FERMENTATION OF SUGARCANE MOLASSES BY SACCHARAMYCES CEREVESIA: EFFECTS OF OPERATING PARAMETERS ON ETHANOL PRODUCTION

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ABSTRACT

Pakistan has more than 70 sugar manufacturing industries which are responsible for massive production of molasses. Typically the molasses is converted to ethanol through fermentation process. A pilot plant has been designed and fabricated to investigate the effect of process parameters for the sustainable production of ethanol from sugar cane molasses. The effect of various experimental parameters such as fermentation time, molasses solution concentration and its pH during ethanol recovery from sugarcane molasses has been investigated. The interaction effects, optimum conditions, and percent contribution of each factor were determined statistically. The maximum ethanol recovery was found to be 24.65 gm/L when pH of molasses solution was 4.5, concentration of molasses solution was 20 gm/kg and fermentation time was 56 hours.

KEYWORDS: *Waste treatment; Fermentation; Ethanol production*

1. INTRODUCTION

The emission of gasses during burning of fossil fuels can cause the environmental impacts and reserves of fossil fuels are declining day by day[1]. While the transportation mainly depends upon non-renewable liquid fuels like as diesel and gasoline and these fuels contribute 40% of the total energy consumption in the world[2]. Therefore, focus of the research has been diverted to investigate renewable energy sources to overcome the energy crises, pollutants emissions and greenhouse gases[3]. Biomass has proven to be a major renewable energy source for the supply of chemicals, materials and energy in future[4].

Ethanol is a best option in the liquid biofuels which can be employed as a substitution for fossil fuels and is strongly recommended by European Union[5, 6]. It is mostly employed as a renewable energy source for the production of pure fuel or it can be blended with gasoline[7]. For the fuel purpose, 10 to 85 % of ethanol by volume can be blended with gasoline[8, 9]. Therefore the addition of ethanol in gasoline has been implemented by many countries[10, 11].

In crop production of sugarcane, Pakistan is the 5th largest country where sugarcane export is 20% [12]. The total ethanol production in the world is about 40 billion liters for which about 80% of world's molasses has been used[13]. Annual production of molasses in Pakistan is around 2 million tons while 1.45 million tons were exported at a nominal rate of 35 dollars per ton, and the earning was about 47 million dollars in 2004. The ethanol production depends upon the quality of molasses while from one ton molasses about 240 to 270 liters ethanol has been produced. If the annually production of molasses in Pakistan (2 million tons) has been processed for ethanol production, so 500 million tones ethanol will be produced[13]. The 497 million liters alcohol is exported from 500 million tons alcohol while the remaining 2.5 million liters is consumed locally[14]. By exporting the same quantity of ethanol with average price of 360 dollars per ton the country can earn around 144 dollars millions per annum[13]. Pakistan is the second largest exporter of sugarcane bio ethanol to the European Union[14]. When the ethanol is used as a fuel instead of petroleum fuel in Pakistan, the emission of greenhouse gas will be reduced from 20% to 50%[15].

The ethanol can be produced from different materials such as wheat, sugarcane, corn, cassava and sugar beet[16, 17]. So there is an urgent need to identify and expand the new technologies using for the production of bio fuels. One way is to enhance the production of ethanol by providing the total use of straw and bagasse in sugarcane. For this purpose the sugarcane is depolymerized by means of hydrolysis, so the fractions of hemicelluloses and cellulose are converted to fermentable sugars which can be later on fermented to ethanol[18].

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The Saccharomyces cerevisiae yeast is mostly used for ethanol production from renewable biomass like as sugar beet, molasses and sugar cane[19]. In bioconversion process the Saccharomyces cerevisiae is mostly used as a biocatalyst and it can be employed for ethanol production under specific conditions from sugar cane molasses[20]. The process for ethanol production may be batch, continuous or fed batch process[21]. The sugarcane molasses is mostly produced in Brazil and the low cost of molasses is an important aspect for the production of substances through fermentation process[22].

This study focuses on the statistical analysis of ethanol recovery from sugar cane molasses. Statistical analyses for experimental results are performed and for the process parameters such as molasses solution concentration, pH of molasses solution and fermentation time the optimum condition are determined. The regression and ANOVA models have been presented for the experimental results. The percentage contributions for the process parameters are determined. The interaction effects, response surface plots and contour plots for the process parameters are presented.

2. MATERIAL AND METHODS

2.1 Microorganism and media

In pre-fermentor, the molasses which generally have 50-55% of sugar content was diluted to the required concentration. The concentration was measured by using refractive meter. The refractive index of the solution was checked regularly after dilution with water in order to decrease its concentration from 50-55% up to desired concentration. After getting the desired concentration, the urea, ammonium phosphate and nitro phosphate was added which acts as a food for yeast and help in their survival and growth. These can be added as each one has two gram per liter. After addition of urea and phosphate the pH was become five but it was further adjusted to the required value of acidity of solution in order to make a suitable environment for the incoming yeast. A few drops of sulfuric acid were added in order to adjust the desired value of pH. Then 4 gm/L of saccharomyces cerevesiae yeast was added. These all processes were carried out in pre-fermentor in order to provide such an environment which will increase the growth rate of saccharomyces yeast up to the maximum level. The pre fermentation process was carried out for eight hours at a temperature range from 20 °C to 25 °C in pre-fermentor. If the temperature was increased from 25 °C in pre-fermentor so the fermentation will be start instead of proper growth of yeast, for this purpose the cold water will exchange heat with solution in summer season. The ethanol production at this stage was controlled because it has adversely affected the growth of yeast.

2.2. Experimental procedures

For experimental analysis a pilot plant was used as shown in Figure 1. It consists of pre- fermentor, fermentor, water storage tank, centrifugal pump, stand, stirrer and cupper coils. Iron pipe of 0.0127 m in diameter is used for the flow of water from the water storage tanks. The materials used for the construction of fermentor and water storage tanks were acrylic glass and galvanized iron respectively. Rotary evaporator was used for distillation of fermentation mixture and refractory meter was used to find out the refractive index of the molasses solution.

In fermentation process, the already grown yeast was used to convert the sugar molecules to ethyl alcohol (ethanol) and carbon dioxide. The reaction was exothermic and the decomposition of each mole of glucose gave -31 kJ/kg of energy as a result the temperature of the solution was increased. The favorable temperature range for the fermentation process is 25 °C to 40 °C. If the temperature was increased from 40 °C, the yeast will be killing down, so for this purpose the cold water will exchange heat in summer season and the hot water will exchange heat in winter season in order to get the desire temperature for favorable fermentation process.

The ethanol concentration in the solution was gradually increased as the fermentation process proceeds, until it was reached up to the maximum or critical level. At that level the ethanol concentration was so enough to stop yeast from fermentation process. The fermentation takes about 40 to 60 hours but time factor is also important for industrial scale. The process was become uneconomical for time above 60 hours. When the fermentation process was completed, the fermentation product undergoes distillation in rotary evaporator in order to separate the ethanol from the water and residue. The mass of the yeast in the forms of solid was obtained

in residue. The ethanol has very low concentration in the solution (almost 10%) which was increased up to 90% by distillation.

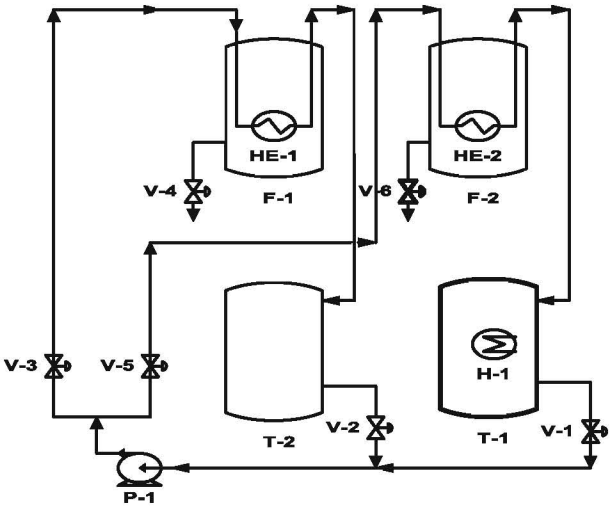

Figure 1 Schematic diagram of ethanol production from sugarcane molasses. T-1 – hot water storage tank; T-2 – cold water storage tank; H-1 – heater; P-1 – centrifugal pump; F-1,2 – Fermentors; HE-1,2 – Heat exchangers; V-1,2,3,4,5,6 – flow control valves

2.3. Statistical analysis

For experimental analysis the three main factors such as concentration of molasses solution, pH of molasses solution and fermentation time were selected. The two levels were selected for each main factor as presented in Table 1.

Two levels factorial was employed for statistical analysis. The experiments which are performed at different conditions of the operating parameters are presented in Table 2. The ethanol recovery obtained after experiments are presented in the last column of the design matrix.

Table 2: Two level factorial design matrix

Runs	Concentration (gm/kg)	pH	Fermentation Time (hours)	Ethanol Recovery(gm/liter)
1	15	4.5	40	16.73
2	15	4	40	11.26
3	15	4	56	12.51
4	15	4.5	56	19.21
5	20	4.5	56	24.65
6	20	4.5	40	21.94
7	20	4	56	13.67
8	20	4	40	12.75

3. EXPERIMENTAL DESIGN

3.1. Screening of experiments

The ethanol recovery is affected by the concentration of molasses solution. For experimental analysis 15 to 20% of molasses concentrations were selected because above 20% concentration will cause fermentation process start in pre-fermentor while below 15% was not enough for yeast. Hafiz et al. (2012)[23] have also observed the same results and they have obtained the maximum yield of alcohol at 20% of molasses solution. The minimum and maximum pH of molasses solutions was taken 4.0 and 4.5 respectively. Above or below the selected pH the growth of yeast will be stop and as a result the ethanol recovery will be reduced. Maiorella et al. (1984)[24] have also predicated the same observations and obtained maximum ethanol at 4.5 pH. For experimental work 40 and 56 hours were selected as a fermentation time. Above 56 hours ethanol recovery will remained constant and production time will increases while the time was not sufficient for optimum ethanol recovery below 40 hours. Kanwal Manzoor, et al. (2012)[25] have also observed the

Table 1: Actual and codified values for each main factor

S.No	Factors	Natural values		Codified values	
		Min	Max	Min	Max
1	Concentration (gm/kg)	15	20	-1	+1
2	pH	4	4.5	-1	+1
3	Fermentation Time (hours)	40	56	-1	+1

same results by considering fermentation time from 24 to 144 hours.

3.2 Regression model

A regression model was obtained through two level factorial design from (Design Expert 8.0.3 trail version) software. This model is given by Equation 1 in terms of coded factors.

$$\text{Ethanol Recovery (gm/L)} = -69.6 + 0.669 * A + 16.2 * B + 0.116 * C \quad (1)$$

The *"R-Squared"* value for this model is 0.945. Where A is the concentration of molasses solution (gm/kg), B is pH of molasses solution, C is fermentation time (hours).

3.3 ANOVA (Analysis of variance) mode

The ANOVA (Analysis of variance) model is shown in Table 3 which was obtained when experimental data was fitted in 2-level factorial design. The ANOVA model shows that concentration of molasses solution and pH of molasses solution are more significant.

The Model F-value of 553.52 indicates that the model was significant. There is only a 3.25% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate that model terms were significant. In this case concentration of molasses and pH of molasses solution were significant model terms.

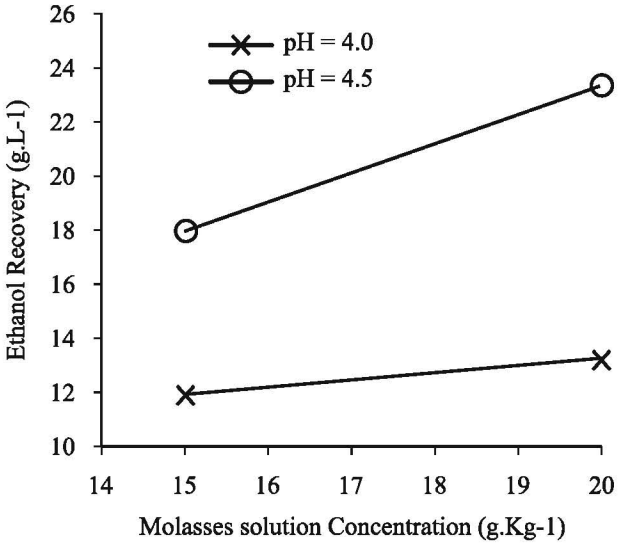

Figure 2: Interaction effect of molasses solution concentration and its pH

4. RESULTS AND DISCUSSION

4.1. Effect of process parameters and their interactions

The effect of main factors and their interactions such as molasses solution concentration and pH of molasses solution is shown in Figure 2. It was observed from Figure 2 that ethanol production increased when pH of

Table 3: ANOVA model

Response 1 Ethanol Recovery						
ANOVA for selected factorial model						
Analysis of variance table [partial sum of squares – Type III]						
Source	Sum of squares	df	Mean square	F value	P-value Prob>F	
Model	170.04	6	28.34	553.52	0.0325	Significant
A-Concentration	22.38	1	22.38	437.07	0.0304	
B-pH	131.38	1	131.38	2566.06	0.0126	
C-Fermentation time	6.92	1	6.92	135.14	0.0546	
AB	8.16	1	8.16	159.39	0.0503	
AC	5.000E-005	1	5.000E-005	9.766E-004	0.9801	
BC	1.20	1	1.20	23.46	0.1296	
Residual	0.051	1	0.051			
Cor total	170.09	7				

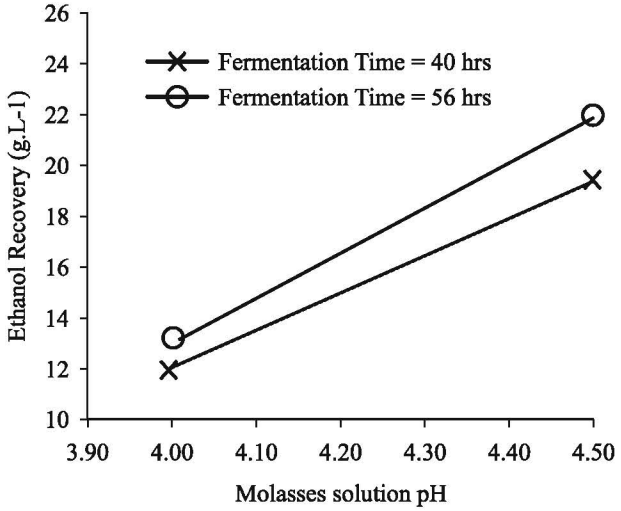

Figure 3: Interaction effect of fermentation time and pH of molasses solution

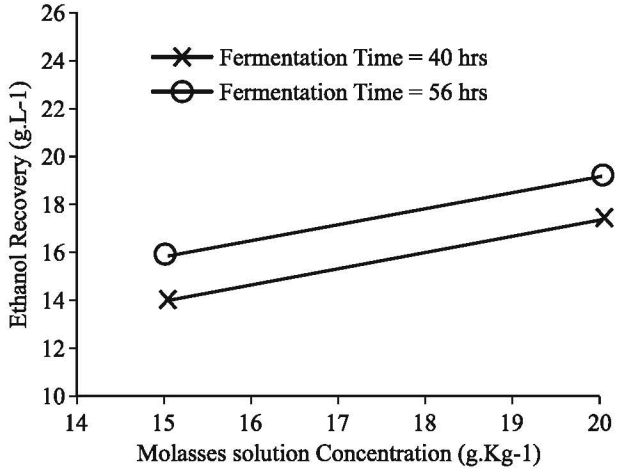

Figure 4: Interaction effect of molasses solution concentration and fermentation time

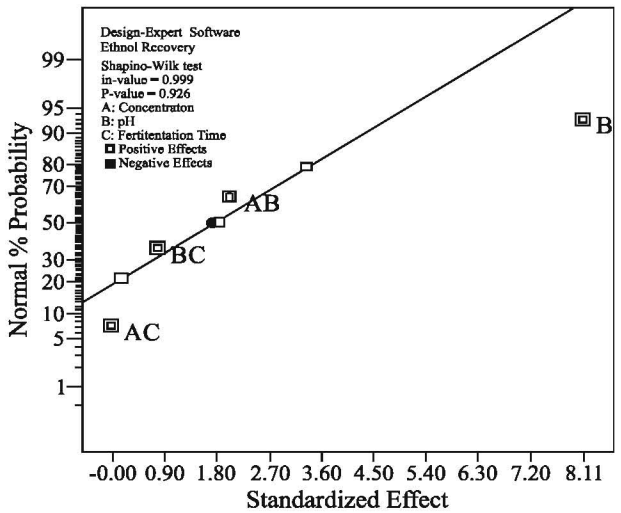

Figure 5: Normal plot from two level factorial design

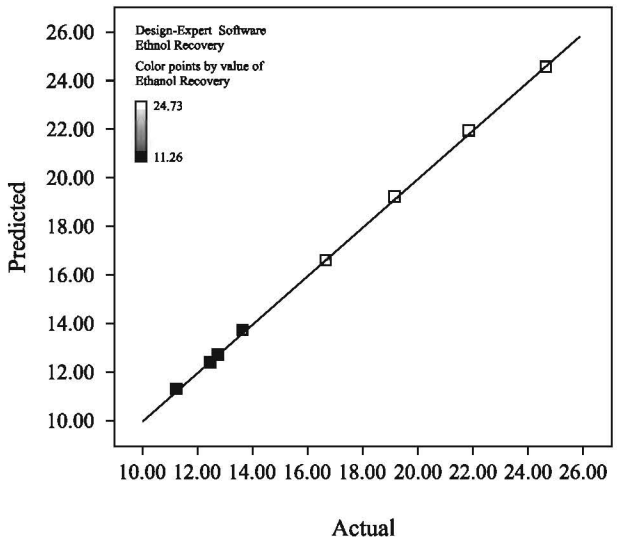

Figure 6: Predicted verses actual plot

the molasses solution was increased from 4.0 to 4.5 and concentration of molasses solution was also increased from 15% to 20%.

The interaction effect of fermentation time and pH of molasses solution is shown in Figure 3. The interaction effect shows that ethanol production increased with the increase of fermentation time from 40 to 56 hours while pH has also a significant effect on ethanol production.

The interaction effect of fermentation time and molasses solution concentration is shown in Figure 4. Figure 4 indicates that ethanol production was increased when concentration of molasses solution was increased from 15 to 20% and fermentation time was increased from 40 to 56 hours.

4.2. Combine effects of process parameters

In the normal plot as shown in Figure 5, the molasses solution concentration its pH and fermentation time were represented by A, B and C respectively. The figure indicates that pH of the molasses solution lies on the right side of the normal plot representing that pH of molasses solution has positive and significant effect on ethanol production when it was increased from 4.0 to 4.5 The interaction effect of fermentation time and molasses solution concentration has significant effect compared to the main effects of fermentation time and molasses solution concentration.

Table 4: Effects of main factors and their interactions

Term	Standardized effects	Sum of squares	% Contribution
A- Concentration	3.34	22.38	13.16
B-pH	8.10	131.38	77.24
C-Fermentation time	1.86	6.92	4.07
AB	2.02	8.16	4.80
AC	-5.000E-003	5.000E-005	2.940E-005
BC	0.78	1.20	0.71
ABC	0.16	0.051	0.030

The predicated verses actual plot as shown in Figure 6 represents that all the experimental data lies on the diagonal and not so scattered, which shows that the experimental data was accurate and there was no noise factor in the experimental data.

The Table 4 illustrates that pH has more percentage contribution (77.24%) and fermentation time has less percentage contribution (4.07%) compared to other main factors. The main factors and interactions which have more standardized effects will be more significant. In Table 4 the concentration, pH and its interaction (AB) were more significant compared to other main factor and interactions because they have more standardized effects.

The residual verses predicated plot shows that all the experimental data were within the range indicates that there were no outliers in the experimental data as shown in Figure 7.

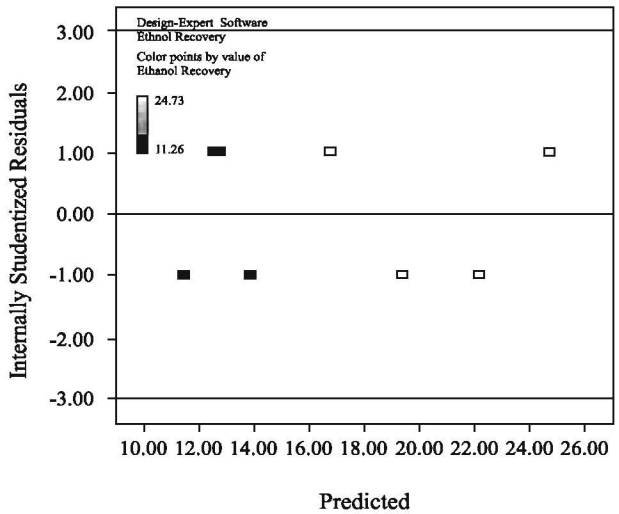

Figure 7: Response plot for residual verses predicated

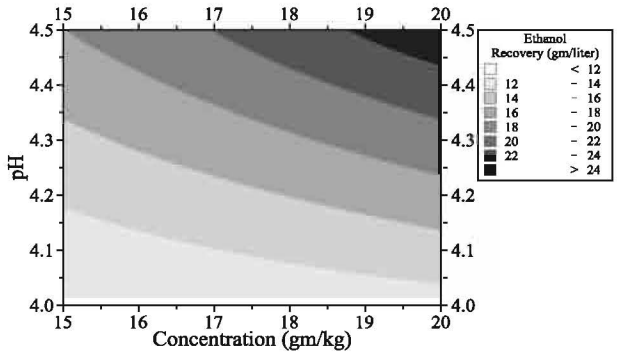

Figure 8: Contour plot for pH and concentration of molasses solution

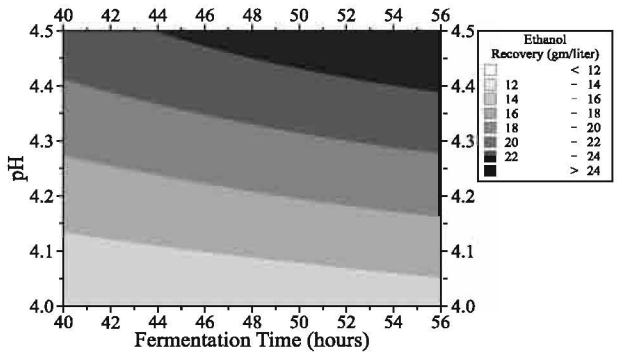

Figure 9: Contour plot for pH of molasses solution and fermentation time

The ethanol recovery was increased when pH of molasses solution and its concentration was increased as shown in counter plot of Figure 8. The maximum ethanol obtained from 22-24 gm/L when pH of molasses solution was in range from 4.45 to 4.5 and concentration was from 19 to 20 percent.

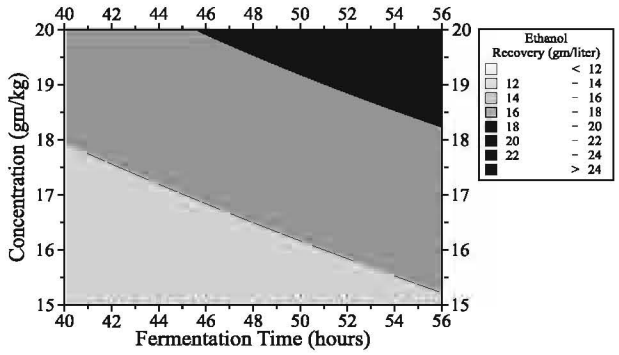

Figure 10: Contour plot for concentration of molasses solution and fermentation time

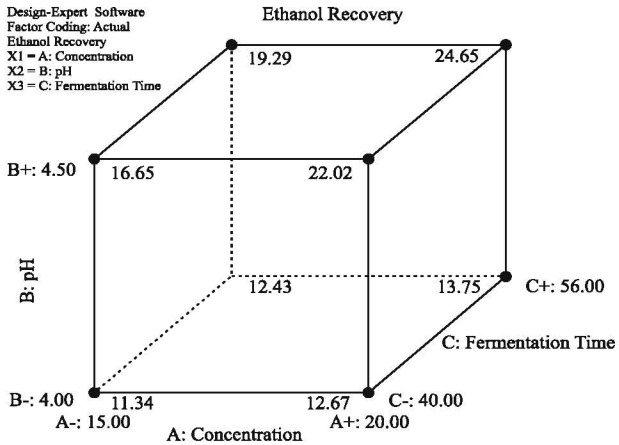

Figure 11: Response cube for experimental results of extraction

The contour plot for pH of molasses solution and fermentation time is shown in Figure 9. The contour plot represents that maximum ethanol recovery was (20-22 gm/L) at a pH of molasses solution (4.4 to 4.5) and fermentation time (44-56 hours).

The contour plot for concentration of molasses solution and fermentation time was obtained as shown in Figure10. The contour plot shows that maximum ethanol recovery was (18 to 20 gm/L) when concentration of molasses solution was (18-20 percent) and fermentation time was (44 to 56 hours). The plot also validate that as the combined effect of concentration and fermentation time has less effect on ethanol recovery so these two factors were less significant compared to pH.

For experimental data the response cube is shown in Figure 11. The ethanol recovery has been shown for the different values of molasses solution pH and its concentration and fermentation time. The response cube for the ethanol recovery presents that maximum ethanol (24.65 gm/L) was obtained when pH of molasses solution was 4.5, concentration of molasses solution was 20 gm/kg and fermentation time was 56 hours.

5. CONCLUSIONS

In this study the process parameters for ethanol recovery from sugar cane molasses were determined experimentally and optimized through statistical analysis. Ethanol recovery from sugarcane molasses was found to be an effective way for the treatment of waste molasses. The amount of ethanol increases significantly with the pH of molasses solution and concentration of molasses solution. (Design Expert 8.0.4 Trial version) software was employed in order to determine the cubic response, counter plots and interaction effects of the significant factors. The ANOVA (Analysis of variance) and regression models were developed for the experimental analysis. The optimum conditions for ethanol recovery were obtained when the pH of molasses solution was 4.5, concentration of molasses solution was 20 % and fermentation time was 60 hours.

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