Dynamic Simulation of Bioethanol Production from Banana Rejected using Flocculating Yeast

J Contreras¹, Y Haro¹ and G Gelves¹
¹Universidad Francisco de Paula Santander, Cúcuta, Colombia
germanricardogz@ufps.edu.co

Abstract. A dynamic model is presented to simulate a fermentation process for bioethanol production from banana rejected using a batch system. The critical model feature is using an inhibition function to relate substrate concentration and specific growth rate. Kinetic parameters were calculated based on previous experimental data, and the optimal substrate concentration is investigated. The Andrews kinetic model was adapted to the concentration profiles, indicating that it could describe the existence of substrate and product inhibition. Different initial substrate concentrations were evaluated (90-400 g/L), and simulated results suggest 200 g/L of rejected banana as a starting point to reach a high and efficient bioethanol production since a maximal ethanol production is reached with a value of 150 g/L. However, when using a higher banana rejected concentration inhibition occurs, and therefore bioethanol production decreases to reach levels lower than 100 g/L. Based on results found, kinetic models allow obtaining important observations on microbial metabolic processes and facilitating a good approximation for further large-scale stages.

1. Introduction
The rapid increase in fossil fuel consumption and uncertainty about global warming over the past decade has undoubtedly increased commercial interest in renewable fuels [1]. Furthermore, due to the magnitude of the pollution that has reached the environment, the public and private sectors have adopted policies adapted to the negative impacts of productive activities. For this reason, alternatives to the use of fossil fuels have been sought. One of these alternatives is obtaining ethanol from the fermentation of carbohydrates. Currently, bioethanol production is synthesized during the anaerobic glucose fermentation present in the biomass of various organic substances such as sugar cane and beets. In addition, in grains such as corn, wheat, and sorghum, starch can also be converted to sugar [2].

Because wholesale or supply centers tend to have a high rate of maturity in their products, competition in the market has helped increase business productivity and add value to both products and production processes to benefit the consumer final.

The industry increasingly envisions new ways of using these resources and, therefore, not wasting the waste obtained, which can have added value as raw material for other processes. Now, bananas ripen faster than other fruits. Therefore, this product must be consumed or sold soon since it is less pleasant to the public for its sale when it matures. Therefore, alternatives are sought to take advantage of this residue, turning losses into profits. The ripe banana fruit has a high carbohydrate content (22-28%), and its production volume currently in Colombia reaches 4,805,629 tons/year [3]. Currently, a third of the fruit that is not exported is destined for domestic consumption. Another third is used as
raw material in producing organic fertilizers for growing the same fruit, and a third is still considered waste. It is precisely these last two-thirds of the residue that can potentially be used in alcoholic fermentation processes. For these reasons, reject banana was identified as a suitable substrate for obtaining bioethanol.

It has been shown in previous studies that it is possible to obtain ferment with high alcohol content from rejected bananas using commercial yeasts. So, isolated yeasts with desired characteristics, such as flocculation property, improve the fermentation process. Therefore, the development of a bioprocess using flocculated cells of *Saccharomyces cerevisiae* and rejected bananas for ethanol production is considered a profitable bioprocess and an alternative to today's problems. Based on the latter, the objective of this study is to simulate the performance of the banana rejected fermentation process and optimize this bioprocess to obtain bioethanol using a batch system. The batch mode was considered since it guarantees data integrity and traceability in production processes. In addition, a mathematical model was taken to simulate this bioprocess based on previous studies [4]. The Matlab software was used, which allows to model and analyze static and dynamic systems. The kinetic parameters were taken from experimental data previously reported [5-6].

2. Methodology

In a bioreactor where the yeast is grown for ethanol production, different conditions can be generated that limit cell viability and growth, such that total cells can be classified into viable cells and dead cells as shown in Eq. (1 and Eq. (2), respectively [4,7].

\[
\frac{dX_v}{dt} = \mu X_v - K_d X_v \quad (1) \\
\frac{dX_d}{dt} = K_d X_v \quad (2)
\]

In which, \(X_v\) and \(X_d\) are alive and dead cell concentrations, respectively. \(K_d\) Moreover, \(\mu\) are the dead and growth rates, respectively. The latter is calculated using the Andrews kinetic model for relating biomass growth to substrate inhibition [8] and is calculated according to Eq. (3).

\[
\mu = \frac{\mu_{max} S}{k_s + S + \frac{S^2}{k_i}} \quad (3)
\]

Where \(\mu_{max}\) is the maximum microbial growth rate and \(k_s\) is a saturation constant and \(k_i\) is the substrate inhibition constant. According to the referenced experimental data [6], glucose concentration from banana rejected is a limiting substrate. That is why glucose impacts in batch mode are simulated using Eq. (4):

\[
\frac{dS}{dt} = -\left(\frac{\mu}{Y_{X/S}} + m_s\right)X_v \quad (4)
\]

Where \(Y_{X/S}\) is the yield of *Saccharomyces cerevisiae* cells produced per unit of glucose consumed, ethanol concentration production in a bioreactor can be simulated using the Eq. (5):

\[
\frac{dP}{dt} = \frac{Y_{P/X}}{\mu}X_v \quad (5)
\]
Where $P$ is the bioethanol concentration. Table 1 shows the kinetic parameters used for all simulations based on experimental results [6].

| Table 1: Kinetic parameter used for simulations |
|------------------------------------------------|
| Parameter | Value   |
| $\mu_{max}$ (h$^{-1}$) | 0.280   |
| $k_s$ (gL$^{-1}$) | 5.750   |
| $Y_{xs}$ (gg$^{-1}$) | 0.076   |
| $Y_{p/x}$ (gg$^{-1}$) | 3.660   |
| $m_s$ (gg$^{-1}$h$^{-1}$) | -1.460  |
| $K_s$ (gL$^{-1}$) | 1403    |
| $K_d$ (h$^{-1}$) | 0.25    |

The initial conditions and parameters for ethanol production are presented in Table 2.

| Table 2: Initial Conditions and parameters used for simulations |
|---------------------------------------------------------------|
| Parameter | Value   |
| $X_{v0}$ (gL$^{-1}$) | 3.43    |
| $X_{d0}$ (gL$^{-1}$) | 3.43    |
| $S_0$ (gL$^{-1}$) | 90-400  |
| $P_0$ (gL$^{-1}$) | 0.00    |

The Matlab R2017b software is used for solving numerically the mathematical framework proposed in this research. In addition, the Runge-Kutta 45 numerical method was used [9-11].

3. Results and Discussions

A dynamic model is presented to simulate a fermentation process for bioethanol production from banana rejects using a batch system. The critical model feature is using an inhibition function to relate substrate concentration and specific growth rate. In addition, kinetic parameters were calculated based on previous experimental data, and the optimal substrate concentration is investigated.

Figures 1-3 show the simulation studies for cell concentration, sugar consumption, and bioethanol production using different initial concentrations of reject banana from values of 90 g/L to a maximum level of 400 g/L.

According to the results obtained in Figure 1, a level of 20 g/L of yeast reached when using 90 g/L as the starting concentration of rejected banana is observed. However, biomass production increases by doubling the levels of rejected bananas, achieving a maximum biomass value of 37 g/L by using around 200 g/L of the substrate as raw material.

These findings indicate a direct relationship between the biomass produced and the degree of raw material invested in yeast production that may be of great importance in technical-economic studies for obtaining single-cell protein on a large scale using non-conventional raw material sources.

Interestingly, this relationship is also maintained in simulation studies carried out at 300 g/L of substrate levels. Consequently, the total biomass reaches a maximum value of 50 g/L, indicating that the conditions studied could be in the region that allows the process optimization in biomass production.

The preceding, taking into account that further experimental studies can be designed based on the interval close to 300 g/L of the substrate. The optimal value of ideal rejected banana concentration allowing maximizing biomass would be determined using statistical optimization techniques such as the response surface methods by step ascent.
Figure 1. Total biomass $X_t$ production ($X_v + X_d$) simulated at different banana rejected concentrations: (a) 90, (b) 200, (c) 300 and (d) 400 g/L, respectively.

In contrast, the results are not so promising when the substrate concentration increases above levels above 300 g/L since the biomass concentration falls to levels below 34 g/L.

Similar trends are also found in the analysis of the substrate consumption kinetics (see Figure 2), in which only less than 20 hours are required to deplete all of the glucose when using an initial concentration of rejected banana of 90 g/L.

Contrarily, this time increases with the elevation of the initial substrate load levels with values close to 40 and 80 hours to exhaust all the glucose available in the bioreactor when loading the equipment with 200 and 300 g/L concentrations, respectively. These data obtained through simulation may be necessary for planning bio-separation unit operations following the fermentation process since operational logistics is vital in large-scale bio-processing [12-13].

However, a concentration of 400 g/L of the initial substrate could trigger problems of inhibition and efficiency in substrate conversion. The above, taking into account that only a sugar consumption of only 50% would be achieved, in such a way that half of the raw material would be wasted, and the cost overruns would be evident during the production of bioethanol from rejected bananas.
Figure 2. Substrate consumption simulated at different banana rejected concentrations: (a) 90, (b) 200, (c) 300 and (d) 400 g/L, respectively.

Figure 3 shows the results obtained from the ethanol production using banana rejected at different concentrations in the range of 90-400 g/L.

According to the data obtained, the best ethanol concentration is achieved by using 300 g/L as raw material to reach maximum levels of 150 g/L of bioethanol. The microorganism growth could be interpreted using different mathematical models. In general, the mathematical model is accepted as long as the Monod kinetics is fulfilled, in which the specific growth rate $\mu$ increases to a maximum $\mu_{\text{max}}$ while increasing the value of the substrate concentration. However, it is well known that high substrate concentration can inhibit ethanol production. At this point, the Monod model does not capture the influence of high substrate concentration in ethanol production. This is why a more precise kinetic expression was used in this research sense based on the Andrews model [8].

According to the results obtained, the proposed mathematical model successfully captures the effects of inhibition per substrate because, at concentrations of 400 g/L of reject banana, a considerable reduction in ethanol concentration is observed. This is since bioethanol levels reach values of only 100 g/L when using the highest concentration of sugars.

A bioreactor operated in batch mode for the production of bioethanol offers certain advantages. It allows dividing the productions into small sections, improving the efficiency, monitoring, flexibility, and manufacturing process consistency. The batch process also allows the input of raw material such as its location, dosage, and release to become part of the system, providing the development of inventories and reports related to production.
Figure 3 Bioethanol production simulated at different banana rejected concentrations: (a) 90, (b) 200, (c) 300 and (d) 400 g/L, respectively.

A batch system is fed, closed, and allowed to react. Therefore, it is discontinuous. The conditions within the system vary with time, so it is non-stationary, and finally, the concentration rates and other reaction variables are different from zero, so the system is dynamic, as shown in Figures 1-3.

In order to establish the advantages and improvements of the fermentation process, the initial concentration of rejected bananas as substrate was varied. The modes presented in this study incorporates control theory tools to determine lipid production so that the processes can be optimized knowing the appropriate setup conditions.

Finally, Figure 4 shows the maximal bioethanol and total biomass concentration for each initial substrate condition evaluated. Undoubtedly, the concentration that can result in promising levels for the bioethanol industry from rejected bananas is operating the batch mode bioreactor at a 300 g/L for a time not exceeding 100 hours of fermentation.
Figure 4. Maximal ethanol (a) and total biomass (b) were simulated for different initial substrate concentrations.

4. Conclusions
The kinetic model proposed to simulate the bioprocess was able to accurately describe the dynamics of bioethanol production by a flocculating yeast, growing on a rejected banana substrate, with a sugar concentration between 90 and 400 g/L, thus deducing that the most optimal initial concentration for this bioprocess is 300 g/L of the substrate. Furthermore, the Andrews kinetic was adapted to the concentration profiles, indicating that it could describe the existence of substrate and product inhibition based on the latter. Therefore, the Andrews kinetic model is the most suitable to simulate this bioprocess. Furthermore, it was possible to optimize the process for obtaining bioethanol. These improvements were obtained based on the parameters of the initial substrate concentration and the time spent in the experimental study of bioethanol production employing reject bananas. Furthermore, the rejected banana presented a high potential for use as a substrate in the fermentation processes for bioethanol production. With this substrate, high yields were achieved concerning the reported experimental values, making it a promising substrate for alcoholic fermentations and obtaining biofuels. Finally, it is concluded that Matlab software could also be used to design and develop different kinetic models.

References
[1] Stoll B 2002 N-3 fatty acids and lipid peroxidation in breast cancer inhibition. British Journal of Nutrition 87 193
[2] Halim R, Harun R, Danquah M and Webley P 2012 Microalgal cell Disruption for biofuel Development. Applied energy 91(1) 116
[3] Vázquez H and Dacosta O 2007 Fermentación Alcohólica: Una Opción Para La Producción De Energía Renovable A Partir De Desechos Agrícolas Ingeniería, Investigación y Tecnología 8(4) 249

[4] Niño L, Acosta A and Gelves G 2013 Evaluación de pretratamientos químicos para la hidrólisis enzimática de residuos lignocelulósicos de yuca (Manihot esculenta Crantz). Revista Facultad de Ingeniería Universidad de Antioquia 69 317

[5] Teles A, Meneses E, Ceccato S, Marques S, Ponte M and Rocha L 2017 Mathematical Modeling Of Cashew Apple Juice Ethanol Fermentation By Flocculating Yeast: Effect Of Initial Substrate Concentration And Temperature Bioprocess and Biosystems Engineering 40(8) 1221

[6] Bello A, Morales K, Sánchez L, Lidueñez V, Leal A and Gelves G 2020 Computational Implementation of Required Industrial Unit Operations for Bio-Plastic Production From Starch Extracted from Banana Peels by Aerobic Fermentation using Rizophus Oryzae Journal of Physics: Conference Series 1655 012078

[7] Guevara C, Acevedo J, Peláez C 2014 Isolation And Characterization Of Flocculant Yeast To Produce Ethanol From Banana Refuse Biotecnología en el sector Agropecuario y Agroindustria 12(2) 151

[8] Araque J, Niño L and Gelves G 2020 Industrial Scale Bioprocess Simulation for Ganoderma Lucidum Production using Superpro Designer Journal of Physics: Conference Series 1655 012077

[9] Andrews J 1968 A Mathematical Model For The Continuous Culture Of Microorganisms Utilizing Inhibitory Substrates Biotechnology and Bioengineering 10(6) 707

[10] Caicedo Y, Suarez C and Gelves G 2020 Evaluation of preliminary plant design for Chlorella vulgaris microalgae production focused on cosmetics purposes Journal of Physics: Conference. Series 1655 012086

[11] Ibañez A, Rolon Y and Gelves G 2020 Evaluating Cost-Effective Culture Media for Nutraceutical Production from Microalgae Using Computer-Aided Large Scale Predictions Journal of Physics: Conference. Series 1655 012082

[12] Nieto L, Rivera C and Gelves G 202 Economic Assessment of Itaconic Acid Production from Aspergillus Terreus using Superpro Designer Journal of Physics: Conference Series 1655 012100

[13] Pacheco S, Niño L and Gelves G 2020 Recombinant Anti-Thrombin Production from Saccharomyces Cerevisiae: Large Scale Trends Based on Computational Predictions Journal of Physics: Conference Series 1655 012081

[14] Hernandez S, Niño L, Gelves L 2020 Simulating of Microbial Growth Scale Up in a Stirred Tank Bioreactor for Aerobic Processes using Computational Fluid Dynamics Journal of Physics: Conference Series 1655 012109