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Bioethanol Production From Shorea Robusta Seeds By Using Chemical Pretreatment

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Abstract

The study explores the impact of acid treatment on bioethanol production by pre-treating S. robusta with varying concentrations of hydrochloric acid (HCI) and sulphuric acid. The solubilization of hemicellulose increases porosity and digestibility with maximum enzymatic hydrolysis. The maximum bioethanol production was observed to be 9.7±0.0% (v/v) with 1.5% concentration of HCI pretreated sample.Alkali-based pretreatment effectively delignifies lignocellulose by disrupting ester bonds between lignin and xylan, resulting in cellulose and hemicellulose fractions. This process increases surface area, allowing water molecules to penetrate inner layers. The study used *S. robusta* pretreated with sodium hydroxide (NaOH) and calcium hydroxide (CaOH)2, and the maximum bioethanol production was 8.7±0.2% with 2% NaOH pretreated sample.

Keywords: Bioethanol, *Shorea Robusta* seeds, hydroxide (NaOH), hydrochloric acid (HCI) and sulphuric acid sodium hydroxide (NaOH), etc.

Introduction

Fossil energy sources such as oil, coal, and natural gas have all led to a significant increase in greenhouse gas levels in the Earth's atmosphere (Ballesteros *et al.* 2006). As a result of this challenge, researchers are looking for environmentally acceptable alternative energy sources. Bioethanol is one of the alternative energy sources. Bioethanol production from agricultural waste is a promising technology; nevertheless, the process has various hurdles and constraints, including biomass transportation, biomass processing, efficient pretreatment procedures for total delignification of lignocellulosics, and appropriate fermentative organisms (Sarkar *et al.* 2012). In 2007, Nigeria's federal government issued an official gazette outlining the country's biofuel strategy and incentives. The gazette covers policies and measures that the federal government and its partners will take to ensure that ethanol and fuel are blended efficiently in Nigeria. This notice encourages all energy stakeholders to conduct research on strategies to ensure efficient indigenous bioethanol production. The feedstocks recommended in the gazette include agricultural waste and other sources where the country has a comparative advantage. Nigeria, from this perspective, offers vast natural resources to assist the development and even commercialisation of bioethanol. Traditionally, ethanol is produced from the processing of starch, utilizing enzymatic liquefaction and saccharification; leading to the production of a relatively clean glucose stream that is then fermented to ethanol by Saccharomyces (Sarkar *et al.* 2012). However, this yeast, Saccharomyces cerevisiae, cannot utilize the main C-5 sugar (xylose) of the hydrolysate resulting from agricultural waste hydrolysis (Xu *et al.* 1998). This limitation has spurred the need for microorganisms with the ability to increase sugar utilisation; *Zymomonas mobilis* is one bacterium that has showed significant potential in this area (Sarkar *et al.* 2012). Sugarcane (Saccharum officinarum) is used worldwide as a feedstock for ethanol and sugar production (Rezende *et al.* 2011). Nigeria is one of the most important producers of the crop, with approximately 500,000 hectares of appropriate cane fields capable of generating more than 3.0 million metric tonnes of sugarcane. (NSDC 2003). After sugarcane is processed for juice extraction, bagasse is obtained as a residue and equates to approximately 25% of the overall weight, containing 60-80% of carbs(Rezende *et al.* 2011). The plant cell wall of sugarcane bagasse is similar to that of other plants; it is composed of two carbohydrate fractions (cellulose and hemicellulose) embedded in a lignin matrix. Lignin is a phenolic macromolecule resistant to enzyme assault and breakdown, therefore its amount and distribution are recognised as the most critical elements regulating cell wall recalcitrance to hydrolysis (Mosier 2005). Bagasse is processed to produce ethanol in the same way that lignocellulosics are converted. The procedure consists of three major steps: pretreatment for delignification, which is required to liberate cellulose and hemicellulose before hydrolysis; hydrolysis of cellulose and hemicellulose to produce fermentable sugars (glucose, xylose, arabinose, galactose, and mannose); and fermentation of sugars to ethanol. The noncarbohydrate components of lignin have value-added applications(Balat *et al.* 2008). Proper pretreatment procedures can enhance fermentable sugar concentrations following enzymatic saccharification, increasing overall process efficiency (*Sarkar et al.* 2012). To make the entire process economically efficient, the conversion of glucose and xylose to ethanol necessitates the development of new fermentation methods and organisms.The aim of present work is, bioethanol production through chemical- acidic and alkali pretreated from Sal seeds using *Zymomonas mobilis MTCC92*.

Materials and methods

Collection of Substrates

Shorearobusta seeds were collected from forest area of Betul and Budhni after that they were identified by the Department of Botany J.H.Govt .P.G.College Betul ,(M.P.) India. The seeds were grinded into a fine powder that serves as a feedstock for the synthesis of bioethanol.

Isolation of bacterial strains from *Shorea robusta* (Sal) seeds during fermentation process:

The serial dilution approach was used to isolate bacteria from *S. robusta,* using fermented sap suspensions. The spread plate approach isolated bacteria on NAM media, and the streak plate approach created pure cultures of each isolated sp.(*Choudhary et al.,* 2015 &Tandon *et al.,* 2018).

Fermentation

Shorea robusta (Sal) seeds by using *Zymomonas mobilis* MTCC 92 (bacteria) was used as a substrate (sole carbon source) for bioethanol production through fermentation process.20 gm powdered seeds was added in 200 ml of distilled water 10% (w/v) in 250 ml conical flask and then autoclaved it at 121°C, 15 lb pressure for 15 min (Al-shorgani *et al.*, 2012). The initial pH of *Shorea robusta* (Sal) seed fermentation media was 6. *Zymomonas mobilis* MTCC 92 broth was inoculated with inoculum size of 1% (v/v) separately in *Shorea robusta* (Sal) seed powder under aseptic condition and incubated for 24 hour at 30 °C for fermentation. After fermentation, fermented sample was distilled. For distillation, 25 ml of fermented sample was mixed with 200 ml of distilled water and then poured in the distillation flask and distillation was performed in distillation apparatus (Pharmacopoeia of India, 1985).

Estimation of Bioethanol

Quantitative estimation of bioethanol

Quantitative estimation of bioethanol was done by specific gravity method. Specific gravity refers to the density of any liquid (Pharmacopoeia of India, 1985). Twenty five millilitres fermented sample was mixed with distilled water (make up the volume 150 ml) and this mixture was distilled on distillation unit. After distillation ethanol percentage was calculated by specific gravity method (Yadav, 2003). Percentage in v/v was obtained from the standard table correlating percentage volume of ethanol with specific gravity at 25 °C. Each step was repeated three times. All the values are mean ± standard error, values differ significantly at 5% as analyzed by Duncan multiple Range Test by SPSS. ρ= W3 - W1 X Density of water at t °

$$\rho = \frac{\boldsymbol{W3-W1}}{\boldsymbol{W1-W3}} \times \text{Density of water at t °C}$$

Where
ρ = specific gravity.
W1 = weight of empty specific gravity bottle.
W2 = weight of empty bottle + distilled water, W3 = weight of empty bottle + fermented liquid.
The bioethanol yield (Yp/s) and fermentation efficiency (%) were calculated by using formula:-

$$\boldsymbol{a})\ \text{Ethanol yield (Yp/s), g/g} = \frac{\text{Mass of ethanol formed}}{\text{Mass of sugar consumed}}\ (\text{Behera } et\ al., 2012)$$

$$\boldsymbol{b})\ \textbf{Fermentation efficiency}\ (\%) = \frac{\text{Ethanol yield obtained (Yp/s)}}{\text{Theoretical maximum ethanol yield from substrate}} \times \mathbf{100}\ (\textbf{Sharma}\ \boldsymbol{et\ al.}, \mathbf{1975})$$

Chemical pretreatment

A) Acid pretreatment

Three different acids, H_2SO_4, HCl, and H_3PO_4 were used for pretreatment of Shorea robusta (Sal) seed powder. For pretreatment, 20 g of *Shorea robusta* (Sal) seed powder was added in 200 ml of acidified distilled water in the ratio 1:10 (w/v) at different concentrations of 0.25, 0.5, 1 and 2% (v/v) separately then incubated for 24 h at 100 rpm under 60°C (Ranjan and Moholkar, 2013). After pretreatment, filtered the substrate and adjusted pH 6 by using 1N NaOH then inoculated with *Zymomonas mobilis MTCC 92* for fermentation at their optimized conditions to produce bioethanol.

B) Alkali pretreatment

Three different alkali NaOH, KOH and $Ca(OH)_2$ were used for pretreatment. 20 g of Deoiled sal seed was added in 200 ml of distilled water containing concentrations of alkali 0.5%, 1.0% and 2% (w/v) at two residence time of 30 and 60 min. in a water bath at 60°C for pretreatment. Then alkali pretreated *Shorea robusta* (Sal) seed powder. was washed with water and after maintained the pH 6 using 1N HCl and then this pretreated substrate was inoculated with *Zymomonas mobilis MTCC 92* for fermentation at their optimized conditions to produce bioethanol (McIntosh and Vancov, 2011).

Results and Discussions

Chemical treatment: The present study aimed to select the best chemical pretreatment among different chemicals at different concentration ranges for the effective pretreatment of *S. robusta* and only the best results were selected for comparing the amount of bioethanol. Each pretreatment was carried out by separate hydrolysis and fermentation methods.

Acid pretreatment

The study investigates the impact of acid treatment on bioethanol production by pre-treating S. robusta with varying concentrations of hydrochloric acid and sulphuric acid. *S. robusta* at a solid loading rate of 10% (w/v) was pretreated with different concentration varying from 0.5%, 1.0%, 1.5% and 2.0% of hydrochloric acid (HCI) and sulphuric acid (H_2SO_4).The solubilization of hemicellulose increases the porosity and digestibility with the maximum enzymatic hydrolysis (Chaturvedi *et al.,* 2013).On quantitative estimation of HCI pretreated sample it was observed that maximum amount of bioethanol production was 9.7± 0.0 % (v/v) that was achieved with 1.5% concentration of HCI pretreated sample On quantitative estimation of HCI pretreated sample it was observed that maximum amount of bioethanol production was 9.7± 0.0 % (v/v) that was achieved with 1.5% concentration of HCI pretreated sample(fig & table 1.1). While On estimation of bioethanol for the H_2SO_4 pretreated sample, it was observed that maximum amount of bioethanol production was 8.4 ± 0.0 % (v/v) produced with 0.5% H_2SO_4 pretreated sample (fig & table 1.2) .Sun and Cheng (2009) studied the dilute acid treatment of rye straw and bermudagrass at a solid loading of 10% (w/w) with dilute sulfuric acid from 0.6, 0.9, 1.2, 1.5% (w/w) and pretreated in an autoclave at 121°C with residence times of 30, 60, and 90 min The. Similarly Akpan *et al,* (2005) studied the production of bioethanol from maize cos ad groundnut shells and pretreated them with 3M, 4M, 4.5M and 5M of H_2SO_4 before saccharification and fermentation experiments. Leon *et al.,* (2018) employed different H_2SO_4 concentration and found 1% best for the hydrolysis of biomass.The best result was obtained with of HCI pretreatment with as significant increase of bioethanol production.Further during the study it was observed that H_2SO_4 pretreated sample also resulted in increased production but it was comparatively lower than results obtained with HCI pretreated sample.

Alkali pretreatment

The research shows that alkali-based pretreatment effectively delignifies lignocellulose by disrupting ester bonds between lignin and xylan, resulting in cellulose and hemicellulose fractions. This process increases surface area, allowing water molecules to penetrate inner layers. The study used *S. robusta* pretreated with sodium hydroxide (NaOH) and calcium hydroxide (CaOH)2, and the maximum bioethanol production was 8.7 ± 0.2% with 2% NaOH pretreated sample(fig & table 1.3). NaOH treatment increases cellulose's internal surface area and reduces polymerization, disrupting the overall lignin structure(Taherzadeh and Karimi 2008).The production decreases after reaching an optimal range due to the dissociation of biomass into hydroxide and sodium ions during pretreatment with NaOH, with higher hydroxide ion concentrations accelerating hydrolysis, making alkali treatment with NaOH more effective(Kumar *et al.* 2009).Wang *et al.,* (2008) studied the effect of NaOH pretreatment using 1%, 2% and 3% (w/v) NaOH for 15, 30, 60 and 90 minutes at 121°C and found that the lower NaOH of 0.5% and 0.75% is optimum for the maximum sugar recovery ad ethanol production. Similar study was performed by Yoswathana and Phuriphipat (2010) studied the pretreatment of dried rice straw with 1, 2, 3, 4, 5% NaOH in ratio of 1:10 (w/v).

On further quantitative estimation of bioethanol by (CaOH)2 showed similar results like (NaOH) it was observed that the maximum production was 8.5 ± 0.2% (v/v) that was achieved with 2% (w/v) (CaOH)2 pretreated sample (fig & table 1.4). The results of present study again showed that a further increase in (CaOH)2 concentration results in decreased bioethanol production. Wang et al., (2008) studied the bioethanol production from alkaline treatment methods and used 0.1 g Ca(OH)2/g for the pretreatment of the biomass at 50 °C, 80 °C, and 121 °C. Based on these results, optimal Ca(OH)2pretreatment concentration was 2%. Similarly, Pothiraj *et al.,* (2014) studied lime pretreatmend of water hyacinth for bioethanol production. Raval *et al.,* (2016) treated the rice mill wastewater with calcium hydroxide in varying quantities of 0.5, 1, 1.5 and 2 gm for the pretreatment. Not many studies have been carried out using lime because it has not been used beyond lab scale investigation.

Table-1.1: Showing Effect of hydrochloric acid pretreatment on bioethanol production

S.NO.	**Percentage Concentration of HCL in (V/V)**	**Percentage of Bioethanol production in(v/v)**
1	0.5	6.5±0.5
2	1	8.2±0.4
3	1.5	9.7±0.0
4	2	7.6±0.1

Fig-1.1: Showing the Effect of hydrochloric acid pretreatment on bioethanol production

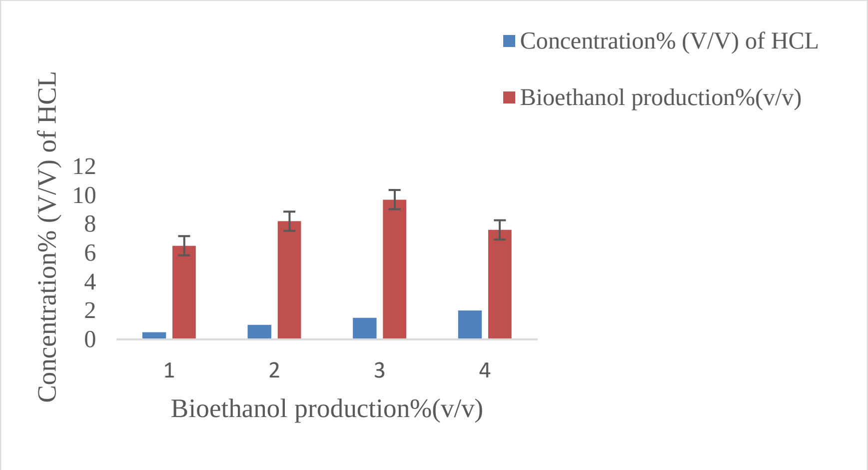


Table:-1.2 Effect of sulphuric acid pretreatment on bioethanol production

S.NO.	**Concentration% (V/V) of $H2SO_4$**	**Bioethanol production%(v/v)**
1	0.5	8.4±0.0
2	1	7.8±0.1
3	1.5	7.2±0.1
4	2	6.2±0.0

Fig-1.2: Showing Effect of sulphuric acid pretreatment on bioethanol production.

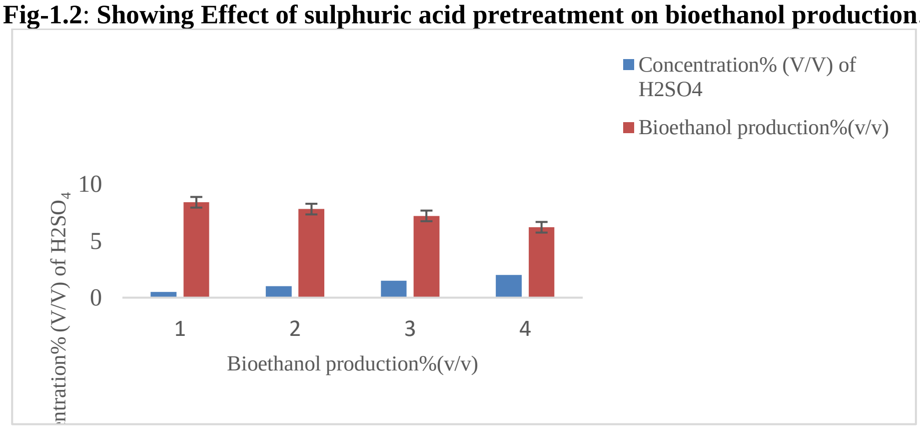


Table 1.3: Effect of sodium hydroxide (NaOH) pretreatment on bioethanol production

S.NO.	**Concentration% (W/V)of NaOH**	**Bioethanol production%(v/v)**
1	1	7± 0.0
2	2	8.7± 0.2
3	3	8.2± 0.1
4	4	6.3± 0.0
5	5	2.2± 0.2

Fig:- 1.3:Showing the effect of sodium hydroxide pretreatment on bioethanol production.

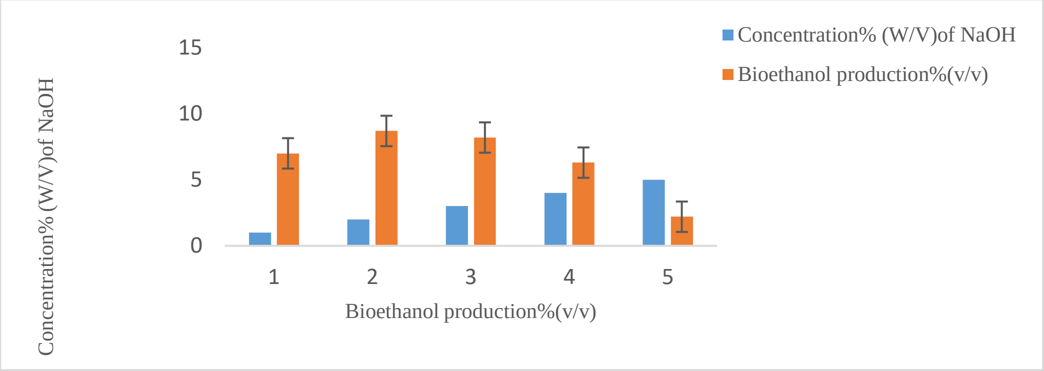
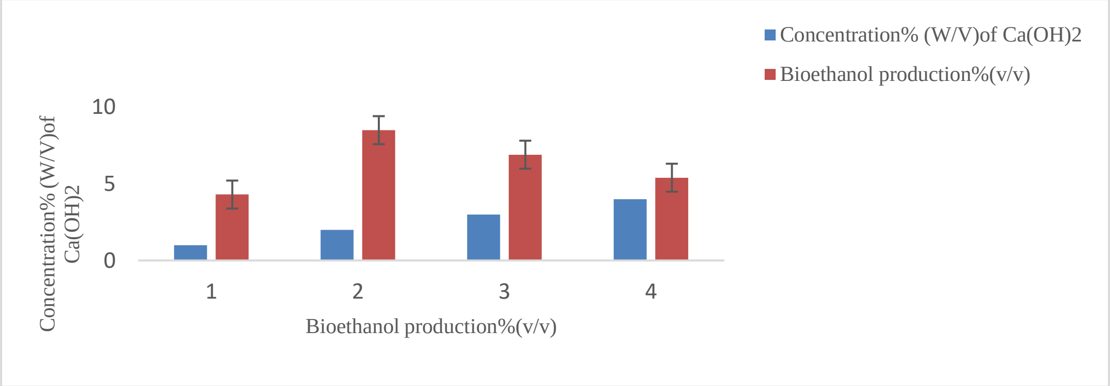


Table:-1.4 :Showing Effect of calcium hydroxide pretreatment on bioethanol production

S.NO.	Concentration% (W/V)of Ca(OH)2	Bioethanol production%(v/v)
1	1	4.3± 0.1
2	2	8.5± 0.2
3	3	6.9± 0.1
4	4	5.4± 0.0

Fig:- 1.4:Showing effect of calcium hydroxide pretreatment on bioethanol production.



Conclusions

The study investigates the impact of acid treatment on bioethanol production by pre-treating S. robusta with varying concentrations of hydrochloric acid (HCI) and sulphuric acid. The solubilization of hemicellulose increases porosity and digestibility, leading to maximum enzymatic hydrolysis. The maximum bioethanol HCI pretreated sample.
Alkali-based pretreatment delignifies lignocellulose by disrupting ester bonds between lignin and xylan, resulting in cellulose and hemicellulose fractions. The study used *S. robusta* pretreated with sodium hydroxide (NaOH) and calcium hydroxide (CaOH)2, with the maximum bioethanol production being NaOH pretreated sample. The production decreases after reaching an optimal range due to the dissociation of biomass into hydroxide and sodium ions during NaOH pretreatment. Lower NaOH concentrations are optimum for maximum sugar recovery ad ethanol production.
Various studies have investigated the use of lime pretreatment of water hyacinth for bioethanol production, but few have used lime beyond lab scale investigation. Overall, the study provides valuable insights into the potential of acid treatment in bioethanol production and the potential benefits of different treatments.

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