic spectrum was performed in the UV-VIS T80 spectrophotometer from PG Instruments (Leicester, LEC, UK) allowing to find the highest absorbance wavelength, which was 570nm. Further, sample dilutions (microalgae consortium and TAP culture medium) were made in 10ml flasks, to reach

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absorbances between 0.1 and 1.0, and the optical density was reread at 570nm. To obtain the biomass concentration, a calibration curve was constructed relating the absorbance to the biomass concentration, obtained by filtering and drying these samples to a constant weight. The biomass concentration was determined by collecting samples of 10mL every 24 hours in all of the culture experiments.

Isolation of the predominant species was performed using the method of successive dilutions in TAP medium, followed by the technique applied in agar nutrient plates. The scratching method was repeated until pure microalgae cultures were obtained. After isolation, individual algae colonies were transferred to 500ml of sterile fresh TAP medium and cultured. These methods were performed under mixotrophic growth conditions.[9]

Identification of the isolated species was carried out based on their morphological characteristics, having as reference works of the authors.[11,10,4] The images were captured using a binocular biological microscope with microphotography equipment coupled to the computer.

All maintenance and inoculation procedures in the culture effluents were performed with sterile glassware and culture medium, inside a vertical laminar flow hood with air filtration system and lamps with UV radiation.

2.2 Wastewater treatment experiments

The same conditions of microalgae cultivation already described were used for growing microalgae in tannery wastewaters and the experiments were performed in two replicates. The wastewaters were 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: raw effluent without treatment (R) and effluent treated (T) by primary physicochemical treatment (coagulation-flocculation-sedimentation) followed by biological secondary treatment (active sludge-sedimentation). Nevertheless, this treated effluent does not meet environment standards for discharge to water bodies, requiring advanced treatment in the wastewater treatment plant (WWTP).

The isolated microalga, *Tetraselmis sp.*, was cultivated with continuous light and at room temperature in 5000mL bottles for 19 days, in the following compositions:

- 50% raw/50% treated effluent (50R50T): (i) 1800mL of raw effluent; (ii) 1800mL of treated effluent; and (iii) 400mL of the microalgae consortium pre-inoculum, totaling 4000mL.
- 75% raw/25% treated effluent (75R25T): (i) 2700 mL of the raw effluent; (ii) 900mL of treated effluent; and (iii) 400mL of the microalgae consortium pre-inoculum, totaling 4000mL.

2.3 Wastewater analyses

Effluents were analysed after the effluent systems were assembled with the pre-inoculum and at the end of the experiment to quantify removal of:

- total nitrogen (TN) (TNM-L Shimadzu and 8-port sampler (OCT-L Shimadzu));
- total organic carbon (TOC), total carbon (TC), inorganic carbon (IC) (TOC-L Shimadzu);
- ammonia ($N\text{-}NH_3$) (Basic IC Plus Package, Metrohm);
- phosphorus ($P\text{-}PO_4$)[15];
- chemical oxygen demand (COD)[15];
- biological oxygen demand (BOD) (VELP Scientifica DBO System 6).

The collected samples were filtered using a vacuum pump and glass fibre microfilters (MN GF-3), with pores of 0.6µm.

Analyses were performed in duplicates and each compound (pollutant) removal was calculated by Equation 1:

$$R(\%) = \frac{(xi-xf)}{xi * 100} \quad (1)$$

Where *xi* is the initial compound concentration, *xf* is the final concentration and R is the compound removal percentage.

3 RESULTS AND DISCUSSION

The plating with the successive dilutions technique proved to be effective for the predominant micro-organism isolation. Figure 1 shows a microscopic image of the microalgae consortium and the isolated predominant microalgae.

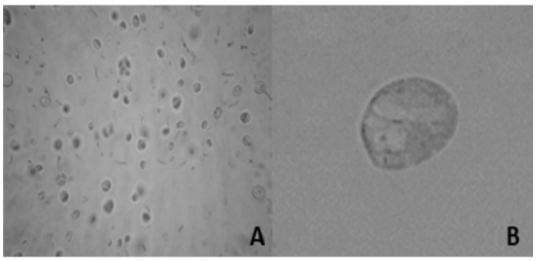

Figure 1. Optical microscopy of the (**A**) microalgae consortium (10x) and (**B**) isolated microalgae (40x).

The isolated microalgae presented the following characteristics: unicellular, with flagella of the same size, monadic, ovoid form, with two flagella and fast movement. Tetraselmis cells are 20 to 24µm inlength, 12-16µm width, are flattened, elliptical in the frontal view, kidney-shaped in lateral view and rounded.[10] The species *Tetraselmis* are cells capable of being compressed, have four flagella which are in opposite pairs. The flagella are thick, of equal length, smaller than the length of the cell and covered by hair. The microalgae found presented a similarity to the microalgae *Tetraselmis sp.*, due to the cited format and the presence of flagella, additionally having characteristics as easy adaptation in various environments and easy locomotion.[7]

Figure 2 presents the biomass growth in mixotrophic cultivation with microalgae *Tetraselmis sp.* for compositions 50R50T and 75R25T. The maximum concentration reached in the effluents was 1.24 ± 0.14gL^{-1} for 50R50T and 0.99 ± 0.19gL^{-1} for 75R25T. The biomass produced was lower for 75R25T effluent

when compared to 50R50T, demonstrating that there was greater stress caused by the higher pollutants concentration in raw (untreated) effluent.

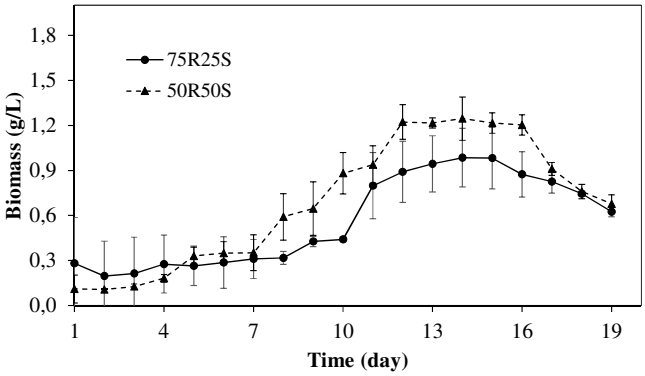

Figure 2. Growth of the microalgae *Tetraselmis sp*. in along cultivation in composite effluents.

TABLE I
Average concentrations in composite effluents 50R50T and 75R25T before and after cultivation with *Tetraselmis sp*.

Parameters		50R50T1		75R25T2	
		Conc.[5] [mg l^{-1}]	Removal %	Conc.[5] [mg l^{-1}]	Removal %
pH	initial	7.45		7,68	
	final	8.58		8,75	
$P\text{-}PO_4$	initial	1.75 ± 0.02	96.59	2.25 ± 0.04	99.81
	final	0.01 ± 0.00		0.06 ± 0.01	
TN	initial	83.13 ± 2.25	89.06	89.91 ± 3.09	54.78
	final	9.09 ± 0.139		40.92 ± 1.64	
$N\text{-}NH_3$	initial	73.90 ± 3.31	99.90	79.30 ± 2.00	89.212
	final	N.D.[6]		8.55 ± 0.35	
COD	initial	991.00 ± 12.73	40.46	1045.0 ± 7.07	43.54
	final	590.00 ± 14.14		590.00 ± 14.14	
TOC	initial	115.52 ± 4.84	59.24	126.31 ± 2.16	57.90
	final	47.57 ± 0.21		53.18 ± 5.37	
BOD_5	initial	1590.0 ± 14.14	32.70	1900.0 ± 28.28	44.73
	final	1070.0 ± 14.14		1050.0 ± 14.14	

Table I presents the initial, final and removal for the mixotrophic growth with the microalgae *Tetraselmis sp*. It also shows the initial and final pH. In the literature,[3] it was found maximum values of ammoniacal nitrogen (85.63%), phosphorus (96.78%) and COD (80.33%) for the microalgae *Scenedesmus sp.* (88.4%) and luminance intensity of 182.5µmol of photons m^{-2} s^{-1}. The values found by these authors are close to the removal values found in this present work for phosphorus (96.59% and 99.81%) and ammoniacal nitrogen (99.90% and 85.63%), however, COD removals were lower (40.46% and 43.54%).

Figures 3 and 4 show total nitrogen and ammonia removal of mixotrophic cultivation with microalgae *Tetraselmis sp*. In the last day of cultivation, there was a short increase of total nitrogen in the medium concomitant with the microalgae decline phase (Fig. 2), when the cells die, releasing nutrients into the medium.

On the 7th day of cultivation, there was an increase of ammonia for both 50R50T and 75R25T compositions. It can be explained by the increase in pH. The higher the pH, the higher the total ammonia percentage present in NH_3^+ form.

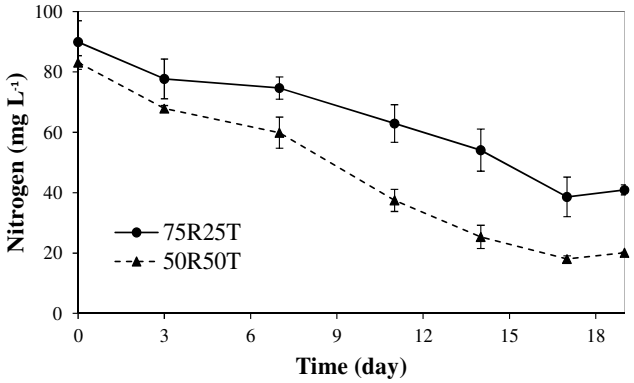

Figure 3. Removal of total nitrogen along *Tetraselmis sp.* cultivation in composite effluents.

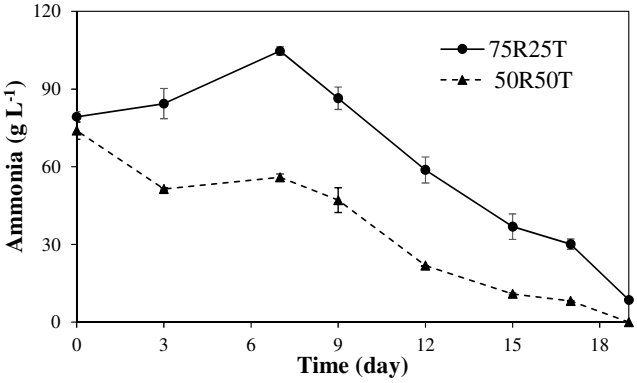

Figure 4. Removal of ammonia along *Tetraselmis sp.* cultivation in composite effluents.

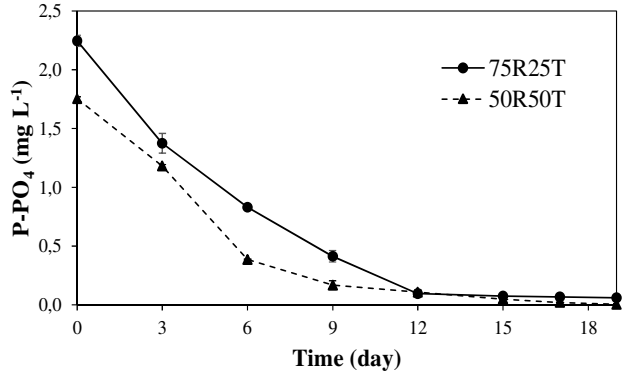

Figure 5. Phosphorus removal along *Tetraselmis sp.* cultivation in composite effluents.

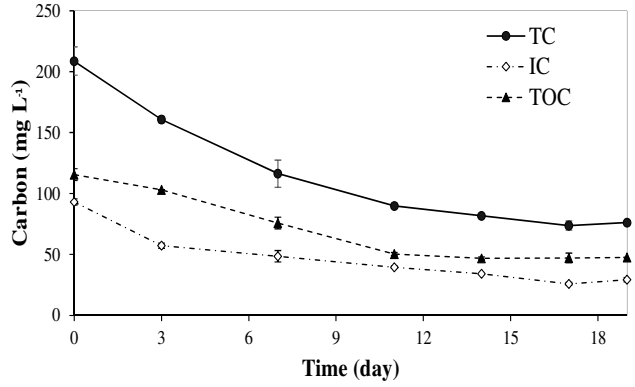

Figure 6. Carbon removal along *Tetraselmis sp.* cultivation in composite effluent (50R50T).

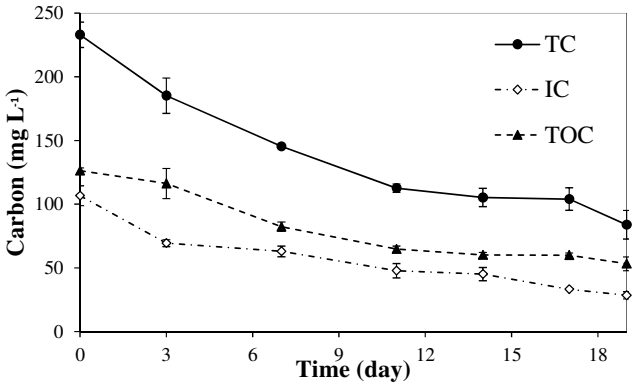

Figure 7. Carbon removal along *Tetraselmis sp.* cultivation in composite effluent (75R25T).

The results presented in the literature[13] applying the microalgae *Arthrospira (Spirulina)* in biomass production and tannery wastewater treatment showed the maximum cellular concentration of 0.78gL^{-1} with 38.2% of ammoniacal nitrogen removal and 91.0% of phosphorus removal.

Phosphorus removals were effective for this experiment (Fig. 5), with removals of 96.59% and 99.81% for compositions 50R50T and 75R25T, respectively.

Figures 6 and 7 show the carbon removals by the microalgae *Tetraselmis sp.* mixotrophic cultures. It is possible to note that both organic carbon and inorganic carbon removal occurs in the 50R50T and 75R25T cultures. This can be explained by the difficulty of light penetration into the effluent due to its darkness, promoting the use of organic carbon as an energy source at some time by the microalgae *Tetraselmis sp*.

4. CONCLUSION

The plating technique and the successive dilutions were efficient for the isolation of the predominant microorganism. Through image identification, it was possible to conclude that the isolated microalgae was *Tetraselmis sp*. The isolated *Tetraselmis sp.* was able to grow in the effluents in the compositions 50R50T and 75R25T, presenting a remarkable growth in the mixotrophic culture with efficient removals of the analysed parameters: 99.9%, 89.06% and 59.24% for N-NH_3, TN and TOC, for the 50R50T composite effluent, respectively, and 99.81%, 43.54% and 44.73% for P-PO_4, COD and BOD, for the 75R25T composite effluent, respectively.

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