PRODUCTION OF FERMENTABLE SUGARS FROM WATERHYACINTH BY HIGH-TEMPERATURE DILUTE-ACID HYDROLYSIS METHOD

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Abstract

A study on acid-catalyzed hydrolysis of Waterhyacinth (WH) biomass for production of monomeric sugars has been done. Waterhyacinth (Eichhornia Crassipes) plants were obtained from Lake Victoria. A yield of 18.5% w/w fermentable sugars was achieved during hydrolysis of oven-dried Waterhyacinth (ODWH) biomass at 220°C and 1.5-% w/w H_2SO_4 acid. The waterhyacinth (WH) hydrolysates obtained from a high-temperature dilute-acid hydrolysis were rich in six-carbon (C_6) sugars (glucose) which could be fermented to ethanol. The production of fermentable sugars from waterhyacinth biomass is a promising new opportunity for its economic value which can in turn accelerate its rate of harvest and utilization.

INTRODUCTION

Waterhyacinth, *Eichhornia crassipes* a free-floating freshwater angiosperm, is the most investigated species of the family *Pontederiaceae*[1-3]. Waterhyacinth has been declared a nuisance and a weed ranking first among the aquatic plants. It has created innumerable problems to man including serious social, economic and environmental problems. It has interfered with the use of water by causing direct obstruction to navigation, by checking water-flow in irrigation channels, and by degrading water quality for domestic use. It has been responsible for drastic changes in the plant and animal communities of the freshwater environments (particularly fish kills), and it has served as an agent for dispersal of several deadly diseases.

A number of alternative methods have been applied in order to control the plant[1]. Mechanical harvesting of the plant was considered in the beginning as an alternative control strategy. Recently, more attractive uses of Waterhyacinth have been found which try to confer upon the plant the status of a resource and hence changing the weed management in concept from "the management for control" to "the management of a resource". However, utilization of Waterhyacinth such as; making compost, a livestock feed supplement, upgrading

wastewater treatment plants, recovery of metals, etc. do not match with the plant's growth. Ecological control of Waterhyacinth growth and its utilization as source of thermal energy and for production of ethanol, have been suggested for sustainable management of the plant growth. Production of ethanol from Waterhyacinth biomass is thought to be one of the approaches that can enhance the economic value of the plant so that it can attract more harvesters. Uses of ethanol for example; as raw material, solvent and liquors would help to recover part of the money currently being injected in various programmes concerning Waterhyacinth infestation on Lake Victoria.

Conversion of biomass into fuels and chemicals requires hydrolysis of the polysaccharide fraction into monomeric sugars. Hydrolysis of biomass can be accomplished by several techniques including the use of mineral acids or enzymes. Although enzymatic hydrolysis of biomass has been found to be inexpensive, acid-catalyzed hydrolysis is faster than enzymatic[4]. Hydrolysis of WH biomass using low-temperature concentrated-acid is reported in Masende et al. (1999)[5]. Hydrolysis of biomass at high temperature and pressure has been reported to minimize acid usage[6-7]. The present work is concerned with the hydrolysis of Waterhyacinth biomass using dilute sulphuric acid at high temperatures and
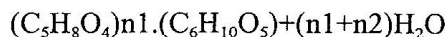
elevated pressure for production of fermentable sugars.
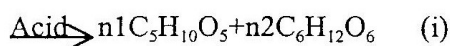
BACKGROUND LITERATURE

Several studies on biomass conversion and cellulose hydrolysis using both concentrated-acid low-temperature and dilute-acid high-temperature hydrolysis has been reported[6,9,10]. The hydrolysis of crystalline cellulose in concentrated acids have been reported to give high sugar yields as compared to dilute-acid hydrolysis. However, a common feature of concentrated acid hydrolysis processes is that a relatively dry substrate is required, otherwise the acid becomes diluted below its initial desired level [11-13]. Also, since the cost of raw material is very sensitive to the acid usage, essentially any loses of acid comparable to the acid usage for dilute-acid process will make the concentrated acid process more expensive than dilute process. In concentrated-acid process both the hydrolyser and acid recovery system must be of suitable corrosion resistant (and therefore expensive) material. The reactor volume per unit production for concentrated acid process is relatively large because the reaction time is of the order of several hours. Generally, the concentrated acid processes suffer from the expenses associated with handling and replacing or recovering of large quantities of acid[11]. Dilute acid processes have relatively low yields (55 -75%) because the rate of sugar degradation is rapid at the high temperature necessary for the hydrolysis of crystalline cellulose. The low acid cost and potential for utilizing simple, lower cost hardware make the dilute sulphuric acid approach an attractive alternative for biomass hydrolysis study.

An adverse situation is also encountered in acid hydrolysis of cellulosic biomass insofar as hemicellulose is concerned. During an acid hydrolysis process aiming at glucose as the main product, hemicellulose is hydrolysed more rapidly than cellulose since the structure of hemicellulose is more open to attack than cellulose. Consequently, when the reaction is carried out under the conditions that favour cellulose hydrolysis, most of the xylose is decomposed to furfural, and very litt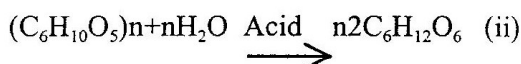le of it is actually recovered[4,7]. The reactions to reduce cellulose and hemicellulose to hexoses and pentoses are given below:

$$(C_5H_8O_4)n1.(C_6H_{10}O_5)+(n1+n2)H_2O$$

Hemicellulose

$$\xrightarrow{Acid} n1C_5H_{10}O_5+n2C_6H_{12}O_6 \quad (i)$$

Pentose   Hexose

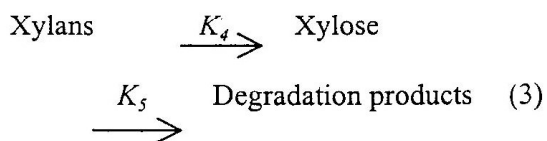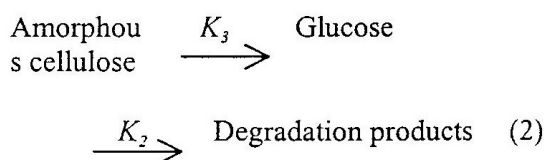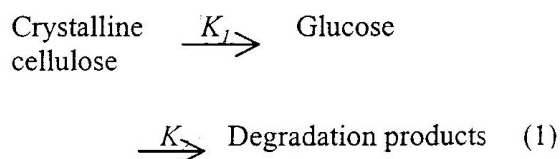$$(C_6H_{10}O_5)n+nH_2O \xrightarrow{Acid} n2C_6H_{12}O_6 \quad (ii)$$

Cellulose   Hexose

Although the hydrolysis reaction is heterogeneous, it could be modelled as a first-order homogeneous reaction where cellulose concentration is expressed as a fraction of the potential glucose. After the glucose is formed, it is then degraded to hydroxymethylfurfural (HMF) in a first-order homogeneous reaction. The hydrolysis of hemicellulose xylans to xylose and furfural can be expressed in a similar manner. The following expressions describe the hydrolysis and degradation reactions:

$$\text{Crystalline cellulose} \xrightarrow{K_1} \text{Glucose}$$

$$\xrightarrow{K_2} \text{Degradation products} \quad (1)$$

$$\text{Amorphous cellulose} \xrightarrow{K_3} \text{Glucose}$$

$$\xrightarrow{K_2} \text{Degradation products} \quad (2)$$

$$\text{Xylans} \xrightarrow{K_4} \text{Xylose}$$

$$\xrightarrow{K_5} \text{Degradation products} \quad (3)$$

Where $K_1 - K_5$ are rate constants.

Since the objective in a hydrolysis process is to maximize the yield of the glucose, the yield of both glucose and xylose depends on the relative rates of reaction or selectivity between the first and second reaction. For glucose, the yield depends on relative magnitude of K_1 (the cellulose decomposition rate) and K_2 (the glucose decomposition rate). Although both K_1

and K_2 increase with increasing temperature and acid concentration, because of the activation energy for the first reaction being greater than that for the second, K_1 increases more quickly than K_2, and therefore the yield of glucose increases with temperature[12]. The fact that the hemicellulose is considerably easier to hydrolyse to monomeric sugars than is the cellulose provides a possibility that the hemicellulosic fraction can be selectively hydrolysed from biomass.

The problem of sugar degradation inherent in dilute acid processes has been dealt with by several researchers[6,10,11]. Different reactor systems have been designed for the purpose of minimizing both the degradation of sugars and residence times. Such systems include; batch, plug flow, percolation, or countercurrent reactor configurations. At high temperatures (approximately 200°C) and 1% H_2SO_4, the hydrolysis reactions are faster than the degradation reactions and yields of about 55% and above could be achieved in shorter reaction times[6,11].

While there are several studies of cellulose hydrolysis using dilute-acid high-temperature process, the use of Waterhyacinth biomass in production of fermentable sugar has not been reported. This paper reports on the production of ethanol using Waterhyacinth biomass from Lake Victoria. Waterhyacinth biomass, like other cellulosic materials, contains cellulose (> 20% w/w dry basis), hemicellulose (> 16.5%), and lignin (> 1%)[1]. The basic problem that has to be solved for an economic process is to overcome the difficulty of hydrolysing the crystalline cellulose and to minimize the decomposition of the sugars, which are also promoted in the acid media. In order to accomplish this task, a high-temperature dilute-acid hydrolysis process was used. Batch reactor configuration was employed in the hydrolysis study. The major parameters studied were temperature, acid concentration and hydrolysis time. The influence of particle size and liquid-to-solid ratios on the yield of sugars is also reported.

EXPERIMENTS

Materials and Chemicals

Waterhyacinth plants for this work were mainly collected from Lake Victoria in Mwanza, Tanzania. At Lake Victoria, the waterhyacinth plants were harvested at a distance of about 5m from the shore to avoid local contamination. The plants were then thoroughly cleaned at the site by water to minimize contaminants. The waterhyacinth plants were packed in plastic bags and stored in cold boxes for preservation and transportation to the laboratory. In the laboratory, the plants were thoroughly cleaned using de-ionised water and then stored at 4°C before use.

All chemicals used in this study were of grade reagent. Anhydrous D(+)-glucose , D(+)-xylose, D(+)-galactose, L(+)-arabinose, D(+)-mannose, D(-)-ribose, Ca(OH)$_2$, CaCO$_3$, NaOH, H$_2$SO$_4$ and chromatography grade CH$_3$CN were from Merck (Darmstadt, Germany). All water was filtered and deionized before use. The yeast strains of *Saccharomyces cervisiae*, namely bakers and brewers yeast were used to convert glucose into ethyl alcohol. The organism is a facultative with optimal temperature and pH for growth being 28.5°C and 4.1, respectively. The yeast strains were stored and maintained at -20°C.

Experimental Plan

Four experimental plans were used to study the acid hydrolysis of waterhyacinth (WH) biomass in a batch reactor system. In the first plan, the influence of temperature in the hydrolysis range was studied at a fixed acid concentration and liquid-to-solid ratio. In the second plan the acid concentration and the residence time were held constant to study the role of liquid-to-solid ratio with temperature variations. In the third plan the temperature and the liquid-to-solid ratio were kept constant in order to investigate the role of acid concentrations. Finally, the fourth experimental series is a factorial plan to investigate the influence of all three principal variables: temperature at three levels, acid concentration and residence time at two levels respectively. The liquid-to-solid ratio was fixed for all experimental points at 30:1 in the

reactor. The hydrolysis experiments were carried at a pressure range of 50–100 bars. Besides the above-described experimental plans, other repetitions were conducted to prove the reproducibility of the results. The data acquisition system allowed the evaluation of the uncertainties related to the measurement of the three basic variables used.

High-Temperature Dilute-Acid Hydrolysis

A schematic diagram for the reactor set-up is shown in Figure 1. The tubular reactors of 16.4-mL were essentially the empty HPLC columns placed inside a GC oven (CARLO

ERBA, Milano, Italy). The reactor had nuts and ferrules of stainless steel at both ends. The reactor was connected by a stainless steel tubing to the constant pressure/flow pump (a positive displacement piston type) (Varian 8500, USA). The GC oven was equipped with an on-off temperature controller. With this provision the reactor temperature was controlled to within 2°C of the set point. In order to prevent evaporation of reactant fluid, the reactor system was put under pressure not less than 50 bars by using a constant-pressure pump.
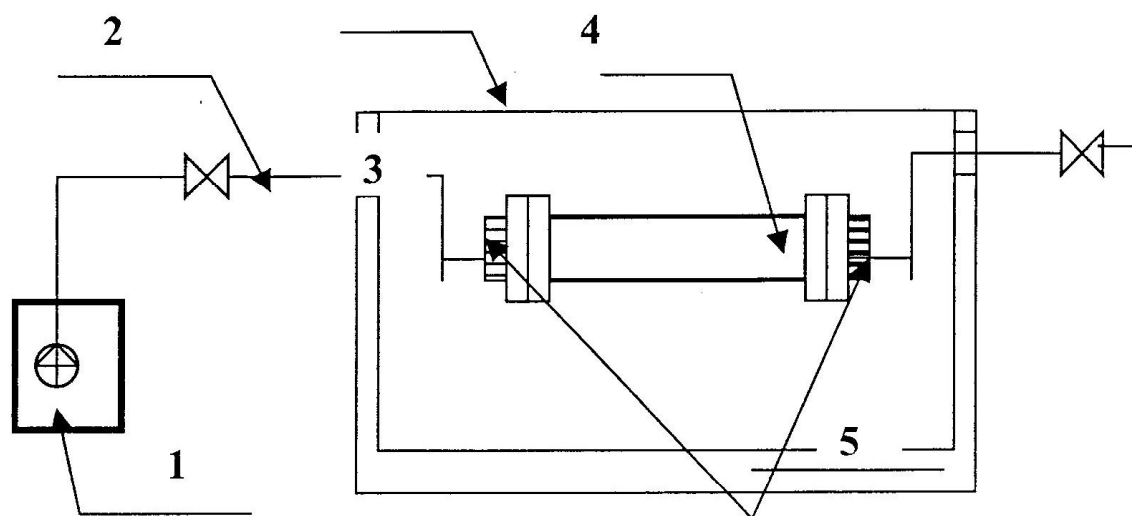
1:    Pump unit (constant pressure pump)   2:   Stainless steel tubing
3:    GC Oven   4:   Tubular reactor (HPLC column)   5:   Nuts and ferrules (SS)

Figure 1: Schematic diagram of a batch reactor for High-Temperature Dilute-Acid hydrolysis of waterhyacinth (WH) biomass

Four different acid concentrations (0.5, 1.0, 1.5 and 2.0% by weight H_2SO_4) were used in the hydrolysis and five to six experiments were carried out for each concentration. The hydrolysis experiments were carried in the temperature range of 180-240°C. In order to study the effect of particle size, a sample of sun-dried or oven-dried waterhyacinth biomass was ground in a laboratory mill. Samples were separated of such particle size as to pass a 6 mm sieve and be retained on a 5 mm screen, pass a 5 mm sieve and be retained on a 1 mm sieve, and pass a 1 mm sieve. The liquid-to-solid ratio was varied between 20:1 and 40:1.

The WH samples were heated for various times in the tubular reactor.

When the desired hydrolysis time was reached, both the oven and the pump were stopped. The reactor was removed from the GC oven and immersed in ice-water mixture in order to terminate the reactions. The reactor was left in ice water for about 5 min until the pressure in the reactor was equal to the atmospheric pressure. The reactor was then quickly opened and the hydrolysis products were collected and immediately filtered through a porous glass filter of medium porosity. Aliquots of the acid

hydrolysates were neutralized by the addition of calcium carbonate. The calcium sulphate cake was filtered off and washed with water. Then, the liquid mixture was filtered through Whatman (grade 202) filter paper without washing the filter cake.

The filtrate was analyzed for glucose and xylose and other sugars using the HPLC equipment. This equipment included a column (ZORBAX NH_2, 4.6 mm x 25 cm, P.N.880952709 chromatographic column) packed with a chemically-bonded aminopropyl goup on silica gel (5 μm). The detector used was a differential refractometer detector (Model R-401, Waters Associates Inc.). Integration of the obtained chromatographs was performed by a Hitachi integrator (Model D-2500 Chromo-Integrator, Japan). The detailed experimental and analytical procedures ca be obtained in Masende (1999)[18]. It should be pointed out that sugar concentrations in acidic liquors should be measured as soon as possible, since sugars otherwise polymerises and erroneously low concentrations are observed by both GC and HPLC methods. In this case also, the residue after filtration was washed with additional water and oven-dried at 105°C or air-dried at 45°C for chemical analyses.

RESULTS AND DISCUSSION

In this paper, the percent yield of sugar was calculated by:

$$Yield \; (\%) = \frac{Amount \quad of \quad sugar \quad obtained}{Amount \quad of \; dry \quad WH \quad biomass \quad Sample} \times 100$$

The yields of fermentable sugars have been calculated on the basis of dry weight of the starting material.

Effect of Temperature

It is of interest to establish whether the hydrolysis experiments reported here are reaction controlled and that the kinetic rates obtained are intrinsic. This is of special importance since the experiments are being carried out at a low L:S ratios and could possibly be mass-transfer limited. Hence four runs were carried out at identical conditions of acid concentration and L:S ratio, namely 1.5%

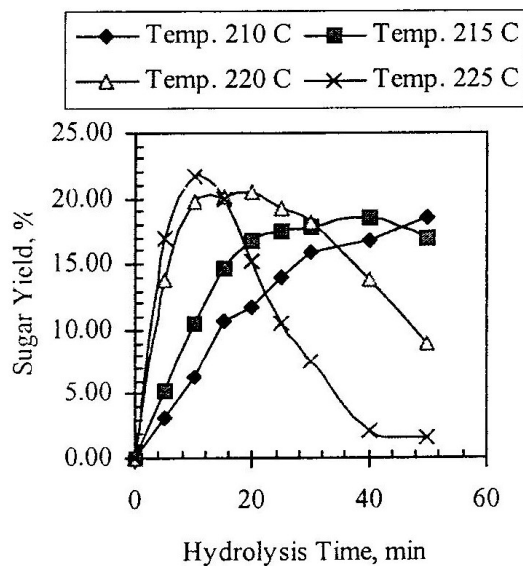and 40:1. The temperature in each experiment was increased by 5°C that is, from 210 to 225 °C.

Figure 2: Effect of Temperature on Hydrolysis rate and Yield at Acid conc. 1.5% and L:S of 40:1

The sugar yield curves for the four experiments are shown in Figure 2. This figure shows the yield (expressed as the ratio of the glucose produced to the oven-dried WH biomass), for the simultaneous hydrolysis of crystalline cellulose and degradation of glucose, as a function of time and temperature. As the temperature increases, the reaction proceeds more quickly and the yield improves. The maximum sugar yield occurs at 225 °C after about 10 min.

At high temperatures the rate of hydrolysis is high and the reaction time to reach the maximum yield becomes shorter. However, the rate of decomposition of sugars also increases with temperature. Furthermore, runs at 230 and 240°C have shown excessive deposition of black carbonaceous residues within the reactor and no increase in glucose yield even when the acid concentration is substantially reduced as it will be illustrated later.

Hydrolysis of WH biomass at low temperatures shows that, the maximum yields

of sugars is achieved at longer reaction times than at high temperatures. Similarly, the rate of degradation of glucose at low temperatures is lower than at high temperatures. For example, at 215°C the yield of sugars becomes significant at 15 min and remains stable for about 30 min. This implies that at lower temperatures the maximum yield is delayed while at high temperatures is reached within the 10 min as for the case of hydrolysis at 225°C. Therefore, another set of experiments was carried within the reaction time of 10 min with various liquid-to-solid ratios.

Effect of Liquid-to-Solid Ratios

The influence of solid concentration in the reactor feed was also investigated. The hydrolysis time and acid concentrations were kept constant at 10 min and 1.5% H_2SO_4 respectively. Figure 3 shows the maximum sugar yield at different hydrolysis temperatures and L:S ratios (ml/g) of 20:1, 30:1 and 40:1. It can be seen from Figure 3 that, at hydrolysis time of 10 min the yield of sugar increases with temperature. The maximum yield of sugar of 21.6% was achieved at 225°C for all L:S ratios; above this temperature, the yield decreases.
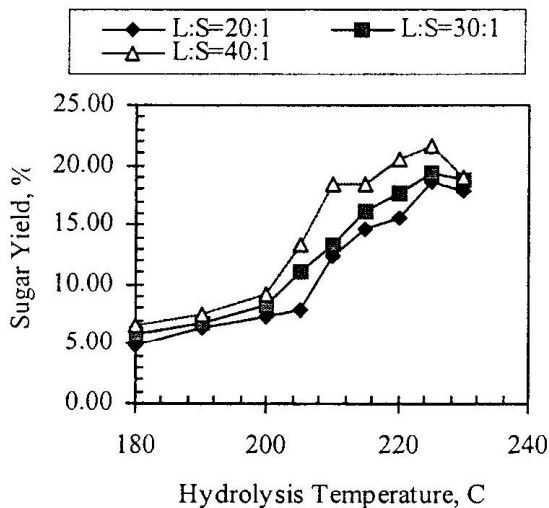

Figure 3: Sugar Yield vs Reaction Temperature for 1.5 % acid, at Reaction Time of 10 min

It can also be noted that, from Figure 3 the liquid-to-solid ratio over the range tested had a small but definite effect on the hydrolysis reaction. At 225°C the glucose yield were 18.6%, 19.3% and 21.6% for L:S ratios of

20:1, 30:1 and 40:1, respectively. Although the yield is high with L:S of 40:1, the sugar concentration in the hydrolysates is low as compared to lower L:S ratios.

The L:S ratio of 40:1 gave a yield of 21.6% and hydrolysates had sugar concentration of 3.9 mg/mL while L:S ratio of 20:1 gave a yield of 18.6% and the concentration was 4.45 mg/mL. This phenomenon could simply be due to large liquid content which leads to low sugar concentration in the WH hydrolysate. However, low liquid–to-solid ratio gives low yield probably because of the difficulty of uniformly wetting fine WH biomass with small quantities of liquid. And because of this observation the study was not carried to liquid-to-solid ratios lower than 10 to 1. These results suggest that, more experiments on acid concentrations be carried out at 220 °C because it gives reliable and stable yields of sugar in a longer hydrolysis time. The liquid-to-solid ratio of 30:1 gives good concentrations of sugar in hydrolysates with minimum reactor problems.
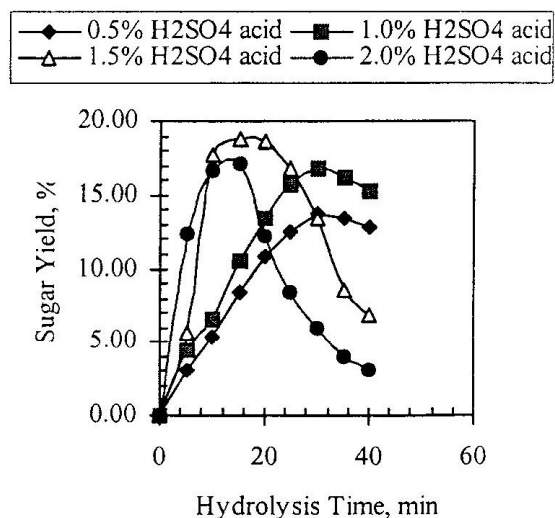
Effect of Acid Concentration

Figure 4: Effect of Acid Concentration at L:S ratio 30 ml:1g, and Temperature of 220 C

The effect of acid concentration on the rate of hydrolysis and the rate of degradation was determined in acid concentration range of 0.5% to 2.0% by weight H_2SO_4. Sulphuric acid

was used in all the above mentioned experiments. In order to verify the effect of acid concentration, four experiments were conducted, each at 220°C with a L:S ratio range of 20:1 to 40:1 and sufficient reaction time to ensure reaction completion. Figure 3 shows the sugar yield curves from the hydrolysis of WH biomass. It can be seen that the rate of hydrolysis increases with increase in acid concentration and the sugar yield improves.

It could however be noted that, with much higher acid concentration, the maximum yield decreases as for the case of hydrolysis with 2.0% acid as compared to the yield with 1.5% at 220°C. Since at higher acid concentrations there are more hydrogen ions than needed to catalyse the reaction, it follows that, the hydrolysis and degradation rate must both increase. The results shown in Figure 4 suggest that 1.5-% H_2SO_4 acid gives a better yield. Stronger acids lead to increased formation of sugar decomposition products. These results are consistent with those of other researchers[10].

Composition of Waterhyacinth (WH) Hydrolysates

The HPLC results of the hydrolysates obtained from high-temperature dilute-acid hydrolysis at 1.5% H_2SO4, 220°C, and L:S ratio of 30:1 are presented in Table 1. Also, reported in the Table are results from a low-temperature concentrated-acid hydrolysis of oven-dried waterhyacinth (ODWH) biomass[5] Table 1 shows that, hydrolysis of ODWH biomass at low temperature gave high concentration of hemicellulose-derived sugars such as mannose and xylose. At high temperature hydrolysis using dilute acid such as 1.5% H_2SO_4, the xylose concentration decreased while glucose concentration increased.

However, since glucose concentration constitutes most of the fermentable sugars, it is advantageous to use the high-temperature dilute-acid hydrolysis to maximise the fermentable sugar concentration. Concentration of arabinose and galactose were not detected at all hydrolysis conditions; while unknown substances were detected. The concentration of unknown matters was high when hydrolysis was carried at high

temperatures; probably these could be by-products as a result of degradation or repolymerisation of hemicellulose-derived
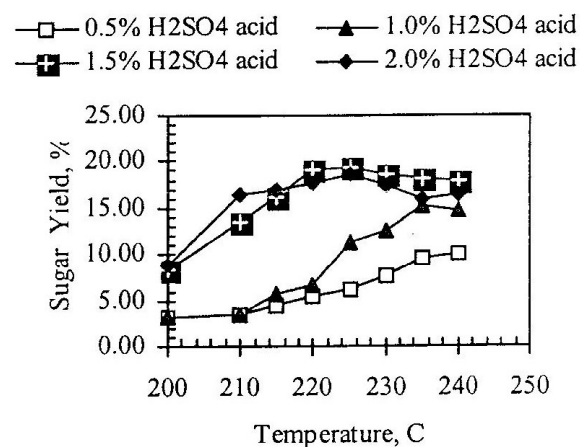

—□— 0.5% H2SO4 acid   —▲— 1.0% H2SO4 acid
—⊞— 1.5% H2SO4 acid   —◆— 2.0% H2SO4 acid

Figure 5: Sugar Yield as a function of Temperature and Acid Concentration at L:S = 30:1 and Reaction Time of 10 min

sugars.

Table 1: Composition of WH Hydrolysates in g/L

Type of hydrolysate	Concentration, g/L			
	Xylose	Glucose	Mannose	Unknown
Sample I		3.2	2.0	2.4
Sample II	3.1	10.6	ND	4.5

Note: *ND:*   *Not detected*

Sample I: Obtained from Low-Temperature Concentrated Acid hydrolysis at 60% H_2SO_4, 70 °C, L:S ratio of 20:1
Sample II: Obtained from High-Temperature Dilute-Acid hydrolysis at 1.5% H_2SO4, 220 °C, L:S ratio of 30:1

CONCLUSIONS AND RECOMMENDATIONS

From the experimental results obtained in this study, the following conclusions were drawn.

1.   A good glucose yield of about 18.5% was obtained by dilute acid hydrolysis at high temperatures. The

best glucose yield was obtained at 220°C. Increasing temperature initially favors glucose formation, but glucose decomposition becomes significant above 225°C. Sugar yield of about 18.5% was achieved at the following experimental conditions; acid concentration, 1.5% H_2SO_4 by weight; temperature, 220°C; liquid-to-solid ratio (ml/g), 30:1; particle size, < 1 mm and hydrolysis time, 10-25 min. The hydrolysates obtained were rich in six-carbon (C6-) sugars (glucose) which could be fermented to ethanol.

2.   The dilute-acid hydrolysis method is preferred for production of fermentable sugars although the yield of glucose is only about 18.5% based on the dry weight of Waterhyacinth (WH) biomass.

3.   Overall, it can be stated that, the production of fermentable sugars from waterhyacinth (WH) biomass is a promising new opportunity for solving the current problem of waterhyacinth in Lake Victoria. It would both reduce the waterhyacinth infestation rate and recover part of the

money being injected in various programmes.

Following the research findings and physical observations in the Lake Victoria shores, it is recommended that:

There is a need to carry out more studies on the hydrolysis of waterhyacinth biomass at higher temperatures even above 230°C and elevated pressures. This would require designing of a prototype scale followed with the optimisation study. There are indications that, reactions at high temperatures and pressure result in improved yields, decreased time, and decreased acid consumption What has been studied in this research is only the beginning, much laboratory and pilot-scale will be needed before production can be carried in a full scale.

Concerning the general waterhyacinth problem, preference should be given to integrating the utilisation of waterhyacinth plant and other methods such as biological, physical and chemical control. The implementation of chemical, physical and biological methods should not jeopardise the waterbody ecosystem.

ACKNOWLEDGEMENT

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