UNIVERSIDAD DISTRITAL
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Visión Electrónica
Más que un estado sólido
https://doi.org/10.14483/issn.2248-4728

VISIÓN ELECTRONICA
A RESEARCH VISION

Design and optimization of a pilot batch fermentation system for the production of bioethanol

Diseño y optimización de un sistema piloto de fermentación en batch para la producción de bioetanol

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INFORMACIÓN DEL ARTICULO

Historia del articulo
Enviado: 21/09/2018
Recibido: 29/09/2018
Aceptado: 19/11/2018

Keywords
Eichhornia Crassipes,
Zymomonas,
bioethanol,
Eichhornia Crassipes mobilis,
downstream.

Palabras clave:
Eichhornia Crassipes
(buchón de agua),
sistema de fermentación
"*in house*",
bioetanol,
Zymomonas mobilis,
baja escala.

ABSTRACT:

An "in house" fermentation system has been designed on a laboratory scale, which would be used in a batch fermentation process of the Zymomonas mobilis to obtain bioethanol from the biomass degradation of the Eichhornia crassipes. This model is proposed starting from simple tools and laboratory equipment that at a small scale allow simulating alcoholic fermentation by controlling the aeration, the pH, the temperature, the amount of glucose, and the concentration of the product so that the model has been used for the future optimization of the same, and reliable bioethanol obtaining as well as production control.

RESUMEN

Se ha diseñado un sistema de fermentación *"in house"* a escala de laboratorio, el cual será utilizado en un proceso de fermentación batch de la bacteria *Zymomonas mobilis*, para obtener bioetanol a partir de la de degradación de biomasa de la *Eichhornia crassipes*. Este modelo se propone partiendo de herramientas y equipos de laboratorio sencillos que a baja escala permiten simular una fermentación alcohólica controlando la aireación, pH, temperatura, cantidad de glucosa y la concentración de producto, de tal manera que el modelo optimizado sea usado para la futura fermentación y obtención confiable de bioetanol, así como el control de la producción.

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Cite this article as: A. León-Agatón, E. Berdugo-Romero, J. C. León-García and A. Ramirez-Valencia, "Design and optimization of a pilot batch fermentation system for the production of bioethanol", Visión electrónica, vol. 2, no. 1, Special edition, January-June 2019 https://doi.org/10.14483/issn.2248-4728.

1. Introduction

Due to the production cost and the fall in fossil fuel reserves (non-renewable resources), the production of bioethanol from renewable resources to be used as fuel has been increasing in interest; in particular, because of its low environmental impact, [1]. One of the production ways is through alcoholic fermentation, which since 1907 has been studied, and was used in Sweden with the use of yeasts such as S. Cerevisiae which converts glucose under anaerobic conditions to biomass and ethanol, [2]. Lignocellulose biomass is considered as the future raw material for ethanol production due to its low cost and high availability, [3]. One of the main lignocelluloses' materials found in large quantities is the Eichhornia Crassipes (water Aubuchon) which dwells in marshes and water bodies where it is not only an environmental inconvenience to cover the surface of water mirrors, it is also an unused renewable resource with high sources of lignin and cellulose which, when transformed by acid hydrolysis is a substrate in alcoholic fermentation processes carried out by Zymomonas mobilis to bioethanol and biomass. In this research, a pilot fermentation system was designed where hydrolysis process and batch fermentation are linked, such that the conditions base for alcoholic fermentation with controlled and reproducible conditions within a system where the substrate is Eichhornia Crassipes and the production of bioethanol is carried out by Zymomonas mobilis. This model is based on the theoretical approach developed by Sayago, U.F; et al. in 2017, Figures 1 and 2, [4].

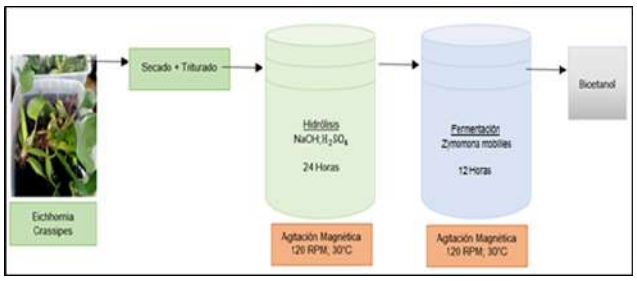

Figure 1. The theoretical model of the bioethanol production process from the hydrolyzed biomass of the Eichhornia crassipes in fermentation pilot systems developed at the Fundación Universitaria Los Libertadores, [5].

Final design of batch fermentation pilot system.

Figure 2. Pilot fermentation system designed for the alcoholic fermentation process.
Source: own.

2. Materials

- 2 glass bottles with a capacity of 4 liters and lids.
- 3 glass bottles with 100 ml capacity and its lids.
- 5 Teflon hydrophobic filters of 0.22 µm.
- 3 meters, silicone hose for peristaltic pump head 15.
- 1 peristaltic pump, head 15.
- 2 magnetic stirrers with control at a stirring speed (R.P. M) and plate temperature (°C).
- 1 syringe of 20 ml.
- 1 air engine for fish tank.
  Silicone.
- Magnetic bars (stir bar) of different dimensions (51,16 mm and 80,3 mm).
- 1 Flexometer.
- Potentiometer with a temperature sensor.

3. Methodology and Design Results

3.1. Design and Optimization Hydrolysis.

The hydrolysis process is carried out on the *Eichhornia crassipes* collection, which has previously been dried in a furnace at 60 C and then crushed to a very fine powder. This powder is exposed to acid hydrolysis where it is expected to break the greater percentage of Lignin polymers and cellulose to obtain monomers or dimers of glucose, which is used as a substrate in the alcoholic fermentation. In this design, the optimization of

hydrolysis is based on the lid or the seal to the system (see images 2 and 3). The biomass of crushed *Eichhornia crassipes* is added to the hydrolysis bottle with distilled water, it will be reacted with 1% (p / v) caustic soda (NaOH) at a temperature of 60ºC and stirring at 250 RPM for 12 hours [5]. For this first stage of hydrolysis, the optimization that lies in the design on the seal or lid allows it to be a contained and closed process; however, with a de-fogging system consisting of in 2 hydrophobic filters made of Teflon with a membrane pore of 0,22 µm, it is allowed that the gases from the hydrolysis process come out without pressurizing the system but keeping control at constant temperature and without external contamination to the system altering the substrate. 3 % (v/v) sulphuric acid (H_2SO_4) is then added at a temperature of 60ºC, for 12 hours, in this case, the optimization of the type of sealing prevents the exothermic reaction that occurs between the caustic soda and the sulphuric acid avoiding any affectedness in the system operator because the filters allow the gas to be released again without losing containment, Figure 3-5.

Figure 3. Hydrolysis content system "in house". Source: own

Figure 4. Hydrolysis Content System Filters "in house". Source: own

Figure 5. Magnetic stirrer with temperature and agitation control. Source: own

3.2. Design and Optimization to agitation system and temperature control

For the processes of hydrolysis and alcoholic fermentation, the constant preservation of variable stirring and temperature is crucial. For this design, it was required to carry out 2 models, one consisting of a heat map to different volumes, where it is demonstrated the constant and little variable heat transfer to all points of the system and another one where it is best stirring bar size (stir bar) modeled that maintains that constant condition heat transfer, therefore, the complete steering system consists of 2 variables: the magnetic stirrer with the 2 control variables, and the magnetic bar (stir bar). In modeling, the first experiment consisted of taking the corresponding measurements has all the magnetic bars (see Figure 6).

Figure 6. Stir Bar. Source: own

The selection of the appropriate magnetic bar for the fermentation system depends on the diameter of the fermentation system "in house" which is 124 mm, therefore, the magnetic bar should be a

diameter¾ for this case the bar of a diameter 51.16 mm is selected, however, it has to challenge the different sizes of magnetic bars in the system within a heat map. For this purpose, the 2 experiments were carried out where temperature measurements were taken at different points in the already assembled system (see figure 7), obtaining the results of Tables 1 and 2.

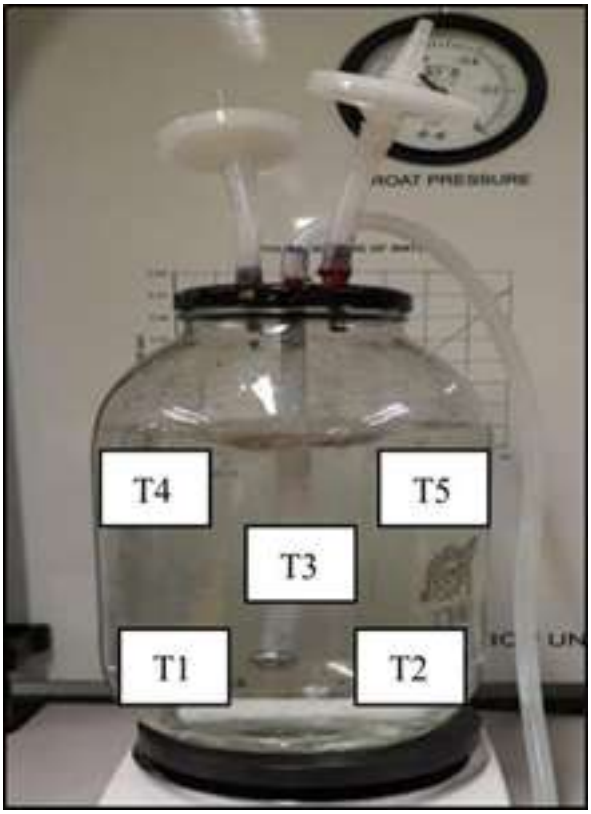

Figure 7. Sample point diagram for heat map. Source: own.

zonas de confort (36°C ± 1°C)	datos del mapa calorifico volumen 1000 ml temperatura de la plancha 60 °C tiempo de estabilización 2 horas			
	stir bar (51,16 mm)	stir bar (51,16 mm)	stir bar (80,3 mm)	stir bar (80,3 mm)
	140 RPM	250 RPM	140 RPM	250 RPM
T1	35,9	35,9	31,2	33,5
T2	35,6	35,8	30,7	33,2
T3	35,7	35,8	30,6	33,1
T4	35,5	35,7	31,0	33,3
T5	35,5	35,7	31,0	33,3
T PROMEDIO	35,64	35,78	30,9	33,28
DIFERENCIA MAX - MIN	0,4	0,2	0,6	0,4
% DIFERENCIA	1,0%	0,6%	14,2%	7,6%
MEJOR OPCION		x		

Table 1. 100ml heat map data. Source: own.

zonas de confort (36°C ± 1°C)	datos del mapa calorifico volumen 200 ml temperatura de la plancha 60 °C tiempo de estabilización 2 horas			
	stir bar (51,16 mm)	stir bar (51,16 mm)	stir bar (80,3 mm)	stir bar (80,3 mm)
	140 RPM	250 RPM	140 RPM	250 RPM
T1	35,9	35,9	31,4	33,2
T2	35,6	35,8	30,6	32,6
T3	35,6	35,7	30,6	32,6
T4	35,5	35,7	31,1	33,0
T5	35,5	35,7	31,1	33,0
T PROMEDIO	35,62	35,76	30,96	32,88
DIFERENCIA MAX - MIN	0,4	0,2	0,8	0,6
% DIFERENCIA	1,1%	0,7%	14,0%	8,7%
MEJOR OPCION		x		

Table 2. Heat map data 2000 ml. Source: own.

As a result of the second experiment, the ideal combination to maintain optimal stirring and temperature conditions is determined to be the magnetic bar from 51.16 mm to 250 R.P.M and with a plate temperature of 60°C.

3.3. Design and optimization to batch fermentation system.

For the design of the pilot fermentation system, it was necessary to consider the following variables: 1. The system must be sealed or contained to avoid contamination that alters alcoholic fermentation by producing instead of bioethanol lactic acid or acetic acid. 2. It must have a gas-defrosting system to allow fermentation without pressuring the system and respiration of the *Zymomonas mobilis.* 3. Since it is a closed system, a sampling system must allow control over the consumption of the substrate and the percentage of alcohol production. 4. The ideal volume of liquid in the system that maintains constant heat transfer in the system and that is homogeneous. The design and optimization of this process is in the lid or seal form (see figure 8), which allows a continuous fermentation and sampling process without opening the system and which loses the fermentation conditions, for this a lid with 3 inputs and 1 output was designed. The entries correspond to 1. To the input of the transfer of the hydrolyzed substrate of *Eichhornia crassipes* from bottle #1 with the aid of a peristaltic pump and a silicone hose. 2. An air-aeration or air-bubbling inlet, consisting of a hydrophobic 0,22 µm Teflon filter coupled to a hose and a fish tank engine as a bubbling system (See figure 9). 3. Inlet coupled to a 100 ml bottle corresponding to the preparation of the inoculum of *Zymomonas mobilis* or another micro-organism which undergoes alcoholic fermentation as *Saccharomyces cerevisiae*, and the output corresponds to a

vacuum system with a 20ml syringe to a 100ml bottle for sampling required to control the process (see Figure 10).

Figure 8. Pilot fermentation system. Source: own

Figure 9. Bubbling instrument. Source: own.

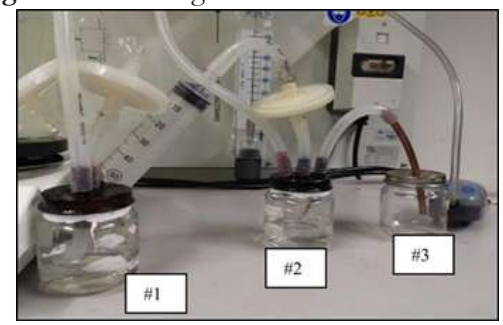

Figure 10. Recipients from left to right #1 inoculated addiction of microorganism, #2 sampling vacuum system, #3 sample bottle. Source: own.

Already with the fermentation system reinforced and optimized and transferred the hydrolysis of bottle #1 to the fermentation bottle, it is possible to start the fermentation in batches, giving the conditions of agitation and temperature mentioned in the point 3.2.

3.4. Design and optimization of alcoholic fermentation control systems.

Any fermentation process must be controlled during every period to determine its yield; in this design, the variables to consider were: pH, concentration of glucose (mg/dl) which is the substrate of the alcoholic fermentation and the result of hydrolysis, % of alcohol which is the product of interest, and temperature.

3.4.1. Measurement of alcohol.

Measuring and controlling alcohol content; which is the product result of fermentation that is conducted with a wide range of alcoholmeter, this is an immersion instrument that was verified in a solution of ethyl alcohol 70% (see figures 11 and 12). For the measurement of alcohol production, it is required to take a sample of 200 ml of fermentation every 1 hour at 2 hours and dilute it in 200 ml a 35% ethyl alcohol solution, so that if you do not have % alcohol produced, its measurement is approximately 17%, and from this value, the increase of % alcohol is measured until the end of fermentation.

Figure 11. Measurement in alcohol. Source: own

Figure 12. Measurement in water. Source: own.

3.4.2. Measurement of glucose:

Measurement and control of glucose which are the result of the hydrolysis process is the most important substrate in fermentation for the production of bioethanol, it is performed by an enzymatic methodology containing equipment such as glucometers, this system determines the value in mg/dl with an analysis range between 700mg/dl and 40 mg/dl. For the optimization of the fermentation process, it is important to note that the determination of efficiency is how much mg glucose mg is transformed into alcohol, Figure 13.

Figure 13. Glucometer. Source: own.

3.4.3. pH measurement

Image 11. Glucometer 3.4.3. pH measurement Within the fermentation processes, this variable is an indirect indication of the metabolic respiration of the fermenting microorganism Zymomonas mobilis or Saccharomyces cerevisiae, this variable indicates the acidification of the fermentation which may be due to growth of biomass or changes from alcoholic fermentation to acidic fermentation. Good control over the pH where it does not change, nor does it change significantly the process demonstrates a stable and well run alcoholic fermentation. Alcoholic fermentation should be carried out at pH s of 3 to 5, Figure 14.

Figure 14. pH strips. Source: own.

3.4.4. Temperature Control

The measurement of this variable is carried out with a potentiometer that has the ability to give a reading at pH and temperature at the same time, as mentioned above, the fermentation must have a homogeneous and constant heat distribution. It must be carried out at temperatures below 30 °C, Figure 15.

Figure 15. pH and temperature. Source: own.

4. Discussion of the Results

The most relevant result of this research is the modeling of the entire system, note that the good yields within a fermentative or productive system depend on the series of variables that accompany it with the set of operations that are performed. Industrially, modeling a process and its optimization only allows changing one variable at a time, since the results are depending on each one of them.

Table 3 is the summary of the complete system modeling, which ideally should not be modified and validated to ensure the success of the system.

TABLE OF RESULTS	
Capacity of the bottle	4000 ml
Volume Test	1000 ml to 2000 ml
Diameter of the jar	124 mm
Diameter of stirring bar	51,16 mm
Agitation of the plate	250 R.P.M
Temperature of the plate	60 °C
Membrane of the filters	0,2 µm
Syringe to make empty	60 ml
Range of the breathalyzer	0% to 100 %
Range of the glucometer	700 mg/dl to 40 mg/dl

Table 3. Complete modeling to fermentation pilot system.
Source: own.

5. Conclusions

The system designed needs to maintain the conditions in the different experiments that are required to carry out an alcoholic fermentation from *Eichhornia crassipes* as a substrate and as fermentation microorganism is possible to use *Zymomonas Mobilis* or *Saccharomyces cerevisiae*; it is recommended that the next step be an alcoholic fermentation using as substrate the common grape and Saccharomyces cerevisiae, to challenge the system in an alcoholic fermentation under well-known and studied conditions that are variables based on the yields of alcoholic fermentation with more complex substrates such as lignins.

The conditions designed and optimized in this research work in case they become modified must be challenged again, as they are variables that change the expected and reported yields.

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