Sustainable integration of biofuel generation and domestic wastewater treatment by *chlorella vulgaris*

Integração sustentável da geração de biocombustíveis e do tratamento de águas residuais domésticas pela *chlorella vulgaris*

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ABSTRACT
The production of microalgae biofuels has become promising because it is a renewable, non-polluting and non-competitive source such as food growing areas. This work has the objective of using the effluents generated by the Lorena Engineering School - EEL / USP as a growth medium for microalgae of the *Chlorella vulgaris* species, aiming at lipid production and treatment of effluents. The laps were completed in photobioreactor with effluent from the collection on alternate days. *C. vulgaris* showed good adaptation to the effluent, reaching a cell density of $1.92x10^7$ cells.mL^{-1} and a dry biomass concentration of 1.74 g.L^{-1}. The lipid content per gram of biomass was 0.095 to 0.164 mg.g^{-1}. Concomitantly, between 54% and 78.74% of nitrate was dismissed and the chemical oxygen demand (COD) fell between 17.73% and 36.13%. With the results of statistical analysis (ANOVA and Tukey's test). Although lipid has been lower, the success of the microalga *Chlorella vulgaris* in this sense must have been similar to that of the same.

Keywords: wasterwater treatment, microalgae, *Chlorella vulgaris*, oil content.

RESUMO
A produção de microalgas biocombustíveis se tornou promissora por ser uma fonte renovável, não poluente e não competitiva, como as áreas de cultivo de alimentos. Este trabalho tem o objetivo de utilizar os efluentes gerados pela Escola de Engenharia de Lorena - EEL / USP como meio de crescimento para microalgas da espécie Chlorella vulgaris, visando a produção de lipídios e tratamento de efluentes. As voltas foram completadas em fotobioreator com efluentes da coleta em dias alternados. A C. vulgaris mostrou boa adaptação ao efluente, atingindo uma densidade celular de 1,92x107 células.mL-1 e uma concentração de biomassa seca de 1,74 g.L-1. O conteúdo lipídico por grama de biomassa era de 0,095 a 0,164 mg.g-1. Concomitantemente, entre 54% e 78,74% de nitrato foi descartado e a demanda química de oxigênio (DQO) caiu entre 17,73% e 36,13%. Com os resultados da análise estatística (ANOVA e o teste de Tukey). Embora os lipídios tenham sido menores, o sucesso da microalga Chlorella vulgaris neste sentido deve ter sido semelhante ao da mesma.

Palavras-chave: tratamento de águas residuais, microalgas, C*lorella vulgaris*, teor de óleo.

1 INTRODUCTION

The Growing concern about environmental issues has been evident in recent years, influencing business leaders' decisions about energy sources. The use of microalgae as raw material for the production of energy and as food is widely known, due to its reach cellular composition in carbohydrates, proteins, lipids, vitamins and pigments. The proportion of these macromolecules varies according to the specie and growth conditions. Microalgae can be cultivated on a water recycling basis and specific tanks, (known as bioreactors) or in lakes [1,2]. In this case, it is not necessary the use of a large growth area, therefore there isn´t competition between energy and food production [3]. Microalgae can be cultivated ir rivers, lakes or in the sea [4,5]. It can also be cultivated in many kinds of effluents and industrials; this alternative is possible only when the cells will not be used on the food industry [2, 6-7].

The domestic effluent is daily produced and, many times, poured in rivers and oceans without been treated. For been rich in organic matter, it favors the microorganism's growth, which can compromise the aquatic ecosystem. The use of the domestic effluent as a way of algae cultivation is a sustainable alternative, whereas it can metabolize the organic material transforming the organic effluent into eutrophic effluent [2]. Although the use of effluent as a culture medium become more attractive than the use of fresh water, since this water is not used immediately for human consumption, it is not used on plant irrigation and it is also not affected of sea seasonality [4].

The energy generated during the growth of microorganisms is stored in the form of lipids, carbohydrates and proteins. These compounds of high added value can be extracted by specific processes, considered green technologies [8]. The accumulated lipids in the biomass can be extracted and converted into biodiesel via transesterification. Lipid synthesis has gained popularity in many parts of the world, stimulating the use of microalgae as a raw material for the production of biofuels, but large gaps in this biotechnology still need to be optimized [9].

In addition to the energy generation capacity, microalgae assimilate water-specific nutrients, such as nitrogen compounds (NH_4^+, NO_2^- and NO_3^-), improving their quality [10]. Most microalgae are classified as photoautotrophic microorganisms, that is, they use sunlight as a source of energy and CO_2 as a source of carbon. However, some species are mixotropic, that is, they act simultaneously with autotrophism and heterotrophism, consuming both inorganic carbon and organic carbon matter present in the medium [11]. The mixotrophic mechanism creates an additive and synergistic effect that increases

biomass productivity and the ability to purify wastewater [12]. Once again, the use of effluents as a culture medium has shown to be a promising strategy, since in addition to reducing the environmental impacts associated with its incorrect disposal in water bodies, it can improve the energy balance of photobioreactors [13].

Considering the depletion of the energy resources of the planet and the taking into account the reduction of the impacts caused by traditional means of energy generation, this work aims to evaluate the feasibility of integrating the generation of biofuels to the water treatment through the cultivation of the microalga *Chlorella vulgaris* com the effluent from the School of Engineering of Lorena (EEL / USP), towards a sustainable process.

2 MATERIALS AND METHODS

2.1 MICROORGANISMS

The microalgae strain *Chlorella vulgaris* (BMAK D1) was obtained from the Aidar & Kutner (Oceanographic Institute) of São Paulo University (USP). The inoculum of the microalgae was maintained in 250 ml Erlenmeyer flasks containing Basal Bold Medium (BBM), with pH in the range of 7.0 to 8.0, in incubators with light intensity of 150 Lux and temperature of 25 °C.

2.2 EXPERIMENTAL DESIGN

The wastewater of the School of Engineering of Lorena / University of São Paulo (EEL / USP) was used as cultivation medium. The EEL / USP Effluent Treatment Station (ETS) applies a biological reactor with decanter, with a capacity to treat all the effluent generated by students and staff (about 3000 people). The effluent is composed of domestic sewage and small concentrations of metals, which are used in laboratories and may reach the effluent after the dish wash. The concentration of the effluent produced varies according to the student's attendance at the university, the period of the day in which it is collected - day or night - and also depending on the hydraulic detention time (HDT) under which the biological reactor operates. Because of these changes, the crops were therefore grown on three different periods as mentioned below (Table 1). The values presented in Table 1 correspond to the parameters analyzed in the treated wastewater of the EEL/USP ETS.

Table 1 Characterization of the effluent at the photobioreactor input.

Parameter	Cultivation 1	Cultivation 2	Cultivation 3
COD ($mgO_2.L^{-1}$)	251. 54 ± 4.10	127.24 ± 12.60	88.36 ± 21.80
Nitrate ($mg.L^{-1}$)	23.23 ± 1.80	15.73 ± 0.80	10.87 ± 1.60

Cultivation 1 in the end of July, Cultivation 2 in the beginning of August, and Cultivation 3 in mid-August. All cultivations were done in triplicate and the cultures were carried out until reached the stationary phase.

2.3 CULTURE CONDITIONS

The inoculum used was maintained in the BBM and had a concentration of approximately 0.325 x 10^7 cells.mL^{-1}. To sterilize the effluent, the method described by Kawachi and Noel was used, adding sodium hypochlorite (0.30 to 0.50 $mL.L^{-1}$) to the medium [9]. After 24 hours, the chlorine was neutralized with sodium thiosulfate (30.00$mg.L^{-1}$). The sterilized effluent was inoculated in a 5.00 L photobioreactor.

The microalgae culture was performed in triplicate in acrylic photobioreactor (in batch) with constant artificial light source (4.8 klux) composed of fluorescent lamps and room temperature, which ranged between 30 °C to 18 °C (overnight). No CO_2 cylinders were used to feed the system, therefore the carbon available for the microalgae in this experiment was the organic carbon found in the wastewater and in the inorganic carbon from the atmosphere, characterizing a mixotrophic cultivation. The compressor used was a BOYU 70W with an output of 50$L.min^{-1}$. This equipment was shared among the triplicate to assure the same air flow and, consequently, same agitation rate. The pH of the medium was in the range of 7,0 to 8,0.

2.4 ANALYSIS

2.4.1 Growth analysis

Microalgae growth was monitored daily by spectrophotometer reading at 680 nm (UV-Vis, Bel Photonics) and cell counting, under an optical microscope in the Neubauer chamber (TNB-01T). To determine the dry biomass weight (DBW), the cells were centrifuged at 2000 g during 10 min. The cell pellet was washed twice with distilled water and dried at 60 °C. The dry biomass weight was used to determine the growth curve, specific growth rate and biomass productivity. Samples were collected at 24 h intervals.

The batch time of the photobioreactors was determined from the growth curve. The batches were interrupted when the stationary growth phase in each condition was

reached. As different substrate concentrations (nitrate) were tested, there were different growth rates (μ), therefore, different batch times.

2.4.2 Chemical analysis

To evaluate the efficiency of the process, the effluent was characterized at the entrance and exit of the reactor through Chemical Oxygen Demand (COD) and nitrate (NO_3^-) analysis according to the methodologies described in *Standard Methods* [14]. Before characterization of the effluent the samples were centrifuged so that the supernatant was collected.

The COD analysis was done according to APHA, by spectrophotometry (ICP-OES, Optima 8000), to verify the amount of organic matter removed from the effluent. Dilutions were then made, and the calibration curve was prepared. Subsequently, the absorbance was converted to the concentration of dissolved oxygen, consumed per liter [15].

The nitrate concentration analysis was performed every 3 days to determine the nitrate consumption profile of *C. vulgaris* in the effluent. The analyses were done in a spectrophotometer (Jenway 7305) at the wavelength of 220 nm, which represents the total amount of nitrogen in the sample. However, the amount of organic nitrogen produced by the microalgae during its growth had to be discarded. Organic nitrogen was determined by reading the sample at wavelength 275 nm. Finally, the concentration of the organic nitrogen of the total nitrogen was subtracted to obtain the amount of inorganic nitrogen absorbed.

2.4.3 Lipid quantification

After each cultivation, the biomass was recovered by flocculation, adding aluminum sulphate (1,0 $Eq.L^{-1}$) at a rate of 3,0 $mL.L^{-1}$ for decantation of the biomass. The sediment biomass was filtered, dried in an oven at 60 °C and grinded to increase the contact surface area. The biomass was then quantified with respect to its lipid content, according to Bligh and Dyer [16].

3 THEORY AND CALCULATION

3.1 SPECIFIC GROWTH RATE

The specific growth rate (μ) at the exponential phase was calculated by:

$$\mu = \frac{(lnX_2 - lnX_1)}{(t_2 - t_1)} \quad (1)$$

Where: X_2 and X_1 were dry biomass weight ($mg.L^{-1}$) at time of t_2 and t_1, respectively.

3.2 BIOMASS PRODUCTIVITY

Biomass productivity (P_{DBW}) was calculated by:

$$(P_{DBW}) = \Delta X/\Delta t \quad (2)$$

Where: Δ_X corresponds to the variation of biomass concentration ($mg.L^{-1}$) over the cultivation time of each test Δt (hours). Data were expressed as mean standard deviation ($\pm SD$).

3.3 LIPID PRODUCTIVITY

The lipid productivity was calculated by equation below:

$$Lipid\ Productivity = (\frac{W_L}{DBW}) * P_{DBW} \quad (3$$

Where W_L is the weight of the extracted lipids (mg), DBW is the dry biomass weight (mg) and P_{DBW} is the biomass productivity ($mg.L^{-1}.h^{-1}$).

3.4 REMOVAL EFFICIENCIES

Wastewater was characterized physico-chemically before and after culturing in the photobioreactor according to the Standard Methods. The parameters evaluated were COD and nitrate ($mg.L^{-1}$) [10]. From the obtained data the nutrient removal efficiencies (RE) of the photobioreactor through Equation 1 below [17].

$$RE(\%) = \frac{(Ci - Cf)}{Ci} * 100 \quad (4)$$

3.5 ANOVA AND TUKEY TEST

The analysis of variance (ANOVA) and the Tukey test were done through the software *Excel 2013*, to verify and identify the existence of significant differences between the biomass productivity of the crops.

3.6 ECONOMICAL ANALYSIS

In this work an economic analysis was made to verify the economy made when using effluents as a culture medium. The economic analysis was performed using *Excel 2013* software.

and Methods should be described with sufficient details to allow others to replicate and build on published results. Please note that publication of your manuscript implicates that you must make all materials, data, computer code, and protocols associated with the publication available to readers. Please disclose at the submission stage any restrictions on the availability of materials or information. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited.

Research manuscripts reporting large datasets that are deposited in a publicly available database should specify where the data have been deposited and provide the relevant accession numbers. If the accession numbers have not yet been obtained at the time of submission, please state that they will be provided during review. They must be provided prior to publication.

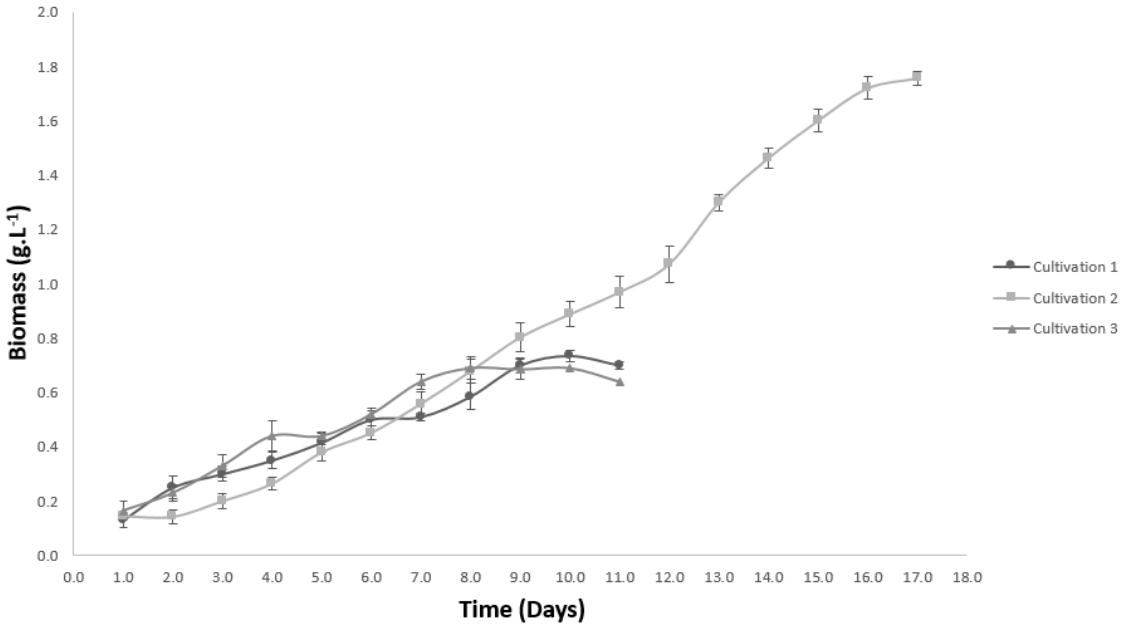
Interventionary studies involving animals or humans, and other studies require ethical approval must list the authority that provided approval and the corresponding ethical approval code.

4 RESULTS

4.1 MICROALGAE GROWTH

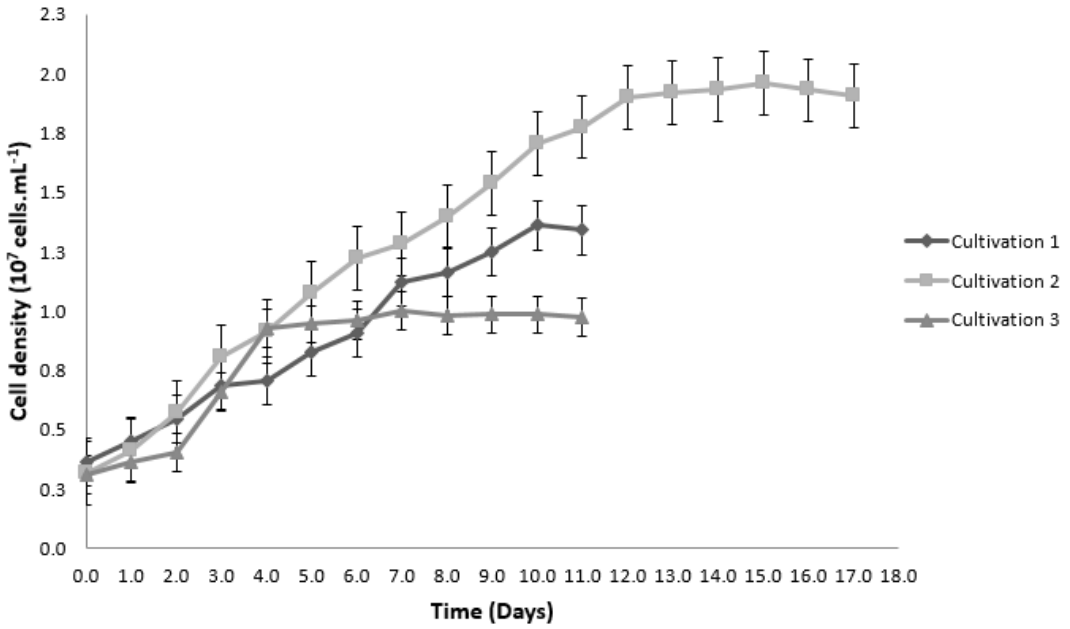
The microalgae growth was evaluated by the concentration of dry biomass and by the number of cells present in the effluent. The concentrations of dry biomass, showed in Fig. 1, were determined using the analytical curve, where the absorbance readings of the culture (abs) were converted into milligrams per liter ($mg.L^{-1}$).

Fig. 1. Concentration of microalgae biomass from different seasoned of EEL / USP ETS wastewater cultivation.



The mean cell density between the reactors for each culture was also determined (Fig. 2).

Fig. 2. Cell density in culture containing wastewaters.



After quantifying the biomass concentrations of the cultures over time, and the accumulated lipid concentration per gram of dry biomass, Fig. 3 and Table 2 were constructed.

Fig. 3. Comparison of cell density using wastewater with the BBM standard.

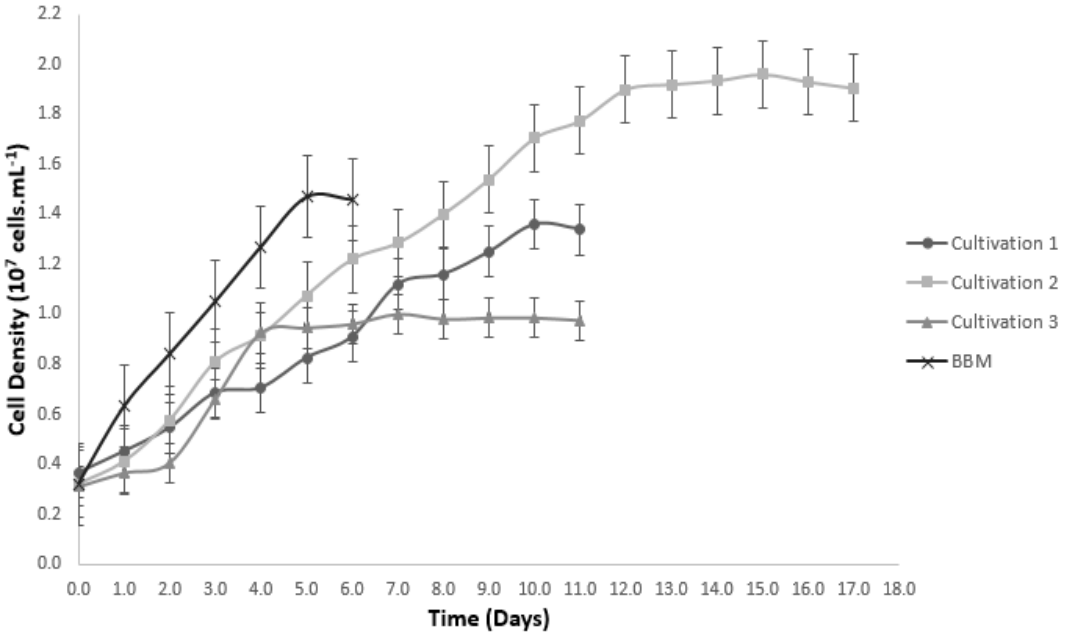


Table 2 Productivity of biomass in different mediums.

Parameter	Cultivation 1	Cultivation 2	Cultivation 3	BBM
Biomass (g.L^{-1})	0.70 ± 0.05	1.74 ± 0.02	0.61 ± 0.03	0.96 ± 0.01
DBW (mg.L^{-1}.h^{-1})	2.16 ± 0.16	4.0 ± 0.03	1.79 ± 0.02	5,71 ± 0.02
μ (d^{-1})	0.15	0.14	0.13	0.31

4.2 NUTRIENT REMOVAL

The effluents were physically and chemically characterized (COD and NO_3^-) after cultivation. With the results obtained, Fig. 4, 5 and 6, that relate the microbial growth to nitrate consumption and the calculations of removal efficiency of the photobioreactor, were made.

Fig. 4. Nitrate consumption of culture 1.

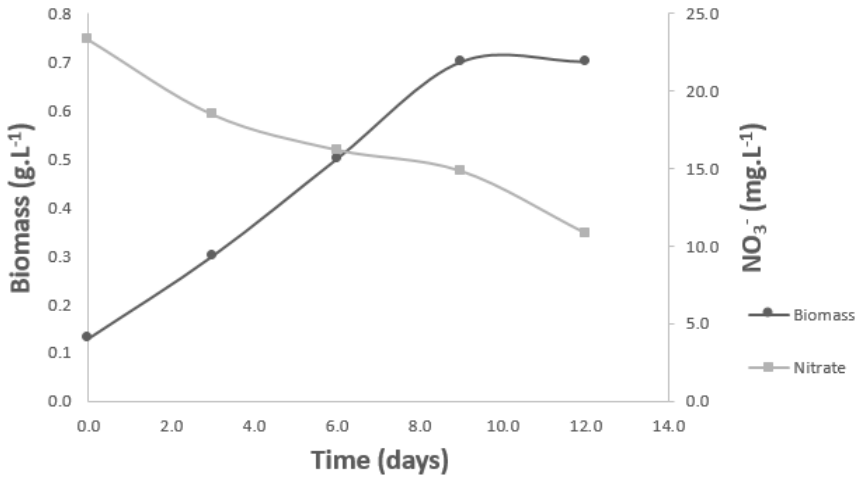


Fig. 5. Nitrate consumption of culture 2.

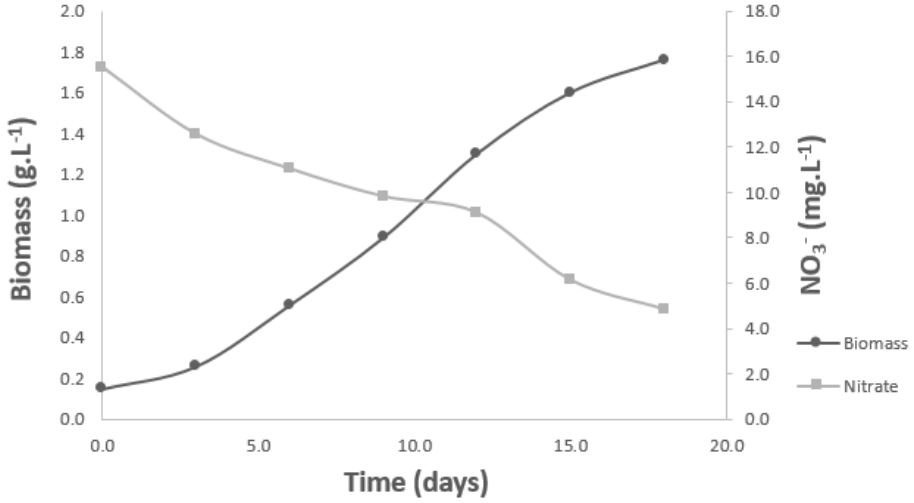
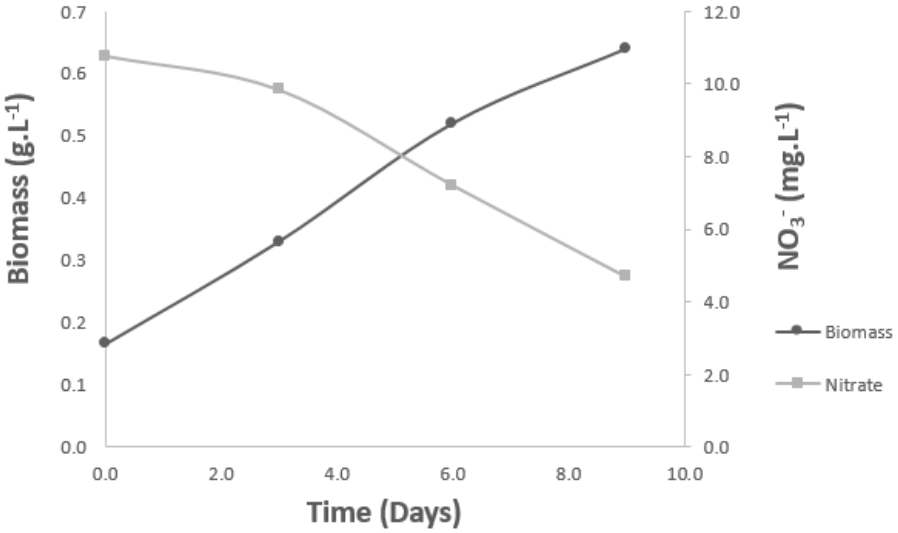


Fig. 6. Nitrate consumption of culture 3.



4.3 LIPID PRODUCTIVITY

The lipid concentration per gram of biomass and the lipid yield of the cultures were calculated and presented in Table 3. The highest accumulation of lipids was achieved with the effluent corresponding to culture 3, which had a lipid ratio per gram of 16.4%.

Table 3 Lipid productivity.

Parameter	Cultivation 1	Cultivation 2	Cultivation 3	BBM
Lipids ($mg.g^{-1}$)	0.10 ± 0.01	0.12 ± 0.01	0.16 ± 0.01	0.12 ± 0.01
Ratio (%)	9.50 ± 0.10	12.40 ± 0.60	16.40 ± 0.70	12.20 ± 0.6
L.P. ($mg.L^{-1}.h^{-1}$)	0.20 ± 0.01	0.49 ± 0.02	0.29 ± 0.01	0.69 ± 0.01

- LP is the Lipidic Productivity

4.4 STATISTICAL ANALYSIS

4.4.1 ANOVA and Tukey test

The analysis of variance (ANOVA) and the Tukey's test were performed with the biomass concentration of the cultures ($cells.mL^{-1}$) in order to verify if there was a significant difference between the biomass yield of the crops and what they are. The statistical results obtained by the ANOVA showed that the biomass productivity among the cultures of *C. vulgaris* represented a significant difference in p-value < 0.05 (Table 4).

Table 4 Analysis of variance (ANOVA).

C.V.	S.S.	D.F.	M.S.	F	p-value
Between	2.90	3	0.97	4.98	0.05
Residue	8.76	45	0.19		
Total	11.66	48			

- C.V is the Cause of Variation; S.S is the Sum of Squares; D.F. is the Degree of Freedom; M.S. is the Medium Square.

By performing the Tukey test to verify what these differences are, a difference in productivity was observed only between Cultivation 2 and 3, probably due to deficiency of trace metals in effluent 3 (Table 5).

Table 5 Tukey test.

Comparation	$\lvert X_A-X_B \rvert$	dms	Conclusion
$\lvert X_1-X_2 \rvert$	0.475	0.499	$\mu_1 = \mu_2$
$\lvert X_1-X_3 \rvert$	0.101	0.499	$\mu_1 = \mu_3$
$\lvert X_1-X_4 \rvert$	0.115	0.499	$\mu_1 = \mu_4$
$\lvert X_2-X_3 \rvert$	0.576	0.499	$\mu_2 \neq \mu_3$
$\lvert X_2-X_4 \rvert$	0.360	0.499	$\mu_2 = \mu_4$
$\lvert X_3-X_4 \rvert$	0.216	0.499	$\mu_3 = \mu_4$

4.4.2 Economic analysis

The economic analysis of each cultivation is illustrated in Table 6, which reveals the percentage of each input on capital costs.

Table 6 Percentage of the inputs on capital cost in each cultivation medium.

Input	Cultivation 1	Cultivation 2	Cultivation 3	BBM
Reactor building (%)	36.90	28.00	43.69	32.98
Inoculum (%)	0.24	0.18	0.28	0.21
Growing medium (%)	0.00	0.00	0.00	1.86
CO_2 delivery (%)	50.59	59.59	43.69	53.72
Harvesting (%)	0.45	0.34	0.54	0.40
Extraction (%)	4.94	3.75	5.85	4.41
Lighting(%)	6.85	8.12	5.87	7.28
Capital cost (U$)	16.80	22.15	14.19	18.80

The efficiency and costs of cultivation are presented in Table 7.

Table 7 Efficiency and cost of crops.

Parameter	Cultivation 1	Cultivation 2	Cultivation 3	BBM
P.B. ($mg.L^{-1}.h^{-1}$)	2.16	4.00	1.79	5.06
L.P. ($mg.L^{-1}.h^{-1}$)	0.20	0.49	0.29	0.617
N.R. (%)	54.00	78.74	74.57	75.22
C.O.Q.R. (%)	17.73	36.13	26.92	0.0
Cost (R$)	16.80	22.15	14.19	18.80

- P.B. is the Productivity of Biomass; L.P. is the Lipid Productivity; N.R. is the Nitrate Removal; C.O.Q.R. is the COQ Removal

5 DISCUSSION

As shown in Figure 1, *Chlorella vulgaris* presented the highest biomass concentration in Cultivation 2 (1.74 ± 0.02 $g.L^{-1}$) after 17 days of culture. Cultivation 1 and 3 showed biomass concentrations of 0.69 ± 0.05 $g.L^{-1}$ and 0.61 ± 0.03 $g.L^{-1}$ at day 10 and 8, respectively.

As observed in Figure 2, The highest growth rate of *C. vulgaris* occurred in cultivation 2 ($1.91 \pm 0.02x10^7$ $cells.mL^{-1}$), followed by cultivation 1 and 3 which showed $1.34 \pm 0.05x10^7$ $cells.mL^{-1}$ and $1.00 \pm 0.05x10^7$ $cells.mL^{-1}$, respectively. Li et al. [18] studied the use of *Chlorella sp*. in a medium containing municipal wastewater of the Metropolitan Wastewater Treatment Plant located in Saint Paul, Minnesota USA. The highest biomass value obtained was $1.18g.L^{-1}$ at 14 days of cultivation, which is a lower value when compared to the biomass produced in culture 2 of the present stufy. However, Cho et al.

[19] obtained 3.0g.L^{-1} of biomass using effluent containing 90% of wastewater and 10% of anaerobic treatment tanks.

According Figure 3 and Table 2, the highest growth rate of *C. vulgaris* occurred in cultivation 2 (1.91 ± 0.02x10^7 cells.mL^{-1}). Cell growth assays 1, 3 and in BBM were lower: 1.34 ± 0.05x10^7 cells.mL^{-1}, 1.00 ± 0.05 cells.mL^{-1} and 1.41 ± 0.05x10^7 cells.mL^{-1} respectively. Tam and Wong [20] performed assays to quantify the specific growth rate (μ) of *C. vulgaris* in culture media with different concentrations of ammoniacal nitrogen. The cultures in this study were batch-treated for 21 days using erlenmeyer flasks with a useful volume of 400mL. The highest specific growth rates were obtained in the 250 and 500mg.L^{-1} ammonium cultures, reaching 0.24 and 0.22 d^{-1}, respectively. Therefore, crop growth rates in the EEL/USP effluent, which reached 0.15, 0.14 and 0.13 d^{-1}, were reasonable, since the availability of available nutrients was lower.

It is possible to observe that the substrate concentration affected the microbial growth kinetics. The higher nitrate concentration in this experiment resulted in a higher specific growth rate. These kinetic parameters are extremely important in the design of more efficient photobioreactor, since the optimization of microalgae growth kinetics and substrate consumption will require less hydraulic holding time (HDT), reducing the costs involved in this treatment process.

As noted in the Figures 4-6, the first crop (at the end of July) showed a removal rate of 17.73% of the COD and 54% of the nitrate concentration of the effluent; the second (at the beginning of August) had a removal rate of 36.13% of the COD and 78.74% of the nitrate concentration of the effluent. Finally, the third crop (mid-August) had a removal rate of 26.92% of the COD and 74.57% of the nitrate concentration of the effluent. Melo et al. [11] cultivated *C. vulgaris* in batched for 14 days, under autotrophic and mixotrophic conditions, using 1.0L erlenmeyer flasks containing agroindustrial effluents. In this study NO_3^- was 100% removed in all growth conditions because the substrates used had low ammonia and nitrite loading, consequently *C. vulgaris* consumed all the NO_3^- available for microalgae. However, Sayadi et al cultivated *C. vulgaris* in erlenmeyer containing municipal domestic effluent prepared with different concentrations of nitrate. The photobioreactor was operated in batch for 8 days, achieving maximum removal efficiency of 89.8% [21]. In this case, the efficiency of nitrate removal achieved in this study was satisfactory.

Regarding Lipid Productivity, the highest accumulation of lipids was obtained with the effluent corresponding to Cultivation 3, which presented a lipid ratio per gram

of 16.40%. Comparing the results presented in Tables 1 and 3, it is possible to observe that the effluent with low nitrate concentration promoted a greater accumulation of lipids, and that the effluent with high nitrate concentrations presented low formation of reserve lipids. Aquarone et al. [22] states that lipid production is related to the concentration of nitrogen present in the medium.

However, in this experiment, *C. vulgaris* presented satisfactory lipid accumulation when compared to standard BBM medium, since the lipid percentage was lower only in the effluent of Cultivation 1, probably due to the deficiency of the medium in some nutritional source, such as iron, for example [23]. Although the lipid concentration per gram of biomass was higher in Cultivation 2 and 3, the lipid productivity of both was lower than that of the artificial medium due to the high productivity of biomass. Feng et al. cultivated *C. vulgaris* in bubble column photobioreactor containing artificial effluent, operated in batch for 14 days, reaching lipid content (42%) and yield (147 $mg.L^{-1}.d^{-1}$). Therefore, accumulated lipid concentrations were low in all cultures made in this experiment [24]. The difference in the biosynthesis of fatty acid algae occurs not only between taxonomically different organisms, but also between groups that share similarities of the same species. The cultivation strategies can be adopted aiming at an increase in lipid productivity, for example, providing a large amount of light and low concentrations of nitrogen [25].

With respect to statistical results (Tables 4 and 5), comparing crops using effluent and the artificial standard medium (BBM), it is observed that there was no significant difference. Therefore, the statistical analysis allows to affirm that the use of EEL/USP effluent proved to be a good alternative when the objective in question is biomass production.

The economic analysis of each cultivation is illustrated in Table 6, reveals that the use of effluent as a culture medium is an interesting alternative, since it eliminates the need to prepare a synthetic medium and purchase reagent, allowing savings per liter when compared to the BBM standard [26]. That is, in large-scale projects and for industrial use, using effluents will bring significant cost reductions, in addition to providing proportional improvements in the water quality, eliminating the need for future investments in effluent treatment.

Tables 6 and 7 enable the comparison of the main parameters evaluated in this project with the cost of implementing each experiment. One can conclude that the use of effluent as a culture medium actually reduces the cost of the project, since all cultivations

with wastewater had lower costs, except for cultivation 2, which due to its high hydraulic retention time of 17 days had a higher energy demand for lighting and more CO_2 release. The costs obtained in the experiments using the standard BBM medium were only relatively low because the tests were carried out on the laboratory scale. So, the cultivation of effluents is therefore a good alternative for the optimization of lipid production, providing considerable cost reductions if it is done at larger scales, such as in industry and characterizing a green and sustainable process.

6 CONCLUSIONS

The EEL/USP effluent is a good alternative to cultivate the microalgae *C. vulgaris*, reaching higher or similar cell densities and biomass concentrations as when using the standard BBM medium. The lipids accumulated by *C. vulgaris* in ETS (EEL/USP) effluent cultures also showed satisfactory lipid concentrations, which may be converted into biodiesel. Lower values than the BBM standard were only obtained in the culture where the effluent had very high nitrate concentrations. In addition, *C. vulgaris* has been shown to be an interesting option in the treatment of water, presenting high rates of nitrate and organic matter removal, which improves the water quality and reduces negative impacts on the environment, such as eutrophication of water bodies. That is, after the cultivation, the effluent returns to the environment with a better quality than the effluent at entry.

Finally, the cultivation of *C. vulgaris* in effluents (instead of using the standard BBM medium) eliminates the preparation stage of reagent and consumption solutions, resulting in reduced expenses with the purchase of nutrients, resulting is a green and sustainable process.

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