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Acid-base pretreatment of lignocellulosic biomass to facilitate recovery of fermentable sugar for anaerobic fermentation

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Abstract

The increase in the consumption the fosil fuel has led to the search for alternative source. But plant which serves as alternative source has lignocellulosic material, which includes cellulose, hemicellulose and lignin (lignocellulosic complex), Biogas produced from various lignocellulosic biomass, such as hardwood, agricultural, or forest residues, can be a useful replacement for the conventional energy production. Several physical, structural and compositional factors has the ability to stop the hydrolysis of cellulose present in biomass to simple sugar and other organic compounds that can later be converted to biofuels. The aim of pretreatment is to make the cellulose accessible to hydrolysis and to get rid of the recalcitrant lignin for conversion to fuels to be possible. Various pretreatment techniques change the physical and chemical structure of the lignocellulosic biomass and improve hydrolysis rates. During the past few years a large number of pretreatment methods have been developed, including alkali treatment, ammonia explosion, and others. Many methods have been shown to result in high sugar yields, above 90% of the theoretical yield for lignocellulosic biomasses such as woods, grasses, corn, and so on. This research work looks at acid-base pretreatment process methods. It also showed that this method works and can recover high percentage of simple sugar for fermentation. It adopts the technique of dilute acid low temperature.

Keywords: Lignocellulose; Pretreatment; Acid-Base; Fermentation and Sugar

1. Introduction

The need for alternative source of energy cannot be overemphasized. Plant cells are mainly composed by lignocellulosic material, which includes cellulose, hemicellulose and lignin (lignocellulosic complex). The hydrolysis of lignocellulose to glucose is a major bottleneck in cellulosic biofuel production processes (5)In nature, microorganisms, especially fungi, are able to degrade the plant cell wall through a set of acting synergistically enzymes. This phenomenon leads to glucose being released in a free form, which can enter the metabolism of the microorganism, providing its energy. A great challenge is to modify the architecture of the plant cell walls and/or the ability of the microorganisms to degrade it, by modifying their genomes (4). For instance, researchers can generate genetically engineered microorganisms able to degrade efficiently the polymers in the plant cells, producing sugars monomers that can be fermented directly by yeasts, generating ethanol. This chapter will describe the composition of plant cell walls and how microorganisms cope with the lignocellulosic material (5).

Conversion of abundant lignocellulosic biomass to biofuels as transportation fuels presents a viable option for improving energy security and reducing greenhouse emissions.(3). Unlike fossil fuels, which come from plants that grew millions of years ago, biofuels are produced from plants grown today. They are cleaner-burning than fossil fuels, and the short cycle of growing plants and burning fuel made from them does not add CO_2 to the atmosphere. It has been

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reported that cellulosic ethanol and ethanol produced from other biomass resources have the potential to cut greenhouse gas emissions by 86%(1) Lignocellulosic materials such as agricultural residues (e.g., wheat straw, sugarcane bagasse, corn stover), forest products (hardwood and softwood), and dedicated crops (switchgrass, salix) are renewable sources of energy. These raw materials are sufficiently abundant and generate very low net greenhouse emissions. Approximately 90% of the dry weight of most plant materials is stored in the form of cellulose, hemicellulose, lignin, and pectin (5). The presence of lignin in lignocelluloses leads to a protective barrier that prevents plant cell destruction by fungi and bacteria for conversion to fuel. For the conversion of biomass to fuel, the cellulose and hemicellulose must be broke.

Hemicellulose can be readily hydrolyzed by dilute acids under moderate conditions, but much more extreme conditions are needed for cellulose hydrolysis. In the dilute-acid process, the reaction is carried out at high temperature and pressure, and because of low yields of glucose from cellulose in the hydrolysis step, the ethanol yield is low. The use of concentrated acid in the hydrolysis process can yield higher quantities of ethanol because of the approximately 100% conversion to glucose from cellulose (11). The dilute-acid hydrolysis process uses high temperatures (160-230°C) and pressures (~10 atm).(Iramahboob (7). The acid concentration in the dilute-acid hydrolysis process is in the range of 2-5%.(2 ,10). The acid concentration used in the concentrated-acid hydrolysis process is in the range of 10-30%.(2). Lower operating temperatures (<50 °C) and atmospheric pressures are required during the concentrated-acid hydrolysis process. The concentrated-acid hydrolysis involves longer retention times and results in higher ethanol yields than the dilute-acid hydrolysis process (12). Enzymes produced by a variety of microorganisms are also capable of breaking down lignocellulosic materials to sugars but require longer retention times. Enzymatic hydrolysis is the most common method of producing ethanol from lignocellulosic biomasses. The digestibility of cellulose present in lignocellulosic biomass is hindered by many physicochemical, structural, and compositional factors. In the conversion of lignocellulosic biomass to fuel, the biomass needs to be treated so that the cellulose in the plant fibers is exposed. Pretreatment uses various techniques, including ammonia fiber explosion, chemical treatment, biological treatment, and steam explosion, to alter the structure of cellulosic biomass to make cellulose more accessible (12). Then, acids or enzymes can be used to break down the cellulose into its constituent sugars.

So, effective pre-treatment is required to liberate the sugar by breaking the complex crystalline structure and by removing lignin content. Pre-treatments are given by physical, chemical, and biological means. Physical treatments reduce the physical size of biomass whereas chemical treatments remove chemical barriers, especially to break recalcitrant components so as to make easy accessibility of cellulose for microbial destruction. Biological pre-treatments utilize various enzymes to achieve the accessibility of cellulose for hydrolysis process (11).

2. Material and methods

Table 1 Biomass used and percentage content of the three compositions

Lignocellulosic material	Cellulose %	Hemicellulose %	Lignin %
Corn cob	45	35	15
Waste Paper	85-90	10	0-10

SOURCE: Reshamala et al (1995), Cheuing and Anderson (1997), Boopathy (1995) and Dewea and Hunshe (1998)

2.1 Pre-treatment process of lignocellulosic material to get starch rich cellulose for anaerobic fermentation

One thousand (1,000) grams each of samples were collected from different sites. The samples collected from these locations were then sundried for three days after which they were milled. The dried-milled samples were divided into two portions; the first portion was pre-treated while the second was not.

2.2 Acid pretreatment (hydrolysis of hemicellulose)

Different concentrations of dilute H_2SO_4 were mixed with the biomass to hydrolyze almost 100% hemicellulose to xylose (a pentose class of plant sugar or a monosaccharide) and other sugars and then continues to break xylose down to form furfural (a dehydration product of xylose). The dilute H_2SO_4 pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis. Dilute acid effectively removes most of the hemicellulose by recovering it as dissolved sugar. Hemicellulose is removed when H_2SO_4 is added and this enhances digestibility of cellulose in the residual solids.

Procedure: Dilution of Tetraoxosulphate VI acid (Conc $= \frac{1dm^3 \times vol}{\text{Molar mass}}$)

Table 2 Concentration of Acid used

Acid (cm^3)	Distilled water (dm^3)	Concentration (mol/dm^3)
2.5	1	0.03
3.5	1	0.04
4.5	1	0.05
5.5	1	0.06
9.8	1	0.1
29.5	1	0.3
68.6	1	0.7

The first - stage process which combines the dilute acid pre-hydrolysis as described by (11) was used. One portion of the dried-milled samples was treated with dilute Tetraoxosulphate VI acid which involved the use of 1.25% (w/v) H_2SO_4 solution in a 1: 8, g : v, solid : liquid ratio (50grams of biomass to $1dm^3$ of dilute acid). This will be done using different dilutions and concentrations as shown above.

The one step dilute acid pre-hydrolysis was performed in an autoclave at 121C at different exposure time for 20, 15, 10mins after which the solids were collected and drained.

2.3 Alkaline delignification (breaking down of lignin)

The effectiveness of alkaline pretreatment of lignocellulose biomass relies on lowering the lignin content of the biomass. This will in turn increase the rate and yield of enzymatic hydrolysis of carbohydrate.

Procedure: Dilution of Sodium Hydroxide (Conc $= \frac{1dm^3 \times \text{mass}}{\text{Molar mass}}$)

Table 3 Concentration of Base used

NaOH (g)	Distilled water (dm^3)	Concentration (mol/dm^3)
15.6	1	0.39
12.6	1	0.32
10.6	1	0.30
6.6	1	0.17
24.0	1	0.6
32.0	1	0.8
36.0	1	0.9

The solid biomass from acid pretreatment were then treated with 2% (w/v) sodium hydroxide solution in a solid: liquid ratio of 1: 20, g: v,(1gram biomass to $20cm^3$ of alkaline) at 120C for 90mins, , 10hrs,, 20hrs. After that, the residual solid material (Cellulose pulp) separated by filtration was washed with water to remove the residual alkali and was dried at 50 ± 5C for 24 hours.

By the use of benedict solution: this was done in seven tubes based on the different concentrations of acid and base as shown in tables below.

But when the untreated biomass is used, it will require a longer heating before the formation of dark red. In most cases, HCl is added to provide acidic condition or Na_2CO_3 will give the needed alkaline condition**.**

Table 4 Test for simple sugar to confirm the presence and percentage of glucose available

Test	Observation in pretreated biomass	Observation in untreated biomass	Inference
About 0.2g of Pretreated biomass mixed with water	Pretreated biomass dissolved given a cloudy solution	Untreated Biomass dissolved to give a cloudy solution	Both biomass are soluble.
Approximately 1ml of the sample is put in a clean test tube and about 2ml (10 drops) of Benedict's reagent added.	There is reaction or colour change. The blue colour of Benedict reagent starts to change	No visible reaction or colour change. Only the blue colour of Benedict reagent is formed	
The solution is then heated in a boiling water bath for 3-10 minutes	There is a colour change. A reduction reaction causes a colour change from blue to green to yellow to orange and dark red or rusty brown.	The blue colour takes a very long heating period to come up.	In pretreated biomass, the reducing sugar is already released, thus fast formation of colour change. But for the untreated, the long heating period and the sodium carbonate in Benedict's reagent will serve as physiocochemical pretreatment first. That accounts for delay in colour change formation

3. Results and interpretation

Spectrophotometric quantitative analysis on the pretreated biomass showed the following based on the concentration of acid and based used as shown in the table 1 below.

From the table and its graph, it shows that the concentration is directly proportional to sugar yield to some extent before we experience sugar loss. Use of inorganic acid like sulphuric acisd which acts as catalyst will almost and completely hydrolyze hemicellulose. It will also distrupt the lignin structure and partially solublize crystalline cellulose (9, 8).

Dilute acid used is the correct form of acid to be used. The main disadvantages of acid pretreatment are the possible formation of inhibitory by product like hydroxymethylfurfural (HMF). This can only be possible if a concentrated acid is used. Another disadvantage is a possible loss of fermentable sugar due to the increase degradation of complex substrate in longer pretreatment time (6). Ibbett (6) also noted that there was relative loss of more than 50% of sugar at the temparature of 140^0C and above.

Dilute acid is the most efficient and commond method of lignocellusoic pretreatment. It will dissolve the hemicellulose content of the biomass. This can be done in low concentration and high temperature or high concentration and low temperature (12).

Based on the test for simple sugar using Benedict solution: it shows that the amount of sugar recovered in the pretreatment is far higher when compared with the non-pretreated biomass.

Table 5 Concentration of Acid and Base against the percentage of sugar recovered

Conc of acid used(mol/dm^3)	Conc of base used(mol/dm^3)	Yield (%/conc)
0.03	0.17	3
0.04	0.30	10
0.05	0.32	15
0.06	0.39	25
0.1	0.6	3
0.3	0.8	0.5
0.7	0.9	0.8

- The colour upon boiling is changed into green, then it means there is about three percent of sugar in the solution measured in %yield/conc.
- The colour upon boiling is changed into yellow, then it means there is about ten percent of sugar in the solution measured in %yield/conc.
- The colour upon boiling is changed into orange, then it means there is about fifteen percent of sugar in the solution measured in %yield/conc.
- The colour upon boiling is changed into red, then it means there is about twenty five percent of sugar in the solution measured in %yield/conc.
- The colour upon boiling is changed into brick red, then it means there is above thirty percent of sugar in the solution measured in %yield/conc.

Figure 1 Colour Change based on percentage of sugar present

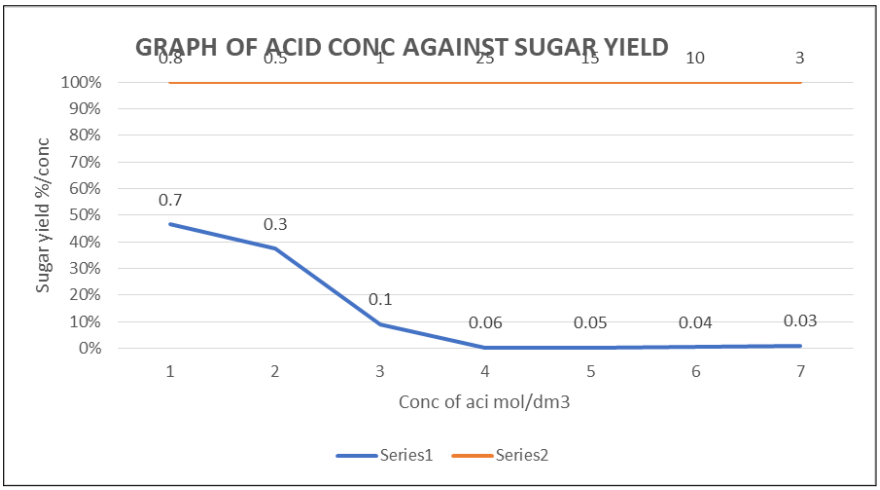

Figure 2 Graph of Percentage yield of Sugar against Conc. Of Acid

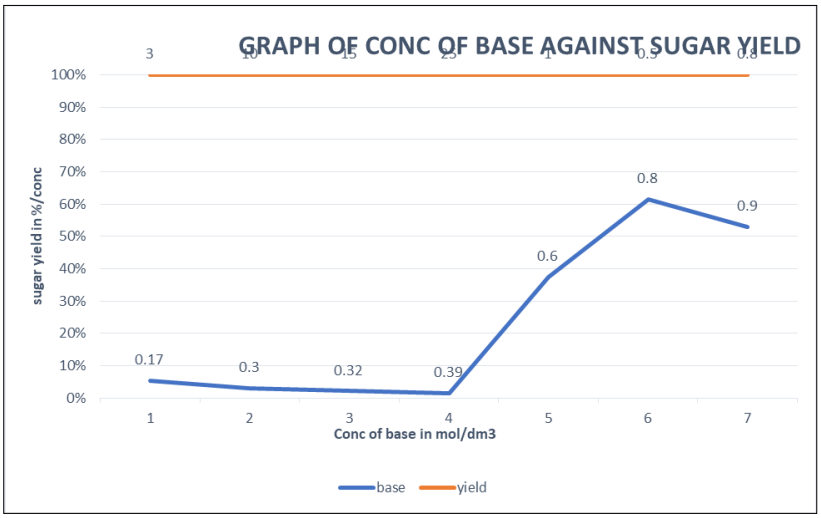

Figure 3 Graph of Percentage yield of Sugar against Conc. Of Base

4. Conclusion

As the recalcitrant nature of lignin has made it important to pretreat using Acid/alkaline method which is easy to operate. As the concentration increases, the yield of sugar increases and the sugar yield decreases due to increase in concentration and temperature. Concentration of 0.06 mol/dm^3 and 0.39 mol/dm^3 of acid and alkaline must be monitored and measured.

Compliance with ethical standards

Acknowledgments

This research has really proved that without pretreatment of lignocellulosoic biomasss, the trapped sugar cannot be released. Concentration of 0.06 mol/dm^3 and 0.39 mol/dm^3 of acid and alkaline respectively must be monitored and measured is acknowledged.

Disclosure of conflict of interest

No conflict of interest

Author's contribution

Osuji M.I was of the view that as the concentrations of both acid and alkaline increases, the percentage of released sugar increases. But the last three authors are of the opinion that a concentration will reach where sugar yield will no longer increase. But the research showed that at 0.06 mol/dm^3 and 0.39 mol/dm^3 of acid and alkaline respectively, no increase.

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