The effect of delignification on the saccharification of abakaliki rice husk

Nneka Blessing Ekwe

Chemical Engineering Department, University of Abuja, Nigeria.

_____

ABSTRACT

Limited world reserves of petroleum have generated great interest in the development of hydrolytic processes for the production of fuels and chemical feedstock from renewable resources. Renewable cellulosic material especially agricultural residues rich in cellulose are one of the most abundant biomass on earth. Due to the uncoordinated channel of waste disposal, these agricultural waste residues such as rice husks, undergo uncontrolled fermentation and putrification where they are dumped, thereby constituting environmental pollution and health hazards. This work is therefore carried out to help reduce the problems caused by the waste disposal. Delignification was carried out using varying concentrations of sodium hydroxide and maximum delignification of 80.41% was achieved using 12% (w/v) sodium hydroxide, which was hydrolysed at 303K for 45 minutes and 83.13% of reducing sugar was obtained. The experiments carried out, demonstrate that delignification is essential for saccharification.

Key Words: Delignification, Saccharification and Rice Husks

_____

INTRODUCTION

The world's clamour for environmental concerns during the late 60s and late 70s of the twentieth century has generated immense interests in the development of new technologies in the fields of energy conservation, and substitutions. The biggest impetus for developing a worldwide effort to monitor and restrict global pollution is the fact that most forms of pollution do not respect national boundaries [1] Chime N.B., *the effect of delignification on the saccharification of Abakaliki rice husks*, (1998) ,M.Eng thesis UNPUBLISHED. However, this necessitates the need to conserve the world finite reserve of petroleum through the development of hydrolytic processes for the production of fuels and chemical feedstock from renewable resources [2] Amar Singh, Kumudeswar Das and Durlibh K. Sharma, *On production of xylose, fufural*, *fermentable sugars and ethanol from agricultural residues,* (1984), journal of Chemical Technology and Biotechnology,34:51-61, and equally to reduce the environmental pollution caused by the uncontrolled fermentation and putrification of the indiscriminately dumped agricultural waste residues from agro-industrial processes. The environmental pollution caused by these waste residues, both on land and in the air, calls for concern.

Numerous and ingenious methods have been proposed for removing the pollutants from the atmosphere once they have been emitted. All have proved impracticable, primarily because of the huge mass of air that must be treated. One reasonable solution to eliminate the environmental pollution and save energy is to combine the conservation strategies with increased use of agro based waste residues in producing alcohols and other volatile compounds to replace conventional automotive fuels. This is achieved by fermenting the simple sugar produced from biomass or by hydrolyzing the polysaccharide present in biomass to yield glucose. The sugar obtained can be fermented to give alcohol.

Renewable cellulosic materials of plant origin such as maize straw, sorghum stalk, rice husks, and other residues are potential substrates for ethanol production particularly since the energy crisis of 1973 and recently as a result of the expanded use of alcohol for blending into automobile gasoline [3] Christakopoullous P, Macris B.J., Kekos D, *on*

_____

the Mechanism of direct conversion of cellulose to ethanol by Fusarium Oxysporum, Effect of cellulase and β Glucosidase, (1990) , Applied microbiology Biotechnology 33:18-20.

Bioconversion of these materials into ethanol with conventional methods involves first enzymatic or acid hydrolysis of biopolymer to monosaccharides (saccharification) and second the fermentation of the monosaccharides to alcohol [4] Christakopoullous P, Koullas D.P, Kekos D, Koukios E.G. and Macris B.J., *Direct ethanol conversion of pre-treated straw by Fusarium Oxysporum*, (1991) , Bioresource Technology 35,297-300.

In Nigeria, rice is grown all over the country but Abakaliki zone of Ebonyi state is the largest producer[5] Aneke I.E., *First scientific Annual Progress report of the* (Nov. 1995), *ICARUS PROJECT*. Tonnes and tonnes of rice husks are produced every year as by product of rice processing at Abakaliki rice mills. These husks are dumped indiscriminately around the rice mills and are left to pollute the environment and also cause a nuisance in many ways because they undergo uncontrolled fermentation and putrification, thereby causing hazards to human beings and their environment and equally reducing the aesthetic value of the environment. Thus, this work is channelled towards enhancing the aesthetic value of the Abakaliki rice mill, reducing the air pollution caused by the waste residues to the environment and equally reducing the health hazards caused by them.

The primary aim of this work is the production of ethanol from rice husks. The production of ethanol is achieved mainly from petroleum and from the fermentation of sugars. With the increasing shortage of petroleum globally, reliance on biomass as a source of chemicals and Fuel has to increase to conserve the world finite reserve of petroleum. Ethanol is a universally known solvent and is second only to water. Industrially, it is used in the pharmaceuticals, antifreeze, celluloid, perfumes, cosmetics, and ester. It is also used in the preservation of organic specimens. When mixed with gasoline it is used as motor fuel (GASOHOL). It is also used as a beverage for human consumption. Its wide industrial applications cannot be overemphasized; that is reason for its massive importation and equally the rationale behind this work.

In Nigeria, ethanol consumption is high [6] Nigeria Trade summary, *January – June 1996*; Federal Office of statics, most of the ethanol used are imported while it produces about 5% of its ethanol requirements locally – mainly from Nigerian Yeasts and Alcohol Manufacturing Company (NYAMCO) based in Bauchi and Kwara State, as well as from numerous small alcohol plants redistilling alcohol from palm wine. This insignificant quantity produced in this country leads to massive importation of ethanol to meet its demand in the country and thereby draining the country's foreign exchange and also making it affordable at a high cost.

This work will help meet the increase in the demand of ethanol and also make it available at a reduced price, and hence help to conserve the country's foreign exchange. It could be commercialized to offer employment to people and at the same time solve the environmental problems created by the uncoordinated disposal of agro-industrial waste and conserve the nation's finite reserve of petroleum.

The extent of ethanol use is multifarious and currently the world is embarking on this technological advancement of bioconversion of richly available cellulose to ethanol. Today, both India and Brazil are expanding their chemical industries based on fermentation ethanol and Brazil is now actively marketing its ethanol technology to less industrialized nations [7] Maorella B.L. Marray M.D.E, *the principles, application and regulation of Biotechnology in industry, Agriculture and Medicine*. (1985), Ethanol comprehensive Biotechnology, Vol. 3, Young Pergamon Press.

Recently, a group of researchers on the Innovative Concepts in Agricultural Residues Utilization for sustainable Development (ICARUS) whose bold philosophy in the utilization of agro-based waste residues which are rich in cellulose in the production of ethanol which would help in alleviating the problems of pollution, energy waste and drain of foreign exchange has come a long way.

Currently, here in Nigeria, the same technology has been adopted in utilizing the widely available cheap and renewable cellulose materials –rice husks –in the production of ethanol and this has enhanced the interest in the saccharification of Abakiliki rice husks.

MATERIALS AND METHODS

Experimental Methodology
• **Delignification of rice husk**
Apparatus
Conical flask, boiling tube, Oven, Filter funnel and paper

Material: 1g of 425µm sample of Abakaliki rice husks
Reagent: 12%, 4%, 20%, and 16% NaOH

Procedure
Sodium hydroxide breaks the lignin bond and dissolves it. Removal of lignin also depletes the hemicelluloses present since it forms strong bond with lignin.

A gram portion of the test material was placed in a boiling tube. It was treated with 12% sodium hydroxide using a solid – liquid ratio of 1: 15 at 120^oC for 30min. The tube was cooled and residue filtered and washed free of the alkali. The residue was dried at 105^oC for 1hr in an oven, cooled and weighed.

The procedure was carried out for other concentrations of NaOH(4%, 8%,16% and 20%)

- **Effect of alkali on delignification of rice husks**
APPARATUS
Beaker, Boiling tubes, Autoclave, Conical flask,Filter paper and funnel
Material: 1g of 425µm sample of Abakaliki rice husks
Reagent: Sodium hydroxide, Sulphuric acid

Procedure
Cellulose material can be delignified with hot sodium hydroxide in an autoclave at 120^oC for 30min. The lignin content of the residue is determined to ascertain the extent of delignification.

1g of the test sample was placed in five test tubes and treated with varying concentrations of sodium hydroxide (4%, 8%, 12%, 16%, and 20%) and placed in autoclave operating at 120^oC for 30minute for the delignification reaction. After the reaction, the boiled materials were cooled, filtered and the residue washed out with water and dried in an oven at 105^oC. The air dried residue was treated with $5cm^3$ of 72% sulphuric acid which was allowed to go on for 2hrs with frequent stirring at ambient temperature. The content of the beaker was transferred to a flask containing 300ml of distilled water. The sulphuric acid was diluted to 3% by adding water to a volume of $575cm^3$. The resulting solution was boiled for 4hrs, maintaining constant volume by using either a reflux condenser or frequent addition of hot water. The insoluble material (lignin) was allowed to settle, keeping the flask in an inclined position overnight. Without stirring the supernatant solution was decanted using a filtered paper. The remaining lignin was washed free of acid using hot water and dried in an oven at 105^oC to constant weight.

- **Effect of delignification on saccharification of Abakaliki rice husks**

APPARATUS
Conical flasks, Autoclave, Filter paper and funnel, Water bath, Small glass container, Stirring rod and cotton wool
Material: 20g of 425µm sample of Abakaliki rice husks

Reagent: 12% NaOH, 72% H_2SO_4, Calcium carbonate

Procedure
20g of test sample was placed in a flask and 300ml of 12% sodium hydroxide was gradually added to it. The mixture was shaken very well and the flask was plugged with cotton wool and placed in an autoclave at 120^oC for 30min for the delignification reaction. After the reaction, the flask was cooled, filtered and washed free of sodium hydroxide with water. The residue was dried in an oven at 105^oC and was used for saccharification. The residue was treated with 200ml of 72% sulphuric acid and cooled at 15^oC. The mixture was stirred thoroughly and placed in water bath maintained at ambient temperature for a period of 45min. At the end of the period, the content was washed with distilled water and placed in an autoclave operating at 15psi for 1hr. After the secondary hydrolysis the resulting liquid was cooled, diluted and neutralized with excess calcium carbonate and filtered. The filtrate was used for the determination of reducing sugars and total sugar.

_____

RESULTS AND DISCUSSION

Delignification

Conc. of NaOH (w/v)	%Residual acid – insoluble lignin content	%Delignification
4	2.375	58.41
8	1.38	75.83
12	1.115	80.47
16	2.07	63.75
20	2.125	62.78

Varying concentrations of sodium hydroxide were used in the treatment of rice husk. The extent of delignification was observed. Maximum delignification of 80.47% was achieved using 12 %(w/v) of sodium hydroxide at a solid: liquid ratio of 1:15. It was observed that increasing the percentage concentration of alkali could not further improve on the yield of delignification. This observation could be attributed to the error resulting from the way the procedure was carried out.

The ability of sodium hydroxide to remove lignin is that it has the potential to break the lignin – lignin bond and dissolves it. The removal of lignin also affects and accounts for the depletion of polysaccharides particularly hemicelluloses to some extent, since it forms strong bond with lignin. This also may insignificantly affect the yield of the reducing sugars during saccharification, since hemicelluloses are hydrolysed by acid to reducing sugar but are mainly hydrolysed to xylose which on further decomposition yields furfural.

Saccharification

Saccharifying rice husk at 303K for 45min at varying concentrations of sulphuric acid at 15psi was carried out. It was observed that the optimum conditions for the primary hydrolysis were at 45min and 4% sulphuric acid. It was noticed that the chemical pre treatments such as solvent extraction and delignification enhanced the yield of reducing sugar which helped in a high yield of alcohol. An encouraging yield of reducing sugar was obtained using delignified rice husk sample. It yielded 83.13% as against untreated rice husk which yielded 56.25% reducing sugar as could be seen from the following tables;

Effect of time of primary acid hydrolysis on saccharification of untreated rice husk

Time (min)	Absorbance	Conc. of reducing sugar (mg)	%Reducing sugar	%Total sugar
30	0.02	2.25	56.25	1.0
45	0.02	2.25	56.25	0.98
60	0.018	2.0	50.00	0.80
90	0.015	1.95	48.75	0.80
120	0.01	1.15	28.75	0.80

Temp. = 303K

Effect of temperature of primary acid hydrolysis on saccharification of untreated rice husk

Temp (K)	Absorbance	Conc. of reducing sugar(mg)	%Reducing sugar	%Total sugar
303	0.02	2.25	56.25	0.98
323	0.01	1.15	28.75	0.80
343	0.01	1.15	28.75	0.80
363	0.00	0.00	0.00	0.80

Temp. = 303K

CONCLUSION

The ultimate goal of this research is the saccharification of rice husks. Various experiments were designed to help achieve this, and one of such is the investigation into the effect of delignification on saccharification of Abakaliki rice husks which this work majorly centred on.

Delignification of rice husk was carried out using varying concentrations of sodium hydroxide. It was found that removal of lignin enhances the yield of alcohol, since it retards the hydrolysis of polysaccharides, shielding the cellulose from reaction.

APPENDIX

APPENDIX I   *EFFECT OF ALKALI ON DELIGNIFICATION OF RICE HUSK*

1(g) NaOH (w/v)						2(g) NaOH (w/v)				
READINGS	**4%**	**8%**	**12%**	**16%**	**20%**	**4%**	**8%**	**12%**	**16%**	**20%**
Residue	0.021	0.012	0.011	0.019	0.016	0.024	0.014	0.010	0.020	0.024
Wt. of oven dry sample	0.942	0.942	0.942	0.942	0.942	0.942	0.942	0.942	0.942	0.942

4% NaOH (w/v)

$\%\text{lignin} = \frac{A}{W} \times 100$

A = weight of lignin
W = weight of oven dry specimen

% residual lignin$_1$ = $\dfrac{0.021 \times 100}{0.942}$

                                                            =   2.20%

% residual lignin$_2$ = $\dfrac{0.024 \times 100}{0.942}$

                                                            =   2.55%

% residual lignin   = $\dfrac{2.20 + 2.55}{2}$

                                                            =   2.375%

%delignification   = $\dfrac{(5.71 - 2.375) \times 100}{5.71}$

                                                            =   58.41%

8% NaOH (w/v)

% residual lignin$_1$ = $\dfrac{0.012 \times 100}{0.942}$ =   1.27%

% residual lignin$_2$ = $\dfrac{0.014 \times 100}{0.942}$ =   1.49%

% residual lignin   = $\dfrac{1.27 + 1.49}{2}$ =   1.38%

%delignification   = $\dfrac{(5.71 - 1.38) \times 100}{5.71}$ =   75.83%

12%NaOH (w/v)

% residual lignin$_1$ = $\dfrac{0.011 \times 1}{0.942}$ =   1.17%

% residual lignin$_2$ = $\dfrac{0.010 \times 10}{0.942}$ =   1.06%

% residual lignin   = $\dfrac{1.17 + 1.06}{2}$ =   1.115%

%delignification   = $\dfrac{(5.71 - 1.115) \times 100}{5.71}$ =   80.47%

16%NaOH (w/v)

% residual lignin$_1$ = $\dfrac{0.019 \times 100}{0.942}$ =   2.02%

% residual lignin$_2$ = $\dfrac{0.020 \times 100}{0.942}$ =   2.12%

% residual lignin   = $\dfrac{2.02 + 2.12}{2}$ =   2.07%

%Delignification   = $\dfrac{(5.71 - 2.07) \times 100}{5.71}$ =   63.75%

20% NaOH (w/v)

% residual lignin$_1$ = $\dfrac{0.016 \times 100}{0.942}$ =   1.70%

% residual lignin$_2$ = $\dfrac{0.024 \times 100}{0.942}$ =   2.55%

_____

| % residual lignin | = | $\dfrac{1.70 + 1.06}{2}$ | = | 1.115% |

| %Delignification | = | $\dfrac{(5.71 - 1.115) \times 100}{5.71}$ | = | 80.47% |

Note: 5.71% is the value of the actual acid insoluble lignin obtained previously in the course of this work.

APPENDIX II: EFFECT OF DELIGNIFICATION ON SACCHARIFICATION OF RICE HUSK

Readings:

Hydrolysis Time:	=	45 minutes
Hydrolysis Temp.	=	303K
Absorbance		0.03
Concentration of Reducing Sugar	=	3.325 mg
Total Sugar	=	0.80%

| % reducing sugar | = | $\dfrac{3.325 \text{ mg} \times 250 \times 1000}{1000}$ |
| | = | 83.13% |

APPENDIX III: EFFECT OF TIME OF PRIMARY ACID HYDROLYSIS ON SACCHARIFICATION OF UNTREATED RICE HUSK.

Hydrolysis time	:	45 min
Hydrolysis temp.	:	303 K
Absorbance	:	0.02
Concentration of reducing sugar	:	2.25 mg

| % Reducing Sugar | | |
| I ml of hydrolysate | = | 2.25 mg RS. |

| Therefore 250ml of hydrolysate | = | 250 x 2.25 mg |

| % R.S | = | $\dfrac{250\% \times 2.25 \times 100 \text{ mg}}{1000 \text{ mg}}$ |
| | = | 56.25% |

Time (min)	Absorbance	Conc. Of RS mg	% RS	% TS
30	0.02	2.25	56.25	1.0
45	0.02	2.25	56.25	0.98
60	0.018	2.0	50.00	0.80
90	0.015	1.95	48.75	0.80
120	0.01	1.15	28.75	0.80

APPENDIX IV: EFFECT OF TEMPERATURE OF PRIMARY ACID HYDROLYSIS ON SACCHARIFICATION OF UNTREARED RICE HUSK

TEMP ^{0}K	Absorbance	Conc. Of Rsmg	% RS	% TS
303	0.02	2.25	56.25	0.98
323	0.01	1.15	28.75	0.80
343	0.01	1.15	28.75	0.80
363	0.00	0.00	0.00	0.80

Time=45 minutes; R.S. =Reducing Sugar; T.S. =Total Sugar.

REFERENCES

[1] Chime N, B. '' the effect of delignification on saccharification of Abakaliki rice husks, (**1998**), M.Eng thesis, unpublished

[2] Amar Singh, Kumudeswar Das and Durlibh K. Sharma, (**1984**) *journal of Chemical Technology and Biotechnology*, 34: 51 - 61

[3] Christakopoullous P, Maacris B.J., Kekos D, (**1990**) , *Applied microbiology Biotechnology* 33:18-20.

[4] Christakopoullous P, Koullas D.P, Kekos D, Koukios E.G. and Macris B.J., (**1991**), *Bioresource Technology* 35, 297 -300

[5] Aneke I.E., "First scientific Annual Progress report of the ICARUS PROJECT",(Nov. **1995**).

[6] Nigeria Trade summary, January – June **1996**; Federal Office of statics.

[7] Maorella – B.L.,Marray M.D.E., the principles, application and regulation of Biotechnology in industry, Agriculture and medicine. (**1985**) *Ethanol comprehensive Biotechnology*, Vol. 3,Young Pergamon Press.