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Optimization of the Fermentation Conditions for the Production of Bioethanol from Cane Sugar Molasses using *Saccharomyces cerevisae*

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ABSTRACT

Optimization of the production of bioethanol from cane sugar molasses was carried out using *Saccharomyces cerevisae*. The molasses sample was obtained from Dangote Sugar Manufacturing Industry in Lagos State, Nigeria. It was subjected to different conditions by varying the following parameters: pH, temperature, substrate concentration, and fermentation period, in order to optimize the influencing parameters that affect the production of bio-ethanol from the substrate.

Fermentation was carried out and then distillation to obtain the bioethanol from the fermentation broth. The optimal values were as follows: temperature, 35 ^{o}C; pH, 4.0; substrate concentration, 300 g/L; and fermentation period, 72 hours. Under these optimal conditions, the maximum yield of bio-ethanol was 50.45%. Descriptive analysis of the optimum conditions for bioethanol production from the results affirmed that there is significant difference between the optimum conditions: pH 4, temperature of 35 ^{o}C, substrate concentration of 300 g/L, fermentation period of 72 h and other conditions with maximum ethanol concentration occurring at the optimum conditions meaning that this conditions favor ethanol productions more which is in line with history.

(Keywords: bioethanol, fermentation, *Saccharomyces cerevisiae*, substrate, sugar molasses)

INTRODUCTION

Bioethanol has received increased attention over the last few years, mainly due to its potential as a substitute for fossil fuels and the need to reduce global economics dependence on fossil (Afenore, 2002). Also, because biomass fuels are renewable, they help reduce greenhouse gas emissions from fossil fuels (Balami *et al*., 2004). Although, at the moment bioethanol is mainly used in blends with gasoline as E10 and E20, the demand has soared. For instance, consumption of bioethanol in most countries of the European Union is far greater than the quantity produced in those countries.

According to a study by Hart's Global Biofuels Center (a division of Hart Energy Publishing LP, one of the world's largest energy industry publishers), the global biofuel use may double by 2015. Actually Brazil and the USA are the world's largest producers of bioethanol, counting with approximately 62% of world production (Dawson and Boopathy, 2007).

The major feedstocks used by these countries are sugar cane and corn, respectively. Since, the price of feedstock contributes more than 55% to the production cost, inexpensive feedstock such as agro-food waste, are being considered to make bioethanol competitive in the open market (Vasconcelos *et al.,* 1998). In addition, the use of food materials will put pressure on the cost with attendant food scarcity. Therefore there is the need for sourcing of ethanol from non-food materials.

Alcohols are regarded as green organic solvents because they have few human health and environmental risks, particularly in comparison to some other solvents such as methylene chloride or benzene. Ethanol may be a benign solvent, but its source still maters. Alcohols are regarded as green organic solvents because they have few human health and environmental risks, particularly in comparison to some other solvents such as methylene chloride or benzene. If the source is petroleum, all we have done is to exchange one hazardous process with another.

In fact, this has been the case until recently. Ethanol has been produced primarily from petroleum. However over the last few decades

other feed stocks have become important sources of ethanol.

One significant and growing source of ethanol is blackstrap molasses. This is a final waste product of sugar production (Kim and Dale, 2004). Sugar comes from two major feedstocks: sugar cane in tropical climates and sugar beets in temperate climates, giving most countries a source of sugar. Both sources of sugar are processed in a similar way. However most of the wastes including banana peels are not as viable as expected and needs to be optimized for large production. Hence this study was carried out to optimize the variables which affect bio-ethanol production from sugar molasses using *Saccharomyces cerevisae* as yeast.

MATERIALS AND METHOD

Sample Collection

All the chemicals were collected from National Center for Energy Research and Development University of Nigeria, Nsukka, and they were of analytical grade. Cane molasses was purchased from Dangote Sugar Industry Lagos state, Nigeria. It was analyzed for total solids, pH, and Specific gravity. It was diluted with distilled water to the required concentration of sugars and the optimum conditions for ethanol production were determined.

Microorganism and Cultural Condition

The yeast (*Saccharomyces cerevisae*) used in this study was obtained from the Mycology Laboratory unit of Department of Microbiology University of Nigeria Nsukka. The yeast was streaked out on sabourand dextrose agar (SDA) plates and incubated at 37^{o}C for 48 h after which the resulting single colonies were picked with a sterile wire loop and inoculated on SDA slant in test tubes as stock cultures and stored in the refrigerator at 4^{o}C until when needed for the study.

Preparation of *Saccharomyces cerevisae* Stock Culture

The *Saccharomyces cerevisae* stock culture was prepared for fermentation by growing it in sabourand broth at 37^{o}C for 24 h. This was done to achieve a high yeast growth, cell density and biomass that can carry out fermentation effectively. The sabourand dextrose broth was prepared by weighing out 5.6 g of the powder into 100 ml of distilled water in a conical flask. The sabourand dextrose broth powder was allowed to completely dissolve in the distilled water and was dispensed into 10 ml bijou bottles for sterilization by autoclaving.

After autoclaving, the bijou bottles were allowed to cool and then inoculated with a wire loop full of the stock culture of the yeast. The bijou bottles were then kept in the incubator at 37 ^{o}C for 24 h, during which the yeast grow and multiply. After the 24 h incubation the yeast cells in the bijou bottles were used to inoculate 1000 ml flask containing the molasses sample (Lees, 1971).

Characterization of Molasses

The molasses were characterized for pH using a Hanna pH meter model No. 02895, total solids were determined by gravimetry, and specific gravity was determined using a specific gravity bottle (Larson *et al*., 2007).

Fermentation of Molasses

A known quantity of sugar molasses (100 ml) and 100 ml of distilled water with 2 ml of culture solution (*Saccharomyces cerevisiae)* were taken in fermentation flask and kept in a constant temperature. An anaerobic condition was maintained for four days and during this period, the strain coverts sugar into bioethanol with the evolution of CO_2. The fermented sample was collected every 24 h. interval. The same procedure was repeated to optimize the parameters needed.

Figure 1: Fermentation of Molasses in an Incubator.

Test for Bioethanol in Fermented Sample

To about 10 ml of fermented sample, a pinch of potassium dichromate, and a few drops of H_2SO_4 were added. The color of the sample turned from pink to green which indicates the presence of bioethanol.

Optimization of pH

The sample was fermented at different pH values between 2.0 and 12.0 to obtain maximum yield of bio-ethanol by adding 2 M NaOH or sulphuric acid. The samples were kept in anaerobic condition for a period of four days and the fermented solution was analyzed for ethanol concentration every 24 h intervals.

Fermentation Temperature Optimization

The sample maintained at an optimum pH (4) was fermented at different temperatures like 25, 30, 35, 40 and 45^0C. The samples were kept for fermentation period of four days and the fermented solution was analyzed every 24 hr intervals.

Optimization of Substrate Concentration

The sample was fermented with different concentrations of the molasses sample (i.e., 50, 100, 200, 300 and 400 g / L) at optimum pH and temperature.

Optimization of Fermentation Period

The fermentation was carried out at different time periods 24, 48, 72 and 96 h under optimum conditions of pH, temperature and substrate concentration. The fermentation broth was distilled to obtain the ethanol produced. The ethanol was redistilled three more times in order to obtain cleaner alcohol.

Distillation of Bioethanol from the Mixture

Volume of bioethanol produced (540mL), Volume of molasses after production of bioethanol (1005mL), Total volume of bioethanol + molasses sample (1545mL) and % Theoretical yield (34.95%).

Characterizing the Bioethanol Produced

The following parameters were determined in the bioethanol produced: density using a density bottle, viscosity using an Ostwald viscometer, flash point using a flash point tester, and boiling point using a thermometer.

RESULTS AND DISCUSSION

Results

Table 1: Percentage Ethanol Concentration at 24 h Interval at various pH of Fermentation.

Time	pH 2		pH 4		pH 6		pH 8		pH 10		pH 12	
(h)	BV	EtOH (Conc) %	BV	EtOH (Conc) %	BV	EtOH (Conc) %	BV	EtOH (Conc) %	BV	EtOH (Conc) %	BV	EtOH (Conc) %
0	9.11	5.10	9.11	5.10	9.11	5.10	9.11	5.10	9.11	5.10	9.11	5.10
24	41.50	23.24	44.10	24.7	43.80	24.53	41.60	23.3	40.40	22.62	39.20	21.95
48	53.50	29.96	73.30	39.93	67.90	38.03	63.70	35.67	66.50	37.24	43.60	24.42
72	58.70	32.87	84.60	47.38	83.60	46.82	71.7	40.15	64.1	35.9	60.6	33.94
96	54.60	30.58	80.50	45.08	79.20	44.35	67.00	37.52	64.30	36.01	52.8	29.57

% EtOH Conc = BV x Constant (0.56)

Where BV = Brix Value

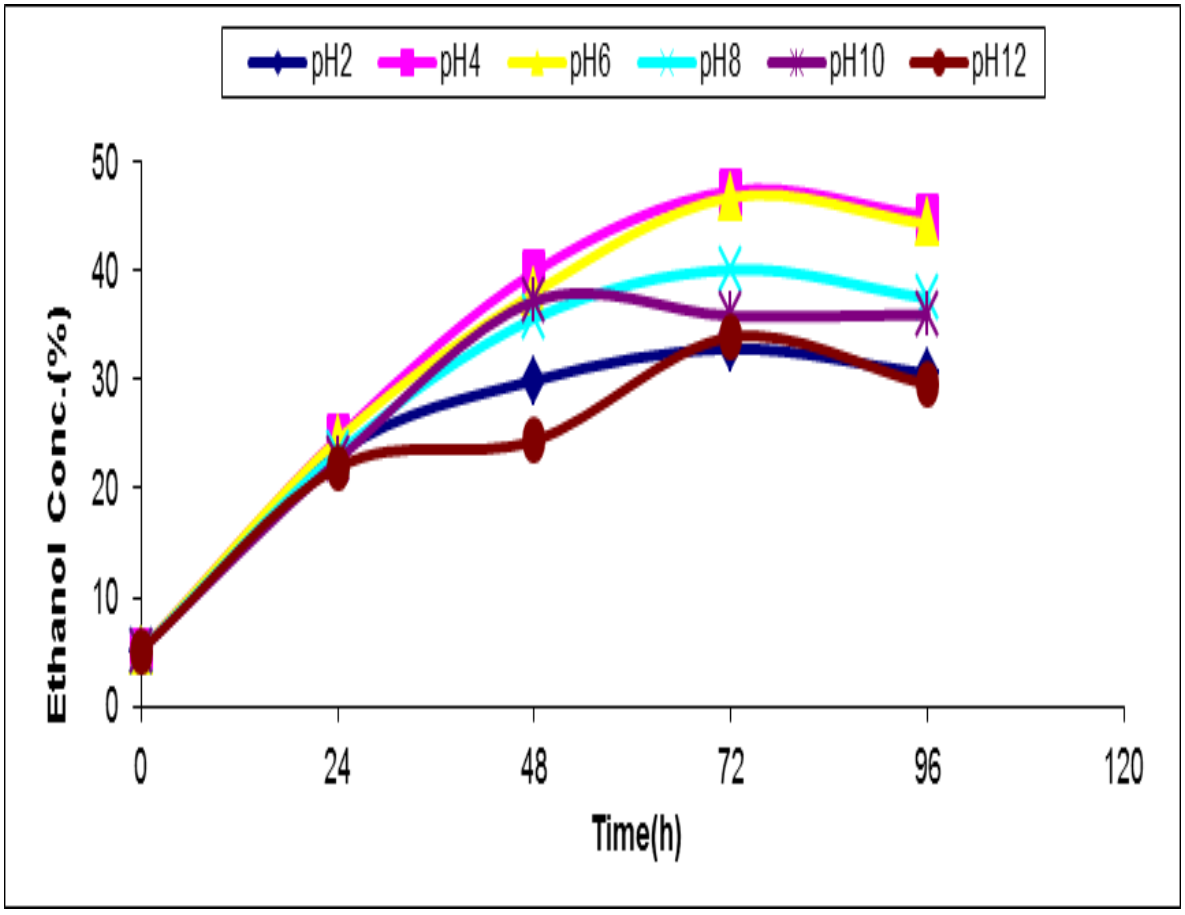

Figure 2: % Ethanol Concentrations at 24 h interval at various pH of fermentation.

Table 2: Percentage Ethanol Concentration at Various Temperatures.

TIME	25 °C		30 °C		35 °C		40 °C		45 °C	
(h)	BV	EtOH (Conc) %	BV	EtOH (Conc) %	BV	EtOH (Conc) %	BV	EtOH (Conc) %	BV	EtOH (Conc) %
0	9.11	5.10	9.11	5.10	9.11	5.10	9.11	5.10	9.11	5.10
24	45.20	25.31	48.50	27.16	49.30	27.61	43.40	24.31	43.29	24.24
48	71.59	40.07	74.20	41.55	76.50	42.84	74.30	41.61	72.90	37.91
72	87.00	48.72	89.20	49.95	89.80	50.29	85.40	47.83	84.20	47.15
96	83.18	46.58	83.20	46.59	84.70	47.43	82.90	46.42	82.80	46.36

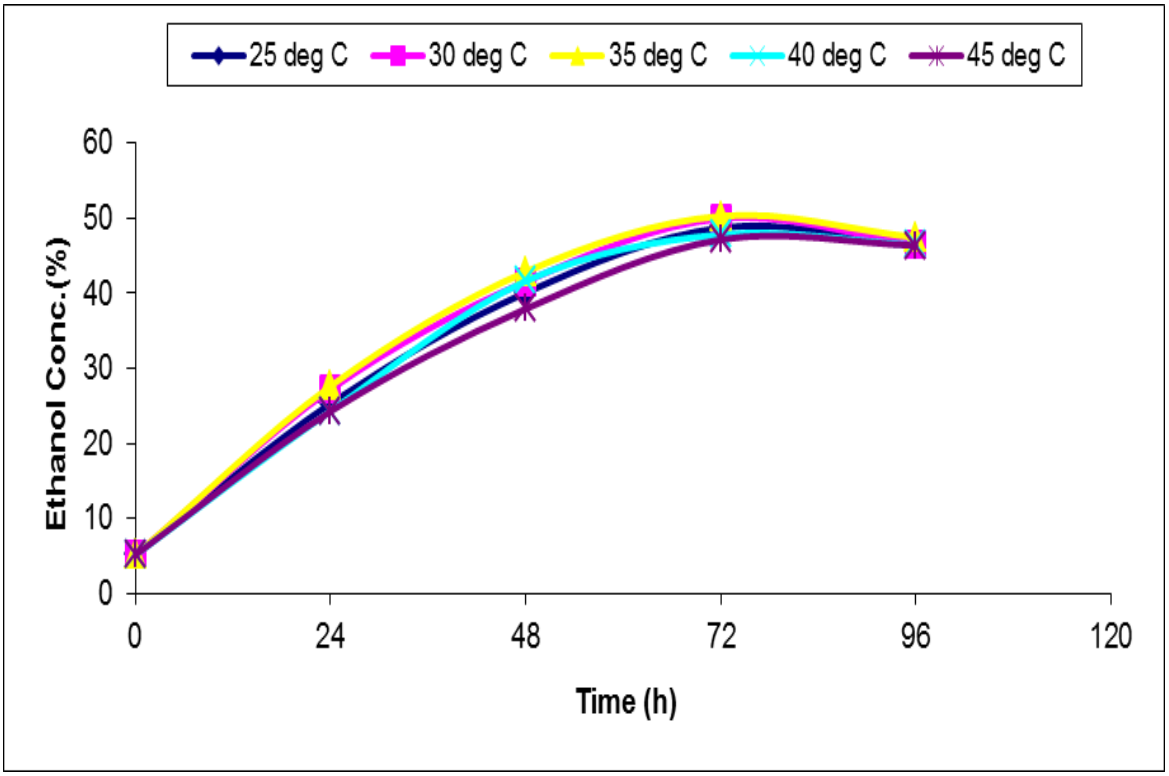

Figure 3: Percentage Ethanol Concentrations at 24 h Interval at various Temperature of Fermentation.

Table 3: Percentage Ethanol Concentration at 24 h Intervals for various Substrate Concentrations.

Time	50g/L		100 g/L		200 g/ L		300 g/ L		400 g/ L	
(h)	Brix	EtOH Conc. (%)	Brix	EtOH Conc. (%)	Brix	EtOH Conc. (%)	Brix	EtOH Conc. (%)	Brix	EtOH Conc. (%)
0	9.11	5.10	9.11	5.10	9.11	5.10	9.11	5.10	9.11	5.10
24	20.80	11.65	33.50	18.76	45.20	25.31	49.50	27.72	47.30	24.49
48	40.55	22.71	55.20	30.91	69.30	38.81	76.72	42.96	70.32	39.38
72	50.32	28.18	62.40	34.94	70.00	39.20	90.10	50.46	82.00	45.92
96	48.22	27.00	59.57	33.36	63.70	35.67	85.00	47.60	70.50	39.48

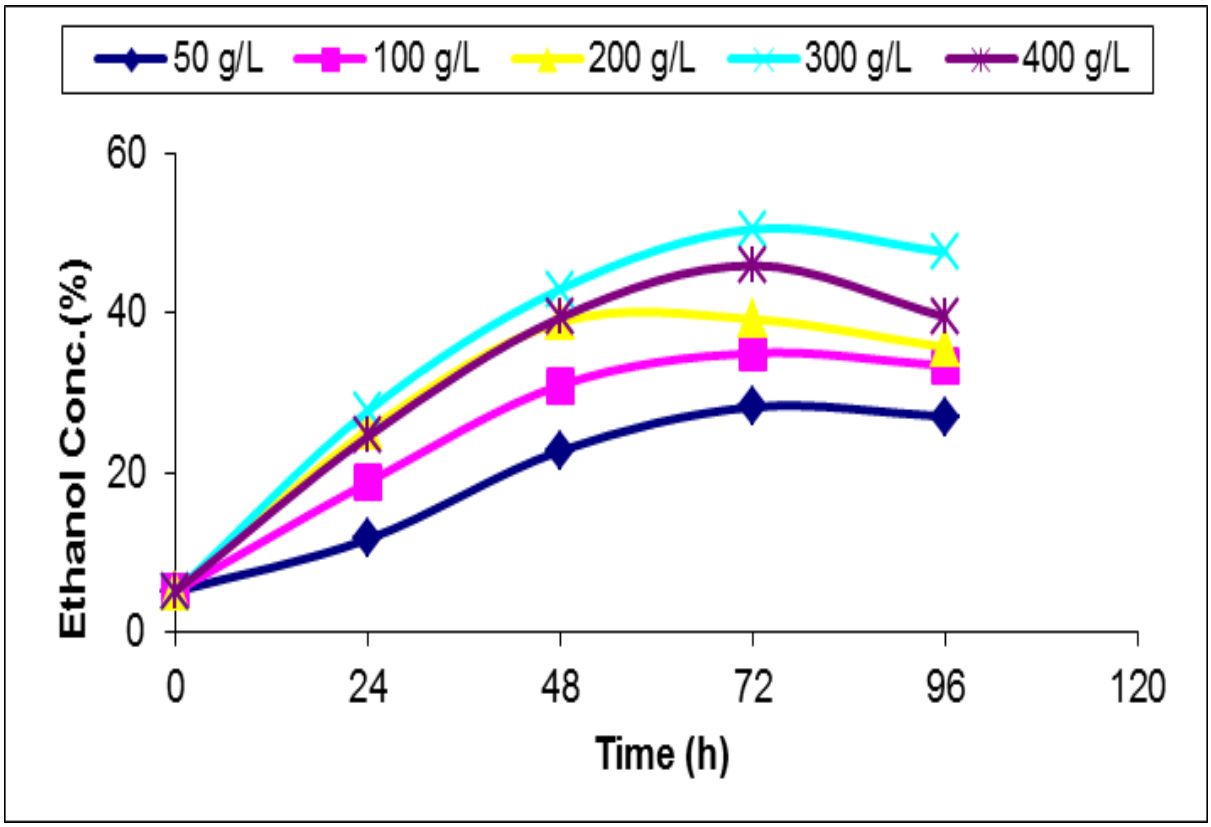

Figure 4: Percentage Ethanol Concentrations at 24 h Interval at various Substrate Concentrations.

Table 4: Percentage Ethanol Concentration and Brix Values at 24 h Interval at the various Optimum Conditions.

TIME	TEMP	pH	SUBSTRATE CONC. (g/L)	BRIX VALUE	EtOH CONC. (%)
0	35	4	300	9.11	5.10
24	,,	,,	300	49.30	27.61
48	,,	,,	300	76.50	42.84
72	,,	,,	300	90.10	50.46
96	,,	,,	300	84.70	47.43

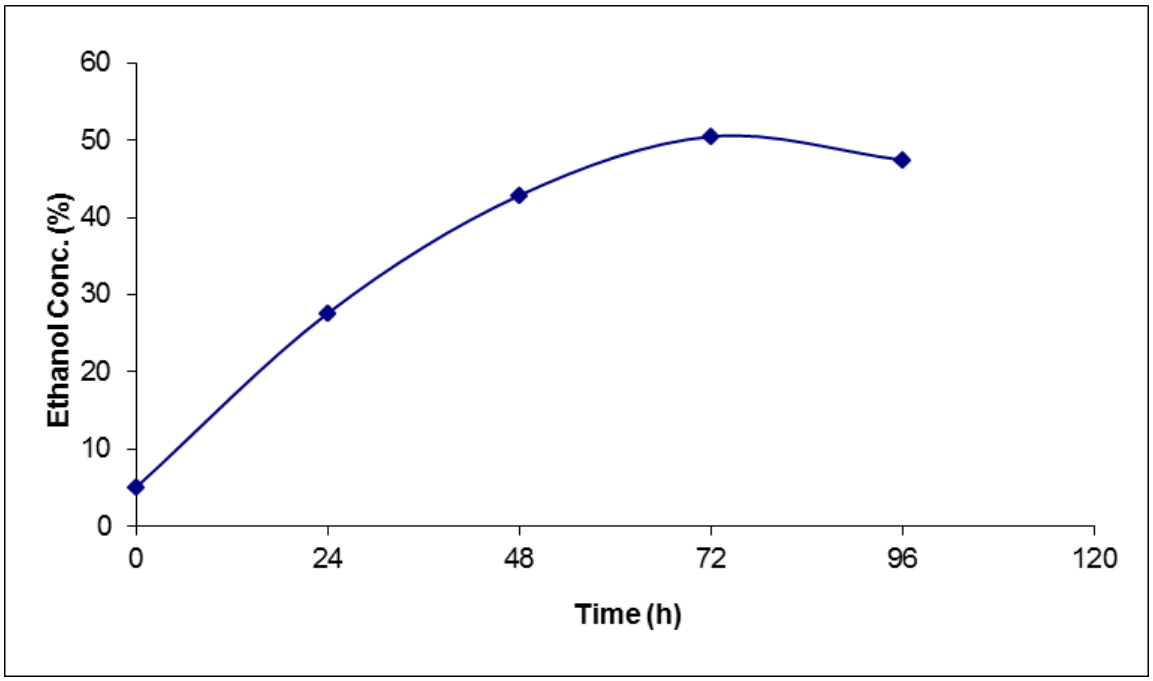

Figure 5: Percentage Ethanol Concentration at 24 h Interval at various Optimum Conditions of Fermentation.

Discussion

From Figure 2, ethanol production is optimal at 72 h after which it begins to decline, for the 4 days of fermentation; various values were obtained at different temperatures at 24 h interval as shown in Table 2. Table 2 showed percentage ethanol concentrations and Brix values at various temperatures at 24 h interval during fermentation, while Figure 3 shows the graphical representation.

The values in Table 4 were also obtained for the various fermentation periods at optimum conditions. The results of the characterization of the molasses were pH, 7.37, specific gravity, 1.37 and total solids, 45.56%. Theoretical yield of the bioethanol produced was 34.95 %. From Table 1, ethanol production is optimal at 72 h after which it begins to decline. The highest yield of bioethanol (50.46 %) was achieved at the optimum conditions: pH 4, temperature 35^{o}C, substrate concentration 300 g/L and fermentation period 72 h. A 72 hr optimal incubation period has been reported (Maris *et al*., 2006). The effect of pH optimization of ethanol production from cane sugar molasses was shown in Table 1. It was observed that ethanol concentration was highest at pH 4.

Control of pH during ethanol fermentation is important as the growth of harmful bacteria is retarded by acidic solution and yeast grows well

in mildly acidic condition. Yeast needs a slightly acid environment in order to grow well, with increase in pH, to basic conditions, yeast produces acid rather than alcohol and this lead to the decrease in alcohol production as the pH increases (Mcmeckin *et al*., 2002). An optimum pH of 4.5 for bio-ethanol production has been stipulated (Nanba and Nagai, 1987). Bio-ethanol production increases with the increase in temperature and reaches maximum value at 35^{o}C (Table 2). Further increase in temperature reduces the percentage of ethanol production and it is mainly due to the denaturing of the yeast cells. Interestingly, the fermentation is faster at 35 ^{o}C (95 F) (Okafor, 2007). The results of this work at 20°C are consistent with that reported previously (Reed, 2001) as a slower growth rate at lower temperature was obtained.

Table 3 showed that the concentration of bio-ethanol increased with increase in substrate concentration and reaches maximum ethanol production at sugar concentration of 300 g/L. This is because the entire enzyme has been saturated with the substrate, then all the enzyme will be in form of complex. In this case, the reaction will be proceeding at the maximum rate and increase in substrate concentration doesn't affect the reaction again. Further increase in sugar molasses concentration inhibits the ethanol productivity. Some researchers observed the maximum ethanol productivity within that range (Nanba and Nagai, 1987; Rass-Hansen *et al.,* 2007; Okafor, 2007).

Descriptive analysis of the optimum conditions for bioethanol productions in the results affirmed that there is significant difference between the optimum conditions pH 4, temperature 35^{o}C and substrate concentration of 300 g/L and others, with maximum ethanol concentration occurring at the optimum conditions. Results of the characterization of the bioethanol to know its suitability as a fuel shows density (0.831g/cm^{3}), flash point (18^{o}C), Viscosity (0.0017 Pas), and boiling point (79.9^{o}C). Comparing these with 0.789 g/cm^{3}, 13-14^{0}C, 0.0012 Pas, 78°C and for 100% ethanol, respectively, it is clear that bioethanol produced still has some level of impurities such as water, higher aldehydes or maybe oil thereby accounting for the deviation from expected values.

CONCLUSION

Saccharomyces cerevisiae has great potential for the production of ethanol from sugarcane molasses. The results indicated that the optimization of cultural conditions, such as sugar concentration, pH, temperature, substrate concentration and time of fermentation can further enhance ethanol production.

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