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Saccharomyces cerevisiae Catalyzed Fermentation of Acid Hydrolyzed Cassava Peels for Third Generation Bio-ethanol Production

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ABSTRACT

The quest for sustainable, renewable and environmentally friendly fuel has led to the discovery and use of bio-ethanol as internal combustion engine fuel. This research work focused on *Saccharomyces cerevisiae* (yeast) fermentation of acid hydrolyzed cassava peel for bio-ethanol production. Bio-ethanol was produced by acid hydrolysis of cassava peel flour and then fermentation of the glucose produced thereof using yeast. Hydrolysis and fermentation processes were optimized using one factor at a time method in other to determine the influence of their parameters on the yield of glucose and bio-ethanol respectively. The physiochemical properties of the bio-ethanol produced were determined based on American Standards for Testing and Materials (ASTM) method. The optimum values of the hydrolysis parameters that gave the highest glucose yield are: hydrolysis temperature, 95 ^{o}C; hydrolysis time, 50 minutes; substrate concentration, 2.5 g/L; and acid concentration, 0.4%. The optimum values of fermentation parameters that gave the highest yield of bio-ethanol are: fermentation temperature, 30 ^{o}C; fermentation time, 6 days; yeast dosage, 10%; glucose concentration, 100%; pH value 4.0 and agitation rate, 180 rpm. The physiochemical properties of the bio-ethanol produced were determined as: kinematic viscosity, 1.55 cst at 40 ^{o}C; density, 780 Kg/m^3; flash point, 13 ^{o}C; refractive index, 1.362; octane number, 102; sulphur content, 0.06%; water content, 0.05 ppm; boiling point, 78.5 ^{o}C; lower heating value, 20.4 KJ/dm^3; and pH value of 6.5.

Key words: Agitation rate**,** bio-ethanol, fermentation, hydrolysis, optimization.

INTRODUCTION

The world energy demand is skyrocketing due to rapid increase in population and high spate of industrialization. Coal, petroleum and natural gas have remained the major world energy resources used as feedstock for industrial production. These energy resources are commonly

termed fossil or nonrenewable resources [1]. These resources are extracted from the earth's crust, processed and burnt as fuel or used as feedstock in the chemical industries. The burning of fossil fuels causes environmental concerns such as greenhouse gas emission, which is the major substance responsible for climate change. Other harmful substances released during fossil fuel production and utilization includes sulphur oxides (SO_X), nitrogen oxides (NO_X) and methane [2]. The problems with the use of fossil fuel include non-degradable, non-renewable, environmentally unfriendly nature, and low octane number. Today bio-fuels have received much attention owing to the problems associated with fossil fuels [3-5].

The world economy depends on energy generation; hence the consequence of inadequate energy could be severe. These have prompted many researchers to look for other sources of energy that are sustainable, renewable, with less negative effect on the environment. Among various options investigated for internal combustion engine fuels, the bio-fuels, bio-ethanol and biodiesel obtained from plants and other sources have been recognized as contenders for reduction of exhaust emission [6]. Bio-ethanol promised to be an alternative to fossil fuel because it has high octane number, high heating value and exhibit complete combustion in automobiles as a result of its high oxygen content which results to low emission of poisonous gases [7, 8].

Bio-ethanol is a biochemical liquid obtained by fermentation of sugar via the catalysis of microorganisms followed by distillation process [9-12]. It is a renewable energy resource produced by fermentation process but also could be chemically produced by reacting ethylene with steam [10]. Bio-ethanol produced from biomass remains the fuel specie that do not exhibit greenhouse gas effect [10, 13-15]. It has the outstanding advantage of not adding to the problem of greenhouse gasses as the oxygen content of the molecules ensures near complete combustion of bio-ethanol resulting in low emission of carbon monoxide when used as a fuel additive. The uses of bio-ethanol include, as a solvent, in cosmetics, as preservative, and also as fuel additive to gasoline.

Bio-ethanol is principally produced from feedstock high in sugar and starch contents. Sugar constitute of first generation feedstock while starch is a second generation feedstock for bio-ethanol production. Recently an inroad is being made for production of bio-ethanol from a third possible feedstock, lignocelluloses biomass named third generation feedstock. The bio-ethanol produced from the first, second and third generation feed stocks are termed first, second

and third generation bio-ethanol respectively. The first generation bio-ethanol is principally produced from energy crops namely maize, sugar cane, corn, barley and wheat [16, 17]. The sugar of the first generation feedstock is easily accessed, thus making fist generation bio-ethanol production simpler. The second generation bio-ethanol is produced from cellulose materials like starch by hydrolysis of the cellulose to sugar before fermentation of the sugar. The third generation bio-ethanol is produced from lignocellulosic biomass consisting of cellulose, hemicellulose and lignin. Though they do not compete with food but the method of their processing is more complicated and therefore involve higher costs. This stemmed from the fact that conversion of hemicelluloses to bio-ethanol involves four processing stages namely pretreatment with acid, hydrolysis of the pre-treated biomass, fermentation of the sugar to bio-ethanol and distillation of the bio-ethanol mixture to pure bio-ethanol. Pre-treatment here involve the breaking of the recalcitrant structure of the lignocellulose for easy access to the cellulose, hemicellulose and lignin components. Hydrolysis entails conversion of the cellulose and hemicelluloses to fermentable sugar like glucose. Fermentation involves the conversion of the hydrolyzed sugar (hydrolysate) to bio-ethanol using microorganism like yeast. Finally, the bio-ethanol supernatant is distilled to obtain pure and concentrated bio-ethanol. Currently the most used feedstock for bio-ethanol production are wheat, corn, sugarcane and sugar beet. The sugar is fermented to bio-ethanol while the starch is first hydrolyzed to obtain sugar which could now be fermented to bio-ethanol. The technology for converting cellulosic material known as third generation feedstock is not yet fully available commercially as it is still being developed.

Researchers have reported that the bulk of bio-ethanol from USA [18] and Brazil [19] come from fermentation of corn glucose and sugar cane respectively. Cassava (*Manihot esculentus)* is a tuberous root crop native to South America, and is cultivated in most parts of the world primarily for its starch as well as serving as low grade animal feed [20]. Cassava can grow in marginal soils where other crops does not thrive [21]. Based on this, some researchers have shown that bio-ethanol production based on cassava is sustainable [22-24]. Therefore, intensive cultivation of cassava coupled with biotechnological improvement of the species will ensure sustainable commercial bio-ethanol production from cassava pulp and the peels as well as serving as food source for Nigeria that has much of the marginal soil lying fallow. The use of cassava peels for bio-ethanol production is advantageous as the feedstock involves little or no

cost in addition to solving the disposal problem and hence enhancing the cleanliness of the environment.

In almost all part of the world where cassava is cultivated, bio ethanol production has been from the pulp of cassava tuber. This practice is envisaged to affect the cost and availability of food. This has necessitated the use of the peels which is usually a waste constituting disposal problem for production of bio-ethanol. This present research work focused on bio-ethanol production by fermentation of cassava peel hydrolysate using yeast. Many researchers have reported on bio-ethanol production by fermentation of sugar using *Saccharomyces cerevisiae* [25-29].

MATERIALS AND METHODS

Materials

Pulverized cassava peels, *Saccharomyces cerevisiae*, distillation unit, digital pH meter, magnetic hot plate, thermostatic water bath, glassware's, autoclave, viscometer, flash point close cup tester.

Experimental Methods

Processing of the cassava peels

Cassava peels disposed as waste after peeling of cassava tubers for garri processing was collected from different garri processing units at Ogwuagor in Enugu, Enugu state, Nigeria. The peels were washed and sundried for 5 days and then oven-dried at 50 ^{o}C for 10 hours. It was then pulverized using a mechanical grinder and sieved to obtain peel flour of particle size of 150 µm. The flour was bagged with polythene and kept for further experimental use.

Production of bio-ethanol from cassava peels

Production of cellulose bio-ethanol typified by cassava peel bio-ethanol involves hydrolysis, fermentation and distillation.

Hydrolysis of the cassava peel flour

The cassava peel flour was hydrolyzed with sulphuric acid of 0.4 M concentration in order to convert the cellulose to fermentable sugar. 25 g of cassava peel flour was introduced into a 250 ml conical flask. 100 ml of distilled water was run unto the cassava peel flour. Then 20ml of 0.4 molar sulphuric acid was run unto the flask content and the mixture well shaken and covered

with cotton wool and aluminum foil to avoid contamination. The flask content was now maintained at a temperature of 95 ^{o}C for the 50 minutes' duration of hydrolysis using thermostatic water bath.

Fermentation of cassava peel hydrolysate

The cassava peel flour hydrolysate was allowed to cool and then filtered using muslin cloth. The concentration of glucose in the flour hydrolysate was measured. The hydrolysate was introduced into another 250 ml conical flask and autoclaved at 121 ^{o}C for 15 minutes. The autoclaved hydrolysate was allowed to cool when the pH was adjusted to 4.0 with calcium hydroxide solution. The hydrolysate was then inoculated with 10% baker's yeast (saccharomyces cerevisiae), agitated at the rate of 180 rpm and then left to ferment at the temperature of 30 ^{o}C for a duration of 6 days using thermostatic water-bath. The supernatant formed contain some bio-ethanol in a mixture of water and other impurities like methanol.

Distillation of the bio-ethanol mixture

The resulting mixture of bio-ethanol, water and other impurities like methanol is separated by distillation of the mixture at 80 ^{o}C using packed distillation column in order to obtain pure bio-ethanol.

Optimization of the process for bio-ethanol production

The two major processes for bio-ethanol production from cassava peel flour hydrolysate are hydrolysis and fermentation. The effect of process parameters for hydrolysis and for fermentation on the yield of glucose and bio-ethanol respectively were investigated and optimized.

Optimization of hydrolysis process parameters

The hydrolysis process parameters, hydrolysis temperature, hydrolysis time, substrate concentration and acid concentration were investigated using classical method of optimization by studying the effect of one factor at a time while the other factors were kept constant. Here the temperature was varied from 45 ^{o}C to 115 ^{o}C, time from 10 to 70 minutes, substrate concentration from 0.5 to 3.5 g/L while acid concentration was varied from 0.1 to 0.7 M.

Optimization of fermentation process parameters

The fermentation process parameters, fermentation temperature, yeast concentration, glucose concentration, pH, fermentation time and agitation rate were optimized using classical method of optimization of studying the effect of one factor at a time while the other factors were kept constant. Here the temperature was varied from 10 to 40 ^{o}C, yeast concentration from 2 to 14%, glucose concentration from 15 to 135%, pH from 4 to 6, fermentation time from 1 to 9 days and agitation rate from 100 to 220 rpm.

RESULTS AND DISCUSSION

Optimization of hydrolysis process

The effect of process parameters on the yield of glucose from hydrolysis of cassava peel was studied in order to obtain the values of optimum parameters that gave the highest glucose yield. The hydrolysis process parameters studied include, hydrolysis temperature, hydrolysis time, substrate concentration and acid concentration.

Effect hydrolysis temperature on the yield of glucose from cassava peels

The effect of hydrolysis temperature on the yield of glucose from cassava peels was determined by varying the temperature from 45 ^{o}C to 115 ^{o}C at a step increase of 10 ^{o}C while keeping other parameters constant. The result for the experiment is as shown in Figure 1. From the figure it could be observed that the glucose concentration increased with increase in hydrolysis temperature till it attained the maximum value of 65 mg/ml at an optimum hydrolysis temperature of 95 °C when it started declining with increase in temperature. Some researchers have reported that increase in temperature is conducive for high yield of glucose [30, 31]. However, on exceeding the optimum hydrolysis temperature, the formation of degradation product of glucose such as furfural and 5-hydroxymethylfurfural were produced due to charring of glucose. The highest glucose yield was obtained at 95^{0}C corresponding with the literature value of 95 ^{o}C reported by Senette and Tando [32].

Effect of hydrolysis time on the yield of glucose from cassava peels

The time of hydrolysis of the cassava peels was varied from 10 to 70 minutes with step increase of 10 minutes and the other parameters kept constant in order to determine its effect on glucose yield. The experimental result is as shown in Figure 2. From the Figure it is seen that glucose

yield increased with increase in hydrolysis time and attained the peak value of 75 mg/ml at optimum time of 50 minutes. On exceeding the optimum time of 50minutes, the glucose yield declined with increase in time. The decrease in glucose concentration on exceeding the optimum hydrolysis time is attributable to decomposition of glucose to degradation product over a long time of hydrolysis. This conforms to the findings of Onyelucheya et al [33].

Effect of substrate concentration on glucose yield from cassava peels

In order to study the effect of substrate concentration on glucose yield, the substrate concentration was varied from 0.5 to 3.5 g/L at a step increase of 0.5 g/L, with the other parameters kept constant. The result obtained is as shown in Figure 3. From the Figure it is seen that the glucose concentration increased with increase in substrate concentration till it attained maximum value of 60 mg/L at an optimum substrate concentration of 2.5 g/L when it started decreasing with increase in substrate concentration. Decrease in glucose yield after the optimum substrate concentration could be that at lower substrate concentration, the viscosity of the mixture was conducive for progressive increase in glucose concentration. However, on exceeding the optimum substrate concentration the viscosity of the mixture increased to a level where it could impede the hydrolysis reaction and thereby decrease the glucose concentration. This fact is buttressed by the findings that low concentration of biomass results in low viscosity, while high concentration give rise to high viscosity [32,34].

Effect of acid concentration on glucose yield from cassava peels

The acid concentration was varied from 0.1 to 0.6 M at a step increase of 0.1 M while the other parameters were kept constant in order to determine its effect on glucose yield. The result obtained is as shown in figure 4. From the Figure, it could be seen that the glucose yield increased with increase in acid concentration till it reached the maximum value of 65 mg/ml at an optimum acid concentration of 0.40 M when it started decreasing with increase of acid concentration. The reduction of glucose yield with high acid concentration results from the fact that low acid concentration is conducive for glucose production during hydrolysis. However the use of high acid concentration for hydrolysis result to browning and charring of the hydrolysate and most times accompanied by formation of undesirable by products like furfural and 5-dehydroxymethyl furfural that inhibit fermentation [35-37].

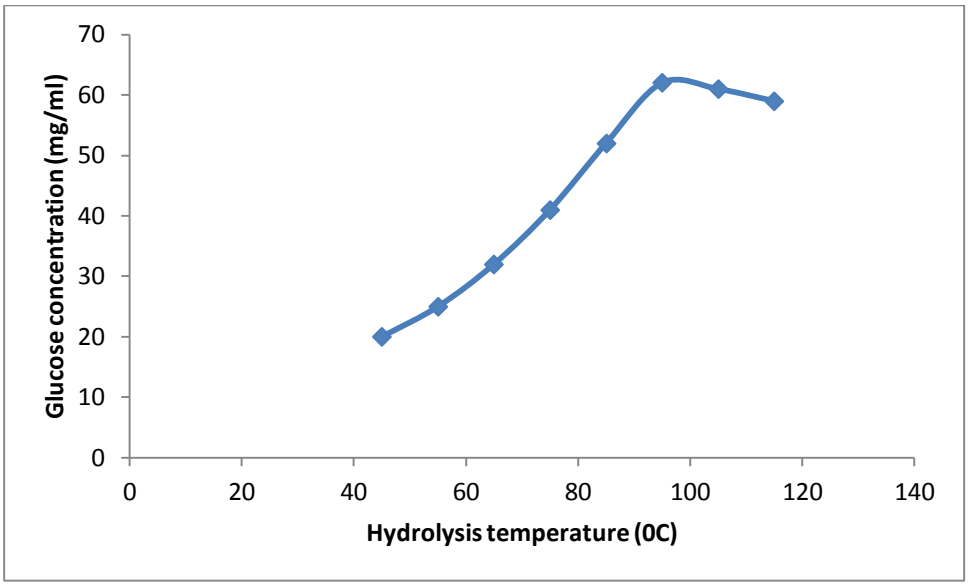

Figure 1: Effect of hydrolysis temperature on the yield of glucose from cassava peels.

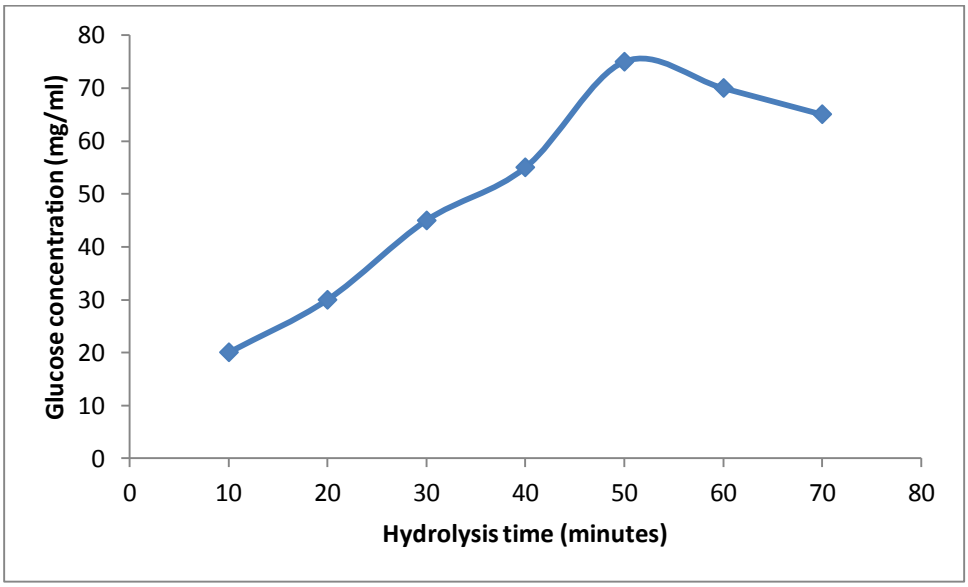

Figure 2: Effect of hydrolysis time on the yield of glucose from cassava peels

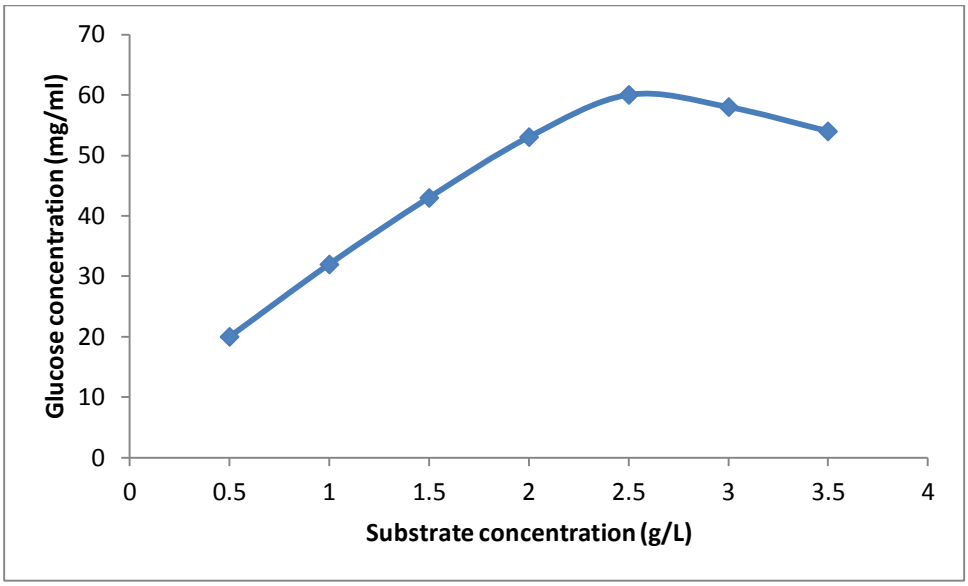

Figure 3: Effect of substrate concentration on the yield of glucose yield from cassava peels

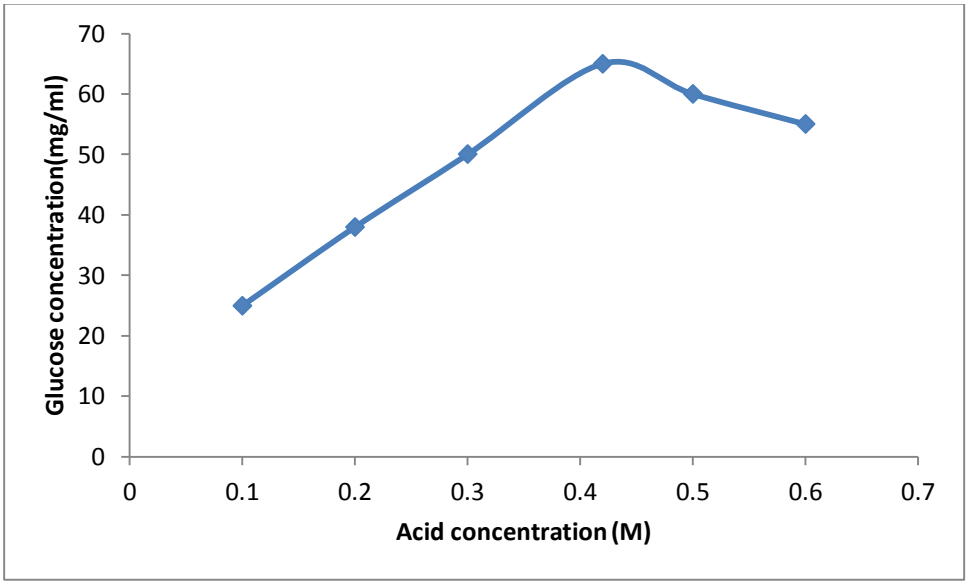

Figure 4: Effect of acid concentration on the yield of glucose from cassava peels

Optimization of fermentation process

The Influence of process parameters on the yield of bio-ethanol from fermentation of cassava peel hydrolysate was studied in order to obtain the values of optimum parameters that gave the highest yield of bio-ethanol. The fermentation process parameters studied include, fermentation

temperature, yeast concentration, glucose concentration, pH, fermentation time and agitation rate.

Influence of fermentation temperature on the yield of bio-ethanol from cassava peel hydrolysate.

The influence of fermentation temperature on the yield of bio-ethanol from cassava peel hydrolysate was studied by varying the fermentation temperature from 10 to 40 ^{o}C at a step increase of 5 ^{o}C while keeping the other parameters, yeast concentration, glucose concentration, pH, fermentation time and agitation rate constant. The result of the experiment is as shown in Figure 5. From the Figure, it could be seen that the bio-ethanol concentration (yield) increased with increase in fermentation temperature until it attained the maximum value of 16% at an optimum temperature of 30 ^{o}C. On exceeding the 30 ^{o}C optimum temperature, the bio-ethanol yield decreased with increase in temperature. One of the characteristics of enzymes and microorganisms is that temperature has a stimulating effect on them over a narrow range. Above the temperature limit, the enzyme may be irreversibly denatured. It has been reported that the optimum fermentation temperature using *Saccharomyces cerevisiae* is 30 ^{o}C which corresponds with the observed optimum fermentation temperature in the present work [38,39]. Carrying out fermentation with *Saccharomyces cerevisiae* above this optimum temperature denatures the protein content of the enzyme or microorganism and ultimately deactivate them [38, 40]. This therefore decreased the bio-ethanol concentration produced.

Influence of yeast dosage (concentration) on the yield of bio-ethanol from cassava peel hydrolysate

Yeast concentration was varied from 2 to 14%, at a step increase of 2% in order to determine its influence on the bio-ethanol yield while keeping the other parameters constant. The results obtained are shown in Figure 6. From the Figure it is clear that the bio-ethanol yield increased with increase in yeast concentration till it reached a maximum value of 16% at an optimum yeast concentration of 10%. On exceeding 10% optimum yeast concentration, the bio-ethanol yield remained constant with increase in yeast dosage. After exceeding the optimum yeast concentration, the inoculums size no longer had significant effect on bio-ethanol yield but rather affected the rate of sugar consumption. Above the optimum yeast concentration, the cells grow

rapidly resulting in rapid consumption of the glucose to produce bio-ethanol at a reduced fermentation time.

Influence of glucose concentration on the yield of bio-ethanol from cassava peel hydrolysate

The influence of glucose concentration on bio-ethanol yield was studied by varying the glucose concentration from 15 to 135% with step increase of 20% while keeping the other parameters constant. The results obtained for the experiment are shown in Figure 7. From the Figure it could be seen that bio-ethanol concentration increased with increase in glucose dosage till it attained the highest value of 16% at the optimum glucose concentration of 95% when the bio-ethanol yield started decreasing with increase in glucose concentration. Decrease in bio-ethanol production after exceeding the optimum glucose concentration stem from the fact that such high concentration of glucose overwhelms the microorganism intake capacity, resulting in the reduction of the activity of the microorganism and hence the bio-ethanol production.

Influence of pH on the yield of bio-ethanol from cassava peel hydrolysate

In order to determine the influence of pH on the bio-ethanol yield from cassava peel hydrolysate, the pH was varied from 4.0 to 6.0 at a step increase of 0.4 while keeping the other parameters constant. The results obtained are shown in Figure 8. From the Figure it could be observed that bio-ethanol yield steeply increased with increase in pH till it attained highest value of 16% at an optimum pH of 5.0 when it started to decrease with increase in pH. Microorganisms have a certain range of pH at which its activity is optimal. It has been reported that pH of the broth is a key factor to be considered in bio-ethanol production as it influences the microorganism directly as well as its cellular processes [35,36]. The optimum pH for bio-ethanol production using *Saccharomyces cerevisiae* has been reported to be 4.0-5.0. On exceeding the pH of 5.0 *Saccharomyces cerevisiae* is denatured and the catalytic activities reduced, which translates to reduction of the bio-ethanol produced.

Influence of fermentation time on bio-ethanol yield from cassava peel hydrolysate

The fermentation time was varied from 1 to 9 days with step increase of 1 day while keeping the other parameters constant in order to determine its influence on bio-ethanol yield. The results obtained are as shown in Figure 9. From the Figure it could be seen that the bio-ethanol yield increased with increase in fermentation time till it attained highest value of 16% at an optimum time of 6 days. On exceeding the optimum fermentation time of 6 days, the bio-ethanol yield

started decreasing with increase in fermentation time. At the initial time of the first day, the bio-ethanol produced was low as the yeast was at its lag phase. As the time progresses, the microorganism enters its exponential period of growth when the bio-ethanol production increase exponentially as well. From the Figure, the optimal period for bio-ethanol production is 6days. On exceeding the optimum fermentation period of 6 days, the bio-ethanol produced remains constant from the 6^{th} to the 7^{th} day when the birth and death rate of the microorganism equal. From the 7^{th} day upwards the bio-ethanol produced continued to decline with increase in time as the death rate of the microorganism become greater than the birth rate.

Influence of agitation rate on bio-ethanol yield from cassava peel hydrolysate

The agitation rate was varied from 100 to 220 rpm at a step increase of 20 rpm while the other parameters were kept constant in order to study the effect of agitation rate on bio-ethanol yield. The result obtained is as shown in Figure 10. From the Figure it could be seen that bio-ethanol increased with increase of agitation rate till it reached maximum production of 16% at an optimal agitation rate of 180 rpm. On exceeding the optimum agitation rate of 180 rpm, the bio-ethanol produced started decreasing. Moderate agitation rate is required for efficient fermentation of glucose to produce ethanol using saccharomyces cerevisiae. Agitation enables permeability of nutrients into the cells and removal of bio-ethanol from the interior of the cells to the fermentation broth. It also increases the level of sugar consumption by the yeast and reduce the inhibitory effect of bio-ethanol on the activities of the cells. Excess agitation is however counterproductive for bio-ethanol production as it lowers the metabolic activities of the cells resulting in lower bio-ethanol yield [39].

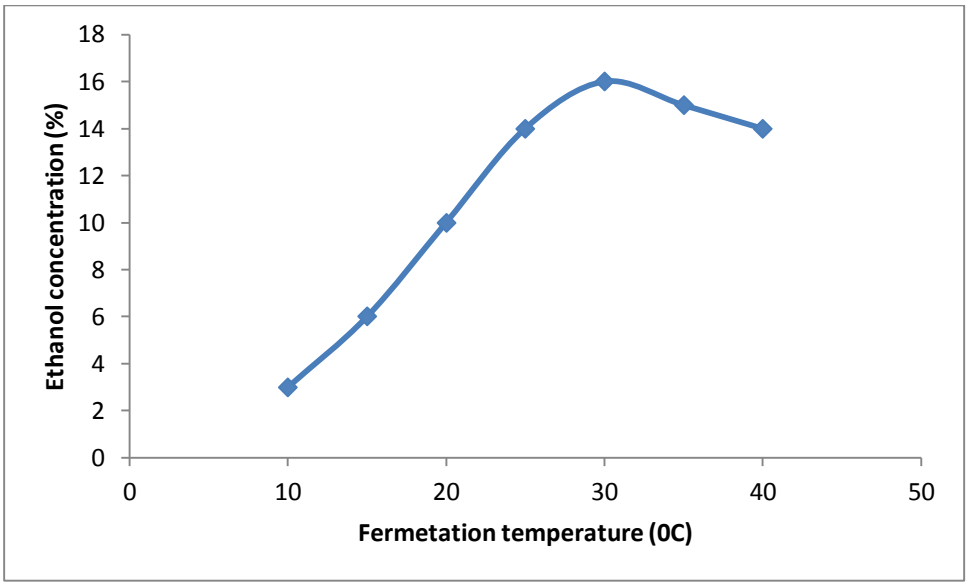

Figure 5: Influence of fermentation temperature on the yield of bio-ethanol from cassava Peel hydrolysate

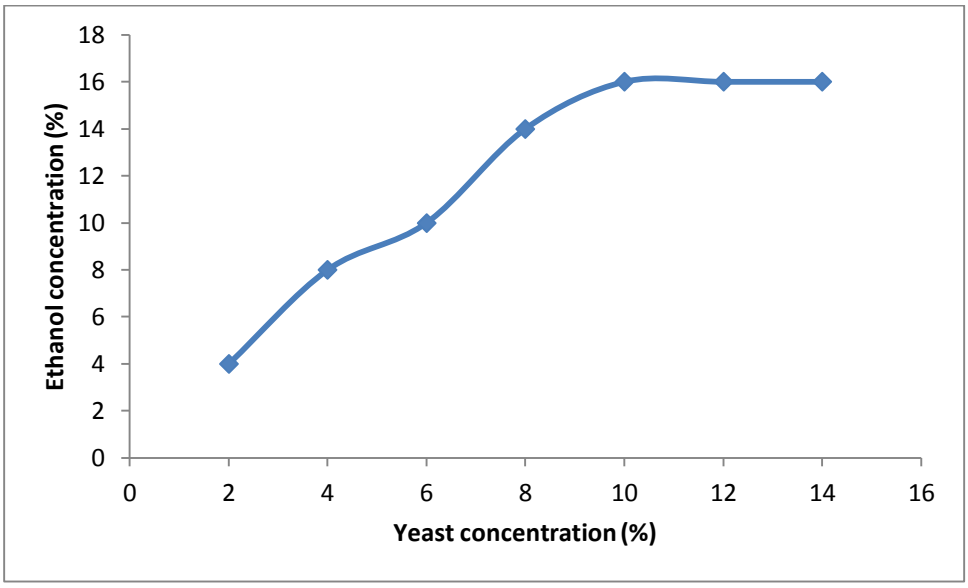

Figure 6: Influence of yeast dosage on the yield of bio-ethanol from cassava Peel hydrolysate

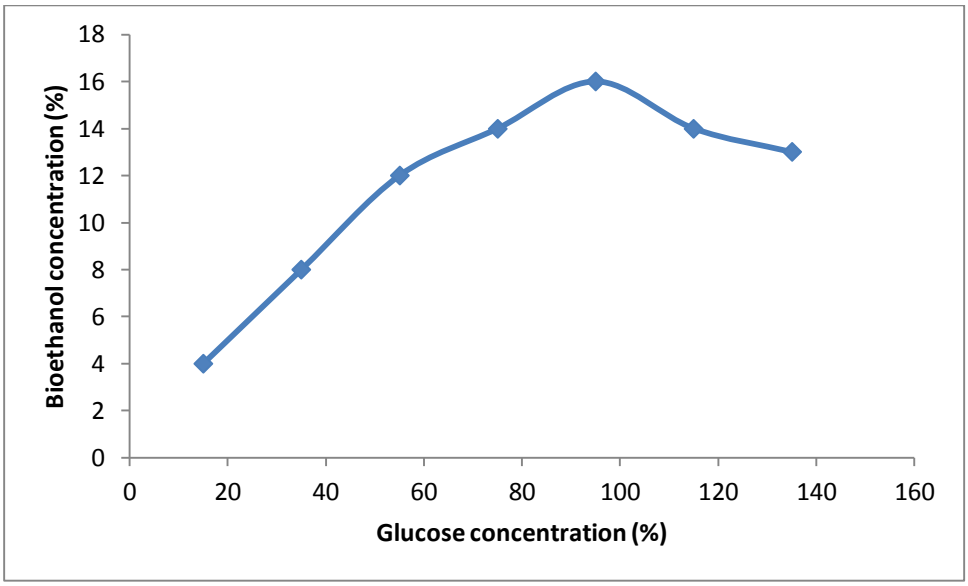

Figure 7: Influence of glucose concentration on the yield of bio-ethanol from Cassava peel hydrolysate

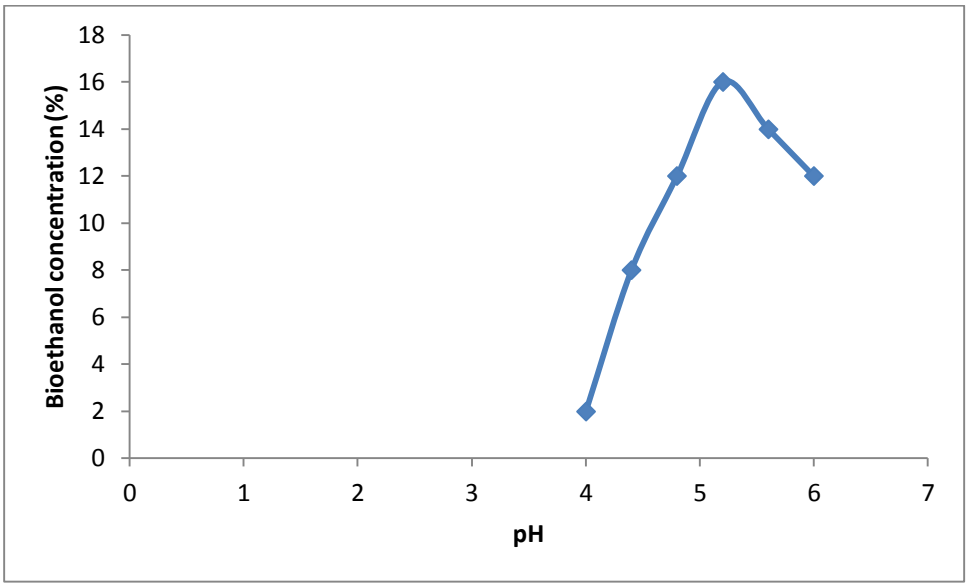

Figure 8: Influence of pH on the yield of bio-ethanol from cassava peel hydrolysate

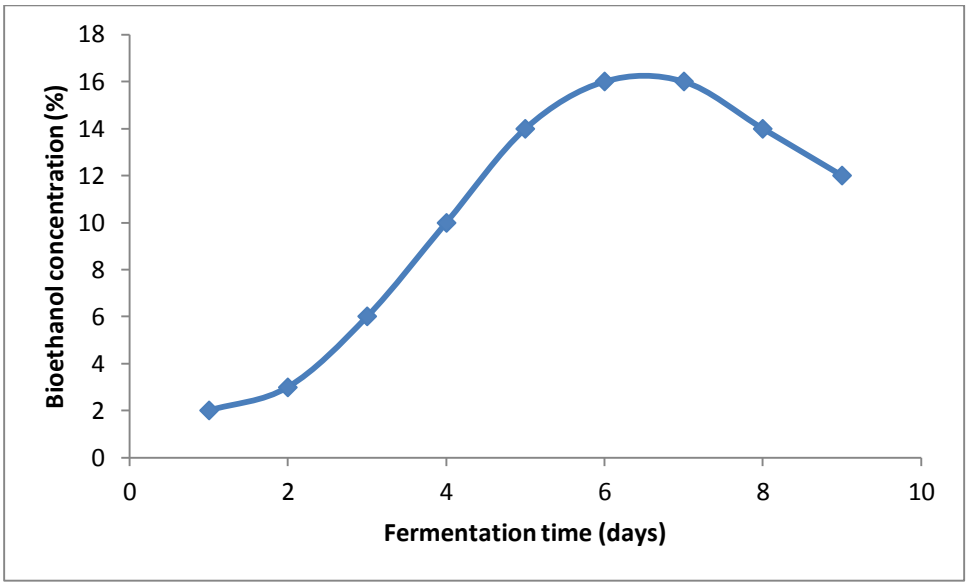

Figure 9: Influence of fermentation time on bio-ethanol yield from cassava Peel hydrolysate

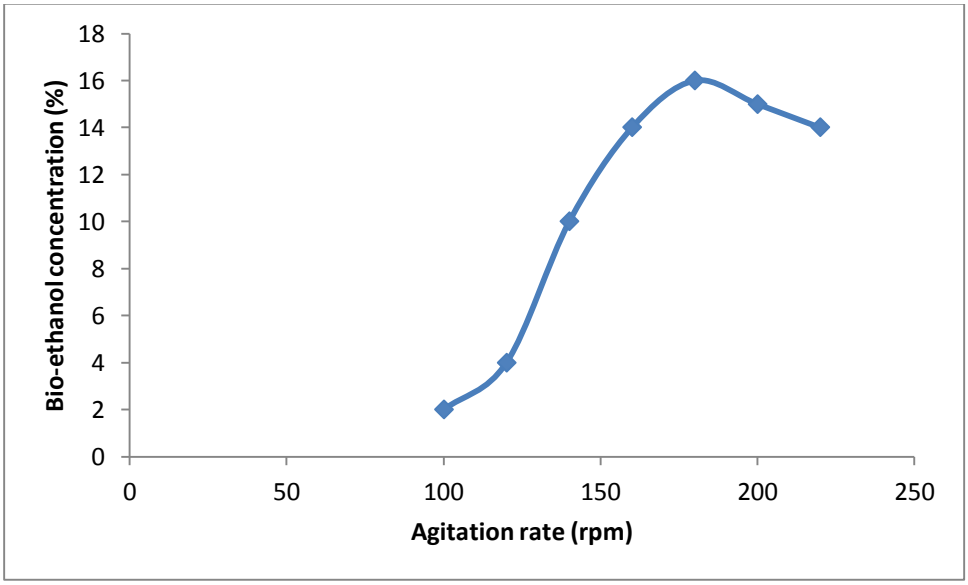

Figure 10: Influence of agitation rate on bio-ethanol yield from cassava peel hydrolysate

Characteristics of produced cassava peel bio-ethanol

The physiochemical properties of the produced bio-ethanol as compared to those of gasoline are presented in Table 1. From the Table, the evaluated density of the bio-ethanol is 780 Kg/dm^3. The value is within the ASTM standard limit for bio-ethanol. Density is the ratio of mass to volume of the fuel which greatly affects the ignition quality of the fuel. It has a significant impact on the fuel consumption as the fuel introduced into the combustion chamber is determined volumetrically. Bio-ethanol is denser than gasoline. This indicates that more volume of gasoline is required to obtain an equivalent mass of ethanol. Also the ignition quality of bio-ethanol should be less than that of the gasoline.

Viscosity is another very important characteristic of fuel. The bio-fuel should neither be too viscous nor too thin. The kinematic viscosity of the bio-ethanol produced was evaluated as 1.55 cst at 40 ^{o}C and is therefore within the ASTM limit. High kinematic viscosity of bio-fuels result in poor atomization and incomplete combustion which give rise to cocking of injector tips and hence engine power loss. This conforms to the findings of Tat and Van Gerpen [41]. On the other hand, very low viscosity fuel produces very subtle spray which cannot properly get into the combustion cylinder, thus forming a fuel rich zone that give rise to sooth formation [42-44]. The viscosity value obtained for the bio-ethanol conformed with the literature value of Muhaj and Sutjahio [45]. The viscosity of bio-ethanol is higher than that of gasoline. This is indicative of the fact that bio-ethanol is prone to less atomization efficiency, incomplete combustion and hence higher engine power loss than gasoline.

Flash point measures the degree of flammability of the fuel. This was evaluated as 13 ^{o}C for the bio-ethanol, and is therefore within the ASTM limit for bio-ethanol. The flash point of bio-ethanol is higher than that of gasoline and so exhibit less flammability than gasoline. The flash point of bio-ethanol 13 ^{o}C is higher than that of gasoline and therefore less dangerous to handle and store. The octane number of fuel expresses the degree of knocking of such fuel in internal combustion engine. Fuels of high octane number do not knock in the engine. The degree of knocking in the engine is a function of the ratio of branched chain molecules like 2,2,4-trimethylpentane to the straight chain molecules like n-pentane contained in the fuel.

Table 1: Physiochemical properties of produced cassava peel bio-ethanol

Properties	Produced Bio-ethanol	ASTM Bio-ethanol Standards	ASTM Gasoline Standards	Test Method
Kinematic viscosity @ 40^0C (cst)	1.55	1.525	1.00	D445
Density (Kg/m^3)	780	750-850	690-790	D1293
Flash point (^{0}C)	13	10-15	-43	D93
Refractive index	1.362	1.360-1.364	1.350-1.355	
Octane number	102	95-110	88-100	
Sulphur content (%)	0.06	0.05		D3452
Water content (ppm)	0.05	0.05	0.05	D203
Boiling point (^{0}C)	78.6	78.5	27-225	
Lower heating value (KJ/dm^3)	20.4	22	30.33	D240
pH	6.7	6.5-9.0		D684
Acidity (As acetic acid)	0.006	0.007max.		D1613
Solubility in water	Soluble	Soluble	Insoluble	

A fuel rated 88% octane number shows that it contained 88% of the branched molecules and 12% of the straight chain molecules. The octane number of the bio-ethanol produce is 102, which is within the ASTM standard limit, indicative of its smooth burning in the engine cylinder. The octane number of gasoline is less than that of bio-ethanol and therefore is prone to more knocking in internal combustion engine.

The water content of the produced bio-ethanol was evaluated as 0.05 ppm which is within the standard limit of ASTM. High water content in bio-ethanol predisposes the oxidation of the fuel as well as being the cause of corrosion in storage tank when stored for a long time. The infinitesimally small water content of bio-ethanol which is equivalent to that of the gasoline is within the ASTM standards. The water content of the bio- ethanol therefore has little or no effect on the degradation of the fuel or on corrosion in tanks during storage. The boiling point of the bio-ethanol was evaluated as 78.5^0C which corresponds with the ASTM standards. Refractive index indicates the state of purity of a substance as any substance has more or less a specific refractive index. The refractive index of the bio-ethanol produce is 1.362 and is within the ASTM standard limit.

CONCLUSION

Production of bio-ethanol by hydrolysis and fermentation requires adequate treatment of the substrate. Acid hydrolysis of cassava peel yielded high glucose concentration (70mg/ml) and fermentation of the hydrolyzed glucose with *Saccharomyces cerevisiae* also yielded high percentage of bio-ethanol (16%). The use of acid hydrolysis for conversion of cassava peel flour to glucose and *Saccharomyces cerevisiae* for fermentation of the hydrolyzed glucose is therefore an efficient means for bio-ethanol production from cassava peel with high yield.

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