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Kinetic Models for the Batch Production of Ethanol from Cassava Whey With *Saccharomyces Cerevisiae*

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Abstract

Saccharomyces cerevisiae has been used to produce ethanol from cassava whey at a yield of about 23 mole % based on glucose. Experimental data on glucose depletion, biomass growth and ethanol production have been fitted to some Kinetic models (Leudeking – Piret; Logistic, Modified Logistic, Logistic with death phase; Leudeking, Product with lag time). In each case, the closeness of the square of the correlation coefficient (R^2) to unity was used to judge how good the predictive model was. The Leudeking – Piret model for substrate consumption was good for glucose depletion. The yield coefficient of the biomass on the substrate $Y_{x/s}$ and the maintenance coefficient m were obtained as 0.169 g/g and 0.08048 g substrate g cell hr^{-1} respectively. The modified logistic model was adopted in predicting the biomass growth; the maximum biomass concentration X_m and the maximum specific growth rate μ_m were computed as 8.264 g/l and 0.6867 hr^{-1} respectively. The Leudeking – Piret model for product formation gave the best fit for ethanol production. The growth and non-growth parameters a and â were calculated as 3.634 and 0.007078 respectively.

Keywords: Cassava-whey, Saccharomyces Cerevisiae, Kinetic, Models, Ethanol, Production

1.0 Introduction

Fossil fuels are currently the mainstay of global economy, but biomass supplies 14% of current global primary energy and will continue to be used in the future (Khesghi *et al*., 2000). The potential for its expanded use for electricity and / or liquid fuels will depend on the demand for the energy form, the economic viability, the technology for conversion, the availability of land, and public policy factors.

Ethanol as a bio-fuel has attracted a lot of attention in recent times due to the following special characteristics:

- it does not cause air pollution or any environmental hazard
- it has a higher octane rating than petrol and as such serves as an anti-knocking agent and as an octane booster.

Thus ethanol can be used to replace or supplement traditional petroleum transportation fuels, and can be used in existing vehicles with little or no modification to engines and fueling systems (Khesghi, *et al.* 2000; Thomas and Kwong, 2001).

Most bio-ethanol is produced by fermenting the sugar or starch portions of agricultural raw materials. The three topmost producers of ethanol from biomass Brazil, United States of America and China rely on sugar cane, grains and maize as their feedstock. Some others have used sugar, cassava, seaweed, among other sources. Though there are economic and environmental benefits of using agricultural plants to produce bio-fuels, there is a worldwide trepidation that a scarce community, food, is converted to fuel at a time when there is food insecurity.

Cassava whey is normally waste liquid effluent resulting from compressing a bag of crushed (grated) cassava to reduce the water content before it is used to produce edible food, for example, garri, etc. The argument against the use of agricultural plants or food items to produce bio-fuels does not apply here. Instead, with the knowledge of converting cassava whey to bio-ethanol, there will be increased rural productivity since there is enhanced value, greater economic growth, increased income and possible reduction in the migration of the rural poor to urban

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areas. The problem that may be associated with cassava whey as a feedstock for producing ethanol may be its starch content. Earlier workers have shown that cassava at 30% starch content would produce about 280 litres of alcohol / tonne while with 20% starch it would only produce 180 litres of alcohol / tonne (Atthasampunna *et al.*, 1987; Alvanblanch, 2009). This information only serves as a guide since there may not be a clear correlation between these data and what is expected from cassava whey.

Saccharomyces cerevisiae also known as ale yeast operates best in acidic medium of pH 4.5 to 5.5. Like all yeasts it has four development stages, namely, the lag phase, growth phase, fermentation phase and the sedimentation / flocculation phase. The development stage in which yeast is, affects the biophysical changes it undergoes in the presence of a food medium (Baei *et al.*, 2008; Weiss and Ollis, 1980). Saccharomyces cerevisiae metabolizes sugar in the absence of oxygen to produce ethanol and carbon dioxide, which is fermentation. Oxygen must be excluded if not water and carbon dioxide will result in place of ethanol. Cassava whey when hydrolyzed with acid (hydrochloric acid) gives sugar which is fermented with yeast to give ethanol. The most ethanol tolerant strains of yeast are known to survive up to approximately 15% ethanol by volume (Atthasampunna *et al.*, 1987; Banat *et al.*, 1998). Fermentation is a complex process and it is not always possible to have a complete picture of what obtains in a particular one. That not withstanding, the study of the kinetics of fermentation is important since it enables us to control and optimize a fermentation process. we show the process description of ethanol production from cassava whey in Figure 1.

2.0 Methodology

Assay methods for fermentation measure the biomass (cell), product (ethanol) and substrate (sugar) concentrations over time. The discontinuous type of assay was used, whereby samples were taken, reaction stopped, and the concentrations of substrates and products determined. Biomass concentration is one of the most critical measurements in fermentation studies and various methods are used to determine it. All the methods have their limitations, but the optical density method was chosen because of its relative low cost. The Dinitrosalicylic acid (DNS) method for glucose assay was adopted because it determines only the amount of reducing sugar present in a sample. Ethanol concentration was measured using a refractometer.

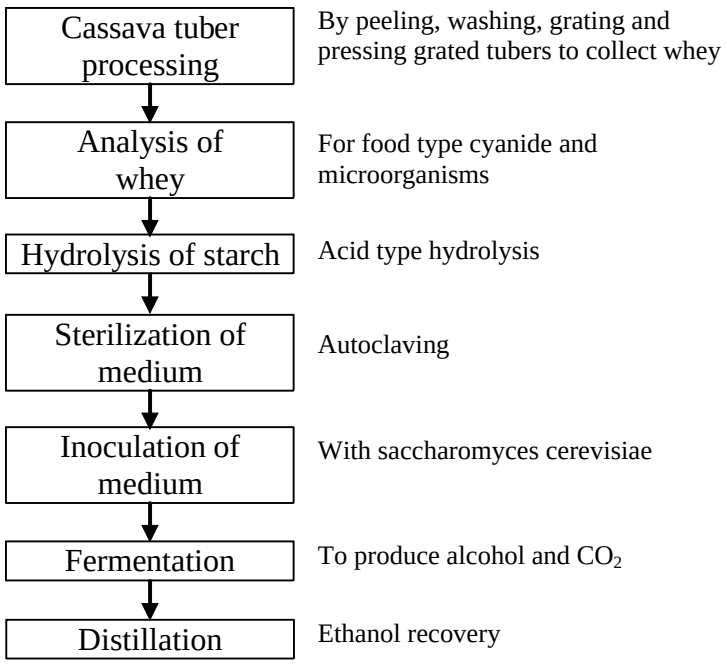

Figure 1: Block diagram of ethanol production from cassava whey

2.1 Kinetic Models Development

i. Biomass Growth Kinetics

The idea of microbial growth kinetics has been dominated by the empirical model originally developed by Monod (Garcia-Ochoa *et al*., 1995). Though variants of the Monod equation exist, most have been found not to describe fermentation processes well. Sigmoidal shaped models, especially the logistic models are widely used to describe microbial growth. In a good number of polysaccharide fermentation processes, biomass growth has been described by the logistic equation (Weiss and Ollis, 1980).

a. Logistic Model

At optimal growth conditions, when also the inhibitory effects of substrate and product play no role, the rate of biomass concentration is given by

$$\frac{dX}{dt} = \mu_m X \qquad ...1$$

where μ_m is the maximum specific growth rate with respect to the fermentation conditions, X is biomass concentration (g/l) and t is time. Equation 1 implies that X increases with time regardless of substrate availability. In reality, cell growth is governed by a hyperbolic relationship given by

$$\frac{dX}{dt} = \mu_m X\left(1-\frac{X}{X_m}\right) \qquad ...2$$

where X_m is the maximum biomass concentration. Equation 2, if integrated with the initial condition X = X_o at t = 0 becomes

$$X = \frac{X_o X_m e^{\mu_m t}}{X_m - X_o + X_o e^{\mu_m t}} = \frac{X_o e^{\mu_m t}}{1-\left(\frac{X_o}{X_m}\right)\left(1-e^{\mu_m t}\right)} \qquad ...3$$

Equation 3, the logistic equation, may represent both an exponential phase and a stationary phase but it does not predict the death phase of microorganisms after the stationary phase. To predict the death phase of bacteria after the stationary phase, the logistic equation becomes:

$$X = \frac{X_o e^{\mu_m t}}{1-\left(\frac{X_o}{X_m}\right)^2\left(\frac{\mu_m}{k+\mu_m}\right)\left(1-e^{(k+\mu_m)t}\right)} \qquad ...4$$

where *k* is a constant associated with a decline or promotion of cell population in the batch culture.

b. Modified Logistic Model

Equation 2 shows that specific growth rate decreases linearly as the cell mass concentration increases. This linear relationship may be applicable in specific cases but may not be valid for all strains. A modified form of the Logistic equation may be used to describe the biomass growth kinetics by introducing an inhibitory effect index r which accounts for the deviation of growth from the exponential relationship. Hence.

$$\frac{dX}{dt} = \mu_m\left(1-\left(\frac{X}{X_m}\right)^r\right)X \qquad r \geq 0 \qquad ...5$$

When r

= 0, there is complete inhibition of cell growth.

= 1, equation 5 is identical to the Logistic equation.

> 1, growth lies between exponential and logistic patterns.

Equation 5 can be rewritten as

$$\frac{dX}{X\left(X_m{}^r - X^r\right)} = \frac{\mu_m}{X_m{}^r}dt \qquad ...6$$

When equation 6 is integrated with the initial condition X=X_o at t = 0, we obtain:

$$X = \frac{X_o{}^r e^{\mu_m r t}}{1-\frac{X_o{}^r}{X_m{}^r}\left(1-e^{\mu_m r t}\right)} \qquad ...7$$

Equation 7 shows that the biomass concentration with respect to time depends on the initial and maximum cell mass concentrations, which vary with the microorganism and fermentation conditions.

ii. Product (Ethanol) Formation Kinetics

a. Leudeking – Piret Model (Leudeking and Piret, 1959)

This model states that the product formation rate varies linearly with both instantaneous cell mass

concentration (X) and growth rate $\left(\frac{dX}{dt}\right)$. That is

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X \quad ...8$$

where α and β are empirical constants that may vary with fermentation, and are growth and non-growth associated terms. When the inclusion of a non-growth associated term is not justified, equation 8 is simplified to

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} \quad ...9$$

$$P = K + \alpha X \quad ...10$$

Thus from equation 3

$$P = K + \alpha\left(\frac{X_o X_m e^{\mu_m t}}{X_m - X_o + X_o e^{\mu_m t}}\right) \quad ...11$$

If there is delay in product formation compared with the cell growth, Δt the lag time is introduced to account for delay in product formation. The product formation rate is modified to

$$\frac{dP}{dt} = Y_{p/x}\frac{dX}{d(t-\Delta t)} \quad ...12$$

where $Y_{p/x}$ is the yield coefficient of product on biomass. Substituting for X from equation 3 and integrating.

$$P = Y_{p/x}\left(\frac{X_o X_m e^{\mu_m (t-\Delta t)}}{X_m - X_o + X_o e^{\mu_m (t-\Delta t)}} - \frac{X_o X_m e^{-\mu_m \Delta t}}{X_m - X_o + X_o e^{-\mu_m \Delta t}}\right) \quad ...13$$

iii. Substrate Consumption Kinetics

Any model of substrate consumption must account for consumption in the formation of biomass and for the maintenance of biomass. The Leudeking–Piret Model for substrate consumption is given by

$$-\frac{dS}{dt} = \frac{1}{Y_{x/s}}\cdot\frac{dX}{dt} + m\cdot X \quad ...14$$

where s is substrate concentration, $Y_{x/s}$ is the yield coefficient of biomass on substrate and m is the maintenance coefficient. When equation 14 is combined with equations 2 and 3, and then integrated, we obtain:

$$S = S_o - \frac{1}{Y_{x/s}}\left(\frac{X_o X_m e^{\mu_m t}}{X_m - X_o + X_o e^{\mu_m t}} - X_o\right) - \frac{X_m^{\ m}}{\mu_m}\ln\left(\frac{X_m - X_o + X_o e^{\mu_m t}}{X_m}\right) \quad ...15$$

3.0 Experimentation

3.1 Sample (Cassava Whey) Collection

Cassava whey was collected from a local garri processing plant. The mixture was left undisturbed for some time so that starch would settle to the bottom. The supernatant water was decanted, the whey mixed thoroughly and subsequently sterilized by boiling at 100°C for 30mins. The whey was continuously stirred as it was heated to prevent excessive foaming as it gelatinized. The sterilized whey was then cooled to room temperature.

3.2 Tests for Starch, Reducing sugar, Protein, Fat and Oil

Standard methods of testing for these components were used. Iodine method was used to indicate the presence of starch. The presence of reducing sugar was identified by the Benedict's solution method. The Biuret solution and Sudan III methods were used for protein and fat / oil respectively.

3.3 Acid Hydrolysis of Sterilized Whey

1 litre of 2M HCl was added to 3 litres of sterilized cassava whey and boiled with continuous mixing. Iodine solution was added periodically. When a brownish red colour was observed after adding iodine solution, it showed complete hydrolysis.

3.4 Fermentation of Hydrolyzed Cassava Whey

NaOH was added to the hydrolyzed cassava whey

in a fermenter to bring the pH to 5.0, and the mixture temperature was kept at 30°C the optimum temperature for the biomass growth. An inoculum was prepared by adding 10g of dried Bakers yeast, Saccharomyces cerevisiae, to 100ml of distilled water in a beaker. A small quantity of glucose was added to the inoculum and the yeast allowed to grow. Bubbles were formed and the water level in the beaker rose indicating biomass growth. The inoculum was then added to 3 litres of hydrolyzed cassava whey held in the fermenter and it was allowed to ferment it for 96hrs. Samples were collected at various times, t = 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 hrs, and the concentrations of yeast, glucose and ethanol determined. A spectrophotometer, model Spectrumlab 23A, was used to determine the concentrations of yeast and glucose, and a refractometer, Abbe Hilger type, for ethanol concentration. The results are shown in Table 1; they represent the average of three independent experiments.

Table 1: Concentration profile of glucose, biomass and ethanol

Time (hrs)	Glucose concentration (g/l)	Biomass concentration (g/l)*	Ethanol concentration (g/l)
0	123.44	3.333	0.000
4	121.52	3.429	2.96
8	113.36	4.717	3.78
12	102.10	5.614	7.10
24	93.10	6.508	10.21
36	85.62	7.218	12.64
48	70.49	7.928	13.68
60	65.78	8.203	14.12
72	53.79	8.235	14.42
84	41.32	8.432	18.33
96	38.60	8.519	19.61

*dry weight

4.0 Results and Discussion

The experimental data shown in Table 1 which reflect the concentrations of glucose, biomass and ethanol over time were fitted to various models proposed in this work using the MATLAB 6.5 software. In each case, the choice of model was based on the value of the basic correlation coefficient (R^2), which is taken as good when its value is close to unity.

Glucose concentration decreased with increase in biomass concentration (Table 1). This is expected since glucose was used for cell growth, cell maintenance, and product formation. Glucose depletion was fitted with the Leudeking–Piret Model for substrate consumption which gave R^2 equal to 0.9894. Figure 2 shows the plot of simulated and experimental data for glucose depletion based on this model. The yield coefficient of the biomass on substrate $Y_{x/s}$ and the maintenance coefficient m, were 0.169 g/g and 0.08048 g substrate g cell hr^{-1}.

The sterilized medium was adjusted to a pH of 5.0 which was the best environment for the fermentation process by the biomass. The lag phase of the biomass in the fermentation process was quite short because the yeast was already adapted. Hence the biomass entered the exponential phase instantly, producing ethanol concurrently. The biomass growth was fitted to the Logistic, Modified Logistic, and Logistic with death phase equations. The basic correlation coefficient R^2 for the three equations was 0.9825, 0.9826 and 0.9476 respectively. Based on these values, the Modified Logistic Model was adopted and the parameters of the equation computed. The maximum biomass concentration X_m and the maximum specific growth rate μ_m for the modified logistic equation were obtained as 8.264 g/l and 0.6867 hr^{-1} respectively. Figures 3, 4 and 5 show simulated and experimental data based on the three models. Predicted is close to experimental.

The data for ethanol production were fitted to the Leudeking, Leudeking-Piret and product with lag time models, and their R-square values computed as 0.9375, 0.9667 and 0.9358 respectively. The simulated and experimental data are plotted in Figures 6, 7 and 8. The Leudeking – Piret model was adopted for ethanol formation. The growth and non-growth associated parameters and were calculated as 3.634 and 0.007078 respectively. It is not clear why the concentration of ethanol between the 40th hour and 70th hour was constant though the biomass concentration increased. It could not have been due to the inhibitory effect of ethanol since the concentration of biomass increased within the period. At this time ethanol was only about 16 mole % of the broth. After the 70th hour the rate of formation of ethanol jumped, becoming almost linear. This may be explained by the small increase in the rate of

biomass growth. Though the Leudeking-Piret model is the best among the three models used here to predict ethanol formation, it has its limitations. The model does not fully describe the product formation as shown by the experimental data, notably after 70 hours. Earlier workers have shown that most ethanol tolerant strains of yeast can survive approximately 15% ethanol by volume (Atthasampunna *et al.*, 1987; Banat *et al.*, 1998). Saccharomyces cerevisiae seems to be one of the more tolerant strains of yeast since it still grew when the concentration of ethanol was above 20% by volume.

Table 2: Some physicochemical properties of raw cassava whey

Moisture content	pH	Density g/cm^3
82.3%	3.84	1.07

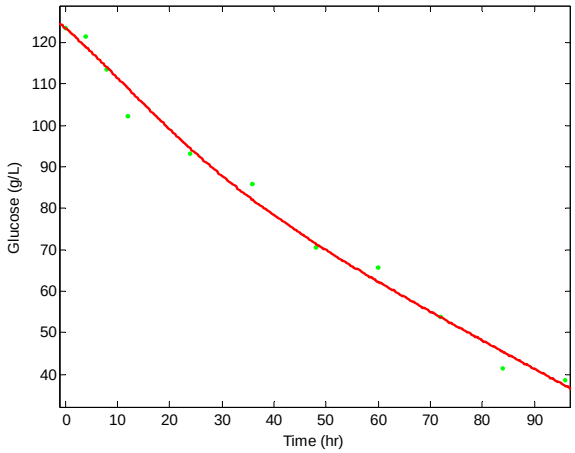

Figure 2: Glucose depletion – experimental and simulated (Leudeking –Piret)

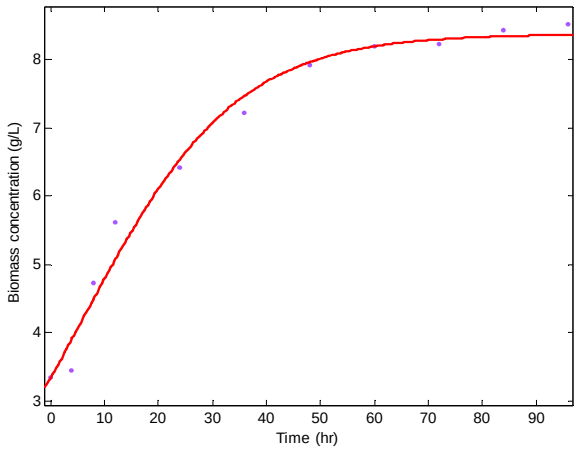

Figure 3: Biomass growth – experimental and simulated (Logistic model)

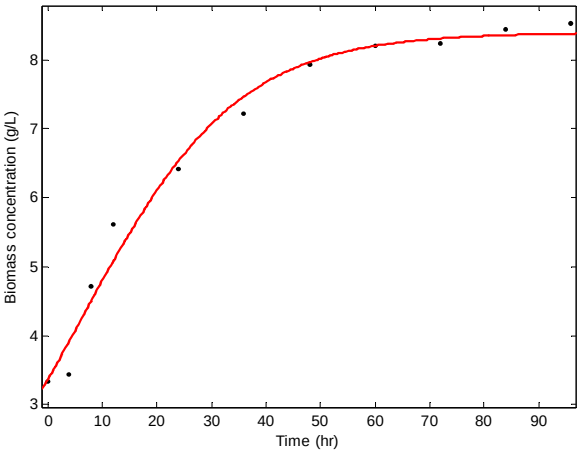

Figure 4: Biomass growth – experimental and simulated (Modified Logistic)

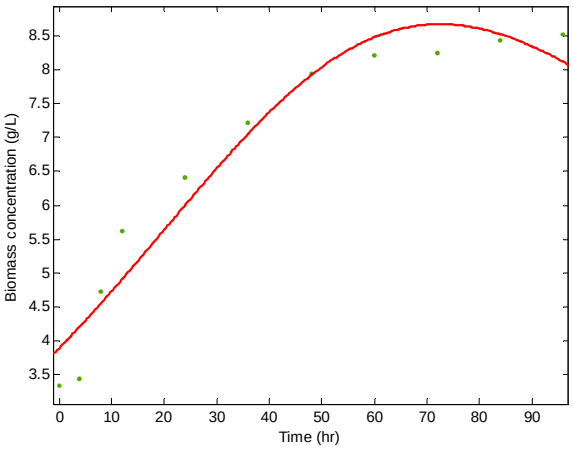

Figure 5: Biomass growth – experimental and simulated (Logistic with death phase)

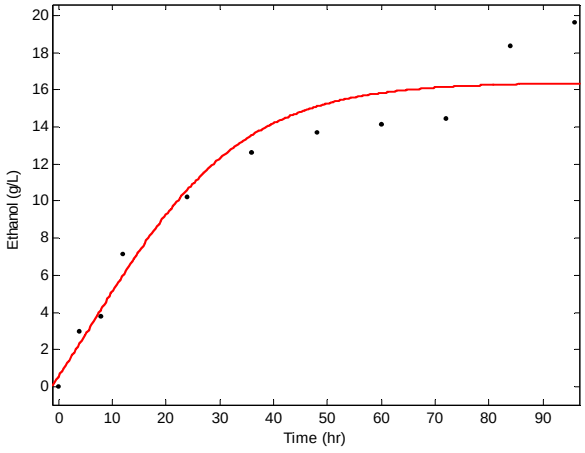

Figure 6: Ethanol production – experimental and stimulated (Leudeking)

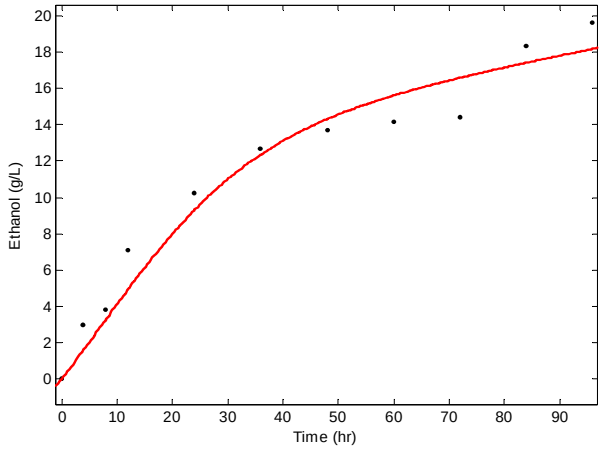

Figure 7: Ethanol production – experimental and simulated (Leudeking –Piret)

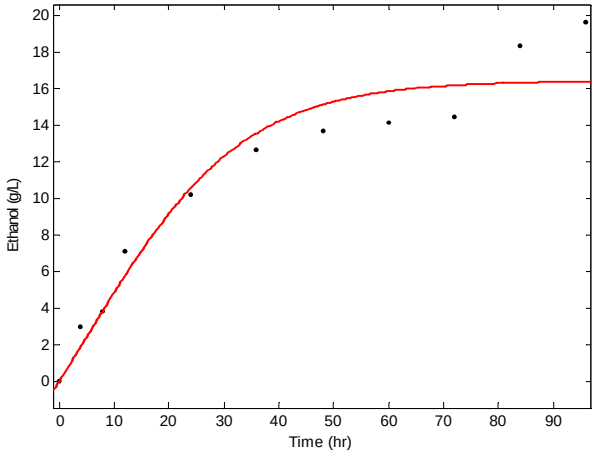

Figure 8: Ethanol production – experimental and simulated (Product with Lag time)

5.0 Conclusion

Saccharomyces cerevisiae has been used to produce ethanol from cassava whey, a liquid effluent resulting from compressing grated cassava to reduce the water content. Unlike most agricultural raw materials for producing bio-ethanol, cassava whey is not a food item, thus the arguments against the use of scarce food commodity to produce bio-ethanol is not relevant here. Kinetic parameters computed for glucose consumption, biomass growth and ethanol production can be used to control and optimize a fermentation process of this nature. However, the predictive ability of the kinetic models tested is limited.

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