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3 **Effect of Dilute Acid and Alkaline Pretreatment of *Typha australis* (Typha grass) for**
4 **Bioethanol Production**

5
6 **ABSTRACT**

7 *Typha australis* (Typha grass) obtained from Kware Lake was used in this research to produce
8 bioethanol. Different pretreatment methods including dilute acid (0.2M H₂SO₄), dilute alkaline
9 (0.2M NaOH) and liquid hot water pretreatments were used to pretreat the Typha grass sample
10 before enzymatic saccharification for 7 days using *Aspergillus niger* and the hydrolysate was
11 seeded with *Sacchromyces cereviceae* to produce bioethanol. HPLC was used to determine
12 bioethanol product. The result showed that pretreatment with 0.2M H₂SO₄ removed more
13 hemicelluloses (7.0%) when compare with other pretreatment methods used, but pretreatment
14 with 0.2M NaOH and liquid hot water remove more lignin (14.29%) than dilute acid
15 pretreatment. The highest percentage reducing sugar concentration of 0.58% was obtained from
16 lower part of the sample pretreated with liquid hot water while Typha grass pretreated with 0.2M
17 H₂SO₄ and 0.2M NaOH produced the highest percentage reducing sugar concentration of 0.32%
18 each from the upper part of the sample. Also, the highest Bioethanol concentration of 2.07% was
19 obtained at day 6 of fermentation from the Typha grass pretreated with liquid hot water while
20 Typha grass pretreated with 0.2M H₂SO₄ and 0.2M NaOH produced highest Bioethanol
21 concentration of 0.43% and 0.54% respectively. The results indicate that Typha grass can be
22 harnessed for bioethanol production thereby reducing their negative impact on Lakes.

23 **Keywords:** Typha grass, Pretreatment, Bioethanol, HPLC,

24 **Introduction**

25 The search for renewable biomass sources that do not serve as human food and animal fodder
26 have focused primarily on plant biomass that possesses mainly cellulose and lignocellulosic
27 materials [1]. Many agricultural waste in the form of plant materials such as grass, wood, crop
28 residues and some plants such as water hyacinth [2], offers the possibility of a renewable energy

29 and relatively greenhouse-gas favoring source of sugars that can be converted to ethanol. The
30 agricultural wastes are abundant and have disposal problems. An alternative is to utilize these
31 agricultural wastes so that the competition between food and fuel can be minimized [3]. *Typha*
32 grass has been identified as a particularly suitable biomass crop for wetland, because of their
33 superiority, productivity, pest resistance, adaptability and chemical composition [4].

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

41 **Methodology**

42 **Collection and Preparation of Samples**

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

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

49 *Aspergillus niger* was isolated from soil sediment collected from kware lake and identified base
50 on microscopic (morphological) and macroscopic characteristics (colour, texture appearance and
51 diameter of colonies) according to Sourza-motta *et al.* [5] and *Saccharomyces cereviceae* was
52 isolated from palm wine sample collected from Giginya Barrack market, Sokoto and identified
53 by the standard morphological and physiological test and identification keys described by
54 Barnett *et al.* [6].

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58 **Determination of Structural Composition of Typha Grass**

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

66 **Dilute Acid Pretreatment**

67 Approximately 2 g of *Typha* grass sample was mixed with 0.2M H₂SO₄ solution in a 250 ml
68 flask with a stopple and then allowed to suck for 24hrs. The mixture was subsequently autoclave
69 at 121 °C for 15mins and then cooled and filtered through a Whatman filter paper to separate the
70 solid residue. The residue was washed with distilled water until neutral pH. The sample was air
71 dried and stored in tightly sealed plastic bag in a refrigerator for further use [7].

72 **Dilute Alkaline Pretreatment**

73 Approximately 2 g of *Typha* grass sample was soaked in 0.2M NaOH solution in a 250 mL flask
74 for 24hrs and then autoclave at 121 °C, for 15min. The solid residue was separated from the
75 mixture by filtration with Whatman filter paper and thoroughly washed with distilled water to
76 neutralize its pH. Finally, the filtrate was dried and stored as above [7].

77 **Liquid Hot Water Pretreatment (LHW)**

78 In liquid hot water pretreatment of the *Typha* grass sample, the grounded powdered of the plant
79 was slurried with distilled water using a solid to liquid ratio of 10% (w/w) and autoclaved at 121
80 °C for 15 min. After autoclaving, the sample was filtered and the solid residue was air dried and
81 stored for further use [8].

82 **Enzymatic Saccharification of Pretreated Typha Grass**

83 The enzymatic saccharification of *Typha* grass was carried out using *Aspergillus niger* isolated
84 from soil sediment as described by Gupta [9]. In this method, the pretreated *Typha* grass samples
85 were inoculated with 0.5 ml suspension of 96 hours culture of *Aspergillus niger*. Hydrolysis was
86 carried out at room temperature for 7 days. Samples were taken daily for reducing sugar
87 determination using 1,4-dinitro salicylic acid (DNS) method to find out the net yield of

88 fermentable sugars. The samples were then filtered using Whatman filter paper No. 1 and the
89 filtrates were used for fermentation.

90 **Fermentation of the Hydrolysate and Bioethanol Production**

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

97 **Distillation**

98 The fermented broth was filtered using whatman filter paper. Each sample was weighed into
99 Microkjeldahl flasks and then heated at 78 °C on the Microkjeldahl apparatus until the solution
100 turned colourless. The presence of bioethanol was determined using high performance liquid
101 chromatography (HPLC).

102 **Results and Discussion**

103 **Structural Composition of Typha Grass Obtained from Kware Lake Before Pretreatment**

104 The structural composition of both the upper and lower part of Typha grass before pretreatment
105 show that, hemicelluloses has a highest composition of 28.70% and 30.00% while Lignin has a
106 composition of 28.15% and 24.93% respectively, and α cellulose has least composition of
107 12.30% and 10.00% respectively in the Typha grass collected from Kware Lake. The result of
108 structural composition of hemicelluloses and lignin is in conformity with the report of Bajpai
109 [11] who reported that hemicelluloses compose between 35-50% and lignin compose 10-30% of
110 grasses. However, this current study disagrees with his report that α -celluloses compose of 25-
111 40%. This research shows that the upper part of Typha grass has higher structural composition
112 than the lower part of the plant (Table 1).

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116 **Table 1:** Structural Composition of Typha Grass Obtained from Kware Lake Before
 117 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

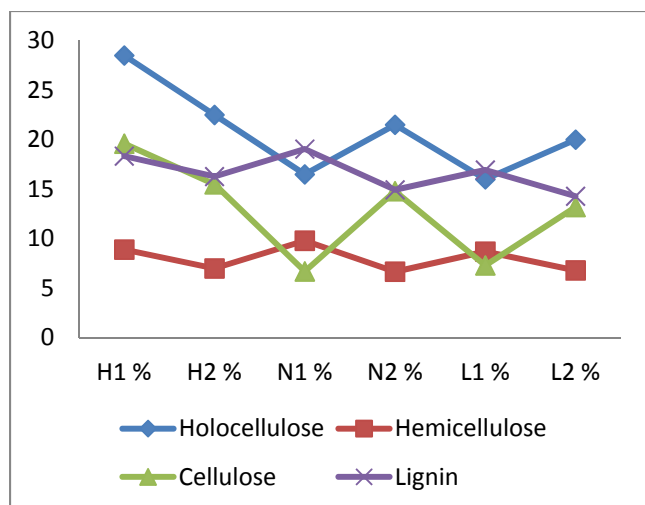
Key: Sample A: Upper part of Typha grass that Grows above water level

Sample B: Lower part of Typha grass that grow inside water

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119 **Structural Composition of Typha grass Obtained from Kware Lake after Pretreatment**

120 Pretreatment of Typha grass with 0.2M H₂SO₄ produced lowest lignin content of 16.28%
 121 cellulose content of 15.5% and hemicelluloses content of 7.0%. Pretreatment with 0.2M NaOH
 122 produced lowest lignin content of 14.94% cellulose content of 13.8% and hemicelluloses content
 123 of 7.67%. Typha grass sample pretreated with liquid hot water has lowest lignin content of
 124 14.29% cellulose content of 12.2% and hemicelluloses content of 7.8%. The result indicates that
 125 0.2M H₂SO₄ remove more hemicelluloses content than the other pretreatment process (figure 1).
 126 This is in agreement with the result of many other researches such as [4] and [13]. Mosier *et al.*,
 127 [12] also reported that hemicelluloses are removed when dilute H₂SO₄ is added and this enhances
 128 digestibility of cellulose in the residual solid. Also, dilute acid pretreatment was not good for
 129 lignin removal when compare with dilute alkaline and liquid hot water pretreatment. Chang *et*
 130 *al.*, [14] reported that Alkaline pretreatment removes amorphous substances lignin, which
 131 increases the crystallinity index of lignocellulosic materials.



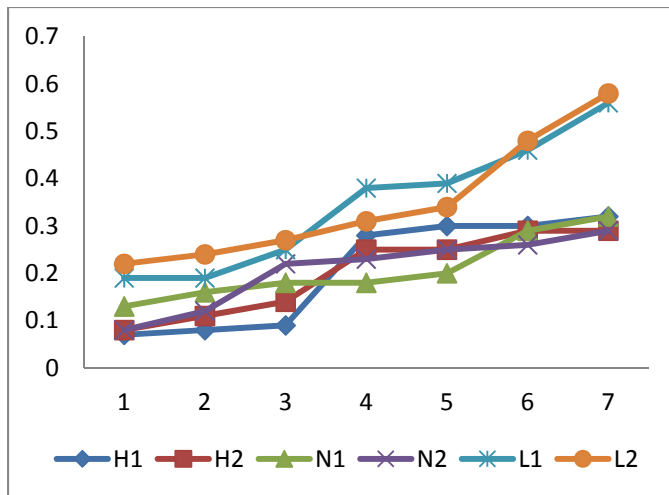
Key: H1: Upper part of Typha grass pretreated with 0.2M H₂SO₄
H2: Lower part of Typha grass pretreated with 0.2M H₂SO₄
N1: Upper part of Typha grass pretreated with 0.2M NaOH
N2: Lower part of Typha grass pretreated with 0.2M NaOH
L1: Upper part of Typha grass pretreated with liquid hot water
L2: Lower part of Typha grass pretreated with liquid hot water

132

133 Figure 1: Structural Composition of Typha Grass after Pretreatment with 0.2M H₂SO₄, 0.2M
134 NaOH, and Liquid Hot Water

135 **Reducing Sugar Concentration of Typha Grass**

136 Pretreatment with 0.2M H₂SO₄, 0.2M NaOH and liquid hot water produces highest reducing
137 sugar concentration of 0.58% at day 7 when liquid hot water was used to pretreat 2g of the
138 sample (figure 2). Chemicals used in this research produce small quantity of reducing sugar. This
139 agrees with the work of Arumugan and Manikandan, [8] who said significant sugar production
140 was not recorded from pretreatment with dilute chemicals, but the current studies disagree with
141 their findings that said more reducing sugar from dilute acid pretreatment was produce than the
142 liquid hot water pretreatment. Also, the production of low reducing sugar in the present study
143 from dilute chemicals can be attributed to the washing of the chemical after pretreatment in order
144 to neutralize their pH. It can also be as result of the solubilization of the carbohydrate by the
145 chemical during pretreatment [13]. According to Ogier *et al.*, [15] and Laser *et al.*, [16], liquid
146 hot water pretreatment can be a promising pretreatment method that present elevated recovery
147 rates of sugars which does not generates inhibitors.



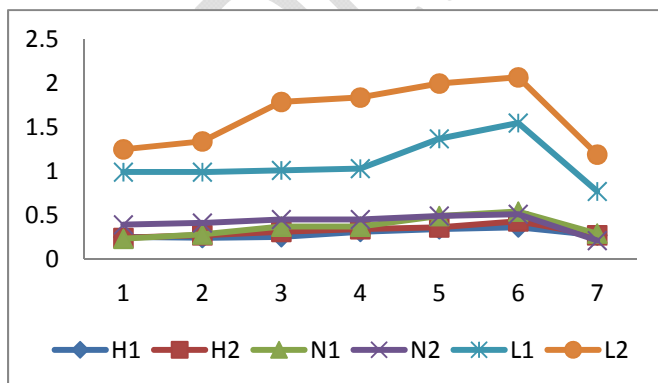
Key: H1: Upper part of Typha grass pretreated with 0.2M H₂SO₄
H2: Lower part of Typha grass pretreated with 0.2M H₂SO₄
N1: Upper part of Typha grass pretreated with 0.2M NaOH
N2: Lower part of Typha grass pretreated with 0.2M NaOH
L1: Upper part of Typha grass pretreated with liquid hot water
L2: Lower part of Typha grass pretreated with liquid hot water

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149 Figure 2: Results of Reducing Sugar Produce after Pretreatment of 2g of Typha Grass with
150 Liquid Hot Water Pretreatment, 0.2M H₂SO₄ and 0.2M NaOH

151 **Bioethanol concentration of Typha grass**

152 A highest bioethanol concentration of 2.07% was produced from the sample that was pretreated
153 with liquid hot water and sample pretreated with dilute NaOH and H₂SO₄ produces highest
154 bioethanol concentration of 0.54% and 0.43% respectively (figure 3). The result of ethanol yield
155 from dilute NaOH and H₂SO₄ pretreated sample is almost the same with 0.5% reported by Fish
156 *et al.*, [17]. Also, Grous *et al.*, [18] reported that 90% efficiency of enzymatic hydrolysis was
157 achieved in 24 h for poplar chips pretreated by liquid hot water, compared to only 15%
158 hydrolysis of untreated chips.



Key: H1: Upper part of Typha grass pretreated with 0.2M H₂SO₄
H2: Lower part of Typha grass pretreated with 0.2M H₂SO₄
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

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160 Figure 3: Percentage Concentration of Bioethanol Produce from the Sample after Pretreatment
161 with Liquid Hot Water, 0.2M H₂SO₄ and 0.2M NaOH

162 **Conclusion**

163 From the result of this research, it has been find out that pretreatment using dilute acid and
164 alkaline were very effective in removing lignin and hemicelluloses from typha grass.
165 Pretreatment with dilute acid removed hemicelluloses by 77% which proved to be better than
166 dilute alkaline which removed hemicelluloses by 75%. Dilute alkaline removed more lignin in
167 typha grass by 60% than dilute acid pretreatment which removed lignin by 35%. However,
168 cellulose content of sample pretreated with dilute H₂SO₄ increased by 59% which is higher than
169 50% increase by sample pretreated with dilute NaOH. Also, sample pretreated with dilute NaOH
170 produce highest bioethanol concentration of 0.54% than H₂SO₄ which produce 0.43%.
171 Determination of bioethanol by HPLC conclude that bioethanol can be produced from Typha
172 grass and pretreatment of the sample can increase accessibility to cellulose and increase reducing
173 sugar production thereby increasing bioethanol production.

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