INCREASE IN GLUCOSE GENERATION FROM THE BIOCONVERSION EFFICIENCY OF SUCCESSIVE PRETREATMENT OF DIFFERENT NIGERIAN SAWDUST LIGNOCELLULOSIC WASTES

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ABSTRACT

Biomass pretreatment plays an important role in lignocellulose utilization for glucose generation and subsequent bioconversion processes. The inherent properties of sawdust lignocellulosic biomass make them recalcitrant to enzymatic catalysis and thereby reduce its bioconversion efficiency. Effective pretreatment option enhances cellulose accessibility and susceptibility to enzymatic hydrolysis into glucose, biofuels and other desired chemicals. Investigation into the bioconversion efficiency of different sawdust lignocellulosic biosubstrates from a successive biomass pretreatment process and the resultant increase in glucose generation has been carried out. The amounts of glucose obtained from sawdust of twenty Nigerian tropical hardwoods at different biomass concentrations of 4, 10, 20, 40 and 60mg/mL after Kraft pretreatment process and Trichoderma viride cellulase interaction were 1.41, 2.29, 2.70, 2.46 and 1.96 mg/mL respectively for M. excelsa wood sawdust. The successive pretreatment of each of the Kraft cellulose with hydrogen peroxide at constant incubation period and enzyme treatment resulted in enhanced glucose concentrations of 3.26, 4.88, 4.65, 8.72 and 7.84mg/mL from the same M. excelsa sawdust to give 131%, 113%, 72%, 225% and 300% increase in glucose generation. The effect of a successive Kraft and hydrogen peroxide pretreatments on the biosubstrates led to large percentage increase in glucose formation from all the twenty-wood sawdust examined.

Keywords: Biomass pretreatment, Lignocellulosic Waste, Bioconversion efficiency, *Trichoderma viride cellulase*, Glucose generation.

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INTRODUCTION

The major prerequisite in the utilization of lignocellulosic biomass for high yield glucose generation is the pretreatment of the biomass feedstock. The bioconversion of lignocellulosic biomass into sugar hydrolytes is significantly hindered by the structural and chemical complexity of cellulosic biomass as the sugars available for fermentation are trapped in cellulose that is cross-linked with the lignin molecule. Thus, to achieve high yield of glucose, lignocellulose must first be pretreated (Zheng *et al.*, 2009).

Pretreatment refers to the solubilisation and separation of one or more components of the lignocellulosic biomass; hemicelluloses, cellulose, and lignin to make the remaining biomass substrate more accessible for further chemical or biological treatment. Primarily, it is a delignification process that converts lignocelluloses into the form most suitable for glucose generation. This is the main processing challenge in the ethanol production from lignocelluloses biomass (Sticklen, 2008). The goal of biomass pretreatment is to overcome lignin barriers, reduce cellulose crystallinity, and increase the porosity of the lignocellulosic biomass to improve the bioconversion efficiency of enzymatic hydrolysis (Mosier *et al.*, 2005; Galbe and Zacchi, 2007). Different pretreatment techniques can affect biomass in very different ways. Pretreatment methods under high pH mainly remove lignin; while those under low pH mainly digest hemicelluloses (Aden *et al.*, 2002).

An effective pretreatment method must ensure the cleavage of lignincarbohydrate linkages leading to the depolymerisation and dissolution of lignin molecule; it should also afford high yields of glucose, a fermentable sugar without carbohydrate degradation (Sun and Cheng, 2002). The technique and processes of its operation must be cost-effective with low energy requirements and must ensure reduction in cellulose crystallinity (Alvira *et al.*, 2010). The Kraft pulping chemicals (Na₂S and NaOH) have been discovered to achieve high percentage lignin removal with wide application on almost all the lignocellulosic biomass to generate about 50% cellulose fibers. The Kraft pretreatment method is quite economical and environmental friendly (Ndukwe *et al.*, 2009; Sixta, 2007). The Kraft pulping chemicals has been reported as an effective pretreatment method as it improves the enzymatic hydrolysis of most hard wood biomaterials with high yield of glucose generation (Ndukwe *et al.*, 2013).

MATERIALS AND METHODS

The twenty sawdust lignocellulosic biomass wastes investigated were designated as samples one to twenty (S1-S20). Their common Nigerian names and botanical names are as follows: Erunobo (*Erythropleum suaveolens*), Okilolo (*Symphona globulifera*), Erimado (*Ricindendron heudelotii*), Oporoporo (*Pterygota macrocarpa*), Iroko (*Milicia excelsa*), Odoko (*Ipomoea asarifolia*), Abura (*Hallea ciliate*), Itara (*Sacoglottis gabonensis*), Akomu (*Pycnanthus angolensis*), Afara (*Terminalia superba*), Ofun (*Avicennia germinans*), Obeche (*Triplochiton scleroxylon*), Akun (*Uapaca guineensis*), Opepe (*Nauclea diderrichii*), Masonia (*Masonia altissima*), Agba (*Entada gigas*), Some (*Ceiba pentadra*), Mahogany (*Khaya ivorensis*), Eki-Eki (*Lophira alata*) and Itako (*Strombosia pustulata*) (Ndukwe *et al.*, 2009).

Kraft Pulping of Wood Sawdust into Cellulose

Two kilograms each of the twenty sawdust wood species of particle size 2.8-5.0 mm was weighed and subjected to Kraft-pulping in a stainless-steel rotary digester. The sawdust was collected at Oko-oba sawmill along the bank of Lagos Lagoon. While, the identification of the different wood species from which the sawdust was produced was carried out at the Forestry Research Institute of Nigeria (FRIN), Ibadan. The white liquor used in pulping was prepared by dissolving NaOH (350 g) and Na₂S (140 g) in 8 L distilled water. The digester temperature was maintained at 170° C and the pressure set at 200 kPa for 1 h 45min. The pulp released in the reaction was washed with deionized water and air-dried. A fluffy, fibrous and dark brown Kraft pulp was obtained after drying (Ndukwe *et al.*, 2009).

Successive Pretreatment of the Kraft Cellulose and Enzymatic Catalysis

The residual lignin was removed by treating the Kraft pulp (10g) with 60 mL of 30% hydrogen peroxide hydrogen at 40°C for 25-30 min. After the Kraft and successive H₂O₂-pretreatment of the Kraft cellulose, each of the biomaterials was subjected to *Trichoderma viride* cellulase hydrolysis by incubating in triplicate the different biomass concentrations of 4, 10, 20, 40 and 60 mg/mL with 0.40 ml Tris (hydroxymethyl aminomethane 0.005 M)buffer solution, pH 4.5 in 100 μ L *T. viride* cellulase (10mg/mL stock solution)at 2h and constant temperature of 40^oC (Van Wyk, 2001).The amount of glucose released during cellulose-cellulase catalysis was determined spectrophotometrically by the dinitrosalicyclic acid (DNS) method at 546 nm (Miller, 1959). The sugar concentration obtained from each of the biomaterials after the bioconversion with *Trichoderma viride* cellulase

was calculated from a standard calibration curve of glucose expressed as mg/mL, using glucose as a standard sugar solution.

RESULTS AND DISCUSSION

During the Trichoderma viride cellulase hydrolysis of the Kraft-pretreated sawdust from the twenty hard wood species examined (Table 1), a progressive increase in glucose formation was observed with the highest glucose concentrations of 9.63, 9.55 and 9.54 mg/mL obtained from Ceiba. pentadra (S17), Lophira alata (S19) and Terminalia. Superb(S10) respectively: while the Avicennia germinans (S11) generated the lowest sugar concentration of 1.34 mg/mL. The successive pretreatment of the Kraft pulp of different biomass concentrations of 4, 10, 20, 40 and 60 mg/mL with alkaline hydrogen led to effective removal of residual lignin still embedded in the cellulose substrate (Table 2). The enzymatic hydrolysis of the H₂O₂-Kraft pretreated sawdust resulted in enhanced glucose concentrations of 3.26, 4.88, 4.65, 8.72 and 7.84 respectively from Milicia excelsa wood sawdust against the poor glucose formation recorded after Trichoderma viride cellulase interaction of the Kraftpretreated sawdust that generated 1.41, 2.29, 2.70, 2.46 and 1.96 mg/mL respectively for the same Milicia. excelsa wood sample. Its bioconversion efficiency gave 131%, 113%, 72%, 225% and 300% increase in the amount of glucose generated. The highest percentage increase in glucose generation after the successive pretreatment of the Kraft pulp at 4 mg/mL biomass concentration was 219% calculated from E. gigas (S16) wood sawdust (Table 3). The highest percentage increase in glucose generation after the successive pretreatment of the Kraft pulp at 10 mg/mL and 20 mg/mL was 232% and 171% from R. heodelotii (S3) and E. gigas (S16) respectively (Tables 4 & 5). The M. excelsa wood sawdust (S5) produced the highest percentage increase in glucose at 40 mg/mL and 60 mg//mL biomass concentrations to give 225% and 300% increase in sugar formation respectively. The bioconversion efficiency of cellulase-cellulose interaction has also been reported in the bioconversion of wastes paper with Trichoderma viride cellulase enzyme (Mokatse and van Wyk, 2003). The amount of glucose released from the Kraft and the peroxide pretreated Kraft cellulose from the twenty different sawdust cellulose with the Trichoderma viride cellulase at 2h incubation period is much more than the highest glucose concentration of 0.0018 mg/mL (Mokatse and van Wyk, 2003) obtained from the bioconversion and efficiency tendencies of biodegraded wastes paper cellulose with Trichorderma viride cellulase. The successive pretreatment process ensures maximum residual lignin removal from each of the Kraft cellulose giving rise to enhanced increase in glucose generation from the biomaterials. The amount of

glucose obtained from the sawdust cellulose also compared favourably with 31.56 mg/mL glucose (Alrumman, 2016) released from the 24 h saccharification of palm cellulosic wastes by *Geobacillus stearothermophilus* cellulase and the 0.9 mg/mL glucose generated from the biodegradation of waste brown envelope cellulose with *Trichoderma viride* at 2 h incubation period and optimum pH-value of 5.0 (Mokatse and van Wyk, 2017)

Table	1:	Glucose generation (mg/mL) from bioconversion of Kraft-
		pretreated sawdust at different concentrations of dry biomass
		(mg) and biomass in enzyme solution (mg/mL)

(mg) and biomass in enzyme solution (mg/mh)						
Wood	(4 mg/mL)	(10 mg/mL)	(20 mg/mL)	(40 mg/mL)	(60 mg/mL)	
sawdust	2 mg	5 mg	10 mg	20 mg	30 mg	
S1	1.93 ± 0.05	$3.4\ 0\pm0.06$	6.54 ± 0.07	6.82 ± 0.02	5.79 ± 0.05	
S2	2.43 ± 0.09	3.41 ± 0.02	5.49 ± 0.06	5.02 ± 0.01	4.38 ± 0.02	
S 3	1.62 ± 0.00	2.83 ± 0.01	5.57 ± 0.05	5.23 ± 0.03	4.56 ± 0.03	
S4	2.26 ± 0.00	3.33 ± 0.01	6.32 ± 0.01	6.06 ± 0.04	4.02 ± 0.01	
S5	1.41 ± 0.00	2.29 ± 0.11	2.70 ± 0.11	2.46 ± 0.01	1.96 ± 0.03	
S6	3.19 ± 0.01	4.34 ± 0.01	7.27 ± 0.09	6.69 ± 0.02	5.43 ± 0.04	
S7	1.92 ± 0.06	2.77 ± 0.00	6.90 ± 0.06	7.34 ± 0.01	7.63 ± 0.02	
S 8	2.28 ± 0.01	3.31 ± 0.02	7.46 ± 0.01	6.19 ± 0.0	7.47 ± 0.02	
S9	2.41 ± 0.00	4.73 ± 0.01	8.48 ± 0.10	8.58 ± 0.01	9.07 ± 0.01	
S10	2.90 ± 0.18	4.99 ± 0.00	9.54 ± 0.08	9.75 ± 0.03	10.48 ± 0.03	
S11	1.34 ± 0.01	2.5 ± 0.002	4.69 ± 0.01	5.17 ± 0.02	4.67 ± 0.05	
S12	3.06 ± 0.03	4.81 ± 0.01	7.40 ± 0.22	7.12 ± 0.05	5.58 ± 0.01	
S13	2.01 ± 0.00	4.28 ± 0.01	8.65 ± 0.08	10.04 ± 0.03	10.30 ± 0.02	
S14	2.64 ± 0.01	5.83 ± 0.02	7.88 ± 0.07	7.69 ± 0.02	7.02 ± 0.05	
S15	1.20 ± 0.01	2.27 ± 0.03	5.65 ± 0.07	7.84 ± 0.04	8.70 ± 0.02	
S16	1.73 ± 0.01	3.92 ± 0.02	4.85 ± 0.02	6.74 ± 0.01	6.64 ± 0.03	
S17	3.36 ± 0.01	4.92 ± 0.04	9.63 ± 0.09	10.46 ± 0.04	11.31 ± 0.01	
S18	2.42 ± 0.01	3.70 ± 0.07	6.49 ± 0.10	7.85 ± 0.01	8.11 ± 0.02	
S19	2.39 ± 0.02	5.21 ± 0.01	9.55 ± 0.30	10.87 ± 0.02	13.30 ± 0.03	
S20	2.34 ± 0.01	3.98 ± 0.06	5.68 ± 0.06	6.88 ± 0.03	6.83 ± 0.01	

Table 2:	pretreated	sawdust at d	lifferent conc	entrations of	of H ₂ O ₂ -Kraft dry biomass
	(mg) and Di	omass in enzy	me solution (n	ng/mL)	
Wood	(4 mg/mL)	(10 mg/mL)	(20 mg/mL)	(40 mg/mL)	(60 mg/mL)
	2	5	10	20	20

Wood	(4 mg/mL)	(10 mg/mL)	(20 mg/mL)	(40 mg/mL)	(60 mg/mL)
sawdust	2 mg	5 mg	10 mg	20 mg	30 mg
S 1	4.792 ± 0.02	10.797 ± 0.05	13.246 ± 0.01	11.119 ± 0.05	14.543 ± 0.02
S2	3.452 ± 0.01	7.285 ± 0.03	7.895 ± 0.03	7.171 ± 0.02	11.079 ± 0.02
S 3	4.344 ± 0.03	9.431 ± 0.01	11.245 ± 0.0	7.737 ± 0.01	9.782 ± 0.03
S4	4.298 ± 0.01	7.935 ± 0.02	8.501 ± 0.04	8.715 ± 0.03	10.087 ± 0.02
S5	3.261 ± 0.03	4.875 ± 0.01	4.649 ± 0.01	8.016 ± 0.04	7.835 ± 0.05
S6	4.477 ± 0.01	8.562 ± 0.04	8.452 ± 0.03	11.565 ± 0.02	8.099 ± 0.01
S 7	3.041 ± 0.04	6.816 ± 0.02	7.719 ± 0.02	11.968 ± 0.05	9.446 ± 0.02
S 8	4.193 ± 0.05	9.074 ± 0.01	9.595 ± 0.03	10.623 ± 0.05	10.102 ± 0.03
S9	5.722 ± 0.01	10.046 ± 0.05	10.76 ± 0.05	12.956 ± 0.03	11.138 ± 0.02
S10	6.936 ± 0.02	10.623 ± 0.04	13.643 ± 0.04	14.612 ± 0.02	14.965 ± 0.01
S11	2.846 ± 0.01	5.834 ± 0.02	6.131 ± 0.02	11.086 ± 0.01	10.884 ± 0.02
S12	4.186 ± 0.03	8.456 ± 0.05	9.375 ± 0.03	15.035 ± 0.04	12.036 ± 0.05
S13	3.458 ± 0.01	8.584 ± 0.03	9.448 ± 0.01	13.74 ± 0.02	13.344 ± 0.04
S14	4.745 ± 0.02	7.401 ± 0.01	8.236 ± 0.02	11.951 ± 0.04	7.868 ± 0.02
S15	2.331 ± 0.01	6.758 ± 0.02	8.927 ± 0.04	16.225 ± 0.02	15.601 ± 0.04
S16	5.531 ± 0.04	11.372 ± 0.01	13.178 ± 0.05	17.68 ± 0.05	17.67 ± 0.02
S17	5.652 ± 0.01	11.499 ± 0.03	12.273 ± 0.02	15.787 ± 0.03	13.932 ± 0.01
S18	3.906 ± 0.02	6.484 ± 0.01	8.667 ± 0.05	15.425 ± 0.02	12.017 ± 0.03
S19	5.054 ± 0.03	10.583 ± 0.03	12.422 ± 0.03	15.147 ± 0.01	16.345 ± 0.02
S20	3.551 ± 0.01	9.674 ± 0.04	9.583 ± 0.02	13.379 ± 0.03	10.913 ± 0.04

Table 3: The percentage increase in glucose generation after the bioconversion of the successive pretreated of H_2O_2 -Kraft sawdust at 4 mg/mL biomass concentration

S/N	Botanical Names	Kraft	H_2O_2	% Increase
S 1	E. suaveolen	1.926	4.792	148.81
S2	S. globulifera	2.433	3.452	41.88
S 3	R. heudelotii	1.617	4.344	168.65
S4	P. macrocarpa	2.264	4.298	89.84
S5	M. excelsa	1.413	3.261	130.79
S6	I. asarifolia	3.186	4.477	40.52
S 7	H. ciliate	1.916	3.041	58.72
S 8	S. gabonensis	2.279	4.193	83.98
S9	P. angolensis	2.414	5.722	137.03
S10	T. superba	2.895	6.936	139.59

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S11 S12	A. germinans T. scleroxylon	1.339 3.059	2.846 4.186	112.55 36.84
S13	U. guineensis	2.013	3.458	71.78
S14	N. diderrichii	2.638	4.745	79.87
S15	M. altissima	1.196	2.331	94.90
S16	E. gigas	1.731	5.531	219.53
S17	C. pentadra	3.364	5.652	68.01
S18	K. ivorensis	2.420	3.906	61.40
S19	L. alata	2.386	5.054	111.82
S20	S. pustulatas	2.241	3.551	58.56

Table 4: The percentage increase in glucose generation after the bioconversion of the successive pretreated H_2O_2 -Kraft sawdust at 10 mg/mL biomass concentration

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S/N	Botanical Names	Kraft	H_2O_2	% Increase
S 1	E. suaveolen	3.404	10.797	217.19
S2	S. globulifera	3.412	7.285	113.51
S 3	R. heudelotii	2.833	9.431	232.90
S4	P. macrocarpa	3.334	7.935	138.00
S5	M. excelsa	2.287	4.875	113.16
S6	I. asarifolia	4.340	8.562	97.28
S 7	H. ciliata	2.767	6.816	146.33
S 8	S. gabonensis	3.309	9.074	174.22
S9	P. angolensis	4.730	10.046	112.39
S10	T. superba	4.990	10.623	112.89
S11	A. germinans	2.499	5.830	133.29
S12	T. scleroxylon	4.807	8.456	75.91
S13	U. guineensis	4.282	8.584	100.47
S14	N. diderrichii	5.834	7.401	26.86
S15	M. altissima	2.269	6.758	197.84
S16	E. gigas	3.920	11.372	190.10
S17	C. pentadra	4.921	11.499	133.67
S18	K. ivorensis	3.694	6.484	75.53
S19	L. alata	5.216	10.583	102.89
S20	S. pustulatas	3.983	9.674	142.88

Table 5:The percentage increase in glucose generation after the
bioconversion of the successive pretreated H2O2-Kraft sawdust at
20 mg/mL biomass concentration

S/N	Botanical Names	Kraft	H_2O_2	% Increase
S 1	E. suaveolen	6.542	13.246	102.48
S2	S. globulifera	5.496	7.895	43.65
S 3	R. heudelotii	5.567	11.245	101.99
S 4	P. macrocarpa	6.316	8.501	34.59
S5	M. excelsa	2.704	4.649	71.93
S 6	I. asarifolia	7.269	8.452	16.27

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S7 S8	H. ciliata S. gabonensis	6.901 7.457	7.719 9.595	11.85 28.67
S9	P. angolensis	8.477	10.760	26.93
S10	T. superba	9.541	13.643	42.99
S11	A. germinans	4.685	6.131	30.86
S12	T. scleroxylon	7.650	9.375	23.50
S13	U. guineensis	8.647	9.448	22.59
S14	N. diderrichii	7.877	8.236	4.56
S15	M. altissima	5.650	8.927	58.00
S16	E. gigas	4.851	13.178	171.66
S17	C. pentadra	9.631	12.273	27.43
S18	K. ivorensis	6.490	8.667	33.54
S19	L. alata	9.548	12.422	30.10
S20	S. pustulatas	5.679	9.583	68.74

Table 6: The percentage increase in glucose generation after the bioconversion of the successive pretreated H_2O_2 -Kraft sawdust at 40 mg/mL biomass concentration

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S/N	Botanical Names	Kraft	H_2O_2	% Increase
S1	E. suaveolen	6.818	11.119	63.08
S 2	S. globulifera	5.015	7.171	42.99
S 3	R. heudelotii	5.216	7.737	49.13
S4	P. macrocarpa	6.063	8.715	43.74
S5	M. excelsa	2.464	8.016	225.32
S6	I. asarifolia	6.694	11.565	72.76
S 7	H. ciliata	7.337	11.968	63.12
S 8	S. gabonensis	6.192	10.623	71.56
S9	P. angolensis	8.578	12.956	51.04
S10	T. superba	9.749	14.612	49.88
S11	A. germinans	5.166	11.086	114.60
S12	T. scleroxylon	7.123	15.035	111.08
S13	U. guineensis	10.039	13.740	36.87
S14	N. diderrichii	7.690	11.951	54.38
S15	M. altissima	7.835	16.225	107.08
S16	E. gigas	6.735	17.680	162.51
S17	C. pentadra	10.436	15.787	51.27
S18	K. ivorensis	7.852	15.425	96.45
S19	L. alata	10.872	15.147	39.32
S20	S. pustulatas	6.889	13.379	94.21

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Table 7:	The percentage		• •					
	bioconversion of the	ne successive p	oretreated H ₂ O ₂	-Kraft sawdust at				
	60 mg/mL biomass concentration							
S/N	Botanical Names	Kraft	H2O2	% Increase				
S 1	E. suaveolen	5.785	14.543	151.39				
S 2	S. globulifera	4.375	11.079	153.23				
S 3	R. heudelotii	4.562	9.782	114.42				
S4	P. macrocarpa	4.002	10.087	152.05				
S5	M. excelsa	1.961	7.835	299.54				
S 6	I. asarifolia	5.430	8.099	49.12				
S 7	H. ciliata	7.634	9.446	23.74				
S 8	S. gabonensis	7.466	10.102	35.31				
S 9	P. angolensis	9.074	11.138	22.74				
S10	T. superba	10.482	14.965	42.77				
S11	A. germinans	4.674	10.884	132.86				
S12	T. scleroxylon	5.581	12.036	115.66				
S13	U. guineensis	10.305	13.344	29.49				
S14	N. diderrichii	7.020	7.868	12.08				
S15	M. altissima	8.701	15.600	79.28				
S16	E. gigas	6.640	17.671	166.13				
S17	C. pentadra	11.310	13.932	23.19				
S18	K. ivorensis	8.113	12.017	48.12				
S19	L. alata	13.298	16.345	22.91				
S20	S. pustulatas	6.826	10.913	59.87				

CONCLUSION

There is a resurgent global interest in the quest for the utilization of lignocellulosic biomass substrates for renewable bioenergy generation, as a means of militating against the increasing economic and environmental concerns associated with fossil fuels consumption. The bioconversion of sawdust cellulose of different biomass concentrations at constant cellulase application and incubation period showed a progressive increase in glucose generation after which further increase in biomass concentration failed to yield a commiserate amount of sugar. The attainment of cellulose-cellulase saturation point accounts for the relative decrease in the amount of fermentable sugar released from the different biomaterials on interaction with cellulase enzyme. The successive pretreatment of the Kraft cellulose with the peroxide chemicals enhanced the bioconversion efficiency of the cellulose due to maximum cellulose-cellulase interaction and digestibility leading to over 140% increase in glucose generation from the bioconversion of the H₂O₂-Kraft cellulose at lowest biomass concentration investigated. The adequate utilization of sawdust lignocellulosic wastes into high glucose generation will reduce the overwhelming effects of environmental degradation caused by sawdust pollution across the Nigerian water

ways. It will also reduce the current starch/ grain ethanol production, thereby making food more available for human consumption.

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