Performance Evaluation of Pre-treatrment Techniques for Bio-butanol Production Using *Arundo donax*

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Abstract: Energy crisis is one of the major global issues of today's world, this can only be addressed by the development of alternative renewable and sustainable forms of energy. Bio-fuels derived from agriculture residue (lignocellulosic feedstock) can be an attractive alternative to fossil fuels. Biobutanol is one such promising liquid fuel, which can be used as gasoline substitute without any change in engine. In the present work various pretreatment techniques has been applied to lignocellulosic biomass of Arundo donax to maximize the availability of fermentable sugar into the filtrate for microorganism. Several pretreatment agents like acid (H_2SO_4), alkali (NaOH), sonication, acids-alkali combine and high temperature has been employed to optimize the pretreatment condition by varying different parameters viz., concentration of acid (1 – 6%) & alkali (1 - 6%), pretreatment time (1 – 6hr), sonication frequency (20 – 60Hz), temperature (50 - 200°C) etc. Maximum 54.4% of cellulose release has been found from cellulosic material treated with 6% NaOH followed by steam hydrolysis for 6 hr.

Keywords: Biofuel, Biobutanol, ABE-fermentation, Arundo donax, Pretreatment techniques.

1. INTRODUCTION

Environmental concerns related to increasing atmospheric carbon dioxide levels and rising average temperatures in recent years have increased concerns regarding use of fossil fuels. For the growth of all the industrial sectors energy is one of the most important input. Lavish modern lifestyle also increases the global demand for energy. According to World energy, technology and climate policy outlook report published in 2003 by the European Union, given the continued dominance of fossil fuels, World CO_2 emissions are expected to increase more rapidly than the energy consumption (2.1% / yr on an average). In 2030, world CO_2 emissions are expected to be more than twice the level of 1990.

These factors have created realization throughout the world that the petroleum sources which are non-renewable are limited and are being consumed at an alarming rate. All these weak factors have increased the interest in alternatives, renewable, sustainable and economically viable energy resources that mitigate the detrimental environmental effects of fossil fuels use [1, 2].

Biodiesel, Bioethanol, biogas and Biobutanol are variety of biofuels which have high potential for replacing petroleum based fuels including. Biofuels are mostly derived from biomass which is abundantly and widely available. Biomass remains to be the oldest sources of energy with very specific properties which can fulfill the mankind's demand for hydrocarbons for manufacturing goods ranging from plastics and chemicals to biofuels and other products usually being derived from modern petroleum refinery.

Biomass has always been a major source of energy for mankind and is presently estimated to contribute to the order 10-14% of the world's energy supply. Among the various products which can be derived from biomass, bio-fuels are considered to be the most important as they are an alternative to petroleum fuels. The processes that convert biomass to energy fall into three major categories. Thermochemical processes such as combustion, pyrolysis, gasification and liquefactions. Biochemical processes include anaerobic digestion and ethanol production. Chemical processes convert organic oils into biodiesel and lubricants.

Comparative evaluation of different pretreatment methods of *Arundo donax* for Biobutanol Production is main objective of this paper. Lignocelluloses are composed of cellulose, hemicellulose and lignin this makes it the most complex polymer. Before the conversion from biomass to butanol, pretreatment process has a crucial role to play, breaking down the hemicellulose part in order to make the accessibility of cellulose much easier [3].

The pretreatment of cellulosic and lingo-cellulosic materials to make them hydrolysable by acid/alkali or enzyme is major limitation in the commercialization of lingo cellulosic based butanol. Efficiency and complexity has so far limited the production of biobutanol from biomass. An effective and economical pretreatment would accomplish the following requirements: release of reactive cellulosic fiber for enzymatic attack, reduction in the destruction of hemicellulose and cellulose, decrease in the formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms, minimization of energy demand, reduction of costs of the feedstock, reduction of costs of material for construction of pre-treatment reactors, production of less residues, consumption of little or no chemicals and utilization of cheap chemicals [4].

A multitude of different pretreatment methods have been suggested during the past few decades. They can be divided into different categories: physical (e.g. milling, grinding and irradiation), chemical (e.g. alkali, dilute acid, oxidizing agents and organic solvents), Physicochemical (e.g. steam pretreatment/auto hydrolysis, hydrothermolysis & wet oxidation) and biological, or combinations of these [5 - 8]. In order to hydrolyze lignocellulosic biomass with enzymes successfully, it is very important to use a suitable pretreatment, because crystallinity of cellulose, degree of polymerization (DP), moisture content, available surface area, and lignin content are factors that hinder the attack of enzymes [9]. Certain kinds of chemical, physical, and biological pretreatments remove lignin, reduce the degree of cellulose crystallinity, and increase the surface area of biomass, resulting in an enhancement of lignocellulosic substrate digestibility [5].

In the present study *Arundo donax* was subjected to various physical and chemical pretreatment techniques in order to identify effective pretreatment technique and the relative importance of individual pretreatment on the rate of enzymatic hydrolysis of the lignocellulose.

2. METHODS & MATERIAL

Experiments were repeated thrice and the arithmetic mean value has been reported.

2.1. Collection and preparation of lignocellulosic biomass

Biomass was collected from agricultural fields from in and around Rajatalab, Varanasi (**Latitude**: 25°15'50"N; **Longitude**: 82°50'43"E). Lignocellulosic biomass of *Arundo donax* mainly contains 30% hemicellulose, 31 - 36% cellulose and 21% insoluble lignin (dry weight basis) [10]. Collected biomass was first dried at 70 ± 2°C for 24 hr and then ground and sieved to get the desired particle size (50 mesh) for effective pretreatment of the material.

2.2. Characterization of lignocellulosic biomass

Some Physico-chemical characterization of lignocellulosic material was done using standard protocol [11]. Characterization of biomass was done in terms of bulk density, solid density, moisture content, ash content and volatile matter content. Fourier Transform Infrared (FTIR) spectroscopy of the pretreated samples has been performed to evaluate the effect of different pretreatment agents.

2.3. Pretreatment of lignocellulosic material

Butanol production from lignocellulosic wastes mainly involves three steps: pretreatment, hydrolysis and fermentation [12]. *Arundo donax* biomass was subjected to acid (H_2SO_4), alkali (NaOH), sonication, acid-alkali pretreatments etc.

2.3.1. Temperature Pretreatment. Temperature treatment is one of the simplest approaches that do not require addition of chemicals. High lignin solubilization has been found in water at higher temperature (e.g. 180°C) [13]. In this context treatment of cellulosic biomass has been performed by varying the temperature in the range of 50 – 200°C by adding 0.5 g of biomass into 50 ml of distilled water for 1 hr. The amount of the residual cellulose was analyzed spectrophotometrically using UV-Vis spectrophotometer (SL 210, ELICO Double Beam UV-VIS Spectrophotometer) at 620 nm following standard protocol [14].

2.3.2. Acidic Treatment. In the present study dilute acid (H_2SO_4) pretreatment of cellulosic biomass has been tested with and without steam hydrolysis. Hydrolysis is the step that increases the availability of fermentable sugar into the filtrate. Acidic treatment of biomass was performed by varying the H_2SO_4 concentration in the range 1 – 6%. 0.5 g of cellulosic biomass was soaked in 50 ml of H_2SO_4 separately and incubated at room temperature (37°C) for 6 hr. Half part of the treated sample was subjected for steam hydrolysis at 121°C for 20 min. The samples were collected and washed with distilled water until neutral pH was achieved then the sample was filtered and dried at 70°C for 24 hr. Residual cellulose content was analyzed spectrophotometrically at 620 nm.

2.3.3. Alkaline Treatment. Among the various bases available, NaOH was used because it increases the porosity and internal surface area of the material by breaking complex polymeric structure & lignin [15]. For alkaline pretreatment various aqueous solution of NaOH differing in concentration in the range of 1 - 6% (w/v) were prepared. Equal amount (0.5 g) of powdered lignocellulosic material was added to each flask containing 50 ml of NaOH solution for 6 hr at room temperature (37±2°C). Further processing of the sample was done in the same manner as mentioned in the section 2.3.2.

2.3.4. Acid-Alkali Treatment. Combined acid-alkali treatment of powdered lignocellulosic substrate has been attempted to enhance the release of fermentable sugar with and without the steam hydrolysis of the treated sample. 0.5 g of powdered biomass was mixed in 50 ml of 6% H_2SO_4 and incubated for 2 hr followed by treating with 6% NaOH for 5 hr at room temperature (37±2°C). The residue was collected and washed extensively with distilled water and dried at 70±2°C for 24 hr. residual cellulose content was analyzed as mentioned above.

2.3.5. Effect of Sonication. Ultrasound affects the physical and chemical structure of lignocellulose by producing sonochemical and mechano-acoustic effect [16]. Ultrasound produces cavitation and heating at low frequency, the powerful vibrations severely damage the biomass structure which increases the yield of hydrolysis [17]. 0.5 g of the powdered lignocellulosic biomass of 50 mesh size was mixed with 50 ml of distilled water and then subjected to sonication

(20 – 60 Hz) for 15 – 60 min. Material was filtered, washed and dried at 70°C for 24 hr and residual cellulose content was analyzed spectrophotometrically.

3. RESULTS & DISCUSSION

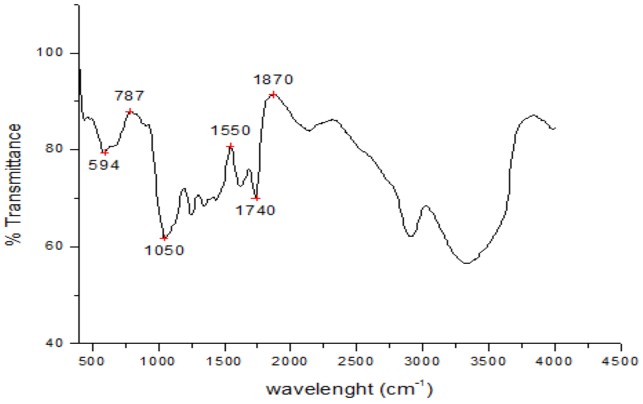
3.1. Characterization of lignocellulosic biomass

The Physico-chemical characterization of lignocellulosic biomass has been performed in terms of bulk density, solid density, moisture content, ash content and volatile matter. Data are shown in Table 1.

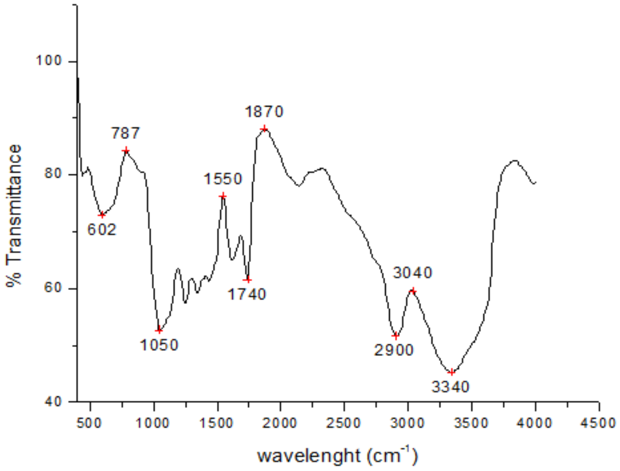
Table 1: Characterization of lignocellulosic biomass

Properties	**Biomass**
Bulk density	135.77 Kg/m^3
Solid density	1279.51 Kg/m^3
Moisture content	3.9%
Ash content	3.0%
Volatile matter	74.1%
Fixed Carbon content	19.0%

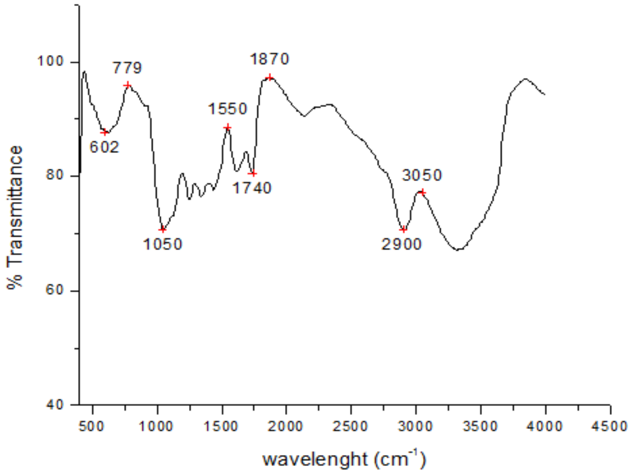
The FTIR spectroscopy has been used to analyze the variations introduced due to the treatment of different pretreatment agents on the chemical structures of cellulosic biomass by examining the changes in vibrational frequency. All spectra were dominated by the peaks at 787, 1050 and 1740 cm^{-1} that correspond to the =C-H bending, C-O and C=O stretching in lignocellulosic material respectively. Figures 1(a) – 1(d), represent FTIR spectra of raw biomass, heat treated, acid treated and alkali treated biomass respectively. Peak value 1550 cm^{-1} shows the presence of lignin in all the material (raw and pretreated biomass). Alteration of position of certain peaks can be observed in all the FTIR spectra. Fig. 1(b) – 1(d) shows certain new peaks in between 2900-3050 cm^{-1} and at 3340 cm^{-1} that confirms the presence of C-H and O-H stretching respectively that is not present in the raw lignocellulosic biomass.



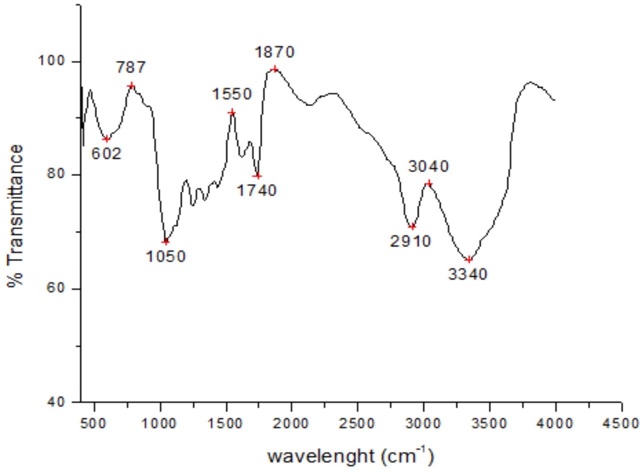
(a)



(b)



(c)



(d)

Fig. 1. FTIR spectra of lignocellulosic biomass: (a) raw biomass, (b) heat treated, (c) acid treated, (d) alkali treated biomass.

3.2. Pretreatment of lignocellulosic material

3.2.1. Temperature Pretreatment. Fig. 2 represents the effect of temperature ranging from 50 to 200°C on the cellulose release from biomass. From Fig. 2, it is clear that the percentage cellulose released is almost same for 150 and 200°C. Maximum 17.67% of cellulose was released at 200°C that is very less so that other pretreatment agents have been used to maximize the cellulose release that can be further processed and utilized for butanol production. Lower cellulose release at this temperature may be due to the less lignin break down.

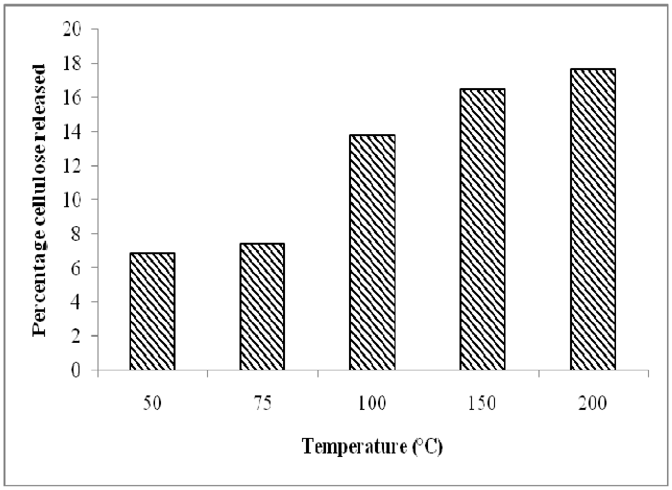


Fig. 2. Percentage cellulose released considering temperature as parameter.

3.2.2. Acidic Treatment. To overcome corrosive nature of conc. acid lower acid (H_2SO_4) concentration was selected ranging from 1 – 6%. From Fig. 3, it is evident that percentage cellulose release is higher with 6% H_2SO_4 treatment followed by steam hydrolysis. Maximum 40.02% cellulose release has been found for this case.

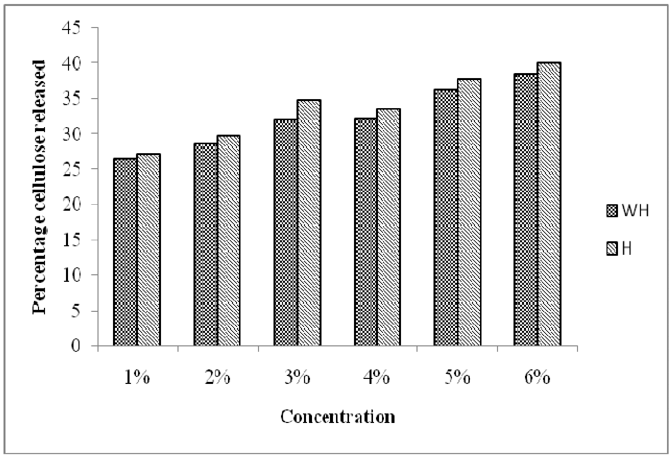


Fig. 3. Percentage cellulose released considering concentration of acid as parameter. WH: without steam hydrolysis; H: Steam hydrolyzed.

3.2.3. Alkaline Treatment. For the treatment of lignocellulosic biomass, alkali (NaOH) concentration has been varied from 1 – 6 %. Higher cellulose release with increasing conc.of alkali can be observed from Fig. 4. Maximim 52.04% cellulose release was obtained from 6% NaOH treatment followed by steam hydrolysis.

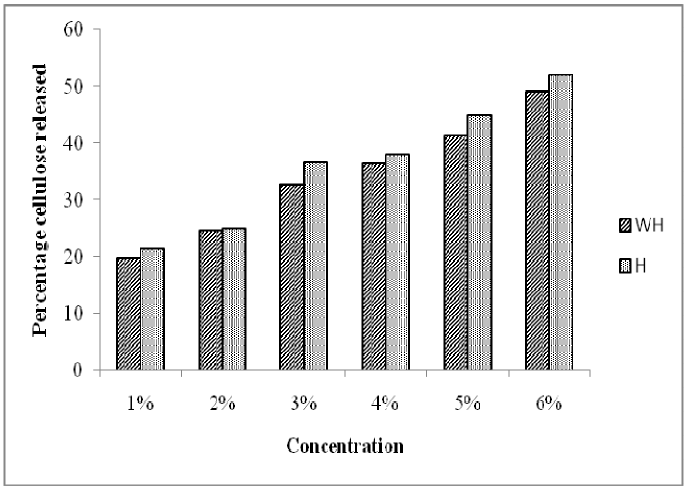


Fig. 4. Percentage cellulose released considering concentration of alkali as parameter.

3.2.4. Comparative study of Acid & Alkali pretreatment considering treatment time as parameter. Fig. 5 shows the effect of treatment time on the cellulose release when the material was treated with 6% acid (H_2SO_4) and 6% alkali (NaOH) separately followed by steam hydrolysis. From figure it is clear that alkali treatment of the lignocellulosic biomass is more efficient than the acid treatment. Maximum 54.5% of cellulose release was found with 6% NaOH in 6 hr.

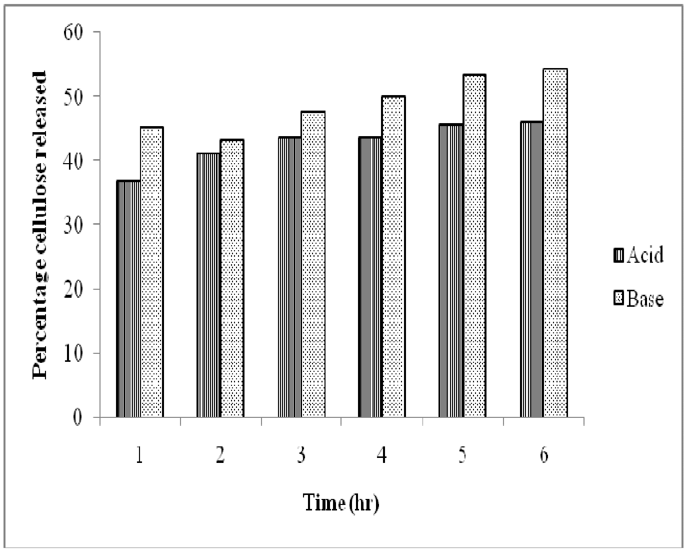


Fig. 5. Percentage cellulose released considering treatment time as parameter.

3.2.5. Acid-Alkali Treatment. Treatment of lignocellulosic biomass with the combination of acid-alkali is another approach to enhance the release of cellulose in the form of fermentable sugar into the filtrate. Maximum 54.87% cellulose release has been found when the material was treated with 6% acid-alkali followed by steam hydrolysis.

3.2.6. Effect of Sonication. Disruption of lignocellulose using ultrasonic frequency affects the chemical structure of the material. Effect of sonication frequency ranging from 20 to 60 Hz on cellulose release has been analyzed. Percentage cellulose released is almost same for 30, 50 and 60 Hz frequency i.e. 23.67%, 24.01% & 24.89% respectively. Less variation has been found with increase in frequency after 30 Hz, thus this frequency has been selected for further study. Fig. 6 shows the effect of treatment time ranging 15 – 60 min with sonication frequency 30 Hz. Maximum 26.87% cellulose release has been found after 60 min.

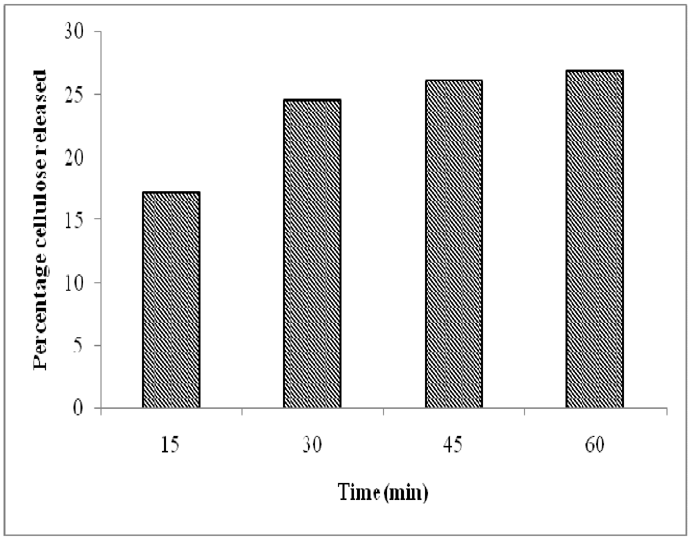


Fig. 6. Percentage cellulose released considering sonication treatment time as parameter.

4. CONCLUSION

Feedstock selection is the most significant parameter for butanol production due to its economic feasibility. It is advantageous to use lignocellulosic biomass as raw material for butanol production because of the rich biodiversity. Lignocellulosic materials are sustainable and renewable source of feedstock. Various pretreatment techniques such as acid (H_2SO_4), alkali (NaOH), sonication, acid-alkali combine and high temperature has been used. The investigation reveals that out of selected pretreatment techniques, alkali treatment followed by steam hydrolysis is a better option. The combination of acid/alkali treatment followed by steam hydrolysis gives nearly similar results. However sonication for the pretreatment of *Arundo donax* is not a viable alternative.

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