OPTIMIZATION AND EFFECT OF PRE-TREATMENT CONDITIONS ON GLUCOSE YIELD FROM SWEET SORGHUM BAGASSE.

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Abstract
Two chemical pre-treatment options, Hydrochloric acid (HCl) and Hydrogen peroxide (H_2O_2) were investigated for effective optimization of glucose yield from the bagasse of two selected Sweet sorghum varieties, one Nigerian (SWSV 2006-3) and one Indian (SPV 422) using glucose oxidase kit. D-Glucose was oxidized by glucose oxidase to produce D-gluconic acid and hydrogen peroxide. The hydrogen peroxide was then oxidatively coupled with 4-aminoantipyrine and phenol substitute, pHBS, in the presence of peroxidase to yield a red quinoneimine dye. The amount of coloured complex formed was proportional to glucose concentration and was photometrically measured. All experiments were performed in triplicates. Analysis of Variance (ANOVA) was used to compare mean of the treatments and results were presented as mean ± standard deviation of glucose concentration for each treatment. The difference between the mean of treatments were tested using the Duncan Multiple Range test at $p<0.05$. The results obtained showed that the final optimal concentration and ratio of sample to pre-treatment solution for acid pre-treatment were 2% HCl concentrations and 1:50 (ratio of sample to 2% HCl) respectively for both varieties. At these conditions (2% HCl and 1:50), the temperature; 121 ^oC released the highest % glucose yield of 15.36 ± 0.51 (from SWSV 2006-3) which is significantly ($p<0.05$) higher than 10.36 ± 0.51 (of same variety) at 138 ^oC at $p<0.05$. These results also revealed that for acid pre-treatment, the Sweet sorghum variety; SWSV 2006-3 performed better than SPV422 at the temperatures investigated. The optimal concentration and ratio of sample to pre-treatment solution for peroxide pre-treatment are 3% H_2O_2 concentrations and 1:200 (ratio of sample to 3% H_2O_2) respectively for both varieties. At these conditions (3% H_2O_2 and 1:200), the temperature; 121^oC also released the highest % glucose yield of 87.14 ± 2.02 (from SPV 422) which is not significantly higher than 85.71 ± 0.00 (of same variety) at 138 ^oC ($p<0.05$). These results also revealed that for peroxide pre-treatment, the Sweet sorghum variety; SPV422 performed better than SWSV 2006-3 at the temperatures investigated. H_2O_2 is a better pre-treatment solution compared to HCl. The % glucose yield released from H_2O_2 pre-treated bagasse showed that 3% H_2O_2 together with the optimized condition can degrade cellulose significantly.

Keywords: Sweet sorghum, Chemical pre-treatment, Optimization, Bioethanol feedstocks, Glucose, Renewable energy

Introduction

Based on the current utililisation rate of fossil fuel the 2015 BP Statistical Review of World Energy has estimated that the global reserves of coal would be exhausted in the next 113 years, while natural gas and crude oil reserves will become entirely used up by the years 2069 and 2067 respectively (Knoema, 2016). The exhaustion in fossil reserve will tremendously affect the technologically driven world especially the developing countries whose industrial and commercial sector depends largely on these forms of fossil fuel to function effectively. In order to minimise the effect of this projected depletion in fossil reserve, research is being intensified in the area of biofuel production (Ijeomah and Nzelibe, 2015).

Bioethanol, a form of biofuel is gotten via alcoholic fermentation of sugar using susceptible microorganisms. Sugar-containing energy crops and lignocellulosic feedstocks/biomass are renewable source of raw material for bioethanol production (Sanchez and Cardona, 2008). Sweet sorghum plant also known as *Sorghum bicolor (L) moench* has been considered as a potential alternative source of sugar and bagasse for bioethanol production. Its short life span, water utilization efficiency, low cost of cultivation confers an added advantage on Sweet sorghum when compared with other sugar crops such as sugarcane (Reddy *et al.*, 2006; Reddy, 2007). The bioethanol production process from sweet sorghum is eco-friendly compared to that from molasses. The ethanol produced from Sweet sorghum has high octane rating and the burning quality is superior with less sulphur than from sugarcane (Reddy *et al.*, 2006). Sweet Sorghum (SS) varieties from India and Nigeria have been screened for Bioethanol production potential, and one **Nigerian Variety** (SWSV 2006-3) and an **Indian Variety** (SPV 422) were selected (Ijeomah and Nzelibe, 2015) based on glucose yield, stem size; % bagasse powder yield; overall carbohydrate content (cellulose and hemicelluloses content) and lignin content; yield in glucose after 1.0 % HCl and 1.0 % H_2O_2; availability and level of pest infestation.

Integration of the sugars from sweet sorghum bagasse (lignocellulosic residue after extraction of sweet sorghum stem juice) with the sugar derived from the Sweet sorghum stem juice will further increase bioethanol yield. Utilisation of the bagasse from sweet sorghum for bioethanol production could lead to product yield maximization.

The nature of plant cell wall polysaccharide poses great difficulty in utilization of lignocellulosic feedstocks for ethanol production (Pandey, 2015), thus optimization of bagasse (Sweet sorghum) pre-treatment conditions then becomes imperative. The aim of this paper, therefore, was to determine the optimum pre-treatment conditions for maximum glucose release from the Sweet sorghum bagasse. These conditions under consideration are: type and concentration of pre-treatment solution (HCl and H_2O_2), the temperature of pre-treatment and ratio of pre-treatment solution to the bagasse to be saccharified.

MATERIALS AND METHODS

Materials

Sweet sorghum varieties used and their origin

Two (2) Sweet sorghum varieties, one Nigerian (SWSV 2006-3) and one Indian (SPV 422) selected by Ijeomah and Nzelibe (2015) as potential bioethanol sources were used for the study.

Sample Collection and Identification

Mature plants of selected varieties of *Sorghum bicolor L. moench* (Sweet sorghum) already identified at Department of Plant Science, Institute for Agricultural Research, Ahmadu Bello University (ABU) were harvested at 9th week from the experimental farm of the Department of Plant Science, Ahmadu Bello University located in Shika Zaria, Kaduna State, Nigeria.

Chemicals and Reagents

The chemicals and reagents used for the study were Glucose oxidase kit (Teco diagnostics), Concentrated Hydrochloric acid, Hydrogen peroxide, Potassium hydroxide.

The instruments used for the study were: measuring balance, colorimeter (Jenway, UK), laboratory mill model 4 (Thomas Wiley), shaker, water bath, oven, incubator, Aluminum pans, and autoclave, desiccators containing desiccants.

Bagasse (pith) preparation

The pith bagasse of the two selected varieties were prepared according to the method described by Ijeomah and Nzelibe (2015).

Methods

Sample Pre-treatment

Acid pre-treatment

Bagasse powders were pre-treated with acid according to the method of Arthe *et al.,* (2008) with a little modification as was reported in Ijeomah and Nzelibe (2015).

Peroxide Pre-treatment

Alkaline peroxide pre-treatment was carried out according to the method of Sridevi *et al.,* (2009) with some modifications as documented by Ijeomah and Nzelibe (2015).

Optimization of pre-treatment conditions:

Optimization of sugar-release from the bagasse powder of two selected Sweet Sorghum varieties was carried out by applying the following conditions below to acid and peroxide pre-treatment methods (Arthe *et al.,* 2008; Sridevi *et al.,*2009; Ijeomah and Nzelibe, 2015).

The conditions are:

- Effect of concentrations ranging from 0 %, 0.5 %, 1 %, 1.5 %, 2 %, 2.5 %, 3 % of the pre-treatment solutions: HCl and H_2O_2 on glucose yield was determined using glucose oxidase reagent assay kit (Teco diagnostics) procedure as documented in Ijeomah and Nzelibe (2015).
- Effect of ratio of the sample to pre-treatment solution on glucose yield was determined for the following ratios: 1:50, 1:100, 1:150 and 1:200 (g of sample/ml of pre-treatment solution).
- Effect of temperature on glucose yield was determined at two different temperatures (121 ^oC and 138 ^oC) in an autoclave.

Instruments

Yield calculations

Percentage glucose yield

This was calculated from experimental results as follows:

$$\%\ \text{saccharification or}\ \%\ \text{Glucose yield} = \frac{\text{Weight of Glucose after pre-treatment} \times 100}{\text{Weight of raw sample}}$$

Data Analysis

Experiments were performed in triplicates and duplicates. Analysis of Variance (ANOVA) was used to compare mean of each treatment. The results were presented as mean ± standard deviation of glucose/reducing sugar concentration for each treatment. The difference between the mean of treatments were tested using the Duncan Multiple Range test at $p<0.05$.

RESULTS

Effect of pre-treating the bagasse of two sweet sorghum varieties with different concentrations of HCl and H_2O_2 on the percentage (%) yield of glucose (w/w)

Table 1 shows the effect of changing concentrations of HCl and H_2O_2 on the amount of glucose released from the bagasse of two S.S. varieties. The result showed an increasing trend in the value of glucose released as the concentration of pre-treatment solution increases for both HCl and H_2O_2 treated S.S. bagasse. For pre-treatment with HCL the highest % glucose yield was observed at the concentration of 2.5 % for SPV 422 and the value was not significantly different ($p>0.05$) from the glucose concentration released at 2.0 % and 1.5 % of HCl. Almost the same trend was observed for HCl pre-treated S.S. variety SWSV2006-3, which released the highest % glucose yield at 2.5 and 3 % of HCl ; acid concentrations that gave numerically the same % glucose yield and are not significantly higher ($p>0.05$) than the glucose values released at 1.5 % and 2 % HCl. For S.S variety SPV 422 pre-treated with different concentrations of H_2O_2, the highest % glucose yield was recorded at 3.0 % H_2O_2 which was not significantly different ($p>0.05$) from glucose released at 2.5 % H_2O_2. For SWSV 2006-3, the highest % glucose yield was at 3.0 % of H_2O_2. The lowest % glucose yield was observed at 0.50 %. No glucose yield was observed for the control in all the groups. Two (2.0) % for HCl and 3.0 % for H_2O_2 were therefore selected and used in the subsequent optimization steps.

Table 1: Effect of pre-treating the bagasse of two sweet sorghum varieties with different concentrations of HCl and H_2O_2 on the %yield of glucose (w/w)

% concentration	Pre-treatment solutions			
	HCl Treated		H_2O_2 Treated	
	SPV422	SWSV2006-3	SPV422	SWSV2006-3
0.00 (Distilled water)	$0.00±0.00^{a}$	$0.00 ± 0.00^{a}$	$0.00±0.00^{a}$	$0.00±0.00^{a}$
0.50	$5.42±0.59^{b}$	$1.67 ± 0.00^{b}$	$1.25±0.00^{a}$	$5.00±0.00^{b}$
1.00	$9.79±1.47^{c}$	$3.33 ± 0.00^{c}$	$6.46±0.88^{b}$	$10.63±0.29^{c}$
1.50	$14.17±0.59^{de}$	$3.75 ± 0.59^{c}$	$11.88±0.29^{c}$	$21.04±2.06^{d}$
2.00	$14.38±0.88^{de}$	$3.75 ± 0.59^{c}$	$13.33±0.00^{c}$	$31.04±0.88^{e}$
2.50	$16.04±3.83^{e}$	$4.17 ± 0.59^{c}$	$23.96±0.88^{d}$	$31.25±3.54^{e}$
3.00	$10.42±0.00^{cd}$	$4.17 ± 0.59^{c}$	$24.17±4.71^{d}$	$46.46±3.24^{f}$

Values are Mean ± SD of triplicate experiments.
Values with different superscripts down the column are significantly different from each other at $p<0.05$

Nigerian Variety	**Indian Variety**
SWSV 2006-3	SPV 422

Effect of ratio of bagasse to pre-treatment solutions (HCl and H_2O_2) on the percentage (%) yield of glucose (w/w) from the bagasse of two sweet sorghum varieties

Table 2 shows the effect of ratio of bagasse to pre-treatment solutions on the amount of glucose released from the bagasse of two S.S. varieties. The highest % glucose yield for HCl pre-treated SPV422 was obtained as 28.57 ± 1.01 % at the ratio of 1:50 which is significantly different ($p<0.05$) from other ratios. Also, the lowest % glucose yield of 9.64 ± 1.52 % was obtained at the ratio of 1:150. Similarly, the highest % glucose yield for HCl pre-treated SWSV2006-3 was 25.71 ± 2.02 % at the ratio of 1:50, which is significantly different from other ratios. The lowest % glucose yield of 17.14 ± 0.00 % was recorded at the ratio of 1:150.

The highest % glucose yield for H_2O_2 pre-treated SPV422 was recorded as 84.29 ± 2.02 % at the ratio of 1:200. This value was significantly different ($p<0.05$) from other ratios. The H_2O_2 pre-treated SWSV2006-3 had the highest % yield of 81.43 ± 2.02% at the ratio of 1:150 which was also significantly different ($p>0.05$) from other values. The lowest value of 26.07 ± 2.53% was recorded for 1:50 ratio. No glucose was released from the control groups.

Table 2: Effect of ratio of bagasse to pre-treatment solutions on the percentage yield of glucose (w/w) released from the bagasse of the two sweet sorghum varieties.

Ratio of sample to pretreatment Solutions	Pre-treatment solutions					
	Control (distilled water)		2.0 % HCL Treated		3.0 % H_2O_2 Treated	
	SPV422	SWSV2006-3	SPV422	SWSV 2006-3	SPV422	SWSV 2006-3
1:50	0.00±0.00	0.00±0.00	28.57±1.01^d	25.71±2.02^c	25.36±0.51^a	26.07±2.53^a
1:100	0.00±0.00	0.00±0.00	25.71±0.00^c	21.43±0.00^b	46.43±5.05^b	45.00±5.05^b
1:150	0.00±0.00	0.00±0.00	9.64±1.52^a	17.14±0.00^a	64.29±0.00^c	62.14±0.00^c
1:200	0.00±0.00	0.00±0.00	14.29 ± 0.00^b	22.86±0.00^b	84.29±2.02^d	81.43±2.02^d

Values are Mean ± SD of triplicate experiments.
Values with different superscripts down the column are significantly at $p<0.05$.

Nigerian Variety	**Indian Variety**
SWSV 2006-3	SPV 422

Effect of temperature on the percentage (%) yield of glucose (w/w) released from the bagasse of two Sweet sorghum varieties

Table 3 shows the effect of temperature on the % yield of glucose released from the bagasse of two sweet sorghum varieties. The highest % glucose yield of 87.14 ± 2.02 % was released at the temperature of 121^oC from S.S. variety SPV422 pre-treated with 3.0 % H_2O_2 at the ratio of 1:200. This value is significantly different ($p<0.05$) from values obtained from SPV422 pre-treated at the ratio of 1:50 with 2.0 % HCl at this temperature.
A similar trend was also recorded in SPV422 pre-treated at the temperature of 138 ^oC. The highest % glucose yield of 85.71 ± 0.00 % was observed when this variety was pre-treated with 3.0 % H_2O_2 at 121 ^oC. Also at the temperature of 121 ^oC, the highest % glucose yield of 84.29 ± 2.02 % was obtained from S.S. variety SWSV2006-3 at the ratio of 1:200 of sample to 3% H_2O_2. This value is significantly different ($p<0.05$) from values obtained from 2.0 % HCl at 1:50. Similarly, the highest % glucose yield of 82.86 ± 0.00 % was observed for SWSV2006-3 at the ratio of 1:200 with 3% H_2O_2 at the temperature of 138 ^oC. This result is significantly different ($p<0.05$) from values obtained for S.S. variety SWSV2006-3 at the ratio of 1:50 with 2.0 % HCl. The control had no value for glucose. The S.S. variety SWSV2006-3 pre-treated at the temperature of 121 ^oC gave a better glucose yield than at 138 ^oC.

Table 3: Effect of temperature on the percentage yield of glucose (w/w) released from the baggase of two Sweet Sorghum varieties.

Ratio of sample to pre-treatment Solutions	Temperature			
	121^{o}C		138^{o}C	
	SPV422	SWSV2006-3	SPV422	SWSV2006-3
1:50 distilled water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1:200 distilled water	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1:50 2% HCl	3.93 ± 1.52^{a}	15.36 ± 0.5^{c}	4.64 ± 0.51^{a}	10.36 ± 0.51^{b}
1:200 3%H_2O_2	87.14 ± 2.02^{b}	84.29 ± 2.02^{ab}	85.71 ± 0.00^{ab}	82.86 ± 0.00^{a}

Values are Mean ± SD of triplicate determinations.
Means with different superscripts across the row are significantly at $p<0.05$.

Nigerian Variety	**Indian Variety**
SWSV 2006-3	SPV 422

DISCUSSION

Effect of different concentration of the pre-treatment solution on the amount glucose released from the bagasse of two sweet sorghum varieties

HCl pre-treatment solution at concentration of 1.5 %, 2.0 % and 2.5 % gave highest yield of glucose from bagasse of SPV 422. Though numerically, the yields differ but the differences were not significant. This implies that either concentrations of 1.5 %, 2.0 % and 2.5 % can be used to obtain high glucose yield from bagasse.

SWSV 2006-3 treated with HCl at concentrations of 1.0 %, 1.5 %, 2.0 %, 2.5 % and 3.0 % gave values that are not significantly different ($p>0.05$) from the numerically highest yield; which also implies that any of the concentrations can be used. The HCl concentration of 1.5 % is far from 2.5 % which gave the numerically highest yield in the case of SPV 422 while 2.0 % is closer to 2.5 % and also gave highest yield that is not significantly different ($p>0.05$) from that obtained with 3.0 % concentration. Apart from the fact that the yield is not significantly ($p>0.05$) different from the numerically highest yield (4.17 ± 0.59), it is closer to it. This trend of result is different from that obtained when cotton fibre was treated with acids. Arthe *et al*. (2008) reported that an increase in acid severity in terms of concentration from 1 % to 3 % resulted in a higher sugar release from cotton fibre treated with dilute sulphuric acid. Dussán *et al*. (2014) reported that 2 % w/v acid (H_2SO_4) at 155 ºC for 10 min gave the highest glucose extraction efficiency (70 %) from Sugarcane bagasse and that the conditions led to extensive hydrolysis of cellulose to glucose. Orozco *et al.* (2007) reported optimum conditions of 2.5 % phosphoric acid at a temperature of 175 °C for grass hydrolysis. Degradation occurred at acid concentrations greater than 2.5 % (v/v) and temperatures greater than 175 °C. H_2O_2 pre-treatment at 2.5 % and 3.0 % gave maximum yield of 23.96 ± 0.88 and 24.17 ± 4.71, respectively which were not significantly different (p>0.05) and therefore implies that any of them can be used to obtain the highest result. However, 3.0 % concentration is preferable since numerically, it gave the highest yield in both SPV 22 and SWSV 2006-3. This corroborates the result of Diaz *et al.* (2013), where the highest sugar yield of 86.48 ± 3.07 % was attained with a peroxide concentration of 7.5 % (at pH 11.5, 90 ºC in 1h) when rice husk was hydrolysed at peroxide concentrations of 2.5, 5 and 7 %. This shows that higher concentration of hydrogen peroxide can further increase glucose release.

Effect of ratio of sample to the pre-treatment solutions on the amount of glucose released from the bagasse of two sweet sorghum varieties

The ratio of sample to pre-treatment solution, 1:50 gave the maximum yield of 28.57 ± 1.01and 25.71 ± 2.02 for SPV 422 and SWSV 2006-3, respectively (Table 2). Increase in the proportion of pre-treatment solution to 1:100 and 1:150 resulted in continuous decrease in glucose yield. This can be attributed to the fact that 1:50 is the ratio of sample to pre-treatment solution that can maximally release glucose from hemicellulose. As the proportion of the pre-treatment solution increases, the glucose released becomes degraded until a point is reached as in 1:200 (Table 2) when the acid starts attacking the cellulose portion of the bagasse (hemicellulose) which will result in further release of glucose. On the other hand, increase in the proportion of the pre-treatment solution of H_2O_2 from 1:50 to 1:200 gave a progressively increasing yield of glucose. Further increase may continue to yield glucose at an increasing rate until a maximum ratio is reached when further increase begins to give reduced yield due to substrate saturation as a result of increased ratio. De Moraes *et al.* (2010) reported that two different solid-to-liquid ratios (1.5:10 and 1:10) were used in the pre-treatment that efficiently hydrolysed the hemicelluloses giving removals above 90%. The extractive components were also effectively solubilised, and lignin was only slightly affected.

Effect of temperature on the amount of glucose released from two sweet sorghum varieties

The effect of temperature on bagasse for all selected best conditions (which are concentration of pre-treatment solutions and the ratio of sample to pre-treatment solution) that released the highest glucose concentration showed that the temperature of 121 ^{o}C was the best for the two varieties. This could be attributed to the fact that 121 ^{o}C is the optimum temperature for glucose release for the selected condition. Any further increase in temperature will likely cause the degradation of glucose. However, the yield obtained at 138 ^{o}C, though numerically different, is still not significantly different (p>0.05) from those obtained at temperature of 121 ^{o}C. Similarly sugarcane bagasse has been successfully delignified at temperature ranges that are close to 121 ^{o}C under different conditions. Gamez *et al*. (2006) used Phosphoric acid at 2-6 % concentrations, time of 0-300 min. and temperature of 122 ^{o}C to delignify sugarcane bagasse (SCB). Chong *et al*. (2004) reported that successfully delignification of SCB was achieved by using nitric acid at variable concentration of 2-6 %, reaction time up to 300 minutes and temperature range of 100-128 ^{o}C. Cardona *et al*. (2010) reported that delignification of SCB with dilute acids (sulphuric, hydrochloric or acetic, typically 1-10 % weight) hydrolysed the hemicellulose (sugars) fraction at temperature range of 100 ^{o}C and 150 ^{o}C).

CONCLUSION

The optimal conditions discovered for HCl treatment are 2.0 % HCl concentrations, 1:50 ratio of sample to 2.0 % HCl, at 138 ^{o}C for SPV 422 and 2.0 % HCl concentrations, 1:50 ratio of sample to 2.0 % HCl at 121^{o}C for SWSV 2006-3.
For H_2O_2 optimal conditions of 3.0 % H_2O_2 concentrations, 1:200 ratio of sample to 3.0 % H_2O_2, at 121^{o}C for both SPV 422 and SWSV 2006-3.
The glucose concentration released from the two sweet sorghum varieties suggests that 3.0 % H_2O_2 together with the optimized condition can degrade cellulose effectively. The pith of sweet sorghum may be more susceptible to hydrolysis than the whole stem. Therefore, the difficulty and cost of hydrolysis can be reduced if the pith alone is used.

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