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Full Length Research Paper

Optimization of ethanol production from *Garcinia kola* (bitter kola) pulp agrowaste

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Optimization options for production of ethanol from bitter kola *(Garcinia kola)* pulp wastes were investigated. The methods are degumming, saccharification, acid hydrolysis, alkaline hydrolysis. Degumming was effectively achieved at 96 h of saccaharification. The concentration of reducing sugar for the treated sample (acid hydrolysed and saccharification) and control sample (saccharification) was maximum at 144 h (86.2 g/100g) and 96 h (31.5 g/100 g), respectively. Ethanol yield from treated sample and control sample using baker's yeast was maximum at 120 h (70.7 g/L) and 192 h (29.3 g/L), respectively. Alkaline hydrolysis with 0.25 M sodium hydroxide has no significant effect on concentration of reducing sugar and ethanol yield. Acid hydrolysis with 2.5 M sulphuric acid and saccharification using *Aspergillus niger* are better methods for optimizing ethanol production from bitter kola pulp waste. Solar drying of the bitter kola pulp waste significantly enhanced ethanol production.

Key words: Bitter kola pulp wastes, degumming, saccharification, acid hydrolysis, alkaline hydrolysis, fermentation.

INTRODUCTION

Ethanol is a renewable energy resource produced through fermentation of simple sugars by yeasts. Ethanol is widely used as a partial gasoline replacement in US and other parts of the world such as Canada. It can also be used in a variety of cooking, heating, and lighting appliances. Fuel ethanol that is produced from corn has been used in gasohol or oxygenated fuels since 1980. Ethanol that is blended directly with gasoline in a mix of 10% ethanol and 90% gasoline is called gasohol. Recently, US automobile manufacturers have announced plans to produce significant numbers of flexible-fueled vehicles that can use ethanol blend – E85 (85% ethanol and 15% gasoline by volume). Demand for ethanol E85 has grown from 144,000 gallons in 1992 to 2 million gallons in 1998. The federal Government of Nigeria has concluded plans to invest 400 Billion Naira (3.5 billion US dollars) in Jigawa State Ethanol production programme to diversify its sources of revenue (THISDAY, 2006). US President, George W Bush, announced in his state of the union speech, an agenda to develop alternative energies such as ethanol from grains and cellulose in order to end Americas dependence on oil (Analyst, 2006). Using ethanol –blended fuel for automobiles can significantly reduce petroleum use and existing greenhouse gas emission (Wang et al., 1999). Ethanol is also a safe alternative to methyl tertiary butyl ether (MTBE), the most common additive to gasoline used to provide cleaner combustion (Mani et. al., 2002). MTBE is a toxic chemical compound and has been found to contaminate ground-water. World's ethanol production was about 29.9 billion liters in 2000, which was less than 31.4 billion liters forecasted (Mani et al., 2002). The benefits of developing biomass-to-ethanol technology are: increased national energy security, reduction in greenhouse gas emissions, use of renewable resources, foundation of a carbohydrate-based chemical process industry, macro-economic benefits for rural communities and society at large (Wayman et al., 1992).

Lignocellulosic material is a less expensive source of carbon, and includes wheat straw (Doppelbauer et al.,

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1987; Abd El-Nasser et al., 1997), sugar cane bagasse (Kawamori et al., 1986; Aiello et al., 1996), aspen wood (San Martin et al., 1986), willow (Reczey et al., 1996) corn cobs (Abd El-Nasser et al., 1997) and waste newspaper (Maheshwari et al., 1994; Chen and Wayman, 1991). However, the necessary pretreatment of raw material also adds to the cost of production (Hsu, 1996). Akin-Osanaiye et al. (2005a) reported ethanol production with *Carica papaya* wastes. Also the wet sample of bitter kola pulp waste has been used for the production of ethanol (Akin-Osanaiye et al. (2005b). The production of ethanol using molasses have also been reported by Bilford (1942) and Bose and Ghose (1973). Most pretreatment approaches do not hydrolyze significant amount of the cellulose fraction of biomass, pretreatment enables more efficient enzymatic hydrolysis of the cellulose by removal of the surrounding hemicellulose and/or lignin along with modification structure. Current pretreatment research and development activities are geared toward identifying, evaluating, development, and demonstrating promising approaches that primarily support the subsequent enzymatic hydrolysis of the treated biomass. Universal pretreatment process is difficult to envision owing to the diverse nature of biomass. Thus, several physical, chemical and biological treatments are under evaluation. The resulting composition of the treated material is independent on the sources of the biomass and the type of treatment, but in general, is much more amenable to enzymatic hydrolysis by cellulases and related enzymes than native biomass. Recently we have reported the effect of acid hydrolysis of *G. kola* pulp waste on the production of CM-cellulase and ß-glucosidase using *Aspergillus niger* (Okafoagu and Nzelibe, 2006). The present study was therefore focused on the methods that could optimize ethanol production from bitter kola pulp wastes.

MATERIALS AND METHODS

G. kola was seperated from the fruit and the pulp waste is discarded after the removal of the seeds. One kilogram of the pulp waste was dried in the solar dryer and ground into powdered form using a blender. The sample was packaged and then stored in the deep freezer at 4°C. The *A. niger* was isolated from the soil and identified in Microbiology Department Laboratory, Ahmadu Bello University, Zaria. *A. niger* was added into a freshly prepared potato dextrose agar (PDA) and left for four days. Tween 80 (2%) solution was prepared and sterilized for one hour. *A. niger* spores were transferred into the solution to get uniform suspension. Number of spores was counted with binocular using haemocytometer, and the spore load per ml was counted to be 9.55×10^7.

Chemicals and reagents

Bakers yeast and EMCEferm instant active yeast were of *Saccharomyces cereviscae*. Bakers yeast was obtained from Kaduna central market while EMCEferm was obtained from Muhlenchemie cmbh & co. kg Ahrensburg, Germany. Hydrochloric acid, sulphuric acid, sodium hydroxide, Tween 80, dinitrosalicylic acid, glucose, arabinose, sucrose and maltose standards, silica gel 60, anhydrous calcium sulphate, chloroform, acetic acid, diphenylamine, acetone, phosphoric acid, aniline potassium sodium tartrate, were of analytical grade and products of British Drug House (BDH) Poole , England and Sigma Chemicals (USA).

Degumming of bitter kola using *A. niger*

Ten grams of the sample were weighed into five 250 cm^3 conical flasks, and 100 cm^3 distilled water added respectively. The flasks were covered with aluminum foil and boiled for 5-10 min. The flasks were allowed to cool and 1.0 ml of 9.33×10^7 spores suspension of *A. niger* were added aseptically into each flask except one which was taken as control and kept immediately in the refrigerator. The remaining four flasks were incubated at 30°C. A flask was removed each day and kept in the refrigerator. At the end of four days the samples and arabinose standard were spotted on thin layer chromatographic plates with the developing solvents (chloroform : acetic acid : water, 6:7:1). After a run, the plates were dried in an oven and the spots were located with the aid of reagent spray. The spotting agent was made up by dissolving one gram diphenylamine and 1 cm^3 aniline in 100 ml of acetone prior to spraying). Acetone solution (10.0 ml) was mixed with 1.0 ml 85% phosphoric acid. The sprayed plates were heated for 10 min at 130°C in an oven.

Acid hydrolysis and saccharification (Dahot and Noomrio, 1996)

One hundred grams of dried sample were weighed into two 2000 cm^3 conical flasks and 1000 ml of sulphuric acid were added to one conical flask (acid hydrolysed) while 1000 ml distilled water were added to the other (control). The flasks were covered with aluminium foil and heated for two hours on flame and autoclaved for 30 min. The flasks were allowed to cool and filtered through Whatman No.1 filter paper. The pH was adjusted to 4.5 with 0.4 M sodium hydroxide. Fifty millilitres of solubilized treated sample were added into nine 250 cm^3 conical flasks. The flasks were covered with aluminium foil and autoclaved for 20 min and were allowed to cool at room temperature. One millilitres of 9.33×10^7 spores suspension of *A. niger* were added to each flask and incubated at 30°C. A flask was removed every twenty-four hour and the concentration of reducing sugar was determined by dinitrosalicylic acid (DNS) method described by Miller (1959). The experiment was carried out in duplicates for nine days. The same was repeated for control sample.

Alkaline hydrolysis

Alkaline hydrolysis of samples was carried out according to Shipnei et al. (1995). One hundred grams of dried sample were weighed into each of the two 2000 cm^3 conical flasks and 1000 cm^3 of 0.25 m sodium hydroxide solution was added to one conical flask (alkaline hydrolysed) while 1000 cm^3 of distilled water were added to the other (control). The flasks were left for one hour, after which the mixtures were neutralized with 0.1 M hydrochloric acid (HCL) to a pH of 4.5. The flasks were allowed to cool at room temperature and filtered through Whatman No.1 filter paper. Fifty millilitres of solubilized alkaline hydrolysed sample was added into nine 250 cm^3 conical flask. The flasks were covered with aluminium foil and autoclaved for 20 min and were allowed to cool at room temperature. One millilitre of 9.33×10^7 spores suspension of *A. niger* were added aseptically to each flask and incubated at 30°C. The experiment was carried out in duplicates for nine days. The procedure was repeated for control sample. A flask was removed every twenty-four hour and the concentration of reducing sugar was determined by DNS method (Miller, 1959).

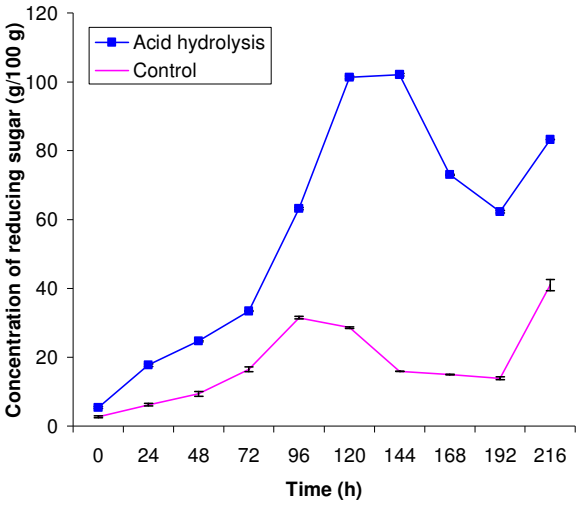

Figure 1. Effect of acid hydrolysis with 2.5 M sulphuric acid, saccharification with *Aspergillus niger* of bitter kola pulp on concentration of reducing sugar.

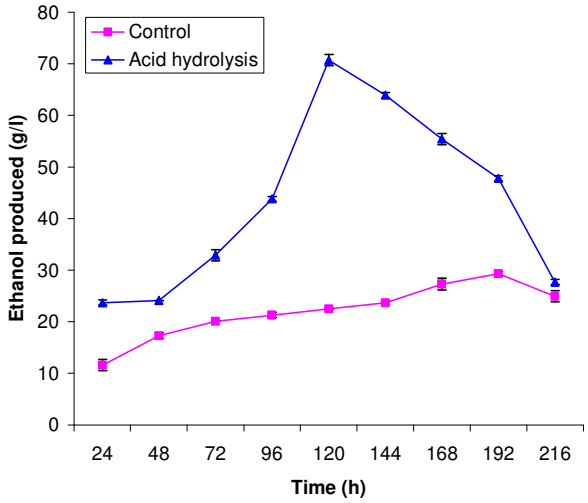

Figure 2. Ethanol produced (g/L) after acid hydrolysis with 2.5 M sulphuric acid, saccharification with *Aspergillus niger* and fermentation of bitter kola pulp using baker's yeast

Fermentation

Ten grams of the dried sample were weighed into nine 250 cm^3 conical flask and 100 ml of distilled water added. The pH was adjusted to 4.5 with 4.0 ml of 0.4 M sodium hydroxide. The flasks were covered with aluminium foil and hydrolysed for 3 h with 2.0 M sulphuric acid. The flasks were allowed to cool and baker's yeast (10 g) was added to each flask and incubated at 30°C for nine days. One conical flask was removed every twenty-four hour and contents distilled using distillation column. The distillate was collected at 78°C over a slow heat and the volume of ethanol determined is expressed as g/L by measuring the volume of distillate at 78°C and multiplying by the density of ethanol (0.8033 g/ml), g/L is equivalent to the yield of 100 g of dried substrate. Fermentation was carried out in duplicates for nine days. The procedure was repeated using EMCEferm yeast.

Invertase supplementation

Hundred grams of dried sample were weighed into 2.0 L conical flasks and 1.0 L 2.5 M sulphuric acid was added to one conical flask (acid hydrolysed) while 1.0 L distilled water was added to the other (control).The flasks were covered with aluminium foil and hydrolysed for three hours on boiling water bath and flasks autoclaved for 30 min were allowed to cool and the sample filtered through Whatman No.1 filter paper. The pH was adjusted to 4.5 with 0.4 M sodium hydroxide. 50 ml of solubilized acid hydrolysed sample was each added into nine 250 cm^3 conical flask.The flasks were covered with aluminium foil and autoclaved for 20 min and were allowed to cool at room temperature. One millilitre of 9.33×10^7 spores suspension of *A. niger* was added aseptically to each flask and incubated at 30°C. A flask was removed every twenty-four hour and 1.0 millilitre of digest added into a test tube. One millilitre of 0.1% invertase was added and the tube was covered with aluminium foil and incubated at 30°C for one hour. The concentration of reducing sugar released was determined by DNS method (Miller, 1959). The experiment was carried out for nine days in duplicates. The same was repeated for control samples. The procedure was repeated using maize and rice husks.

RESULTS AND DISCUSSION

Degumming was optimal at the 96 h (result not shown). Effect of acid hydrolysis with 2.5 M sulphuric acid and saccharification of bitter kola pulp with *A. niger* on concentration of reducing sugar is shown on Figure 1. The concentration of reducing sugar for treated sample (acid hydrolysed and saccharification) and control sample (saccharification) was maximum at 144 h (86.2 g/100 g) and 96 h (31.5 g/100 g), respectively. The result obtained with the control sample was higher than what was reported by Akin-Osanaiye et al. (2005b). This could be due to the fact that dried sample was used in this study instead of wet sample. Wet sample might have contained some compounds which act like inhibitors and affects the performance of the micro-organism. The treated sample released the highest fermentable sugar. Perhaps bitter kola pulp waste might have high cellulose and hemicellulose contents and low lignin content. Cellulose is placed beneath the network of lignin and hemicellulose components. Pretreatment or hydrolysis with sulphuric acid may have removed and hydrolysed hemicellulose to their monomeric constituent and lignin-hemicellulose-cellulose interactions partially disrupted (Ladisch, 1989; Days, 1989). This pretreatment therefore solubilized the cellulose making it amenable to attack by the cellulase produced by *A. niger.* The acid disrupts the hydrogen bonding between cellulose chains, converting it to a completely amorphous state, thereby forming a homogenous gelatin with the acid. At this point the cellulose is extremely susceptible to hydrolysis (Hsu, 1996). This shows acid hydrolysis significantly ($P<0.05$) enhanced saccharification of bitter kola pulp waste with *A. niger.*

Ethanol produced after acid hydrolysis with 2.5 M sulphuric acid and saccharification with *A. niger* and fermentation of bitter kola pulp wastes using baker's yeast is shown on Figure 2. Ethanol produced from treated sam-

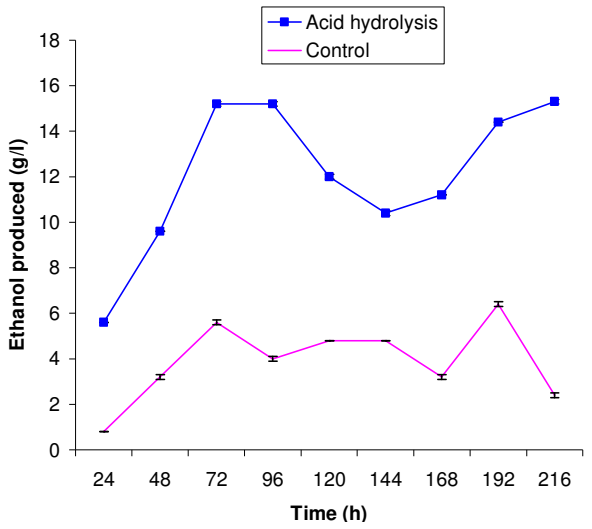

Figure 3. Ethanol produced (g/L) after acid hydrolysis with 2.5 M sulphuric acid, saccharification with *Aspergillus niger* and fermentation of bitter kola pulp using EMCEferm yeast.

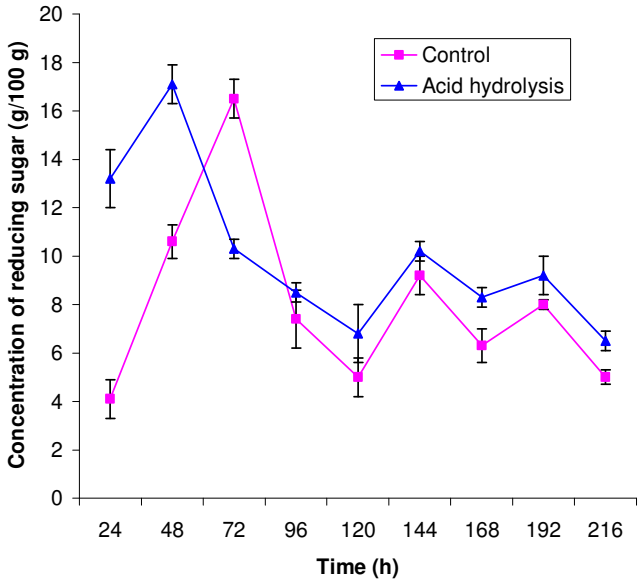

Figure 4. Effect of alkaline hydrolysis (0.2 M sodium hydroxide) and saccharification with *Aspergillus niger* of bitter kola pulp on concentration of reducing sugar.

ple was maximum (70.7 g/L) at 120 h while control value was maximum (29.3 g/L) at 192 h. The result obtained with the control sample was higher than that reported by Akin-Osanaiye et al. (2005b). This could be due to the fact that dried sample was used in this study instead of wet sample. Acid hydrolysis significantly ($P<0.05$) enhanced ethanol yield. Ethanol produced after acid hydrolysis with 2.5 M sulphuric acid and saccharification with *A. niger* and fermentation of bitter kola pulp wastes using EMCEferm yeast is shown on Figure 3. Two peaks were observed for both treated and control samples. Ethanol produced from treated sample was 11.2 g/L at 96 h and 12.9 g/L at 216 h while control was 5.6 g/L at 72 h and 6.4 g/L at 192 h. These values were lower than those produced with baker's yeast. This showed that baker's yeast is a better yeast for fermentation of bitter kola pulp waste. This is in line with the report of Akin-Osanaiye et al. (2005a). The decline and stabilization in ethanol noticed at some stages may be due to the inhibitory effect of ethanol on growth and transport metabolism of the yeast (D'AMarc and Stewart, 1987; Xu, 1996). Conventional yeast can ferment glucose to ethanol, but it cannot ferment xylose. Xylose makes up 30% of the sugar from agricultural residues, and inability to ferment xylose would constitute a major loss of ethanol yield. Use of genetically modified yeast that can simultaneously convert glucose and xylose to ethanol has been reported (Ho, 2004). Presently we are focusing this new research direction.

Effect of alkaline hydrolysis using 0.25 M sodium hydroxide and saccharification of bitter kola pulp wastes on concentration of reducing sugar is presented in Figure 4. Alkaline hydrolysis had no significant effect on concentration of reducing sugar. This could be due to the fact that sodium hydroxide was more effective at solubilizing a greater fraction of lignin while leaving behind much of the hemicellulose in insoluble polymeric form. Ethanol was also produced after two hours alkaline hydrolysis of bitter kola pulp wastes and saccharification with *A. niger* using EMCEferm yeast. Ethanol produced (g/L) for both alkaline hydrolysed and control increased to maximum value of 5.6 g/L at 96 h and then increased to maximum of 6.4 g/L at 144 h and 168 h respectively. Alkaline hydrolysis has no significant effect on ethanol yield.

Effect of invertase supplementation is presented in Table 1. Invertase supplementation enhanced the production of reducing sugar from bitter kola pulp wastes with time. Acid treatment decreased the time of optimal sugar production to 48 h for the bitter kola pulp wastes and maize husks. However rice husks had optimal sugar production at 120 h

.

Conclusion

This study revealed that acid hydrolysis with 2.5 M sulphuric acid and saccharification using *A. niger* significantly optimized ethanol production from bitter kola *(G. Kola)* pulp wastes. Alkaline hydrolysis had no significant effect on ethanol yield. Bakers yeast performed better than EMCE-ferm yeast. Furthermore, solar drying of the agro-waste has significant positive effect on the optimization of ethanol yield.

Table 1. Effect of invertase supplementation after acid hydrolysis with sulphuric acid and saccharification with *Aspergillus niger* on bitter kola pulp compared with maize and rice husks.

Time (h)	Control			Acid hydrolysed		
	Bitter kola pulp	Maize husks	Rice husks	Bitter kola pulp	Maize husks	Rice husks
24	13.2±0.4	8.5±0.4	5.6±0.4	22.1±0.4	17.4±0.4	14.4±0.4
48	13.8±0.2	10.3±0.3	10.9±0.3	23.2±0.3	22.7±0.3	15.6±0.3
72	14.4±0.4	7.9±0.4	11.5±0.4	20.3±0.4	18.0±0.4	16.8±0.4
96	15.6±0.4	7.4±0.4	13.2±0.4	18.0±0.4	16.8±0.4	20.3±0.4
120	16.8±0.3	6.8±0.3	14.4±0.3	16.2±0.4	14.4±0.3	21.5±0.3
144	17.4±0.4	8.5±0.4	16.8±0.4	15.0±0.3	13.8±0.4	18.0±0.4
168	18.0±0.4	10.3±0.4	17.4±0.4	14.4±0.3	15.6±0.4	19.1±0.4
192	19.1±0.3	10.9±0.3	18.5±0.4	12.7±0.4	12.7±0.3	17.4±0.3
216	17.4±0.4	11.5±0.4	18.5±0.4	15.6±0.4	13.2±0.4	15.9±0.4

Results are expressed as means ± SD of duplicate analysis.

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