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Articles

Development of a sustainable system of phytoremediation and bioethanol with *E. crassipes*

Desarrollo de un sistema sostenible de fitorremediación y bioetanol con *E. crassipes*

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Abstract

Eichhornia crassipes is an aquatic plant, present in tropical climates and due to poor management of domestic wastewater in aquatic ecosystems, its reproduction is prominent, therefore, in contaminated river, wetland, between other, the biomass of this plant is very abundant and hence its final disposal entails great economic costs. Moreover, all this biomass is currently not used in a sustainable way. This plant could use for different process of type environmental, due to its proven use in the recuperations of waters contaminated with heavy metals and also it is could use in the productions of biofuels, due to high content of cellulose in your biomass. The objective of this research is to develop a sustainable process between phytoremediation and bioethanol production with the biomass of *Eichhornia crassipes*, evaluating the incidence of lead (II) adhered to the biomass of this plant in the production of bioethanol. Materials and methods: a system was installed to evaluate phytoremediation with *E.* crassipes with water loaded with Lead (II), determining the effectiveness of this plant to remove this heavy metal even if it is alive in a water body. After this process, it proceeded to take the lead (II) loaded biomass to the bioreactors to evaluate the bioethanol production, evaluating three types of biomass, one without lead (II) attached and the other two with lead (II) attached to the structure of its plant. There was a 30 % decrease in ethanol production from *Eichhornia crassipes* due to the presence of lead (II). Concluding that *E. crassipes* biomass could be fully used for phytoremediation processes of heavy metal contaminated water.

Keywords: *Eichhornia crassipes*, biomass, phytoremediation, bioethanol.

Resumen

Eichhornia crassipes es una planta acuática, presente en climas tropicales. Debido al mal manejo de aguas residuales domésticas en ecosistemas acuáticos su reproducción es prominente, por lo tanto, en cuerpos de aguas contaminados, la biomasa de esta planta es muy abundante y por ende su disposición final conlleva grandes gastos económicos. Además, toda esta biomasa en la actualidad no se aprovecha de manera sostenible. Esta planta podría utilizarse para diferentes usos de tipo ambiental, debido a su comprobado uso en la recuperación de aguas contaminadas con metales pesados y también se podría utilizar en la producción de biocombustibles debido a su alto contenido de celulosa en su biomasa. El objetivo de esta investigación es desarrollar un proceso sostenible entre fitorremediación y producción de bioetanol con la biomasa de *Eichhornia crassipes*, evaluando la incidencia de plomo (II) adherido a la biomasa de esta planta en la producción de bioetanol. Materiales y métodos: se instaló un sistema para evaluar la fitorremediación con *E. crassipes* con agua cargada con plomo (II) y la determinación de la eficacia de esta planta para eliminar este metal pesado incluso si está viva en un cuerpo de agua. Después de este proceso se procedió a tomar la biomasa cargada con plomo (II); se llevó a los biorreactores para evaluar la producción de bioetanol así como para la evaluación de tres tipos de biomasa: una sin plomo (II) adherido y los otros dos con plomo (II) adherido a la estructura de su planta. Hubo una disminución del 30 % en la producción de etanol de *Eichhornia crassipes* debido a la presencia de plomo (II). Se concluye que la biomasa de *E. crassipes* podría utilizarse totalmente para los procesos de fitorremediación de aguas contaminadas con metales pesados.

Palabras clave: *Eichhornia crassipes*, biomasa, fitorremediación, bioetanol.

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Introduction

The macrophyte E *crassipes*, is considered an invasive species, due to its adaptability to a wide range of ecosystems, considerably affecting the natural balance of ponds, lakes etc. (Vankar & Bajpai, 2008; Vásquez 2012). A single E. *crassipes* plant that reaches a wetland contaminated by domestic wastewater, with excess nutrients, is eutrified, therefore, the plant has the best nutritional conditions to grow and multiply, and on average, every 3 days it reproduces by stolons, reaching a total of more than 100,000 specimens in the same wetland in 6 months. The best way to control the growth of the E. *crassipes* plant is not to contaminate the wetlands (Carreño-Sayago & Rodríguez-Parra, 2019; Sayago, Castro, Rivera, & Mariaca, 2020).

It is a plant that has been the source of many investigations in the world, such as in phytoremediation of contaminated water and the production of bio-energy. In recent years it has been demonstrated that this species can be managed in a sustainable manner and design simple but interesting solutions in different industries that pollute water with heavy metals and also a contribution to the energy problem facing the world (Zimmels, Kirzhner, & Malkovskaja, 2006; Kasturiarachchi, Jayaweera, Wijeyekoon, Hirimburegama, & Fernando, 2014; Atehortua & Gartner, 2003).

The decontamination of water from industries that discharge heavy metals and pollute water resources, is an efficient and economic option with the use of biomass from E *crassipes* (Eid *et al*., 2019; Victor, Ladji, Adjiri, Cyrille, & Sanogo, 2016). The presence in *E. crassipes* cellulose of hydroxyl (OH), amino (NH_2) and carboxyl (C=O) groups facilitates the adsorption of different heavy metals through cation exchange (Lin, Yang, Na, & Lin, 2018; Eyley & Thielemans, 2014; Han, Zhou, French, Han, & Wu, 2013; Man *et al*., 2011; Sayago *et al*., 2020; Carreño-Sayago, 2021).

The design and development of treatment systems with the biomass of E *crassipes*, has had significant results in the adsorption of zinc (Atehortua & Gartner, 2003); copper (Ibrahim, Ammar, Soylak, & Ibrahi, 2012); chromium (Atehortua & Gartner, 2003); arsenic (Sarkar, Rahman, & Bhoumik, 2017; Lin *et al*., 2018); and lead (Borker, Mane, Saratale, & Pathade, 2013; Zhou *et al*., 2011; Ammar, Elhaes, Ibrahim, & Ibrahim, 2014; Deng *et al*., 2012).

The biomass generated in these treatments, involves a series of costly final disposals due to the type of waste, an alternative is to continue the sustainable process, since this biomass contains even the main polysaccharides for bioethanol production.

E. crassipes contains large contents of cellulose and hemicellulose, making it a significant plant as biomass for large-scale production of ethanol and hydrogen (Cuervo, Folch, & Quiroz, 2009; Porous, Siregar, &

Salem, 2012; Pizarro *et al*., 2016; Lee, Park, Cho, & Kim, 2018; Zabed, Sahu, Boyce, & Faruq, 2016; Kouwanou *et al*., 2018).

In the present investigation a sustainable system was developed, with the biomass of E *crassipes*, where a system of phytoremediation was built for the decontamination of lead (II) in a synthetic water, the biomass product of this process, was taken to a process of production of bioethanol, in order to evaluate the capacity of production of bioethanol of this biomass with adhered lead used in this process.

Materials and methods

E. crassipes was taken in the municipality of Mosquera, near the city of Bogota, then washed with water to remove traces of mud in that wetland there is a high degree of contamination. Two important processes were carried out in this investigation, a phytoremediation process where *E. crassipes* were used to treat water contaminated with Lead (II). After this experiment, the biomass used to treat the water was used to create a system composed of two bioreactors for the production of bioethanol.

Development of the phytoremediation experiment

The dimensions of the experimental model of the phytoremediation are 45 cm long, 10 cm high and 12 cm wide, where each one had 10 L of water. This design is to pilot scale and had 200 grams of E. *crassipes* (approximately two plants). Experiments were conducted in duplicate:

- (2) 500 (mg/l) of Lead.
- (2) 750 (mg/l) of Lead.

Where the mean, standard deviations and correlation coefficients were obtained, in the figure 1, it is to appreciate the experiments.

Figure 1. Phytoremediation Process

These lead solutions are standardized for testing and resemble industrial effluents, where lead (II) solutions were prepared by dissolving anhydrous lead nitrate salt in deionized water. The proposed evaluation of this treatment system took approximately 15 days. For the evaluations of this treatment system, lead concentrations in water (mg/l) were measured at first and then every two days.

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Development of the experimental model of bioethanol production

The biomass used in the previous phytoremediation process was used in this biofuel production process. In Figure 2, lead (II) is shown attached to the plant structure.

Figure 2. Plant of E *crassipes*.

Three experiments were carried out with 3 different types of biomass:

- x1 is the biomass of 500 (mg/l).
- x2 is the biomass of 750 (mg/l).
- x0 is the biomass without process of phytoremediation.

Evaluating Lead (II) affects the production of bioethanol from this type of biomass. Represented in Table 1.

Table 1. Different types of biomass.

Experimental	Representation
1. Biomass with 500 (mg/l) lead (II)	E1
2. Biomass with 700 (mg/l) lead (II)	E2
3. Biomass outwith lead (II)	E0

The design of the bioethanol generation process consists, for each experiment, of the construction of two bioreactors: a bioreactor to make the hydrolysate and a bioreactor for fermentation. For all the experiments, 50 gr of dry biomass used in the phytoremediation process was counted.

Hydrolysis bioreactor. It is 1 liter in glass, has a lid for the evaporation of gases and for some samples of pH and temperature, also has a magnetic agitation heater at 120 rpm at a temperature of 60 ºC, this operation is carried out continuously.

Dry *E. crassipes* was taken to this bioreactor, in a quantity of 100 gr, where it was mix with distilled water. The samples were made to react in 1 % (weight/volume) of caustic soda (NaOH) at a temperature of 60 °C, during 12 h, the samples were wash with tap water until reaching the pH value of water.

Subsequently, sulfuric acid (H_2SO_4) 3 % (volume/volume) was add at a temperature of 60 °C, during 12 h, the samples were wash with tap water until they reached the pH value of water. The content of reducing sugars was determined by the dinitro-salicylic acid (DNS) method (Cuervo *et al*., 2009), which indirectly quantifies substrate consumption. Two liters of *E. crassipes* hydrolyzed solution were obtained for the continuation of bioethanol production.

Fermentation bioreactor. 2.5 liters in glass, with the same characteristics of the bioreactor for the hydrolyzed. *Sacharomices sereciciae* was use as inoculum for the hydrolyzed *E. crassipes* fermenter. According to studies by Cuervo *et al*. (2009), and Ganguly, Chatterjee and Dey (2012), *Sacharomyces cerevisiae* has been determined to be suitable for ethanol production from plant biomass.

40 g of the hydrolysate was taken to the fermentation bioreactor, where it was mix with distilled water and 50 g of the inoculum was adding, the initial pH was adjusted to 5.5. The bioreactors were hermetically

sealed with rubber septa and aluminum caps. During the fermentation of the hydrolysis of the biomass of each type of biomass, the tests of the ethanol percentages were carried out by gas chromatography at different time intervals.

Phytoremediation results

The tests were carried out during 15 days. In Table 2 and Table 3, the results of the two tests of each experiment, the averages and standard deviation of each experiment are shown.

Table 2. Result of Experiment with 500 (mg/l) lead (II).

	Experiment 1 (mg/l)	Experiment 2 (mg/l)	Media (mg/l)	Deviations Standard (mg/l)	(%) remotion of lead (II)
Day (0)	500	500	500	-	0
Day (1)	405	433	419	14	16
Day (3)	305	298	302	3,5	40
Day (5)	182	179	181	1,5	64

Day (7)	151	121	136	15	73
Day (11)	121	111	116	5	77
Day (13)	120	105	113	7,5	77
Day (15)	93	95	94	1,5	81

Table 3. Result of Experiment with 750 (mg/l) lead (II).

	Experiment 1 (mg/l)	Experiment 2 (mg/l)	Medium (mg/l)	Deviations Standard (mg/l)	(%) Remotion Lead (II)
Day (0)	750	750	-	-	0
Day 1)	599	622	611	12	19
Day 3)	480	514	497	17	34
Day 5)	388	366	377	11	50
Day (7)	288	255	272	16	64
Day (11)	179	155	167	12	78
Day (13)	155	145	150	5	80
Day (15)	145	159	152	7	80

The results of Table 2 and Table 3 show a homogeneity of the data, the three experiments show a significant average due to the low standard deviation, the averages are sufficiently represented in the experiments, because the higher the coefficient of variation, the higher the heterogeneity of the values. From the mean and lower coefficient of variation, greater homogeneity in the mean values (Lin *et al*., 2018).

The eliminations of the phytoremediation process, with 500 (mg/l) of Lead (II) initially, shows a continuous decrease in this metal, it can be observed, the stabilization after 15 days of treatment. The two tests showed a similar behavior during the whole process and the removals were above 80 %, these results could be compared with Borker *et al*. (2013), where they obtained 72 % of Pb (II) removals. In Table 3, he presents a summary of the data of the experiment with initial lead concentrations of 750 (mg/l).

Like the initial experiment of 500 (mg/l), where the plant was able to adsorb 100 (mg/l) of lead (II), in this experiment also representative results were achieved at the first day of treatment and in general in the whole treatment process in removals of Lead (II) by *E. crassipes,* where they reached final concentrations of 152 (mg/l) of this heavy metal, reaching a removal of 80 %.

The cellulose of the roots attracts the lead present in the water, this is the mechanism by which *E. crassipes,* is a wide retention of heavy metals, especially lead (II) (Pizarro *et al*., 2016; Ibrahim *et al*., 2012).

Results of bioethanol production

The sugar production through the hydrolysis of the three experiments was significant, the Table 4 shows the results of the production of each type of biomass.

Table 4. Productivity of reducing sugars with three types of biomass.

	(g/l) Sugar primary	**Yield after 25 hours**
Biomass 500 (mg/l) (E1)	8	110
Biomass 750 (mg/l) (E2)	6	75
Biomass of *E. crassipes* (E0)	14	150

There was a constant production in the hydrolysis process, it can be seen that the lead (II) concentrations adhered to the plant structure doesn`t affect the production of reducing sugars in this process. This hydrolysate is passed on to the next ethanol production bioreactor.

Figure 3 shows a higher ethanol production for the *E. crassipes* sample without phytoremediation (E0) compared to 500 (E1) and 750 (E2) biomass samples, in a 24-hour time frame.

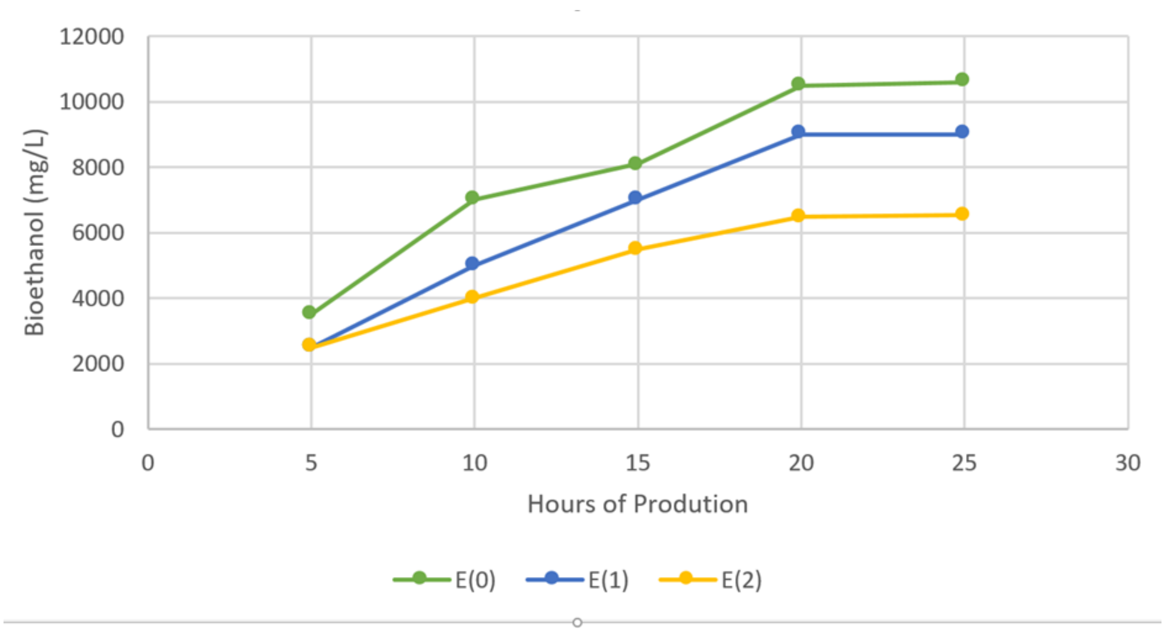

Figure 3. Production of ethanol with different biomass hydrolyzed.

Treatments with the *E. crassipes* biomass with lead (II) attached started in the first 5 hours to produce ethanol, in a smaller amount than the biomass without Lead (II). Figure 3 shows the growth curves and stabilization of each amount of biomass.

In conducting the mass balance, it was established that ethanol production from hydrolyzed *E. crassipes* (E0) biomass is cost effective with no lead attached, at 10 000 (mg/l) in 25 hours. These different results are similar to the study conducted by Lee *et al*. (2018), Zabed *et al*. (2016) and Kouwanou *et al*. (2018), where bioethanol production from *E. crassipes* biomass is produced with different types of hydrolysis and with a different fermentation agent. These investigations reported effective results but none with heavy metal loaded biomass.

The biomass of the 750 (mg/l) (E2) Lead (II) experiment produced an amount of 4 000 (mg/l) in 10 hours, less than the other two samples, then stabilized at 6 000 (mg/l) bioethanol in the 15th hour. While the biomass of the experiment of 500 (mg/l) (E1), reaches the highest values, reaching 7 000 (mg/l) of bioethanol at hour 15. This biomass was less affected than (E2), but both experiments showed some degree of affectation, but produced bioethanol despite having large amounts of Lead (II) attached.

Discussion

In different investigations, the capacities of *E. crassipes* to bio-adsorb heavy metals have been proven, but it is not discussed which is the final disposition of this biomass with polluting material adhered to it, also different investigations, have concluded that and designed the experiments for the production of biofuels with *E. crassipes* biomass.

For this reason, a sustainable process was developed, due to the bad use that is given to the plant E *crassipes,* which abounds in the humid zones of different cities of Latin America. This project demonstrated that phytoremediation with this plant is efficient and also the biomass used in this treatment could be used in hydrolysis processes and the subsequent production of biofuels.

The experimentation with two types of *E. crassipes* biomass loaded with different concentrations of Lead (II) showed that it could be used to produce bioethanol or other biofuels because their affectation is not significant. All *E. crassipes* biomass can be used for both phytoremediation and later for the production of bioethanol or other biofuels such as biohydrogen.

The use of the *E. crassipes* plant for phytoremediation processes and the subsequent utilization of this biomass for large scale biofuel production should be promoted, the costs of this macro project and the market for its use in phytoremediation and bioethanol should be determined.

Conclusions

The biomass without lead (II) charge obtained interesting results in the production of bioethanol, with an amount of 10 000 (mg/l) in 25 hours. For the samples with 500 (mg/l) lead (II) attached (E1), 10 % less ethanol was produced, being approximately 9 000 (mg/l) in the same 25 hours. While the *E. crassipes* biomass experiment used in the initial lead (II) removals of 750 (mg/l) yielded 40 % less bioethanol production, this biomass was affected by the amount of lead (II) attached. All *E. crassipes* biomass can be used for both phytoremediation and bioethanol production.

The presence of this plant in the wetlands should be used for large phytoremediation and biofuel production projects. It is recommended to formulate a sustainable phytoremediation and bioenergy macro-project, using the potential of the E. *crassipes* plants.

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