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Copper removal by *Botryococcus braunii* biomass with associated production of hydrocarbons

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Abstract.
The goal of the present article is to evaluate the potential of copper(II) removal from acidic wastewater, associated with the production of hydrocarbons by the microalgae *Botryococcus braunii*.
Results demonstrate that the growth of *B. braunii* is correlated with the hydrocarbon production as well as with alcalinization and copper removal from the medium. Even though *B. braunii* did not present high rates of copper adsorption, the increase in the pH of the media promotes the precipitation of the metal. In this way copper can be removed from solution by both, adsorption and precipitation. Results suggest that metabolic active biomass of *B. braunii* could be used for copper removal from solution while it produces appreciable quantities of hydrocarbons. This fact is very interesting in order to develop new remediation processes of waste water with coupled energy production.

Introduction
Society has developed on the bases of the advances in the industry and technology. These advances are associated with an increased use of energy resources, particularly fossil fuels, as well as an increase in environmental pollution. There are many scientific papers that states that in the years to come the petroleum reserves will decrease considerably [1-3]. The increasing waste products as well as the prediction that oil reserves are decreasing, generate the need to improve technologies to remove trace metals from polluted waters and to develop alternative and renewable energy sources that can fill, in a near future, the energy and the environmental quality that world demands [4, 5].
In recent years, many researchers have studied the capacity of microalgae to generate high quantities of hydrocarbons [6-7] as well as to remove heavy metals from waste waters [8-9].
Botryococcus braunii is a freshwater colonial planktonic alga with a worldwide distribution. This alga is characterized by the excretion of oil to the outer layers of the cell wall [10]. Even though *B. braunii* have a great capacity of synthesize hydrocarbons, its biotechnological potential resides also in the capacity of revert the acid conditions of an effluent, and the capacity to remediate the presence of heavy metals.
The aim of the present article is to evaluate the potential of copper(II) removal from contaminated water, associated with the production of hydrocarbons by the microalgae *Botryococcus braunii*.

Materials and Methods
For the cultivation of *B. braunii* a strain (B-race) from the Algal Culture Center of the University of Texas was used. Stock cultures were prepared in modified Chu 13 medium [11].
In order to determine the hydrocarbons production by *B. braunii* as a function of time, known amounts of biomass were added to 7 Erlenmeyer flasks (250 ml) with 100 ml of modified Chu 13 medium in each, then samples were incubated at different times (7, 9, 13, 16, 25, 30 and 40 days).

Afterwards, they were centrifugated at 3500 rpm during 15 minutes. The pellet were separated, dried in an oven at 60°C till constant weight in order to make the separation and gravimetric quantification of the hydrocarbons by successive extractions with n-hexane [12].
Prior to evaluate the copper removal potential of growing cultures of *B. braunii*, toxicity of the metal on the algae was determinate. With this purpose the cultures were incubated during 40 ± 3 days at 25°C in modified Chu 13 medium and exposed to different copper concentrations (1, 2, 3, 4, 10 and 20 ppm). Aliquots were taken at different time intervals in order to evaluate the pH variation, the culture growth, the hydrocarbon production as well as the final copper concentration in solution.
Copper removal experiments were conducted in batch mode at an initial pH of 4.5± 0.5. The copper removal efficiency was evaluated as a function of contact time and initial metal concentration. Known amounts of biomass were added to 4 Erlenmeyers with 100 ml of modified Chu 13 medium and different copper concentrations (control, 2, 4, and 10 ppm). Samples were incubated during 38 days under the same conditions as toxicity experiments. Aliquots were taken at different time intervals in order to evaluate the pH variation, the culture growth and the final copper concentration in solution. To evaluate the capacity of *B. braunii* to continue removing the metal in solution after 24 days, when the copper concentration in solution decreased, an aliquot of concentrated copper solution (1000 ppm) was added to the medium in order to reload the same initial metal concentration.
In all experiments the culture growth were determined by turbidimetry, measuring optical density (O.D.) at 680 nm or by gravimetry, when correspond.

Results and Discussion.

Results demonstrated that after certain stage of growth (O.D. = 0.35), the hydrocarbons production is coupled with the growth of the biomass of *B. braunii* (Fig. 1)

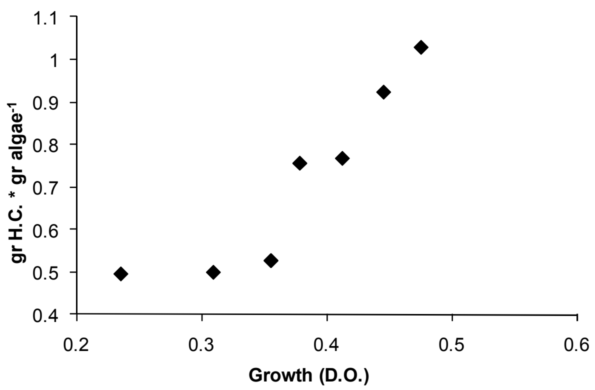

Figure 1: Hydrocarbon production as a function of the growth of *B. braunii*.

Toxicity results demonstrate that copper is toxic for the alga at concentrations over 10 ppm (Fig. 2. A). Although the cellular viability was maintained under this concentration values, the cultures did not grow in the presence of copper when concentrations where higher than 3 ppm (Fig. 2.A). Even though at concentration of 1 and 2 ppm the cultures grew, these growth were much lower than the growth of the culture in the absence of copper (control).

The variation of the pH througout the experiments were affected by the copper concentration in solution (Fig. 2.B). At copper concentrations of 20 ppm the pH value first decrease and then kept constant in time, this is probably because the alga died due to high copper concentration and there were no fisiological activity that can affect the pH of the medium. At copper concentrations of 3, 4 and 10 ppm the pH values riced from 5.5 til 6.0 ± 0.3; below 2 ppm, the pH values raced from 5.5 til 7.0± 0.3. These experiments demonstrate that the rise in the pH of the solution is clearly proportional to the growth of *B. braunii*, since at lower copper concentrations the variation of the pH values were higher than the variations of the pH where the copper concentrations were high and the alga did not grow.

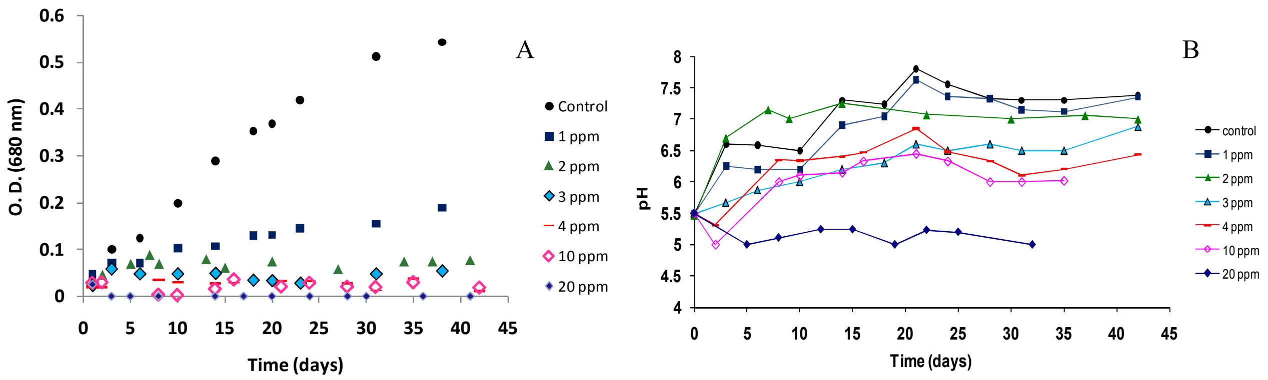

Figure 2. Growth of *B. braunii* (A) and the variation of the pH (B) in modified Chu 13 (•) medium and exposed to different copper concentrations: 1 ppm (■), 2 ppm (▲), 3 ppm (♦), 4 ppm (-), 10 ppm (◊) and 20 ppm (♦).

The results of hydrocarbons production by *B. braunii* in each of the experiements are shown in table 1. The amount of hydrocarbons produced by *B. braunii* in the presence of Cu(II) at concentrations over 4 ppm, decreased with respect to the amount generated by those cultures where there is no Cu(II) at all. Despite the fact that the hydrocarbon production diminsh when metal concentration in the medium increases, at concentrations of 10 ppm *B. Braunii* keeps on producing almost 70% of hydrocarbons respect to the control; at 4 ppm it produces 80% with respect to the control and at lower copper concentration (2 ppm) the amount of hydrocarbon generated in the presence and absence of the metal does not present signigicant diferences.
These results are correlated with the effect of different copper concentration on the growth of *B. braunii*.

Table 1: Amount of hydrocarbon generated by *B. braunii* per gram of algae cultivated under diferent copper concentrations.

Culture medium	Initial Cu(II) concentration [ppm]	Hydrocarbon production [gr H.C. * gr algae^{-1}]
Modified Chu 13 (control)		0.95
Modified Chu 13 with Cu(II)	2	0.97
Modified Chu 13 (control)		0.96
Modified Chu 13 with Cu(II)	4	0.81
Modified Chu 13 (control)		0.97
Modified Chu 13 with Cu(II)	10	0.72
Modified Chu 13 (control)		0.90
Modified Chu 13 with Cu(II)	20	0.39

Figure 3 show the removal of copper from solution by *B. braunii* at different initial metal concentrations. As it can be seen, at initial metal concentration of 10 ppm, the copper in solution decreased till concentrations below 6 ppm at day 22, when the culture died. By this time there was a rise in copper concentration in solution. This could be due to the fact that when the culture was alive it physiological activity affected the pH of solution, increasing the pH till values above 5 where the copper might precipitate. When the culture died, the pH of solution droped and part of the precipitated copper was resuspended.
At initial copper concentrations of 4 ppm and 2 ppm there was a constant decrese in the metal concentration of the solution (Fig. 3) as well as an increase in the pH of the medium. In the experiment where the initial metal concentration was 4 ppm, after 24 days this concentrations decrease till less than 2 ppm while the pH of solution increased till values above 5.5. In the experiment where the initial copper concentraion was 2 ppm, after 24 days there was almost no copper remaining in solution. So after 24 days, an aliquot of concentrated copper solution was added to both experiments in order to restablish the initial metal concentration. As it can be seen in figure 3, *B. braunii* kept on removing the metal from solution even when more metal was added to the medium, as well as the pH kept on increasing till values above 6.

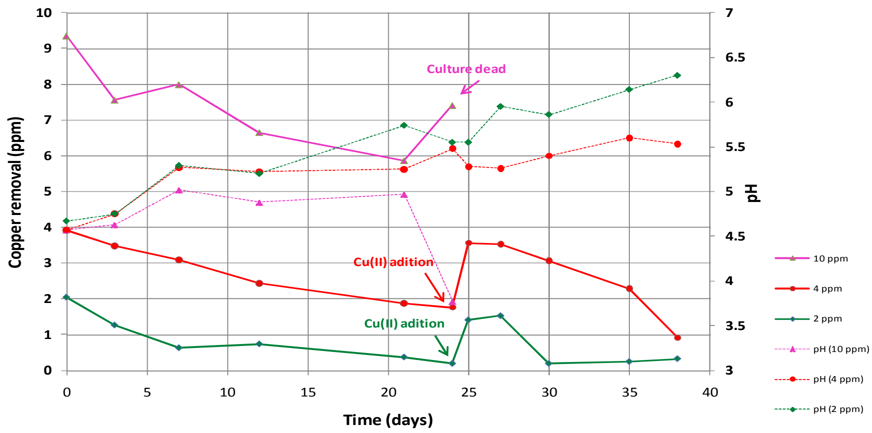

Figure 4: Removal of copper from solution by *B. braunii* at different initial metal concentrations: 2 ppm (♦), 4 ppm (●) and 10 ppm (▲) correlated with each pH variation.

Conclusions.

As shown, these results state many of the parameters necessaryto use *B. braunii* for the remediation of copper from effluents and demonstrate its potentiallity to be used in the remediation of acidic wastewaters containing this metal.as well as to be used as a natural energy source.

The copper concentration range at which *B. braunii* is viable, the amount of hydrocarbons obtained under these conditions as well as the amount of copper removed from solution were determined.

Results obtained in the present study demonstrate the biotechnological potential that *B. Braunii* posses, as the importance of studiyng new remediation techniques and the development of alternative environmentaly friendly energy sources.

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