

Design, Modeling and Adjustment of the Parameters of a Bioethanol Production System with the Eichhornia Crassipes Biomass

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Abstract

Colombia is in a major backlog of alternative energy production. An interesting option is the production of biofuels with biomass of *Eichhornia crassipes*. This plant is abundant in rivers, wetlands and other aquatic ecosystems and generates problems in ecological equilibria. In the current research, the parameters of a bioethanol production system with this biomass were designed, modeled and adjusted to find the best parameters when building bioethanol production with this plant. Taking into account the designs, it is concluded that this bioethanol production system has a minimum cost in its assembly compared to other similar systems. The ethanol's production time is made in 2 days including the hydrolysis as well as the fermentation, and it would produce for each 500 gr of dry biomass 100 ml of bioethanol, represented in the production of *Sacharomyces*, finding that scenario 3 is the most ideal at the time of the assembly.

Keywords: *Eichhornia crassipes*; Bioethanol; *Sacharomyces*.

1. INTRODUCTION

The production of fuel ethanol from lignocellulosic material has become an interesting alternative in the use of this type of waste that could open new markets for its revalorization (Benítez, 2010). In the production of bioethanol from lignocellulosic material several physical, chemical and biological processes take place, such as: reduction of size, lignin removal, acid hydrolysis, fermentation and distillation (Riaño, A. M. S., 2010).

A more feasible alternative to oil, coal and nuclear reactors in developing countries is the direct and indirect use of energy from plant residues; it is a renewable, abundant, decentralized and clean energy (Gil, De Pérez, V. I. M., & Colorado, 2006). One of the options to produce ethanol is by fermentation from raw materials rich in carbohydrates (sugar, starch, cellulose,

etc.). For this reason, it is common to designate as "bioethanol" the one obtained through this way (Vázquez, H. J., & Dacosta, O., 2007).

Currently, bioethanol is produced by alcoholic fermentation of sugars present in renewable materials. This fermentation is influenced by factors such as the concentration of sugars in the substrate and the fermenter microorganism that is used. According to previous reports (Peña, c., & Arango, r, 2008) and based on the studies of (Holcberg, i. B. And Margalith, P., 1981); (Converti, 1985) when *Sacharomyces cerevisiae* is grown at high concentrations of sugar (less than 30-40%) increases the production of ethanol.

One of the main drawbacks when producing ethanol is the use of human and animal foods as raw material. (Cuervo, Folch, & Quiroz, 2009) characterized different materials to produce bioethanol, finding that the wood and walnut shell have high percentages of cellulose and low levels of lignin. *S. cerevisiae* continues to be the microorganism used industrially, but to improve its yield, the possibility of creating genetically modified strains to increase the yield of ethanol has been explored.

A viable alternative to produce bioethanol is the *Eichhornia crassipes* known in Colombia as "Buchón de agua" (water horn), this aquatic plant is an indicator of pollution in wetlands, rivers, etc. due to its high reproductivity in polluted waters. Currently, there are large accumulations of this plant in the Juan Amarillo and the rabbit hut wetlands, among others.

Eichhornia Crassipes meets the criteria to produce bioenergy, due to its permanence because there are large amounts of available, biodegradable plants, and it has a high content of cellulose (Porous & Dhahiyat, 2012). However, its strong disadvantage is that it has more than 90% water content, which complicates the harvesting and processing process. Biomass can be subjected to the production of biogas to generate energy for domestic use in rural areas (Chuang, Lay, Sen,

Golapalakisnan, & Wu, 2012). Due to its morphology *Eichhornia crassipes* has also been investigated about its high energetic power, as in the study by (Yeong-Song, Chyi-how, Chi, Biswarup, & Gopalakrishnan, 2014), where they experimented on the production of hydrogen from the water horn using microflora of pig dung.

Currently, much attention is focused on the development of methods to produce ethanol from biomass with high cellulose content, and *Eichhornia crassipes* is an abundant plant that has essential characteristics for this purpose (Hossain, Chowdhury, & Yeasmin S, 2010); (Ahmend, Abdel, Moahmed, & Abdel, 2012); (Ganguly A, P.K. Chatterjee, A. Dey, 2012).

(Magdum, More, & Nadaf, 2012) performed on *Eichhornia crassipes*, an acid hydrolysis using sulfuric acid. The resulting hydrolysed 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. produced bioethanol from the *E. crassipes* using a two-stage process: an acid hydrolysis, followed by an alcoholic fermentation by implementing the yeast xylo-fermentative: *Candida Sheatae*, obtaining a performance comparable to that obtained by enzymatic hydrolysis, demonstrating that a simple and accessible procedure can be generated at the moment of thinking on an industrial scale.

The production of fuel ethanol from biomass involves prehydrolysis, hydrolysis, fermentation and distillation. The resulting hydrolyzate after prehydrolysis and hydrolysis contains varying amounts of monosaccharides, both pentoses and hexoses, and a wide range of substances, either derived from the raw material or resulting as reaction products from sugar and degradation of lignin. Many of these substances can have an inhibitory effect on microorganisms in subsequent fermentation stages (Nigam, 2002).

(Bronzato, 2016), determined that sulfuric acid hydrolysis is the most effective pre-treatment for the treatment of *Eichhornia crassipes*. In one year, from one hectare covered by water hyacinth, it is possible to produce 265 liters of ethanol. Also (Pattra & Surewan, 2015), optimized the hydrolyzate parameters of the *Eichhornia crassipes*.

(Kuldiloke, Eshtiaghi, & Peeploy, 2010), investigated different compounds to degrade the sugar of *Eichhornia crassipes*,

finding that *Saccharomyces cerevisiae* increased the alcohol content in the process. Saprativ (2016) also developed a bioreactor to produce bioethanol from the *Eichhornia crassipes*, providing the design parameters for this experimentation.

In the present investigation, the parameters of a bioethanol production system with the biomass of the *Eichhornia crassipes* were designed, modeled and adjusted. These parameters were optimized by determining the viable time for experimentation, the amount of biomass of the hydrolyzed *Eichhornia crassipes* and *Sacharomices* to be used.

2. MATERIALS AND METHODS

For the design of the bioreactor, there was a worldwide literature review on the design of bioreactors, hydrolysis of lignocellulolytic material and characterization of the biomass of the *Eichhornia crassipes*.

2.1 *Eichhornia crassipes*' Hydrolysate and Fermentation Process Design

The bioethanol generation process design consists of two bioreactors: a bioreactor to make the hydrolysate and a bioreactor for the fermentation where it will have all the mathematical component in this article.

The hydrolyzate bioreactor will be 5 liters in glass, it will have a lid for the evolution of gases, PH & T sampling, and it will be placed in a heater with magnetic stirring at 120 RPM at a temperature of 60° C.

In this bioreactor the *Eichhornia crassipes* will be dried and grinded in an amount of 200 gr, where it will be mixed with distilled water. The samples will be reacted in 1% (w / v) of caustic soda (NaOH) at a temperature of 60°C, and during 12 h, the samples will be washed with tap water until reaching the pH value of the water. Subsequently, sulfuric acid (H₂SO₄) 3% (v / v) will be added at a temperature of 60°C, and during 12 h, the samples will be washed with tap water until reaching the pH value of the water. The content of reducing sugars will be determined by the Dinitro Salicylic Acid (DSA) method (Peña, c., & Arango,r, 2008), which indirectly quantifies substrate consumption. 4 Liters of *Eichhornia crassipes* hydrolysate solution will be obtained for the continuation of the production of Bioethanol. This component will henceforth be called (S) the substrate.

The bioreactor of the fermentation will also be 5 liters in glass, it will have a lid for the evolution of gases, sampling of PH and

T, and it will be placed in a heater with magnetic stirring at 120 RPM at a temperature of 60° C. Figure 1 will expose the two bioreactors proposed in this research.

Saccharomyces cerevisiae (X) is used for the fermentation process and will be inoculated in the hydrolyzate of *Eichhornia crassipes* (S).

2.2 Mathematical model of bioethanol production through fermentation

In this section we will present the most important equations for the transformation of the hydrolyzed biomass of the *Eichhornia crassipes* bioethanol. Equation # 1 represents the rate of microbial growth.

$$\frac{dx}{dt} = \mu X \quad (1)$$

μ = Specific Growth Rate

X = Microorganisms' Concentration (mg/L)

At time $t = t_0$ the substrate will be the hydrolyzate of the *Eichhornia crassipes* ($S = S_0$), when the time increases the substrate tends to decrease, $X = X_0$ the microorganisms increase with time.

Sorting equation 1 results in:

$$\frac{dx}{x} = \mu t \quad (2)$$

The biomass growth rate within the bioreactor is proportional to the microorganism's concentration and is represented by the following first order equation.

$$\int_x^x \frac{dx}{x} = \mu \int_t^t t \quad (3)$$

$$\ln \frac{x}{x_0} = \mu t$$

Solving for X

$$X = X_0 * e^{\mu t} \quad (4)$$

X = Final microorganisms' concentration. Mg/L

X₀ = Initial microorganisms' concentration. Mg/L

With this equation (4) the speed of microbial growth can be determined. For the present experiment we will have the *Saccharomyces cerevisiae* represented by X.

This growth of microorganisms are those that will decompose the hydrolyzate of *Eichhornia crassipes*. The specific rate of microbial growth was established by Monod in 1942, and it is used to date for describing the microbial growth in a system, represented herebelow:

$$u = \frac{U_{mS}}{K_s + S} \quad (5)$$

Where:

μ = Specific growth speed

μ_m = maximum growth speed

K_s = Average speed constant. Substrate concentration at half the maximum growth rate. Mass / volume unit.

S = Concentración del Hidrolizado de *Eichhornia crassipes*. Masa/unidad de volumen

The relationship between the microbial growth rate is represented by the substrate utilization rate represented as follows:

$$-\frac{ds}{dt} = \frac{dx}{dt} \quad (5)$$

The microbial growth rate in the logarithmic growth phase, for this growth is needed the ideal conditions of nutrients and oxygenation requirements, is represented in equation 6.

$$\frac{dx}{dt} = -Y \frac{ds}{dt} \quad (6)$$

The measured maximum performance coefficient during a finite period in the logarithmic growth phase is introduced. It is defined as the mass of formed cells / mass of substrate consumed.

Y = Maximum performance coefficient measured during a finite period in the logarithmic growth phase. It is defined as the mass of formed cells / mass of substrate consumed.

$\frac{ds}{dt}$ = Substrate use speed. Mass / volume unit × time.

Substituting equation 2 in equation 5, results in the representative equations for the design of reactors in the balance of substrate (s) and microorganisms (x).

$$\frac{ds}{dt} = -\frac{UmSX}{Y(Ks+S)} \quad (7)$$

$$\frac{dx}{dt} = \frac{UmSX}{Ks+S} \quad (8)$$

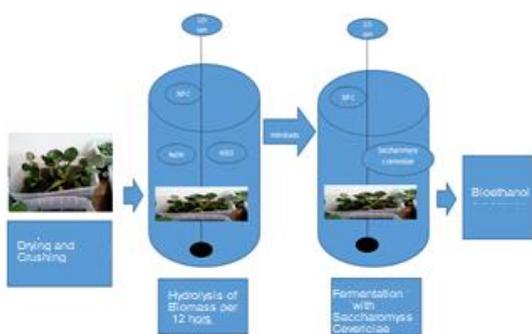
These equations 7 and 8 are those that describe the internal balance of microbial growth, where there is an important multiplication in the reactor and therefore it also shows a microbial decay.

The following figure 1 shows the complete process, the hydrolysis and the fermentation with the entrances and exits, the raw material that will be the dried *Eichhornia crassipes*, grinded and later hydrolyzed.

For the optimal design, a modeling of the bioreactor counts was made. The following equation of material balance shows the incoming biomass (S), the conversion to ethanol (P) and the incorporation of the microorganism *Sacharomyces cerevisiae* (x).

In the following equation the balance of microorganisms in the reactor is represented, you can see a discontinuous bioreactor therefore the inputs and outputs are 0.

Figure 1. Incoming biomass (*Eichhornia* crushed) + incorporation of the microorganism *Sacharomyces cerevisiae* (x) = biomass produced (bioethanol)



Source: The Authors

Substrate Balance “*Eichhornia crassipes*”

In the present substrate balance, equation (7) will be used

$$\frac{ds}{dt} = \left(-\frac{UmS}{Y(Ks+S)}\right)X \quad (10)$$

With this equation the use of substrate in function with the biomass is represented, they could not be integrated because the substrate changes and the concentration of microorganisms also changes.

Microorganisms Balance “*Sacharomyces cerevisiae*”

$$\frac{dx}{dt} = \left(\frac{UmS}{(Ks+S)}\right)X \quad (11)$$

Due to the non-linearity of these equations, cell death is not taken into account since in this type of discontinuous reactor this data is negligible. An equation that results from the analysis of the bioreactor and the correlation between X and S, and the term Y = maximum efficiency coefficient measured during a finite period in the logarithmic growth phase. The following equation (12) remains

$$X = Xo * Y\Delta S \quad (12)$$

$$X = Xo * Y(So - S). \quad (13)$$

In discontinuous bioreactors, the Y value in fermentation of sugars is 0.18. (Rittmann & Mc Carty, 2001). In this article this value will be used.

By replacing (13) in (10), equation (14) remains.

$$\frac{ds}{dt} = \left(\frac{UmS}{Y(Ks+S)}\right)Xo * Y(So - S). \quad (14)$$

Equation (14) was integrated, yielding equation (15) to be the equation where the optimal times and combinations of substrate versus quantity of microorganisms to be used in the bioreactor will be modeled.

$$t = \frac{Um}{y} \left\{ \left(\frac{Ks}{Xo+YSo} + \frac{1}{y} \right) \ln(Xo + YSo - YS) - \left(\frac{Ks}{Xo+ySo} \right) \ln \frac{SXo}{So} - \frac{1}{y} \ln Xo \right\} \quad (15).$$

3. RESULTS

A mathematical modeling procedure was designed to find the best combination by combining the variables proposed by (Rittmann & Mc Carty, 2001) where the variables U, Um, Ks, q and Y represented in the following table 1 were used.

Table 1. Variables proposed by (Rittmann and McCARTY, 2000)

Growth rate	U	1/T	0.6
Max microbial growth rate.	Um	1/T	1.764
Constant half	K	mg/L	0.882
Maximum substrate use rate	q	mg/mg*H	9.8
Cell Synthesis Real Performance	Y	mg/mg*H	0.8

SOURCE Modified: (Rittmann & Mc Carty, 2001).

The fermentation process was implemented according to the modeling elaborated by (Cuchimaque, 2018), figure 2 in the Los Libertadores University Foundation, using a glass bioreactor with a capacity of 5 L, with a lid for gas evolution, and pH samples, this being a pressurized system which guarantees a constant temperature during the process. The fermentation and bioethanol production system is shown below.



Figure. 2. Pilot batch fermentation system

Source: (Cuchimaque, 2018).

40 g of the hydrolyzate were mixed with 2 L of water and added to bioreactor # 1, on the other hand to bioreactor # 2 7 g of the fermenting agent (*Saccharomyces Cerevisiae*) diluted in 20 ml of water were added. The process is started and the hydrolyzate contained in bioreactor # 1 is transported to bioreactor # 2 which contains the fermenting inoculum, as shown in figure 3.



Figure 3. Starter the fermentation

Source: The Authors

Different scenarios of initial and final concentrations of *Eichhornia crassipes* hydrolyzate (S) and the initial and final concentrations of *Sacharomyces cerevisiae* (X) were made calculating the time and maximum productivity of ethanol for the construction of the bioreactors. They are shown in the table 2.

Table 2 Variable Data

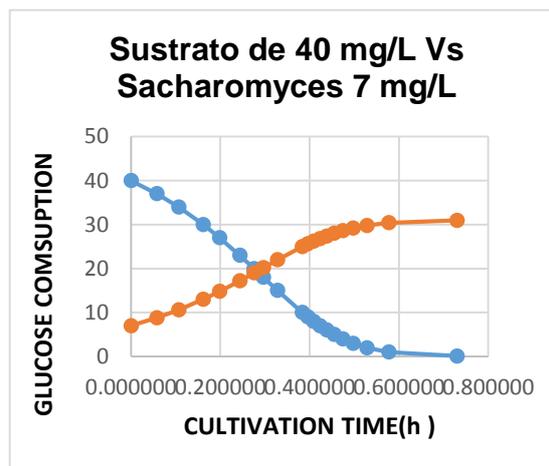
Eichhornia Molida	So	Ms/L	40
Eichhornia Molida	S	Ms/L	19
saccharomyces cerevisiae	Xo	Mx/L	7
saccharomyces cerevisiae	X	Mx/L	19,6
Tasa de crecimiento	U	1/T	0,60
Max tasa de Crec	Um	1/T	5,88
Constante mitad	K	Ms/L	20
Maxima tasa de utilización de susutrato	q	Ms/Mx*T	9,8
Rendimiento Real de síntesis de celulas	Y	Ms/Mx*T	0,6
Tiempo	t	dia	1
Maxima tasa de utilización de susutrato	q	Ms/Mx*T	9,80
Tiempo simulado	t	dia	1,0
Tiempo simulado			0,291897

7		
t	S	a
0,000000	40	7
0,059116	37	8,8
0,108585	34	10,6
0,164971	30	13
0,202498	27	14,8
0,248506	23	17,2
0,291897	19	19,6
0,334708	15	22
0,367951	12	23,8
0,391557	10	25
0,404088	9	25,6
0,417307	8	26,2
0,431439	7	26,8
0,446811	6	27,4
0,463923	5	28
0,483615	4	28,6
0,507452	3	29,2
0,538943	2	29,8
0,589277	1	30,4
0,745016	0,1	30,94

The times were calculated with equation (15) by varying the amount of substrate (S) and the amount of microorganisms (X) was calculated according to equation (13). In the previous table you can see a start of the Eichhornia hydrolyzate for a scenario of 40 mg / L.

It can be observed in graph 1 that a total consumption of the hydrolysed substrate (S) was made, thus generating an increase of the microorganisms 6 times compared to its onset. The used amounts of substrate and fermentor inoculum used are shown to be viable because a glucose consumption is generated in a time of 0.29 days or 6.96 hours in the bioreactor # 2, this being its equilibrium point for bioethanol production.

Graph 1 Substrate with 40 mg/L Vs Sacharomyces 7 mg/L



4. CONCLUSIONS

In the present research, the parameters of a bioethanol production system with the biomass of the Eichhornia crassipes were designed, modeled and adjusted. Considering the designs, it is concluded that this bioethanol production system has a minimum assembly cost compared to other bioethanol production systems. This aquatic plant to be so abundant would have an economic and very efficient raw material.

The ethanol's production time is 2 days, counting the hydrolysis together with the fermentation, and it would be produced for each 500 g of dry biomass, resulting a Liter of bioethanol represented in the production of Sacharomyces, and therefore finding that scenario 3 is the most ideal at the time of the assembly.

In the experimental assembly that will be carried out by this research group an experimentation on the quantification of the maximum Y will be addressed.

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