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International Journal of Current Research
Vol. 3, Issue, 09, pp.017-022, September, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EFFECT OF PRETREATMENT ON THE SACCHARIFICATION OF AGROWASTES

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ARTICLE INFO

Article History:
Received 24th June, 2011
Received in revised form 27th July, 2011
Accepted 18th August, 2011
Published online 17th September, 2011

Key words:
Agro waste,
Pretreatment,
Cellulose,
Physicochemical.

ABSTRACT

Biomass burning has a significant impact on global atmospheric chemistry since it provides large sources of carbon monoxide, nitrogen oxides, and hydrocarbons, primarily in the tropics. Rural areas of developing countries depend primarily on biomass for fuel. Biofuels include the wood fuels, and agricultural waste, such as crop residues and dung. The recent thrust in bioconversion of agricultural and industrial wastes to chemical feedstock has led to extensive studies on the pretreatment methods. The main aim of this study was to determine the effective pretreatment method, therefore, to alter or remove structural and compositional impediments to hydrolysis and subsequent degradation processes. The agrowastes collected were subjected to acid and alkali hydrolysis and thereby the release of sugar was studied both before and after pretreatment. The results showed that compared to the acid pretreated samples, more release of sugar was achieved from the alkali-pretreated samples. Also, it was found that as the concentration of alkali increases, the release of glucose also increased. Of the different agrowastes used in the study, groundnut shell (387 mg glucose / g of agrowaste) was found to be the best in releasing the sugars. The released sugar could help in the bioethanol production.

INTRODUCTION

Waste management is one of the biggest problems faced by the agro based industries. Renewable fuels, such as bio-ethanol, are becoming increasingly important. It is the impact of heightened concern for the greenhouse effect, depleting oil reserves, and rising oil prices (Ohgren *et al*., 2007). Most ethanol is currently produced by fermentation of either cornstarch or sucrose. The United States, Brazil and China are in the top of countries producing the largest quantities of fuel ethanol. If the oil crisis continues to develop, ethanol is one of the most promising biofuels. It can replace gasoline for transportation vehicles (Claassen *et al*., 1999; Sun and Cheng, 2002). Woody materials include wood, bark, and mixtures of forest residues. Lignocellulosic material comprises the fibrous structural component of plants - the roots, stem and branches. Lignocellulosics consist of some two-thirds carbohydrate and one-third lignin. The lignin supplies the majority of the structural rigidity of the plant. In annual plants lignin is less abundant than in perennials. The carbohydrate component is mostly cellulose but hemicellulose is a substantial constituent. Lignocellulosic biomass consists primarily of cellulose, hemicellulose, and lignin, at a typical weight ratio of 50:25:25. Although rice in carbohydrates (cellulose and hemicellulose), lignocellulose is an insoluble substrate with a complex structure, which makes its conversion into fermentable sugars and subsequently into ethanol difficult. Nevertheless, using cellulose-hydrolyzing enzymes (cellulases) and ethanol – producing microorganisms, cellulose and hemicellulose can be converted first to fermentable sugars (glucose and xylose, respectively) and eventually to ethanol. Cellulose (40-60% of the dry biomass) is a linear polymer of glucose, the orientation of the linkages and additional hydrogen bonding make the polymer rigid and difficult to break. In hydrolysis the polysaccharide is broken down to free sugar molecules by the addition of water. The product, glucose is a six-carbon sugar or hexose. It provides the major source for hexose in woody biomass. Unlike cellulose, hemicellulose has a random and amorphous structure and is easily hydrolyzed by dilute acid or base. Lignin is the third major component in wood and comprises the glue that protects woody biomass from foreign invasion. It is mainly composed of phenolic units. It represents the part of biopolymer that cannot be converted into ethanol

directly or indirectly using the current technology.
Excluding lignin, the optimistic situation is to preserve and utilize all the carbohydrates. Pretreatments constitute the means to separate carbohydrates and lignin. It disrupts the crystalline region of these materials. On the same hand it should also downgrade carbohydrates as little as possible. Different pretreatment methods have been explored in order to achieve the optimistic situation (Taherzadeh and Karimi, 2007). In principle, the sugar chains can be hydrolyzed to monomeric sugars, most of which can be fermented to alcohol using yeast. Research is being done to improve the efficiency of transforming these sugars to ethanol. The main difference between process alternatives is the hydrolysis, which can be acid hydrolysis, either dilute or concentrated, or enzymatic hydrolysis (Galbe and Zacchi, 2002). To help the enzymes to perform well and degrade the lignocellulose efficiently, the fibres in the raw material need to be accessible to the enzymes. A pretreatment in some way is needed to expose the fibres. If the pretreatment is too harsh, liberated sugars can be degraded to enzyme- and yeast-inhibiting compounds lowering the overall yields. On the other hand, if too weak pretreatment conditions are used this will result in low enzyme accessibility and the same drawbacks (Sendelius, 2003). Industrially, the pretreated material is mainly thought to be hydrolyzed and fermented in two different steps: separate hydrolysis and fermentation (SHF); or in one single step: simultaneous saccharification and fermentation (SSF).

Enzymatic hydrolysis has been thought to have the potential for higher yields and reduced formation of toxic compounds. To date, a number of pretreatment technologies have been proposed and investigated, yet mostly on a laboratory scale. Some of the most widely recognized techniques include steam explosion, alkaline treatment, treatment with sulphur dioxide, organosolve pretreatment, treatment with hydrogen peroxide, treatment with supercritical ammonia, dilute acid pretreatment and the ammonia freeze explosion process. Part of the reason is that the cellulose is well protected by hemicellulose and lignin (Oh *et al.,* 2002). Pretreatment is one of the necessary elements in the biomass – to – ethanol conversion process. The primary purpose of pretreatment is to make the cellulosic biomass amenable to the action of the cellulase enzyme. Agro wastes can be used as a fermentation feedstock only after being subjected to an effective pretreatment. Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields. Pretreatment affects the structure of biomass by solubilizing hemicellulose, reducing crystallinity and increase the available surface area and pore volume of the substrate. Pretreatment has been considered as one of the most important processing steps in biomass to fermentable sugar conversion (Gupta, 2009). An ideal pretreatment should fractionate the biomass into three main streams: cellulose, hemicellulose and lignin. To qualify as effective, the pretreatment must meet the following criteria: 1) maximize fermentable sugar yields, 2) avoid, or minimize degradation of carbohydrates, 3) avoid, or minimize the formation of microbial growth-inhibiting byproducts, and 4) be energetically, and most importantly, economically efficient. In simpler terms, the purpose of a pretreatment is to breakdown the lignocellulosic structure to its monosaccharide components for use as fermentation substrates (Oh *et al*., 2002). It therefore entails pretreatment processes that expose cellulose in such materials or modify the pore structures so that enzymes can penetrate into fibers and hydrolyze cellulose more readily. After pretreatment, the hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved faster and with greater yields. In the present study, various agrowastes were collected, processed and used for the pretreatment and saccharification experiments. The study was focused on determining the efficiency of acid and alkali pretreatment on the release of glucose from various cellulosic agrowastes. The released glucose is now readily available for bioethanol production.

MATERIALS AND METHODS

Collection of cellulosic agrowastes

The various cellulosic agrowastes used in the present study were collected from various mills and fields, Coimbatore, stored in sealed containers, transported to the laboratory and stored at room temperature until usage. The agrowastes include various cotton industry wastes such as sweeping waste, dropping waste, gutter waste, comber noil waste, V X L flat strips, mud waste and hard waste; banian waste, cotton plant waste, sugarcane bagasse, ground nut shell, forest leaf litters, paddy straw, corn straw and corncobs.

Substrate purification

All the cotton wastes were processed mechanically to reduce the length of fibres and remove the debris material contained in it. The fibers were then cleaned by hand to remove impurities, such as seed fragments. They were conditioned at 65% relative humidity and 21°C for 24 hours prior to pretreatment. All the other wastes were cleaned by hand, cut into pieces of small size for the study. The amount of cellulose present in each agrowastes was studied by estimating the total cellulose content by anthrone method.

Estimation of cellulose by anthrone method

Cellulose, a major structural polysaccharide in plants, is the most abundant organic compound in nature, and is composed of glucose units joined together in the form of the repeating units of the disaccharide cellobiose with numerous cross linkages. The amounts of cellulose present in the agrowastes were determined by subjecting to digestion, followed by estimation by anthrone method.

Estimation of glucose by DNSA Method

Sugars with reducing property (arising out of the presence of a potential aldehyde or keto group) are called reducing sugars. The glucose released was estimated by DNSA method and the yields of glucose from various agrowastes were compared.

Effect of pretreatment of substrate: Physicochemical treatment

After analyzing the broad physical and chemical compositions of the raw cotton wastes, the substrates were pretreated using physicochemical treatments and then subjected to enzymatic

Table 1. Cellulose and glucose estimation in various raw agrowastes

S. No.	Agrowaste	Amount of cellulose (mg / g waste)	Amount of glucose (mg / g waste)
1	Sweeping cotton waste	750	60
2	Dropping cotton waste	800	80
3	Comber noil waste	920	56
4	Gutter cotton waste	950	63
5	V X L flat strips waste	700	71
6	Hard waste	960	3
7	Mud waste	840	36
8	Banian waste	960	2
9	Cotton plant waste	600	89
10	Groundnut shell waste	940	120
11	Corn cobs	610	33
12	Corn straw	580	94
13	Paddy straw	540	52
14	Forest leaf litters	980	58
15	Sugarcane bagasse	860	67

Figure 1. Agrowaste used in the present study

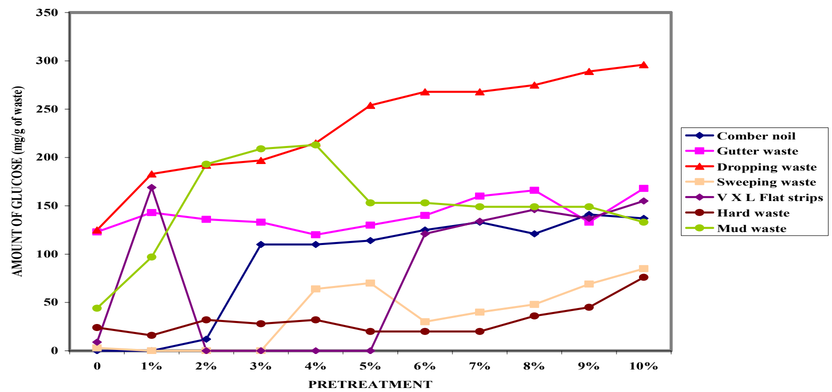

Figure 2. Estimation of glucose in alkali (NaOH) pretreated cotton wastes

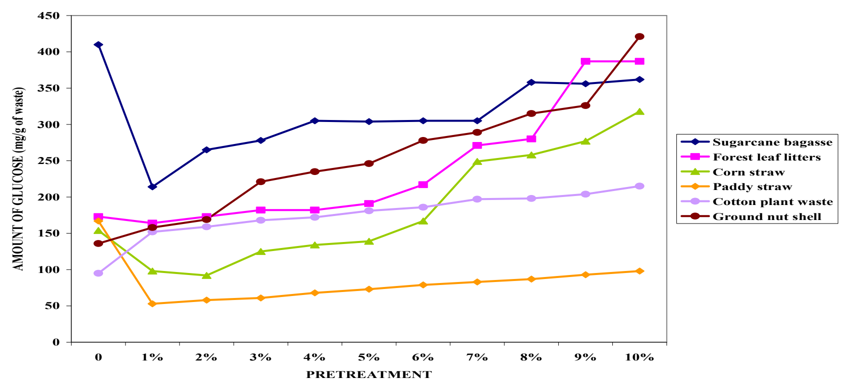

Figure 3. Estimation of glucose in alkali (NaOH) pretreated agro wastes

hydrolysis to release the sugars using enzymes produced and standardized under laboratory conditions.

Acid pretreatment

About 50 ml of dilute sulphuric acid was prepared with a concentration range of 0 - 10% in separate 100 ml Erlenmeyer flasks. The flasks were added with 0.2g of processed cotton separately and autoclaved at 121°C for 30 minutes. The flasks containing the pre-treated waste were then neutralized with distilled water. The acid insoluble residues were dried for further use.

Alkali pretreatment

About 50 ml of sodium hydroxide was prepared with a concentration range of 0 - 10% in separate 100 ml Erlenmeyer flasks. The flasks were added with 0.2g of processed cotton separately and autoclaved at 121°C for 30 minutes. The flasks containing the pre-treated waste were then neutralized with distilled water. The alkali insoluble residues were dried for further use. After pretreatment trials, all the samples were subjected to glucose estimation as given in section 2.4.

RESULTS AND DISCUSSION

Collection of agrowastes and substrate purification

out. The agrowastes were processed mechanically to remove the impurities and conditioned before pretreatment. Various agrowastes used in the study are presented in figure 1.

Estimation of cellulose and glucose

The amount of cellulose as estimated by anthrone method present in each agrowaste and the amount of glucose as estimated by DNSA method present in each waste are presented in table 1. It was clear that the amount of free glucose present in the agrowastes were minimum compared to the amount of cellulose present in the wastes. The amount of glucose present in the untreated wastes showed that groundnut shell waste had maximum glucose compared to all other wastes.

Estimation of glucose in pretreated agro wastes

The amount of free glucose in the pretreated agrowastes was estimated after treating the various agrowastes with varying concentration of acid and alkali. The results showed that compared to the alkali pretreated agrowastes; the acid treated agrowastes did not have any effect on the release of glucose upon pretreatment. Except for the bannian wastes, all the other wastes showed better glucose release for the alkali-pretreated wastes. The amounts of glucose released from the alkali-pretreated agrowastes are presented in the figures 2 and 3.

maximum glucose release upon pretreatment with alkali. Also, it was showed that with increase in the concentration of alkali there was increase in the amount of glucose released except for mud waste and gutter waste.

The result of the figure 3 showed that there was gradual increase in the glucose release with increase in the alkali concentration. For most of the wastes studied, 10% alkali treatment was found to be best compared to other lower concentration of alkali. Groundnut shell waste showed maximum glucose release (387 mg glucose / g of agrowaste) at 10% alkali treatment. But the pretreatment studies of sugarcane bagasse revealed that maximum glucose was released for the treatment without any acid or alkali. Production of ethanol is historically a well-known process and in principal it is carried out by fermentation of plant sugars into ethanol using strains of yeast. However, plant biomass is made of polymers of sugars, ordered in a matrix called lignocellulose, which is not as easily fermented. In order to produce ethanol, this material must undergo degradation to for the yeast more accessible components, e.g. mono- and dimers of sugars. This degradation can be made by hydrolysis of biomass using added enzymes, called enzymatic hydrolysis (EH). To help the enzymes to perform well and degrade the lignocellulose efficiently, the fibres in the raw material need to be accessible to the enzymes. A pretreatment in some way is needed to expose the fibres. If the pretreatment is too harsh, liberated sugars can be degraded to enzyme- and yeast-inhibiting compounds lowering the overall yields. On the other hand, if too weak pretreatment conditions are used this will result in low enzyme accessibility and the same drawbacks (Hinman *et al*., 1992).

Gupta (2009) concluded that pretreatment is the first step required to fractionate lignocellulosic materials into its major plant components of lignin, cellulose and hemicellulose. The mechanisms by which pretreatments improve the digestibility of lignocellulose are however not well understood. An important goal of pretreatment is to increase the surface area of lignocellulosic material, making the polysaccharides more susceptible to hydrolysis. Along with an increase in surface area, pretreatment effectiveness and hydrolysis improvement has been correlated with removal of hemicellulose and lignin and the reduction of cellulose crystallinity. Some workers carried out H_2SO_4 pretreatments in a Batch reactor at 121°C. The treatment times were from 30 to 120 min with acid concentrations ranging from 0 to 2% (w/v) at a solid concentration of 5% (w/v). The results showed that performance of H_2SO_4 pretreatment (hemicellulose recovery and cellulose digestibility) was significantly better than that obtained with H_3PO_4. Alkali (NaOH, $Ca(OH)_2$, NaOH-urea, Na_2CO_3) hydrolyses of rice straw (Carrillo *et al*., 2005); spruce wood waste (Zhao *et al*., 2007); sugarcane, cassava and peanuts wastes (Thomsen and Belinda, 2007); corn cob (Torre *et al*., 2008); organic fraction of municipal solid waste (Torres and Lloréns, 2008) have been investigated. When these pretreatments are performed by using 0.5 - 2 M alkali at 120 - 200°C, they substantially facilitate saccharification and improve enzymic hydrolysis of lignocellulosic wastes (Mtui, 2009).

CONCLUSION

environmental pollution. Their conversion into useful products may ameliorate the problems they cause. These wastes which include cereals, straw, leaves, corncobs etc are highly underutilized. Cellulose which forms about 40-50% of plants' composition is the most abundant organic matter on earth. Proper biotechnological utilization of these wastes in the environment will eliminate pollution and convert them into useful by-products. In the present study, various cellulosic agrowastes were collected, processed and the amount of cellulose and free glucose present in these agrowastes were studied. The effect of acid and alkali pretreatments were studied after treatment by measuring the amount of free glucose released upon hydrolysis. The results showed that maximum glucose release was found in groundnut shell waste (421 mg / g of waste), followed by forest leaf litters and sugarcane bagasse. Also, the alkali pretreatment was better compared to the acid pretreatment in the glucose release. The released glucose will serve as an effective substrate for various processes like biofuels production.

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