

Research Article

Utilization of *Typha* grass (*Typha australis*) for bioethanol productionA. Bala*¹, A. A. Farouq¹, A. D. Ibrahim¹, C. Muhammad² and A. M. Gada¹¹Department of Microbiology, Faculty of Science, Sokoto State University, Sokoto, Nigeria²Department of Chemistry, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Nigeria

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Abstract: The ability to utilize *Typha* grass as a potential substrate for bioethanol production was analyzed in this research, the sample of *Typha* grass collected from Kware Lake was pretreated by autoclaving after it was initially dried and grounded into powdered form. The pretreated sample was enzymatically hydrolyzed using *Aspergillus niger* for 7 days. The hydrolyzate was used to produce bioethanol by fermentation using *Saccharomyces cerevisiae* which was determined by HPLC analysis. The results indicated that pretreated *Typha* grass sample that was having higher lignin and hemicellulose composition of 28.15 % and 30.0 % was reduced to 14.29 % and 7.8 % respectively. Also, the α -cellulose composition was increased to a highest composition of 23.2 % from a lowest composition of 10.0 %. The pretreated sample that was hydrolyzed produced a highest reducing sugar yield of 19.14 g/L and the fermented hydrolyzate produced a bioethanol concentration of 2.07 % at day 6 of the fermentation period. From the results of this research, it is concluded that bioethanol can be produced from *Typha* grass and pretreatment can lessen crystallinity and expose cellulose thereby escalating reducing sugar yield for maximum bioethanol production.

Keywords: *Typha* grass, Kware lake, *Aspergillus niger*, HPLC.

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INTRODUCTION

Typha grass is an aquatic or semi-aquatic, rhizomatous, herbaceous plants (Stance, 2010). The leaves are smooth, linear and mostly basal on an easy, joint-less stem that bear the flowering spike. *Typha* are habitually among the initial wetland plants to colonize areas of newly uncovered wet mud, with their plentiful wind-dispersed seeds. Buried seeds can stay alive in the soil for long periods of time. They germinate excellently with sunlight and fluctuating temperatures, which is typical of many wetland plants that revive on mud flats. The plants also extend by rhizomes, forming large, unified stands (Shipley *et al.*, 1989).

Although *Typha* are inhabitant wetland plants, they can be violent in their competition with other inhabitant species. They have been wrapping many ponds in several regions of North America preventing farming activities, from the grand Lakes to the Everglades (Keddy, 2010). Native sedges are displaced and wet meadows minimize, likely as a response to distorted hydrology of the wetlands and improved nutrient levels. An introduced hybrid species could also be contributing to the problem (Boers *et al.*, 2007). The most successful approach appears to be mowing or burning to take away the aerenchymous stalks, followed by prolonged flooding. It may be more important to avoid invasion by preserving water level

fluctuations, including periods of drought, and to uphold infertile environment (Boers *et al.*, 2007).

Under the right condition, *Typha* grass can grow and extend vigorously at the edge of a pond, river or lake. Due to this, many pond owners see cattail with uncertainty because of their inclination to grow thick, nearly impassable stands, blocking the view of open water and raising distress that they will take over and cover the pond (Zhang *et al.*, 2012). This reduced the value, conservation and sustainability of the plant. This research is aimed at utilizing *Typha* grass for bioethanol production thereby adding more value to the grass, increase conservation and sustainability of the plant, contribute towards alternative energy supply and also create profit and jobs opportunity. The objective of this research is to breakdown lignin, hemicelluloses and cellulose from *Typha* grass to produce bioethanol.

METHODOLOGY

Collection and Preparation of Samples

Typha grass sample was collected from Kware Lake in Kware local government area, Sokoto State, Nigeria. The sample was washed with tap water and then cut into two, separating the upper part that grow on top of the water level and the lower part that grow inside water. The two portions were dried out separately for 14 days. The dried samples were then grind into

powder and stored at room temperature for further analysis.

Isolation and Identification of *Aspergillus niger* and *Saccharomyces cereviceae*

Aspergillus niger was isolated from mud collected from kware lake and identified base on microscopic (morphological) and macroscopic characters (colour, texture, appearance and diameter of colonies) according to Sourza-motta *et al.*, (2003). The soil was sequentially diluted; a sample suspension was formed by adding 1.0g of sample to 10ml of distilled water and mixed well for 10 minutes. The suspension was diluted sequentially into 10^{-1} , 10^{-2} and 10^{-3} . 1ml (from the third dilution factor) was carefully measured using a syringe and inoculated into a Sabraud Dextrose Agar (SDA) plate and incubated at 37 °C for five days. The first white color of the colonies that later turns black at the top, with pale yellow color at the bottom prove the organism to be *Aspergillus niger*.

Saccharomyces cereviceae was isolated from palm wine sample collected from Giginya Barrack market, Sokoto and identified by the typical morphological and physiological test and identification keys described by Barnett *et al.*, (1990). The palm wine sample was sequentially diluted; a sample suspension was formed by adding 1.0 ml of sample to 10ml of distilled water and mixed. The suspension was diluted sequentially into 10^{-1} , 10^{-2} and 10^{-3} . 1 ml (from the third dilution factor) was carefully measured using a syringe and inoculated into a Sabraud Dextrose Agar (SDA) plate and incubated at 28 °C for five days. Capability of the organism to hydrolyze starch and form bud under microscope was used to prove the organism as *Sacchromyces cerevisiae*.

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 mixture of hemicellulose and cellulose, was determined in order to find the total quantity 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 resources 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.

Pretreatment of the Sample

The grounded powdered of Typha grass was slurried with distilled water using a solid to liquid proportion of 10% (w/w) and autoclaved at 121 °C for 15 min. After autoclaving, the sample was filtered and the solid residue was air dried and stored for further analysis (Arumugam and Manikandan, 2011).

Hydrolysis of Pretreated Typha Grass

Hydrolysis of Typha grass for reducing sugar generation was carried out using *Aspergillus niger* isolated from soil sediment as described by Gupta (2006). 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 utilizable sugars during fermentation. The samples were then filtered using Whatman filter paper No. 1 and the filtrates were used for fermentation.

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 121 °C 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 (Mojovic, *et al.*, 2006). 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 amount using UV-visible spectrophotometer.

Distillation

The fermented broth was filtered using whatman filter paper no. 1. Each sample was measured into Microkjeldahl flasks and then heated at 78 °C on the Microkjeldahl apparatus until the solution turned colourless. The presence of bioethanol was determined using high performance liquid chromatography (HPLC).

RESULTS AND DISCUSSIONS

Structural composition of Typha grass before pretreatment (table 1) indicates that both the upper and lower part of Typha grass has high hemicelluloses composition of 28.70% and 30.00% and Lignin composition of 28.15% and 24.93% respectively. α -cellulose has the least composition with 12.30% and 10.00% from the upper and lower part of Typha respectively. Lignin is the integral part of the resulting cell wall of plants. They are the second most abundant organic polymers that require a defined composition because of their heterogeneity and help to strengthen xylems in plants. The branches in lignin make it hard to measure its level of polymerization (Keweiet *al.*, 2012). The result is in conformity with the report of Bajpai (2016) who reported that hemicelluloses compose between 35-50% and lignins 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: Structural Composition of Typha Grass 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

Table 2 shows the result of structural composition of *Typha* grass after pretreatment. The result shows that lignin and hemicellulose content of the upper part of *Typha* has decrease to 14.29% and 7.8% while cellulose content has increased to 23.2% The result indicates that 0.2M H_2SO_4 remove more hemicelluloses content than other pretreatment process. This is in agreement with the result of many other researches such as (Ahmadu *et al.*, 2017, Zhang *et al.*,

2012). Mosier *et al.*, (2005) also reported that hemicelluloses are removed when dilute H_2SO_4 is added and this enhances digestibility of cellulose in an outstanding solid. Also, dilute acid pretreatment was not good for lignin removal when compare with dilute alkaline and liquid hot water pretreatment. Chang *et al.*, (2000) reported that Alkaline pretreatment removes amorphous substance lignin, which increase the crystallinity index of lignocellulosic resources.

Table 2: Structural Composition of Typha Grass after Pretreatment

Contents	Sample A	Sample B
Holocellulose	26	31
Hemicellulose	8.7	7.8
Cellulose	17.3	23.2
Lignin	16.94	14.29

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

Pretreatment with 0.2M H_2SO_4 , 0.2M NaOH and liquid hot water produces highest reducing sugar yield of 19.14 g/L at day 7 when liquid hot water was used to pretreat 2g of the sample (Table 3). Chemicals used in this research produce small quantity of reducing sugar. This agrees with the work of Arumugan and Manikandan (2011) 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 (Ahmadu *et al.*, 2017). According to Ogier *et al.*, (1999) and Laser *et al.*, (2002), liquid hot water pretreatment can be a promising pretreatment method that present eminent recovery rates of sugars which does not generates inhibitors.

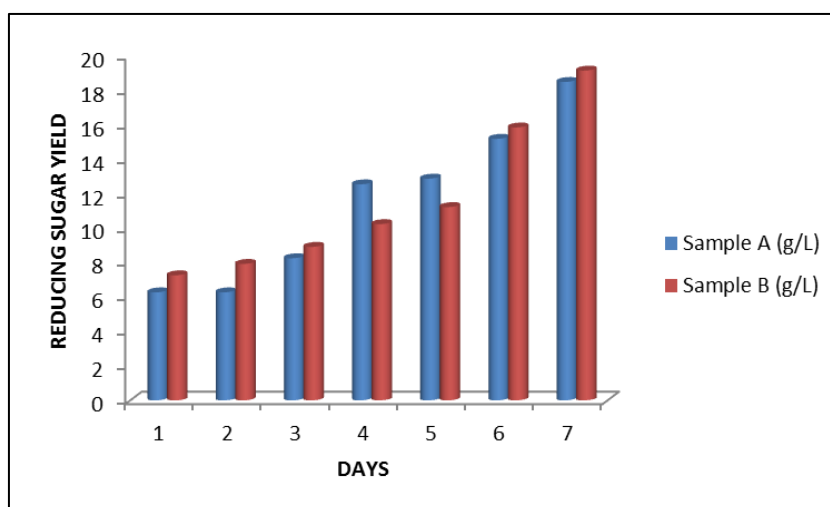


Figure 1: Reducing Sugar yield of Typha grass

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

A 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 H₂SO₄ produces highest bioethanol concentration of 0.54% and 0.43% respectively (Table 4). The result of ethanol yield from chemically pretreated sample is almost the same with 0.5% reported by Fish *et al.*, (2009). Also, Grous *et al.*, (1986) 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. Elimination of

hemicelluloses from the microfibrils is believed to expose the cellulose surface and increase enzyme accessibility to the cellulose microfibrils. Lignin is removed only to a narrow extent during the pretreatment but is redistributed on the fiber surfaces as a result of melting and depolymerization reactions (Li *et al.*, 2007). The removal and redistribution of hemicellulose and lignin increase the amount of the pretreated sample. Rapid flashing of a substance to atmospheric pressure and turbulent flow can cause fragmentation of the substance, thereby increasing the accessible surface area (Duff and Murray, 1996).

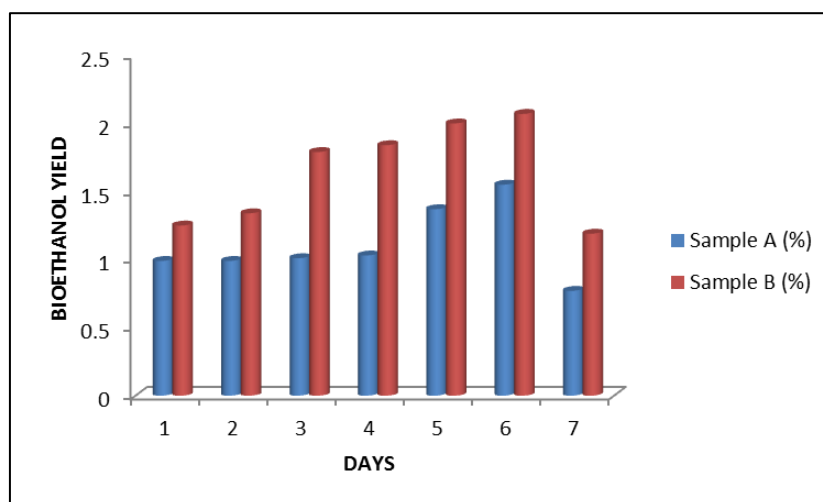


Figure 2: Percentage Concentration of Bioethanol Produced

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

CONCLUSION

The result of this research show that *Typha* grass can be used as a potential substrate for bioethanol production. The pretreatment of the sample of *Typha* has reduced lignin composition of the grass by 11.21% and 10.64% in both the upper and lower part of the grass respectively. Also the cellulose composition of the grass was increased by 5% and 13.2% in the upper and lower part of the grass respectively. This has contributed to the maximum reducing yield of 18.48 g/L and 19.14 g/L. both the upper and lower part of the grass produced a maximum bioethanol concentration of 2.07% and 1.55% at day 6 of fermentation by *Saccromyces cereviceae*. It is concluded in this research that bioethanol can be produce from *Typha* grass and pretreatment of the sample can reduce lignin content thereby increasing cellulose composition and maximum reducing sugar yield for ethanol production.

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