Culture of *Scenedesmus acuminatus* in Corn Steep Liquor

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Abstract: Microalgae have been evaluated as a source of lipids for biodiesel production. They can be grown on effluent and produce biomass while removing organic and mineral components from the medium. The use of agro-industrial wastes for the production of microalgae is an alternative to reduce the costs related to the composition of the culture medium, thus reducing operational costs considerably. This work was conceived as a research about the cultivation of microalgae *Scenedesmus acuminatus* on the substrate composed by corn steep liquor. A central composite design (CCD) was carried to study the influence of the effluent and inoculum concentration on the chemical oxygen demand (COD) of the culture. Statistical analysis indicated that the percentage of inoculum and effluent had an influence on the COD. The cultivation, together with the coagulation procedure, was efficient for the removal of organic matter, verified through the COD reduction and changing of pH value. These two parameters reached the appropriate level according to the standard required by current legislation.

Key words: *Scenedesmus*, corn steep liquor, chemical oxygen demand, biomass, microalgae.

1. Introduction

New alternatives for obtaining raw materials for biodiesel synthesis have been researched, among them, microalgae.

The microalgae contain lipids in membrane components, reserve product, metabolites and also energy source [1]. Some genera have high percentage of triglycerides, diglycerides and monoglycerides in their lipid profile and have great potential for their use as raw material for the production of biodiesel [2].

They have several advantages in relation to the cultivation of oleaginous plants for biodiesel production: they can be grown in areas not suitable for agriculture and also in saline water, yielding high productivity in a minimum surface or volumetric area [3, 4]. According to Halim et al. [5], even in a conservative scenario, it is estimated that microalgae can produce about 10 times more biodiesel per unit area of land than a typical terrestrial oleaginous crop.

The use of microalgae for wastewater treatment has been suggested for several years because of its ability to effectively and inexpensively remove excess nutrients and other contaminants, which are the causes of eutrophication and pollution of water bodies [6].

Microalgae can grow in effluents and produce biomass while removing organic and mineral components from the environment. It is possible to combine the benefits of biofuel production, CO₂ mitigation and wastewater treatment [2, 7].

In addition, the use of agro-industrial wastes for the production of microalgae is an alternative to reduce the costs associated with the composition of the culture medium, thus reducing operational costs considerably [8]. Corn steep liquor is a by-product of the corn starch production process; in some industries, like the case in this study, it is considered an effluent. However, in some studies, it has been used as a relatively inexpensive source of nutrients to make up the culture medium of various microorganisms, such as bacteria and yeast [9-14].

For the definition of the technology or treatment

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that will remove the organic load existing in the effluents, the physical and chemical characterizations of this effluent are necessary. An example of characterization, which applies to almost all branches of industrial activity, is performed through analysis of chemical oxygen demand (COD) and pH (hydrogenation potential) of the sample [15]. COD is a leading indicator of organic pollution [16].

In this context, the objective of this research was to investigate the cultivation of *Scenedesmus acuminatus* microalgae on a substrate composed of corn maceration water, evaluating the efficiency of the treatment of this industrial effluent through COD and pH analysis.

2. Materials and Methods

2.1 Location and Samples

The corn steep liquor was collected at the Cassava and Corn Starch Industrial Unit of the Agro-industrial Cooperative Lar (UIM), located in the district of Dom Armando, in the municipality of Missal (PR-Brazil). The samples were transported to the laboratory and stored in a freezer at -10 °C. The microalgae culture and the COD analysis were carried out at the environmental and chemical laboratories of Itaipu Binacional (Foz do Iguaçu—PR), respectively.

2.2 Cultivation

The cultivation of microalgae received 24 h of artificial lighting (fluorescent lamps—8,000 lx). The aeration was performed through aquarium pumps and silicone hoses with the capacity to pump 0.8 L of air per reactor. Inoculum with cell density of $3,200 \times 10^4$ cells/mL was used. This inoculum was originated in a culture using the effluent under study at 30% concentration (preliminary test).

2.3 Experimental Planning

A central composite design (CCD) (Table 1) was used to evaluate the effects of effluent concentration on the medium and initial percentage of cells (inoculum) on the COD of the culture medium (output variable). Eleven tests were established from the combination of five levels (-1.41, -1, 0, 1 and +1.41). Among the proposed tests, four of them were factorial (combination between levels -1 and +1), four axial (combination between levels -1.41, +1.41 and 0) and three repetitions at the central point (all variables in the level 0).

2.4 Treatment Efficiency

The efficiency of the treatment was calculated from the COD data obtained from the culture medium before and after finishing the culture, through Eq. (1) [17, 18].

Table 1 Central composite design.

Test	Coded variables		Real variables	
	X_1	X_2	Effluent (%)	Inoculum (%)
1	-1	-1	23.1	1.9
2	1	-1	86.9	1.9
3	-1	1	23.1	8.6
4	1	1	86.9	8.6
5	-1.41	0	10.0	5.3
6	1.41	0	100.0	5.3
7	0	-1.41	55.0	0.5
8	0	1.41	55.0	10.0
9	0	0	55.0	5.3
10	0	0	55.0	5.3
11	0	0	55.0	5.3

$$E = \frac{DQO(i) - DQO(f)}{DQO(i)} \times 100 \quad (1)$$

where, E = treatment efficiency (%); $DQO(i)$ = initial COD value of the culture medium; $DQO(f)$ = COD value of the culture medium after treatment.

2.5 Effluent Characterization

For COD analysis, the digestion methodology was used in the reactor adapted from Jirka and Carter [19] for the analysis of wastewater. The pH was also monitored with a pH meter (Oakton), previously calibrated with buffer solution of 4.00, 7.00 and 10.0 [20]. Samples of 5 mL of the culture were daily measured.

3. Results and Discussion

From the second day of cultivation, it was already possible to observe an increase in the pH value. At the end of the treatment, all tests indicated a pH in the range of 8.12-8.57. These values are within the range allowed by the National Council for the Environment (CONAMA) [21], which establishes an acceptable range of 5-9 pH values for effluent discharge, for this case.

The characterization of the maceration water showed a COD value of 2,861.11 mg/L and a pH of 4.43. In the state of Paraná (Brazil), the State Environmental Council (CEMA), through Resolution No. 70/2009 [22], establishes the value of 200 mg/L

of COD as the standard for the discharge of liquid effluents into water body receivers (category “other activities”). Therefore, it is observed that the corn steep liquor presented a value about 14 times higher than the maximum limit allowed.

Table 2 showed the COD results obtained after the microalgae culture was finished. It is observed that only test 4 presented a final COD higher than 200 mg/L. This is, the other tests demonstrated that the microalgae were efficient in the organic matter consumption in relation to COD, reaching a value that meets with the current resolution [22].

The test that resulted in the highest removal efficiency was number 6, which started with 100% effluent, 5% inoculum and initial COD of 2,861.11 mg/L, with 96% efficiency in the treatment. The least efficient test was the number 5, with only 55% efficiency; however, the final COD value of the culture medium was 71.0 mg/L, which is within the established limit.

In a study conducted by Lopes et al. [18] for *Chlorella* sp., this microalgae was grown in vinasse and reached a COD removal efficiency of approximately 87% (initial COD of 1,528 mg/L and final COD of 190 mg/L), in a medium composed of Bold's Basal Medium (BBM) and only 5% vinasse, with the addition of 15% vinasse in the medium, the treatment efficiency decreased to about 84%. However, the authors of the study state that “studies with *Chlorella vulgaris* in

Table 2 Results of the CCD considering COD as the response variable.

Test	Effluent percentage in culture medium (%)	Percentage of inoculum (%)	Final COD (mg/L)	Percent of COD removal (%)
1	23	2	85.5	81
2	87	2	78.0	96
3	23	9	38.7	91
4	87	9	230.0	87
5	10.0	5	71.0	55
6	100.0	5	109.5	96
7	55.0	1	76.5	92
8	55.0	10	78.0	92
9	55.0	5	117.0	88
10	55.0	5	97.0	90
11	55.0	5	116.0	88

Table 3 Analysis of variance (ANOVA).

Source	SS	DF	MS	$F_{\text{calculated}}$	$F_{\text{tabulated}}$
Model	16,992.36455	1	16,992.36455	22.214	3.360
Error	6,884.433429	9	764.9370477		
Total	23,876.79798	10			

SS: sum of squares; DF: degree of freedom; MS: mean sum of squares.

100% vinasse obtained only 25% COD removal and low cell growth” [18]. Torres et al. [4], who also cultivated *Scenedesmus* sp. on an effluent (in this case, upflow anaerobic sludge blanket (UASB) reactor) obtained a final COD of 76.24 mg/L with removal efficiency of 59% in 10 d of cultivation of this microalga.

ANOVA was performed at a 90% confidence interval, the results of which were shown in Table 3. The Fisher test pointed to the significance of the model against error, since calculated F (22.214) was six times higher than tabulated F (3.360). The regression coefficient (R^2) obtained was 0.79702. In the analysis of the effects of the COD variables at the 90% confidence level ($p < 0.1$), it was verified that the interaction term and effluent percentage had an influence on the COD results.

The CCD results showed that the optimal point of the experiment was within the limits of the ranges under study; and so, it would be possible to obtain even lower COD values under other experimental conditions, as well as increasing the culture time.

4. Conclusions

The cultivation of *S. acuminatus* proved to be efficient for removal of the organic load from corn steep liquor, in reference to the adequacy of pH and COD to the corresponding legislation. In addition, the biomass produced can be used as a source of lipids for the production of biodiesel. It should be noted that the final COD analysis of the culture was performed after coagulation of the medium by aluminum sulphate, so the results of COD removal are the result of the action of the microalgae and also of the coagulant. The cultivation of *S. acuminatus* in effluent “maceration

water” is a sustainable and renewable approach to energy production.

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