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PRODUCTION OF BIOETHANOL FROM (*Chrysophyllum albidum*) SEEDS.

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ABSTRACT

Studies on bio-ethanol production using *Crysophyllum albidum* seed as substrates were conducted. *C. albidum* seed powder was subjected to hydrolysis using *A. niger* and the amount of reducing sugar liberated was monitored using benedict method while bio-ethonol concentration using *Saccharomyces cereviaes* and amount of bio-ethanol concentration

INTRODUCTION

Production of bio-ethanol from lignocelluloses materials such as agricultural waste though faces challenges, can substitute bio- ethanol production from edible food substances. Maize (*Zea mays*) is the most abundant cereals produced in Ghana (Asante, 2004). Subsequentely, there is large quantity of maize agro wastes which is underutilized. Currently the corncobs are burnt fuel in household of peasant rural farmers. Production of bio-ethanol from maize agro waste has been attempted with enzymes from different sources for hydrolysis of lignocelluloses and with diferrent organisms for fermentation (Eken-Saracoglu and Arslan, 2000. Wayman *et al*,1992).

Ethanol is a renewable energy resources produced through fermentation of simple sugar by yeast. Ethanol is widely used as a partial gasoline replacement in US and parts of the worldsuch as Canada. It can also be use in a variety of cooking, heating and lighting appliances. Ethanol that is blended directly with gasoline in a mix of 10% ethanol and 90% of gasoline is called gas oil. Recently,

liberated was monitored using U.V-visible spectrophotometer. The results obtained from the test for reducing sugar were recorded as follows:47.36mmol/1, 43.30mol/, 51.63mmol/1 respectively. Ethanol produced was recorded at interval of 1 day after the fermentation. The ethanol yields were as follows: 0.122%. 0.209%. 0.146%, 0.172% and 0.4458 percent respectively. Day five gave maximum yield of bio-ethanol while minimum yield was on day 1. The results suggest that. *C. albidum* seed could be harnessed for the production of bio-ethanol, considering the appreciable bio-ethanol yield.

Keywords: Bio-ethanol, seeds, Sokoto, reducing sugar, fermentation. lignocelluloses materials.

US automobile manufacturers have announced plans to produce significant number of flexible- fueled that can use ethanol blended-E85 (85% ethanol and 15 % gasoline by volume).

The federal government of Nigeria has concluded plans to invest 3.5 billion US dollars in jigawa states towards ethanol programme to diversify its sources (thisday 2006). Using ethanol blended fuel for automobile s can significantly reduce petroleum use and existing green house emission (Wang *et al.,* 1999). Ethanol is safe alternative to ethyl tertiary butyl ether (MTBE), the most common additive to gasoline used to provide cleaner combustion (Mani *et al.,* 2002). The benefits of developing biomass- to-ethanol are many, including: 1) increased national energy security, 2). Reduction in green house industry gas emission, 3).used of renewable resources foundation of a carbohydrate- based chemical process, 4) macro-economic benefits for rural communities and society at large (Wayman *et al.,* 1992).

The recent thrust in conversion of agricultural and industrial wastes to chemical feed stock has led to extensive studies on cellulose enzymes produced by fungi and bacteria. Celluloce is potentiality valuable resources of fibre, fuel and feed. Investigation into the ability of microbes to degrade native and modified cellulose so far have revealed that only a few fungi possess ability to degrade native cellulose. Majority of microbes can however degrade modified cellulose. A lot of emphases had given to screening of the agricultural waste for release of sugars

produced by hydrolysis of lignocellulosics. Therefore this Research aims to achieve the production of bio- ethanol by *Aspergillus niger* and *Saccharomyces cerevisies* cultured on *crysophyllum albidum*-seed, and to have optimization of the production process of the bio-ethanol production.

MATERIALS AND METHODS

Samples collection

Seed of *chrysophyllum albidum* were collected from kasuwar Daji Market Sokoto.

Sample processing

The dried seeds of *chrysophyllum albidum* were ground into powder using griding machine. The samples were further sieved in order to obtain very fine powder. The samples were later taken into drying machine for complete drying.

Media preparation

Potato dextrose agar (PDA) was the media used for fungi growth. 39gram of the powder were suspended in 100ml of distilled water in a sterilize conical flask, the mixture was then heated gently until it was absolutely dissolved. The conical flask was corked with cotton and aluminium foil. It was then sterilized by autoclave at 121 ^{o}c for 15minutes, allowed to cool at 45 before pouring into sterile petridish and was allowed to solidify. The yeast dextrose agar are use to isolate yeast.1 gram of the powder were suspended in 100ml of distilled water in a sterilize conical flask, the mixture were heated gently until it was absolutely dissolved. The conical flask was corked with cotton and alluminium foil. It

Isolation of aspergillus niger

The fungus, aspergillus niger was isolated from the soil in main compus of usman danfodio university, sokoto. Serial dilution were made as 10^{-1} 10^{-2} 10^{-3} and 10^{-4}. The $10^{-4,}$ was inoculated into nutrient media and incubate for 24hrs. then, it was grown into petri dishes containing potato dextrose agar (PDA) medium maintaining aseptic condition. Pure culture was inoculated on PDA in a petri dishes and grown at room temperature, 25c for 2-3 days for mycelia.

Isolation of *Saccharomyces cerevisiae*

S. cerevisiae which was used as fermentation organisms was obtained from chopped sugar cane solution exposed for 24 hr, the yeast was isolated on yeast

dextrose ager after 48hrs. the growth yield monitored and identified, and then further sub cultured into yeast extract and incubated for 24hrs.

Hydrolysis of *chrysophyllum albidum* seeds.

Ten (10)grams of C.albidum powder substrate was weighed and prepare into 15 different conical flasks labeled as A, B, AND C 5 FOR each, different volume of distilled water was dispensed into the substrate as 150, 200, and 250mls into A, B and C respectively and shaken gently to mix. The mixture was then auclaved at 121c for 15 minutes for sterilization as well as heat treatment in order to release sugar. After cooling at room temperature, the *Aspergillus niger* culture inoculums of 1%, 2% and 3% were introduced into each flask of A, B and C respectively. The flasks were incubated at different temperatures 40c, 35c and room temperature (37c were gently shaken each day for seven days.

Determination of reducing sugar

The reducing sugar was determined using benedics quanative test with glucose as standard. A standard glucose concentration of 1.0mmol/1, 2.5mmol/1, 5.00mmol/1, 7.50/1 and 10.00mmol/1 were prepared. Then a blank (5.00mmol/1) was put in 2ml of benedict solution and was used to zero the spectrophotometer. Then 5ml of each of the inoculated *Chrysophyllum albidum* sample was put in a test tubes and 2mls of benedicts reagent added. The mixtures were boiled in a water bath for 5mins. The absorbance of each sample was taken at 477nm using the spectrometer (6100 model, jenway uk), and the values were used to plot graph of absorbance against concentration. The reducing sugar concentration was extrapolated from the standard glucose curve.

Production of ethanol/seeding with *S. cereviasiae*

The inoculated *Chrysophyllum albidum* powder solutions were filtered using cotton wool and autoclave in order to kill the *A.niger* prior not to compete with *S. cereviae*. Seeding with *S.cereviae* culture inoculums of 1%, 2%, 3% were introduced into each flasks of A, B and C respectively. The flasks were reincubated at maintained temperature 40, 30, and room temperature (37c).each day, three samples of incubated flasks were distilled. Sample was weigh into kjeldahl flasks. The sample were then heated carefully at 75-78c until the solution turned colorless.

Determination of ethanol concentration using UV – visible spectrophometer
Ethanol concentrations of the fermentation were determine at 1, 2, 3, 4. And 5 days. Iml of fermented distilled aliquot was poured into a test tube, 7ml of distilled water added and 2ml of acidified potassium dichromate were also added. The solutions were then heated at 40mins in water bath. The absorbance read at 580nm using UV- visible spectrophometer.

Results:
The results of production of bio-ethanol from *chrysophyllum albidum* seeds are presented in the table 1 and 2. The result revealed that bio-ethanol was produced within 24hours after the fermentation. The analysis was carried out after 1 day interval. The result obtained from the test of sugar was presented in the table 1. From the results,sample c had the maximum (51.63mmol/1) concentration of reducing sugar while sample b had the minimum (43.30mmol/) concentration.in sample A ,sample A_1 had the highest concentration of reducing sugar (10.85mmol/1) and sample A_5 had the lowest concentration (7.78mmol/1). In sample group B, sample B_1 had the highest concentration of reducing sugar (10.01mmol/1) and sample B_5 had the lowest concentration (6.53mmol/1). And in the sample C, sample C_1 had the lowest concentration (15.56) and sample C, sample C_5 had the lowest concentration (7.63mmol/1).

Table 1: Sample and their corresponding concentration of reducing sugar (produced after the analysis)

Samples	H_2O:substrate **concentration**	Conc. Of reducing **sugar mmol/**	Group total
A_1	1:3	10.85	
A_2	1:3	10.49	
A_3	1:3	9.42	47.36
A_4	1:3	8.84	
A_5	1:3	7.78	
B_1	1:4	10.01	
B_2	1:4	9.59	
B_3	1:4	9.50	43.30
B_4	1:4	7.67	

B_5	1:4	6.53	
C_1	1:5	15.56	
C_2	1:5	10.49	
C_3	1:5	9.49	51.63
C_4	1:5	8.46	
C_5	1:5	7.63	
Total=15		142.29	142.29

Key: A,B, and C = designation sample group A, B, C.

The result of bio-ethanol analysis was recorded using U.V. spectrophotometer, every day for five day (5) days. Table 2 shows the detailed recording in yield of ethanolat day 1, sample C2 had the highest concentration of 0.114% while sample A2 had least (0.003). the group total stood at 0.122%. at day 2, sample B3, C2 had a concentration of 0.095 percent followed by sample B3 (0.070), while the group total was 0.209 percent. On day 3, sample B1 had the highest concentration of 0.101, with C1 having the lowest (0.004%).the group total stood at 0.146, showing a decrease from the previous day. On forth day sample B3 recorded the highest yield (0.082). Followed by sample C5 (0.057) with their group total of 0.172 percent. This shows a slight increase over the previous day. Day 5 saw the increase in each, with B4 recording the highest yield of 0.226, followed by C4 recording 0.192 percent. Their group total was highest (0.458) in all other group. The total ethanol yield in 5 days was 1.107 percent.

Table 2: the sample and the total concentration of ethanol yield after the analysis

Samples	Fermentation period (Days)	Ethanol concentration (%)	Ethanol yield (%)
A_2		0.003	
B_2	1	0.005	0.122
C_2		0.114	
A_3		0.044	
B_3	2	0.070	0.209
C_3		0.095	
A_1		0.041	
B_1	3	0.101	0.146
C_1		0.004	

A_5		0.033	
B_5	4	0.082	0.172
C_5		0.057	
A_4		0.040	
B_4	5	0.226	0.458
C_4		0.192	
Total=15		1.107	1.107

Key: A, B and C= designating sample group A, B and C

Discussion

Analysis on hydrolysis of *chrysophyllum albidum* seeds using A. niger was conducted and the highest group total for reducing sugar concentration was 51.63mmol/1 for group C. This was followed by group A and lastly by group B with 43.30mmol/1. The result might attribute to the fact that water increases the solubility of nutrient in the seed powder of C*.albidum*. there by making substrate susceptible to attack the microbial enzyme to release the higher amount of reducing sugar, while on the other hand lower moisture levels can cause a reduction in solubility of nutrient, thereby causing streak hindrances on A. niger enzyme, which might subsequently result to lower concentration of reducing sugar (Zahangir *et al,* 2005).

Production of bio-ethanol from chrysopyllum albidum seeds, hydrolysate seeded with saccharomyces cerevisiae were evaluated at 1,2, 3,4 and 5 days after fermentation. The highest bio-ethanol yield of 0.458% was obtained on day 5, followed by 0.209%, 0.172% and 0.122% on day 2, 4, 1 respectively. The result of bio-ethanol obtained on day 1 could be attributed to the fact that yeast cells might be in their lag phase trying to synthesize enzyme that could convert readily available sugar to ethanol. Similarly, observation has been made by Nester et al., (2001). The higher yields of bio- ethanol concentration are toxic to yeast cells. Similar observation has made by Ibeans and Menez, (1997).

Comparison of similar works in literature might be difficult because bio- ethanol concentration was not cited and they differ in either the type of pretreatment, if any detoxification, substrate concentration, fermentation strain, temperature or mode of operation which affects the final ethanol concentration as reported by Olofsson et al., (2008). This result suggest that C. albidum seed could be harnessed for the production of bio- ethanol, considering the appreciation ethanol yield.

Conclusion

Production of bio=ethanol from *chrysopyllum albidum* seeds was conducted. The results obtained from the test for reducing sugar were recorded as follows: 47.36mmol/1, 443.30mmol/1 and 51.63mmol/1 respectively. Sample group C gave

the maximum yield of reducing sugar, while minimum yield was on sample group B.

Bio- ethanol produced was recorded at intervals of 1 after fermentation. The ethanol yield are as follows: 0.122%, 0.209%, 0.146%, 0.172% and 0.458 percent respectively. Day 5 gave the maximum yield of ethanol while minimum yield was on day 1. These results suggest that *C. albidum* seed could be harnessed for the production of bio- ethanol, considering the appreciation ethanol yield.

Recommendation

i) the utilization of *Chrysophyllum albidum* seeds powder should be encourage for the large scale of Bio-ethanol by *A. niger* and *Sacharomyces cerevisiaes*.

ii) The government should give more emphasis on planting more of this tree for quantitative production of this useful product.

References

Asante,A. (2004).Assesment of food import and food against support for Agricultural development, the case of Ghana. Draft report for FAO Regional office, p.10.

Eken-saracoglu, N., Arslan, Y.(2000). Comparison of different pretreatments in ethanol fermentation using corn cob hemocellulosic hydrolyate with Pichia stipitis and Candida shehayaae. Biotechnology letter. **22:**855-858.

Ibeas,J.I. and Jimenez, j (1997).Mitochondrial DNA loss caused by ethanol in Saccharomyces flour yeast Appl. Environmental Microbiology **63**:7-12.

Mani,S., Tabil, L.G., Opoku, A. (2002). Ethanol from Agricultural crop residues. An overview. The society for engineering food and biological systems. MBSK 02-217. An ASAE/CSAE meeting presentation.

Neste, E.W, Anderson, D.G, Roberts, C.E, Pearsall, N.N, Nester, M.T. (2004). Dynamics of Prokaryotic growth In: Microbiology: A human perspective. 4th ed. McGraw- hill, York. PP. 87-108.

Olofsson, K., Bertiloson, M.Liden, G. (2008). A short review on SSF an interesting process option for ethanol production from Lignocelluloce fedstocks Biotechnology for Biofeuel **1**(7):1-14.

Thisday (2006). Federal government of Nigeria takes over 400 billion Naira Ethanol project.
Thisday newspaper Thursday feb. 2 Vol. **11** (3939):11

Wang, M.C., Saricks, D.,Santini. (1999). Effects on fuel ethanol use on fuel cycle energy and green house gas emissions. Agronne national laboratory, agrone, il.

Wayman,M. Chen, Doan, K. (1992). Bioconversion of waste paper to ethanol, process Biochemical. **27:** 239-245.

Zahangir, M.D., Nurdina , A.M.and Mohd. E.M. (2005). Production of celluloce from oil palm Biomass as substrate by solid state bioconversion. American, journal. Appl. Sci. **2(**2**):** 569-572.