PRODUCTION OF FERMENTABLE SUGARS FROM WATERHYACINTH BY LOW-TEMPERATURE CONCENTRATED-ACID HYDROLYSIS METHOD

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Abstract

Conversion of Waterhyacinth (WH) biomass to monomeric sugars using acid-catalyzed hydrolysis has been studied. Waterhyacinth (Eichhornia Crassipes) plants were obtained from Lake Victoria. The hydrolysis of Waterhyacinth hemicellulose at 100°C and 4% w/w H_2SO_4 gave a yield of 5.8% w/w sugar from the oven-dried (ODWH) samples. Hydrolysis of WH biomas at 70°C and 60%w/w H_2SO_4 acid gave a yield of 23.2% w/w sugar, however, the hydrolysates were rich in five-carbon (C_5-) sugars (xylose) which could not be converted to ethanol. Also reported are the results of some experiments in which the effect of acid concentration, temperature, hydrolysis time and liquid-to-solid ratios were investigated. It has therefore been concluded that concentrated acid hydrolysis is not suitable means for hydrolysis of WH cellulose as it results into C_5- sugars which are not readily fermentable to ethanol.

INTRODUCTION

Waterhyacinth, *Eichhornia crassipes* (Mart) Solms, a freshwater macrophyte, is the world's worst aquatic weed. The free-floating freshwater angiosperm is a member of the family *Pontederiaceae*. It infests rivers, dams, lakes and irrigation channels on every continent; it costs billions of dollars worldwide every year in control costs and economic losses; and it disrupts and makes more burdensome the lives of millions of people especially in the third world. Waterhyacinth has been such a successful weed because it propagates extremely fast, both vegetatively and by seed. Waterhyacinth doubles its weight in only 6-20 days, depending upon the levels of nutrients, temperature, and light where its population is established[1] . A native of tropical South America, with a particular abundance in Northern Brazil, Venezuela and Amazon basin, the Waterhyacinth (WH) is now widespread in the tropics and sub-tropics[1,2 . Originally introduced for its decorative value, Waterhyacinth is now scheduled as a dangerous weed throughout Eastern Africa. The fast-spreading Waterhyacinth is encroaching on some of the region's major inland waterways, threatening communities on their shores.

Waterhyacinth (WH) 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.

Several methods have been studied 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 trea tment plants, recovery of metals, etc. do not match with the plant's growth. Ecological control of nutrient loading into the lake and Waterhyacinth utilization as source of thermal energy and for production of ethanol, have been suggested as being potential for sustainable benefits of the plant.

Conversion of biomass into fuels and chemicals requires breakdown of the polysaccharide fraction into monomeric sugars. A method that can be used to process a large amount of cellulosic materials at a high rate is acid-catalyzed hydrolysis.

LITERATURE REVIEW

A number of researchers[3,4,5] have investigated on different parameters and conditions for acid hydrolysis of cellulosic materials. Grethlein (1978)[6] presented hydrolysis data for acid concentrations ranging from 0.5 - 2% and temperatures 210 to 300°C in slurry concentration range from 5 to 13.5%. Mok and Antal (1992)[4] reported high glucose yields (around 71%) using 0.05% H_2SO_4 at 488 K and 34.5 MPa. However, the concentration of glucose obtained by dilute acid hydrolysis have always been lower than those obtained with concentrated-acid processes due to both changes in mechanism of hydrolysis as well as reduction in rate of glucose degradation.

In acid hydrolysis of cellulosic biomass, a difficulty situation is also encountered in the recovery of easily hydrolysable hemicellulose. During an acid hydrolysis process aiming at glucose as the main product, hemicellulose is hydrolysed more rapidly 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 little of it is actually recovered[7,8]. 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. Since the objective in a hydrolysis process is to maximize the yield of the glucose it is important to note that, the yield of both glucose and xylose depends on the relative rates of reaction or selectivity. For glucose, the yield depends on relative magnitude of the cellulose decomposition rate and the glucose decomposition rate.

While there are several studies of cellulose hydrolysis in both concentrated-acid low-temperature and dilute-acid high-temperature hydrolysis, the use of Waterhyacinth biomass in production of fermentable sugar has not been reported. It was therefore, the main task of this study to investigate on the production of ethanol using Waterhyacinth biomass from Lake Victoria. 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. Both low-temperature concentrated acid and high-temperature dilute-acid hydrolysis were investigated as methods of selectively recovering monomeric sugars from Waterhyacinth biomass.

In this paper, research findings on Low-temperature concentrated-acid hydrolysis of waterhyacinth biomass are presented. Also, recovery of sugars from easily hydrolyzable hemicellulose is reported. The major parameters studied were temperature, acid concentration, hydrolysis time, particle size and the liquid-to-solid ratio.

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 water hyacinth 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_2SO_4 and chromatography grade CH_3CN were from Merck (Darmstadt, Germany). The water used in making the sample solutions and during the experiments was distilled and deionized. The yeast strains of *Saccharomyces cervisiae*, namely bakers and brewers yeast was 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[9]. The yeast strains were stored and maintained at -20°C.

Experiments

Three sets of experimental studies were carried out in an effort to achieve quantitative yields of monomeric sugars by hydrolysing Waterhyacinth (WH) biomass using mineral acids. The first set of experiments were basically screening experiments aimed at studying the suitability of mineral acids namely; sulphuric acid (H_2SO_4) and hydrochloric acid (HCl). The physical and chemical properties of waterhyacinth biomass were also determined. The second set of experiments involved low-temperature acid hydrolysis of the Waterhyacinth biomass for production of fermentable sugars. The major variables whose effects were studied include hydrolysis temperature, hydrolysis time or residence time, acid concentration, particle size and liquid-to-solid (L:S) ratios. Another set of the experiments was aimed at studying the fermentability of the hydrolysates obtained from the hydrolysis of Waterhyacinth biomass. In this case two types of yeast strains namely, bakers and brewers yeast were used in the fermentation experiments. Besides the above-described experimental plans, other repetitions were conducted to prove the reproducibility of the results.

The hydrolysis experiments were performed at temperatures between 50 to 100°C and atmospheric pressure. The temperature was controlled a water bath. The liquid-to-solid ratio was varied between 10:1 and 30:1. The hydrolysis time was recorded from the time at which the reactor content reached the desired temperature. Four different acid concentrations (50, 60, 70 and 80% w/w) were used and five to six experiments were carried out for each concentration. The liquid sample (acid hydrolysate) was neutralised to a pH between 5 and 7 before injection into the HPLC column by the addition of calcium carbonate. The hydrolysate was then filtered through a 0.45-μm filter using a 1-mL syringe-type filtration unit supplied by SABRE Corporation.

The analysis of the liquid products was made by a High Performance Liquid Chromatography (HPLC) equipped with a high-pressure pump (Model 100A BECKMAN, The Netherlands). This equipment included a column (ZORBAX NH2, 4.6 mm x 25-cm (5 µm), P.N.880952708 chromatographic column) packed with a chemically-bonded aminopropyl group 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 procedure can be obtained in Masende (1999)[10].

RESULTS AND DISCUSSION

The yields of fermentable sugars in this study have been calculated on the basis of dry weight of the starting material i.e. waterhyacinth biomass. The percent yield of sugar was calculated by:

$$Yield(\%) = 100x \frac{Amount\ of\ sugar\ obtained}{Amount\ of\ dry\ WH\ biomass\ sample}$$

Table 1 shows the summary of the average moisture content and hence the dry-matter in the waterhyacinth (WH) biomass.

Table 1: Moisture Content in Fresh Waterhyacinth (FWH) Biomass

Plant Part	% w/w Moisture	%w/w Dry matter
Leaf	84.3 ± 0.8	15.7 ± 0.8
Stem	92.8 ± 0.7	7.2 ± 0.7
Whole Plant	91.9 ± 1.0	8.1 ± 1.0

It can be seen that, fresh waterhyacinth plant contains about 92% of moisture. The high moisture content in the FWH biomass affects the concentration level of acid since the acid becomes diluted below its initial desired level. Otherwise, a relatively dry substrate is required for a concentrated-acid low-temperature hydrolysis process.

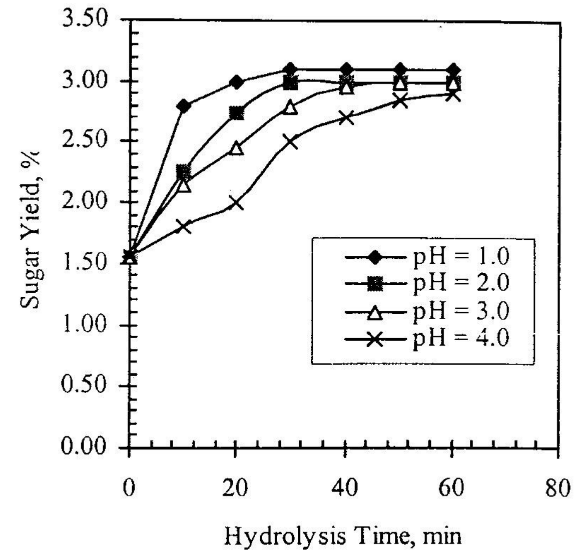

Figure 1: Hydrolysis of WH Juice using H2SO4 at 100C and various pH levels

Screening Studies

A set of experiments was carried out to study the availability of reducing sugars in waterhyacinth plant. Fresh waterhyacinth juice (FWH-Juice) was hydrolysed at 100°C using H_2SO_4 acid at various pH levels. Figure 1 presents the results of hydrolysis of FWH-Juice whereby the acid pH was varied from 1.0 to 4.0. As it can be seen in Figure 1, a maximum yield of 3.10% was attained with acid pH of 1.0 in 30 min. This maximum value remained the same up to 40 min, thereafter the yield started to decrease. With pH levels of 2.0, 3.0 and 4.0 different yields were attained at longer reaction times.

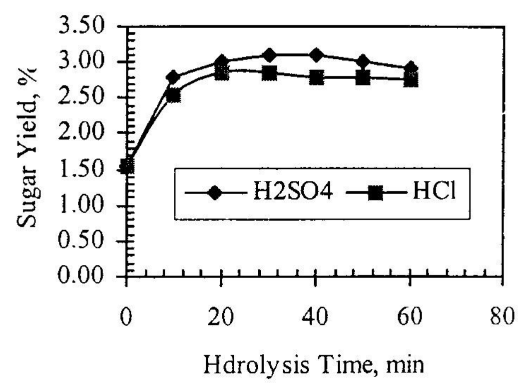

Figure 2: Comparison of Acids on Sugar Yields during WH Juice Hydrolysis at 100C and pH = 1.0

Based on the results of the samples tested, the yield of monomeric sugars from FWH juice have been very low. This may be attributed to of low content of water-soluble carbohydrates in the fresh waterhyacinth biomass. Gopal (1987)[1] reported that the total available carbohydrates (TAC) in waterhyacinth biomass were in the range 7.8-9.5% w/w dry weight. Since one of the objectives in hydrolysis reaction is to reduce the reaction time, the acid pH of 1.0 seemed to give better results and this was selected for further experimentation of the FWH-Juice.

In order to examine the effect of different mineral acids on the yield of sugar from FWH-Juice, a number of experiments were carried out using sulphuric (H_2SO_4) and hydrochloric (HCl) acids at a fixed pH of 1.0 and reaction temperature of 100°C. As is shown in Figure 2, a maximum yield of 3.1% was attained with H_2SO_4 acid in 30 min while only about 2.85% yield was attained with HCl acid in 20 min. The hydrolysing action of HCl and H_2SO_4 acids give different yields probably because they have been compared on the basis of acid concentration in weight by volume percent.

The hydrolysis results of HCl might be of the same order of magnitude as that obtained with H_2SO_4 acid when the concentrations are expressed in moles as observed by Ullal et al. (1984)[10]. It can also be seen that, the maximum yield of sugar is reached in a shorter reaction time with HCl acid (20 min) than when using H_2SO_4 acid (30 min). It was also found that, FWH-Juice contains very little amount of reducing sugars when it was analysed before the hydrolysis. These results suggest that, hydrolysis studies be focused on WH biomass instead of FWH-juice.

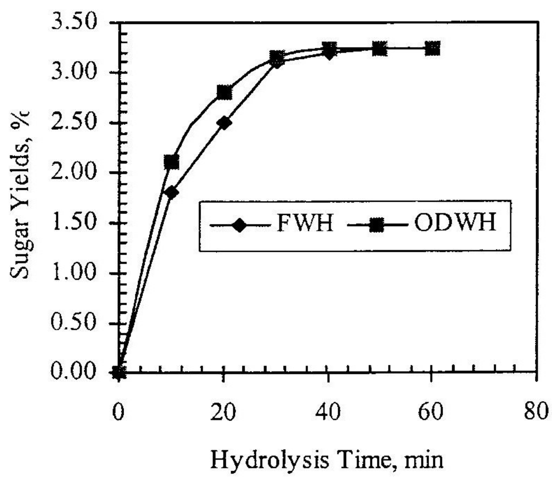

Figure 3: Sugar Yields from Water-Soluble Carbohydrates of Fresh WH biomass at 100C and pH of 6.5 (i.e. no acid)

Fresh waterhyacinth (FWH) biomass and oven-dried waterhyacinth (ODWH) biomass were studied using acid concentration of 4% by weight. The size range of biomass used was below 1 mm sieve and the L:S ratio (ml/g) was fixed at 20:1. A comparison of the sugar yield at different experimental conditions is made in Figures 3, 4, and 5. It can be seen that a good yield of reducing sugar was obtained from hydrolysis of oven-dried waterhyacinth (ODWH) biomass as compared to fresh waterhyacinth (FWH) biomass. However, at longer hydrolysis time, sugar yields on WSC are the same for both ODWH and FWH biomass as could be seen in Figure 3. With exception of Figure 3, increasing reaction times gave no appreciable change on the general trend of the results. It could also be noted in Figures 4 and 5 that, H_2SO_4 acid gave a better yield in both FWH and ODWH biomass than when HCl acid was used. These results suggest that, with any kind of waterhyacinth biomass one could achieve good yield when H_2SO_4 acid is used for hydrolysis.

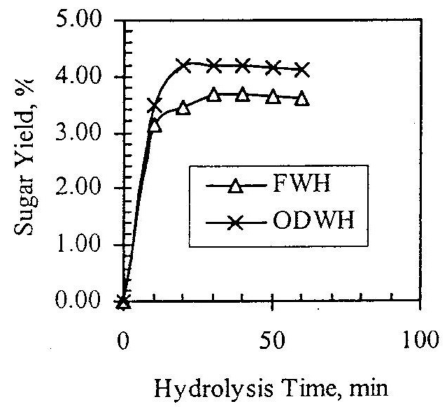

Figure 4: Effect of HCl acid on the Yield of Sugar during WH biomass Hydrolysis at 100 C and pH of 1.0

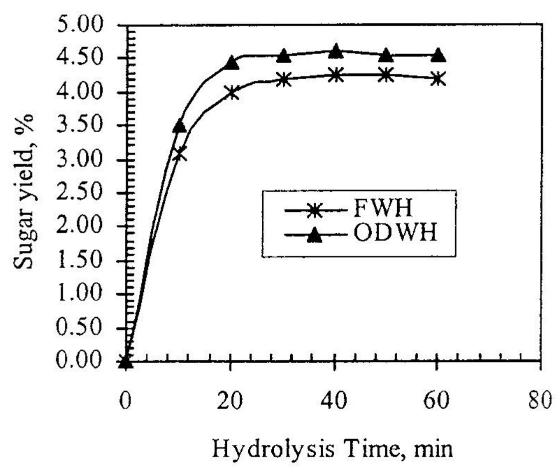

Figure 5: Effect of H2SO4 acid on the Yield of Sugar during WH biomass Hydrolysis at 100 C and pH of 1.0

Figure 6 gives the summary of the comparative studies. The results show that oven-dried waterhyacinth (ODWH) biomass gives good yield of reducing sugar during acid hydrolysis. It can be noted further that hydrolysis of WH biomass using H_2SO_4 acid gives better results than HCl acid. In addition, the use of H_2SO_4 acid in hydrolysis has the advantage that, during sample treatment, it is easier to neutralise the acid using either $CaCO_3$ or $Ca(OH)_2$ to get $CaSO_4$ which could

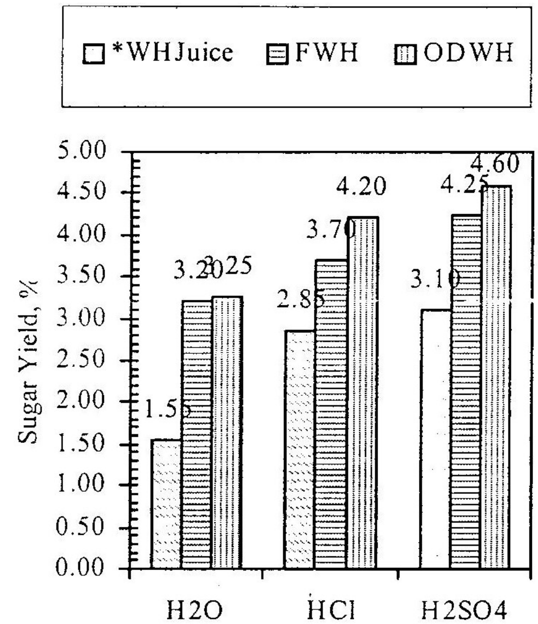

Figure 6: Comparative Yield of Easily Hydrolyzable Sugar from WH biomass

be separated by simple filtration while $CaCl_2$ could be obtained when HCl acid is used.

The results of these studies discourage the use of FWH-Juice for production of reducing sugar because of its low yield. Furthermore, the hydrolysate obtained from hydrolysis of WH-Juice has been very dilute such that it requires too much energy to concentrate the solution. With these results and observation it

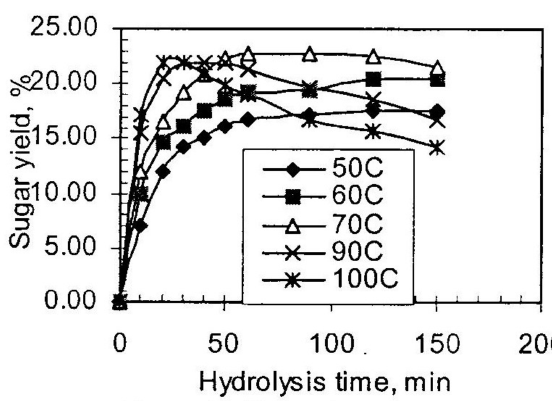

Figure 7: Effect of Temperature on ODWH biomass hydrolysis with 50% H2SO4 and L:S ratio of 20:1 for ODWH Biomass

was decided to carry out studies on cellulose hydrolysis using H_2SO_4 acid.

Low-Temperature Concentrated-Acid Hydrolysis of Waterhyacinth(WH) Biomass Effect of hydrolysis temperature

The results of the experiments utilising 50% H_2SO_4 and varying reaction temperatures and times with a fixed L:S ratio (ml/g) of 20:1 are shown in Figures 7 and 8. It can be seen in Figure 7, . that the maximum yield of 22.8% was attained at a reaction temperature of 70°C and reaction time of 60 min. Increasing the reaction temperature actually decreased the yield. This can be attributed to degradation of sugars to by-products or repolymerisation of the monomeric sugars. However, an increase in reaction time gave no substantial increase in the yield. Figure 8 shows similar results when fresh waterhyacinth (FWH) biomass was used under the same conditions. Reducing sugar yield presented in Figure 8, were less than 22.8% at all conditions. The maximum yield of

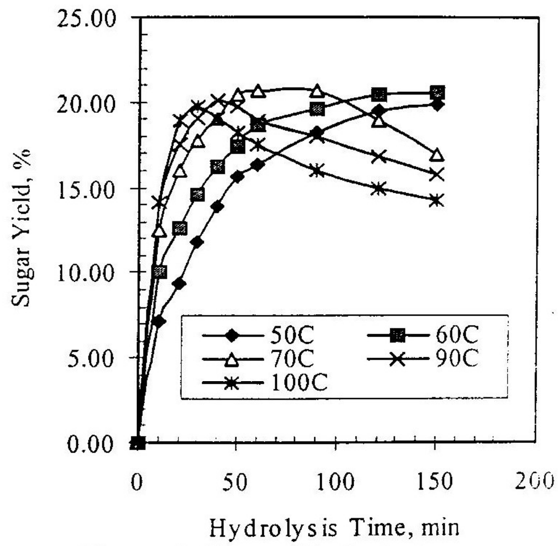

Figure 8: Effect of hydrolysis temperature on the yield with 50% H2SO4 L:S ratio of 20:1 for FWH Biomass

20.7% was attained at 70°C with 50% H_2SO_4 acid and 60 min reaction time. This value is lower than that obtained with ODWH biomass as depicted in Figure 7. Thus, the results confirm the observations from the previous experiments that ODWH biomass is more suitable for hydrolysis than FWH biomass. On the basis of these studies it was concluded that the hydrolysis temperature of about 70°C was

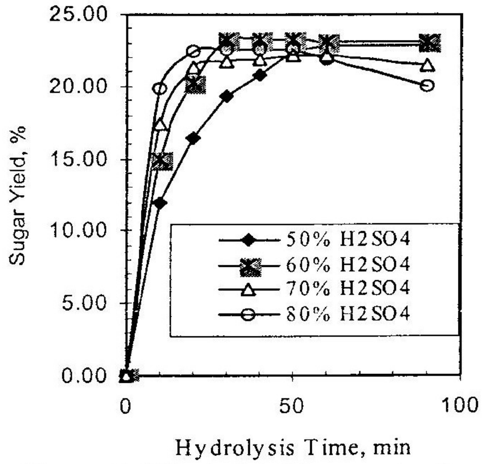

Figure 9: Effect of Acid concentration on hydrolysis of ODWH biomass at a Temperature of 70C and L:S ratio of 20:1

appropriate for concentrated-acid hydrolysis. At higher temperatures low sugar yield was probably due to decrease of sugar as a result of degradation to by-products or repolymerisation of the monomeric sugars.

Effect of acid concentration

During another set of experiments, the temperature of hydrolysis was set at 70°C and the L:S ratio was fixed at 20:1. In this case the acid concentration was varied from 50% to 80% by weight H_2SO_4. The results of this study are presented in Figure 9. It was found that, a maximum yield of 23.2% was obtained with 60% H_2SO_4 in 30-min. High acid concentrations gave low yield of sugar even when the reaction time was increased. However, as the acid concentration was increased, the time required to reach maximum yield in each case was shortened.

With low acid concentrations, low yield of sugars was obtained even at longer reaction times. At longer reaction time, however, there was no significant difference on the yield for 60% and 50% H_2SO_4 acid. These data suggest that, although increased acid concentration could shorten the time required for hydrolysis, it is not always true that the yields will improve. This is because at high concentration and high L:S ratio, the rate of formation of monomeric sugars competes with degradation into by-products or repolymerisation of monomeric sugars. From the results, it can be seen that at 70°C the acid concentration of 60% H_2SO_4 acid seemed to give better results.

Effect of liquid-to-solid (L:S) ratio

In the hydrolyses studies, it is desirable to obtain as much of the sugar produced in each condition as possible. One potential method for increasing the yield of monomeric sugars is to utilise lower solids concentrations in the feed in order to have more water available for conversion of polymeric sugars to monomers.

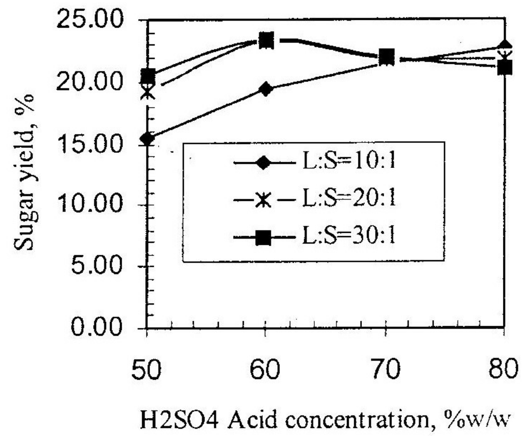

Figure 10: Effect of L:S ratios at different Acid concentration on hydrolysis of ODWH biomass at 70C and reaction time of 30 min

Figure 10 shows the yield of monomeric sugars during hydrolysis at 70°C and fixed reaction time of 30 min with varying acid concentration and varied liquid-to-solid (L:S) ratios. It can be seen that, with a L:S ratio of 30:1, the yield increased from 20.5% at acid concentration of 50% H_2SO_4 to 23.4% at 60% H_2SO_4, and then decreased to 22% at 70% H_2SO_4. Similar results were obtained with a L:S ratio of 20:1; in fact, there is no significant difference on the yield between the two ratios.

With L:S ratio of 10:1, however, the maximum yields were 15.5%, 18.6% and 22.2% at 50, 60, and 70% H_2SO_4 respectively. And then the yield increased when the hydrolysis was carried out using more concentrated acid. For example, at 80% H_2SO_4 acid, L:S ratio of 10:1 gave the highest sugar yield of 23.0% as compared to those obtained with other L:S ratios. The results in Figure 10 show that, good yields of sugars could be achieved when a high L:S ratio (i.e. low solids concentration) was used with 60% H_2SO_4. However this may result into hydrolysates with low sugar concentrations as compared to the low L:S ratios (i.e. high solids concentration). The hydrolysis of low L:S ratios (i.e. high solids concentration) using low acid concentration such as 50% H_2SO_4 on one hand gave low sugar yield (Figure 10). The low yield was probably due to the difficulty of uniformly wetting fine WH biomass with small quantities of liquid (low L:S ratios).

It seems reasonable to speculate that, the decrease in sugar yield occurs because there are insufficient hydrogen ions to catalyse the reaction[14]. With low L:S ratios like 10:1 and below, i.e. high solids concentrations, good yields could be obtained when using a more concentrated acids such as 70% H_2SO_4 or above. On the basis of these studies, it was decided that a L:S ratio of 20:1 was satisfactory for the hydrolysis of ODWH biomass using concentrated acid of about 60% H_2SO_4, and this confirms the choice of L:S ratio of 20:1 in the previous experiments.

CONCLUSION

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

1. Little amount of fermentable /reducing sugars could be obtained from waterhyacinth juice. Furthermore, hydrolysis of water-soluble carbohydrates (WSC) gave a yield of 4.6% sugar, which is relatively low as compared to other biomass hydrolysis.

2. Hydrolysis of waterhyacinth hemicellulose shows that oven-dried waterhyacinth (ODWH) biomass gives a better sugar yield as compared to fresh waterhyacinth (FWH) biomass. Sugar yield of 5.8% was achieved at the following conditions: acid concentration, 4.0% H_2SO_4 by weight; temperature, 100°C; liquid-to-solid ratio (ml/g), 20:1; particle size, < 1 mm and reaction time, 40 min. Furthermore, H_2SO_4 acid was found to give better hydrolysis results as compared to HCl acid.

3. When waterhyacinth biomass was hydrolyzed using concentrated H_2SO_4 acid at low temperatures, suitable experimental conditions that gave a yield of 23.2% were: acid concentration, 60.0% H_2SO_4 by weight; temperature, 70°C; liquid-to-solid ratio (ml/g), 20:1; particle size, < 1 mm and reaction time, 30 min. The hydrolysates obtained were rich in five-carbon (C_5-) sugars (xylose) which could not be converted to ethanol.

4. High concentration acid hydrolysis is not a suitable means for hydrolysis of WH cellulose as it results into sugars with five-carbon (C_5-) which are not readily fermentable to ethanol.

It is thus recommended to undertake hydrolysis studies using dilute acid at high temperatures and pressures in order to improve hydrolysis time and also prevent degradation or decomposition of monomeric sugars and, produce sugars which are suitable for ethanol production.

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