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LABORATORY PRODUCTION OF ALCOHOL FROM RICE AND MAIZE CHAFFS

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ABSTRACT

Alcohol fermentation of two agricultural wastes (rice husk from rice (Oryza satira) and cobs of maize (zea mays) were converted to fermentable sugar by hydrolysis with two cellulase enzyme producing fungi – *Aspergillus niger* and *Penicillium digitatum*. The agricultural chaffs used were dilignified by combined alkaline and steam pretreatments to enhance sugar production by the organisms. The reducing sugar produced from the chaffs were fermented to alcohol using baker's yeast (*Saccharomyces cerevisiae*). The alcohol content of the samples were determined by distillation method. The concentration of alcohol content in MSRH and MSCC ranges between 126.5mg/ml – 479.3mg/ml and 331.6mg/ml – 521.1mg/ml respectively by *A. niger* and *Penicillium digitatum*. The highest reducing sugar obtained from MSCC medium degraded by *A. niger* and *P. digitatum* were 1560 x 10^3mg/ml and 1,300 x 10^3 mg/ml respectively. The results of this research work highlight the industrial potentials of these agricultural chaffs as substrates for alcohol production.

KEYWORDS: Chaffs, *oryzae sativa, zea mays,* fermentation, alcohol*, Aspergillus, Penicillium.*

INTRODUCTION

Alcohol is an important industrial chemical. It is one of the product of yeast metabolism. The production of alcohol by fermentation is an ancient act and is often considered to be one of the first microbial process used by man. Production of industrial alcohol by fermentation draws heavily upon the accumulated knowledge of the brewer and distiller. Alcohol is a chemical substance with molecular formular C_2H_5OH(ROH) containing only carbon, hydrogen and oxygen and the molecular mass is 46.

Alcohol is produced from sugar containing materials such as starch, molasses by a fermentation process utilizing yeast to convert the sugar to alcohol (Brien and Craig, 1996). The most widely used raw materials is blackstrap molasses. This is the waste syrup (mother liquor) remaining after the extraction of crystallized sugar from the evaporated sugar cane juice obtained in commercial cane – sugar mill – operations (Palmer, 1989). Alcohol is also produced by fermentation of starch and sulphite waste liquor, via the ethylsulphate route; by the direct hydration of ethylene; and from various grains and agricultural products (Lyons *et al;* 1995). Alcohol of appreciable yield had been produced from both cellulose and lignocellulose materials using special yeast strain called *Pnachysolen tannophillus* for fermentation (Lynd, 1990). Lee *et al;* (1987) produced alcohol from sargo starch using mobilized amyloglucosidase and zymomonas mobilis. The success of various cellutolisic and starchy materials for alcohol production depends on the development of pre-treatment procedures and highly effective enzymic system for conversion of pre-treated material for fermentable sugars (Agboola, 2006; 2009). Alcohol is a clear, colourless, flammable liquid miscible with water and many organic solvent in all ratio.

Economic Importance of Alcohol and the Nigeria Situation

The industrial uses of alcohol are generally on the increase. It enjoys wide application in the chemical industry as a chemical feed stock. Alcohol serves as an intermediate in many chemical processes due to its great reactivity. For example, it serves as a raw material in chemical manufacture like glycol-ethers, ethylchloride, amines, ethyl acetate, vinegar, acetic acid and in the production of acetaldehyde. Alcohol is also widely used in industry as a solvent for dyes, oils, waxes, explosives and cosmetics. It serves as a disinfectant in hospitals for cleaning and lighting in the home and in the laboratory second only to water as a solvent. Alcohol is mixed with petrol or gasoline up to 10% and this is called gasohol and used in automobile.

Due to usefulness of alcohol in Nigeria over abundant agricultural chaffs and wastes can be used to produce alcohol which can be exported to gain foreign reserve. Thus, also controlling the environmental pollution currently experienced all over the country by these useful agricultural and domestic wastes. In chemical and pharmaceutical industries, alcohol has prospective application which are found all over Nigeria. As energy cost increases and petrochemical raw materials become scarce, production of alcohol from agricultural waste materials could become a viable alternative (Dominguez *et al;* 2000).

Following the realization of the great economic importance of alcohol in the nation and throughout the world, this study was undertaken to examine the method of producing alcohol under laboratory conditions using

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some selected agricultural chaffs; with the aim of determining the role of microorganisms in the production process. Corn and rice chaffs were possible substrates for alcohol production using *Aspergillus niger* (Cs_9) and *Penicillium digitatum* (YF_{50}) for degradation and *Saccharomyces cerevisiae* for fermentation to yield alcohol. These agricultural chaffs are thus converted to useful products as well as achieve environmental pollution control of these chaffs. Local production of alcohol using these agricultural chaffs would certainly help to conserve foreign exchange and probably help in increasing local contents indigenous industries.

MATERIALS AND METHODS

Sample Collection

The rice and corn chaffs used in this work were collected from Ipata Market in Kwara State.

Sources of Organisms

Aspergillus niger and *Penicillium digitatum* were isolated from yam flour and compost soil. They were maintained on Potato dextrose Agar (PDA) slants and kept at 4^0C prior to use. The baker's yeast was obtained from Baboko market in Ilorin.

Sample Treatment

The pretreatment of substrates was carried out in the laboratory. The methods of Ali *et al* (1991) and Lyons, *et al* (1995) were adopted. Rice husk was sorted to remove stones and washed thoroughly with water to remove surface dust. It was dried and ground with domestic blender. (250W Philips Holland). Corn cob was sun dried and chopped into pieces and was ground into pellets.

Combined Alkaline and Steam Pretreatment

The ground rice husk and corn cob were autoclaved at 121^0C with 5% (W/V) NaOH (20ml per gramme of substrate) in separate conical flasks for degradation. The autoclaved materials were filtered through muslin cloth and the substrates washed thoroughly with water and neutralized with dilute HCl to pH7 and finally washed with distilled water and dried at 80^0C.

Corn chaff or corn cob used in this study is referred to the cobs left after removal of the maize fruits. Rice chaff means rice husks. This method generally involved the alkaline and steam pre-treatments of the substrates.

RICE CHAFF

Rice chaff was sorted to remove stones and washed thoroughly with water to remove surface dust. It was then dried and ground with domestic blender (250W Philips Holland).

CORN CHAFF

Corn chaff was sun dried, chopped into pieces and was ground.

COMBINED ALKALINE AND STEAM PRE-TREATMENT

The ground rice chaff and corn chaff were autoclaved at 121^0C with 5% (W/V) NaOH (20ml per gramme of substrate) in separate conical flasks for degradation. The autoclaved materials were filtered through muslin cloth and the substrates washed thoroughly with water and neutralized with dilute HCl to PH 7. The substrates were finally washed with distilled water and dried at 80^0C.

MEDIA (MSRC): Mineral – Salts Rice Chaff Medium

Mineral salts – rice chaff medium containing (g/L) KH_2P0_4, 10.0; $(NH_4)_2SO_4$, 10.5; $MgSO_4.7H_20$, 0.3. $CaCl_2$, 0.5; $FeSO_4.7H_20$, 0.013; $MnSO_4.H_2O$, 0.004; $Z_nSO_4.7H_2O$, 0.004; $CaCl_2.6H_20$, 0.0067; Yeast extract, 0.5, rice chaff powder, 40.0.

MINERAL SALTS CORN CHAFF MEDIUM (CCM)

Mineral salts-corn chaff medium containing (g/L), KH_2P04, 10.0; $(NH_4)_2SO_4$, 10.5; $MgSO_4.7H_2O$.0.013; $MnSO_4.H_2O$,0.004; $ZnSO_4.7H_2O$,0.004;$CaCl_2.6H_20$,0.0067; Yeast extract, 0.5, Corn chaff powder, 40.0.

Cultural Condition for Reducing Sugar Production

A modified method of Sanni *et al;* (1992) was used in which spores of 48 hours old cultures of *Aspergillus niger* (CS_9) and *Penicillium digitatum* (YF_{50}) were harvested by washing slants with 10ml of sterile distilled water. An aliquot of 1.5 ml of spores suspension was inoculated into 150ml of each of MSRC and MSCC medium and incubated at 28^0C in an orbital shaker maker set at 100rpm.

Assay for Reducing Sugar Production

Reducing sugar production was determined colorimetrically by measuring the increase in reducing groups in the culture media (Miller, 1959). Cultured samples were centrifugal at 3,000 rpm for 15 minutes to remove the mycelia and the supernatant fluid used to assay for reducing sugar. The same volume of the supernant and the dinitrosalicylic acid reagent (1.5ml) were measured out and added together inside the test tube. The blank was prepared by adding equal volume of dinitrosalicylic acid reagent and distilled water (1.5ml). The mixture was heated in boiling water bath for 15 minutes and then cooled under running tap water at ambient temperature. The resulting orange coloured solution was decanted into colorimeter tubes and absorbance was read at 575nm using a spectrophotometer (MILTON ROY COMPANY SPECTRONIC – 20D).

pH Determination

pH was determined using a pH meter (PYE ELECTRONIC INSTRUMENT LTD.).

Preparation of Yeast Solution

Yeast solution was prepared by dissolving 20 gramme of the dry baker's yeast in 100ml of distilled warm water and kept for 15 minutes prior to use. 15% (22.5ml) was inoculated into 150ml of fermented wort. The control experiment was void of yeast inoculum.

Fermentation

The supernatant of the fermentation product of MSRC and MSCC was used as medium for yeast fermentation. One hundred and twenty millimeter (120 ml) of each of the worts (Supernant Solution) was

transferred into the separate flasks (fermentor sodium metabisulphite 20% was added to eliminate any contaminant. 15g/L KH_2PO_4, 2g/L NH_4CL, 1gm/L $NaHPO_4$ and 1gm/L $(NH_4)_2$ NO_3 was as nitrogen and phosphorus sources (Adeniran and Adeleye, 1994). The worts were then seeded with baker's yeast (*Saccharomyces cerevisiae* (22.5ml).

Fermentation was allowed to continue for 5,10,14, and 20 days at 28^0C. Each fermentor was shaken three times daily to prevent the yeast from settling to the bottom of the wort and to ensure even distribution of nutrients and substrates to the yeast. At the end of the fermentation days, the fermented worts were filtered and the filtrates was distilled by fractional distillation method and the alcohol was collected into clean bottles which were stored for further analysis.

Analysis of Distillates

A modified method of Pearson (1976) was used to determine alcohol yield (% W/V) of each distillate using specific gravity bottle at ambient temperature. The alcoholic content of the sample (rice chaff fermented solution and corn chaff fermented solution) were determined by measuring out 100ml in a volumetric flask at ambient temperature and washed into the distillation flask with 50 ml of distilled water in a distiller. The acidity of each sample were neutralized with 1ml of sodium hydroxide. The samples were distilled slowly on hot plate at (B.P. 78 – 80^0C) until 50ml of distillates were collected; cooled and the specific gravity were determined. Their corresponding alcoholic content by volume was obtained from reference table 1 (Pearson, 1976). The control experiment was done by adding 100ml of water and 50ml of distilled water into a volumetric flask and the distillation was carried out.

Calculation

Weight of density bottle = ag
Weight of density bottle + water = bg
Weight of density bottle + distillate = cg
Weight of water = b–a
Weight of distillate from sample = c–a
Specific gravity of distillate

$$= \frac{\text{Weight of distillate}}{\text{Weight of equal volume of water}}$$

$$= \frac{c - a}{b - a}$$ (Pearson, 1976).

Test for Alcohol (Distillates)

The method of Murray (1974) was used to confirm the presence of alcohol in the distillate. This was done by using various methods.

First Test: To 2ml of the distillates, 2ml of iodine solution (saturated) was added and 1.5ml of dilute sodium hydroxide solution.

Second Test: About 5ml of distillates was placed in an evaporating dish and a small piece (about 50mg) of clean freshly cut dry sodium metal was added.

Thirdly: 2ml of distillates was placed in a petridish and 2ml of potassium permanganate was added, the mixture was allowed to stay for 15 minutes, then about one gramme of sodium bicarbonate was added.

Determination of Sugar Concentration

Preparation of Standard Solution

Zero point one gramme (0.1g) of glucose was dissolved in 100ml of distilled water. 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml of the solution was dispensed into different test tubes. Each solution was then adjusted to 1ml with corresponding volume of distilled water.

One point five (1.5ml) of dinitrosalicylic acid reagent was added into each test tube and mixed together, the blank was prepared by adding 1.5ml of dinitrosalicylic acid reagent to 1.5ml of distilled water instead of glucose. The mixture was heated in boiling water bath for 15 minutes and then cooled under running tap water at room temperature. The reading obtained was used to plot glucose standard curve.

RESULTS

From the study, highest concentration of reducing sugar and alcohol were obtained from mineral salt corn cob (MSCC) medium degraded by *A. niger* cs9 (1560 x 10^3mg/ml) table 5, (696 mg/ml) table 2. This was followed by mineral salt corn cob medium degraded by *P. digitatum* yF_{50} (1300 x 10^3mg/ml) table 5, (653.8mg/ml) table 2.

Therefore, the concentration of alcohol content in MSRH and MSCC were (126.5 – 479.3mg/ml); (331 – 521mg/ml); (384.2 – 696.0mg/ml) and (331.6 – 653.8 mg/ml) respectively.

Table 1: Concentration of alcohol by *S. Cerevisiae* in MSRH.

DAYS	MSRH WORT FROM *A. niger* Cs_9 (mg/ml)	MSRH WORT FROM *P. digitatum* YF_{50} (mg/ml)
5	126.5	331.6 (mg/ml)
10	413.1	521.1 (mg/ml)
14	479.3	521.1 (mg/ml)
20	479.3	479.3 (mg/ml)

MSRH = Mineral Salt Rice Husk.

Table 2: Concentration of Alcohol by *S. Cerevisiae* in MSCC (mg/ml).

DAYS	MSCC WORT FROM *A. niger* Cs_9 (mg/ml)	MSCC WORT FROM *P. digitatum* YF_{50} (mg/ml)
5	384.2	331.6
10	653.8	568.0
14	696.0	653.8
20	272.5	331.6

MSCC = Mineral Salt Corn Cob.

Table 3: Test for Alcohol

TEST	OBSERVATION	INFERENCE
2ml of distillate +2ml of iodine solution + 1.5ml of dilute NaOH	Decolourization of the mixture Colourless	Alcohol is suspected
5ml of distillate + clean freshly cut dry sodium metal	Colourless and produced characteristic odour.	Alcohol is suspected.
2ml of Acid Permanganate + 2ml of Distillate	Changing from purple colour to colourless.	Alcohol is confirmed.
2ml of Distillate + 2ml of Potassium dichromate.	Turned from orange colour to green colour.	Alcohol is confirmed.

From this results, only chemical methods were employed to test the alcohol at the end of distillation. Further work needs to be carried out using other confirmatory methods.

The use of baker's yeast instead of yeast isolated from natural source did not have negative effect on the result. The percentage of alcohol obtained in this study was similar to that of other researchers who isolated the yeast from natural sources and that is to confirm the efficacy of the baker's yeast.

Table 4: The yield of reducing sugar in mg/ml from fermentation of rice husk/chaff.

Hours	**Concentration of reducing sugar from MSRH by *A. niger* (mg/ml)**	***Concentration of reducing sugar from MSRH by P. digitatum* (mg/ml)**
0	*0*	*0*
24	120×10^3	100×10^3
48	310×10^3	480×10^3
72	500×10^3	520×10^3
96	890×10^3	780×10^3
120	890×10^3	520×10^3
144	530×10^3	400×10^3
168	410×10^3	360×10^3
192	400×10^3	350×10^3
216	400×10^3	310×10^3

Table 5: The yield of reducing sugar in mg/ml from fermentation of corn cob/chaff.

Hours	Concentration of reducing sugar from MSCC by A. niger (mg/ml)	Concentration of reducing sugar from MSCC by P. digitatum (mg/ml)
0	*0*	*0*
24	*430 x 10^3*	*390 x 10^3*
48	*650 x 10^3*	*410 x 10^3*
72	*960 x 10^3*	*450 x 10^3*
96	*1560 x 10^3*	*1300 x 10^3*
120	*800 x 10^3*	*1000 x 10^3*
144	*680 x 10^3*	*820 x 10^3*
168	*630 x 10^3*	*400 x 10^3*
192	*450 x 10^3*	*400 x 10^3*
216	*350 x 10^3*	*400 x 10^3*

DISCUSSION

This study was carried out to determine the potential use of rice chaff and corn chaff (i.e. rice husk corn cob) (that are agricultural waste products) as substrates for alcohol production using A. niger Cs_9 and Penicillium digitatum YF_{50} for degradation. The effectiveness of the chemical that was used for hydrolysis and the enzymes (cellulose) produced into the medium by these fungi resulted into the production of reducing sugar that was used for alcohol production. This was similar to the report of Lyons *et al;* 1995. This was also favourably compared with the report of Gharpuray *et al;* (1983) who stated that alkaline treatment permits greater accessibility of the cellulose to cellulolytic enzyme. The blending of the chaffs used was to increase the accessibility of the substrate to the organisms. This was similar to the observation of Doppelbauer *et al* (1987) who reported similarly that increase in enzyme production that resulted in increase in fermentable sugar production could be attributable to increase in surface area, pore size, and altered physical nature of lignin. Maheswari *et al* (1993) and Agboola (2009) reported similar cases.

The high concentration of sugar produced in the medium by the fungi was in line with what Marjailmen *et al;* (1997) and Agboola (2006) observed that high levels of enzymes and reducing sugar were produced in the medium containing cellulose or complex plant material degraded by fungi. The presence of water in the fermented substrates was important and it was an indication of occurrence of continued active fermentation, this supported the findings of Ullah *et al;* (1994). The low sugar concentration after optimum was in line with what Brien and Craig (1996) observed.

The alcohol yield (% W/V) was outlined in the table 1 and 2 and it was found to vary. The high level of alcohol production from these substrates were similar to those that was reported by other researchers on yeast fermentation of starchy and agricultural materials. Adeniran and Adeleye (1994) reported that about 96.17% alcohol (W/V) was distillated from the fermentation of potato mash by yeast. Engelbart and Dellweg (1977); Zikmanis *et al* (1988); Asghari *et al* (1990), Brian and Craig (1996) reported similar observations.

The production of high percentage of alcohol in this study indicated that, the sugar in the fermented wort did not lead to a decrease in water activity (^aw) as it was noticed by Kenyon *et al;* (1986). Since these substrates were converted to alcohol by fermentation and yield high concentrations, this shows that hydrolysed lignocellulose represents a potential source of renewable energy which was similar to the report of Lynd, 1990; Lebean *et al;* (1998).

Nutrient (yeast food) that was included in the fermentation media might also accounted for high yield of alcohol. The same trend was observed by Thomas and Ingledew, (1990); Brien and Craig (1996) who reported that in yeast fermentation rate of fermentation and alcohol yields are enhanced with appropriate nutrient in the medium. The production of alcohol from those substrates was also in line with the findings of Belkacemi, *et al;* (1998) who observed that about 40 – 60% of alcohol was produced after 24 hours with yeast fermentation of forages and agricultural residues such as corn stalks and barley straw.

The high yield of alcohol in this study was also favourably compared with the Dominguez *et al;* (2000) who reported that about 88.1% (alcohol by weight) and 72.6% of alcohol was obtained with yeast fermentation of xylose.

Progressive decreased in alcohol concentrations as the duration of fermentation increases might have resulted in decreased on nutrient value of the fermented wort. In addition, production of toxic by-products in the medium that could cause death of yeast cells and also serve as inhibitor of cell growth. This observation supports that of Leroi and Ridoux (1993). It also agrees with Zaldivar and Ingram (1999) who observed that both aliphatic and mononuclear organic acids inhibit growth and alcohol production.

The high concentration of sugar obtained in this study was similar to the report of Agboola (2009) who observed the high production of sugar from waste paper and sugar cane wastes. This study has clearly demonstrated the importance of these agricultural chaffs.

CONCLUSION

The economic importance of this work are: alcohol can serve as source of renewable energy. The use of those selected chaffs assist to solve

environmental waste problems. The country can save her foreign reserve used in importing alcohol i.e. alcohol serve as source of revenues for both individual and the government.

Despite an enormous and worldwide utilization of different wastes, there are still abundant quantities of agricultural chaffs that could be used more efficiently. Better processing methods that would be more economically profitable are therefore suggested.

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APPENDIX I
REFERENCE TABLE 1

Specific gravity at 20^0C	% Alcohol by Weight	By Volume	% Proof Spirit
0.7904	100.00	100.00	175.35
0.7936	98.98	99.37	174.25
0.8000	96.85	98.02	171.88
0.8100	93.38	95.69	167.77
0.8200	89.71	93.06	163.15
0.8300	85.89	90.18	158.08
0.8400	81.94	87.07	152.61
0.8500	77.89	83.75	146.76
0.8600	73.77	80.29	140.80
0.8700	69.60	76.59	134.19
0.8800	65.38	72.78	127.46
0.8900	61.12	68.80	120.47
0.9000	56.80	64.66	113.19
0.9100	52.11	60.33	105.57
0.9200	47.93	55.77	97.57
0.9300	43.31	50.94	89.09
0.9400	38.42	45.88	79.86
0.9500	33.16	39.85	69.60
0.9600	27.25	33.09	57.73
0.9700	20.28	24.88	43.34
0.9800	12.65	15.88	27.27
0.9900	5.71	17.18	12.44
1.0000	0.00	0.00	0.00