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Effect of Dilute Acid and Alkaline Pretreatment of *Typha australis* (Typha Grass) for Bioethanol Production

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Typha australis (Typha grass) obtained from Kware Lake was used in this research to produce bioethanol. Different pretreatment methods including dilute acid (0.2M H_2SO_4), dilute alkaline (0.2M NaOH) and liquid hot water pretreatments were used to pretreat the Typha grass sample before enzymatic saccharification for 7 days using *Aspergillus niger* isolated from soil sediment and the hydrolysate was seeded with *Saccharomyces cerevisiae* isolated from palm wine to produce bioethanol. HPLC was used to analyze bioethanol product. The result showed that pretreatment with 0.2M H_2SO_4 removed more hemicelluloses (7.0%) when compared with other pretreatment methods used, but pretreatment with 0.2M NaOH and liquid hot water removed more lignin (14.29%) than dilute acid pretreatment. The highest percentage reducing sugar concentration of 0.58% was obtained from lower part of the sample pretreated with liquid hot water while Typha grass pretreated with 0.2M H_2SO_4 and 0.2M NaOH produced the highest percentage reducing sugar concentration of 0.32% each from the upper part of the sample. Also, the highest Bioethanol concentration of 2.07% was obtained at day 6 of fermentation from the Typha grass pretreated with

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liquid hot water while Typha grass pretreated with 0.2M H_2SO_4 and 0.2M NaOH produced highest Bioethanol concentration of 0.43% and 0.54% respectively. The results indicate that Typha grass can be harnessed for bioethanol production thereby reducing their negative impact on Lakes.

Keywords: Typha grass; pretreatment; bioethanol; HPLC.

1. INTRODUCTION

Consumption of energy has increased steadily over the last few decades as the population of the world has grown, and more countries have become industrialized. Crude oils have been the major natural resource to meet the growing demand for energy. Along with this, the usage of the fuels direct to global warming, environmental pollution, and other related hazards [1]. Production of liquid biofuels from lignocellulosic biomass will significantly reduce the dependence on petroleum-based fuels and therefore it has become a research area of great interest to many research scientists and government agencies [2]. Potential source intended for low-cost ethanol production is the utilization of lignocellulosic biomass such as grasses, agricultural residues, wood chips, and sawdust [3].

Typha grass has been identified as a particularly suitable biomass crop for wetland, because of their superiority, productivity, pest resistance, adaptability and chemical composition [4].

Kware lake serves a lot of functions to the nearby communities including a source of water for domestic activities such as washing and bathing. Typha grass has prolific vigorously, nearly impenetrable stand, getting to close the view of the water and take over the lake. This has raised concern that it may take over the lake and prevent a lot of activities from the lake. The present research is aimed at pretreating and hydrolyzing *Typha grass* for bioethanol production thereby adding more value to the grass, increase conservation and sustainability of the plant and lake, contributing towards alternative energy supply and also create profit and jobs opportunity.

2. METHODOLOGY

2.1 Collection and Preparation of Samples

The *Typha* grass sample was collected from Kware Lake in Kware local government area, Sokoto State, Nigeria. The sample was washed under tap water and then cut into two separating the upper part that grows above the water level and the lower part that grow inside water. The two portions were shade dried separately for 14 days. The dried samples were then grounded into powder using and stored at room temperature for further analysis.

2.2 Isolation and Identification of *Aspergilus niger* and *Saccharomyces cerevisiae*

Aspergillus niger was isolated from soil sediment collected from kware lake and identified base on microscopic (morphological) and macroscopic characteristics (colour, texture appearance and diameter of colonies) according to Sourza et al. [5]. The soil was serially diluted; a sample suspension was prepared by adding 1.0g of sample to 10ml of distilled water and mixed well for 10 minutes. The suspension was diluted serially 10^{-1}, 10^{-2} and 10^{-3}. 1ml (from the third dilution factor) was measured using a syringe and inoculated into a Sabraud Dextrose Agar (SDA) plate and incubated at 37 ^{O}C for five days. The initial white color of the colonies that later turns black at the top with pale yellow color at the bottom confirm the organism to be *Aspergillus niger* and *Saccharomyces cerevisiae* was isolated from palm wine sample collected from Giginya Barrack market, Sokoto and identified by the standard morphological and physiological test and identification keys described by Barnett et al. [6]. The palm wine sample was serially diluted; a sample suspension was prepared by adding 1.0 ml of sample to 10ml of distilled water and mixed. The suspension was diluted serially 10^{-1}, 10^{-2} and 10^{-3}. 1ml (from the third dilution factor) was measured using a syringe and inoculated into a Sabraud Dextrose Agar (SDA) plate and incubated at 28 ^{O}C for five days. Capability of the organism to hydrolyze starch and form bud under microscope was used to confirm the organism as *Sacchromyces cerevisiae.*

2.3 Determination of Structural Composition of Typha Grass

The percentage of acid – insoluble lignin, which is defined as the residue, was determined according to TAPPI procedure (T224 om-88). The holocellulose content, which is the combination of hemicellulose and cellulose, was determined in order to find the total amount of cellulose and hemicellulose in Typha grass. The holocellulose content was determined according to DIN 2403. α-Cellulose is the pure cellulose content of the materials which was extracted from holocellulose using alkali solution. The α-Cellulose content of Typha grass was determined as the residue insoluble in the 17.5 % NaOH solutions according to TAPPI 203 om-93 method.

2.4 Dilute Acid Pretreatment

2 g of *Typha* grass sample was mixed with 0.2M H_2SO_4 solution in a 250 mL flask with a stopple and then allowed to suck for 24hrs. The mixture was subsequently autoclave at 121 ^{O}C for 15mins and then cooled and filtered through a Whatman filter paper no. 1 to separate the solid residue. The residue was washed with distilled water until neutral pH. The sample was air dried and stored in tightly sealed plastic bag in a refrigerator for further use [7].

2.5 Dilute Alkaline Pretreatment

Approximately 2 g of *Typha* grass sample was soaked in 0.2M NaOH solution in a 250 mL flask for 24hrs and then autoclaved at 121°C, for 15 minutes. The solid residue was separated from the mixture by filtration with Whatman filter paper no. 1 and thoroughly washed with distilled water to neutralize its pH. Finally, the filtrate was dried and stored as above [7].

2.6 Liquid Hot Water Pretreatment (LHW)

In liquid hot water pretreatment of the *Typha* grass sample, the grounded powdered of the plant was slurried with distilled water using a solid to liquid ratio of 10% (w/w) and autoclaved at 121ºC for 15 minutes. After autoclaving, the sample was filtered using Whatman filter paper no. 1 and the solid residue was air dried and stored for further use [8].

2.7 Enzymatic Saccharification of Pretreated Typha Grass

The enzymatic saccharification of *Typha* grass was carried out using *Aspergillus niger* isolated from soil sediment as described by Gupta [9]. In this method, the pretreated *Typha* grass samples were inoculated with 0.5 ml suspension of 96 hours culture of *Aspergillus niger.* Hydrolysis was carried out at room temperature for 7 days. Samples were taken daily for reducing sugar determination using 1,4-dinitro salicylic acid (DNS) method to find out the net yield of fermentable sugars. The samples were then filtered using Whatman filter paper No. 1 and the filtrates were used for fermentation.

2.8 Fermentation of the Hydrolysate and Bioethanol Production

The fermentation studies were carried out using *Saccharomyces cerevisiae* isolated from palm wine. The hydrolysates were autoclaved at 121ºC for 15 min and the flasks were then cooled to room temperature. The pH of the fermentation medium was adjusted to 6.5 and then 1ml of prepared suspension of yeast isolated was added in the hydrolysate [10]. The fermentation was allowed for 7 days and samples from the medium were withdrawn periodically at 24 hrs interval from the flasks to determine ethanol quantity using UV-visible spectrophotometer at 540 nm.

2.9 Distillation

The fermented broth was filtered using Whatman filter paper no. 1. Each sample was weighed into Microkjeldahl flasks and then heated at 78 ^{0}C on the Microkjeldahl apparatus until the solution turned colourless. The presence of bioethanol was determined using high performance liquid chromatography (HPLC) [11].

2.10 Statistical Data Analysis

All the experiment were carried out in triplicate and their mean was expressed as the result of the experiment. Analysis of variance (ANOVA) was used for statistical analysis at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Structural Composition of Typha Grass Obtained from Kware Lake before Pretreatment

The structural composition of both the upper and lower part of Typha grass before pretreatment

show that, hemicelluloses has the highest composition of 28.70% and 30.00% while Lignin has a composition of 28.15% and 24.93% respectively, and α cellulose has least composition of 12.30% and 10.00% respectively in the Typha grass collected from Kware Lake. The result of structural composition of hemicelluloses and lignin is in conformity with the report of Bajpai [12] who reported that hemicelluloses compose between 35-50% and lignin compose 10-30% of grasses. However, this current study disagrees with his report that α-celluloses compose of 25-40%. This research shows that the upper part of Typha grass has higher structural composition than the lower part of the plant (Table 1).

Table 1. Structural composition of Typha grass obtained from Kware Lake before pretreatment

Contents	Sample A (%)	Sample B (%)
Holocellulose	41.00	40.00
Hemicelluloses	28.70	30.00
α cellulose	12.30	10.00
Lignin	28.15	24.93

Key: Sample A: Upper part of Typha grass that Grows above water level; Sample B: Lower part of Typha grass that grow inside water

3.2 Structural Composition of Typha Grass Obtained from Kware Lake after Pretreatment

Pretreatment of Typha grass with 0.2M H_2SO_4 produced lowest lignin content of 16.28% cellulose content of 15.5% and hemicelluloses content of 7.0%. Pretreatment with 0.2M NaOH produced lowest lignin content of 14.94% cellulose content of 13.8% and hemicelluloses content of 7.67%. Typha grass sample pretreated with liquid hot water has lowest lignin content of 14.29% cellulose content of 12.2% and hemicelluloses content of 7.8%. The result indicates that 0.2M H_2SO_4 remove more hemicelluloses content than the other pretreatment process (Fig. 1). This is in agreement with the result of many other researches such as [4] and [13]. Mosier et al. [14] also reported that hemicelluloses are removed when dilute H_2SO_4 is added and this enhances digestibility of cellulose in the residual solid. Also, dilute acid pretreatment was not good for lignin removal when compared with dilute alkaline and liquid hot water pretreatment. Chang et al. [15] reported that Alkaline pretreatment removes amorphous substances such as lignin, which increases the crystallinity index of lignocellulosic materials.

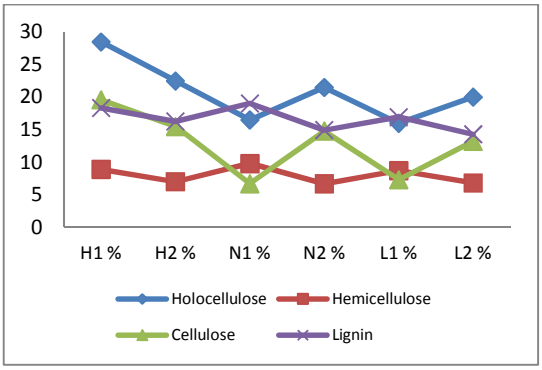

Fig. 1. Structural composition of Typha grass after pretreatment with 0.2M H_2SO_4, 0.2M NaOH, and liquid hot water

Key: H1: Upper part of Typha grass pretreated with 0.2M H_2SO_4; H2: Lower part of Typha grass pretreated with 0.2M H_2SO_4; N1: Upper part of Typha grass pretreated with 0.2M NaOH; N2: Lower part of Typha grass pretreated with 0.2M NaOH; L1: Upper part of Typha grass pretreated with liquid hot water; L2: Lower part of Typha grass pretreated with liquid hot water

3.3 Reducing Sugar Concentration of Typha Grass

Pretreatment with 0.2M H_2SO_4, 0.2M NaOH and liquid hot water produces highest reducing sugar concentration of 0.58% at day 7 when liquid hot water was used to pretreat 2 g of the sample (Fig. 2). Chemicals used in this research produce small quantity of reducing sugar. This agrees with the work of Arumugan and Manikandan, [8] who said significant sugar production was not recorded from pretreatment with dilute chemicals, but the current studies disagree with their findings that said more reducing sugar from dilute acid pretreatment was produce than the liquid hot water pretreatment. Also, the production of low reducing sugar in the present study from dilute chemicals can be attributed to the washing of the chemical after pretreatment in order to neutralize their pH. It can also be as result of the solubilization of the carbohydrate by the chemical during pretreatment [13]. According to Ogier et al. [16] and Laser et al. [17], liquid hot water pretreatment can be a promising pretreatment method that presented elevated recovery rates of sugars which does not generates inhibitors.

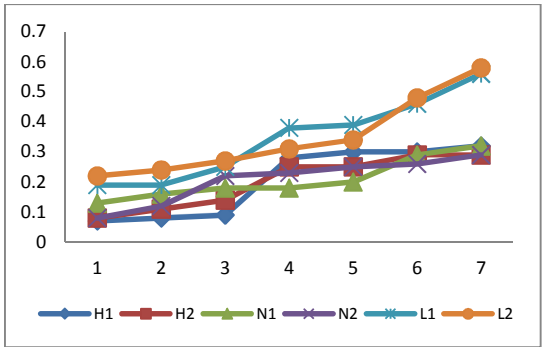

Fig. 2. Results of reducing sugar produce after pretreatment of 2g of Typha grass with liquid hot water pretreatment, 0.2M H_2SO_4 and 0.2M NaOH

Key:
H1: Upper part of Typha grass pretreated with 0.2M H_2SO_4
H2: Lower part of Typha grass pretreated with 0.2M H_2SO_4
N1: Upper part of Typha grass pretreated with 0.2M NaOH
N2: Lower part of Typha grass pretreated with 0.2M NaOH
L1: Upper part of Typha grass pretreated with liquid hot water
L2: Lower part of Typha grass pretreated with liquid hot water

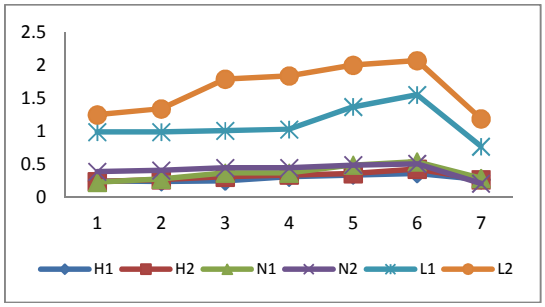

Fig. 3. Percentage concentration of bioethanol produce from the sample after pretreatment with liquid hot water, 0.2M H_2SO_4 and 0.2M NaOH

Key:
H1: Upper part of Typha grass pretreated with 0.2M H_2SO_4
H2: Lower part of Typha grass pretreated with 0.2M H_2SO_4
N1: Upper part of Typha grass pretreated with 0.2M NaOH
N2: Lower part of Typha grass pretreated with 0.2M NaOH
L1: Upper part of Typha grass pretreated with liquid hot water
L2: Lower part of Typha grass pretreated with liquid hot water

3.4 Bioethanol Concentration of Typha Grass

The highest bioethanol concentration of 2.07% was produced from the sample that was pretreated with liquid hot water and sample pretreated with dilute NaOH and H_2SO_4 produces highest bioethanol concentration of 0.54% and 0.43% respectively (Fig. 3). The result of ethanol yield from dilute NaOH and H_2SO_4 pretreated sample is almost the same with 0.5% reported by Fish et al. [18]. Also, Grous et al. [19] reported that 90% efficiency of enzymatic hydrolysis was achieved in 24 h for poplar chips pretreated by liquid hot water, compared to only 15% hydrolysis of untreated chips.

4. CONCLUSION

From the result of this research, it has been find out that pretreatment using dilute acid and alkaline were very effective in removing lignin and hemicelluloses from typha grass. Pretreatment with dilute acid removed hemicelluloses by 77% which proved to be better than dilute alkaline which removed hemicelluloses by 75%. Dilute alkaline removed more lignin in typha grass by 60% than dilute acid pretreatment which removed lignin by 35%. However, cellulose content of sample pretreated with dilute H_2SO_4 increased by 59% which is higher than 50% increase by sample pretreated with dilute NaOH. Also, sample pretreated with dilute NaOH produce highest bioethanol concentration of 0.54% than H_2SO_4 which produce 0.43%. Determination of bioethanol by HPLC conclude that bioethanol can be produced from Typha grass and pretreatment of the sample can increase accessibility to cellulose and increase reducing sugar production thereby increasing bioethanol production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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