Novel Pretreatment Methods to Improve Enzymatic Saccharification of Sugarcane Bagasse: A Report

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ABSTRACT: *A lot of recent research in the biomass sector is focusing on how to improve the efficiency of biomass resources. Pretreatment of biomass resources is a novel approach and has gained a lot of attention in the last decade. A review of modern methods and the latest technologies of enhancing the enzymatic scarification of sugarcane bagasse are presented in this work. This paper looks at the very recent developments in this field. Use of such advanced methods as coupling ionic liquid pretreatment with supercritical fluids and ultrasound, irradiation is taking us swiftly towards the ultimate goal which is achieving 100% yield at minimum cost with no adverse environmental effects. However, Optimum process conditions for these methods are yet to be discovered. There is a need to optimize the processes and learn completely about the reaction mechanism in order to shift from lab scale to pilot scale and ultimately to the industrial level. An effort is made to report the latest work and because of it, this paper contains about 95% citations from papers within the last five years. In the end, useful recommendations are given in the conclusion section.*

KEYWORDS: *Pretreatment methods; Sugarcane bagasse; Enzymatic saccharification; Ionic liquids; Ultrasound irradiation; Microwave irradiation.*

INTRODUCTION

The hydrolysis of polysaccharides to soluble sugars is called "saccharification". In the past, lignocellulosic material was broken down into monosaccharides by using acid-catalyzed or alkali-catalyzed hydrolysis. Acid hydrolysis can be done in two ways; using dilute acid or concentrated acid. Dilute acid hydrolysis produces very low sugar yield and requires high size reduction of feedstock for adequate acid penetration. Using concentrated acid hydrolysis removes these demerits but handling and recovery become a problem. Corrosion is also a major drawback of acid hydrolysis. Alkali hydrolysis results in efficient removal of lignin and low inhibitor formation but this technique is costly and results in altering the lignin structure. However, it causes the corrosion of gas equipment and produces byproducts that inhibit subsequent fermentation. Enzymatic hydrolysis is the unit operation in the lignocellulose conversion process that utilizes enzymes to depolymerize lignocellulosic biomass. Enzymatic hydrolysis has attracted interest because it could overcome the demerits of acid and alkali catalyzed hydrolysis. Enzymatic hydrolysis of cellulose happens

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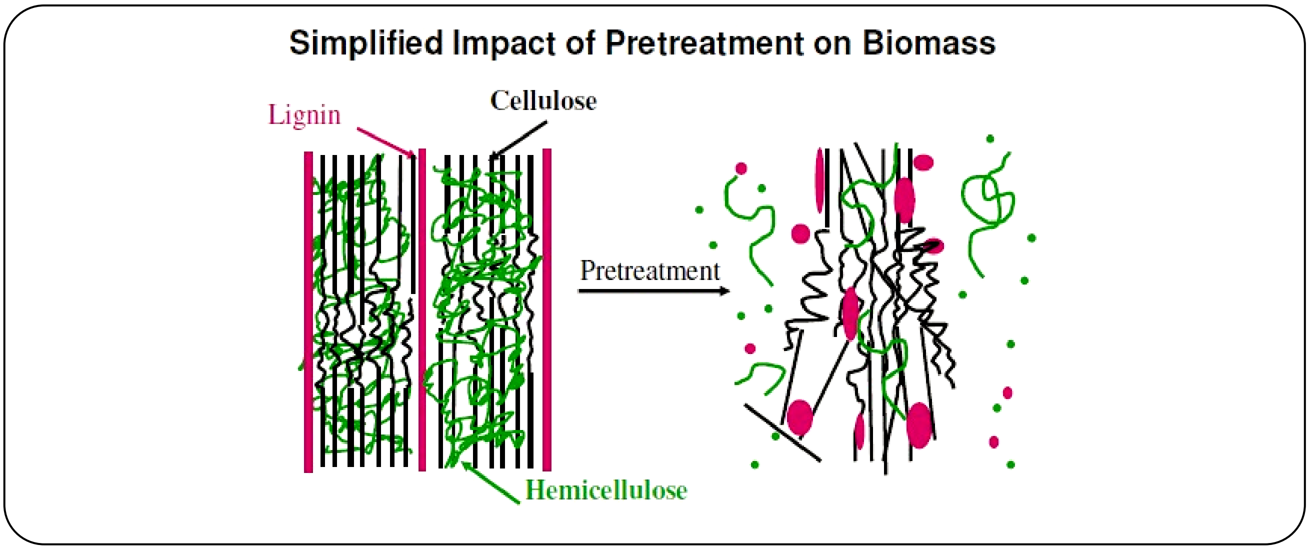

Fig. 1: Loosening of structure on pretreatment [5].

by a complex system of reactions involving several steps which are influenced by the mode of interaction between the cellulases and the cellulose fiber, nature of the cellulases employed, enzyme vulnerability to product inhibition as well as structural features of cellulose. However, there are still some drawbacks such as a slow reaction rate and the limited enzymatic accessibility to polysaccharides. Pretreatment is a necessary step required to open the cell wall structure of biomass, which would increase enzymatic accessibility during enzymatic hydrolysis. A suitable pretreatment method should avoid the size reduction of biomass particles, preserve the hemicellulose fraction, avoid the formation of inhibitory by-products and be cost-effective. The enzymatic digestibility of cellulose present in cellulosic biomass is hindered by many compositional and structural factors [1, 2]. Fig. 1 shows a simplified impact of pretreatment on biomass. These methods alter the physical and chemical makeup of the biomass material by loosening the cellulose and hemicellulose and lowering the crystallinity. Fig. 2 shows the difference in the microscopic structure of treated and untreated sugarcane bagasse. They also enhance the specific surface area of biomass and improve the rate of reaction. This significantly increases the accessibility of enzymes and as a result, enhances glucose yield. Fig. 3 shows an electron microscope image of how to roll milling destructs the structure of sugarcane bagasse. This paper will focus only on the modern pretreatment methods employed to improve the enzymatic hydrolysis of sugarcane bagasse. Composition of sugarcane bagasse is shown in Table 1.

Numerous physical, biological, physicochemical and chemical pretreatment methods were developed from the early 1980's till the start of the millennium but most of them were not cost effective. This was mainly due to the lack of predictive pretreatment models needed for optimization as well as the economic feasibility of processes [4]. Conventional pretreatment methods such as using an alkali or acid are becoming out of fashion due to high energy input, low efficiency, and environmental hazards. New pretreatment methods are being investigated which are energy efficient, cost-effective as well as environment friendly. Pretreatment techniques discussed in this paper include ionic liquid, ultrasound, microwave irradiation, supercritical fluids as well as a combination of two or more of these techniques.

IONIC LIQUIDS

Ionic liquids are salts having melting point temperature below 373K. They have low volatility, high thermal as well as chemical stability, excellent solubility, non-flammability and ease of recovery. Physicochemical properties of these ionic liquids, i.e. viscosity, polarity, density, melting point, and solubility vary a lot which

Makes them extremely useful [6-11]. They can also be recycled easily by flash evaporation.

Ionic liquids as green solvents have been studied extensively [12, 13]. Their application in the chemical

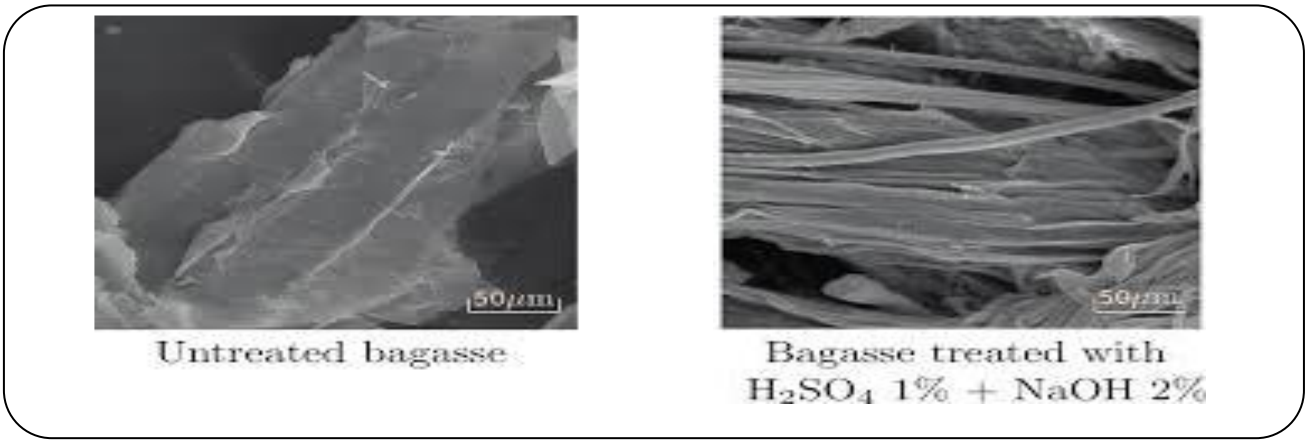

Fig. 2: Showing microscopic changes in untreated and treated sugarcane bagasse [18].

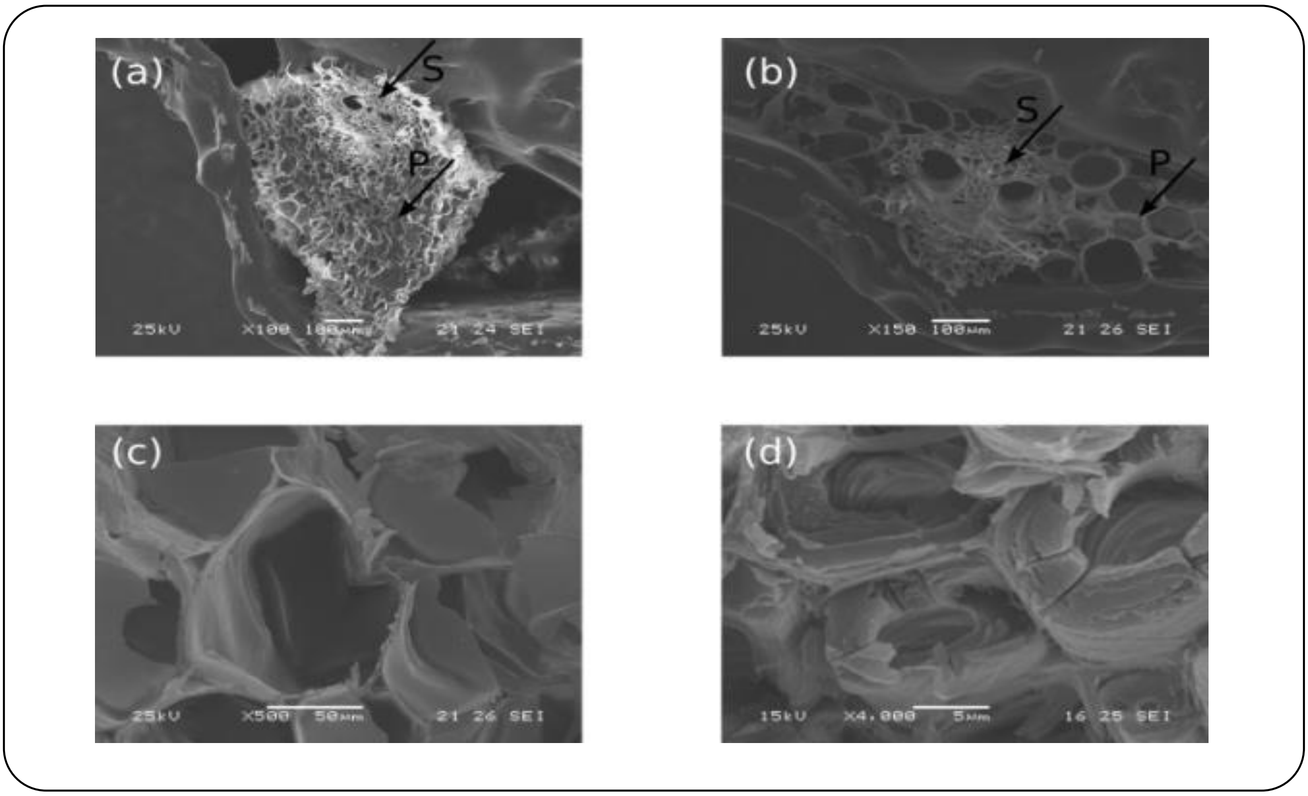

Fig. 3: Scanning electron microscopy images obtained from fractures of sugarcane bagasse undergoing roll milling only [18].

industry are being explored [14]. They have the potential to replace many organic and inorganic chemicals currently employed in most of the industrial applications. Dissolution of lignocellulosic material using ionic liquids was investigated and it was found that 1-allyl-3-methyl imidazolium chloride [Amim][Cl] and 1-ethyl-3-methyl imidazolium acetate [emim][Ac] were the best solvents for cellulose as well as lignocellulosic material [15, 16] Showed that pretreatment of wheat straw by ionic liquid, [emim][dep], can greatly enhance enzymatic hydrolysis. Similar work was reported by [17].

Combination of ammonia and the ionic liquid was employed for pretreatment of rice straw [19] and switch grass [20]. *Li et al.* compared untreated, dilute acid treated and ionic liquid treated biomass based on crystallinity index, rate enhancement, and digestibility (see Table 2). Woody biomass has also been subjected to ionic liquid pretreatment [21-23].

Table 1: Composition of Sugarcane Bagasse (% w/w) [3].

Component	Percentage
Moisture	8.34
Total Solids	91.66
Ash	1.9
Total Nitrogen	0.448
Cellulose	30.2
Hemicellulose	56.7
Lignin	13.4
Total Sugars	0.55
Total Carbon	36.45

**%w/w=Percentage based on a dry weight*

Two imidazolium based ionic liquids have been used in most sugarcane juice pretreatment methods, i.e. 1-ethyl-3-methylimidazolium acetate [Emim][Ac] and 1-butyl-3-methylimidazolium chloride [Bmim][Cl].

Silva et al. Compared six ionic liquids for pretreating sugarcane bagasse and observed that the ability of [Emim][Ac] to significantly reduce the crystallinity and increase the specific surface area was responsible for improved rate and yield of enzymatic saccharification. IL-bagasse ratios, pretreatment temperatures and time were taken constantly for all the ionic liquids used [24]. Structural changes in bagasse when exposed to pretreatment by [Emim][Ac] were investigated by advanced analytical tools such as FTIR, XRD, SEM, and NMR and it was observed that the original cellulose experienced an increase in glucose content from 80.0–83.3% to 91.6–92.8%, a decrease in the degree of polymerization from 974–1039 to 511–521 as well as an increase of surface area during pretreatment [25]. Similar results were reported in [26] and [27]. [28] Studied the effect of recycled [Emim][Ac] on enzymatic hydrolysis of bagasse and found that enzymatic digestibility suffered as the number of recycles increased. *Yoon et al.* Found the optimum values for [Emim][Ac] pretreatment of bagasse by applying Central Composite Design (CCD) of Response Surface Methodology (RSM). Results after modeling showed optimum conditions for pretreatment were 145°C, 15min, and 14wt % of solid loading with an optimum RS (reduced sugar) yield of 69.7% [29].

Zhang et al. and *Jiang et al.* Investigated acid-catalyzed pretreatment of bagasse in aqueous ionic liquid solutions. It was observed that enzymatic digestibility reached 94-100% after 72 hrs. Of enzymatic hydrolysis. Aqueous ionic liquid pretreatment method was observed to be a good way of reducing IL solvent cost. Similar work was reported by [32] with almost identical results [33]. Obtained optimum process conditions for sugarcane bagasse pretreated in 1-butyl-3-methyl imidazolium chloride [Bmim][Cl]. It was determined that without complete dissolution, maximum enzymatic saccharification can be achieved at 150°C and a reaction time of 90 mins. [34] Found the optimum temperature (120°C) and time (2 hours) for aqueous ionic liquid pretreatment of bagasse. Ionic liquid used was 1,3-dimethyl imidazolium dimethyl phosphate [Mmim][dmp]. [35] Observed that regenerated bagasse after NH_4OH-H_2O_2 and ionic liquid combined pretreatment exhibited efficiency of 91.4% after 12 hours of hydrolysis.

Gao et al. Showed that sequence of pretreatment processes had an impact on overall efficiency. It was observed that pretreatment by liquid hot water followed by NaOH gave better results than vice versa [36].

SURFACTANT-ASSISTED PRETREATMENT OF BAGASSE

Surfactant-assisted pretreatment of sugarcane bagasse was performed by [37] by using a combination of Tween 80 (surfactant) and Polyethylene glycol with 1-butyl-3-methyl imidazolium chloride [Bmim][Cl] as an ionic liquid. Tween 80 is a 20 mole ethoxylate of sorbitan mono oleate. 3% w/w PEG was found to be optimum concentration achieving an efficiency of 96.2% after only 12 hours of enzymatic hydrolysis.

COMPLETELY BIO-DERIVED IONIC LIQUIDS

Recent research works have reported the use of completely bio-derived ionic liquids for pretreating sugarcane bagasse. *An et al.* [38] reported that lignocellulosic biomass can be treated with bio-derived ionic liquid. These bio-derived ILs are cheaper, more biocompatible, biodegradable and bio renewable than other imidazolium based ionic liquids. *Ninomiya et al.* [39] showed that pretreatment by a Choline acetate [Cho][Ac], a complete bio derived ionic liquid, presented same results as the one by [Emim][Ac]. *Benazzi* [40] used Choline acetate [Cho][Ac] for pretreating sugarcane bagasse and compared the results

Table 2: Comparison of various pretreatment methods [20].

Pretreatment Method	Crystallinity Index	Initial enzymatic rate (mg/L/min)	Rate enhancement	Enzymatic hydrolysis	
				Reducing Sugar (mg)	Digestibility (%)
Untreated	26.2	0.3 +/- 0.03	-	0.8 +/- 0.1	2.7
Dilute acid	39.1	1.1 +/- 0.1	3.3	13.2 +/- 1.1	47.6
Ionic liquid	2.6	18.5 +/- 1.6	54.4	24 +/- 2.1	96

Table 3: Comparative analysis of pretreatment methods based on energy ratio.

Pretreatment method	Glucose yield	Energy Production (KJ/g-bagasse)	Energy Requirement (KJ/g-bagasse)	EPR
Untreated	0.091	1.38	0.45	3.07
[Cho][Ac] (110°C, 6 hrs.)	0.346	5.25	1.30	4.04
[Cho][Ac] (130°C, 3 hrs.)	0.355	5.38	1.45	3.72
Comminution	0.415	6.29	13.50	0.47
Microwave	0.380	5.77	14.54	0.40
Alkaline	0400	6.06	8.99	0.67

with other pretreatment methods. Pretreatment temperature of 110°C and time of 6hrs. Were used. The conversion was found to be 98.7% after 72 hrs. Of enzymatic hydrolysis. *Asakawa et al.* [40] compared pretreatment by [Cho][Ac], comminution, microwave and alkali based on energy output/ energy input ratio and established that pretreatment by [Cho][Ac] was the most energy saving and cost-effective pretreatment method (see Table 3). It was also reported that ultrasound-assisted Choline acetate pretreatment of bagasse offered better results than with [Emim][Ac]. In addition to this, separation also becomes much easier.

SUPERCRITICAL FLUIDS

Because of its ability to dissolve organic compounds selectively and assist the functioning of enzymes, supercritical CO_2 can be an effective green solvent suitable for biomass pretreatment [41]. *Benazzi et al.* showed that the use of supercritical carbon dioxide increased enzymatic hydrolysis by 280% than without it. *Silvera et al.* [43] reported enhanced glucose yield and enzymatic saccharification by using supercritical carbon dioxide and ethanol. Time for pretreatment was 2 hrs. and the temperature was kept between 110-180°C. Delignification degree was reported at 41%. This process greatly reduces the amount of ionic liquid to be used. Glucose yield reached 70.7% by weight when sugarcane bagasse was exposed to enzymatic hydrolysis for 12 h.

Structural changes during pretreatment of sugarcane bagasse with supercritical fluids need to be explored with analytical techniques. Operating conditions for pretreatment with supercritical fluids have yet not been optimized. Other supercritical fluids also need to be investigated.

ULTRASOUND IRRADIATION

Ultrasound irradiation is gaining an increasing interest in all fields of research. It has been discovered that ultrasound irradiation can increase the rate of reaction by nearly a million fold *Li et al.* and *Karimi et al.* gave a comprehensive review on the use of ultrasound irradiation on the production of ethanol from biomass [45, 46]. *Ninomiya et al.* [47] showed that ionic liquid assisted pretreatment of lignocellulosic biomass is enhanced by ultrasound irradiation in comparison with conventional heating. Ultrasound irradiation has been found to be another promising technique for enhancing enzymatic saccharification of sugarcane bagasse. High intensity ultrasound irradiation of sugarcane bagasse in 1-butyl-3-methyl imidazolium chloride [Bmim][Cl] can catalyze such modification processes as glutarylation [48]. Rate of reaction has a direct relationship with irradiation time. Temperature also has a direct relationship with modification but above 90°C, reverse effect has also been observed [49]. *Benazzi et al.* [42] reported that ultrasound irradiation increased the

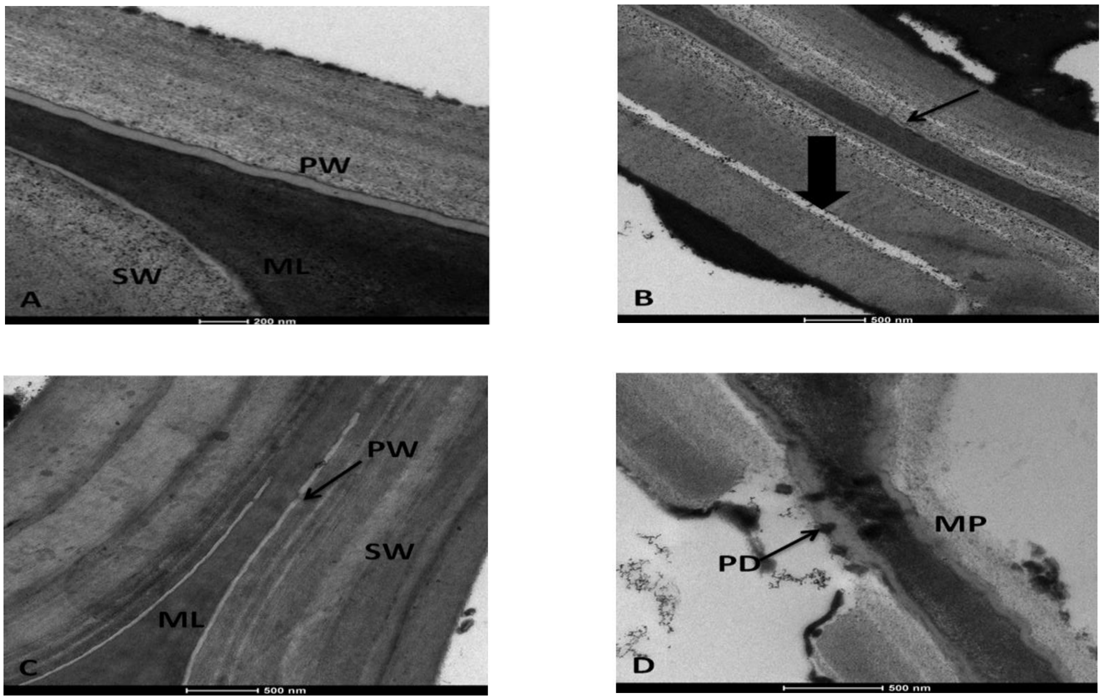

Fig. 4: Electromicrography from TEM of microwave plus glycerol treated sugar cane bagasse [44].

efficiency by 16% while *Lanelli et al.* [50] demonstrated that glucose yield in the presence of ultrasound irradiation doubled when compared to without it. *Ninamiya et al.* [39] showed that ultrasound-assisted bio derived ionic liquid pretreatment of biomass offered much better destruction of cellulose and hemicellulose than by IL alone.

There is a need to optimize ultrasound pretreatment method and explore the structural changes taking place in biomass exposed to ultrasound irradiation to understand the process in a better way. Economic considerations are also stumbling block to the use of this technology on a large scale.

MICROWAVE IRRADIATION

Xu [51] Terms microwave irradiation as one of the most promising methods for biomass pretreatment. Microwave irradiation can directly interact with the material, thereby accelerating chemical, physical and biological reactions. *Binod et al.* [52] showed that combined alkali-acid-microwave treatment of sugarcane bagasse increased yield up to 90%. Microwave heat used was 600 W for 1% NaOH and 450 W for a combined mixture of 1% alkali and 1% acid. However optimum heat values were not explored in the study. *Moretti et al.* [44] studied the structural changes on microwave pretreated bagasse but no effort was made to find optimum parameters. Fig. 4 shows electro micrography taken from TEM of microwave irradiation assisted glycerol pretreated sugarcane bagasse. *Chen et al.* [53] pretreated sugarcane bagasse with dilute sulfuric acid and microwave irradiation. The highest temperature used was 190°C and it was found that as temperature increased, the specific surface area of pretreated biomass became more and more pronounced. However, the optimum value of temperature was not investigated. Time of heating was 5min and 10min and it was reported that increasing time didn’t have a significant impact on increasing efficiency. *Chen et al.* [54] made some progress in understanding structural changes when sugarcane bagasse is exposed to microwave assisted alkali pretreatment by using FT-IR, XRD, HPLC, and TGA. It was found that crystalline cellulose and lignin remained unaffected by pretreatment while hydrolyzed hemicellulose was 80-98%. 0.005M acid concentration was suggested to be optimum value for maximum yield. A lot of research work is still needed to find optimum operating conditions such as heating rate, and temperature. Also, microwave assisted ionic

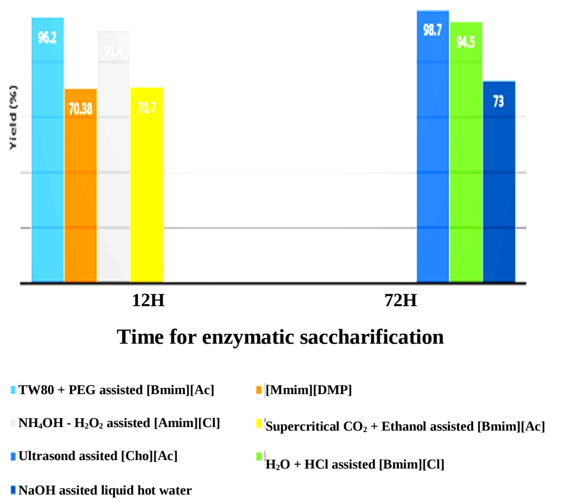

***Fig. 5:** Comparison of pretreatment methods at different enzymatic saccharification times.*

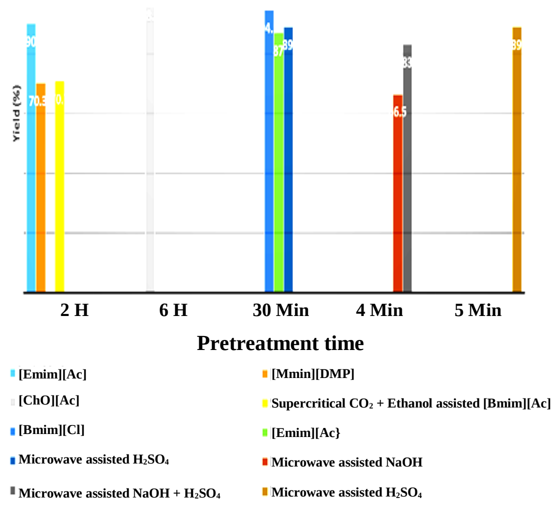

***Fig. 6:** Comparison of pretreatment methods at different pretreatment times.*

liquid pretreatment of sugarcane bagasse is an area yet to be explored.

CONCLUSIONS

Pretreatment is an essential step before sugarcane bagasse is subjected to enzymatic saccharification. A lot of research work is taking place on employing modern methods and technologies to enhance enzymatic saccharification of bagasse. Fig. 5 and Fig. 6 show the comparison of various pretreatment methods on the basis of glucose yield w.r.t enzymatic saccharification and pretreatment times respectively. Following conclusions are made:

- We can safely say that not a single pretreatment method can claim to perform complete delignification of bagasse in an economic & environment friendly manner.
- There is a need to find the optimum operating conditions of these pretreatments methods. Regression analysis should be used more often to find the optimum parameters.
- Lack of understanding of reaction mechanism and non-availability of industrial scale process design are major obstacles in adopting new and innovative pretreatment methods on a large scale.
- There is a need to perform economic feasibility of various pretreatment methods and make a comparison based on yield and economy.
- Only imidazolium based ionic liquids have been used in pretreatment methods so far. Research can be extended to using Pyridinium, Carboxylate and Ammonium based ionic liquids.
- Combination of two or more pretreatment methods can be an option for the future.
- Sequence of pretreatment processes can have a significant effect on efficiency. Another promising research area is to compare different sequences of processes and to understand the mechanism of each process to be in a better position to make a judgment on their sequence.

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