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Design and Construction of a Biohydrogen and Bioethanol Production System from the Biomass of the *Eichhornia Crassipes*

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Abstract: Introduction: Biofuels, biohydrogen and bioethanol have properties that stand out from other fossil fuels, are colorless, odorless, and insipid, therefore, they are free of contaminants. Its production can be generated from the biomass of the aquatic plant *Eichhornia crassipes*, since this alternative is timely and viable due to its high energy composition. This plant is a problem in wetlands, rivers and other hydrosystems, due to its abundance and dominance over other species of aquatic plants, and which in effect causes an ecological imbalance. Objective: To evaluate the production of biohydrogen and bioethanol from the biomass of the *E. crassipes* plant. Materials and methods: Lignocellulosic material was used, realizing different physical, chemical and biological processes such as: reduction of size, lignin removal, acid hydrolysis, fermentation and distillation. a laboratory scale production system was designed and built where two bioreactors were used in the process. Results and discussion: the production of bioethanol and biohydrogen showed a production index of more than 40 mg of bioethanol per gram of bio-mass of *E. crassipes* and in relation to biohydrogen, 65 mmol / L was obtained. Conclusion: With the biomass of *E. crassipes* it is possible to obtain a high index of bio-fuel production, since it is possible to take advantage of its physical characteristics and its high proliferation in hydrosystems, in this way it constitutes an optimal alternative to produce biohydrogen and bioethanol at a high scale.

Keywords: bioreactor; hydrolysis; fermentation; biofuels

1. Introduction

An alternative to the use of oil, coal and nuclear reactors, as an energy source, is the use of biofuels produced from vegetable waste, as they are a renewable, abundant and clean energy. Due, the importance of the production of energies such as biohydrogen and bioethanol is generated.

Hydrogen has unusual properties because it is a clean and green biofuel, it is colorless, odorless, and insipid, therefore it is free of contaminants such as CH₄ (metano) and CO₂ (carbon dioxide), it is used in different industries of chemical processes, since its only compound is water [1-2].

Its production through dark fermentation is a promising path from raw materials rich in carbohydrates (such as wastewater, food waste and agricultural waste) [3].

Dark fermentation to produce hydrogen from various types of biomass has been widely studied and generally demonstrated with mixed microbial cultures, since they are more productive and therefore more applicable to large-scale operations than pure crops. to its greater capacity to use mixtures of substrates and to allow the balanced metabolic flow, in addition the production of biohydrogen with mixed cultures does not require sterilization, which is beneficial for the industrialization [4-8].

On the other hand, the production of fuel ethanol from lignocellulosic material has become a highly viable alternative that could open new markets for its revaluation [9] In this production, different physical, chemical and biological processes take place, such as: size reduction, lignin removal, acid hydrolysis, fermentation and distillation [10]

One of the ways to produce ethanol is by fermentation, from raw materials rich in carbohydrates (sugar, starch, cellulose, etc.). For this reason, it is common to designate the ethanol obtained by this route: "bioethanol" [11]

Currently, bioethanol is produced by alcoholic fermentation of sugars present in renewable materials, which is influenced by factors such as the concentration of sugars in the substrate and the fermenting microorganism that is used. According to the studies of [12] together with [13] it has been determined that when *Sacharomyces cerevisiae* is cultivated at high concentrations of sugar (less than 30 - 40%) the production of ethanol is increased.

One of the main drawbacks, when producing biofuels, is the use of food for humans and animals as raw material, therefore, in different studies have been characterized some materials such as wood and nutshell that have high percentages of cellulose and low levels of lignin.

According to the above, a plant that has the necessary amount of cellulose and hemicellulose, and that is not human food, which is *Eichhornia crassipes*, was investigated.

From the biomass of the *E. crassipes*, in the present investigation was designed and built, and analyzed the biohydrogen and bioethanol production processes at pilot laboratory scale. Developing a bioreactor for the hydrolysis of the dry biomass of this plant and two bioreactors were designed, one for dark fermentation and the other for alcohol fermentation.

2. Materials and Methods

After having carried out a bibliographic review worldwide, about the design of bioreactors, they gave way to the development of these in the present investigation, in order to obtain a clean production of biofuels.

To obtain the biofuels, first, the hydrolysis of lignocellulolytic material was carried out and then the production of bioethanol and biohydrogen was carried out.

The hydrolyzed bioreactor is made of glass, its capacity is 5 liters, it has a lid for gas evolution, sampling of pH and temperature, together with a magnetic stirring heater at 120 RPM at a temperature of 60° C .

To this bioreactor the *E. crassipes* was dried and crushed in an amount of 200 gr where it was mixed with distilled water. The samples reacted in 1% (w / v) of caustic soda (NaOH) at a temperature of 60°C, during 12 h, afterwards the samples were washed with tap water until reaching the pH value of the water. Then sulfuric acid (H₂SO₄) 3% (v / v) was added at a temperature of 60°C, during 12 h, the samples were washed with distilled water until reaching a neutral pH.

The content of reducing sugars was determined by the Salicylic Dinitro Acid (DNS) method [14] which indirectly quantifies substrate consumption. In this way, 4 liters of hydrolyzed solution of *E. crassipes* were obtained for the continuation of bioethanol and biohydrogen production.

The production of sugar through the hydrolysis of 12 hours for NaOH and 12 hours of HSO₄ was 60 grams / 4L. A relevant production of sugar and registering similar datas in [15] There was a constant production of hydrolysis.

Production of bioethanol. After carrying out the hydrolyzate process in the bioreactor, the bioethanol production process is carried out in the alcohol fermentation bioreactor.

The alcoholic fermentation bioreactor is manufactured in glass and its storage capacity is 5 liters, it is made up of a lid for the evolution of gases, sampling of pH and temperature, with heater. The magnetic stirring is carried out at 120 RPM at a temperature of 60° C. In this process, *S. cerevisiae* was used as inoculum of the fermentor of the hydrolyzate of *E. crassipes*. Then concentrations of 200 g / L, 250 g / L and 300 g / L of biomass were evaluated, in order to determine which of these represents the highest percentage of ethanol.

So, 100 g of the hydrolyzate were taken to the bioreactor where it was mixed with distilled water and 100 g of the inoculum were added, then the initial pH was adjusted to 5.5. The bioreactor was hermetically sealed with rubber septums and aluminum plugs. During the fermentation of the hydrolysis of the biomass, ethanol percentage tests were performed by gas chromatography at time intervals of 5 hours, as shown in graph 1, in the results.

According to the previous procedure, the steps for the other two concentrations (250 g / L and 300 g / L) were repeated and the results obtained in the same graph 1 can be observed.

Production of biohydrogen. The same bioethanol procedure was carried out in the hydrolyzate bioreactor for this production and the dark fermentation bioreactor was used at a temperature of 30°C.

The bioreactors were hermetically sealed with rubber septa and aluminum plugs, in addition the orifices of the bottles were purged with nitrogen for 5 minutes to ensure the anaerobic condition. In each time interval, the volume of biogas was measured by the displacement of the plunger method. The hydrogen gas was de-terminated by gas chromatography using a TCD detector in a GC-Agilent 7890 brand chromatograph.

The biosolids generated in the Wastewater Treatment Plant (WWTP), the saltpeter from the city of Bogotá, were taken as inoculants. These biosolids are obtained after digestion after 20 days of treatment and were used due to the appropriate conditions. of its bacterial profile (Chuang et al., 2011).

Two assemblies were made in the bioreactors, for continuous production of biohydrogen in the same conditions to establish productivity. 200 g of the hydrolyzate were taken to each bioreactor where they were mixed with distilled water and 100 g of the inoculum (biosolid) was added, and the initial pH was adjusted to 5.5.

Four different tests were carried out in the two bioreactors, establishing the productivity and standardizing the ideal time in the bioreactor.

3. Results

A greater production of ethanol is observed for the 300 g / L samples compared to the 250 g / L and 200 g / L samples in a time of 25 hours. The final determination of ethanol obtained is shown in graph 1.

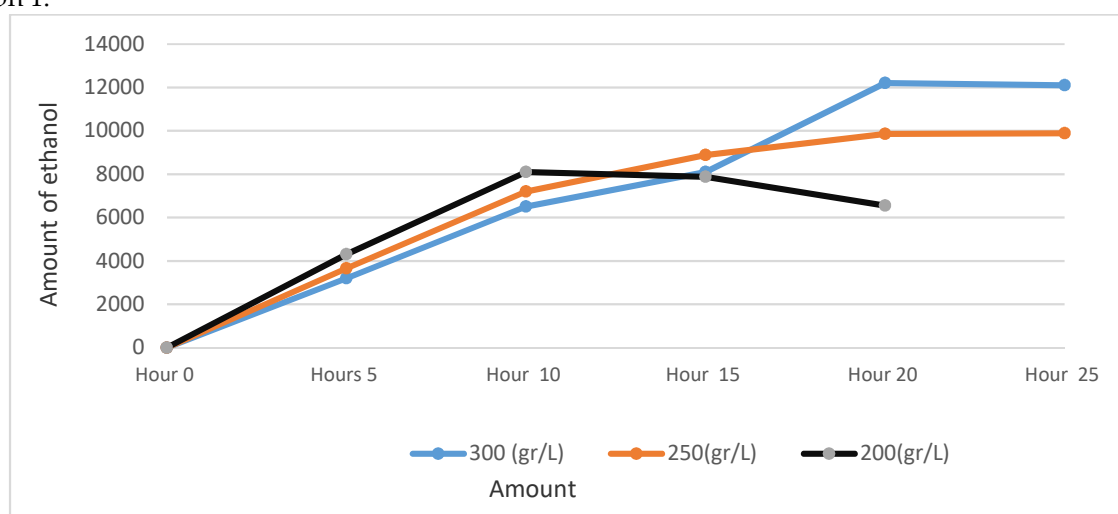


Figure 1. Ethanol productivity with different amounts of hydrolysed biomass

Concentrations with less amount of hydrolysed biomass started to produce more ethanol than the amount of 300 gr / L. during the first 10 hours, maintaining a constant production during that

time, under the design conditions but with different types of hydrolyzing agents [16], obtained similar results.

The concentration of 200 g / L decreases in its entirety at hour 20 with a final ethanol production of about 6,552 mg / L, and a yield of 32.76 mg of ethanol is obtained for each gram of *E. crassipes*. These results were similar to the experiments of [17], where they used *Clostridium thermo-cellum* to produce 9.72 mg / L.

The concentration of 250 gr / L continues its production after 10 o'clock until 25 hours of production with an ethanol result of 9,888 mg / L and a yield of 39.6 mg of ethanol per gram of *E. crassipes*. [18], obtained results with similar bioreactors but with *P. tannophilus* reaching a maximum ethanol concentration of 4.3 g / L, followed by 2.1 g / L, results well below those reported in this investigation.

The concentration of 300 gr / L obtained a production of 12,100 mg / L of ethanol in the total time of the process, and a yield of 40.3 mg of ethanol is obtained for each gram of *E. crassipes*.

[19], they used *Aspergillus Niger* celulasas as transforming agent of the hydrolyzed biomass of *E. crassipes*, reporting 20% more than those experimented in this research, but this bacterium is more difficult to isolate than the *Sacharomy-ces*.

Table 1 shows the biohydrogen production of the two bioreactors called x1 and x2. 14 samples were taken every 12 hours, where the samples represent the productivity tests, obtaining an arithmetic average and a standard deviation of each process.

In the bioreactor x2 there was an imbalance and only 9 samples were taken from the biohydrogen production process.

As can be seen in Table 1, an average production value of 68.5 mmol H₂ / L was obtained in the bioreactor x1 and in the bioreactor x2 an average production of 65.6 mmol / H₂ was obtained.

In these bioreactors, the Ptar biosolids were used as a microbial inoculum for the production of biohydrogen.

Table 1 Production of Biohydrogen in bioreactors.

	X1(mmol)	X2(mmol)
sample 1	98	100
sample 2	52	98
sample 3	87	111
sample 4	80,3	85
sample 5	72	63
sample 6	63	52
sample 7	59	53,3
sample 8	59,6	23
sample 9	58,6	1
sample 10	55,2	
sample 11	45	

sample 12	32	
sample 13	21	
sample 14	2	
Average	68,5	65,1
standard deviation	14,5	35,0

From the above we can observe a constant productivity of biohydrogen in reactor 1 and 2 with similar averages and a very low standard deviation, demonstrating that the process was done with a high reliability.

In the following Figure 2 you can see the samples taken and how the productivity of biohydrogen decreases in the two reactors

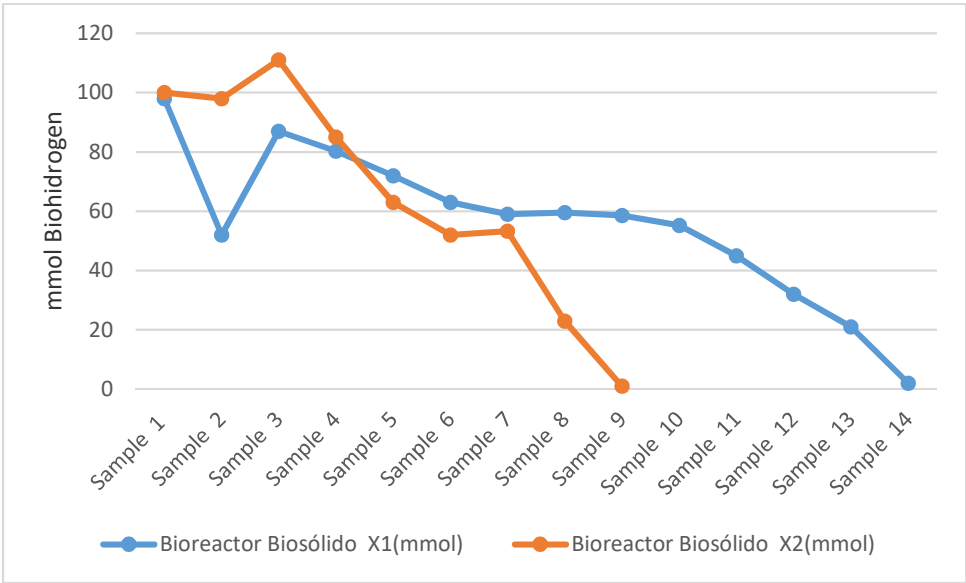


Figure 2. Continuous production of biohydrogen

Subsequently, a biohydrogen production was carried out for 100 g of hydrolyzed biomass, an approximate 65 mmol / L of biohydrogen is produced with the inoculum of biosolids. There is a standard productivity of biohydrogen from the Eichhornia crassipes of 7 days. [20], obtained results similar to this research using also PTAR sludge.

[21] Obtained results above 50% of this research, showing that the hydrolysis process was better, but Mechery and Syllas, (2016), also obtained results due to the use of Pseudomonas.

5. Conclusions

It is possible to establish that the hydrolyzed biomass of *E. crassipes* has a high percentage of reducing sugars and consequently its glucosed syrups produce a high content of bioethanol and biohydrogen.

The yield in obtaining these biofuels, from this biomass is relevant for the production of energy, taking into account that the *E. crassipes* is one of the waste not used and of high quantity in the wetlands, rivers and de-more hydrosystems, so it could be located in an alternative of high interest for the large-scale production of biofuels.

Biofuels have a high potential for the development of energy systems, and their advantage is that they do not present environmental hazards in their production. This production with *E. crassipes* has a high energy efficiency, the environmental impact it produces is great and its profitability is effective.

It is proposed to carry out a greater production of biohydrogen, to establish prevailing statistical models in order to optimize the production of this compound, so that it becomes a strong potential for alternative energy.

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