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UTILIZATION OF SWEET POTATO PEELS FOR BIOETHANOL GENERATION BY *ZYMOMONAS MOBILIS* AND *SACCHAROMYCES CEREVISIAE* USING RESPONSE METHODOLOGY

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ABSTRACT
Ethanol is one of the bioenergy sources with high efficiency and low environmental impact. In this study, fermentation conditions were optimized based on the central composite design of response surface methodology for maximum ethanol recovery. Under these optimized process conditions, *Zymomonas mobilis* and *Saccharomyces cerevisiae* were used to ferment potato peel waste as a sole carbon source. The test variables in a defined range of inoculum concentration (6 % - 14 %), pH (4.0 – 6.0), sugar concentration (14 – 22^{o}Brix), temperature (24 – 32 ^{o}C) and the inoculation time (30 – 54hrs) were modeled using the response surface methodology (RSM). The experimental data indicated that the maximum ethanol production observed by the model was 11.49 mL with optimum inoculum concentration at 10 %, pH of 5, 18°Brix of sugar concentration, 28 °C of temperature at 42 h. All factor variables evaluated had a significant effect on the bioethanol production with probability less than 0.05. The bioethanol has a potential to be used as solid fuel, and furthermore, ethanol yield may have inhibitory impact on the fermentation microorganisms hence lowering product formation.

Keywords: Response Surface Methodology, Fermentation, Bioethanol, *Zymomonas mobilis* and *Saccharomyces cerevisiae*.

INTRODUCTION
Environmental deterioration, depletion of fossil fuel reserves, reduction of dependence on energy imports and increasing energy costs, are a few of the critical challenges that have spurred efforts into research for a promising alternative to conventional fossil based biofuels (Mittelbach *et al*., 1983; Wingren *et al*., 2003; Meher *et al*., 2006; Demirbas, 2007; Campbell, 2008). Biofuels produced from renewable resources are attracting attention as the public concerns on the risk of fuel loss, oil prices, climate change and environmental impacts mounts (Matsakas *et al*., 2014). Worldwide, these concerns has engendered a renewed commitment in exploiting other alternative renewable biomass-derived biofuels (Inui *et al*., 2008; Demirbas, 2009). Among these broadly classified biofuels, bioethanol is currently under the spotlight as a new-generation biofuels, whose economic and environmental benefits can revolutionize the global energy market (Gupta and Verma, 2015). This is due to its competitive energy density over other petrochemical fuels, energy efficiency, less corrosion, less water-soluble, and flexibility in blends with gasoline/diesel fuels for internal combustion engine (Demirbas, 2009). Starch and sugar-based biomass feedstock are currently exploited as most significant materials for bioethanol production through fermentation process, yielding favorable results in terms of energy balance and environmental sustainability (Shankar *et al*., 2015; Nigam and Singh, 2011; Balat and Balat, 2009). Additionally, bioethanol has promising potential to augment current energy demand as fossil fuel production declines, more so mitigating climate change.

Yeasts are most often the common microorganisms used in the fermentation process and can obtain energy from various carbon sources. The most commonly used yeast are strains of *Saccharomyces cerevisiae* which, has short germination time and can easily be cultured in large scale processes (Siqueira *et al*., 2008). However, the genus *Zymomonas* is known to acquire a great social implications as an ethanol producer, with its unique biochemical properties. The *Zymomonas mobilis* subsp. mobilis can better ferment sugars to ethanol, CO_2 and some lactic acid (Swings and De Ley, 1977). When grown under anaerobic conditions, the ethanol tolerance and yield productivity makes *Zymomonas mobilis* a suitable choice over *Saccharomyces cerevisiae* (Rastogi and Shrivastava, 2017). The anaerobic cultivation of *Saccharomyces cerevisiae* and *Zymomonas mobilis* to generate bioethanol, can be cumbersome when one-at-a-time-parameter are implemented in medium composition. Therefore, variations in the composition of fermentation media, can influenced the product yield and metabolic properties of the microorganisms (Scherlach and Hertweck, 2009). The optimization of the parameters that defines such variations are achieved by different kinds of designs available for optimization of significant fermentation factors such as the Response surface methodology. This allows relatedness of the best combination of parameters and prediction of responses in choosing the modeled factors (Ahsan, Chen, Wu, and Irfan, 2017; Kumar, Singh, Beniwal, and Salar, 2016).

As the world production of bioethanol increased simultaneously year by year; to about 25.6 billion liters in 2016 as reported by the Renewable Energy Association (REA) (RFA, 2016), the need for exploiting particularly non-edible feedstock for bioethanol production is apparent. In the current study, experimental design that employs the use of statistical tools offers efficient, and reproducible approaches for determining the favourable fermentation conditions for such processes. In this regards, the central composite design (CCD) of response surface methodology (RSM) was utilized in optimizing the production and growth factors of *S. cerevisiae* and *Z. mobilis* towards bioethanol production. Furthermore, cheap carbon sources such as sweet potato biomass which is mostly incinerated in Nigeria, was utilized to examine the yield of bioethanol based on the bioprocess parameters. The

accuracy of the estimated data was defined based on the prediction evaluation of pH, operational mode and Temperature etc.

MATERIALS AND METHODS

Materials

The potato peel molasses which was collected from local restaurants was used as feedstock for bioethanol production. Aqueous ethanol (Fisher Scientific, Waltham, MA, USA) used for delignification process and other solvents were of analytical grade. The yeast extract, peptone, and glucose, Yeast peptone glucose (YPG) agar media (Sigma-Aldrich, St. Louis, MO, USA) were used for the isolation of *Z. mobilis* and potato dextrose agar (Lifesave Biotech USA) was also used for yeast isolation. Wallerstein Laboratory Nutrient Agar (WL) was from Merck and all reagents and sugars used are of analytical grades.

Microorganisms and Inoculum Preparation.

The yeast and bacteria used in the present study were isolated from the palm tree sap as isolated from palm wine and identified as *Saccharomyces cerevisiae* and *Zymomonas mobilis*, respectively on the basis of morphological and biochemical properties. The stock culture of the yeast was maintained on yeast peptone glucose (YPG) agar medium while the stock of the bacteria was maintained on peptone glucose agar (PGA) medium at 4 ^{o}C. Inoculum was prepared by growing a loopful of bacterium in WL nutrient broth with 0.2 % of $(NH_4)_2SO_4$ and incubated for 48 h at 30 ^{o}C with shaking at 150 rpm. Harvested cells were washed with sterile saline and the cells used as standard inoculum.

Substrates Preparation

Agro-residues molasses of sweet potato were collected from the local restaurants in Nigeria aseptically in sample bags. These potato peels were first dried at 105 °C in an oven for about 4h, and then pulverized to powdered form in a grinder mixer. The power was then sieved to obtain a finely pulverized powder of mesh size 0.5 mm and packed in dry sterilized bottles at room temperature and used for bioethanol production in a submerged fermentation.

Batch Fermentation Experiments

Batch fermentation was done using 100 mL of culture media supplemented with molasses of potato peels (10 g) in Erlenmeyer flasks fitted with rubber stoppers. The pH with other variables were adjusted according to the required experimental conditions (Table 1). The fermentation media was then inoculated with 10 % (v/v) yeast and bacteria suspension (≈10^8 cells/mL), respectively. The medium was supplemented with 0.2 % of $(NH_4)_2SO_4$ to replace nitrogen for the organisms (Yan *et al*., 2013; Swain *et al*., 2013). Incubation was performed at different temperatures and at different times depending on the experimental conditions required (Shankar et al., 2015). The biomass was separated by centrifugation and supernatant was collected at intervals for analyses.

Experimental Design

The optimization function of the MINITAB 14 software was employed to optimize bioethanol production process from potato peels molasses by employing. The central composite design CCD of the response surface methodology (RSM) applied to investigate the influence of fermentation process variables on the bioethanol yield according to the experimental runs carried out to a 2^3 factorial design, 6 axial points (α =2,366). The 6 central point was also applied for the five identified design independent variables to fit a second-order polynomial model for, sugar concentration (16/20 °Brix), inoculum concentration (8 %/12 % v/v), temperature (26 °C-30 °C) and the fermentation time (36/48 hours). Each variable to be optimized was coded at 5 levels giving sugar concentration (14-22 °Brix), inoculum concentration range (6 %-14 % v/v), pH (4.0-6), temperature (24 ^{o}C-32 ^{o}C) and time (30 to 54 hrs) (**Table 1)**.

Table 1: The experimental variables based on the actual and coded levels of the second-order polynomial model

Variables	Symbols	Coded levels				
		-α (-2.366)	-1	0	+1	+α (+2.366)
Inoculum (%)	X_1	6	8	10	12	14
pH	X_2	4	4.5	4.8	5.5	6
Sugar Concentration (^{o}brix)	X_3	14	16	18	20	22
Temperature (^{o}C)	X_4	24	26	28	30	32
Time (hrs)	X_5	30	36	42	48	54

The second order model was chosen to predict the optimal point and was expressed by:

$$\hat{Y} = \boldsymbol{\beta_0} + \boldsymbol{\beta_1 X_1} + \boldsymbol{\beta_2 X_2} + \boldsymbol{\beta_3 X_3} + \boldsymbol{\beta_4 X_4} + \boldsymbol{\beta_5 X_5} + \boldsymbol{\beta_{11} {X_1}^2} + \boldsymbol{\beta_{22} {X_2}^2} + \boldsymbol{\beta_{33} {X_3}^2} + \boldsymbol{\beta_{44} {X_4}^2} + \boldsymbol{\beta_{55} {X_5}^2} + \boldsymbol{\beta_{12} X_1 X_2} + \boldsymbol{\beta_{13} X_1 X_3} + \boldsymbol{\beta_{14} X_1 X_4} + \boldsymbol{\beta_{15} X_1 X_5} + \boldsymbol{\beta_{23} X_2 X_3} + \boldsymbol{\beta_{24} X_2 X_4} + \boldsymbol{\beta_{25} X_2 X_5} + \boldsymbol{\beta_{34} X_3 X_4} + \boldsymbol{\beta_{35} X_3 X_5} + \boldsymbol{\beta_{45} X_4 X_5}$$

where $\hat{Y}$ represents response variable sugars concentration, β_{11}, β_{22}, β_{33}, β_{44} and β_{55} are quadratic terms, β_1, β_2, β_3 β_4 and β_5 are linear terms, X_1, X_2, X_3, X_4 and X_5 are test variables studied and β_{13}, β_{14}, β_{15}, β_{23}, β_{24}, β_{25}, β_{34}, β_{35} and β_{45} are interaction terms.

Estimation of ethanol

The analysis of ethanol from samples is tested by the Caputie method (Caputie, 1986). Culture supernatant (1 mL) was taken and the volume to made up to 5 mL with sterile distilled water then followed by 1 mL of $K_2Cr_2O_7$ solution and 4 mL of Conc.H_2SO_4 solution. The intensity of colour was read at 660nm in UV/VIS spectrophotometer (Biobase). Ethanol production was assayed by fractional distillation on a heating mantle under running tap water, and the distillate collected at 78 ^{o}C (Bekele *et al*., 2015).

Statistical analysis

Regression method of response surface methodology and analysis of variance (ANOVA) were used for the statistical analysis of the model. The determination coefficient R^2, Fisher's F-test, associated probability P(F), and correlation coefficient R were determined, and contour plots were representatives of the quadratic models of variables. The level of mathematical significance test was set at 5 % (Gao, He, Jiang, and Huang, 2016 ; Ahsan *et al*., 2017)

RESULTS

Isolation of yeast and bacterial strains

Culture of yeast and bacteria were determined and observed out of which one isolate each, representing *Saccharomyces cerevisiae* and *Zymomonas mobilis* was selected based on their microbial

characteristics and carbohydrate fermentation. The results of the microorganism screening test shows that the yeast demonstrated ethanol tolerance of 15 %, while the bacteria had 12 %. Since the strains of these microorganism were the preselected ab initio, thus they were chosen for the inoculum preparation and subsequent fermentation tests with potatoes peels molasses.

Regression Model Validation and Experimentation

The yield of bioethanol relative to the amount of total sugars in the waste potato peels had significant correlation to the factors predicted and observed. Results presented in **Table 2** show the complete design matrix with experimental and predicted values of the produced bioethanol yield (%). An adequate description of the different modeled variables as obtained by multiple regression analysis to the experimental data can be represented according to the following second-order quadratic polynomial equation:

$$\textbf{Bioethanol yield } \left(\frac{\mathbf{U}}{\mathbf{mL}}\right) = \beta_0 + 11.488 + 0.966X_1 - 1.606X_2 + 1.145X_3 + 0.443X_4 - 0.342X_5 + 5.889{X_1}^2 + 0.183{X_2}^2 + 1.963{X_3}^2 - 1.834{X_4}^2 - 0.284{X_5}^2 - 5.455X_1X_2 + 5.986X_1X_3 - 0.308X_1X_4 - 10.252X_1X_5 - 11.61X_2X_3 - 1.951X_2X_4 + 11.183X_2X_5 - 2.614X_3X_4 - 5.478X_3X_5 + 0.476X_4X_5$$

Where Y is the bioethanol yield (mL), X_1= Inoculum (%), X_2= pH, X_3= Sugar Concentration (^{o}Brix), X_4= Temperature (^{o}C), X_5= Time (hours) and synergetic and antagonistic effect are represented by the positive and negative sign in front of the terms, respectively (Hamouda et al., 2015). Validation experiments carried out to check the accuracy of the models indicated that the predicted values somewhat agreed with the experimental values. However, the residual difference between the observed and the estimated bioethanol yield had small margin when 10 % inoculum concentration, pH value of 5, 18 Brix of sugar concentration, 28°C of temperature and 42 hours of time was used as shown in **Table 2**. The only significant drop in fermentation yield occurs for the initial concentration of 16 Brix (2.8 %), the highest value being 3.5 %, obtained at 20 Brix. fermentation time decreases in direct proportion to initial soluble solids concentration. However, decrease in productivity was observed only at 16 Brix. It is reported that high osmotic pressure resulting from high substrate concentrations inhibits growth and fermentation of yeasts during such bioprocess which was observed in this study (Takeshige & Ouchi, 1995).

Table 2: Central composite design plan of coded value, the observed value and predicted value of the fermentation

Run Order	Inoculum	pH	Sugar Conc.	Temp.	Time	Bioethanol (mL)		RESID
						Observed	Predicted	
1	8	4.5	16	26	48	8.8	9.35	-0.55
2	12	4.5	16	26	36	14.3	13.64	0.66
3	8	5.5	16	26	36	10.2	9.97	0.23
4	12	5.5	16	26	48	14.0	13.12	0.88
5	8	4.5	20	26	36	15.0	15.66	-0.66
6	12	4.5	20	26	48	12.5	13.59	-1.09
7	8	5.5	20	26	48	13.1	13.76	-0.66
8	12	5.5	20	26	36	12.6	12.87	-0.27
9	8	4.5	16	30	48	13.0	12.47	0.53
10	12	4.5	16	30	48	8.0	7.89	0.11
11	8	5.5	16	30	36	11.2	10.66	0.54
12	12	5.5	16	30	36	12.2	10.88	1.32
13	8	4.5	22	30	48	13.0	13.81	-0.81
14	12	4.5	20	30	48	14.0	13.79	0.21
15	8	5	20	30	36	9.8	10.02	-0.22
16	12	5	20	30	48	11.2	10.98	0.22
17	6	4	18	28	42	13.6	12.74	0.86
18	14	6	18	28	42	10.5	11.46	-0.96
19	10	5	18	28	42	11.2	11.49	-0.29
20	10	5	18	28	42	9.0	11.49	-2.49
21	10	5	14	28	42	10.6	12.31	-1.71
22	10	5	22	28	42	16.8	14.60	2.20
23	10	5	18	24	42	10.0	9.21	0.79
24	10	5	18	32	42	9.2	10.10	-0.90
25	10	5	18	28	30	10.8	11.55	-0.75
26	10	5	18	28	54	11.5	10.86	0.64
27	10	5	18	28	42	11.2	11.49	-0.29
28	10	5	18	28	42	10.0	11.49	-1.49
29	10	5	18	28	42	12.2	11.49	0.71
30	10	5	18	28	42	15.0	11.49	3.51
31	10	5	18	28	42	11.5	11.49	0.01
32	10	5	18	28	42	11.2	11.49	-0.29

The regression coefficients has a 5 % level significance and justifies the strength of the model as well as the potentials of good fit. Within the studied range of experimental variable models, the inoculum size had observable impact on the fermentation process followed by the molasses concentration, and incubation temperature. While the initial pH has a slight positive impact on the bioethanol yield (%), its quadratic effect has negative impact on the fermentation process, followed by the negative quadratic effect of inoculum with time, pH with molasses concentration and inoculum with pH. The quadratic effects of the molasses concentration, inoculum, temperature, and mixing rate revealed a positive impact on the fermentation process (**Table 3)**.

Table 3: Optimization of bioethanol production in second-order polynomial model

Model Term	R. Coefficient	SE Coefficient	T	P
Constant	11.488	0.674	17.050	0.000
Inoculum	0.966	1.018	3.949	0.004
pH	-1.606	1.381	-4.163	0.003
Sugar Conc.	1.145	0.923	4.241	0.002
Temp.	0.443	1.038	3.427	0.007
Time	-0.342	1.065	-3.321	0.008
Inoculum*Inoculum	5.889	9.663	4.609	0.006
pH*pH	0.183	8.905	2.021	0.010
Sugar Conc.*Sugar Conc.	1.963	1.465	3.340	0.002
Temp.*Temp.	-1.834	1.496	-3.225	0.002
Time*Time	-0.284	1.496	-4.190	0.009
Inoculum*pH	-5.455	3.554	-3.535	0.002
Inoculum*Sugar Conc.	5.986	2.888	2.072	0.001
Inoculum*Temp.	-0.308	2.108	-3.146	0.009
Inoculum*Time	-10.252	5.133	-2.997	0.001
pH*Sugar Conc.	-11.610	5.243	-2.214	0.000
pH*Temp.	-1.951	3.378	-3.578	0.006
pH*Time	11.183	3.715	3.011	0.000
Sugar Conc.*Temp.	-2.614	2.418	-3.081	0.003
Sugar Conc.*Time	-5.478	4.201	-4.304	0.002
Temp.*Time	0.476	2.896	3.164	0.009

Evaluation of Significant Factors on Bioethanol Yield

Analysis of variance for the selected factorial model showed that the model was significant with a Model F-value of 35.542 and p value of 0.000, implying reliability of the model to describe the quantitative relation between bioethanol production and the selected parameters **(Table 4)**. Furthermore, the determination coefficient suggest accuracy and general good fit of the polynomial model (Gao *et al*., 2016). The ANOVA of the quadratic regression model demonstrates that the model is significant, as is evident from the low P-value of the Fisher's F-test. This proves that the model equation is adequate for describing the responses in the bioethanol yield. The model was found to be adequate for prediction within the range of variables employed.

Table 4: ANOVA for the Quadratic Polynomial Model on the Bioethanol Production

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	20	87.70	87.698	4.385	3.200	0.004
Linear	5	26.98	16.365	3.273	3.900	0.005
Inoculum	1	0.09	3.288	3.288	3.900	0.004
pH	1	2.87	4.936	4.936	4.350	0.003
Sugar Conc.	1	18.29	5.619	5.619	4.540	0.002
Temp.	1	5.16	0.666	0.666	4.180	0.007
Time	1	0.56	0.375	0.375	4.100	0.008
Square	5	22.20	26.070	5.214	5.430	0.003
Inoculum*Inoculum	1	1.23	1.356	1.356	3.370	0.006
pH*pH	1	7.40	0.002	0.002	4.000	0.010
Sugar *Sugar Conc.	1	9.29	6.556	6.556	5.800	0.002
Temp.*Temp.	1	4.28	5.483	5.483	5.500	0.002
Time*Time	1	0.00	0.131	0.131	4.040	0.009
Interaction	10	38.52	38.521	3.852	5.060	0.005
Inoculum*pH	1	0.01	8.604	8.604	5.360	0.002
Inoculum*Sugar Conc	1	0.11	15.679	15.679	6.290	0.001
Inoculum*Temp.	1	1.71	0.078	0.078	3.020	0.009
Inoculum*Time	1	0.01	14.560	14.560	3.990	0.001
pH*Sugar Conc.	1	1.05	17.900	17.900	4.900	0.000
pH*Temp.	1	0.04	1.218	1.218	4.330	0.006
pH*Time	1	23.08	33.091	33.091	9.060	0.000
Sugar Conc.*Temp.	1	6.30	4.266	4.266	5.170	0.003
Sugar Conc.*Time	1	6.12	6.209	6.209	5.700	0.002
Temp.*Time	1	0.10	0.098	0.098	4.030	0.009
Residual Error	11	40.16	40.157	3.651		
Lack-of-Fit	4	18.71	18.708	4.677	5.530	0.003
Pure Error	7	21.45	21.449	3.064		
Total	31	127.86				0.000

Coefficient of determination (R^2) = 68.59 %; adjusted R^2 = 11.49 %.

The contour and surface plots (**Figure 1a - 1f)** showed variability of the relationships between independent and dependent variables. Usually, observable circular contour plot were not prominent, which would have resulted in negligible interactions between the corresponding variables. However, the observed elliptical contours suggest the presence of a significant interaction between the corresponding variables (Gao *et al*., 2016). In the present study, two variables were depicted in the contour plots, while the other variables were fixed at coded levels. The effect of the interaction between temperature and pH on bioethanol yield was obtained at fixed inoculum size (10), sugar (18 brix), and time (42h). Increasing the temperature up to 30 ^oC and pH 6 resulted in maximal yield as the interactions between variables are significant. However, the observed bioethanol yield when time is varied at a fixed inoculum (10), sugar (18 brix) and Time (28h), provided significant increase in bioethanol production. The surface and contour plots proposes that there were well defined optimum operating conditions. On the other hand, increasing inoculum size and pH, while fixing sugar (18 brix), temperature (28 ^oC) and time (42h) impacted on bioethanol production (**Figure 2a, 2b**).

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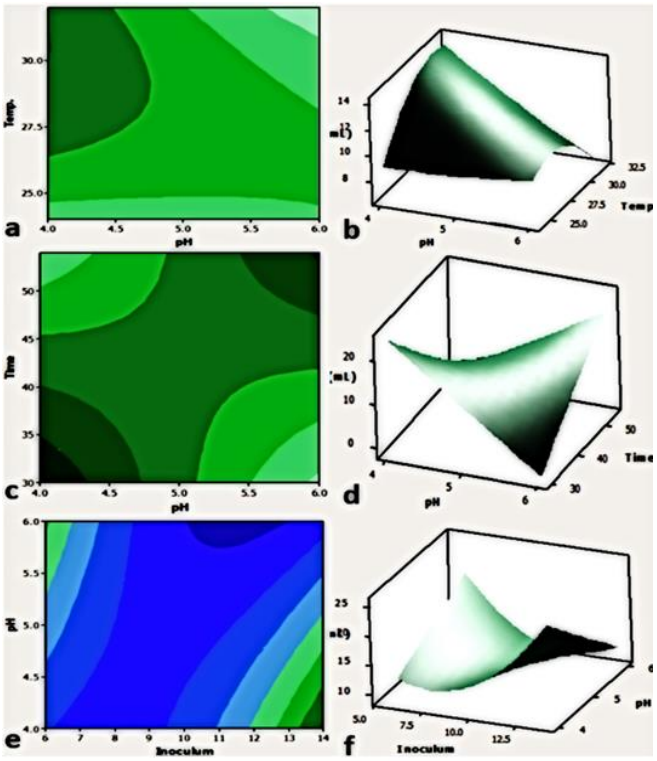

Figure 1: Contour plot (a) and surface plot (b) of bioethanol yield observed (ml) vs temperature, pH

Furthermore, increasing inoculum concentration at a fixed pH (5), temperature (28 ^{o}C), and time (42h) resulted in good yield of bioethanol. It may be also observed that higher sugar concentration combined with increasing inoculum concentration resulted in an increase in bioethanol yield. From the contour and surface plots enhancement effects of higher levels with strong interactions effect on bioethanol yield could be said ultimately liked to sugar concentrations and inoculum size (**Figure 2a, 2b)**. From the contour plots it can be concluded that higher levels of sugars and inoculum at lower pH range resulted in an improved bioethanol production.

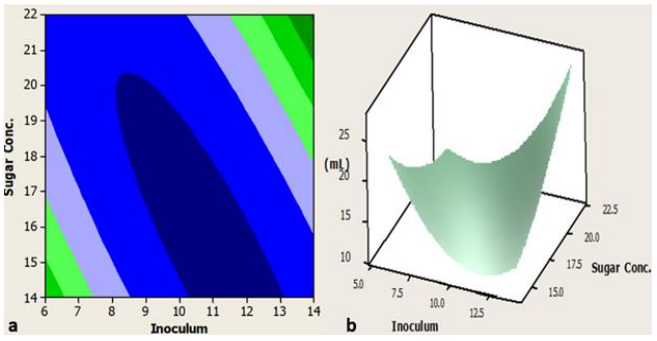

Figure 2: Contour plot (a) and surface plot (b) of bioethanol yield observed (ml) vs time, pH.

DISCUSSION

Bioenergy or alternative renewable energy have been found to be a suitable alternative toward solving energy crisis. In the current study, the response surface methodology was used to optimize the production of bioethanol from potato peels using the combined effect of *Zymomonas mobilis* and *Saccharomyces cerevisiae* which showed greater potential in enhancing the yield of the ethanol. Potato peels is an organic compound and composed of carbohydrates, protein, fat, ash, carotene, thiamine, riboflavin and ascorbic acid which promotes the cell growth and product formation during bioethanol formation by the fermentative activity of these microbes (Sheikh, Al-Bar, and Soliman, 2016).

Both *Saccharomyces cerevisiae* and *Zymomonas mobilis* exhibited similar properties in their fermentation potentials particularly where the pH played a significant role on fermentation parameters influencing growth of the microorganism, fermentation rate and by-product formation. Maintenance of pH at constant state influenced the yield of bioethanol as other conditions become variated. Although high or low pH are known to also cause chemical stress on organism cell (Wang *et al*., 2013). As reported by Hwang *et al*. (2008) the difference demonstrated by *Zymomonas mobilis* and *Saccharomyces cerevisiae* has a resultant effect on the parameters modulation which was observed in the cause of this study in the pH variation. There was a significant correlation observed between both *Zymomonas mobilis* and *Saccharomyces cerevisiae* as the substrate is been fermented due to the availability of the sugars and also undesired products like organic acid, glycerol as a trade off to the produced bioethanol. Swings and De Ley. (1977) described the destabilization of the molecular structures of these organisms as pH approaches optimum within the substrate medium. However in the present study, it was observed that the pH supported the growth of *Zymomonas mobilis* and *Saccharomyces cerevisiae* as reported by Hossain *et al*. (2010) which described the tolerance of such organisms to variation in pH from acidity to neutrality.

Temperature is known to affect the metabolism of microorganism which results to the formation of secondary metabolites (Fleet and Heard. (1993). Temperature is also known to have influence on fermentation which to some extent influences microbial growth, speed of enzyme action, cell sensitivity, and increased membrane fluidity (Wang *et al*., 2013). Increasing temperature significantly impacted on the production of bioethanol for both *Zymomonas mobilis* and *Saccharomyces cerevisiae* as revealed by the decreased in bioethanol yield. The decrease in the yield might be due to thermal sensitivity of the cells. It is also reported that decrease in the membrane phospholipid content can play a role in the unique thermal sensitivity of *Zymomonas mobilis* cells grown at temperatures above 30 ^{o}C (Panesar et al., (2007) which indicated that. For *Saccharomyces cerevisiae* there could be low enzymes activity at higher temperature which could also lead to formation of other secondary metabolites leading to lower bioethanol production (Panesar., Marwaha., and Kennedy, 2007).

The study revealed that the time course of ethanol production by both *Zymomonas mobilis* and *Saccharomyces cerevisiae* followed an initial upward duration with a decline in bioethanol production as the time is lowered. This decline could arise from the build-up of toxic product within the fermentation medium (Zakpaa *et al*., 2009). *Saccharomyces cerevisiae* is known to employ the EMP pathway

to metabolize glucose, whereas *Zymomonas mobilis* employing the ED pathway producing 1mole of ATP from 1 mole of glucose. Therefore, the growth rate of *Z. mobilis* may proceed faster in utilizing a significant amount of the carbon source in the waste potato peels hence outperforming *S. cerevisiae* in maintaining a high level of glucose flux (Reinis, Kalnenieks, and Stalidzans, 2013). However, *Zymomonas mobilis* perform less biomass formation and efficient production of bioethanol as compared to *Saccharomyces cerevisiae* (Sootsuwa *et al*., 2013). Exploiting an inexpensive and cost effective means of biofuel production is the hallmark of alternative energy generation (Tao *et al*., 2005, Aggarwal *et al*., (2001). This work therefore shows that under appropriate conditions waste potato peel can be used as alternative and cost effective feed stock for the production of bioethanol.

Conclusion

Large scale industrial application of potato peels is very limited as biomass for bioethanol owing to its low cultivation. In the recent past, several studies have provided other alternatives, however, the present study focuses on the need to revisit the cost effective production and statistical optimization of bioethanol of agro-residues like potato peels. *Zymomonas mobilis* and *Saccharomyces cerevisiae* can be said to be good candidates in bioethanol production using potato peels as the substrates. The results obtained in this investigation indicate that Potato peels is suitable for bioethanol fermentation due to high content of fermentable sugars present. The response surface model was used to validate 32 sets of random experiments which were dependent on the use of a developed quadratic model, response surface plots and contour plots. The model equations was able to provide a proposed description to the behavior of the production of bioethanol in the fermentation by the microbes employed. Hence the findings of the study revealed the potential use of alternative biomass by optimization of the components and culture requirements, and also the chosen method of optimization, which proves to be somewhat efficient. Further studies are required to encourage cultivation of potato for commercial utilization in bioethanol production.

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