

# Optimization of Fermentation Process Parameters for Bioethanol Production from Sri Lankan Overripe Fruits

I.W. Kularathne, C.A. Gunathilaka, A.C. Ratnaweera, C.S. Kalpage, S. Rajapakse and P. Gamage

**Abstract:** Bioethanol produced by biomaterials is used in many countries around the world as an alternative option for gasoline, mainly due to its better emission characteristics. Presently, Sri Lanka depends on imported fossil fuel to fulfil the majority of its transportation fuel requirement and also committed to reduce green house gas emission. Therefore, searching for alternative freely available and low-cost bio resources to produce bioethanol is a timely requirement. This research aims to study the possibility of producing bioethanol from Sri Lankan rotten fruits (without skin) using batch fermentation followed by optimization of the fermentation process parameters. Optimization is carried out by using Response Surface Methodology (RSM) and Genetic Algorithm (GA), and Particle Swarm Optimization (PSO). Bioethanol production is carried out using three different overripe fruits: Banana (*Musa ssp.*) embul kesel variety, Papaya (*Carica-papaya*) and Jackfruit (*Artocarpus heterophyllus*) with two different microorganisms (inoculum) at three different fermentation conditions. The maximum ethanol yield (%) obtained with RMS, GA and PSO were 13.39, 13.39, and 13.36 vol.%, respectively, by using Banana (embul kesel variety) fermentation with *Pseudomonas mendocina* (PM) as inoculum, 1:1 substrate concentration, 5.1 pH and 35°C temperature conditions.

**Keywords:** Bioethanol, Overripe Fruits, Microorganisms, Fermentation, Optimization

## 1. Introduction

Energy crisis and environmental issues catalysed the researchers to discover alternative and renewable energy sources as a replacement for fossil energy sources. As a result, biofuel produced by biomass has been identified as the best alternative energy source due to several merits [1][2]. Presently, Sri Lanka depends on imported fossil fuel for the transportation fuel requirement, even though sugar and starch-based bioethanol feedstocks are well grown in Sri Lanka. Considering the limited cultivable lands and food security issues in Sri Lanka, bioethanol production using such feedstocks is discouraged [3]. Although lignocellulose materials such as agricultural residues, wood, paper and municipal solid waste are cheaper than sugar-containing biomaterials and feely available in Sri Lanka, the usage of them in bioethanol production is limited due to low ethanol yield and high processing cost [1][4]. From the perspective of the above-mentioned issues, any freely available proven waste material containing a considerable amount of fermentable sugar with low conversion cost, has gained wide attention.

Presently many researchers working on producing bioethanol aim towards vegetable and fruit wastes as a feasible substrate, which

contains a high amount of fermentable sugar with limited processing steps [5][6].

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Overripe fruit mass (waste fruits) has been identified as an ideal substrate for bioethanol production with higher bioethanol yield [7], and *Pseudomonas mendocina* PM microorganism has been identified as an efficient microorganism to produce bioethanol through fermentation [8]. Sri Lanka being a tropical country, sugar-rich, mostly available fruits such as Banana, Papaya, Jackfruit, Watermelon, Star fruit, Sour soup, etc. are grown well in Sri Lanka. Out of those fruits, Banana, Papaya, and Jackfruit have been identified as the most sugar-rich fruits for the fermentation ethanol production [7]. It has been estimated that approximately 270000 tons of the harvested fruit in Sri Lanka is wasted annually due to improper post-harvesting activities [9] [10] [11]. Reference to Sarananda [9], about 20% of Banana, 46% of Papaya, 18% of Pineapple, 40% of Lime, 41% of Avocado, and 90% of Jackfruit of the annual production in Sri Lanka are wasted [9].

Commonly, bioethanol is produced by the fermentation of the substrate using a bacterium. During the fermentation process, the growth of bacterial cells takes place, which involves various biochemical networks and chemical reactions [12][13]. By varying various fermentation factors such as agitation, aeration temperature, pH, biomass concentration, type of microorganism, etc. the fermentation processes can be optimized [14] [15]. Thus, it is necessary to have a prior study on the optimization of the fermentation parameters to maximize the bioethanol yield. The conventional method of optimization is based on the change in one factor at one time while keeping the other factor constant. But the disadvantages of this method are a low degree of accuracy in the output, the necessity of a large number of experiments, waste of materials, and an increase in unreliability, etc. [16] [17].

Considering the drawbacks of the conventional optimization methods, some complex statistical oriented techniques such as Plackett-Burman, Central Composite Design, and Box-Behnken Design, etc. are available to predict, control, and optimize the bioprocess systems. Many researches have been reported on the optimization of ethanol production using response surface methodology with minimum numbers of tests. Using the test results, a surface plot is generated using advanced statistical software, which predicts the optimum values of each parameter referring to

the generated surface [18][19]. In addition, some other advanced techniques, such as GA and PSO, are recently used for optimization. In GA and PSO, a regression equation is employed as a fitness function for the optimization of the function in accordance with the constraints provided [20][21].

Accordingly, the present study was carried out by fermentation of selected freely available Sri Lankan rotten fruits without skin in sample scale to maximize the ethanol yield using optimization tools. Fermentation was carried out by varying several fermentation parameters according to a design model: Box-Behnken Design for response surface optimization in order to select the best fermentation parameters for maximum ethanol yield [22]. GA and PSO based optimization techniques were carried out for the validation of the results.

## 2. Materials and Methods

### 2.1 Selection of Fermentation Parameters

Banana, Papaya, and Jackfruit were selected by using a prior experiment as the most sugar-rich three types of fruits for the fermentation process and ethanol production [7]. The best fermentation temperature for many fruits using *Saccharomyces cerevisiae* microorganism is 27°C to 30°C [23][24][25]. In order to study the ethanol yield at higher temperatures, three different fermentation temperatures, 27, 32, and 35°C, were selected. Reference to the literature, it was identified that the best pH values for the fermentation of fruits are in the range of 4.0 to 6.0 [23]. Accordingly, three different pH values :4.3, 5.0, and 5.7 were selected from that range. Three different concentrations of the fruit mass were selected as 1:1, 1:1.5 and 1:2 (w:w) of fruit to distilled water ratio [24][25][26].

### 2.2 Raw Materials

Banana (embul variety), Papaya, and Jackfruit were obtained from the local market. The best ripening stage of each fruit was determined by a prior experiment, and fruits were selected accordingly [7].

### 2.3 Chemicals

Yeast extracts, peptone and dextrose, were obtained from Techno Pharma Chem (Delhi, India). Glucose was obtained from the local market. Agar, Ammonium sulfate and Magnesium sulfate heptahydrate were obtained from Marine Chemicals (India). Potassium dihydrogen phosphate was obtained from Loba Chemie (Pvt) Ltd. (India). Potassium iodide and potassium dichromate were obtained from BDH Limited (England), concentrated

sulphuric and sodium hydroxide were obtained from Dae-Jung Chemical & Metal Co., Ltd Korea, and sodium thiosulfate was obtained from British Drug Houses Ltd. (London, UK).

#### 2.4 Use of Microorganism and Media

Two types of microorganisms, baker's yeast and newly isolated naturally grown microorganism (PM) by a researcher of the Department of Molecular Biology and Biotechnology of University of Peradeniya (Gen Bank number of KU056820.1), were used for the fermentation. Pure cultures of baker's yeast were obtained by growing in standard Yeast Extract–Peptone–Dextrose (YPD) medium and pure cultures of PM were obtained by growing in standard glucose media [8].

#### 2.5 Sample Preparation

Fruit juices with different solid contents were prepared by blending rotten fruit mass (without skin) with distilled water. Fruit pulps of different fruit to water ratios (1:1, 1:1.5 and 1:2 (w:w)) were blended using a domestic blender (Panasonic mixer grinder, model number - MX-AC300). 100 ml of each juice were poured into sterile 250 ml conical flasks. pH (4.3, 5, 5.7) of each sample was adjusted using 0.1 NH<sub>2</sub>SO<sub>4</sub> and 0.1 N NaOH.

#### 2.6 Fermentation

Bacterial suspension of 1ml (0.5 McFarland) was added into the fruit samples in 250 ml conical flasks inside a laminar flow. The conical flasks were then air tightened with sterile cotton wool and aluminium foil to ensure the anaerobic condition, and incubated in a shaking incubator (AT 12 R) for the fermentation. Fermentation was carried out at three different pH values: 4.3, 5.0 and 5.7, three different temperatures: 29, 32, and 35°C at 85 rpm for about 96 hours with yeast and 144 hours with PM microorganism. 5 ml sample was taken out from each conical flask at 24hour intervals. The collected samples were centrifuged using a centrifuge (TOMY, Model No. suprema 21) for 20 min at -4 °C at 10,000 rpm to separate solid particles. The filtrate was collected to analyse

the amount of reducing sugar and the concentration of ethanol. Duplicate samples were fermented, and ethanol and sugar concentration were measured.

#### 2.7 Sugar and Ethanol Concentration

Sugar concentration of the filtrate was measured using a calibrated refractometer (ATAGO, Model number -1T) while the concentration of ethanol of the filtrate was analysed using Gas Chromatography (SHIMADZU, Model No. C 114850). The average results of the ethanol concentration are tabulated in Table 3.

#### 2.8 Validation of Ethanol Concentration

The ethanol concentration of few arbitrarily selected samples was then validated using dichromate reagent method and noted that the difference in readings between the two results was very small [27].

#### 2.9 Statistical Analysis

Box–Behnken type Response Surface Method was used as the experimental method to optimize the ethanol yield obtained by fermentation of different fruits with each microorganism (Table 1). The three factors (temperature, pH and concentration of fruit) with two levels (-1 for low level and +1 for high level) and each factor with one centre point (0-center points) were selected as the most influential factors to see their effect on ethanol yield (Table 2) [22].

**Table 1 – Layout of the Experiment**

Experiment No.	Fruit	Inoculum
1	Banana	<i>Saccharomyces cerevisiae</i>
2		PM
3	Papaya	<i>Saccharomyces cerevisiae</i>
4		PM
5	Jackfruit	<i>Saccharomyces cerevisiae</i>
6		PM

**Table 2 - Data Used for the Experimental Design**

Symbol	Test variable	Coded level		
		-1	0	1
X <sub>1</sub>	Temperature	Room (29°C)	32°C	35°C
X <sub>2</sub>	pH	4.3	5	5.7
X <sub>3</sub>	Fruit to water concentration/(w:w)	1:1	1:1.5	1:2



**Table 3 - Box-Behnken Design with Three Levels, the Observed and Predicted Ethanol Concentrations**

Run order	Ethanol concentration / (vol%)											
	Banana				Papaya				Jackfruit			
	Yeast		PM		Yeast		PM		Yeast		PM	
	Obs.	Pre.	Obs.	Pre.	Obs.	Pre.	Obs.	Pre.	Obs.	Pre.	Obs.	Pre.
1	10.3	10.1	12.6	13.0	4.0	3.9	4.4	4.3	5.6	5.3	6.9	7.0
2	8.4	8.5	10.3	10.7	4.2	4.3	4.7	4.8	5.5	5.9	5.1	4.9
3	7.5	7.4	10.9	10.3	4.4	4.2	4.4	4.5	5.8	5.8	5.8	5.8
4	9.4	9.6	10.7	10.5	4.7	4.8	4.7	4.7	6.4	6.0	7.2	7.2
5	7.1	7.0	10.3	10.1	4.9	4.8	5.4	5.0	5.8	5.6	5.9	6.1
6	8.1	8.1	10.9	11.3	4.8	4.9	4.8	4.9	5	5.4	4.6	4.7
7	9.6	9.5	12.3	12.4	3.8	3.9	4.5	4.8	5.9	5.7	6.8	7.1
8	9.7	9.6	12.1	11.8	4.4	4.5	4.7	4.8	5.5	6.0	6.3	5.7
9	9.2	9.2	11.7	12.0	4.6	4.5	5	4.9	6.6	6.5	7	6.9
10	9.2	9.2	12.0	12.0	4.1	4.5	5	4.9	6.5	6.5	6.9	6.9
11	7.3	7.4	10.8	10.3	4.1	4.2	4.4	4.5	6	5.8	5.8	5.8
12	8.7	8.7	11.9	11.9	4.6	4.6	5	5.0	5.9	5.9	5.6	6.4
13	9.5	9.0	12.3	11.2	4.1	4.7	4.4	5.0	5.8	6.3	6.9	7.2
14	8.1	8.1	11.2	11.3	5.1	4.9	4.9	5.1	5.1	5.4	4.8	4.7
15	9.2	9.2	12.0	12.0	4.5	4.5	5	4.9	6.3	6.5	6.9	6.9
16	9.7	9.6	10.1	10.5	4.7	4.8	4.7	4.7	6.2	6.0	7.2	7.2
17	8.5	8.5	13.3	13.2	3.5	3.6	4.8	4.4	5.4	5.6	6.5	6.5
18	8.9	9.0	13.2	13.0	3.6	3.5	4.8	4.6	4.9	5.4	7.1	6.8
19	9.6	9.6	11.9	12.0	3.9	4.0	4.6	4.5	5.6	5.9	6.6	6.5
20	8.9	9.0	13.2	13.0	3.4	3.5	4.8	4.6	5.1	5.4	7.1	6.8
21	8.5	8.5	10.3	10.7	4.3	4.3	4.6	4.8	5.6	5.9	5.2	4.9
22	8.7	8.7	11.9	11.9	4.5	4.6	5.1	5.0	6.1	5.9	5.8	6.4
23	9.9	10.1	12.7	13.0	3.7	3.9	4.5	4.3	5.3	5.3	7	7.0
24	9.6	9.6	12.5	11.8	4.5	4.5	4.8	4.8	5.5	6.0	6.3	5.7
25	8.6	8.5	13.5	13.2	3.6	3.6	4.7	4.9	5.3	5.6	6.5	6.5
26	9.1	9.2	11.9	12.0	4.6	4.5	4.8	4.9	6.5	6.5	6.7	6.9
27	9.2	9.2	12.1	12.0	4.7	4.5	5	4.9	6.3	6.5	7	6.9
28	7	7.0	10.2	10.1	4.9	4.8	5.2	5.0	5.8	5.6	5.8	6.1
29	9.1	9.2	11.8	13.1	4.5	3.7	4.8	4.7	6.5	5.8	6.7	7.1
30	9.6	9.6	11.7	13.3	4.1	3.5	4.3	4.0	5.8	4.8	6.5	6.7

In this experiment, the design matrix was setup using Box-Behnken three-factor design matrix with three centre runs and two replicates (30 experimental runs per one fruit type) with two types of microorganism, counting 180 experimental runs (Table 3) [28]. Table 3 shows the experimental ethanol concentrations, denoted as "Obs", with the predicted ethanol concentration denoted as "Pre". Predicted ethanol concentrations were obtained from the regression equations, obtained from statistical analysis.

Box-Behnken Response Surface Design was analysed using MINITAB17 software to fit models to the different sets of experimental data while identifying the significant factors and their optimum levels to find the maximum yield. In addition, regression analysis, analysis of variance (ANOVA), and response optimizer

were also carried out to determine the optimized condition. Multiple box plots were taken from the same software to study the variability in the different data sets. Finally, 3-D surface plots were taken to study the effect of different factors on the ethanol concentration. Optimization was reconfirmed using GA analysis and PSO optimization tools by using the regression equations, obtained from RSM analysis as the fitness functions. GA optimization was carried out using MATLAB R2015a GA optimization tool, while PSO optimization was carried out with MATLAB R2015a software by programming with MATLAB. GA optimization was carried out by taking the lower bound as -1 and upper bound as +1 for all the factors and repeated several times with different initial conditions to see whether the optimization results vary with different initial conditions. After a different

number of iterations ranging from 64 to 124, with different fruit types, the global maximum of ethanol yield was obtained. PSO optimization was carried out with 500 iterations taking the lower and upper bound of the test variables as -1 and +1 for every three variables. ANOVA results for the Banana data are tabulated in Table 4 and obtained regression coefficients, P-values, and correlations ( $R^2$ ) for the three types of fruit, with two different microorganisms are tabulated in Table 5. Optimization results obtained by different optimization tools are tabulated in Table 6.

### 3. Results and Discussion

Optimization results obtained by three different optimization tools: RSM, GA, and PSO, are shown in Table 6. Reference to the results obtained from three different optimization tools, all the results seem similar, and the results of RSM and GA tools are exactly the same. Considering the overall optimization results in Table 6, the maximum predicted ethanol concentration of 13.39 vol% was determined by optimization with RMS from Banana (embul kesel variety) by fermentation with PM microorganism, under 1:1(w:w) substrate concentration, pH 5.1 and 35°C temperature conditions. The optimization results using RMS have reconfirmed GA optimization results, however, it can be noted that optimum ethanol concentration has been reduced by optimization using PSO with a concentration of 13.36 vol% with the same fermentation conditions only with a small reduction of pH value to 5.07. Significant regression models at the 5% level with high correlation values ( $R^2$ ) were obtained as shown

in Table 5. High correlation coefficient confirmed that the predicted ethanol concentration and the actual concentration in Table 3 are very close to each other. It further reveals that the experimental data could be accepted [28].

#### 3.1 Analysis of RSM Results

##### 3.1.1 Analysis of Boxplot

Figure 1 shows the boxplot of variation of the experimental ethanol concentrations of different fruits with different microorganisms. Reference to the Figure 1, the highest ethanol concentration has been achieved from Banana fruit by fermentation with PM microorganism. Second highest ethanol concentration could also be observed in Banana fruit fermentation with traditional yeast. Papaya and Jackfruit show very low ethanol concentration for the fermentation with both PM and traditional yeast. The results obtained for Papaya and Jackfruit can be comparable to the results discussed in the literature [24][25][26].

##### 3.1.2 ANOVA Results

Table 4 shows the ANOVA analysis results for the Banana fruit by fermentation with PM microorganism and traditional yeast. Statistical analysis results show that three main factors (PH, Temperature, and fruit to water concentration) show a significant effect on ethanol concentration of Banana fruit fermentation with both microorganisms (*Saccharomyces cerevisiae* and PM) at a 5% significant level. Furthermore, reference to the Table 5, ANOVA results show that two-way interactions ( $X_1X_2$  and  $X_2X_3$ ) and a square term

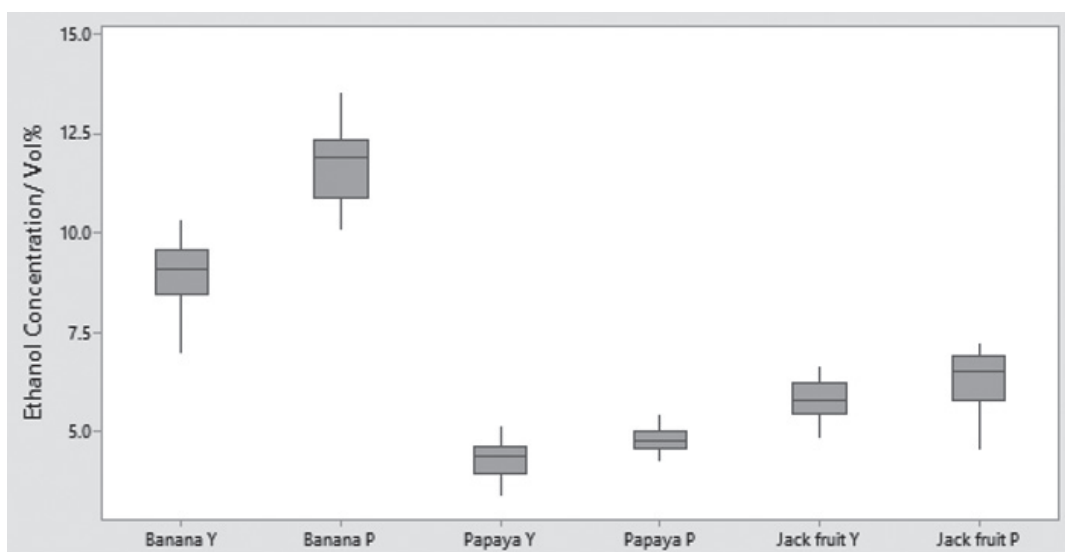


Figure 1 - Boxplot of Variation of Ethanol Concentration with Different Fruits and Microorganisms



**Table 4 - ANOVA for the Quadratic Polynomial Model on the Ethanol Production - Banana**

Source	Degrees of freedom (DF)		Sum of squares		Mean square (MS)		F-value		P-value	
	Yeast	PM	Yeast	PM	Yeast	PM	Yeast	PM	Yeast	PM
Regression	9	6	20.4091	26.8212	2.26768	4.4707	216.40	79.55	0.000	0.000
Linear	3	3	10.7488	23.3100	3.58292	7.7700	341.91	138.27	0.000	0.000
Square	3	1	4.6228	1.2590	1.54094	1.2590	147.05	22.40	0.000	0.000
Interaction	3	2	5.0375	1.6029	1.67917	0.8015	160.24	14.26	0.000	0.000
Residual error	20	23	0.2096	1.2924	0.01048	0.0562	-	-	-	-
Lack of fit	3	6	0.0262	0.5774	0.00875	0.0962	0.81	2.29	0.505	0.084
Pure error	17	17	0.1833	0.7150	0.01078	0.0421				
Total	29	29	20.6118	28.1137						

**Table 5 - Regression Coefficients and P Values**

Model term	Banana				Papaya				Jackfruit			
	Yeast		PM		Yeast		PM		Yeast		PM	
	Regre. Coef.	P-value	Regre. Coef.	P-value	Regre. Coef.	P-value	Regre. Coef.	P-value	Regre. Coef.	P-value	Regre. Coef.	P-value
X <sub>1</sub>	0.7625	0.000	1.1375	0.000	-0.4362	0.000	-0.0938	0.003	-0.0812	0.016	0.7375	0.000
X <sub>2</sub>	0.2188	0.000	0.3291	0.000	-0.2363	0.000	-0.1063	0.001	-0.2750	0.000	-0.0625	0.024
X <sub>3</sub>	0.2063	0.000	-0.2901	0.000	-0.0875	0.030	-0.1750	0.000	0.1813	0.000	0.4125	0.000
X <sub>1</sub> X <sub>1</sub>	-0.7583	0.000	-	-	-0.3084	0.000	-0.1288	0.005	-0.6688	0.000	-0.5396	0.000
X <sub>2</sub> X <sub>2</sub>	0.0792	0.048	-0.4155	0.000	-	-	-	-	-0.2312	0.000	-0.0896	0.027
X <sub>3</sub> X <sub>3</sub>	0.1292	0.003	-	-	-	-	-0.1913	0.000	-0.3437	0.000	-0.3396	0.000
X <sub>1</sub> X <sub>2</sub>	-0.5125	0.000	0.2125	0.018	-	-	0.2500	0.000	-0.1500	0.003	0.2375	0.003
X <sub>1</sub> X <sub>3</sub>	0.5625	0.000	-	-	0.2500	0.000	-	-	-	-	-0.1625	0.000
X <sub>2</sub> X <sub>3</sub>	-0.2250	0.000	0.4073	0.000	0.1500	0.010	-	-	-0.1875	0.000	-0.3125	0.000
R <sup>2</sup>	98.98 %		95.40%		91.23 %		85.31 %		95.17%		98.69%	
Adj R <sup>2</sup>	98.53 %		94.20 %		88.94 %		81.48 %		93.33 %		98.11 %	
Pre R <sup>2</sup>	97.47 %		91.28 %		96.10 %		73.61 %		89.92 %		97.31 %	

**Table 6 - Optimization Results**

Fruit	Inoculum	Optimization tool	Temperature /°C	pH	Concentration /(w:w)	Predicted max. vol% ethanol concentration
Banana	<i>Saccharomyces cerevisiae</i>	RSM	35.0	4.3	1:2	10.67
		GA	35.0	4.3	1:2	10.67
		PSO	35.0	4.3	1:2	10.67
	PM	RSM	35.0	5.10	1:1	13.39
		GA	35.0	5.11	1:1	13.39
		PSO	35.0	5.07	1:1	13.36
Papaya	<i>Saccharomyces cerevisiae</i>	RSM	Room (29)	4.3	1:1	5.01
		GA	Room (29)	4.3	1:1	5.01
		PSO	Room (29)	4.3	1:1	5.01
	PM	RSM	Room (29)	4.3	1:1.3	5.29
		GA	Room (29)	4.3	1:1.3	5.29
		PSO	Room (29)	4.3	1:1.4	5.25
Jackfruit	<i>Saccharomyces cerevisiae</i>	RSM	31.9	4.6	1:1.6	6.56
		GA	31.9	4.6	1:1.6	6.56
		PSO	31.0	4.6	1:2	6.33
	PM	RSM	32.9	4.3	1:2	7.27
		GA	32.9	4.3	1:2	7.27
		PSO	32.8	4.3	1:2	7.23

( $X_2X_2$ ) have a significant effect on the ethanol concentration of Banana fruit by fermentation with PM microorganism at a 5% significance level. There is a significant effect from all three-square terms ( $X_1X_1$ ,  $X_2X_2$ , and  $X_3X_3$ ) and three two-way interactions ( $X_1X_2$ ,  $X_1X_3$ , and  $X_2X_3$ ) on ethanol concentration of Banana fruit fermentation with traditional yeast at 5% significance level.

### 3.1.3 Regression Equations

Regression coefficients and the p values obtained from ANOVA analysis for the fermentation of three fruit types with both microorganisms, PM and traditional yeast (six sets of experimental data), are tabulated in Table 5. Reference to Table 5, it could be noticed that all three main factors ( $X_1$ ,  $X_2$  and  $X_3$ ) have a significant effect on ethanol concentration of three types of fruit fermentation, with both microorganisms at 5% significance level. The square term  $X_1X_1$  shows a significant effect on all three fruits except Banana fermentation with PM. The square term  $X_2X_2$  has a significant effect on Banana and Jackfruit fermentation with both microorganisms. The  $X_3X_3$  square term also shows a significant effect on all fruits except Banana fermentation with PM and Papaya fermentation with yeast. The two-way interaction term,  $X_1X_2$  shows a significant effect on all three types of fruits except Papaya fermentation with yeast. The  $X_1X_3$  two-way interaction has a significant effect on Banana and Papaya fermentation with yeast, and Jackfruit fermentation with PM. The  $X_2X_3$  two-way interaction has a significant effect on all three types of fruit except Papaya fermentation with PM. Furthermore, the final regression models explain the majority of the variation at a satisfactory level with  $R^2 > 85.31\%$ . Model adequacy was tested for all the models by checking residual plots, four in one plot, etc. which have been produced from MINITAB 17 statistical software. It was noted that all the models fulfilled the assumptions: homoscedasticity, normally distributed residuals, and independence of residuals (no autocorrelation), and therefore it implies the "model adequacy" [28].

Predicted ethanol concentration in Table 3 was obtained by substituting the relevant coded levels to the six equations. Reference to Table 3, it clearly shows that the highest ethanol concentration can be obtained using Banana fermentation with PM. Second highest ethanol concentration can be obtained using Banana

with traditional yeast. The negligible difference in the predicted and the observed ethanol concentration could be expected due to experimental errors.

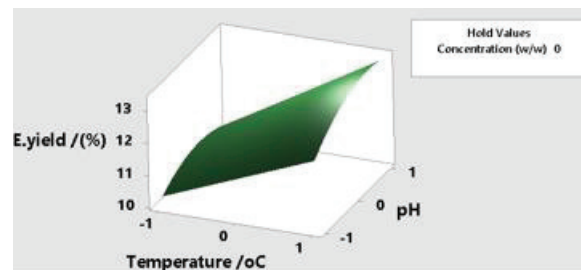
Out of the obtained six regression equations from regression analysis, the regression equation of the selected fruit: Banana by fermentation with PM microorganism is given in equation 1.

$$Y_{(\text{Banana, PM})} = 11.9571 + 1.1375X_1 + 0.3291X_2 - 0.2901X_3 - 0.4155X_2^2 + 0.2125X_1X_2 + 0.4073X_2X_3 + \epsilon \quad \dots(1)$$

where  $\epsilon$  is the random error.

### 3.1.4 Three-dimensional surface plots for the ethanol yield of Banana fruit by fermentation with PM

Three-dimensional surface plots were generated for the pair-wise combination for ethanol production using Banana fruit with PM microorganism for each test variable: temperature, pH, and biomass concentration, as shown in Figure 2, Figure 3, and Figure 4. pH range (the hydrogen ion concentration in the fruit pulp) is an important factor affecting cell growth and metabolite production and thereby the rate of fermentation [29]. Figure 2 shows that ethanol concentration significantly increases with increasing temperature.



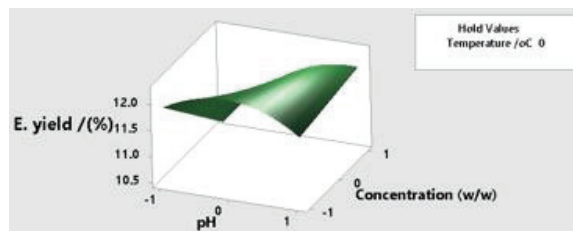
**Figure 2 - Surface Plot for the Effect of Type of Temperature and pH on the Ethanol Concentration**

The highest ethanol yield was observed at 35°C temperature, and pH ~5. Further, it could be noted that both pH and temperature are crucial factors for the fermentation of fruits for ethanol production [30]. Reference to the surface plot in Figure 3, the highest ethanol yield could be obtained at pH ~ 5, and fruit to water concentration of 1:1 (w:w).

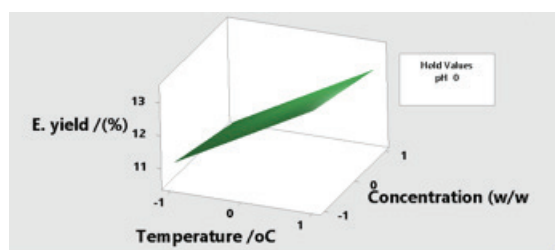
Reference to Figure 4, ethanol yield is increasing linearly with increasing fermentation temperature. The highest ethanol concentration could be obtained at 35°C temperature and 1:1



(w:w) fruit to water concentration. Finally, the highest ethanol concentration could be obtained by fermentation Banana fruit using PM when the temperature is set at 35°C, pH value is set at 5, and fruit to water concentration is set at 1:1 (w:w).



**Figure 3 - Surface Plot for the Effect of pH and Biomass Concentration on the Ethanol Concentration**



**Figure 4 - Surface Plot for the Effect of Temperature and Biomass Concentration on the Ethanol Concentration**

### 3.2 Optimization with Genetic Algorithm (GA)

GA optimization results obtained with different initial conditions were compared to see whether the initial parameters have an effect on the optimization results and noted that the maximum ethanol concentration is the same with different initial conditions for all the fruit types with two different microorganisms, studied. By comparing the overall optimization results in Table 6, it could be noted that the optimization results obtained from GA and RSM are almost the same.

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### 3.3 Particle Swarm Optimization

For PSO, optimization was carried out with 500 iterations to obtain global maximum values for the maximum ethanol concentrations. Reference to Table 6, the optimum ethanol concentrations obtained from PSO optimization are quite close to the results obtained from RSM and GA.

## 4. Conclusion

This study investigated ethanol production using three different, freely available, Sri Lankan fruits by fermentation with two different micro-organisms under different fermentation conditions to optimize the ethanol yield. Considering the experimental results, the following conclusions could be made.

1. Overripe Banana (embul kesel variety) fruit mass could be used for ethanol production by fermentation with PM, a novel microorganism.
2. A maximum ethanol concentration of 13.39 % (v/v) could be obtained by fermentation of Banana (embul kesel variety) fruit mass with PM microorganism at 35°C, pH=5 with fruit to water concentration of 1:1 (w:w) .
3. Relatively same optimization results were obtained by optimization of the ethanol production process using RSM GA and PSO.
4. Commercial-scale ethanol production could be recommended from Banana (embul kesel variety) by fermentation using PM microorganism.

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Financial assistance by the University Grant Commission (UGC) is highly appreciated (*Grant No. UGC/VC/DRIC/PG2015(III)/PDN/01*).



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