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PRETREATMENT OF YAM PEELS WITH ALKALINE HYDROGEN PEROXIDE TO ENHANCE ENZYMATIC HYDROLYSIS FOR BIOETHANOL PRODUCTION

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ABSTRACT
The effectiveness of alkaline hydrogen peroxide (AHP) as a suitable choice of pretreatment for the conversion of yam peels to reducing sugars using cellulase enzyme for hydrolysis and subsequent ethanol production was determined. The effects of three variables on the chemical composition and reducing sugar production from yam peels were determined using one factor at a time (OFAT) method namely; hydrogen peroxide concentration (%v/v), pretreatment time (minutes) and pretreatment temperature (°C). From the results obtained, it was observed that a significant (P<0.05) amount of reducing sugars were lost during pretreatment of yam peels. The untreated group which was only physically pretreated (milled), however, yielded a significantly higher (P<0.05) reducing sugar concentration of 21.33±0.02mg/ml after 48hours enzymatic hydrolysis, while the highest reducing sugar concentration of 4.22±0.02mg/ml was liberated using 0.75%v/v alkaline hydrogen peroxide concentration for 60minutes at 50°C. Therefore, pretreatment of biomass with alkaline hydrogen peroxide may be more suitable for feedstocks with higher lignin contents than yam peels.
Key words: Pretreatment, hydrolysis, optimization, yam peels, alkaline hydrogen peroxide (AHP)

INTRODUCTION

In order to improve the standard of living, there is a need to search for sustainable energy sources to meet up with the increased demand for energy worldwide. Fossil fuels have been used for a long time as the main sources of energy. However, its use has been associated with environmental pollution and global warming. As a result, a search for more environmentally favorable, renewable and sustainable source of energy is on the rise. Liquid bio-fuels contribute about 40% of energy consumption worldwide and have been said to contribute to a reduction in green house gases, increased job creation and economic development (Mohd *et al.*, 2017).

Lignocelluloses biomasses are renewable and abundant resources which are used for the production of bio based materials such as bioethanol. Lignocelluloses feed stocks are mainly made up of a complex structure of cellulose, hemicelluloses and lignin and a small extractive and ash fractions which are often resistant to enzymatic hydrolysis (Kim *et al.*, 2016). Cellulose and hemicelluloses hydrolysis releases fermentable sugars for subsequent ethanol production. One of the best technologies used for the conversion of lignocellulosic biomass to fermentable sugars involves the use of enzymes due to its low energy requirement and less environmental pollution. One major problem however is the low accessibility of cellulose to cellulase enzyme because of the rigid association of cellulose with lignin. This often causes setbacks during the hydrolysis step. Therefore, an efficient way of disrupting the rigid structure of lignin to improve cellulose accessibility to enzymatic attack remains a critical aim of pretreatment (El-Naggar, 2014).

There are different types of pretreatments being employed which are; chemical, physical and biological methods or a combination of them (Dutra *et al.*, 2017). In all, pretreatments have advantages and disadvantages. It is therefore important to create a criterion for the cost effective pretreatment given that this stage is one of the most expensive for lignocellulosic ethanol production (Dutra *et al.*, 2017).

For pretreatment efficiency, it is required that the following requirements are met: the production of reactive cellulose fibers, little or no loss of cellulose and hemicelluloses fractions, absence of possible inhibitors that may be generated during the hydrolysis and fermentation steps and least energy use (Dutra *et al*., 2017).

Most of the various the different pretreatment methods currently in use are conducted under high energy and/or pressure which may not be cost effective. More successful pretreatment methods which can be carried out at lower temperatures and pressure are still under investigation. This has caused an increased interest in using alkaline hydrogen peroxide (AHP) as a pretreatment choice for the pretreatment of different feed stocks. AHP is an oxidative pretreatment method. It exerts its effects by delignification of the lignocelluloses biomass thereby increasing the accessibility of cellulose to the hydrolyzing effects of cellulase enzyme. This pretreatment requires low-energy and does not generate inhibitors like hydroxymethyl furfural and furfural (Dutra *et al*., 20 17).

Therefore, for pretreatment efficiency to be achieved using alkaline hydrogen peroxide, the effects of the most important factors must be optimized (Dutra *et al*., 2017). This research focused therefore, on determining the effects of: (i) H_2O_2 concentration in the pretreatment solution; (ii) pretreatment time; and (iii) pretreatment temperature on reducing sugar production from millet husk using cellulase enzyme for hydrolysis.

MATERIALS AND METHODS

Preparation of raw biomass

Yam peelss were collected from domestic wastes, washed and sun dried. The samples were subjected to physical pretreatment (grinding) to reduce the size and increase the surface area of contact of the biomass. The sample was then used for compositional analysis and chemical pretreatment.

Compositional analysis of biomass

Chemical composition of cellulose, hemicelluloses and lignin of yam peels was determined according to the method of Hernawan *et al*., (2017). The sample 1g (a) was added to 150ml of water and boiled for 1hour at 100^{o}C. It was washed with 300ml of hot water, dried and weighed (b). To the dried residue, 150ml of 1N H_2SO_4 was added and boiled at 100^{o}C for 1hour. It was then washed with 300ml of hot water, dried and weighed (c). A quantity (10ml) of 72% H_2SO_4 was added to the dried residue and soaked at room temperature for 4 hours and then filtered. A quantity (150ml) of 1N H_2SO_4 was added to the residue and boiled in a water bath for 1 hour, filtered, washed with 800ml of water and dried at 105^{o}C and weighed (d). The residue was ashed and weighed (e). The % of cellulose, hemicelluloses and lignin were determined using the formula below:

Lignin content = (d-e)/a*100

Cellulose content = (c-d)/a*100

Hemicellulo se content = (b-c)/a*100

Biomass pretreatment

Chemical pretreatment was carried out according to the method of Diaz *et al.* (2013) but with some modifications in the concentrations of pretreatment solutions where lower concentrations were used. It was carried out using alkaline hydrogen peroxide (pH 11.5) with varied concentrations of 0.375%v/v, 0.75%v/v, 1.5%v/v and 3.0%v/v for 2hours at 90^{o}C.

Yam peelss (6%w/v) were pretreated in 100ml peroxide solutions of different concentrations (0.375, 0.75, 1.5, 3% v/v), depending on the experiment in 250ml flasks and the pH was adjusted to11.5 with NaOH (1M) which was added dropwise. Each experiment was carried out in triplicate.

Flasks were covered with aluminum foil and incubated in a water bath at 90^{o}C for two (2) hours. The solid residue was collected by filtration and washed thoroughly until neutral pH of the filtrate was obtained. The residue was dried at 60^{o}C overnight. After drying, it was weighed to determine mass loss, which corresponds to the lignin content and other solubilized compounds.

After each pretreatment, a liquid sample (pretreatment supernatant) was taken and tested for the presence of reducing sugars using DNS method. Before the analysis, samples were centrifuged for ten (10) minutes at 5000 rpm and the pH was neutralized by adding 2% H_2SO_4. The same procedure as above was repeated for the optimizations of pretreatment time (30, 60, 90 and 120 minutes) and pretreatment temperature (25, 50, 75 and 90^{o}C) where time was varied while H_2O_2 concentration and temperature were kept constant. Likewise, temperature was varied and H_2O_2 concentration and time were kept constant.

Analytical methods

To determine the chemical pretreatment efficiency, reducing sugar concentrations were determined colorimetrically after pretreatment (pretreatment supernatant) and after subsequent enzymatic hydrolysis (with cellulase enzyme) using dinitrosalicylic (DNS) method.

Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out according to the method of Karagoz and Ozkan, (2014).

Effects of pretreatments were evaluated through enzymatic hydrolysis of pretreated yam peels by analyzing the reducing sugars produced after the hydrolysis of cellulose and hemicelluloses using DNS method. Cellulase enzyme (3mg/ml/g of dry substrate) was used for hydrolysis with citrate-Na_2HPO_4 buffer (0.05M, pH5.0) (Li *et al.*, 2019). The washed and dried residues of yam peels obtained after pretreatment were hydrolyzed in 250ml flasks at a temperature of 50^oC with a solid loading of 5% (w/v). Enzymatic hydrolysis was performed on an orbital shaker at 150 rpm.

Dinitro-salicylic acid method

Solution A: DNS (1g) was dissolved in 2M NaOH (20ml). Solution B: Sodium and potassium tartrate tetrahydrate (Rochelle salt) (30g) was dissolved in distilled water (50ml). Solutions A and B were mixed and heated in a water bath to homogenize. The volume was completed to 100ml with distilled water. The solution was stored in an amber bottle at 4^oC.

Test tubes were labeled as blank and test**.** Dilutions of glucose standards with concentrations of 40, 80, 120, 160, 200 µg per 200 µl by transferring respective amount of glucose from the standard glucose solution (1mg/ml) and adjusting it to a total volume of 200 µl by adding distilled water were made (for standard curve)**.** Samples obtained each from pretreatment supernatant and hydrolysis of yam peels were added (200 µl). DNSA reagent (1ml) was added to all the test tubes and mixed well. It was kept in a boiling water bath for 15minutes. Absorbance of the blank was read first at 540nm using 1ml cuvettes and it was made zero. The absorbance of all the tubes was read (Miller, 1959). The cuvettes were rinsed each time after taking the absorbance. A standard curve was plotted for absorbance at 540nm on Y axis versus concentration of glucose in µg/200µl on X axis. The value of unknown was recorded from the graph corresponding to the absorbance reading of the test sample. Sugar concentration was calculated using the following formula: Sugar concentration in Test sample = concentration of unknown in µg/200µl x 5µg/ml.

Results

Table 1: Effect of varied H_2O_2 concentration on the chemical composition and reducing sugar concentration (mg/ml) produced from yam peels pretreated at constant time (120 minutes) and temperature (90°C)

[H_2O_2] %v/v	Solubilized compounds (g)	Reducing sugar concentration (mg/ml) in pretreatment hydrolysate	Reducing sugar concentration (mg/ml) after enzymatic hydrolysis (48hrs)	Hemicellulose (%)	Lignin (%)	Cellulose (%)
Untreated	0.00$^{a, b, c, d}$	0.00 $^{a, b, c, d}$	21.33±0.02 $^{a, b, c, d}$	25.96 ± 0.03$^{a, b, c, d}$	19.55 ±0.3 $^{a, b, c, d}$	40.39±0.53 $^{a. b, c, d}$
0.375	0.75±0.03$^{a, e}$	6.97±0.01 $^{a, e}$	3.89±0.08 $^{a, e}$	20.33± 1.0$^{a, e}$	17.01± 0.06$^{a, e}$	32.22± 0.67$^{a, e}$
0.75	1.11±0.05$^{b, e, f}$	7.69±0.02 $^{b, e, f}$	4.22±0.02 $^{b, e, f}$	17.67± 0.2 $^{b, e, f}$	14.94± 0.06 $^{b, e}$	25.53± 0.03$^{b, e, f}$
1.5	1.33±0.04$^{c, e, f}$	9.25±0.01 $^{c, e, f}$	3.13±0.02 $^{c, e, f, g}$	12.61± 0.36 $^{c, e, f, g}$	14.24± 0.24 $^{c, e}$	21.62± 0.98 $^{c, e, f, g}$
3.0	1.38±0.06$^{d, e, f}$	9.62±0.01 $^{d, e, f}$	1.81±0.11 $^{d, e, f, g}$	8.61± 0.19 $^{d, e, f, g}$	14.05± 0.13 $^{d, e}$	16.29± 0.05 $^{d, e, f, g}$

Values in the Table represent mean ± SD, same superscripts along columns indicate significant differences between the groups at $P < 0.05$, n =3 for each group.

Table 2: Effect of varied pretreatment time (minutes) on the chemical composition and reducing sugar concentration (mg/ml) produced from yam peels pretreated with constant H_2O_2 concentration (0.75% v/v) and temperature (90°C)

Time (minutes)	Solubilized compounds (g)	Reducing sugar concentration (mg/ml) in pretreatment hydrolysate	Reducing sugar concentration (mg/ml) after enzymatic hydrolysis (48hrs)	Hemicellulose (%)	Lignin (%)	Cellulose (%)
Untreated	0.00$^{a, b, c, d}$	0.00 $^{a, b, c, d}$	21.33±0.02 $^{a, b, c, d}$	25.96 ± 0.03$^{a, b, c, d}$	19.55 ±0.30 $^{a, b, c, d}$	40.39±0.53 $^{a. b, c, d}$
30	0.74±0.05$^{a, e}$	4.71±0.06 $^{a, e}$	3.29±0.33 $^{a, e}$	20.33± 0.08$^{a, e}$	17.95± 0.02$^{a, e}$	37.82± 0.07$^{a, e}$
60	0.93±0.05$^{b, e, f}$	5.68±0.05 $^{b, e, f}$	4.02±0.75 $^{b, e, f}$	18.07± 0.07 $^{b, e, f}$	15.40± 0.05 $^{b, e, f}$	35.30± 0.08$^{b, e, f}$
90	1.02±0.05$^{c, e, f, g}$	5.16±0.08 $^{c, e, f, g}$	3.84±0.05 $^{c, e, f, g}$	17.59± 0.01 $^{c, e, f}$	14.33± 0.02 $^{c, e, f}$	27.05± 0.05 $^{c, e, f, g}$
120	1.12±0.06$^{d, e, f, g}$	7.64±0.04 $^{d, e, f, g}$	3.08±0.86 $^{d, e, f, g}$	17.52± 0.02 $^{d, e, f}$	14.15± 0.77 $^{d, e, f}$	16.08± 0.07 $^{d, e, f, g}$

Values in the Table represent mean ± SD, same superscripts along columns indicate significant differences between the groups at $P < 0.05$, n =3 for each group.

Table 3: Effect of varied pretreatment temperature (°C) on the chemical composition and reducing sugar concentration (mg/ml) produced from yam peels pretreated with constant H_2O_2 concentration (0.75% v/v) and time (60 minutes)

Temperature (°C)	Solubilized compounds (g)	Reducing sugar concentration (mg/ml) in pretreatment hydrolysate	Reducing sugar concentration (mg/ml) after enzymatic hydrolysis (48hrs)	Hemicellulose (%)	Lignin (%)	Cellulose (%)
Untreated	0.00[a, b, c, d]	0.00 [a, b, c, d]	21.33±0.02 [a, b, c, d]	25.96 ± 0.03[a, b, c, d]	19.55 ±0.30 [a, b, c, d]	40.39±0.53 [a. b, c, d]
25	0.34±0.04[a, e]	2.76±0.06 [a, e]	3.21±0.05 [a, e]	19.92± 0.42[a, e]	17.25± 0.23[a, e]	38.39± 0.1[a, e]
50	0.54±0.05[b, e, f]	3.32±0.07 [b, e, f]	4.19±0.05 [b, e]	18.66± 0.26 [b, e]	15.83± 0.06 [b, e]	32.41± 0.08[b, e, f]
75	0.81±0.07[c, e, f, g]	3.89±0.06 [c, e, f, g]	3.59±0.08 [c, e, f]	18.71± 0.11 [c, e]	14.33± 0.10 [c, e, f]	31.91± 0.06 [c, e, g]
90	1.06±0.07[d, e, f, g]	7.59±0.08 [d, e, f, g]	3.33±0.11 [d, f]	18.62± 0.22 [d, e]	15.53± 0.13 [b, e, f]	25.01± 0.13[b, e, f, g]

Values in the Table represent mean ± SD, same superscripts along columns indicate significant differences between groups at $P < 0.05$, n =3 for each group.

DISCUSSION

The effect of pretreatment with varied alkaline hydrogen peroxide concentrations, pretreatment time and pretreatment temperature on the chemical composition and reducing sugar production after 48 hours hydrolysis of yam peels using cellulase enzyme was determined. H_2O_2 concentration has an important role in improving the biomass accessibility to cellulase enzyme hydrolysis. The concentrations of hydrogen peroxide used for the pretreatment of lignocelluloses biomass may vary widely which depends on the nature of biomass, whether it contains more amorphous or crystalline cellulose (Dutra *et al.*, 2017). From the results of different studies, it was observed that concentrations of hydrogen peroxide used for pretreatment range between 1 to 10% (v/v). The main challenge encountered when using H_2O_2 as a pretreatment choice is associated with the adequate concentration that should be used. High concentrations have been reported to be more efficient at short periods of time (Rabelo *et al.*, 2011). Most of the pretreatment studies carried out were conducted using high concentrations of H_2O_2, therefore, it is important to determine the optimum pretreatment concentrations for different types of biomass, preferably using lower H_2O_2 concentrations, because this parameter plays an important role in determining the cost effectiveness of the pretreatment method (Juarez *et al.*, 2016).

Table 1 shows the results of the chemical compositional analysis of yam peels before and after pretreatment with varied concentrations of alkaline hydrogen peroxide solution (pH11. 5) for 120minutes at 90°C under static condition. In order to study the sole effect of concentration, time and temperature, a control group made up of yam peels which was only physically pretreated (milled) was analyzed to determine the chemical composition. It was also enzymatically hydrolysed under the same conditions. It was observed that, the untreated yam peels group had significantly *(P<0.05)* higher percentages of hemicelluloses, cellulose and lignin than the other pretreated groups (Tables 1, 2 and 3). Also, loss in weights of yam peels significantly (*P<0.05*) increased with increase in hydrogen peroxide concentration in all the pretreated groups with the highest loss observed in the group pretreated with 3.0%v/v. This corresponds with the loss of reducing sugars in the pretreatment solution, which was also observed to increase with increase in hydrogen peroxide concentration. The concentrations of cellulose, hemicelluloses and lignin also decreased significantly with increase in AHP concentration. This finding corresponds with that of Diaz *et al.*, (2013) where rice husks were pretreated with alkaline hydrogen peroxide. Higher concentration of pretreatment solution may have allowed for the production of hydro-peroxide anion which may have further reacted with H_2O_2 thereby leading to the formation of superoxide and hydroxyl radicals responsible for lignin removal (Dutra *et al.*, 2017).

These free radicals that may have been produced may also be responsible for the dissociation of cellulose and hemicelluloses fractions of yam peels and millet husk as observed in this study leading to higher reducing sugars concentration lost during pretreatment. However, in this study (Table I), the untreated group was observed to release a significantly high *(P<0.05)* concentration of reducing sugars (21.33±0.31mg/ml) than the AHP pretreated groups. This observation may be due to no loss in weight and therefore no loss of reducing sugars during pretreatment. The significant *(P<0.05)* differences observed in the reducing sugar concentrations between the alkaline hydrogen peroxide pretreated groups and the untreated group may be due to loss of some fractions of cellulose and hemicelluloses during pretreatment and washing of yam peels. Lignocellulosic biomass compositions of plants vary depending on their locality and seasonal changes (Joshi *et al*., 2018). Therefore, reducing sugars produced after pretreatment of biomass may also vary. The group pretreated with 0.75%v/v hydrogen peroxide concentration was observed to liberate a significantly *(P<0.05)* higher reducing sugar concentration of 4.22±0.02mg/ml after 48hrs enzymatic hydrolysis. In a similar study, Saha and Cotta (2007) determined the effects of H_2O_2 concentration in the pretreatment of rice husks for increasing reducing sugar concentration using enzymes. They reported obtaining a high concentration of reducing sugars when 7.5% (v/v) H_2O_2 was used for pretreatment. At a concentration of 10% (v/v) however, they reported a decrease in the concentration of reducing sugars. It has also been reported that pretreatment of biomasses that have an initial sugar concentration with a high concentration of H_2O_2, such as microalgae, might result in the degradation of polysaccharides leading to subsequent byproducts and inhibitors production. A hydrogen peroxide concentration of 0.75%v/v was therefore used for further optimizations.

The time taken to pretreat a biomass which is the contact time of the biomass with the pretreatment solution has been reported to vary depending on the choice of pretreatment method. Pretreatment of lignocelluloses feed stocks with alkaline hydrogen peroxide have been reported to vary between 1 to 24hours. This depends on the biomass and the conditions being optimized (Dutra *et al*., 2017).

In this study, Pretreatment time was observed to also increase the total reducing sugar concentration in the pretreated groups. However, the groups pretreated for 60minutes had a significantly *(P<0.05)* higher reducing sugar concentration of 4.02±0.75mg/ml than the groups pretreated for 30, 90 and 120 minutes (Table 2). Also, a significant *(P<0.05)* decrease in cellulose, hemicelluloses and lignin were observed. This may be due to longer contact time allowed between the biomasses and hydrogen peroxide which may have allowed for the sufficient oxidizing effects of H_2O_2 on the biomasses (Dutra *et al*., 2017). In a similar study, Saha and Cotta, (2006) reported that wheat straw pretreated with 2.15% H_2O_2 (v/v) which had a total solid content of 8.6% (w/v), at a temperature of 24^{o}C and pH of 11.5, an increase from 3 to 24 h in the pretreatment time yielded a higher concentration of reducing sugars after enzymatic hydrolysis.

The pretreatment temperature is an important factor which is directly associated with the cost effectiveness of the pretreatment method. Pretreatments using alkaline H_2O_2 were reported to be conducted at low temperatures ranging from 25-70^{o}C. Pretreatment temperatures have been reported to having varying effects depending on the biomass being pretreated.

In this work, high pretreatment temperature of 90^{o}C was observed to dissolve a significantly *(P<0.05)* higher mass of yam peels (1.06±0.07g) than the 25, 50 and 75^{o}C pretreated groups. Thus, a significantly *(P<0.05)* high amount of reducing sugars may have been lost during pretreatment, recovery and washing of the biomass. This may be due to the oxidizing effects of H_2O_2 and the solubilizing effect of high temperatures on yam peels. After 48hrs enzymatic hydrolysis, the highest reducing sugar concentration of 4.19±0.05mgml was produced in the group pretreated at 50^{o}C which was significantly *(P<0.05)* lower than the concentration produced in the untreated group. In a similar study, at high pretreatment temperatures, 2% (w/v) H_2O_2 pH 11.5, 12 h of reaction, rye straw biomass was reported to have an increased solubilization effect on the lignin and hemicelluloses fractions of the biomass. Sun *et al*., (2000) investigated the effect of pretreatment temperature on sugarcane bagasse as a feedstock. High temperatures were reported to have no effect in the release of reducing sugars. The optimum conditions were reported at a temperature 20^{o}C, H_2O_2 concentration of 5% (v/v) H_2O_2, and 4% total solids (w/v) for 6 h.

In summary, the best conditions for the chemically pretreated yam peels group were; a hydrogen peroxide concentration of 0.75%v/v, pretreatment time of 60 minutes and pretreatment temperature of 50^{o}C.

CONCLUSION

From this work, it was observed that physically pretreated yam peels has a higher bioethanol

producing potential due to the high concentration of reducing sugars produced than the AHP treated groups. It may therefore be concluded that, the concentration of reducing sugars produced from yam peels after pretreatment were lower than those produced in the untreated groups. This observation may be due to the oxidizing effects of hydrogen peroxide. Alkaline hydrogen peroxide may however be more effective in the pretreatment of feedstock that has a higher lignin concentration.

RECOMMENDATION

From the findings of this study, it may be recommended that, pretreatment optimization studies should be carried out for each feedstock using milder reaction conditions such as; low pretreatment temperatures, low chemical concentrations and short reaction times since feedstocks vary in chemical compositions, in order to establish an effective pretreatment criterion.

Author's contributions

Zeenat Ibrahim Saulawa conducted the research
Dr. Nura Lawal Co supervised the work
Professor Muntari Bala Co supervised the work
Dr. Abdullahi Abdulkadir Imam Supervised the work

Conflict of interest

The authors declare no conflict of interest

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