CIEEMAT`17

III Congresso Ibero-Americano de Empreendedorismo, Energia, Ambiente e Tecnologia: Livro de atas

12 a 14 de julho de 2017

Instituto Politécnico de Bragança

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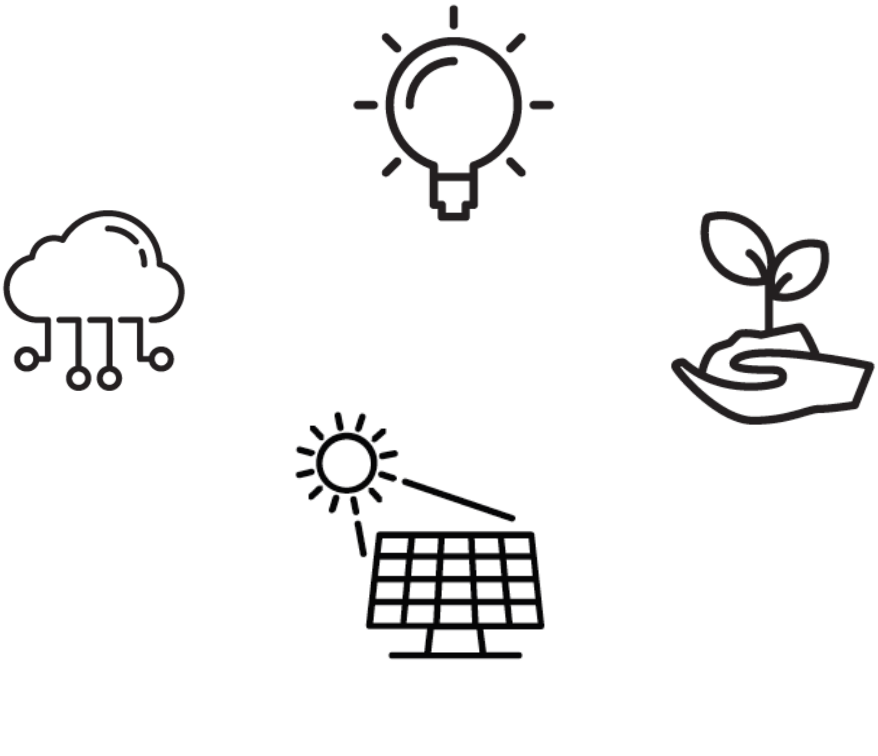

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Cassava-processing wastewaters: bioremediation potential of immobilized *Chlorella vulgaris* (Chlorophyceae)

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Abstract— Most cassava processing industry generates a large volume of wastewater effluent with high contaminating load of COD and BOD and cyanide concentration, posing to a serious threat to the environment. A variety of processes has been tested to treat this wastewater effluent, where the effectiveness in reducing the toxicity varies greatly. Microalgae are photosynthetic microorganisms that can rapidly generate biomass from solar energy, CO_2 and nutrients in water, namely those present in wastewater effluents. Also, algal biomass and algae-derived compounds are potentially useful in industrial applications, as well as for biodiesel production.

Taking this into account, the aim of this work was evaluated the ability of *Chlorella vulgaris* to growth on cassava-processing effluent and assess the bioremediation potential by evaluating toxicity of effluent.

Batch cultures, with *C. vulgaris* in suspended and in immobilized-cell systems, under different dilutions (20-50%) of cassava effluent were tested. The effectiveness of the process has been assessed by phytotoxicity, in germination trials of *Lactuca sativa.* Best results were obtained with *C. vulgaris* in immobilized-systems and carried out with higher initial cell concentrations. Regarding the phytotoxicity, after biotreatment we can conclude that microalgae can reduce the toxicity of cassava effluent, leading to Germination Increase index (GI) range of 88-100%.

Keywords— **cassava wastewaters; bioremediation; *Chlorella vulgaris;* phytotoxicity; *Lactuca sativa*.**

I. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important food, being produced by more than 80 countries [1]. Most cassava processing industry generates a large volume of wastewater effluent with high contaminating load of COD and BOD and cyanide concentration, posing to a serious threat to the environment. Because disposal of effluents from cassava processing is becoming an increasing problem, several treatments were carried out [2-6]. Nevertheless, the cost of implementing the technology is, in many cases, prohibitive [6], or are not very viable for the treatment.

Microalgae are photosynthetic microorganisms that can rapidly generate biomass from solar energy, CO_2 and nutrients in bodies of water. Algal biomass and algae-derived compounds are potentially useful in industrial applications, as well as for biodiesel production [7-11]. Other applications from microalgae are due to their ability to wastewater treatments [12-17].

Therefore, the aim of this work was evaluated the ability of *Chlorella vulgaris*, a single-cell Chlorophyceae, to growth in cassava-processing effluent and assess the bioremediation potential by evaluating the toxicity of treated effluent.

II. Material and Methods

Effluent sampling: Samples of cassava effluent were collected in Norwest of Brazil using a 5L plastic bottle in December of 2016. At laboratory arrive the cassava effluent was filtrated, for removal of suspended solids and frozen until use for batch cultures.

Microalgae cultures: *C. vulgaris* (CBSC 15-2075), was obtained from the Carolina Biological Supply Company (USA) and was pre-cultured in 250 mL flasks with sterilized Bold´s Basal medium (BB) in a controlled chamber, under the following conditions: temperature of 22 ± 0.5 ºC, light intensity of 4500 lux (Gro-Lux fluorescent lamps), 16:8 h light:dark photoperiod, with continuous aeration, until reach exponential growth phase and to be able to be used for essays.

Effluent biotreatment: The assays were carried out in batch cultures, with *C. vulgaris* in suspended and in immobilized-cell systems, under different dilutions of cassava effluent. This later consists of effluent dissolved on BB medium in concentrations range between 20% to 50% and sterilized by autoclaving (121^{0}C, 0.1 Mpa, 20 min). The microalgal immobilization was made in sodium alginate solution at 1.5% and previously washed in distilled water before inoculation.

Batch experiments were developed in 100 mL flasks under no axenic conditions, for 4 days in the following conditions: (i) two distinct initial cell concentrations, approximately $7{\times}10^6$ cells mL^{-1} and $12{\times}10^6$ cells mL^{-1}; (ii) a constant ratio of total culture volume to beads volume of 5 (v/v) for suspended system; (iii) initial pH of 4.45±0.17; (iv) room temperature (approximately 25.0±1.0 °C); (v) light intensity of 5000 lux and 16:8h light:dark photoperiod and (vi) continuous aeration with the injection of atmospheric air at the bottom of the flasks. For each condition, two independent experiments were performed.

Toxicity evaluation: The evaluation of the cassava effluent toxicity, after biotreatment, was made by germination tests. These tests were performed with lettuce (*Lactuca sativa*) seeds, incubated in a growing chamber, in the dark, at 26°C±0.1, for 3 days. Six lettuce seeds were disposed on each Petri dishes (diameter of 55 mm), lined with filter paper and watered with 2 mL of sample collected after final biotreatment. For each dilution of cassava effluent, the positive control was distilled water and the negative control was water:BB medium at same dilution than effluent. The effect of biotreatment was made by comparisons of biotoxicity of cassava effluent with and without biotreatment. All the essays were done in triplicates. Results were expressed as a number of Germinated seeds (G) and Germination Increase index (GI) according to the formula:

$$GI\ (\%) = (Gtest - GcontW) \div Gtest \times 100$$

(Gtest = nº of seeds germinated after biotreatment; GcontW = nº of seeds germinated without biotreatment).

Statistical analysis: Results were expressed as mean values ± standard error.

III. Results and Discussion

To evaluate the growth ability of *C. vulgaris* under the presence of cassava effluent, several effluent dilutions were prepared with BB medium and inoculated with suspended cells or with immobilized cells. Best results were obtained with *C. vulgaris* in immobilized-systems. In fact, results showed that, in generally, *C. vulgaris* in suspended-cell systems can not growth in the tested dilutions of the effluent. Reports have shown that cassava effluent contains harmful cyanides, copper, mercury and nickel which have the capacity to affect native micro-biota [18], and therefore certainly also affects the microalgae, inhibiting their grow. On the other hand, *C. vulgaris* in immobilized-systems apparently could grow in all tested dilutions of the effluent. The time-course evolution of pH of these cultures, also suggests that the pH increase observed, from 4.45 to 8.00, could be related to cyanide remove, since their presence produces an acidic effect. In cell entrapment, microalgal cells are confined to the polymeric matrix and substrates and products diffuse to and from the cells through the pores present in the matrix. This system had some advantages, since immobilization matrix confers cells higher resistance to harsh environments [7,13,19], such as with the cassava effluent.

Table 1- Germinated seeds (G) and number of days obtained for controls and for the cassava effluent dilutions, with and without biotreatment (higher inoculum).

<table>
<tr><th colspan="2" rowspan="2"></th><th colspan="2">Without Biotreatment</th><th colspan="3">With Biotreatment</th></tr>
<tr><th>Days</th><th>G (%)</th><th>Days</th><th>G (%)</th><th>GI (%)</th></tr>
<tr><td colspan="2">Control⁺</td><td>2</td><td>94±9.62</td><td>2</td><td>100</td><td rowspan="2"></td></tr>
<tr><td colspan="2">Control⁻</td><td>2</td><td>100</td><td>2</td><td>94±9.62</td></tr>
<tr><td rowspan="3">Effluent dilutions</td><td>20%</td><td>2</td><td>11±9.62</td><td>2</td><td>94±9.62</td><td>88±10.72</td></tr>
<tr><td>40%</td><td>2</td><td>5.56±9.62</td><td>3</td><td>78±9.62</td><td>93±11.55</td></tr>
<tr><td>50%</td><td>3</td><td>0</td><td>3</td><td>56±19.25</td><td>100</td></tr>
</table>

The bioremediation potential of *C. vulgaris* in immobilized-systems was evaluated by germination tests and results showed that the toxicity of the effluent after treatment was diminished considerably. Also, results showed that batch cultures carried out with higher initial cell concentrations in alginate beads ($12{\times}10^6$ cells mL^{-1}) showed best results, comparing with lower initial cell concentrations (table 1). As expected, biotreatment carried out in cassava effluent leads to an increase in the number of germinated seeds, comparing with cassava effluent without treatment. This result was expressed as Germination Increase index (GI), which reached 100% for the treatments of cassava effluent diluted at 50%. In general, the increase of effluent concentration affects the seeds germination, inhibiting the number of germinated seeds (G), namely, cassava effluent diluted at 50% fully inhibits seeds germination. The negative control was prepared to access the effect of BB medium on seeds germination and since there are no differences between dilutions, the results presented here concerning the water:BB medium at 50 % dilution. These results confirmed that *C. vulgaris* can reduce phytotoxic compounds present in cassava effluent that inhibits germination of *L. sativa*, thus showing a good biotreatment potential.

IV. Conclusions

In generally, results from this work showed that immobilized *C. vulgaris* could be an alternative to bioremediation of cassava effluent, but more detailed studies are still needed to optimize the process. Biotreatments of wastes using living organisms is an environmentally friendly, relatively simple and cost-effective alternative to physico-chemical processes. Furthermore, the biotechnology of growing microalgae in wastewater is getting importance as biomass production for many other valuable applications.

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