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Design and Implementation of a Sustainable Development Process Between Fitorremediation and Production of Bioethanol with E. *crassipes*

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Abstract: A E. *Crassipes* is considered a problem in different aquatic ecosystems, due to its abundance could become a solution to design and build economic and efficient treatment plants, and especially for the production of biofuels such as bioethanol. The objective of this research is to design and implement a sustainable development process between phytoremediation and bioethanol production with E. *crassipes*, evaluating the incidence of chromium adhered to the biomass of this plant in the production of bioethanol. Materials and methods: A system was installed to evaluate the phytoremediation with E crassipes with water loaded with chromium, determining the effectiveness of this plant to remove this heavy metal even if it is alive in a body of water. After this process, we proceeded to bring the biomass loaded with chromium to bioreactors to evaluate the production of bioethanol, assessing three types of biomass, one without chromium adhered and the other two with chromium adhered to its plant structure. There was an impact of the ethanol production of the E crassipes due to the presence of chromium, but this production can be taken into account for the assembly of an integral system of phytoremediation and bioethanol production, making the most of this biomass.

Keywords: E *crassipes*; biomass; phytoremediation; bioethanol

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1. Introduction

The macrophyte E *crassipes*, also known as "Water hyacinth", or "Water hyacinth" is considered an invasive species due to its adaptability to a wide variety of ecosystems, considerably affecting the natural balance of lagoons, lakes, etc. [1, 2], An example of the above is its strong presence in contaminated wetlands such as Juan Amarillo in the city of Bogotá, where they removed almost 30 tons in 2016 [3].

It is a plant that has been the source of many investigations in the world, such as the phytoremediation of contaminated water and the production of energy. In recent years it has been shown that this species can be manipulated in a sustainable manner and create practical solutions in different industries that pollute water and also a contribution to the energy problems facing the country. [4-6]

Phytoremediation with this plant represents an efficient and economical technology for the treatment of water contaminated with nutrients, heavy metals and high contents of organic matter, since it does not require sophisticated infrastructure [7- 9].

Different investigations have exposed the biomass of this plant with heavy metals, removing important quantities [10-13]; concluding that the biomass of E. *crassipes* is suitable as effective absorbent of different heavy metals, such as chromium, mercury and aluminum.

[14] sifted the E. *crassipes*, to build a biological filter, treating the industrial waters contaminated with chromium and lead, removing 60% of these metals. Also, [9]), screened E *crassipes* to treat industrial effluents, yielding efficiencies of over 90% in heavy metals. In the study by [15], he analyzed the adsorption capacity of dry E. *crassipes* by means of flow tests and it was found that this capacity depends on variables such as the flow rate, the pH of the solution and the size of the solution particles.

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But one of the problems generated by this type of phytoremediation treatment is the amount of biomass contaminated with heavy metals among others. An alternative is to generate with this material a type of biofuel such as bioethanol, since this biomass of the E *crassipes* has large contents of cellulose and hemicellulose, which makes it a significant plant as biomass for the large-scale production of ethanol and hydrogen [16, 17].

[18], performed on E crassipes, an acid hydrolysis, with sulfuric acid. The resulting hydrolyzed solution was found to be rich in hexoses and pentoses which were used directly as a substrate for the production of alcohol by means of batch fermentation using the Pichia stipitis NCIM 3497. [19], produced bioethanol from E *crassipes* using a two-stage process: an acid hydrolysis, followed by an alcoholic fermentation, implementing the yeast xylo-fermentative: Candida Sheatae, obtaining a performance comparable to that obtained by enzymatic hydrolysis, demonstrating a simple and accessible procedure can be generated when thinking about scale industrial.

[20], determined that sulfuric acid hydrolysis is the most effective pre-treatment for the treatment of E crassipes. In one year, from one hectare covered by this plant, it is possible to produce 265 liters of ethanol.

[21], investigated different compounds to degrade the sugar of the E *crassipes*. Finding that Saccharomyces cerevisiae increased the alcohol content in the process. Also [22], developed a bioreactor to produce bioethanol from E. *crassipes* providing the design parameters for this experimentation.

The objective of this research is to design and implement a sustainable development process between phytoremediation and bioethanol production with E.*crassipes*, evaluating the incidence of chromium adhered to the biomass of this plant in the production of bioethanol. Introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance.

2. Materials and Methods

The E. *crassipes* was taken in the municipality of Mosquera, near the city of Bogotá, later it was washed with water to eliminate mud traces since this wetland is in a high degree of contamination. Two significant processes were carried out in this investigation, a phytoremediation process where the E *crassipes* were used to treat the water contaminated with chromium. After this experimentation, the biomass that was used to treat the water was used to create a system made up of two bioreactors for the production of bioethanol.

The dimensions of the experimental model of phytoremediation is 40 cm long, 15 cm high and 15 cm wide, where each one had 10 L of water. This design is pilot scale and had 180 grams of E *crassipes*, which is the equivalent of two plants. There were 6 experimental assemblies, 3 with 620 mg / L of initial chromium and 3 with 740 mg / L of initial chromium.

These chromium solutions are standardized for testing and resemble those of a tannery. The proposed evaluation of this treatment system lasted approximately 1 month. For the evaluations of this treatment system the concentrations in the chromium water in mg / L were measured. At the beginning and later every two days. In the following figure 1. It shows a treatment system.

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Figure 1. Phytoremediation

Development of the experimental model of bioethanol production.

The biomass used in the previous phytoremediation process was used in this biofuel production process. In the following figure 2, the chromium adhered to the plant structure is shown.

Figure 2. Biomass used in the production of bioethanol.

Three experiments were carried out with 3 different types of biomass: 1. biomass of the treatment of 610 mg / L of chromium, 2. biomass of 714 mg / L of chromium and 3. biomass without the process of phytoremediation, evaluating whether chromium affects the production of bioethanol from this type of biomass. Represented in the following table 1:

Table 1. Different types of biomass

Experiment	Representation
1. biomass of treatmnet of 610 mg/L of crhome	X1
2. biomass of 714 mg/L of crhome	X2
3. biomass without the process of phytoremediation	X0

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The design of the bioethanol generation process consists, for each experiment, of the construction of two bioreactors: a bioreactor to make the hydrolyzate and a bioreactor for the fermentation where the mathematical component will have in this article. For all the experiments, 100 g of dry biomass used in the phytoremediation process was counted.

The hydrolyzed bioreactor is 2 liters in glass, has a lid for the evolution of gases, taking samples of pH and temperature, together with a magnetic stirring heater at 120 RPM at a temperature of 60º C. In the following figure 3, bioreactors of hydrolysis and bioethanol production is shown.

Figure 3. Bioreactors of hydrolysis and bioethanol production.

In the bioreactor of the hydrolyzate the dry E. *crassipes* was taken, in an amount of 100 gr, where it was mixed with distilled water. The samples were reacted in 1% (w / v) of caustic soda (NaOH) at a temperature of 60 ° C, during 12 h, the samples were washed with tap water until reaching the pH value of the water. Subsequently, sulfuric acid (H2SO4) 3% (v / v) was added at a temperature of 60ºC, during 12 h, the samples were washed with tap water until they reached the pH value of the water. The content of reducing sugars was determined by the Dinitro Salicylic Acid (DNS) method [23], which indirectly quantifies substrate consumption. 4 Liters of hydrolyzed solution of E. *crassipes* were obtained for the continuation of bioethanol production.

The bioreactor of the fermentation is 5 liters in glass, with a lid for the evolution of gases, sampling of PH and Temperature, with heater and magnetic stirring at 120 RPM at a temperature of 60º C. Sacharomices sereciciae was used as inoculum of the fermentor of the hydrolyzate of E. *crassipes.*

100 g of the hydrolyzate was taken to each bioreactor where it was mixed with distilled water and 100 gr of the inoculum was added, the initial pH was adjusted to 5.5. The bioreactors were hermetically sealed with rubber septa and aluminum plugs. During the fermentation of the hydrolysis of the biomass of each type of biomass, tests of the ethanol percentages were carried out by gas chromatography at different time intervals.

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3.1 Results of phytoremediation

It can be seen in Figure 4, the removals of the process of phytoremediation, showing a continuous decrease in this metal, stabilizing after 24 days of treatment. The three tests showed a similar behavior during the whole process and removals were obtained above 70%, these results could be compared with those of [24], where they obtained removals of Cr (VI) in 72%, from the root biomass E. *crassipes.*

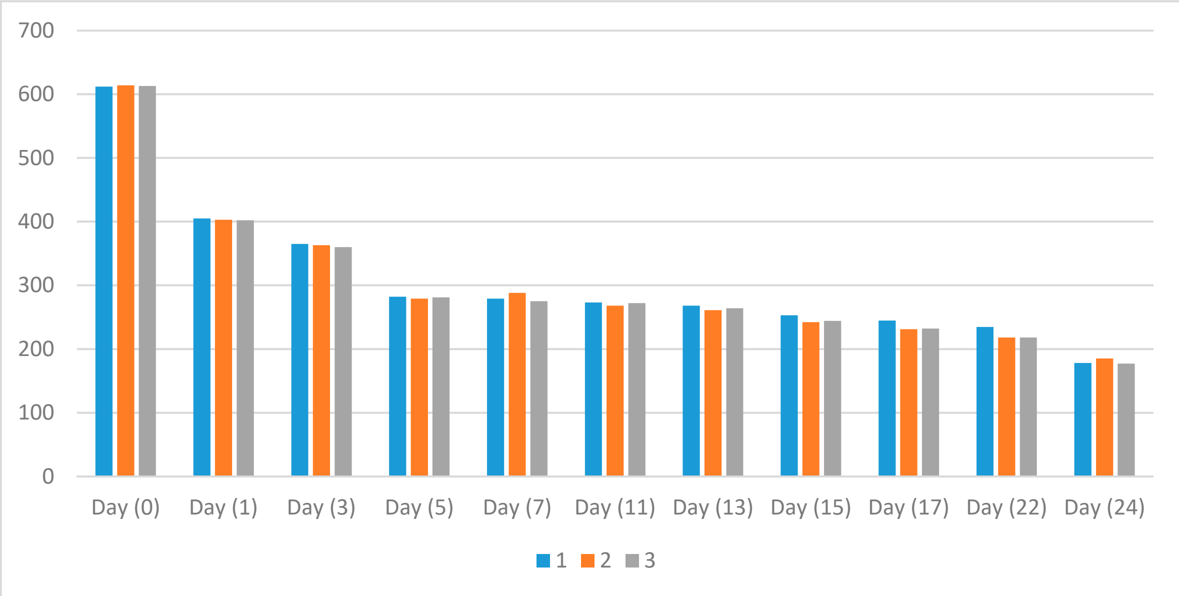

Figure 4 Phytoremediation with 620 mg / L of Chromium

It can be seen in the following Figure 2, that the initial concentrations showed a similar behavior during the whole process and removals were obtained above 60%.

Unlike the previous treatment of 620 mg / L, these treatments with 714 mg / L, the plant found it difficult to adapt and after 3 days removals of more than 30% were obtained, stabilizing the following days. In the end he obtained a 58% removals.

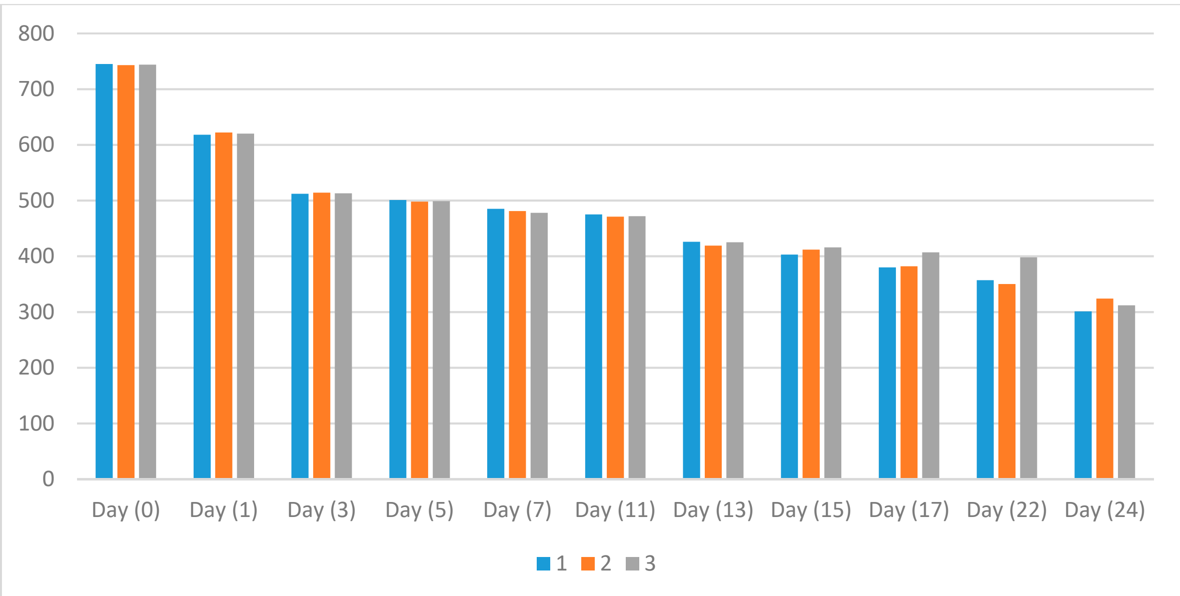

Figure 5. Phytoremediation with 714 mg / L of Chromium.

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3.2 Results of bioethanol production.

The production of sugar through the hydrolysis of the 3 experiments was significant, in the following table shows the results of the production of each type of biomass.

Table 2. Productivity of reducing sugars with 3 types of biomass.

	gr /L of sugars	Performance after 12 hours
Biomass 610 mg/L (x1)	10	110
Biomass 714 mg/L) (x2)	8	75
Biomass of Eichhornia (x0)	15	150

There was a constant production in the hydrolysis process, it can be observed that the chromium concentrations adhered to the plant structure if it affects the production of reducing sugars in this process. This hydrolyzate passed to the next ethanol production bioreactor.

Figure 3 shows a higher ethanol production for the sample of E. *crassipes* without phytoremediation (x0) compared to biomass samples of 610 (x1) and 714 (x2), in a time of 24 hours.

The treatments with biomass of E. *crassipes* with chromium adhered began in the first 5 hours to produce ethanol, in smaller quantity than the biomass without chromium. In the following graph 3, we observe the growth and stabilization curves of each biomass quantity.

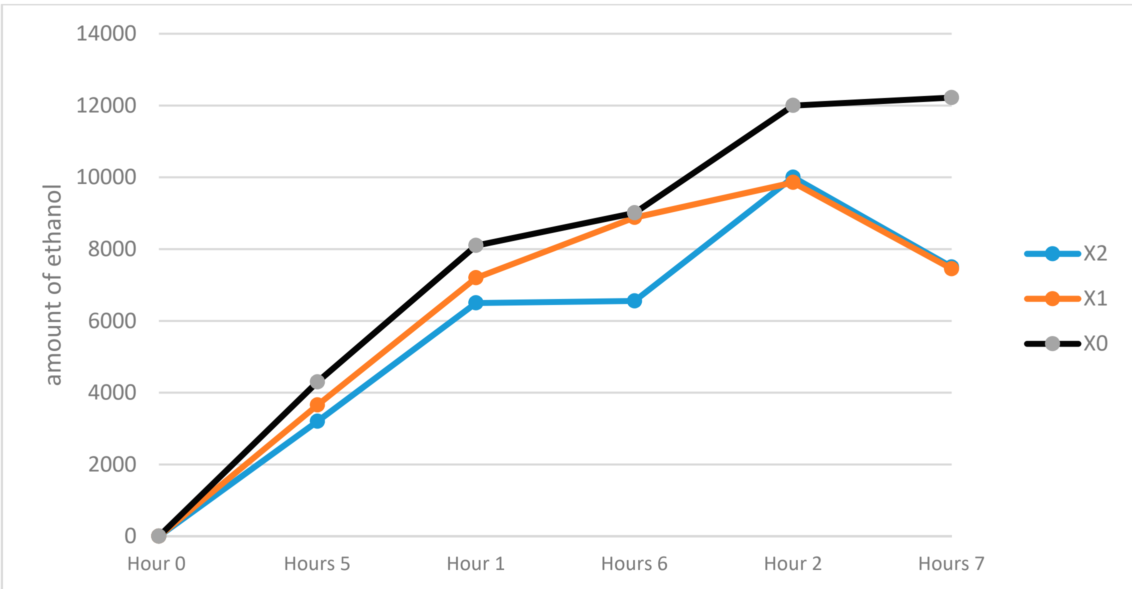

Figure 6. Ethanol productivity with different amounts of hydrolysed biomass

When carrying out the mass balance, it was established that the production of ethanol from hydrolyzed biomass of E. crassipes (x0) is profitable without chromium adhered, with an amount of 12100 mg / l in 25 hours. For the samples with chromium adhered (x1) and (x2), 25% less ethanol was produced close to 8000 mg / L in the same 25 hours, reaching the conclusion that if there is an impairment of ethanol production . These different results are similar to the study carried out by [25] where he establishes the relationship and correlation of the biomass of E. *crassipes* loaded with heavy

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metals with the fermentation process. Concluding that there is an affectation in this production of bioethanol due to the fact that heavy metals do not let consume all the sugar of the plant, but it is an alternative for the construction of an integral system of sustainability of phytoremediation and biofuels.

4. Discussion

In different investigations in the world, the capacities of E *crassipes* to retain heavy metals has been remarkably verified [23-27], but it is not disputed what is the final disposition of this biomass loaded with different heavy metals, also different researches in the world have established the procedures and quantification of results on the biomass capacity of E *crassipes* to become biofuels but with their biomass intact [28-30]. The results of this investigation intertwine these two feats of the plant E. *crassipes* because a system of use of the biomass of this plant was designed and developed after the process of phytoremediation of polluting chromium, defining that if it is viable the use of this biomass loaded with chromium to produce bioethanol. The adhered chromium affects the production of bioethanol but in minimal percentages, this chromium remains in the waste of the bioreactor and was finally disposed as small hazardous waste.

5. Conclusions

It was observed that the hydrolyzed biomass of E *crassipes*, have a higher percentage of reducing sugars compared to the biomass with chromium adhered but this last biomass also has a good yield in the production of sugars through the hydrolyzate.

The yield in obtaining ethanol from the 3 evaluated biomasses is interesting, taking into account that the E *crassipes* is unused waste of high quantity in wetlands, rivers and other hydrosystems, should continue with technical feasibility studies and economic in the construction of a larger refinery with this biomass.

There was an effect on the ethanol production of the E *crassipes* compared to the biomass that did not have chromium adhered after the phytoremediation process, but this productivity can be taken into account for the design and assembly of an integral phytoremediation and production system. Bioethanol, taking full advantage of the biomass of E *crassipes.*

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