

## Kinetics Of Ethanol Production From Nypa Palm (Mangroves Palm) Through Fermentation Process

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### Abstract

The kinetics for production of ethanol from Nypa palm by fermentation was studied. The pH and initial glucose concentration of sap were found to be 4.96 and 108.387 g/L respectively with 5.0 g/L *saccharomyces cerevisiae*. Biomass, ethanol and glucose concentrations were measured at different time interval during fermentation. The experimental data obtained were fitted using different models for yeast growth. The logistic model gave the best fitting and was the basis for the development of the overall kinetic model. For ethanol formation, different model based on the logistic model for yeast growth were used to fit the experimental data and the leudeking – piret model was adopted because of its good fit. The leudeking – piret model was also adopted for substrate consumption. The estimated values of the kinetic parameters in the developed model were  $\mu_m = 0.0372 \text{ hr}^{-1}$ ,  $X_m = 6.932 \text{ g/L}$ ,  $a = 23.64 \text{ g/g.hr}$ ,  $Y_{x/s} = 0.1739 \text{ g/g}$  and  $m = 0.006613 \text{ g/g.hr}$ . Therefore, a model based on the logistic equation of yeast growth, growth associated production of ethanol, and consumption of glucose for biomass and maintenance was found to accurately fit the production of ethanol from Nypa palm.

**Keywords:** concentration, ethanol, fermentation, kinetics, model, Nypa palm,

### 1.0 Introduction

Ethanol has been described as one of the most exotic synthetic oxygen – containing organic chemicals because of its unique combination of properties as a solvent, a germicide, a beverage, an antifreeze, a fuel, a depressant, and especially because of its versatility as a chemical intermediate for other organic chemicals. Ethanol burns more completely than gasoline and diesel, which implies that it is environmentally friendly. It is estimated that one litre of ethanol produces 70 % less carbon dioxide emissions than one litre of gasoline fuel, thus, ethanol help to reduce air pollution. Fermentation is one of the oldest chemical processes known to man. It is use to make a variety of products including foods, flavorings, beverage, pharmaceuticals and chemicals. All beverage alcohols and much of that used in industry is formed through fermentation of a variety of products including grains such as corn, potatoes masses, fruit

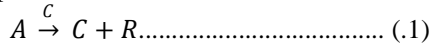
molasses (Kompala,[1]; Wikipedia,[2]). Assay methods for fermentation measure the biomass (cell), product (ethanol) and substrate (sugar) concentration over time. These assays are very useful in obtaining kinetic models for fermentation processes as they provide the kinetic data for the fermentation. Assay can be divided into two groups: continuous and discontinuous assays according to their sampling method. In continuous assay, a continuous reading of activity is taken, while in discontinuous assay, the samples are taken, the reaction stopped and then the concentration of substrate or products determined. The discontinuous assay was used in this study ([www.eng.und.edu/nsw/nch485/lab9c.htm](http://www.eng.und.edu/nsw/nch485/lab9c.htm); wang,n.s)[3]

Nypa palm is a tropical plant, has a horizontal trunk that grows beneath the ground and only the leaves and flowers stalk grow upwards above the surface. It is a large creeping unarmed, pleonastic palm stem prostrate or subtenanean rhizome up to 45cm in diameters; branching dichotomously at regular intervals, with curved leaf scars above and roots along the underside. Nypa palm occupies a unique position in the palmae, it is considered an advanced palm species. It is one of the oldest angiosperm plants and probably the oldest palm species. It is mainly found in the equatorial zone, 100N – 100S. The largest natural Nypa stands are found in Indonesia (7,000,000 ha), New Guinea (500,000

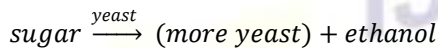
ha)([www.worldagroforestry.org/products/specieninfo.asp](http://www.worldagroforestry.org/products/specieninfo.asp))[4]. The optimum climate for Nypa palm is sub humid to humid. It thrives in brackish water environment. It is rarely seen directly on the seashore. The average minimum temperature is 20 °C, average maximum temperature is 32 – 55 °C, rainfall is 100mm per month throughout the year. Nypa palm sap can be tapped as a source of sugar, alcohol or vinegar slightly fermented sap is used as local bear in Malaysia. As a medicine, the juice from young shoots is used against herpes; it is used as a material for salt extraction. Fresh Nypa sap has an average density of 1.076 g per litre and a dry matter content of 17 %. The sucrose content of the sap is 15 %. The fresh sap has a pH of 7.5. In Malaysia, an ethanol and biodiesel plant was to be completed in 2009 using alcohol from Nypa palm ([www.wikipedia.com/nypafructians/agroforestry/edu](http://www.wikipedia.com/nypafructians/agroforestry/edu))[5]

## 2.0 Models for microbial fermentation

In a simplest situation, we have one type of microbial, C acting called the cell and one type of food needed, A, called the substrate. If the food is right the microbe feeds on it, multiplies, and in the process produces waste material R.



In some cases, the presence of product R inhibits the action of cells, and we have what is called poisoning by product. Ethanol fermentation is a good example of such situation. (Amenaghawon et al)[6]



As the concentration of ethanol rises, the cells multiply more slowly, and at about 12% ethanol concentration, the yeast cells quit and begin to die. Kinetic models for microbial fermentation describe processes of a more complex nature in which many enzymes are involved. The overall kinetic for microbial growth (biomass concentration), substrate utilization and product formation can be found in (Wang et al)[7].

### 2.1 Kinetic Models for Microbial Growth

#### 2.1.1 Monod Model

Monod model relates the specific growth rate,  $\mu$ , and the limiting substrate X, concentrations, S, and was described by the Monod equation Blanch, and Clark [8]; Levespiel [9].

$$\mu = \frac{\mu_{max} S}{K_s + S} \dots\dots\dots (2)$$

Where  $\mu_{max}$  (g/L) is the maximum specific growth rate (the rate at which the substrate concentration is not limiting) and K (hr<sup>-1</sup>) is the half saturation constant (Concentration of S when  $\mu_{max}$  /2).

#### 2.1.2 Logistic Model

The rate of biomass concentration is given by:

$$\frac{dx}{dt} = \mu_m X \dots\dots\dots (3)$$

This equation, which is known as the unstructured Malthus model equation, implies that X increases with time, regardless of substrate availability. The growth of cell was governed by a hyperbolic relationship given as:

$$\frac{dx}{dt} = \mu_m X \left(1 - \frac{X}{X_m}\right) \dots\dots\dots (4)$$

This equation is known as the Riccati equation. With the initial condition,  $X = X_0$  at  $t = 0$ , equation 4 can be easily integrated to give:

$$X = \frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} = \frac{X_0 e^{\mu_m t}}{1 - \left(\frac{X_0}{X_m}\right) (1 - e^{\mu_m t})} \dots\dots (5)$$

The kinetic parameter,  $\mu_m$  can be determined by rearranging equation (5) as:

$$\ln \frac{X_m}{X_0} = X_m t - \ln \left(\frac{X}{1-X}\right) \dots\dots\dots (6)$$

Where  $\bar{X} = \frac{X}{X_m}$ . If the logistic equation describes the data suitably, then a plot of  $\ln \left(\frac{X}{1-X}\right)$  versus time should give a straight line of slope,  $\mu_m$ , and intercept  $-\ln \left(\frac{X_m}{X_0}\right)$ .

The logistic equation presented above does not predict the death phase of microorganisms after the stationary phase in a batch culture. To predict the death phase of bacteria after the stationary phase, the equation becomes:

$$X = \frac{X_0 e^{\mu_m t}}{1 - \left(\frac{X_0}{X_m}\right)^2 \left(\frac{\mu_m}{k + \mu_m}\right) [1 - e^{k + \mu_m} t]} \dots\dots\dots (7)$$

#### 2.1.3 Modified Logistic Model

A modified form of the logistic equation was used to describe the cell growth kinetics by introducing an index of the inhibitory effect 'r' which accounts for the deviation of growth from the exponential relationship as:

$$\frac{dx}{dt} = \mu_m X \left[1 - \left(\frac{X}{X_{max}}\right)^r\right] X \text{ for } r = 0 \dots\dots\dots (8)$$

When  $r = 0$  will be a complete inhibition of cell growth. When  $r = 1$ , equation (8) reduces to the logistic model equation. When  $r$  ranges between 0 – 1, equation 8 describes a higher degree of inhibition compared to logistic growth. When  $r > 1$  the growth lies between exponential and logistic patterns. Equation 8 can be written as:

$$\frac{dx}{X[x_m r - x^r]} = \frac{\mu_m}{X_m r} \dots\dots\dots (9)$$

Equation 9 was integrated by using partial fraction method with the initial condition  $X = X_0$  at  $t = 0$  to give:

$$X = \frac{X_0^r e^{\mu_m r t}}{1 - \frac{X_0}{X_m} (1 - e^{\mu_m r t})} \dots\dots\dots (10)$$

The cell mass concentration with respect to time depends on the initial and final cell mass concentrations, which varies with microorganisms used and fermentation conditions.

#### 2.1.4 Kinetic Models for Product formation

Leudeking – Piret model

The Leudeking – Piret model (Leudeking and Piret[10] states that the product formation rate varies linearly with both the instantaneous cell mass concentration (X) and growth rate  $\left(\frac{dx}{dt}\right)$  as:

$$dP/dt = \alpha \frac{dx}{dt} + \beta X \dots\dots\dots (11)$$

Where  $\alpha$  and  $\beta$  are empirical constants that may vary with fermentation conditions. The

Luedeking – Piret model includes a growth associated and a non-growth associated term. For growth associated product formation (i. e. when  $\alpha \neq 0$ ,  $\beta = 0$ ), which is the case when the inclusion of a non-growth associated term is not justified, equation 11 can be simplified as follows:

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} \dots\dots\dots(12)$$

$$P = K + \alpha X$$

$$P = K - \alpha \left( \frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} \right) \dots\dots\dots(13)$$

When there is a delay of product formation compared with the cell growth a parameter, the lag time,  $\Delta t$ , is introduced to describe the delay of formation to cell growth. Equation (12) is modified as:

$$\frac{dP}{dt} = Y_{p/x} \frac{dX}{d(t-\Delta t)} \dots\dots\dots(14)$$

Where  $Y_{p/x}$  is the yield coefficient of product on biomass. Substituting equation (5) into equation (14), integrating gives equation (15):

$$P = \frac{Y_p}{X_m - X_0 + X_0 e^{\mu_m(t-\Delta t)}} - \frac{X_0 X_m e^{-\mu_m \Delta t}}{X_m - X_0 + X_0 e^{-\mu_m \Delta t}} \dots\dots\dots(15)$$

**2.1.5 Logistic incorporated Leudeking – Piret model**

Logistic incorporated Leudeking – Piret model and was developed by rearranging equation (11), using equation 4 for  $\frac{dX}{dt}$  and equation (5) for X gives:

$$\frac{dP}{dt} = \alpha + \frac{\beta}{\mu_m(1-X/X_m)} \dots\dots\dots(16)$$

Integrating equation (16) with two initial conditions  $X = X_0$  ( $t = 0$ ) and  $P = P_0$  ( $t = 0$ ). Substituting for X from equation (5) and upon rearranging gives:

$$P = P_0 + \alpha X_0 \left[ \frac{e^{\mu_m t}}{1 - \left(\frac{X_0}{X_m}\right)(1 - e^{\mu_m t})} - 1 \right] + \beta \frac{X_m}{\mu_m} \ln \left[ 1 - \frac{X_0 X_m (1 - e^{\mu_m t})}{X_m - X_0 + X_0 e^{\mu_m t}} \right] \dots\dots\dots(17)$$

**2.1.6 Models for substrate consumption**

The substrate consumption rate takes into account two aspects, the substrate consumption in the formation of biomass and the maintenance of biomass, the rate of substrate consumption is written as:

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

Where  $Y_{s/x}$  is the yield coefficient of biomass on substrate and m is the maintenance coefficient. Combined with equation (4) and equation (5), this equation can be integrated and the substrate consumption equation can be obtained as:

$$S = S_0 - \frac{1}{Y_{s/x}} \left[ \frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} - X_0 \right] - \frac{X_m}{\mu_m} \ln \frac{X_m - X_0 + X_0 e^{\mu_m t}}{X_m} \dots\dots\dots(18)$$

**2.1.7 Modified Leudeking – Piret model**

This substrate utilization kinetic given below was a modified form of the Leudeking – Piret model. Substrate consumption depends on the magnitude of three sink terms, the instantaneous cell mass growth rate, the instantaneous product formation rate and a cell mass maintenance function. The assumed kinetic form was a linear combination of these terms (Weiss and Ollis)[11]

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \frac{dX}{dt} - \frac{1}{Y_{P/S}} \frac{dP}{dt} - K_c X$$

Substituting equation (10) into equation (15), the substrate material balance can be rewritten as:

$$-\frac{dS}{dt} = \left[ \frac{1}{Y_{X/S}} + \frac{\alpha}{Y_{P/S}} \right] \frac{dX}{dt} + \left[ \frac{\beta}{Y_{P/S}} + K_c \right] X$$

$$\frac{dS}{dt} = -Y \frac{dX}{dt} - \eta X$$

Where  $Y = \frac{1}{Y_{X/S}} + \frac{\alpha}{Y_{P/S}}$ ,  $\eta = \frac{\beta}{Y_{P/S}} + K_c$

**3.0 Experimentation**

The list of reagents used in this work are NYPA SAP, freshly cultured aspergillus niger, dried baker's yeast (Saccharomyces cerevisiae), distilled water, D- glucose, 2.5N sodium hydroxide (NaOH), standard glucose solution, Sodium metabisulphite, dinitrosalicylic acid reagent solution, and potassium sodium tartrate solution

**3.1 Preparation of Sample**

The sap was mixed thoroughly and 2 litres of it was measured and poured into a pot. It was sterilized by boiling at 40°C for 30 minutes. During the heating, the sap was stirred continuously to prevent excessive foaming. The sterilized sap was allowed to cool to room temperature.

**3.1.1 Fermentation of the Broth**

The temperature was adjusted to 300 °C to provide the optimum growth conditions for growth of the saccharomyces cerevisiae. An inoculum was prepared by adding 10 g of dried baker's yeast, saccharomyces cerevisiae, to 100 ml of distilled water in a beaker. A small quantity of glucose was added to the inoculum and the yeast was allowed to grow. The growth of the yeast was indicated by the formation of bubbles and the water level in the beaker was noted. The inoculum was added into 2 litres of Nypa sap contained in the fermenter and allowed to ferment for 96 hours. A sample was collected at the beginning of the fermentation (time = 0 hr) and subsequently at time t = 12, 24, 36, 48,

60, 72, 84 and 96 hrs. A total of 9 samples were collected and in each of them, the concentrations of yeast, glucose and ethanol were determined.

### 3.1.2 Determination of Yeast, Glucose and Ethanol Concentration

The spectrophotometer was zeroed and the wavelength was set to 630 nm. In determining cell concentration, 4 ml of the sample was transferred to the cuvette. The cell concentration was then determined from a calibration curve of cell concentration against absorbance. The glucose content of the sample was determined using the DNS method (Miller) [12]. Three ml of DNS reagent was added to 3 ml of sample in a test tube. The mixture was heated at 90 °C for 5 minutes to develop the red-brown colour. The glucose concentration was then determined from a calibration curve of cell concentration against absorbance. Fifty ml of the sample (Nypa sap) was poured into a round-bottom flask used as a boiling chamber. Cold water for the condenser was turned on to flow through the condenser.

10ml of distillate was distilled out of 15ml of sample. Afterwards, a few drops of the distillate were placed in the refractometer and the lid was closed. With a bright light source shining on the refractometer, the refractive index was located and the corresponding percentage by volume (concentration) of ethanol was read

## 4.0 Results and Discussion

### 4.1 Experimental Data Obtained from Fermentation

The data obtained were plotted in Figures 1,2 and 3 below for the concentration of biomass, ethanol and glucose respectively.

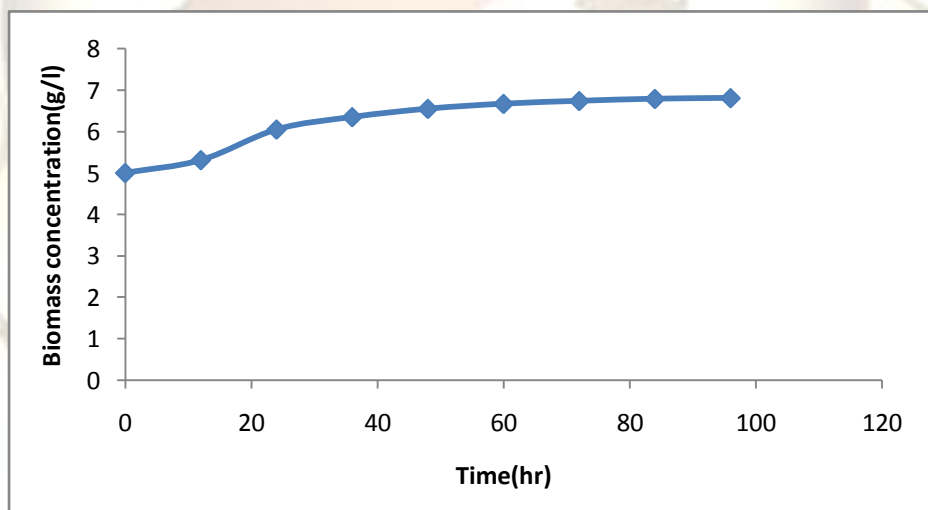


Figure 1:Plot of glucose concentration versus time

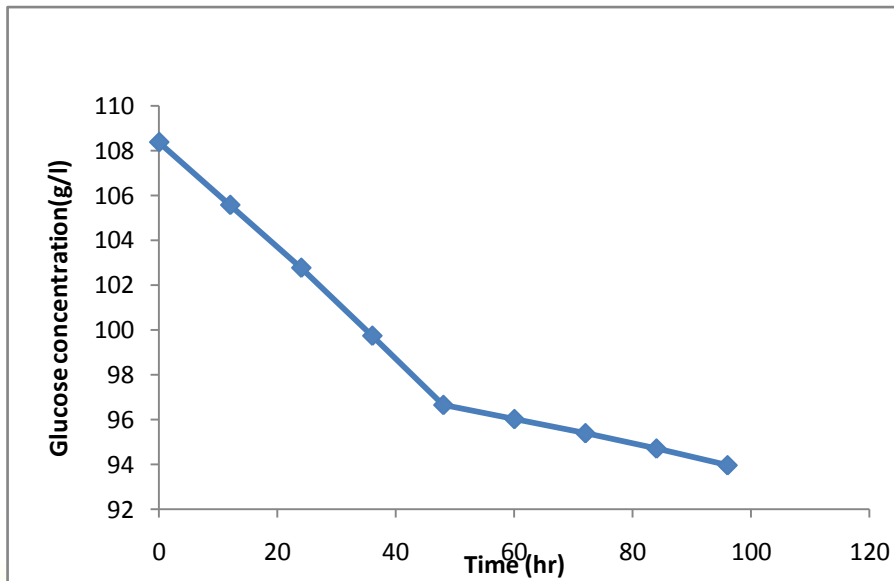


Figure 2: Plot of biomass concentration versus time.

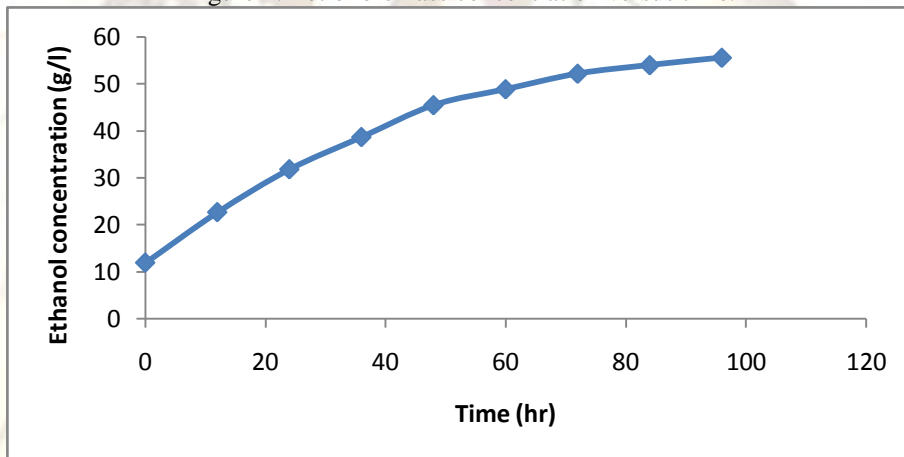


Figure 3: Plot of ethanol concentration versus time

#### 4.2 Model fitting of Experimental data

Model simulation and parameter evaluation

The parameters were estimated with 95% confidence bounds. The initial values of  $X_0$ ,  $S_0$  and  $P_0$  were fixed by experimental conditions. The experimental versus simulated values obtained are represented in Figures 4 to 9, while the model parameter are shown in Tables 1 to 6 respectively.

**Table 1: Logistic Model – For Cell Growth**

$$f(x) = 5 * m * \exp(u * x) / (m - 5 + 5 * \exp(u * x))$$

Parameters	Values
M	6.932
U	0.03722
R –square	0.9797
Adjusted R – square	0.9768
RMSE	0.1028

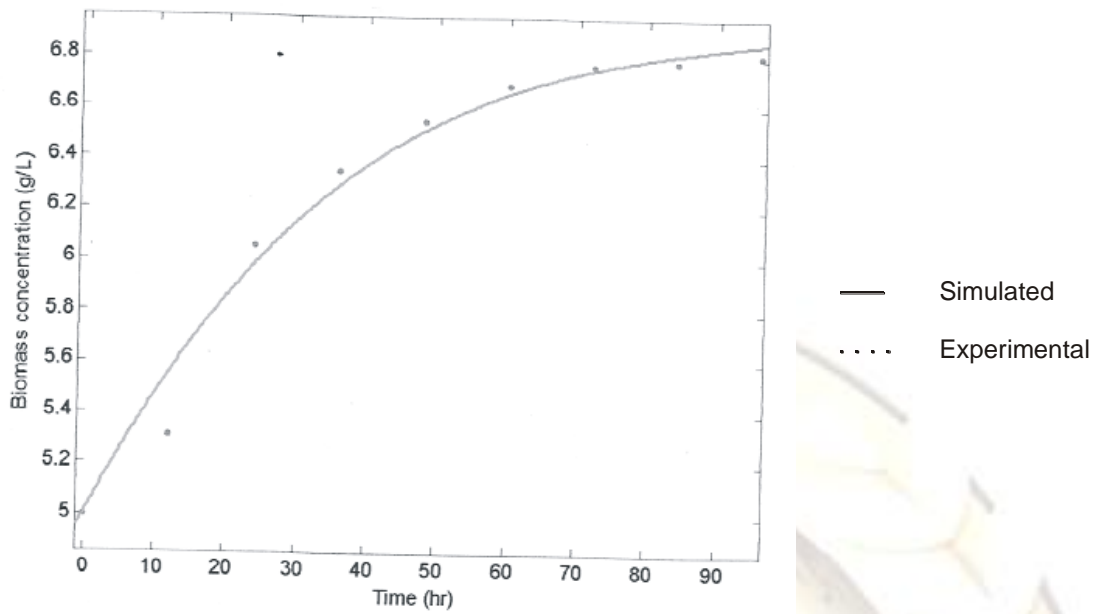


Figure 4: Plot of experimental and simulated values versus time using logistic model.

**Table 2: Modified Logistic Model – For Cell Growth**

$$f(x) = (5^r) * \exp(b * r * x) / ((1 - (5^r) / m^r) * (1 - \exp(b * r * x)))$$

Parameters	Values
B	0.04087
M	7.069
R	0.0881
R –square	0.977
Adjusted R – square	0.977
RMSE	0.1023

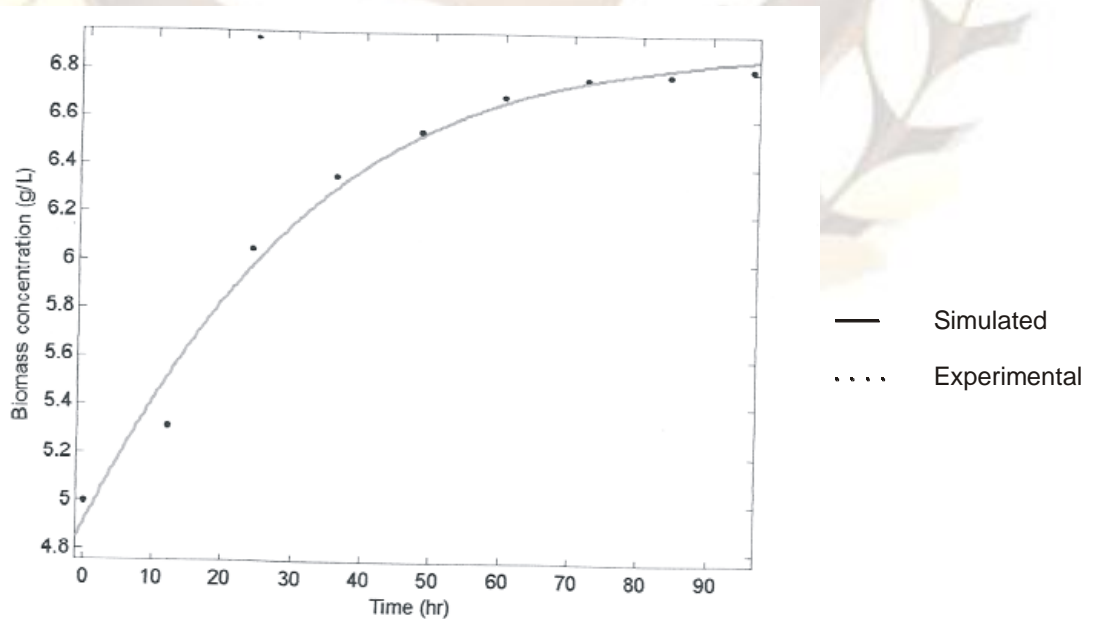


Figure 5 Plot of experimental and simulated values versus time using modified logistic model.

**Table 3: Logistic with death Phase Model – For Cell Growth**  
 $f(x) = 0 * \exp(u * x) / (1 - ((0/6.812)^2) * (u/(k+u)) * (1 - \exp((k+u) * x)))$

Parameters	Values
K	0.004017
O	4.967
U	0.01903
R –square	0.9839
Adjusted R – square	0.9785
RMSE	0.09898

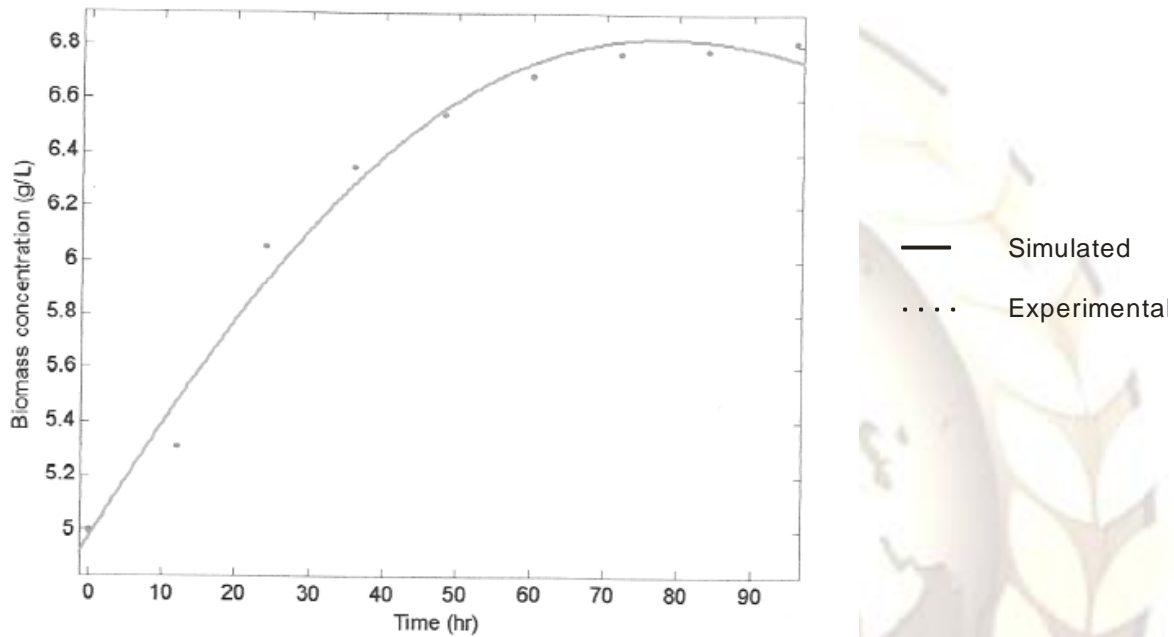


Figure 6 Plot of experimental and simulated values verses time using logistic with death phase.

**Table 4: Growth Associated Product formation Model – For Ethanol Formation**  
 $f(x) = k + a * (5 * 6.932 * \exp(0.03722 * x) / (6.932 - 5 + 5 * \exp(0.03722 * x)))$

Parameters	Values
A	23.64
K	-108.1
R –square	0.9923
Adjusted R – square	0.9912
RMSE	1.431

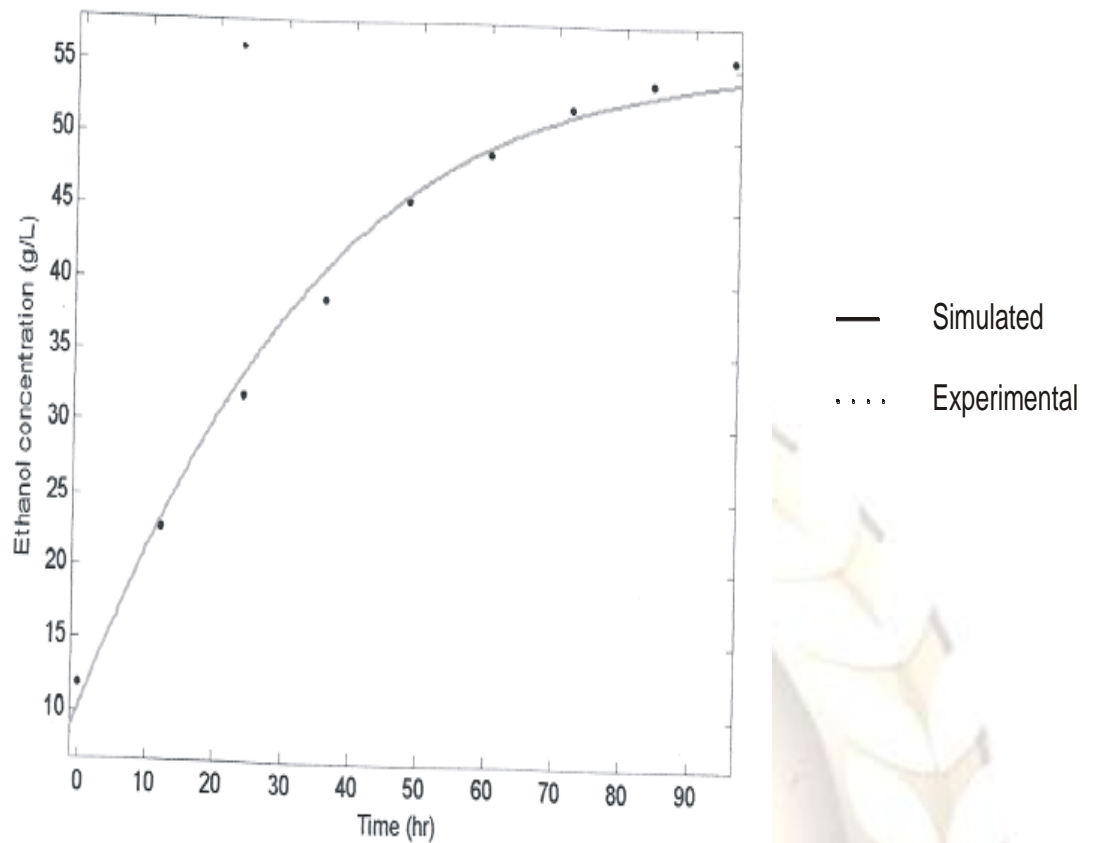


Figure 7 Plot of experimental and simulated values verses time using growth associated product formation model.

**Table 5: Product with Lag Time Model – For Ethanol Formation**

$$f(x) = Y * ((5 * 6.932 * \exp(0.03722 * (x - t)) / (6.932 - 5 + 5 * \exp(0.03722 * (x - t))) - (5 * 6.932 * \exp(-0.03722 * t) / (6.932 - 5 + 5 * \exp(-0.03722 * t))))$$

Parameters	Values
Y	88.65
T	-35.99
R –square	0.9124
Adjusted R– square	0.8999
RMSE	4.813



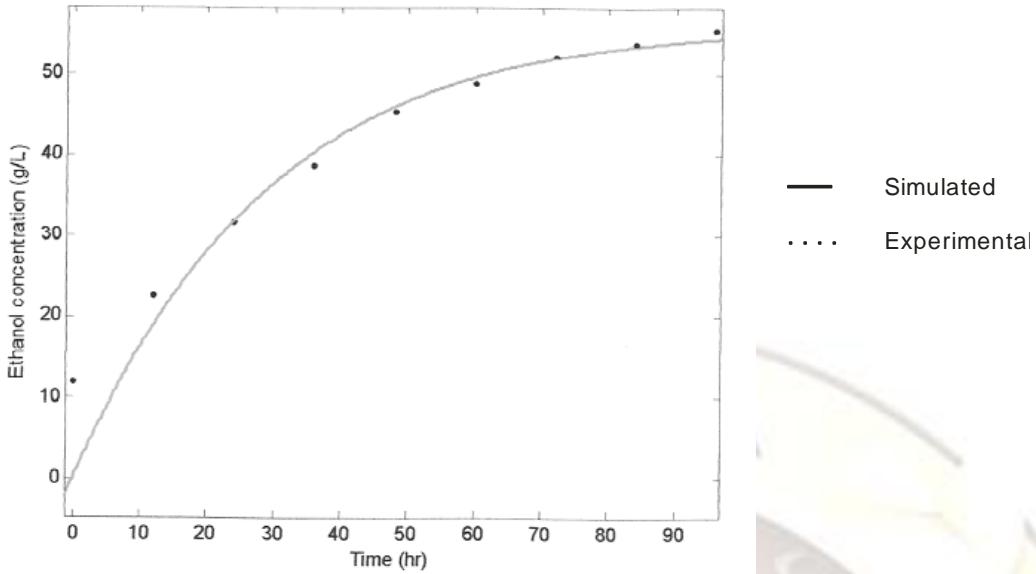


Figure 8 Plot of experimental and simulated values of product versus time with lag time.

**Table 6: Leudeking – Piret Model for Substrate Model**

$$f(x) = 108.39 - (5/Y)*((\exp(0.03722*x)/(1-(5/6.932)*(1-\exp(0.03722*x))))-1)-(m*6.932/0.03722)*\text{reallog}(1-(5/6.932)*(1-\exp(0.03722*x)))$$

Parameters	Values
Y	0.1739
M	0.006613
R-square	0.6849
Adjusted R-square	0.9827
RMSE	0.648

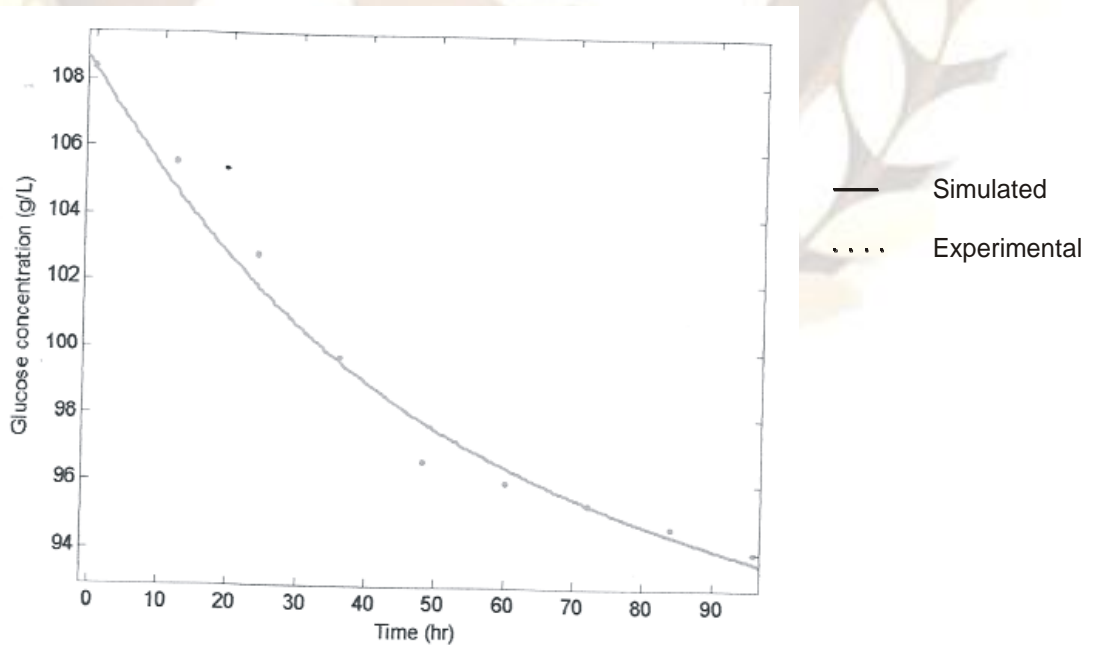


Figure 9 Plot of experimental and simulated values for substrate versus time using leudeking - Piret model.

The yeast growth, ethanol production and glucose consumption experimental data were each fitted with several different model equations and the equation that gave the best fit was adopted as the kinetic model for this work. The concentration profile for glucose consumption, yeast cell growth and ethanol production are shown Figures 1, 2 and 3 respectively. It was clearly shown that as glucose The initial cell concentration in the experimental data was 5.0 g/L and the maximum cell concentration was 6.81 g/L. By fitting the experimental data for cell growth into the logistic equation and the modified logistic equation the correlation coefficients (R-square) obtained were 0.9797 and 0.977 respectively (Table 1 and 2). The simulated and experimental plot is shown in Figures 4 and 5 and there was a close relationship. The logistic equation was preferred because of its simplicity and realistic accuracy. Since the logistic equation gave the best fitting, it was adopted as the developed model for yeast growth. Based on the developed model (logistic model), the calculated values of the parameters for yeast growth are maximum specific growth rate  $\mu = 0.03722 \text{ hr}^{-1}$ , maximum cell concentration,  $X_m = 6.932 \text{ g/L}$ . The correlation coefficients, R – square and adjusted R – square, of the model are 0.9797 and 0.9768 respectively and indicates that the model is able to predict the growth of the yeast in this case as shown in Table 1.

The experimental data for ethanol production was fitted with growth associated product model and product with lag phase model. The correlation coefficients (R-square) were, 0.9923 and 0.9124 for the fits respectively (Table 4 and 5). Growth associated product model gave the best fit for the production of ethanol (Figure 7). Based on the adopted model, the values of the estimated parameter was  $a = 23.64 \text{ g/g hr}$ . The correlation coefficients R-square and adopted R-square are 0.9923 and 0.9912 respectively (Table 4). This means that the adopted model fitted the production of ethanol very well. One model (Luedeking Piret) was used for substrate consumption. The experimental data for glucose consumption was fitted into it (Figure 9), the values of the estimated parameters are  $Y_{x/s} = 0.1739 \text{ g/g}$  and  $m = 0.006613 \text{ g/g. hr}$  as shown in Table 6. The correlation coefficients, R-square and adjusted R-square, are 0.6849 and 0.9827 respectively which indicates that this model fits the experimental data very well. There was a close agreement between the experiment concentration and simulated concentration for both biomass, glucose and product based on Logistic, Leudeking-Piret and growth associated model respectively.

The production of ethanol from Nypa palm is feasible and the kinetic parameters necessary for its production through fermentation have been obtained in the developed model.

decreases in concentration, the amount of biomass and ethanol increases considerably. At any point, the amount of ethanol produce was more than biomass formed. Based on experimental data, some kinetic parameters were estimated by mathematical software

#### MATLAB 7.0.

#### NOMENCLATURE

k	death phase constant ( $\text{hr}^{-1}$ )
Kc	cell mass maintenance constant (g glucose/g biomass hr)
m	maintenance coefficient (g glucose/g biomass hr)
P	ethanol concentration (g/l)
Po	initial ethanol concentration (g/l)
S	glucose concentration (g/l)
So	initial glucose concentration (g/l)
r	inhibition index
t	time (hr)
X	biomass concentration (g/l)
Xo	initial biomass concentration (g/l)
Xm	maximum biomass concentration (g/l)
Yp/s	yield coefficient of ethanol on glucose (g ethanol/g glucose)
Yp/x	yield coefficient ethanol on biomass (g ethanol/g biomass)
Yx/s	yield coefficient of biomass on glucose (g biomass/g glucose)
$\alpha$	growth associated product formation coefficient (g product/g biomass hr)
$\mu$	Specific growth rate ( $\text{hr}^{-1}$ )
$\mu_m$	Maximum Specific growth rate ( $\text{h}^{-1}$ )
$\Delta t$	lag time (hr)

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