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Vinegar production from vegetable waste: Optimization of physical condition and kinetic modeling of fermentation process

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Vegetable waste, as a low cost natural product, has been used as a source of vinegar production. In the present study, the physical conditions for fermentation by *Acetobacter aceti* (NCIM 2116) have been optimized and fermentation kinetics of the acetification has been studied in a batch system. The highest acetic acid production of 5.98% occured when *p*H is 6.2, temperature was 30°C and time is 90 h. Analysis of eigen values predict *p*H is the most significant factor for production. FTIR study confirmed the presence of C=O, O-H, N-H, CH_3, CH_2 groups in acetic acid. A simple kinetic model has been suggested using logistic equation for growth and the Luedeking - Piret equation for vinegar production and substrate utilization. The model parameters are μ_m= 0.0554 h^{-1}, α= 3.3903 g/g of biomass, β= 0.0219 g/g of biomass.h^{-1}, S_0=70.538 g/L. A significant inhibitory effect of accumulation of acetic acid on growth of *A.aceti* has been found. It is presumed that end product limits growth by acting as an uncoupling agent.

Keywords: Vegetable waste, RSM, Kinetics, FTIR, Uncoupling effect

Foods, mostly of cereal, fruit, vegetable and tuber origin, are rejected throughout the supply chain owing to lack of their eating value, as a standard processing and culinary custom[1]. The absence of a diversified product range and sufficient beneficial reuse not only worsens the wastage problem but also makes it worthless. In India, the problem of disposal of organic wastes such as food waste in municipal landfills is aggravated by legal restrictions[2].

Vegetables such as potato, pumpkin, carrot, parwal are well-known for their taste and health benefits and they are widely consumed in India. Wastes from these vegetables are available throughout the year. Vegetable waste commonly includes peel, skin, seed, fiber and other parts that are inedible by the human. Like its source, vegetable waste is rich in phytochemicals, polysaccharides, pectin that have shown significant health benefiting capability besides properties bestowed by physical and chemical characteristics. Hence, vegetable waste represents a loss of precious biomass and nutritional values that can be recovered and reintegrated into a human nutritional system. As an integral part of clean manufacturing initiative, recovery, recycling and production of innovative and high-value products using vegetable wastes have fostered research in past decades. Previously, important phytochemicals, pectin, polysaccharides and pigments were recovered from vegetable wastes[3-6]. Although rich in cellulose and hemicelluloses, these vegetable peels contain fermentable sugars such as glucose, sucrose essential for the production of ethanol, the precursor substrate of vinegar, but the amount is lower than many substances known as promoter of *A. aceti* growth. Studies have indicated that sugars adhering to the fruit processing waste material are an ideal ingredient for alcohol and vinegar production[7]. From the extensive literature analysis, no research report has been found on the vinegar production for vegetable peel.

Response surface methodology (RSM) is an efficient experimental strategy to determine optimal conditions for a multivariate system rather than conventional method of one factor at a time (OFAT) which is incapable of determining true optimum. As industrial fermentation is rapidly approaching towards a highly controlled process, designing and optimization of it requires a quantitative understanding of the production kinetics. Building of kinetic models consists of comparing traditional models with experimental data to find most relevant models. Mathematical models together with carefully designed experiments allow rapid evaluation of the behavior of systems than with laboratory experiments only[8]. In this study, vegetable peels were used

to produce vinegar in a batch culture fermentation process. The production parameters- time, temperature and *p*H were optimized for highest production and experimental data from production process were examined to form the basis of a kinetic model of the process.

Experimental Section

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.

Culture preparation

The stock cultures of *Saccharomyces cerevisiae* (NCIM 3315) and *Acetobacter aceti* (NCIM 2116) were obtained from the National Chemical Laboratory (NCL), Pune, India and maintained on MGYP medium (yeast culture) and tryptone medium respectively. The formulation of MGYP medium per liter was Malt extract (3 g), Glucose (10 g), Yeast extract (3 g), Peptone (5 g) and Agar (20 g); *p*H was maintained at 6.5. The organism was grown at 30°C for 45 h in the solid agar media in 250 mL Erlenmeyer flasks. The composition of the culture medium of *Acetobacteraceti* per liter was Tryptone (10 g), Yeast extract (10 g), Glucose (10 g), Calcium carbonate (10 g) and Agar (20 g); *p*H was maintained at 6.0. The organism was grown at 30°C in the solid agar media in 250 mL Erlenmeyer flasks for 24 h. After incubation, the culture was stored at 4°C in the refrigerator.

Preparation of vinegar

Parwal (*Trichosanthesdioica*), potato (*Solanumtuberosum*), pumpkin (*Cucurbita pepo*) and carrot (*Daucuscarota*) were purchased from market in Kolkata. These were preserved at -50°C in an ultra-low temperature Freezer (Model C340, New Brunswick Scientific, England). The fermentation medium composition per liter was Glucose (10 g), Urea (3 g), KH_2PO_4(0.5 g), K_2HPO_4(0.5 g), $MgSO_4$, $7H_2O$(0.5 g), $FeSO_4$, $7H_2O$ (0.01 g). Vegetable peeling (1kg) was mixed with 500 mL of boiling water (100°C for 15 min) and waste slurry was obtained after manual pressing; the obtained slurry was concentrated to 20° Brix by evaporation prior to fermentation for necessary amount of dry matter for production of vinegar with high acetic degree[7]. The fermentation process was carried out in a 250 mL flask; 100 mL of fermentation media were inoculated with 100 µL of fresh culture of *S. cerevisiae* (10^7cfu/mL). The *p*H and temperature were adjusted to 5.5 and 32°C for each experiment. The incubation time was 3 days and the flask was made airtight by paraffin paper for maintaining anaerobic conditions. After alcoholic fermentation, 59.9 gm/L ethanol was obtained as measured by Gas chromatography (GC). It was then inoculated with 100 µL of a fresh culture of *Acetobacter aceti* (10^7cfu/mL). The mixture was incubated for 90 h. for acetic fermentation to be completed and monitored for microbial population, ethanol utilization and acetic acid production by withdrawing samples with a sterile syringe at predetermined intervals.

Assay

Fermented sample (5 mL) was centrifuged (Remi C-24, Mumbai, India) at 3500 g for 10 min. The supernatant solution was used to determine the ethanol concentration by gas chromatography (Agilent Technologies: GC system-7890A gas chromatography, column-Agilent JK WDB-624 with column ID- 250 µm, length- 60 m and film length-1.4 µm). The ethanol content was calculated by the GC peak areas.

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.

The dry weight of mycelium were obtained after centrifuging the broth samples at 1100 g for 20 min. The harvested biomass was then washed with deionized water, dried for 8 h at 105°C, cooled in desiccators and weighed[9].

Experimental design of RSM

A three-level-three-factor, Center Composite design was employed in the optimization of vinegar production from vegetable peel. Central Composite Design (CCD) contains a factorial matrix with a center point and axial point around the center point that allow the curvature of the model to be established. The distance from the center point to the factorial point is ±1 unit for each factor, and the distance space from the center to the axial point is ±α.

The variables optimized were *p*H, time and temperature with 3 different coded level [+α, 0, -α; α=1]. For *p*H, the coded levels -1, 0, +1 are 4.2, 6.2 and 8.2 respectively. For time, the coded levels -1, 0, +1 are 70, 90 and 1 h respectively, whereas for temperature, the coded levels -1, 0, +1 are 26, 30 and 34°C respectively. The yield of acetic acid (g/L) (Y) was taken as the response of the design experiments.

The relation between the coded forms of the input variable and the actual value of the *p*H, time and temperature are described in Eq (1)

$$X_a = X_r - X_0 / \Delta X_r \quad \ldots (1)$$

where X_a is a coded value, X_r is the actual value of the factor, X_o is the actual value of the same variable at the centre point and ΔX_r the step change of the variable. According to the CCD model, total number of the experimental run is determined by the following Eq (2)

$$N = 2^k + 2k + n_0 \quad \ldots (2)$$

where k is the number of independent variable and n_0 is the number of repetitions of the experiments at the center point. Total number of experimental runs was 20 with 8 factorial, 6 axial and 6 centre point runs.

A nonlinear quadratic model was fitted to correlate the response variable (vinegar production) to the independent variables. The general form of the quadratic polynomial equation is given below in Eq (3):

$$Y = b_0 + \sum_{i=1}^{3} b_i x_i + \sum_{i=1}^{3} b_{ii} x_i^2 + \sum_{i>j}^{3} b_{ij} x_i x_j \quad \ldots (3)$$

where Y is the measured response associated with each factor level combination; b_0, b_i, b_{ii} and b_{ij} are the regression coefficients for intercept, linearity, square, and interaction, respectively, and X_1, X_2 and X_3 are the independent variables. The statistically significant terms were found for analysis of variance (ANOVA) for each response.

The adequacy of the model was checked accounting for R^2, adjusted R^2 and PRESS respectively Eqs (4)-(6).

$$R^2 = 1 - \frac{ss_{residual}}{ss_{residual} + ss_{model}} \quad \ldots (4)$$

$$R^2_{adj} = 1 - \frac{ss_{residual}/df_{residual}}{((ss_{residual} + ss_{model})/(df_{residual} + df_{model}))} \quad \ldots (5)$$

$$PRESS = \sqrt{\sum_{i=1}^{N} (y_{pred,i} - y_{exp,i})^2} \quad \ldots (6)$$

In Eqs. (4)-(6) ss is the sum of squares, df is the degrees of freedom, $y_{exp,i}$ is the experimental responses, $y_{Pred,i}$ the predicted responses and n is the number of experiments.

Numerical optimization technique of RSM (2.7-3) package for R 3.1.0 (R development core team, 2014) was used for optimization of responses.

FTIR study

A Fourier-transform infrared (FT-IR) spectrum of the fermented vinegar on KBr discs was recorded in FTIR-8400S (Shimadzu, Japan). The scanning range covered 400-4000 cm^{-1} with resolution of 4 cm^{-1} (Ref. 10).

Microbial kinetics modeling

Microbial kinetics analyses the effect of different physical and chemical parameters on the growth rate of microorganism. Unlike substrate dependent models such as Monod and Moser Equation, which explicitly requires limiting substrate concentration to be the sole influencing variable, substrate independent models can analyze growth in a medium that contains substances having inhibitory effect on the growth of microorganism or the lag time is significant.

An empirical substrate independent unstructured batch growth model, logistic equation, was used in the study of bacterial cell growth kinetics. It includes an inhibiting factor, proportional to x^2, the square of the cell growth

$$\frac{dx}{dt} = \mu_m x \left(1 - \frac{x}{x_m}\right) \quad \ldots (7)$$

where μ_m is the maximum specific growth rate (h^{-1}) and x_m is the maximum attainable biomass concentration (gm dry wt. l^{-1}). The integrated form of (3) using $x = x_o$ ($t = 0$) gives a sigmoidal variation of x as a function of t which may represent both exponential and stationary phase.

$$x = \frac{x_0 e^{\mu_m t}}{\left(1 - \left(\frac{x_m}{x_0}\right)(1 - e^{\mu_m t})\right)} \quad \ldots (8)$$

Rearrangement of Eq. (8) yields Eq. (9)

$$\ln \frac{x}{(x_m - x)} = \mu_m t - \ln\left(\frac{x_m}{x_0} - 1\right) \quad \ldots (9)$$

A plot $\ln \frac{x}{(x_m - x)}$ of vs t will give a line of slope μ_m and y intercept equal to, $\ln\left(\frac{x_m}{x_0} - 1\right)$ from which initial approximation of initial viable inoculam size x_0 can be found.

Substrates are consumed by microorganism for cell mass, metabolite production and maintenance. Substrate consumption and product formation kinetics are based on Luedeking -Piret equation which considers both growth and non-growth associated contributions[11].

$$\frac{dp}{dt} = \alpha \frac{dx}{dt} + \beta x \quad \ldots (10)$$

$$\frac{ds}{dt} = -\chi \frac{dx}{dt} - \delta x \quad \ldots (11)$$

Negative sign for substrate indicates decrease in concentration with time.

Integrating the Equation (10) and (11) over time t=0 to T yields Eq (12) and (13)

$$P = \alpha \int_{t=0}^{T} dxdt + \beta \int_{t=0}^{T} x(t)dt \quad \ldots (12)$$

$$S = S_0 - \chi \int_{t=0}^{T} dxdt - \delta \int_{t=0}^{T} x(t)dt \quad \ldots (13)$$

(where and.

$$\int_{t=0}^{T} dxdt = x \quad \int_{t=0}^{T} x(t)dt = \left(\frac{x_m}{\mu_m}\right) \ln\left(1 - \left(x_m / x_0\right)(1 - e^{\mu_m t})\right)$$

The initial values for P and S at t=0 is O (0) and S_0 respectively. In Eqs. (13), γ and δ are equal to $1/Y_{x/s}$ and m_s where $Y_{x/s}$ and m_s are biomass yield based on substrate consumption and maintenance coefficient[12].

The above equations can be simplified as:

$$\text{P or S} = Ax + k \quad \ldots (14)$$

For P and S, A is equal to α and -γ respectively and k is a constant.

Results and Discussion

Effect of experiment parameters on acetic acid concentration

Duration of fermentation (time) has profound influence on the acetic acid level as *Acetobacteraceti* has the capability to catabolise acetic acid by soluble ADH and ALDHduring acetate oxidation phase. After exhaustion of ethanol, the concentration of acetic acid falls from the highest level obtained during stationary phase as *A. aceti* starts to utilize acetic acid as chief carbon source[13]. During this experiment, production culminated at 90 h and the concentration was gradually decreased on the both sides; at the initial phases, low acetic acid is a result of high unutilized ethanol concentration; after stationary phases, the lowered acetic acid concentration is an indication of over-oxidation. Over-oxidation is key defect in vinegar production caused by α-ketogluterate dehydrogenase and succinate dehydogenase of TCA cycle[14]. Highest relevancy of time among three variables, as indicated by lowest p value for Time[2], could rises from significant effect of over-oxidation on the process.

In this experiment, the highest acetic acid concentration occured around 30°C which is similar to the optimal condition for industrial submerged vinegar fermentations. An increase in temperature causes deactivation of bacteria by enzyme denaturation and membrane damage that makes the bacteria more susceptible to the acetic acid toxicity[15]. The maximum and minimum temperature for bacterial growth are 35°C and 8°C respectively with the optimum ranges between 25°C and 30°C. Hence, the average production level at 34°C is lower than that of 26°C.

The growth of Acetic acid bacteria is optimum in the *p*H range of 5.0-6.5, but, it can grow at a *p*H lower than 3.5. Tolerance to a low *p*H strongly depends on the ethanol concentration and oxygen availability; a high ethanol concentration (12.5%) and low oxygen availability at a *p*H below 3.4, increases the *p*H sensitivity of *A. aceti*[14]. The acetic acid concentration was highest at a *p*H 6.0 and falls off on the both sides; the larger reduction of yield at higher *p*H is a result of bacteria's ability to sustain at *p*H 4.0. Therefore, 90 h fermentation duration, 30°C temperature and *p*H 6.0 were considered zero point for optimization study of vinegar production.

Analysis

20 runs of vinegar production from vegetable waste were carried out following the experimental design by CCD. The maximum amount of acetic acid was produced in run 2 and 16, the amount was 5.98% at *p*H 6.2 for 90 h of fermentation at 30°C, whereas minimum production was occurred in run 1 and

Table 1 — Central composite design matrix of 3 test variables, the observed response and predicted values

Time (x_1)	Temperature (x_2)	*p*H (x_3)	Acetic acid	
			Experimental value	Predicted value
90.00	30.00	6.20	5.79	5.73
90.00	30.00	6.20	5.98	5.73
70.00	26.00	8.20	3.62	3.59
110.00	34.00	4.20	3.57	3.51
90.00	30.00	6.20	5.66	5.73
110.00	26.00	4.20	3.66	3.64
110.00	34.00	8.20	3.73	3.72
70.00	34.00	4.20	3.55	3.57
90.00	30.00	4.20	4.89	5.01
90.00	30.00	6.20	5.70	5.73
70.00	34.00	8.20	3.65	3.58
110.00	30.00	6.20	4.82	5.01
70.00	30.00	6.20	4.79	4.94
90.00	26.00	6.20	4.87	5.09
110.00	26.00	8.20	3.90	3.79
90.00	30.00	6.20	5.98	5.73
90.00	30.00	6.20	5.96	5.73
90.00	30.00	8.20	4.89	5.02
90.00	34.00	6.20	4.89	5.10
70.00	26.00	4.20	3.72	3.64

Table 2 — Analysis of variance for the fitted quadratic polynomial model of production of vinegar from vegetable waste

	Estimate	Std error	T value
Model	5.730***	0.068	83.253
x^1	0.035	0.063	0.553
x^2	-0.038	0.063	-0.600
x^3	0.040	0.063	0.632
$x^{1:}x^2$	-0.015	0.071	-0.212
$x^{1:}x^3$	0.050	0.071	0.706
$x^{2:}x^3$	0.015	0.071	0.212
X_1^2	-0.753***	0.121	-6.2347
X_2^2	-0.678***	0.121	-5.6135
X_3^2	-0.668***	0.121	-5.5307

	Sum of squares	Df	R Square	F value	P value
Total model	16.217	9	0.9759	44.95	<0.0001
residual			Mean square		
Lack of fit	0.293	5	0.0586	2.71	0.1487
Pure error	0.108	5	0.022		
Total error	0.401	10	0.040		
CV	4.28		R^2	0.9759	
R^2_{adj}	0.9542		PRESS	1.588	

the amount was 3.55% at *p*H 4.20 for 70 h of fermentation at 34°C (Table 1). Analysis of the experimental data for the yield of vinegar using the RSM (2.7-3) package for R 3.1.0 (R development core team, 2014) produced the following mathematical model:

$$Y=5.73+0.035\ x_1-0.038\ x_2+0.040\ x_3-0.015\ x_1x_2+0.050\ x_1x_3+0.015\ x_2x_3-0.753\ x_1^2-0.678\ x_2^2-0.668\ x_3^2$$

Positive regression coefficients of the model terms indicate a synergistic effect whereas negative coefficients indicate an antagonistic effect on the dependent response variable.

Analysis of variance (ANOVA) validated that the selected quadratic model adequately represented the data obtained for vinegar production. The result of ANOVA shows that model was adequate and explained most of the variability in vinegar production (Table 2). For the fitted model, using R^2, F value and significant probabilities one can justify the significance of each experimental variable. Unless an adequate fit is obtained, optimization of a fitted response surface may produce misleading results. A R^2 value of 0.9759 indicates an excellent fit between predicted and experimental values and 97.59% of the variability in the response can be explained by the model. Closeness of adjusted R^2 value to R^2 indicates excellent relationship between predicted and experimental values (Table 1). The regression models are highly significant as indicated by $p<0.0001$. For a model to become significant, it should have a high model F value and low lack-of fit F value. Lack-of fit compares the residual error to pure error and it is not desirable. So, a small F value and high P value for lack-of fit term are desired. The obtained model has F value of 44.95 and lack-of fit F value of 2.713, both of these values indicate the suitability of model. Additionally, Coefficient of variation (CV) describes the extent to which the data were scattered. A CV value of 4.277 is within the acceptable range. By studying the regression coefficients for vinegar production, it can be concluded that only Time^2 (x_1^2), temperature^2 (x_2^2) and *p*H^2 (x_3^2) are the only significant variables as each of them has a p value<0.005; quadratic terms have higher significance than linear and crossproduct terms. Among the significant variable, Time^2 is the most important term followed by *p*H^2 and temperature^2, as it has the highest t value[16].

On the basis of coded data, canonical analysis was performed which showed the stationary point is a maximum one with eigenvalues of negative sign (-0.6593, -0.6783, -0.7606). The largest eigenvalue (-0.7606) corresponds to the eigenvector (0.957,

***-significant at P<0.05, **-significant at P<0.005, ***-significant at P<0.0005**

0.111,-0.267), the largest component of which (0.957) is associated with time; similarly, the second and third largest eigenvalue is associated with temp (-0.6783) and *p*H (-0.6593).

The response surface plots and contour plots describe by polynomial regression models were generated to investigate the interaction between independent and dependent variables (Fig. 1a-c). Change in acetic acid concentration is similar for all variables.

Optimization and model validification study

A numerical optimization was carried out in the final part of this study to identify the overall optimal conditions for vinegar production from vegetable waste. From the study of the response surface plots, the *p*H, temperature and time of 6.27, 29.88°C and 90.51 h were found to be optimum for maximum acetic acid concentration of 5.72%. The values are similar to palm juice vinegar and rice vinegar[9,17]. To verify the predicted results, the triplicate verification experiments were performed under the optimized parameter levels. Since, the mean value of the acetic acid was 5.79%, which was well agreed with the predicted value (5.72%), this result confirms the validity of the optimization approach. As a result, the models developed were considered to be accurate and reliable for predicting the production of vinegar from vegetable waste.

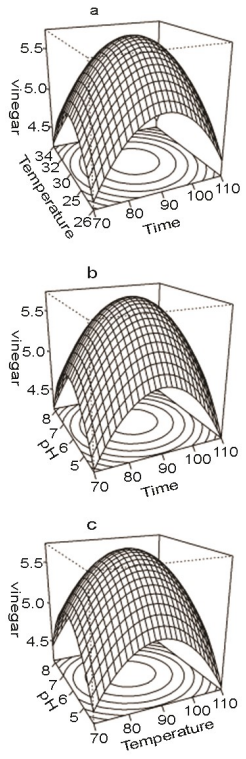

Fig. 1(a)-(c) — 3D response surface plots showing the effect of *p*H, temperature and time on the production of vinegar from vegetable waste by *Acetobacteraceti*.

FTIR analysis

The FTIR spectrum of sample is shown in Fig. 2 and the IR bands are assigned according to Coates and Meyers (2000)[18]. The band at 2517 cm^{-1} represented O-H vibration. The band at 1801 cm^{-1} was probably due to open chain acid anhydride. The band present at 2924.5 and 713.63 represented CH_2 stretch and rock vibrations. The band present at 1426.6 cm^{-1} was probably due to asymmetric band frequency of CH_3 group. The bands at 3415.8, 1639.3, 1106, 875.4 cm^{-1} were due to primary amine group. These N-H groups come from biogenic amine, formed by microbial decarboxylation of amino acids, present in vinegar. Formation of biogenic amine depends on bacterial strain, level of carboxilase activity and availability of amino acids substrate[19,20]. Presence of these groups signifies that the fermented product is vinegar.

Microbial kinetics

At optimized condition, kinetics of the vinegar production by *Acetobacter aceti* was analyzed. *Aceto-bacteraceti* showed a classical growth trend consisting of a well-defined exponential growth phase and stationary phase. Ethanol was converted to acetic acid by *Acetobacter aceti* during exponential growth phase which had lasted for 90 h; after that, ethanol content of media was almost exhausted. Taking maximum biomass concentration X_m=18.307g/L from the experimental biomass data, and fitting the experimental data to yielded the value of parameters as follows:

X_0=7.102 g/L, μ_m=0.0554 h^{-1}.

Low residual standard error (0.1842) and high r^2 value (0.9883) of the experimental data/ fitted model

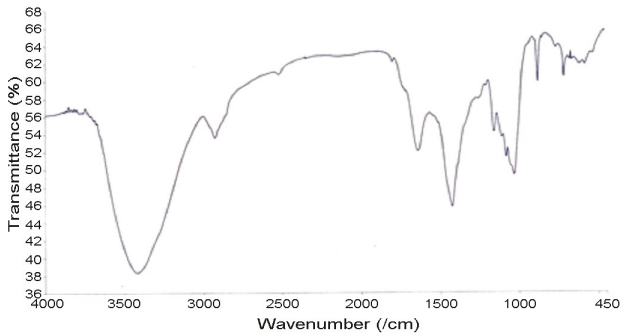

Fig. 2 — FTIR spectra of vinegar produced from vegetable waste.

implies appropriateness of the logistic model for analysis of the *Acetobacter* growth and presence of inhibition of growth by substances. According to the fitted model, calculated value of X_0=7.102 g/L was lower than that of experimental value which can be attributed to the viability of cells. Lower viability of cells may yield X_0 value less than the experimentally determined initial cell concentration.

Fitting the experimental product and substrate data to Luedeking-Piret kinetics Equation (10) and (13) yielded the value of parameters as follows: α=3.39031 g/g of biomass, β= 0.02194 g/g of biomass.h^{-1}, S_0= 70.538 g/l, $Y_{x/s}$= 0.25511 gg^{-1}, m_s= 0.015664 $gg^{-1}h^{-1}$ (Table 3). Large value of α compared to that of β indicates that acetic acid is a primary metabolite and produced in growth phase. Fermentation of 58.9 g/L ethanol yielded 10.308 g (dry wt.) of biomass which translates to a very low biomass yield ($Y_{x/s}$) of 0.175 g/g substrate.

The equation representing the relationship between the rate of product formation and microbial growth is given as:

P=5.92159X-54.29287

Comparison of the amount of substrate consumed and product formed at each time interval reveals the formation of few byproducts as both magnitudes corresponded each other very closely on a molar basis. Therefore, bio-synthesis of vinegar can be practically defined as a stoichiometric conversion of 1 mole of ethanol to 1 mole of acetic acid and rate of substrate utilization (q_s) almost equals that of product formation (q_p). Vegetable peels are low in starch and not good substrate for vinegar production. In this experiment, considerable amount of vinegar was produced from low starch vegetable peels.

The microbial growth, product formation and substrate utilization models were tested using parameters evaluated before. A comparison of calculated functions X(t), P(t) and S(t) along with the experimental values is given in Fig. 3. The comparison was made only for exponential growth phase as the model is invalid beyond that phase.

A careful examination of experimental data reveals a subtle inhibition of *Acetobacteraceti* growth rate by acetic acid produced as depicted by Fig. 4. If alcohol is responsible for the inhibition, a decrease in alcohol concentration would result in a proportional increase in biomass concentration. However, this phenomenon did not occur. Additionally, it is a well known fact that weak acids, such as lactic acid and acetic acid, can

Table 3 — Kinetic parameters for vinegar production from vegetable waste

Parameter	Value	Equation No.
X_0	7.102 g/L	(9)
μ_m	0.0554 h^{-1}	(9)
α	3.3903 g/g of biomass	(12)
β	0.0219 g/g of biomass .h	(12)
S_0	70.538 g/L	(13)
$Y_{x/s}$	0.2551 g/g	(13)
δ	0.01566g/g.h	(13)

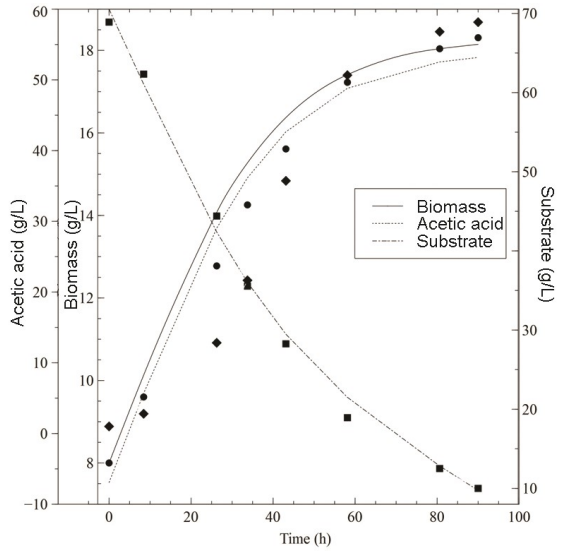

Fig. 3 — Comparison between calculated and experimental data for vinegar production.

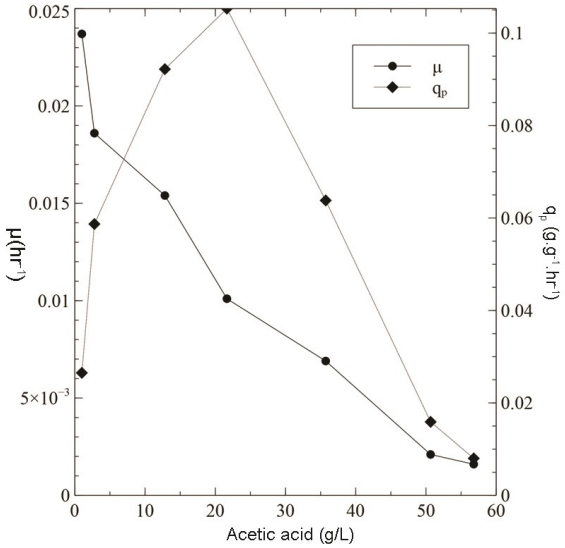

Fig. 4 — Effect of acetic acid concentration on the specific growth rate μ of *A. aceti* and on the specific acetic acid productivity (q_p) in a batch vinegar fermentation process.

exert a strong growth uncoupling action on various microorganisms, including bacteria[21,22]. These weak acids have more inhibitory power in undissociated acid form than the anion of acids[23].

This phenomenon can be explained by considering Δ*p*H, the difference between internal *p*H of bacteria and external *p*H, and characteristics of acid molecule under consideration. Bacteria usually maintains a cytoplasmic *p*H closer to neutrality than the external medium i.e., bacteria have interiors more alkaline than the medium. Bacterial cells resist any effect of a change in *p*H of the environment by extruding H^+ by means of the membrane H^+-ATPase in a process energized by glycolytically generated ATP[24,25]. Studies have found Δ*p*H value for *Acetobacter aceti* ranges from 0.1 to 0.4[26]. Upon accumulation of acid in cytosol, $pH_{cytosol}$ decreases; when $pH_{cytosol}$ drops below a certain level, proton motive force, that maintains Δ*p*H level, collapses as metabolism is unable to supply adequate ATP required for proton extrusion by H^+-ATPase. It results in ceasation of growth[22]. Thus maintaining an optimal interior *p*H is necessary for the optimum growth rate.

Undissociated acid molecules, being non-polar in nature, can penetrate lipid membranes of microbial cells as they are lipophilic compounds. During vinegar bio-synthesis, acetic acid is the sole product and can penetrate into cytosol from initial production phase i.e. early exponential growth phase. Acetic acid can diffuse across the cell membrane even when membrane transport carrier is absent. This leads to accumulation of acetic acid in cytosol resulting in a decrease in *p*H. Under this condition, growth rate will diminish as consumption of substrate will increase for producing more ATP to sustain proton pump that will maintain Δ*p*H. Furthermore, any change in *p*H will have an adverse effect on activity of enzymes and growth will be affected. This uncoupling effect is expected to be decreased during stationary phase. Anions of acid molecules, being polar and lipophobic, have lower lipid membrane penetration capability and thus possess lower inhibition capacity. Thus, product formation rate will increase at first until reaching a certain point; after that, it will decrease as biomass growth rate reduces below the level required to maintain high product formation rate. Acetic acid, being a growth associated product, only forms during growth phase and if inhibition of growth rate shortens the exponential phase duration, it will also reduce the amount of acetic acid produced[27].

Conclusion

Vinegar can be produced effectively from waste material such as vegetable peel using *Acetobacter aceti*. The optimum *p*H, temperature and time were 6.27, 29.88°C and 90.51 h respectively for the highest yield of acetic acid (5.73%). FTIR study confirmed the presence of C=O, O-H, N-H, CH_3, CH_2 groups. The model proposed in this study provides a good description of biomass, product and substrate concentrations versus batch fermentation time. The model parameters X_m, X_0, μ_m, α, β, S_0, $Y_{x/s}$, m_s were determined. Model has established that acetic acid is a growth associated product as it has high α value. Analysis of data substantiated the inhibitory effect of the vinegar on the growth of *Acetobacter aceti*. Growth uncoupling effect of this weak acid is mainly responsible for this inhibitory action.

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References

1 Bond M, Meacham T, Bhunnoo R & Benton T G, *A Global Food Security report* (2013).
2 Mohdaly A AA, Sarhan M A, Smetanska I & Mahmoud A, *J Sci Food Agr*, 90 (2010) 218.
3 Singh A, Sabally K, Kubow S, Donnelly D, Gariepy Y, Orsat V & Raghavan G S V, *Molecules*, 16 (2011) 2218.
4 Maran J P, Mekala V & Manikandan S, *Carbohyd Polym*, 92 (2013) 2018.
5 Maran J P & Prakash K A, *Int J Biol Macromolec*, 73 (2015) 202.
6 Maran J P & Priya B, *J Food Sci Tech*, (2015).
7 Sossou S K, Ameyapoh Y, Karou S D & De Souza C, *Pakistan J Biol Sci*, 12 (2009) 859.
8 Elibol M & Mavituna F, *Process Biochem*, 34 (1999) 615.
9 Ghosh S, Chakraborty R, Chatterjee G & Raychaudhuri U, *Braz J Chem Eng*, 29 (2012) 461.
10 Pal R, Panigrahi S, Bhattacharyya D & Chakraborti A S, *J Mol Struct*, 1046 (2013) 153.
11 Luedeking R & Piret E L, *J Biochem Microbiol Tech Eng*, 1 (1959) 393.
12 Liu J Z, Weng L P, Zhang Q L, Xu H & Ji L N, *Biochem Eng J*, 14 (2003) 137.
13 Sengun I Y & Karabiyili S, *Food Control*, 22 (2011) 647.
14 Gullo M & Giudici P, *Int J Food Microbial*, 125 (2008) 46.
15 De Ory I, Romero L E & Cantero D, *Appl Microbiol Biotechnol*, 49 (1998) 189.

16 Maran J P, Mekala V & Manikandan S, *Ind Crops Prod*, 49 (2013) 304.
17 Chen C & Chen F, *Food Bioprod Process*, 87 (2009) 334.
18 Coates J & Meyers R A, (John Wiley & Sons Ltd.), 2000, 10815.
19 Ordonez J L, Callejon R M, Morales M L & Garcia-Parrilla M C, *Food Chem*, 141 (2013) 2713.
20 Cozzolino D, Smyth H E, Lattey K A, Cynkar W, Janik L, Dambergs R G, Francis I L & Gishen M, *Anal Chim Acta*, 539 (2005) 341.
21 Bar R, Gainer J L & Kirwan D J, *Biotechnol Bioeng*, 29 (1986) 796.
22 Baronofsky J J, Schreurs W J A & Kashket E R, *Appl Environ Microb*, 48 (1984) 1134.
23 Wang G & Wang D I C, *Appl Environ Microb*, 47 (1984) 294.
24 Herrero A A, *Trends Biotechnol*, 1 (1983) 49.
25 Clarke D J, Fuller F M & Morris J G, *Eur J Biochem*, 98 (1979) 597.
26 Menzel U & Gottschalk G, *Arch Microbiol*, 143 (1985) 47.
27 Narendranath N V, Thomas K C & Ingledew W M, *J Ind Microbiol Biot*, 26 (2001) 171.