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Research article

Treatment of Industrial Effluents by the Microalgae Selenastrum Sp.

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Abstract.

High consumption of clean water results in the generation of effluents that need to be treated and then safely discarded. Conventional methods for such treatment often do not offer an economical and sustainable result; therefore, new methods are needed, such as microalgae usage. Microalgae are unicellular beings capable of rapid adaptation, growth, and production of compounds of interest (pharmaceuticals, biofuels and others). This work aimed to study the effectiveness of the microalgae *Selenastrum sp.* in the treatment of effluents from the textile and pulp & paper industries, as well as the respective effects on its biomass development and accumulation of compounds. Four types of culture were carried out (for each type of effluent, a control, and a control with addition of glucose) lasting eight days, in duplicate, all with the addition of a standard culture medium and controlled abiotic factors. Analyses for compound removal (chemical oxygen demand and colour readings on the 200–800nm range) and biomass development (cell number, its dimensions, and weight) were performed four times during the process. At the end of the experiments, the average removal in effluents for COD and colour were 56.6% and 32.7% respectively, in addition to a biomass accumulation of 0.45 g/L. These results were comparable to those obtained for the control cultivation using glucose as a carbon source (70.0% COD removal and 0.51 g/L biomass accumulation). These results demonstrate the effectiveness of *Selenastrum sp.* in the treatment of industrial effluents, its resilience in stressful environments and the potential use of its accumulated compounds for biotechnological purposes.

Keywords: microalgae, *Selenastrum sp.*, industrial effluent, textile effluent, pulp effluent, effluent treatment

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1. Introduction

In 09/2015, the United Nations approved the 2030 Agenda for global sustainable development, with some of its main prerogatives being access to efficient and sustainable water sources for all and decrease in water pollution **[1]**. With the energy and clean water needs, growing continually, the environmental impact generated and the need to reverse them, to find alternatives that are ecological, efficient, and low cost have been sought, being microalgae one of those alternatives. Microalgae are unicellular microorganisms found in almost all types of aqueous media, capable of adapt and

change their environment, to reproduce quickly and are sources of various compounds of interest for a myriad of different sectors and industries **[2]**.

Water is essential in large volumes in the industry mainly to be used in manufacturing processes or washing and with such function and degree of consumption an appropriated method for cleaning and discharge of the effluent generated needs to be applied. Nowadays, conventional systems used for treatment of industrial effluents are effective if well implemented and operated, but are often expensive, complex, and sometimes doesn't eliminate all its contaminants, therefore not achieving the minimum conditions appropriated for discharge. The use of microalgae for the treatment of industrial effluents can be an effective, economical, and simple solution for such situations since its effectiveness has been extensively studied and applied **[3-10]**.

The present work aims to analyse the acclimatization of the microalgae *Selenastrum sp.* in effluents derived from the textile and paper pulp industry, evaluate the treatment and elimination of biodegradable organic matter, and observe the microalgae accumulation of compounds of interest.

2. Material and methods

Cultures were carried out in 1L reactors for 8 days, with control of light intensity (195 $\mu mol.m^{-2}.s^{-1}$, 12h/day), temperature (23ºC) and filtered air flow (0.4L/L/min). Cultures with 90% (v/v) of filtered effluent from the textile and pulp industries, and control (with and without glucose) assays were carried out. Control cultures consisted in 900mL of distilled water, 60mL of microalgae and 40mL of standard nutrients (and 0,5g of glucose for the control with glucose). Due to the low inorganic nutrient content in the effluents, 40mL of GoldMedium Fresh-Water Species were added to all cultures. Samples for cell counting, chemical oxygen demand (COD) analysis, dry weight, spectrophotometer colour reading (200 – 800nm) and cell dimensions (through microscopic observation coupled with specialized software for image processing and analysis) were taken at 4 different times of cultivation.

The Figure 1 illustrates how the dimensions were considered for microalgae cell volume determination (volume was estimated considering the sum of the volumes of two semi-spheres and one cylinder).

For the characterization of the effluents, measurements were made utilizing a multiparameter sensor for the determination of pH, dissolved O_2 and redox potential, along with a nutrient test kit for determination of nitrate and phosphate, standard methods for the COD calculation and spectrophotometer for colour readings of filtered samples in

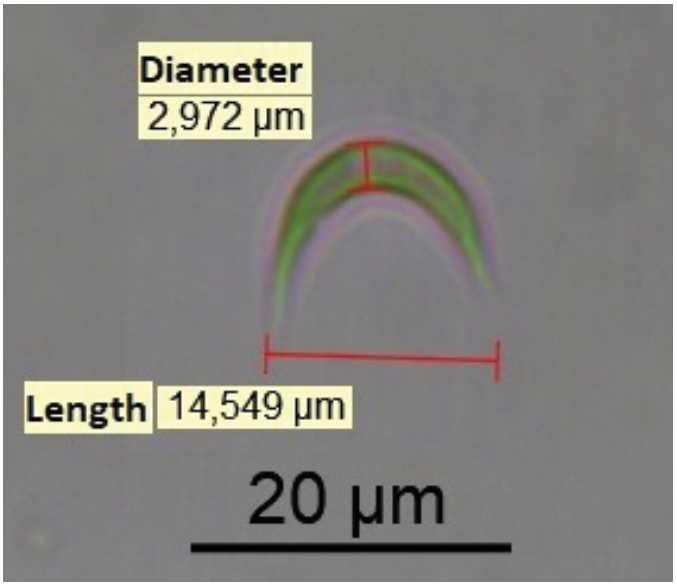

Figure 1: *Selenastrum spp.* dimensions.

the UV-Visible spectrum (200-800nm range). The COD standard method chosen was the closed reflux, titrimetric method.

The characteristics of both industrial effluents (as well as commonly dissolved constituents found in such effluents) are indicated in the table 1.

TABLE 1: Characteristics measured for both industrial effluents.

	Textile effluent	Paper pulp effluent
pH	8.4	7.0
Dissolved O_2 (mg/mL)	4.1	1.5
Nitrate (mg/L)	2.9	4.1
Phosphate (mg/L)	3.0	7.0
Redox potential (mV)	163	-433
Integral of the UV-Visible spectrum	785.5	836.6
COD (mgO_2/L)	434	462
BOD (mgO_2/L)*	103	178
BOD/COD*	0.238	0.385
Other common constituents**	chlorinated compounds, heavy metals, dyes, sulphates, nitrogen oxides, amines	Resins, fatty acids, sterols (β-sitosterol), waxes, adsorbable organic halides

* COD/BOD ratio for both effluents were based on previous works with similar effluents (BODs values were also acquired through this ratio) [3] [4]. ** Common constituents found in both effluents [5] [6].

3. Results and discussion

Figures 2, 3, 4 and 5 demonstrate the COD variation, the integral of spectrophotometer colour readings, cell growth and dry biomass accumulation values for all the tests respectively (time periods for all results shown are the 2h, 24h, 96h and 196h of culture respectively).

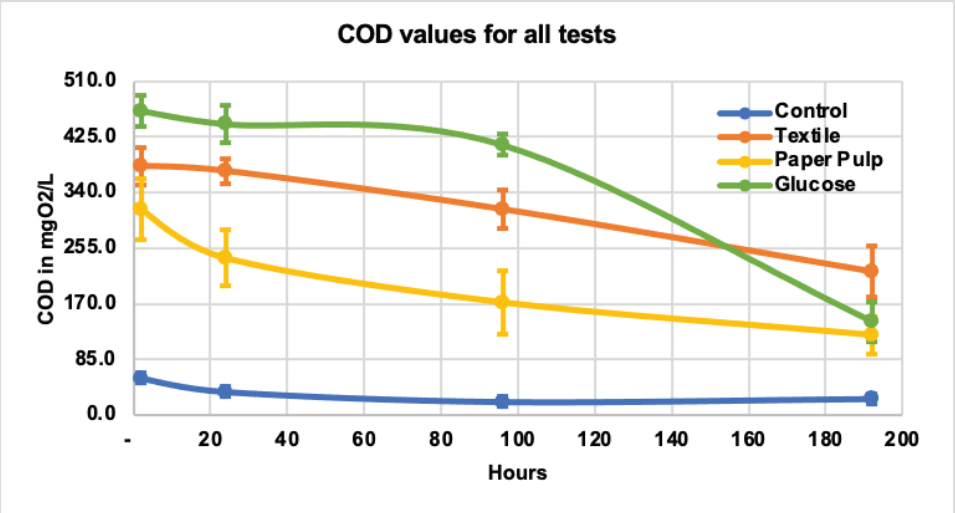

Figure 2: COD removal rate and extent for all cultures.

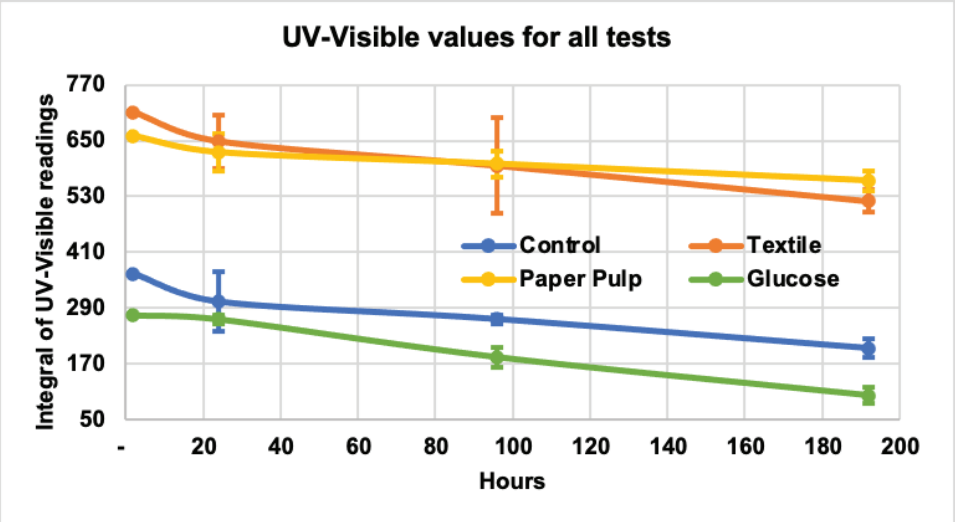

Figure 3: Results for the integral of UV-Visible colour readings for all cultures.

Regarding COD, it is important to comment that the COD values in control culture comes from the organic compounds present in the microalgae inoculum themselves. It is also interesting to observe that in the glucose culture, the organic matter only started to be consumed after about 80 hours, suggesting that the microalgae shifted from a

metabolic state to another, possibly from an autotrophic regime to a heterotopic one **[11]**. On the other hand, considering that both effluents (paper pulp and textile) had approximately the same initial COD, the microalgae assimilation of the two industrial effluents was significantly different; the COD removal for the paper pulp effluent is higher than for the textile effluents, which is in good agreement with the higher biodegradability (BOD/COD ratio) of the pulp effluent (table 1). However, the rapid decrease in COD concentrations in pulp effluents after 2 h is somewhat unexpected and can tentatively explained by the adsorption of these compounds on the surfaces of microalgae, thus, removing them suddenly as the microalgae are introduced in the medium.

Regarding the COD removal, there was a reduction in COD levels of 70.0%, 64.3% and 49.2% for cultures with glucose, pulp effluent and textile effluent respectively, after 8 days of cultivation. On the other hand, considering the both the colour removal (400-800 nm) and other aromatic compounds (200-400 nm) (Figure 3), an average reduction of 31.7% was observed for the industrial effluents, which indicates a reduction of colour in both effluents and the removal of dyes in the textile effluent. Such values demonstrate the potential of *Selenastrum sp.* microalgae to degrade both the pulp and textile effluents. In addition, these results are in good agreement with it was observed by other authors working with effluents derived from the same kind of industries and other species of microalgae. Working with textile effluent, El-Kassas & Mohamed (2014) **[7]** reported a mean value of COD removal around 61.6% in a 14-day culture with *Chlorella vulgaris,* whereas Lim et al. (2010) **[8]**, obtained a mean value of 50.3% for the COD removal in a 12-day culture with the same microalgae. In relation to the microalgae grown in the paper pulp effluents, Gentili (2014) **[9]**, obtained a mean value of 79.8% for the COD removal in a 6-day culture in a paper pulp effluent mixed with other effluents (dairy and municipal) using 3 different microalgae (2 *Scenedesmus* and *Selenastrum*) and Usha et al. (2016) **[10]**, had a 75.0% of COD removal in an outdoor open pound culture (28 days) using two species of *Scenedesmus sp*.

The biochemical reaction conditions resulted in the highest cell production for the controlled cultures (with and without glucose) with an average of 12.3 million cells per mL in contrast with the 7.4 million cells per mL in the cultures with industrial effluents. However, regarding the weight of accumulated biomass, the cultures with dissolved organic carbon (effluents and control with glucose) resulted in higher biomass concentration when compared with the control culture (0.29mg/mL), obtaining 0.42mg/mL in the textile effluent (46.5% higher than the control without glucose), 0.47mg/mL for the pulp effluent (62.8% higher) and 0.51mg/mL for the glucose culture (76.7% higher than the control without glucose).

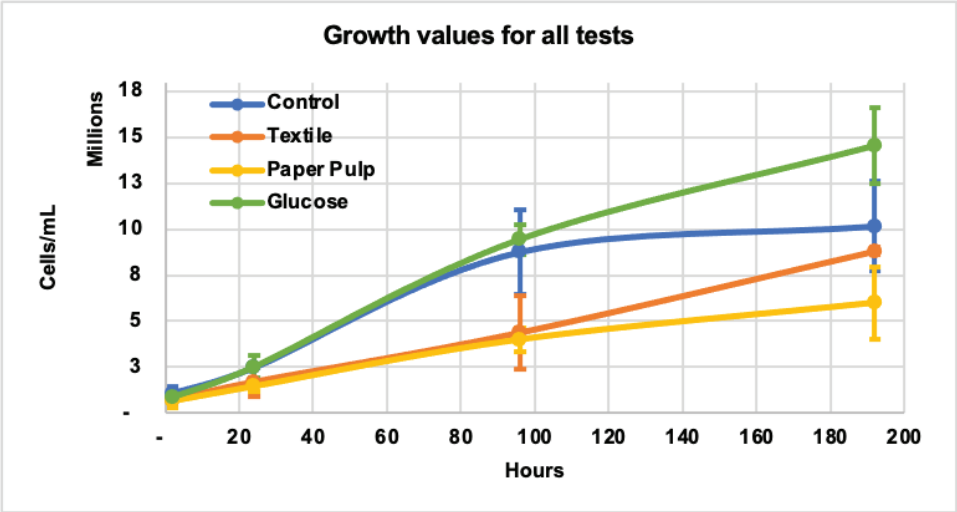

Figure 4: Growth rate for all cultures.

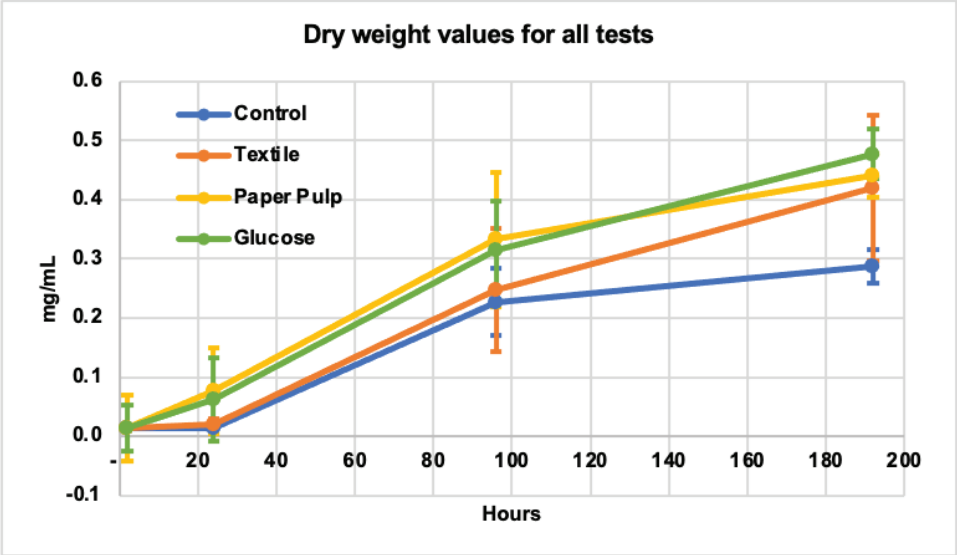

Figure 5: Dry biomass values for all cultures.

Overall, it is noticed that the more controlled and less stressful the environment, the greater the number of cells, while the more stressful environments or those with higher COD content determine a greater dry biomass. Such growth of microalgae biomass in industrial effluents was also observed by Lim et al. (2010) **[8]** and El-Kassas & Mohamed (2014) **[7]** in textile effluents, obtaining the mean values of 0.155 mg/mL (12 days) and 1.325mg/mL (14 days and addition of sodium bicarbonate) of dry weight respectively, both using the microalga *Chlorella vulgaris*. For paper pulp effluent, Gentili (2014) **[9]** reported a mean value of biomass concentration of 0.93mg/mL (mix of effluents, lasting 6 days) using 3 different microalgae (2 *Scenedesmus* and *Selenastrum*).

The contrast between cell number and dry biomass weight can be observed in Figure 6, which shows the value of the mass per cell. A clear difference is revealed, suggesting that stressed cells growing in effluent show higher values. The time profile of this parameter is also very interesting and deserves further studies, namely, to investigate the metabolites accumulated. Based on the results, we can speculate that 4 days culture maximise the metabolites production, namely for culture in the pulp effluent.

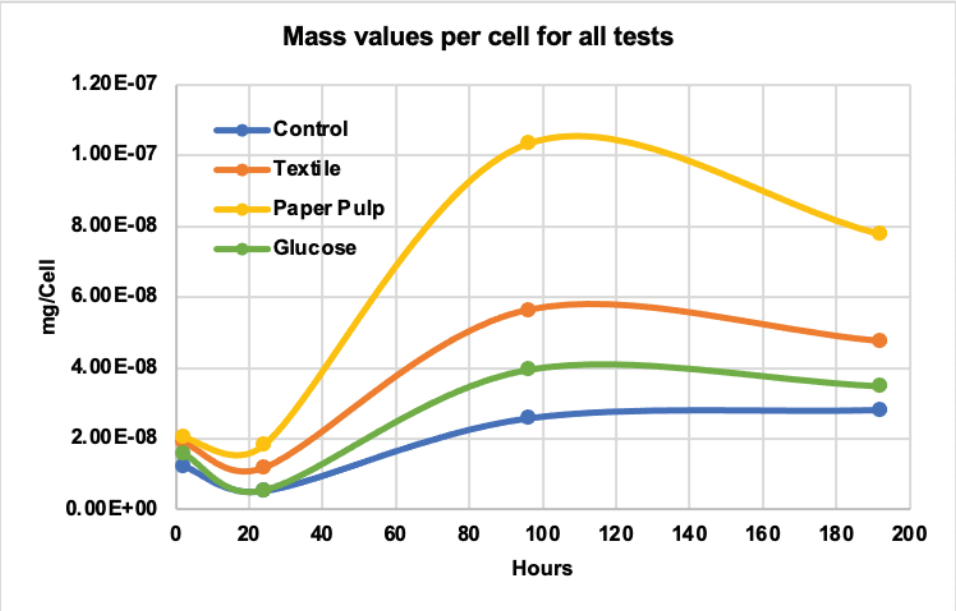

Figure 6: Mass per cell values for all cultures (time periods shown are the 2h, 24h, 96h and 196h respectively).

Figure 7 shows the estimated cell volume. Comparing the control essays with and without glucose, we can conclude the cell with glucose are bigger than the corresponding cell cultivated with inorganic carbon only. On the other hand, comparing the cell cultivated with glucose and effluents, the cell volume is of the same magnitude, but the mass of each cell is much higher in the effluents (Figure 6), suggesting higher accumulation of compounds inside the cell. Figure 7 also shows the cell volume significantly increase along the culture period. Comparing the estimated cell volume variation from the beginning to the end of culture period, the control essays had an increase of 31.9% (from 82.01 to 108.24μm^3), the textile effluent essay an increase of 149% (from 123.18 to 149.05μm^3), the pulp effluent essay an increase of 167.8% (115.09 to 167.82μm^3) and an increase of 201.2% was observed for the microalgae grown in the controlled culture with glucose (127.61 to 201.24μm^3).

The obtained results put in evidence the effect of the carbon source (organic vs inorganic; glucose vs complex molecules from effluents) and the culture condition, namely the stress environment, on the cell characteristics (dimensions and accumulated

metabolites). This phenomenon was also observed by Chioccioli et al. (2014) **[12]**, where addition of organic carbon (glucose) resulted in an increase of 0.25mg/mL in dry weight of microalgae cultures using the species of *Chlorella vulgaris* and *Chlamydomonas reinhardtii*.

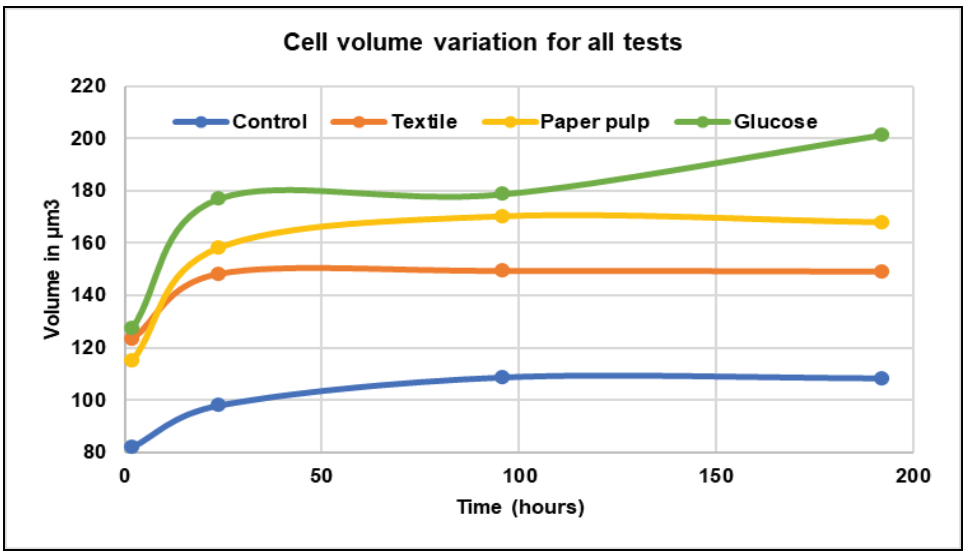

Figure 7: Cell volume values for all cultures (time periods shown are the 2h, 24h, 96h and 196h respectively).

Considering the results for dry biomass obtained in this work and the biomass lipid content reported (14%) by Chakravarty & Mallick in 2019 **[13]**, it is expected that this biomass has higher potential for lipid production. Although other species can provide better lipid results **[14]**, it is possible that changing a few parameters on the cultures **[15]** (like temperature or salt concentration) or mixing effluent with more nutrients **[9]** (like dairy or food effluents), even better results in lipid concentrations can be achieved.

4. Conclusions

The study demonstrates the microalgae *Selenastrum sp* has the potential to treat effluents from the pulp and textile industries. For these effluents, the average COD and colour removal were 56.7% and 32.7%, respectively. In addition, the biochemical conversion of the effluent compounds resulted in a biomass accumulation of 0.45 g/L, comparable with the obtained in the control essay using glucose as carbon source. These experimental results demonstrated that *Selenastrum sp*. is an efficient alternative for the treatment of industrial effluents. Its growth and high biomass accumulation suggest high potential for reusing its compounds for biotechnological purposes.

5. Acknowledgements

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