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Effective Gravimetric Characterization for Lignocellulosic Biomass: Comparison of NaOH-H_2O_2 and $Ca(OH)_2$-H_2O_2 Oxidation Pretreated Sugarcane Bagasse

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Abstract–In this study, alkaline peroxide oxidation pretreatment was evaluated for sugarcane bagasse, a lignocellulosic biomass. By comparing the effects of NaOH-H_2O_2 and $Ca(OH)_2$ on pretreatments at specified reaction time periods (3, 6, 9, and 12 h) and reaction temperatures (60, 70, 80, and 90 h), optimum responses in term of cellulose content, hemicellulose solubilization, and lignin removal were established. Optimum pretreatment conditions of 80 ^oC reaction temperature, 3 h reaction time, and 30 mL/L of water hydrogen peroxide concentration (1%H_2O_2) solubilized 69.5%(w/w) hemicellulose for the sodium hydroxide peroxide (SHP) pretreatments, 75.8%(w/w) lignin removal was also achieved with 59.2%(w/w) cellulose retained in the solid fraction. In addition, the responses for the optimum conditions for the calcium hydroxide peroxide (CHP) pretreatments, the cellulose content, hemicellulose solubilization, and lignin removal were 50.3%, 66.6%, and 65.4%(w/w) respectively. Pretreatments showed both NaOH-H_2O_2 and $Ca(OH)_2$-H_2O_2 to be useful pretreatment agents for the disruption of the polysaccharide complex. The study also revealed that NaOH-H_2O_2 pretreatment stands as a better choice to $Ca(OH)_2$-H_2O_2 pretreatment.

Keywords–**Pretreatment, sugarcane bagasse, Saccharum officinarium, gravimetry**

1. Introduction

The world demand for energy is expected to double between years 2000 and 2050. This demand cannot be satisfied by crude oil, natural gas, coal and nuclear energy combined. By its reliance on oil and gas, the world economy is becoming very dependent on a limited number of exporting countries. Renewable energies will therefore have to play an increasing role in securing energy throughout the century. Lignocellulosic biomass (in which sugarcane bagasse is included) is currently considered the most promising long-term feedstock for production of bioethanol. The recalcitrance of the feedstock is the major hurdle in the bioconversion process. In the plant cell structure, cellulose fibrils are embedded in a matrix of lignin and hemicellulose. This forms a chemical and structural barrier for enzymatic degradation of cell wall sugars and subsequent microbial fermentation to ethanol [1,2]. Among the various agricultural crop residues, sugarcane bagasse is the most abundant lignocellulosic material in tropical countries[3]. Pretreatment is one of the most expensive and least technologically-mature steps in the process of converting biomass to fermentable sugars [3]. Pretreatment, therefore offers a great potential for improvement in efficiency and the reduction of costs through research and development [4]. Hydrogen peroxide and alkaline oxidative pretreatment (Alkaline peroxide oxidation (APO)) is known to decrystallize cellulose [5]. It is also known that under proper conditions hydrogen peroxide (H_2O_2) will react readily with lignin and related phenolic to yield an array of low molecular weight, water-soluble oxidation products. APO pretreatment technology has been used previously to investigate actions on some lignocellulosic biomass [6-8]. Cellulose, hemicelluloses, lignin and the other components are ordered in varying composition in the different parts of the fibre wall depending on the species of lignocellulosic biomass. Extractives include non-structural components that are non-chemically bound components of biomass such as sucrose, nitrate/nitrite, protein, ash, chlorophyll, waxes. The extractives are removed because they potentially interfere with downstream analysis of biomass sample. Gravimetric analysis describes a set of methods for the quantitative determination of a sample or material based on the mass of a solid. Mostly, collected dried solids are weighed with an analytical balance. When carefully followed especially during weighing, gravimetric method can provide precise analysis. It provides very little room for instrumental error. It does not require expensive equipment. Determination of the composition of lignocellulosic substrates using gravimetric analysis exists in scientific literature. These included the monoethanolamine method [9], the trifluoroacetic acid method [10], concentrated sulphuric acid method [11], acid and neutral detergent method [12]. These methods are directly applicable to specific lignocelluloses, and the sources of the materials [13].

This study investigated the actions of sodium hydroxide and calcium hydroxide under oxidative conditions (using hydrogen peroxide) on pretreatment of sugarcane bagasse in order to cause appreciable enzymatic digestibility of treated biomass. Optimum conditions were established for the pretreated samples for each catalyst considered by considering the cellulose content remaining after pretreatment. In addition, lignin and hemicellulose solubilized were also estimated gravimetrically. The more the cellulose is retained in the solid fraction the greater efficiency for reducing sugar to be liberated from cellulose when acted upon by the cellulase enzymes.

2. Materials and Methods

2.1 Raw material

Sugarcane (*Saccharum officinarum*) was purchased from an open market in late December, 2013 from Ota Town, Ogun State, Western Nigeria. The juice was extracted from the sugarcane stalks at a local mill in Ota, Ogun state, Nigeria. The bagasse obtained was air-dried in an open space for three days (about 8 h per day). Milling was carried out on the air-dried bagasse for size reduction. The bagasse was screened into different size particles using a sieve shaker. The size distribution showed that the particles sizes varied between 0.10 and 1.00 mm. After screening, the samples were manually mixed together with hands for 15 min. The mixed samples were dried in a convection oven at 105 ^{o}C for 3 h to a dry matter content of 85% of dry raw biomass. The dried materials were stored in plastic bottles capped tightly and kept at room temperature. The materials were used shortly after.

2.2 Raw material pretreatment

6 ml of 35%(w/v) H_2O_2 was added to 194 ml distilled water to make a total working mixture of 200 ml. This resulted in a 1%v/v H_2O_2 in total working volume. All other working volumes were based on this initial estimation. The pH of the mixture was adjusted to 11.5 by adding either NaOH or $Ca(OH)_2$ as the case may be. A slurry was made with the 200 ml working volume and 10 g of dried raw sample. With the aid of magnetic stirrer, reaction was made to occur for the specified time periods (3, 6, 9, and 12 h) and temperatures (60, 70, 80, and 90 h) (Tables 1, 2, and 3). After pretreatment, the mixture was cooled to room temperature and the slurry was separated into solid and liquid fractions by vaccum filtration. The solid fraction was properly washed with distilled water to a pH of 7. Small portion of the liquid fraction was used to estimate the soluble lignin content ([14]. The wet weight of the solid fraction was weighed and 2 g out of this was dried at 105 ^{o}C until constant weight was achieved in order to estimate the total solids left after pretreatment (It is expected that during pretreatment there will be weight loss due to lignin removal and hemicellulose solubilization/hydrolysis).

2.3 Analysis of raw and pretreated biomass

Compositional analysis on the raw and pretreated samples are as previously described by [15]. The dry solid content was estimated by weighing 1 g of wet biomass after pretreatment and drying to a constant weight in a convection oven at 105 ^{o}C. Extractives were determined by means of the Soxhlet extractor on 5 g of dry biomass with residence times for the boiling and rising stages equal to 70 ^{o}C and 25 min respectively for a 4 h run period. The sample was air dried for few minutes at room temperature and further dried at 105 ^{o}C in a convection oven. The extractives content was calculated as the difference in weight between the raw and extracted material [6,15]. Mineral components were determined by ashing at 575 ^{o}C for 6 h. The hemicellulose content was determined by placing 1 g of dried biomass from the extractive analysis into a 250 mL Erlenmeyer flask and then 150 mL of 500 mol m^{-3} NaOH solution was added. The mixture was boiled for 3 h and 30 min with distilled water. The residue was dried to a constant weight at 105 ^{o}C and later cooled in a desiccator and weighed. The difference between the sample weight before and after this treatment is the hemicellulose. Lignin composition was determined by weighing into glass test tubes 300 mg of dry extracted biomass and adding 3 mL of 72% H_2SO_4. Acid hydrolysis was made to occur by keeping the samples at room temperature for 2 h with mixing of samples every 30 min. 84 mL of distilled water was added to each test tube after the 2 h acid hydrolysis step bringing the total volume to 87 mL. The samples were autoclaved for 1 h at 121 ^{o}C [14]. After the second weak acid hydrolysis step, the hydrolyzates were cooled to room temperature and filtered through vacuum using a filtering crucible. The acid insoluble lignin was determined by drying the residue at 105 ^{o}C and accounting for ash by incinerating the hydrolyzed samples at 575 ^{o}C in a muffle furnace. The acid soluble lignin fraction was determined by measuring the absorbance of the acid hydrolyzed samples at 320 nm [14]. The cellulose content was calculated by difference, assuming that extractives, hemicellulose, lignin, ash, and cellulose are the only components of the entire biomass [16]. The material balance for the lignin removal (%w/w) and hemicellulose solubilization (%w/w) were estimated based on the following equations;

$$\text{Lignin removal (\%w/w)} = 100 - [(LCP/LCU) \times TS] \quad \ldots 1$$

Where,
LCP = Lignin content remaining after pretreatment
LCU = Lignin content in the untreated biomass
TS = Total dry solids remaining after pretreatment

$$\text{Hemicellulose Solubilization (\%w/w)} = 100 - [(HCP/HCU) \times TS] \quad \ldots 2$$

Where,
HCP = Hemicellulose content remaining after pretreatment
HCU = Hemicellulose content in the untreated biomass

3. Results and Discussions

3.1 Effect of pretreatment on the composition of solid material

The aim of the alkaline peroxide oxidation (alkaline-hydrogen peroxide oxidation) is to fractionate the lignocellulosic biomass into a solid fraction containing as much polysaccharides (importantly as cellulose) and as less lignin as possible; and a liquid fraction containing solubilized hemicellulose the best preserved as possible [8]. Cellulose content increased from an initial 41.3% untreated biomass to the highest values of 59.8%(w/w) and 53.3%(w/w) for NaOH-H_2O_2 (shown as SHP, sodium hydroxide peroxide) and $Ca(OH)_2$-H_2O_2(shown as CHP, Calcium hydroxide peroxide) pretreatments respectively (Table 1). Generally, the NaOH-H_2O_2 pretreatments retained more of the cellulose fractions than the $Ca(OH)_2$-H_2O_2 pretreatments. An efficient pretreatment step should provide an increase in the cellulose contents in the solid fraction. The degradation of cellulose should be avoided so as to make more of the solid fraction amenable to enzymatic conversion by

cellulase enzymes. Cellulose enrichment was due majorly to hemicellulose solubilization (hydrolysis) and a percentage of lignin removal.

Table 1. Cellulose content remaining after pretreatments (%w/w)

Time (h)		60 °C		70 °C		80 °C		90 °C	
	RB	SHP	CHP	SHP	CHP	SHP	CHP	SHP	CHP
0	41.3								
3		54.2	44.7	53.7	45.3	59.2	50.3	56.7	46.8
6		51.8	53.3	52.3	51.8	51.6	46.4	56.3	51.9
9		59.3	49.5	52.4	50.3	59.8	51.2	47.0	49.9
12		57.5	53.5	46.6	49.2	54.0	43.4	49.6	51.3

SHP = Sodium hydroxide-hydrogen peroxide pretreated samples.
CHP = Calcium hydroxide-hydrogen peroxide pretreated samples.
RB = Raw biomass

The hemicelluloses content decreased from the initial raw biomass value of 31.7%(w/w) to the lowest values of 19.9%(w/w) and 18.7%(w/w) for NaOH-H_2O_2 and $Ca(OH)_2$-H_2O_2 pretreatments respectively (Table 2). Hemicelluloses in the lignocellulosic complex are easily hydrolysed in alkaline medium due to their amorphous structure unlike the cellulose fibril made up of the crystalline structure. A good pretreatment method should provide a more hydrolysed hemicelluloses fraction than the hydrolysis of the cellulose fraction. NaOH-H_2O_2 pretreatments hydrolysed more of the hemicelluloses fractions than the $Ca(OH)_2$-H_2O_2 pretreatments. The pretreatments resulted in modifications of the compositions of the solid fraction.

The lignin content showed an appreciable decrease in the pretreated biomass to the initial value of 18.7%(w/w) of the raw biomass (Table 3). At 90 °C reaction temperature and 3 h reaction time, the lignin content decreased to the lowest value of 10.1%(w/w) for the NaOH-H_2O_2 pretreatment. The mechanism by which alkaline peroxide pretreatment enhances enzymatic saccharification appears to involve both a release of lignin from the lignocellulosic matrix and a dramatic increase in the degree of hydration of the cellulose polymer [17].

Table 2. Hemicellulose content remaining after pretreatments (%w/w)

Time (h)		60 °C		70 °C		80 °C		90 °C	
	RB	SHP	CHP	SHP	CHP	SHP	CHP	SHP	CHP
0	31.7								
3		23.9	25.2	21.3	24.6	21.8	23.9	22.9	24.4
6		22.3	21.4	20.8	18.8	21.1	22.7	23.9	18.7
9		20.2	20.6	19.9	24.2	20.9	22.2	24.6	20.7
12		20.9	23.9	23.9	21.9	23.9	23.9	23.8	23.1

Table 3 – Lignin content remaining after pretreatments (%w/w)

Time (h)		60 °C		70 °C		80 °C		90 °C	
	RB	SHP	CHP	SHP	CHP	SHP	CHP	SHP	CHP
0	18.7								
3		12.5	19.4	14.5	16.1	10.2	14.6	12.3	15.3
6		12.9	14.1	16.2	17.1	18.2	16.1	10.1	13.2
9		12.5	15.1	13.4	12.9	10.8	12.6	15.1	16.1
12		11.9	13.8	14.5	16.1	11.6	19.2	14.7	15.2

3.2 Optimization of the pretreatment conditions

In determining the optimum APO conditions, the pretreatment (considering only the solid fraction) with the best combinations of the highest cellulose content, highest hemicellulose solubilization (hydrolysis), and highest lignin removal (based on both NaOH-H_2O_2 and $Ca(OH)_2$-H_2O_2 pretreatments) were considered. With all these constraints in mind, the optimum responses (cellulose content, hemicellulose solubilization, and lignin removal) were obtained at 80 °C, 3 h (Figures 1 to 3). The optimized responses for the cellulose content, hemicellulose solubilization, and lignin removal for the sodium hydroxide peroxide, SHP, pretreatments were 59.2%, 69.5%, and 75.8%(w/w) respectively. In addition, the optimized responses for the cellulose content, hemicellulose solubilization, and lignin removal for the calcium hydroxide peroxide, CHP, pretreatments were 50.3%, 66.6%, and 65.4%(w/w) respectively. Results in this study showed both NaOH-H_2O_2 and $Ca(OH)_2$-H_2O_2 to be useful pretreatment agents for the disruption of the both NaOH-H_2O_2 and $Ca(OH)_2$-H_2O_2 to be useful pretreatment agents for the disruption of the polysaccharide complex. However, NaOH-H_2O_2 pretreatment stands as a better choice to $Ca(OH)_2$-H_2O_2 pretreatment.

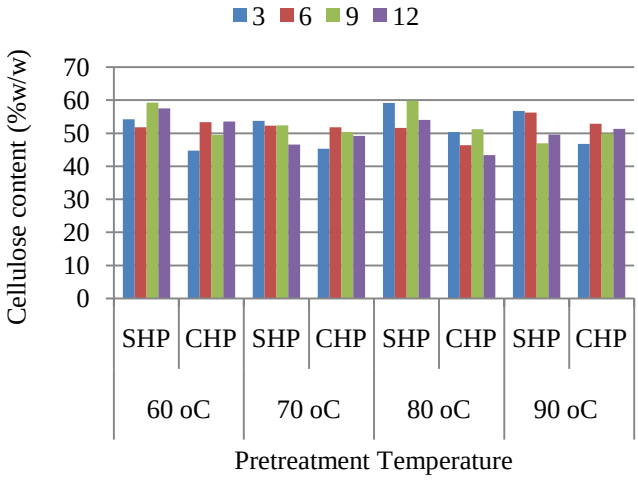

Fig. 1. Effect of time and temperature on cellulose content during NaOH-H_2O_2 (SHP) and $Ca(OH)_2$-H_2O_2 (CHP) pretreatments

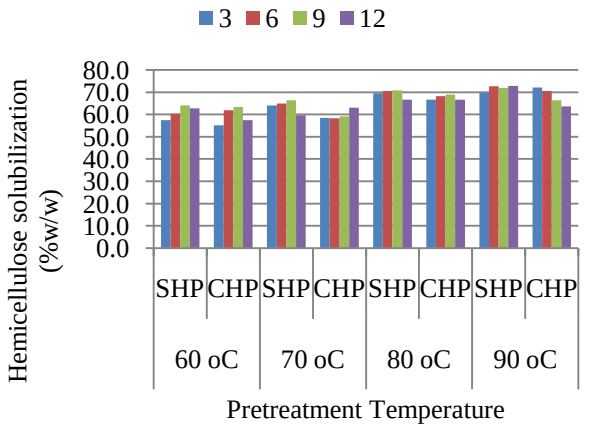

Fig. 2. Effect of time and temperature on hemicellulose solubilization during NaOH-H_2O_2 (SHP) and $Ca(OH)_2$-H_2O_2 (CHP) pretreatments

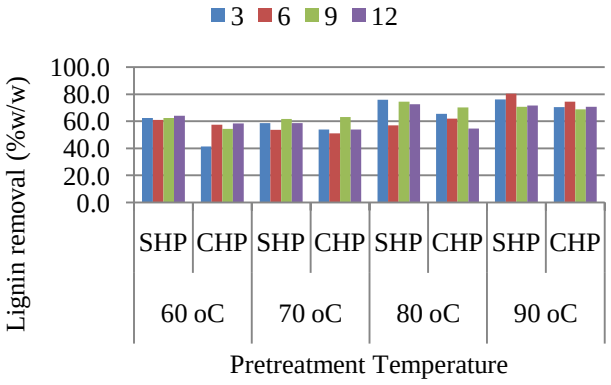

Fig. 3. Effect of time and temperature on lignin removal during NaOH-H_2O_2 (SHP) and $Ca(OH)_2$-H_2O_2 (CHP) pretreatments

4. Conclusion

This study evaluated oxidative effect of hydrogen peroxide in combination with either sodium hydroxide or calcium hydroxide suitability to cause appreciable disruption to the lignocellulosic biomass (sugarcane bagasse) complex such that the treated material could be amenable to further enzymatic hydrolysis during downstream processing. Pretreatments at specified time periods (3, 6, 9, and 12 h) and temperatures (60, 70, 80, and 90 h) resulted in optimized conditions of 3 h and 80 ^oC. The optimized responses for the cellulose content, hemicellulose solubilization, and lignin removal for the sodium hydroxide peroxide, SHP, pretreatments were 59.2%, 69.5%, and 75.8%(w/w) respectively. On the other hand, the responses for the cellulose content, hemicellulose solubilization, and lignin removal for the calcium hydroxide peroxide, CHP, pretreatments were 50.3%, 66.6%, and 65.4%(w/w) respectively. The low lignin content (18.7%(w/w)) provided an easy disruption of the polysaccharide complex thereby causing high lignin removal and hemicellulose solubilization. Further investigations of the enzymatic hydrolysis process of the treated samples are required in order to study the efficiency of this pretreatment method on sugarcane bagasse potential as a source for bio-ethanol production.

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