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STUDY ON FERMENTATION CONDITIONS OF PALM JUICE VINEGAR BY RESPONSE SURFACE METHODOLOGY AND DEVELOPMENT OF A KINETIC MODEL

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Abstract - Natural vinegar is one of the fermented products which has some potentiality with respect to a nutraceutical standpoint. The present study is an optimization of the fermentation conditions for palm juice vinegar production from palm juice (*Borassus flabellifer)* wine, this biochemical process being aided by *Acetobacter aceti* (NCIM 2251). The physical parameters of the fermentation conditions such as temperature, pH, and time were investigated by Response Surface Methodology (RSM) with 2^3 factorial central composite designs (CCD). The optimum pH, temperature and time were 5.5, 30 °C and 72 hrs for the highest yield of acetic acid (68.12 g / L). The quadratic model equation had a R^2 value of 0.992. RSM played an important role in elucidating the basic mechanisms in a complex situation, thus providing better process control by maximizing acetic acid production with the respective physical parameters. At the optimized conditions of temperature, pH and time and with the help of mathematical kinetic equations, the Monod specific growth rate (μ_{max}= 0.021 h^{-1}), maximum Logistic specific growth rate (μ'_{max} = 0.027 h^{-1}) and various other kinetic parameters were calculated, which helped in validation of the experimental data. Therefore, the established kinetic models may be applied for the production of natural vinegar by fermentation of low cost palm juice.
Keywords: Acetic acid bacteria; Fermentation; Kinetic model; Natural vinegar; Palm juice; RSM.

INTRODUCTION

Natural vinegar is a fermentative product of ethanol, its key ingredient being acetic acid. Natural vinegar also contains small amounts of tartaric acid, citric acid, and other organic acids. Acetic acid fermentation is an aerobic biological oxidation process that is thermodynamically favorable (de Ory *et al.,* 1998). The ethanol, as substrate, is partially oxidized by the acetic acid bacteria to produce acetic acid and water. The stoichiometry for the conversion of substrate into product is 1:1 (de Ory *et al.,* 2002).

Natural vinegar has been made from different sources of derived ethanol such as wine, cider, beer, fermented fruit juice, vinegar can also be made synthetically from natural gas and petroleum derivatives. Traditional balsamic vinegar is a natural product prepared from grape must. It contains polyphenol compounds, which show antioxidant activity (Tagliazucchi *et al.,* 2008). In Japan, two rice vinegars, i.e., Komesu and Kurosu are produced by a traditional static fermentation process. Komesu is produced from polished amber rice and Kurosu from unpolished black rice. These vinegars are

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known for their health benefits via the prevention of inflammation and hypertension (Murooka *et al.,* 2008). In recent years, researchers have produced natural vinegar from sources such as cashew, Indian Jujube (*Zizyphus mauritiana*) and pineapple (Silva *et al.,* 2007; Krusong *et al.,* 2010; Sossou *et al.,* 2009; Vithlani *et al.,* 2010).

In acetic acid fermentation, the important physical parameters that affect the growth of *A. aceti* are temperature and pH. It is believed that at the lower pH of wine the growth of *A. aceti* is inhibited. It has been found that cell numbers of *A. aceti* decreased faster at pH 3.4 than at pH 3.8 under strict anaerobic conditions (Joyeux *et al.,* 1984). The optimum pH for the growth of *A. aceti* is 5.5 -6.3. The temperature of 25 – 30 °C is optimum for *A. Aceti's* growth (Holt *et al.,* 1994). Thermo-tolerant *A. aceti* is also able to grow at 37 – 40 °C (Saski *et al.,* 1997). At lower temperatures *A. aceti* can remain active and there is a 30-40 fold increase in cell numbers in wine stored at 18 °C for one week (Joyeux *et al.,* 1984). It has been observed that *A. aceti* does not grow below 8 °C (de Ory *et al.,* 1998).

Palm wine, also called palm toddy or simply toddy, is an alcoholic beverage created from the sap of various species of palm trees. Palm toddy is a refreshing beverage enjoyed by people in parts of Africa, Asia and South America (Jirovetz *et al.,* 2001). Palm juice contains carbohydrate (110 – 130 g/L), protein (150 – 190 mg/L), fat (0.4 – 0.8 g/L), various minerals (Na, K, Ca, Fe), polyphenols and ascorbic acid (30 – 40 mg/L) (Barh *et al.,* 2008). It also has antioxidant properties and therefore can be considered to be a healthy food drink. A bibliographical search on the current production levels of palm toddy and juice in parts of the world revealed the following; Kenya's production level was estimated at 5 x 10^6 L per year (Kadere *et al.,* 2009), the Seychelles Islands (Indian Ocean) has an estimated production level of 10 x 10^6 L per year (Perdrix, *et al.,* 1999) and both Sri Lanka and India has reported production levels of 9 x 10^6 L per year (Lasekan, *et al.,* 2010).

The objective of this study was to optimize the conditions of fermentation for the production of palm juice vinegar. Response Surface Methodology (RSM) was used in this study to treat the aggregate effects of several parameters such as temperature, time and pH to set optimum conditions for a multi-variable system. The central composite design (Ambati *et al.,* 2001; Murthy *et al.,* 2000) was used for the optimization of the different factors that play an important role in palm juice vinegar production. Experimental data on substrate utilization, biomass formation and product formation were obtained from the optimized conditions. These values were then validated using a kinetic model.

MATERIALS AND METHOD

Chemicals

Dextrose, calcium carbonate (GR), KH_2PO_4, K_2HPO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, and urea were purchased from Merck, India. Yeast extract, malt extract, tryptone, agar and peptone were obtained from Himedia, India. 3,5-Dinitrosalicylic acid was from Loba Chemie, India.

Yeast Culture Preparation

Stock culture of *Saccharomyces cerivisiae* (NCIM 3045) was obtained from the National Chemical Laboratory (NCL), Pune, India. The culture medium consisted of 3 malt extract, 10 glucose, 3 yeast extract and 5 peptone (g/L). The organisms were grown at a temperature of 30 °C and pH 6.5. The incubation period was 48 hours. After incubation, the culture was stored at 4° C in a refrigerator.

Acetobacter Aceti Culture Preparation

Stock culture of *Acetobacter aceti* (NCIM 2251) was obtained from the National Chemical Laboratory (NCL), Pune, India. The composition of the culture medium: 10 tryptone, 10 yeast extract, 10 glucose, 10 calcium carbonate, 20 agars (g/L). The organisms were grown at a temperature of 30 °C and pH 6.0. The incubation period was 24 hours. After incubation, the culture was stored at 4 °C in the refrigerator.

Preparation of Fermentation Medium for Ethanol Production

The palm juice (*Borassus flabellifer*) was collected from rural areas of West Bengal, India. It was preserved at -50 °C in an ultra-low temperature Freezer (Model C340, New Brunswick Scientific, England). For the ethanol fermentation, carbon, nitrogen and other trace elements were added to the palm juice at appropriate levels. The composition of the fermentation medium (g/L) was: glucose 10, urea 3, KH_2PO_4 0.5, K_2HPO_4 0.5, $MgSO_4.7H_2O$ 0.5, $FeSO_4.7H_2O$ 0.01.

The fermentation process was performed in a 250 mL flask; 100 mL of fermentation media were inoculated with yeast culture, the concentration of cells corresponding to an OD of 1.3. The pH and temperature were adjusted to 5.5 and 32 °C for each experiment. The incubation time was 2 days and the flask was made air tight by paraffin paper for maintaining anaerobic conditions.

Preparation of Fermentation Medium for Vinegar Production

After ethanol fermentation, 150 g/L of sterile sugar was added to the medium and inoculated with *Acetobacter aceti* starter culture. The concentration of the *Acetobacter aceti* in the fermentation medium was 2.0 x 10^5 cells/mL. The temperature and pH were adjusted as per the experiments. The incubation time was 9 days and an aerobic condition was maintained by shaking the flask at 150 rpm. Samples were withdrawn at 24 hr time intervals with a sterile injection syringe for analysis.

Analytical Methods

Determination of Ethanol and Sugar Concentration

A 5 mL fermented sample was centrifuged (Remi C-24, Mumbai, India) at 6500 g for 10 minutes. The supernatant solution was used to determine the ethanol concentration by gas chromatography (Perichrom SGE D11, column BP1-dimethyl polysiloxane). The absorbance of the sugar solution was determined in a spectrophotometer (Model 2800, Hitachi, Japan) at 540 nm by the DNS method (Wilson *et al*., 2000).

Determination of Acid

Acetic acid concentration was quantified by a HPLC system (JASCO, MD 2015 Plus, Multiwave length Detector) equipped with absorbance detectors set to 210 nm. The column (ODS-3) was eluted with 0.01 (N) H_2SO_4 as the mobile phase at a flow rate of 0.5 mL/min and a sample injection volume of 20 µL. Standard acetic acid (Merck, India) was used as an external standard.

Viable and Total Cell Counts

The total and viable cell counts were determined with a Microscope (Kruss, Optronic, Germany) at 100X magnification; 5 µL of diluted sample was placed in a nebular chamber and a Gram staining was done. *A. aceti* was observed as rod shaped Gram negative bacteria. Five of the 25 squares in the nebular chamber were counted and the result was multiplied by 5 to give the total cell count.

Estimation of Biomass Concentration

The biomass concentration of *A. aceti* was determined by the dry weight method. The cells were separated by centrifuging at 1200 g for 20 minutes. After collection, the pelletes were consecutively washed twice with water (Membra pure, Aquinity, Germany). The total cell count was done by the Gram staining procedure. The pure cells were dried at 65 °C for 2 days. The calibration curve was prepared by slight modification of the method of Raychaudhury *et al.* (2003), The calibration curve correlating the number of cells and dry weight gave a straight line.

Experimental Design of the RSM

Response surface methodology (RSM), an empirical modeling technique, was used to estimate the relationship between a set of controllable experimental factors and the observed results. In the RSM, the Central Composite Design (CCD) is optimized for fitting quadratic models and the number of experimental points in the CCD is sufficient to test the statistical validity of the fitted model and the lack of fit of the model (Li *et al*., 2002; Gacula *et al*., 1984). In this study, RSM was used to find the optimum conditions for the factors affecting the fermentation process. The input variables such as temperature (X_1), pH (X_2), and time (X_3) are shown in Table 1. Twenty experiments were performed according to Table 3. The experimental values were fitted according to Equation (1) as a second order polynomial equation including the linear and cross effect of the variables.

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i<j}^{n} \beta_{ij} X_i X_j + \sum_{j=1}^{n} \beta_{jj} X_j^2 \qquad (1)$$

where Y represents the predicted response, i and j are linear and quadratic coefficients respectively, β is a regression coefficient, and n is the number of variables studied in the experiments.

Table 1: Range of alpha values for variables in the experimental design

Variables	Coded levels		
	-1	0	+1
Temperature	28	30	32
pH	45	5.5	6.5
Time	1	3	5

In our present study, the statistical software Design Expert (Version 7.1.6, Sat-Ease, Inc, USA) was used for regression analysis of the data and to estimate the significance of each coefficient of the regression equation. The fit of the regression model was determined by adjusted coefficient (R_{adj}). Appropriate model significance was determined by Fischer's F-test. The three dimensional graphical representation and the respective contour plots were determined by the interaction of the dependent and independent variables.

Kinetic Modeling for Fermentation

Various structured and unstructured kinetic models have been reported in the scientific literature for fermentative production of acetic acid by bacteria. Unstructured, non-segregated kinetic models play an important role in monitoring and predicting the batch fermentation process (Shuler *et al.,* 1992). Unstructured models are much easier to use and have been proven for the description of a wide range of experimental conditions and media. Therefore, different models have been taken into consideration for our present study and the experimental data were analyzed with the help of the reported model.

Kinetic Study of Microbial Growth

Under optimal growth conditions and when the inhibitory effect of substrate and product were neglected, the rate of cell growth follows an exponential relation (Liu, *et al.,* 2003). The simplest relationship described is the unstructured Malthus model (Najafpour, 2007; Najafpour, *et al.,* 2005; Zinatizadeh, *et al.,* 2006)

$$\frac{dX}{dt} = \mu X \tag{2}$$

Equation (2) thus implies that X increases with respect to time regardless of the substrate available and the growth is governed by a hyperbolic relationship.

By separation of the variables and integrating, Equation (2) yields:

$$\ln\frac{X}{X_0} = \mu t \tag{3}$$

An unstructured model, which is frequently used in the kinetic description of microbial growth, is the Monod equation (Takamatsu *et al.,* 1981). The relationship between μ and the residual growth-limiting substrate is given below:

$$\mu = \mu_{max}\left(\frac{S}{K_s + S}\right) \tag{4}$$

K_s is the substrate utilization constant, numerically equal to the substrate concentration where $\mu=\mu_{max}/2$. This model (Equation (4)) expresses that the specific growth rate of microorganisms decreases if the substrate concentration is decreased and vice versa.

The growth equation becomes (Suscovic *et al.,* 1992):

$$\frac{dX}{dt} = \mu_{max}\left(\frac{S}{K_S + S}\right)X - K_d X \tag{5}$$

In order to establish the relationship between microbial growth and substrate consumption, the yield of biomass ($Y_{X/S}$) based on utilized substrate is defined as follows:

$$Y_{X/S} = -\frac{X - X_0}{S - S_o} \tag{6}$$

Maximum cell dry weight is equal to sum of the inoculum size and the coefficient yield multiplied by the substrate concentration with the assumption that a portion of substrate is converted to biomass.

The Riccati equation (Najafpour 2007) uses the boundary condition $X(t=0) = X_0$ and gives a sigmoidal variation of X as a function of time.

$$\frac{dX}{dt} = K_S.X_m\left(1 - \frac{X}{X_m}\right) \tag{7}$$

Equation (7) contributes to the postulated model for the population growth rate, which is induced by an inhibition factor. Assuming that the inhibition is second order with respect to cell dry weight (X^2), the equation then becomes:

$$\frac{dX}{dt} = \mu_m\left[1 - \frac{X}{X_m}\right]X \tag{8}$$

Equation (7) can easily be integrated to give a logistic equation that represents an exponential and a stationary phase.

$$X = \frac{X_0 X_m e^{\mu_m t}}{X_m - X_0 + X_0 e^{\mu_m t}} = \frac{X_0 e^{\mu_m t}}{1 - \frac{X_0}{X_m}\left(1 - e^{\mu_m t}\right)} \quad (9)$$

Being a closed system, the culture can only maintain cell viability for a limited time and the growth cycle changes progressively from one phase to another in the remaining medium and environment conditions. The advantage of using this model is that the sigmodial curve of X as a function of t can represent growth in both the exponential and stationary phases.

Kinetic Study of Substrate Utilization

The substrate utilization kinetics for acetic acid fermentation can be expressed by the equation proposed by Monteagudo *et al.,* (1997), which consider both substrate consumption for maintenance and substrate conversion to biomass and product. The rate of substrate utilization is related stoichiometrically to the rates of biomass and acetic acid production. The substrate requirement to provide energy for maintenance is usually assumed to be first order with respect to biomass concentration.

The equation is expressed as follows:

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} + \frac{1}{Y_{P/S}}\frac{dP}{dt} + m_S X \quad (10)$$

These parameters are estimated by non-linear regression analysis. The model neglects the effect of substrate concentration on growth rate.

Kinetic Study of Product Formation

The Luedeking – Piret equation describes the mixed growth associated product formation model in the fermentation process (Luedeking *et al.,* 1959). The product formation rate is written as a linear function of the growth rate and cell concentration.

$$r_p = \frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X = (\alpha\mu_g + \beta)X \quad (11)$$

where α and β are two estimated parameters for kinetic expression. This equation has proven to be extremely useful and versatile for fitting product formation data for many fermentation processes.

RESULTS AND DISCUSSION

We studied natural vinegar production from palm juice fermentation and proposed a statistical method of RSM for process optimization using the changes of the physical parameters such as temperature, pH and time. These physical parameters have been optimized on the basis of the highest yield of natural vinegar from the palm juice. We have estimated the biomass production, sugar utilization and highest yield of vinegar concentration under these optimized conditions. These experimental data were validated with the help of mathematical equations of the kinetic model and compared with reported results.

Optimization Procedure Using RSM

Using RSM, the experimental responses along with the predicted response obtained from the regression equation, are shown in Table 2. Twenty runs were performed and a polynomial (Equation (1)) was used to approximate the response of the data. The maximum amount of acetic acid was obtained in runs 1, 10, 12 and 13 and the concentration is 68.12 g/L at pH 5.5 for 3 days for fermentation (Table 2).

Table 2: Interactions of the independent variables as shown by the central composite design matrix.

Temperature	pH	Day	Aetic acid g/L	
			Predicted	Observed
30.00	5.50	3.00	67.70	68.12 ± 3.1
30.00	5.50	3.00	67.70	67.12 ± 3.3
32.00	4.50	5.00	56.50	56.09 ± 2.9
28.00	4.50	1.00	12.19	12.03 ± 3.4
28.00	5.50	3.00	60.20	59.95 ± 4.2
30.00	5.50	5.00	65.74	65.98 ± 2.5
30.00	4.50	3.00	64.28	65.45 ± 3.1
30.00	5.50	3.00	67.70	66.12 ± 3.5
30.00	6.50	3.00	67.53	66.59 ± 3.1
30.00	5.50	3.00	67.70	68.12 ± 2.1
30.00	5.50	1.00	24.22	24.21 ± 3.1
30.00	5.50	3.00	67.40	68.12 ± 3.4
30.00	5.50	3.00	67.40	68.12 ± 2.5
32.00	6.50	1.00	18.99	19.17 ± 2.7
32.00	4.50	1.00	20.72	20.36 ± 3.4
28.00	6.50	5.00	62.04	62.35 ± 3.6
28.00	4.50	5.00	53.80	53.56 ± 3.2
32.00	6.50	5.00	60.42	60.52 ± 3.5
28.00	6.50	1.00	14.77	15.12 ± 1.9
32.00	5.50	3.00	63.66	64.14 ± 4.2

*Experimental results were the average of three replicates
± Standard Deviation

Results of ANOVA are shown in Table 3; the F value was 1353.24 which implies that the model was

significant. The quality of fit of the model was checked by the lack-of-fit F value; it was 0.94, which was not significant. The insignificant lack-of-fit value indicated that the model was suitable. The R^2 was found to be 0.9992. This value indicated that 99.92% of the variability in the response could be explained by the model.

The value of the prob> F (0.05) indicate that the model terms were significant (Table 4). The temperature, pH, time, temperature X pH, temperature X time and pH X time and temperature^2, pH^2, and time^2 were the linear, interactive and quadratic terms of the model, respectively. In this case, F values of these three variables were significant model terms because the prob>F values were less than 0.1. All the variables were significant model terms so the whole model was significant. The small standard error (Std Err) indicates a good significance of the model. The variance inflation factor (VIF) is measured as the variance of the model inflated by the lack of orthogonality in the design matrix. The VIF is 1.0 when the design is orthogonal, a VIF above 10 indicates that the factors are also correlated together and are not independent. Most of the VIF values of our model were 1.0, indicating that our model was also an orthogonal design matrix (Table 4).

Figure 1(a)-(c) shows the surface response plot for optimization of the conditions for acetic acid fermentation. The 2D contour and surface plots were based on the regression equation, holding three variables constant at the level of zero while varying the other two within their experimental range. The effect of temperature and pH on acetic acid production is show in Fig. 1(a). The graph shows that the optimum point for highest production was 68.12 g/L, the optimum pH and temperature being 5.5 and 30 °C. It has been reported that the optimum pH for the growth of acetic acid bacteria is 5.5-6.3 (Holt *et al*., 1994). *A. aceti* can adapt to high acetic acid conditions by producing 35 proteins specifically induced during acetate adaptation (Steiner *et al.,* 2001). The literature reports that cell numbers of *A. aceti* decreased faster at pH 3.4 under strictly anaerobic conditions (Joyeux, *et al.,* 1984). From our experimental data, it can be seen that initially the acetic acid concentration was almost the same for all pHs at the optimum temperature for up to 12-24 hrs (data not shown). However, outside the optimum temperature, the pH significantly affected the initial acetic acid concentration for each flask, as shown in Table 2.

Figure 1(b) shows that the temperature of 30 °C and time of 3 days were optimum conditions for maximum acetic acid production. According to Holt *et al.* (1994), *Acetobacter* sp. grow at an optimum temperature of 25-30 °C. In the case of *A. aceti*, it was found that the maximum temperature for its growth was 35 °C (de Ory *et al.,* 1998). At temperatures of 25- 30 °C, *A. aceti* is typically able to oxidize ethanol to acetic acid and subsequently to carbon dioxide and water (Maal *et al.,* 2010). For our selected strain of *A. aceti* (NCIM 2251) the optimum temperature for highest acetic acid production was also found to be 30 °C.

Table 3: Analysis of variance (ANOVA) used for the palm juice (wine) vinegar fermentation.

Source	SS	df	MS	F-value	Prob(P)>F
Model	8261.89	9	946.50	1353.24	<0.0001
Residual (error)	6.78	10	0.68		
Lack of fit	3.28	5	0.66	0.94	0.5271
Pure error	3.50	5	0.70		
Total	8268.68	19			
[R^2 = 0.9992,	R_{adj}^2 = 0.9984,		Predicted R^2 = 0.9864]		

*SS – Sum of Squares, df – degree of Freedom, MS- Mean of Squares, P- probability

Table 4: Statistical significance of the regression coefficients for palm juice (wine) vinegar production.

Source	F value	Prob>F	Std Err	VIF
Temperature	43.97	0.0001	0.26	1.00
pH	38.97	0.0001	0.26	1.00
Time	6353.81	0.0001	0.26	1.00
Temperature X pH	13.76	0.0040	0.29	1.00
Temperature X Time	25.14	0.0005	0.29	1.00
pH X time	23.61	0.0007	0.29	1.00
Temperature^2	12.98	0.0001	0.50	1.82
pH^2	134.69	0.0048	0.50	1.82
Time^2	2091.52	0.0001	0.50	1.82

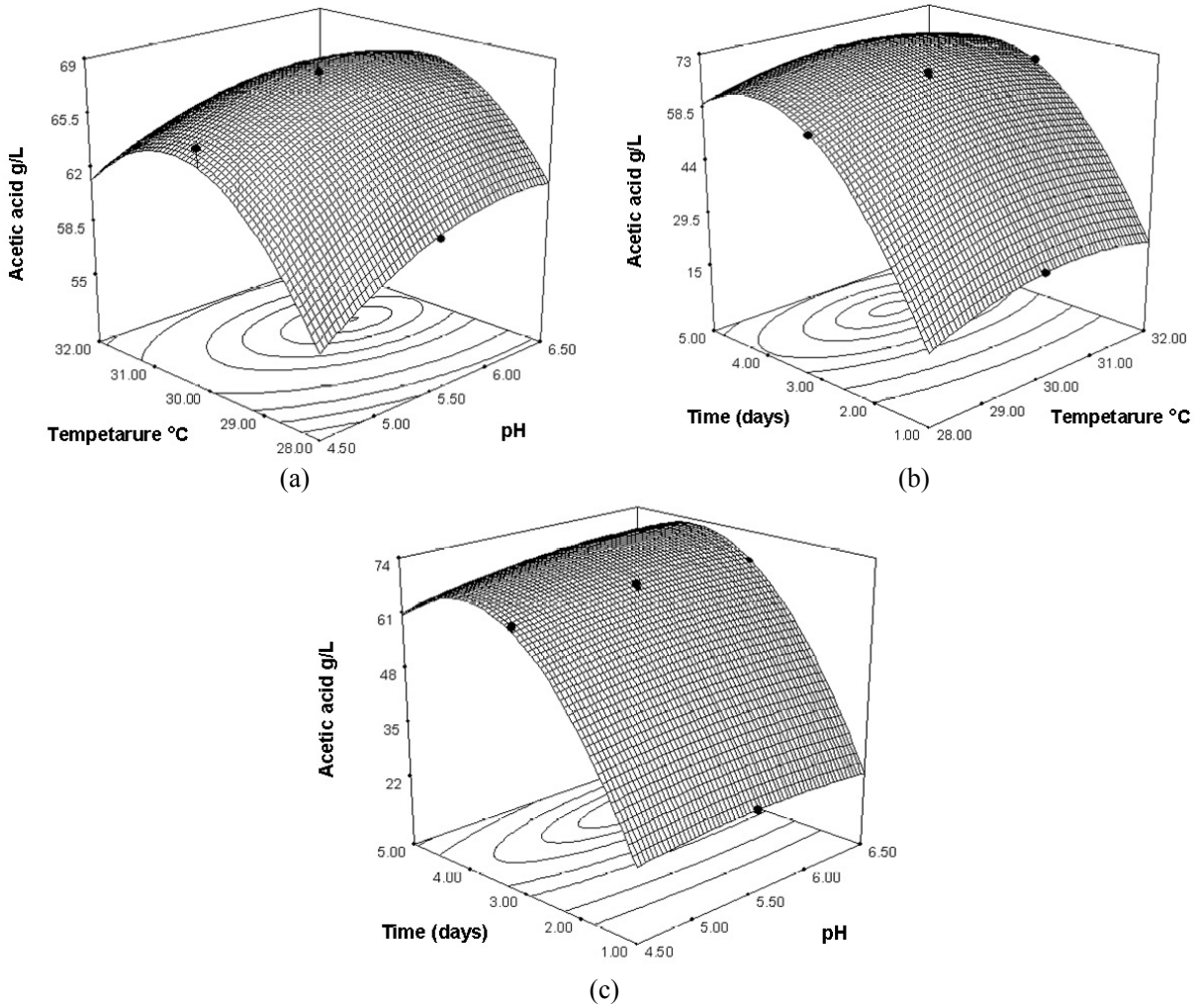

Figure 1: (a)-(c) 3D Response surface and contour plots showing the effect of pH, temperature and time on the production of palm juice (wine) vinegar by *Acetobacter aceti.*
(Experimental results were the average of three replicates).

Figure 1(c) shows that the maximum acetic acid production was obtained at pH 5.5 and a time of 3 days. The 3-5 day incubation time was considered to be sufficient for acid production using *A. aceti*, but it could vary with the fermentation conditions, strain specification and also the process. In our case the optimum incubation time was 3 days.

Studies of Kinetic Parameters

After optimizing conditions for palm juice vinegar fermentation through RSM, the maximum yield conditions were used for the kinetic study. Figure 2 shows that, at pH 5.5 and a temperature of 30 °C, the maximum acetic acid production is 68.12 g/L after 72 hrs with 150 g/L initial sugar concentration. The long incubation time for the fermentation led to the accumulation of product, which has an inhibitory effect on production, but cell growth was observed up to nine days. The highest biomass concentration formed was 35 g/L on the seventh day.

The value of the maximum Monod specific growth rate (μ_{max}) obtained from the parametric estimation was 0.021 h^{-1} by using Equation (4). The Monod kinetic model was plotted as a double reciprocal graph based on the experimental data obtained for substrate consumption and incubation time. The model was validated by the R^2 value of 0.934 (Figure 3). The value of the saturation constant (K_s) is shown in Table 5.

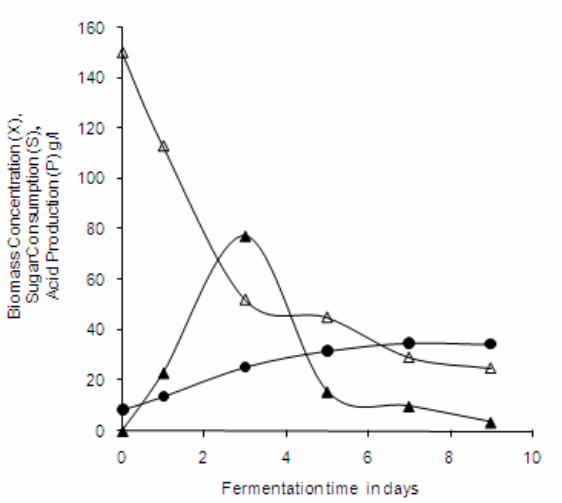

Figure 2: Production of acetic acid, biomass and sugar utilization in palm juice vinegar fermentation at pH 5.5 and temperature 30 °C. Sugar consumption profile (g/l) (△), acetic acid production profile (g/l) (▲), biomass production profile (g/l) (●).
*Experimental results were the average of three replicates.

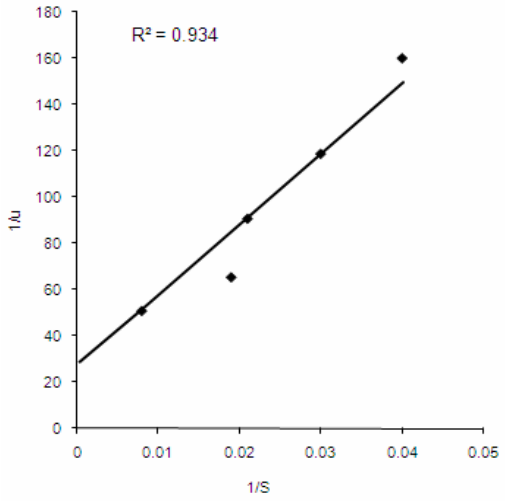

Figure 3: Experimental data fitted to the Monod kinetic model
*Experimental results were the average of three replicates.

Table 5: Kinetic parameters for palm juice (wine) vinegar production.

Estimated kinetic parameter		Parametric value
q_{Pmax}	maximum specific acetic acid production rate (g/g)h^{-1}	0.0359 ± 0.001
μ	Specific growth rate (h^{-1})	0.016 ± 0.001
μ_{max}	maximum Monod specific growth rate (h^{-1})	0.021 ± 0.001
μ'_{max}	maximum Logistic specific growth rate (h^{-1})	0.027± 0.001
α	growth-associated constant in the Luedeking–Piret model (g/g)	0.136 ± 0.01
β	non-growth associated product formation (h^{-1})	0.00016
$Y_{X/S}$	biomass yield based on sugar consumption (g/g)	0.21 ± 0.01
$Y_{P/x}$	acetic acid yield based on growth of biomass (g/g)	0.69 ± 0.01
m_s	maintenance coefficient	0.018 ± 0.001
%	of sugar utilized	83.36 ± 2.4
μ_{net}	maximum specific growth rate (h^{-1})	0.010 ± 0.001
K_s	Monod constant (g/L)	64.4 ± 2.1

± Standard Deviation

Figure 4 depicts the exponential growth with inhibition incorporated as projected by the Logistic model (Equation (9)) for cell growth determination. The simulated data and our experimental data fitted well. The maximum Logistic specific growth rate μ'_{max} was found to be 0.027 h^{-1} by using Equation (8). The Malthus kinetic model (Equation (2)) represents the variation of the logarithm of the cell concentration with respect to incubation time (Fig. 5). The experimental data fitted with this model were validated by an R^2 value of 0.958. The slope of the curve gives the specific growth rate (Table 5).

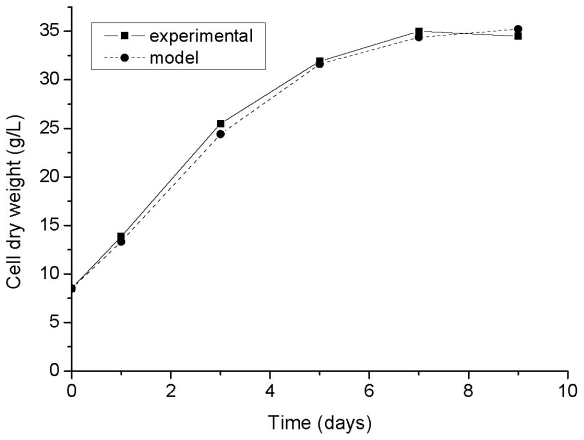

Figure 4: Experimental data fitted to the Logistic kinetic model, where the figure shows the logistic nature of the growth curve

*Experimental results were the average of three replicates.

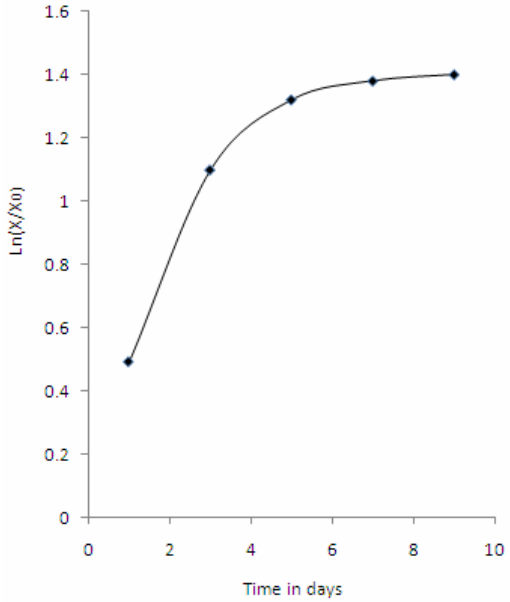

Figure 5: Experimental data fitted to the Malthus kinetic model

*Experimental results were the average of three replicates.

In order to take into account the decrease in the biomass concentration towards the end of some batch fermentations, a cell death coefficient K_d was calculated from Equation (5) using experimental values (Table not shown). The estimated value of K_d was 0.0062 h^{-1}; this suggests a relatively small effect of cell death rate. The "maintenance coefficient" (m_s) term was defined by Pirt (1965) as an extra substrate consumption not used for growth purposes. It has been also considered to be a constant for a species and has been used as such in numerous models describing microbial dynamics (Bodegom 2007). It was observed that the m_s value (Table 5) calculated from Equation (10) of our model was more or less similar to that reported by Hill *et al*. (1999) for the same bacteria.

In this fermentation process, substrate utilization in terms of sugar is 83.36 %. Growth-associated product formation means that products are produced simultaneously with microbial growth. According to the Luedeking–Piret Equation (11), α is the growth-associated constant and β the non-growth associated constant. When α is zero the product is non-growth associated and when β is zero the product is only growth associated. Our experimental values of the growth-associated product formation term (α) and non-growth associated term (β) are 0.136 g/g and 0.00016 h^{-1}, respectively, based on Equation (11). Therefore, the kinetic pattern of growth and product formation in our acetic acid batch fermentation conformed to the growth-associated product formation model because the β value was very small and close to zero. The literature also supports the fact that acetic acid fermentation by *Acetobacter* follows the growth associated product formation model (Tsuchiya, 1983).

The maximum specific acetic acid production rate (q_{Pmax}) was 0.0359 g/(g.h) and, from the above calculated data, it can be predicted that the production of acetic acid is very high during the growth associated phase. Biomass yield ($Y_{X/S}$) and acetic acid yield ($Y_{P/S}$) based on consumed sugar are found to be 0.21 g/g and 0.69 g/g, respectively. Other kinetic parameter values are summarized in Table 5.

CONCLUSION

A kinetic model for the production of natural vinegar from palm juice (wine) using *Acetobacter aceti* has been established. Before the experimental data were fitted to the kinetic model, the fermentation process was optimized by a 2^3 factorial RSM. The

optimum pH, temperature, and time were 5.5, 30 °C and 72 hrs for the highest yield of acetic acid (68.12 g/L). The different values of the various kinetic parameters such as μ_{max}, α, β, μ'_{max} were explained by the validation of our experimental data. Therefore, this model can be applied for the production of natural vinegar using palm juice (wine).

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NOMENCLATURE

$\frac{dP}{dt}$	Volumetric product formation rate	g L^{-1} h^{-1}
K_d	death rate constant	g/L
K_s	substrate utilization constant	
M	the specific growth rate	hr^{-1}
m_s	Maintenance coefficient	
P	Product concentration	g/L
t	fermentation time	hr
X	microbial biomass concentration	g/L
X	biomass concentration at the time	t
X_o	biomass concentration at initial time	
X_m	maximum biomass concentration	g/L
$Y_{X/S}$	Biomass Yield	
$Y_{P/S}$	Product Yield based on the substrate utilized	

Greek Letters

α	growth-associated product formation constant	h^{-1}
β	non-growth associated product formation constant	h^{-1}
μg	apparent growth yield	h^{-1}
μm	max specific growth rate	h^{-1}

REFERECES

Ambati, P. and Ayyanna, C., Optimizing medium constituents and fermentation conditions for citric acid production from palmyra jaggery using response surface method. World Journal of Microbiology and Biotechnology, v. 17, 331-335 (2001).

Barh, D. and Mazumdar, B. C., Comparative nutritive values of palm saps before and after their partial fermentation and effective use of wild date (*Phoenix sylvestris* Roxb.) Sap in treatment of anemia. Research Journal of Medicine and Medical Sciences, v. 3, 173-176 (2008).

Bodegom, P. V., Microbial maintenance: A critical review on its quantification. Microbial Ecology, v. 53, 513-523 (2007).

de Ory, I., Romero, L. E. and Cantero, D., Modeling the kinetics of growth of *Acetobacter aceti* in discontinuous culture: influence of the temperature of operation. Applied Microbiology Biotechnology, v. 49, 189-193 (1998).

de Ory, I., Romero, L. E. and Cantero, D., Optimum starting–up protocol of a pilot plant scale acetifier for vinegar production. Journal Food Engineering, v. 52, 31-37 (2002).

Gacula, M. C. and Sing, J., Statistical Methods in Food and Consumer Research. Academic Press Inc., London (1984).

Hill, G. A., Daugulis, A. J., Phenol inhibition kinetics for growth of *Acetobacter aceti* on ethanol. Applied Microbiology Biotechnology, v. 51, 841-846 (1999).

Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams S. T., Genus *Acetobacter* and *Gluconobacter*. In: Bergey's Manual of Determinative Bacteriology, 9th Ed., Williams and Wilkens, Maryland, U.S.A., pp. 71-84 (1994).

Jirovetz, L., Buchbauer, G., Fleischhacker, W. and Ngassoum, M. B., Analysis of the aroma compounds of two different palm wine species ('Matango and Raffia') from Cameroon using SPMEGC-FID, SPME-GC-MS & olfactometry. Ernahrung / Nutrition, 67-71 (2001).

Joyeux, A., Lafon, L. S. and Ribereau, G. P., Evolution of acetic acid bacteria during fermentation and storage of wine. Applied Environmental Microbiology, v. 48, 153-156 (1984).

Kadere, T. T., Oniang'O, R. K., Kutima, P. M. and Njoroge. S. M., Marketing and economic importance of palm wine (mnazi) and other

coconut-based products in Kenya. Research Journal of Agricultural and Biological Sciences, 5, 815-822 (2009).

Krusong, W. and Vichitraka, A., An investigation of simultaneous pineapple vinegar fermentation interaction between acetic acid bacteria and yeast. Asian Journal of Food and Agro-Industry, v. 3, 192-203 (2010).

Lasekan, O. and. Abbas, K. A flavour chemistry of palm toddy and palm juice: A review. Trends in Food Science and Technology, v. 21, 494-501 (2010).

Li, C., Bai, J., Cai, Z. and Ouwang, F., Optimization of a cultural medium for bacteriocin production by *Lactococcus lactis* using response surface methodology. Journal of Biotechnology, v. 93, 27-34 (2002).

Liu, J. Z., Weng, L. P., Hang, Z. Q. L., Xu, H. and Ji, L. N., A mathematical model for gluconinc acid fermentation by *Aspergillus niger*. Biochemical Engineering Journal, v. 14, 15-64 (2003).

Luedeking, R. and Piret, E. L., A kinetic study of the lactic acid fermentation. Journal of Biochemical and Microbiological Technology and Engineering, v. 1, 393-412 (1959).

Maal, K. B., Shafiee, R., Characterization of an *Acetobacter* strain isolated from iranian peach that tolerates high temperatures and ethanol concentrations. World Academy of Science Engineering and Technology, v. 62, 141-145 (2010).

Monteagudo, J. M., Rodríguez, L., Rincón, J. and Fuertes, J., Kinetics of lactic acid fermentation by *Lactobacillus delbrueckii* grown on beet molasses. Journal of Chemical Technology and Biotechnology, v. 68, 271-276 (1997).

Murooka, Y. and Yamshita, M., Traditioanal healthful fermented product of Japan. Journal of Industrial Microbiology Biotechnology, v. 35, 791-798 (2008).

Murthy, M. S. R. C., Rakshit, T. S. S. K. and Kosugi, Y., Statistical optimization of lipase catalyzed hydrolysis of methyloleate by response surface methodology. Bioprocess Engineering, v. 22, 35-43 (2000).

Najafpour, G. D., Biochemical Engineering and Biotechnology. Amsterdam, Elsevier Science, pp. 51-66 (2007).

Najafpour, G. D. and Yap, Y. M., VOCs and HAP removal from contaminated air in biofilter using *Pseudomonas putida* and isolated strain NTPM1. Asian Journal of Chemistry, v. 17, 1171-1184 (2005).

Perdrix, J., Bovet, P., Larue, D., Yersin, B., Burnand, B. and Paccaud, F., Patterns of alcohol consumption in the Seychelles Islands (Indian Ocean). Alcohol and Alcoholism, v. 34, 773- 785 (1999).

Pirt, S. J., The maintenance energy of bacteria in growing cultures. Proceeding of the Royal Society B: Biological Science, v. 163, 224-231 (1965).

Raychaudhury, B., Chakraborty, R. and Raychaudhuri U., Modeling and simulation of diffusional mass transfer of glucose during fermentative production of pediocin AcH from *Pediococcus acidilactici* H. Biochemical Engineering Journal, v. 16, 237-243 (2003).

Silva, M. E., Torres Neto, A. B., Silva, W. B., Silva, F. L. H. and Swarnakar, R., Cashew wine vinegar production: Alcoholic and acetic fermentation. Brazilian Journal of Chemical Engineering, v. 24, 163-169 (2007).

Saski, A., Theeragool, G., Matsushita, K., Toyama, H., Lotong, N. and Adachi, O., Development of thermotolerant acetic caid bacteria useful for vinegar fermentation at higher temperature. Bioscience Biotechnology and Biochemistry, v. 61, 138-145 (1997).

Shuler, M. L. and Kargi, F., Bioprocess Engineering Basic Concept. New Jersey, Prentice-Hall Inc., 155-206 (1992).

Sossou, S. K., Ameyapoh, Y., Karou, S. D. and de-Souza, C., Study of pineapple peeling processing into vinegar by biotechnology. Pakistan Journal of Biological Science, v. 12, 859-865 (2009).

Steiner, P. and Sauer, U., Proteins inducing during adaptation of acetobacter aceti to high acetate concentrations. Applied Environmental Microbiology, v. 67, 5474-5481 (2001).

Suscovic, J., Beluhan, D. and Kurtanjek, Z., Mathematical Model and Estimation of Kinetic Parameters for Production Lactic Acid by *Lactobacillus delbrueckii*. Chemical and Biochemical Engineering Quarterly, v. 6, 127-132 (1992).

Tagliazucchi, D., Verzelloni, E. and Conte, A., Antioxidant properties of traditional balsamic vinegar and boiled must model systems. European Food Research Technology, v. 227, 835-843 (2008).

Takamatsu, T., Shioya, S. and Furuya, T., Mathematical model of gluconic acid fermentation by *Aspergillus niger*. Journal of Chemical Technology and Biotechnology, v. 31, 697-704 (1981).

Tsuchiya, H. M., The holding time in pure and mixed culture fermentations. Annals of the New York Academy of Sciences, v. 413, 184-92 (1983).

Vithlani, A. V. and Patel V. H., Production of Functional vinegar from India Jujube (*Zizyphus mauritiana*) and its antioxidant properties. Journal of Food Tecnology, v. 8, 143-149 (2010).

Wilson, K. and Walker, L., Practical Biochemistry: Principles and Technique. Cambrige University Press (2000).

Zinatizadeh, A. A. L., Mohamed, A. R., Najafpour, G. D., Isa, M. H. and Nasrollahzadeh, H., Kinetic evaluation of palm oil mill effluent digestion in a high rate up-flow anaerobic sludge fixed film bioreactor. Process Biochemistry, v. 41, 1038-1046 (2006).