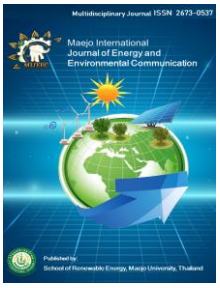
Maejo International Journal of Energy and Environmental Communication

Journal homepage: https://ph02.tci-thaijo.org/index.php/MIJEEC



ARTICLE

Transformation of lignocellulose from corn stove for bioethanol production

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ARTICLE INFO

Article history:
Received 12 March 2021
Received in revised form
2 April 2021
Accepted 10 April 2021

Keywords:
Sustainable approach
Closterium sp.
Transesterification
Biodiesel

ABSTRACT

The use of fossil fuels, as well as the environmental issues associated with their burning, has pushed for the development of clean, renewable energy sources. Biofuels made from lignocellulosic biomass are considered a carbon-neutral and sustainable method. As the demand for non-petroleum fuels grows, more attention will be placed on developing a cost-competitive liquid transportation biofuel like ethanol. This study was conducted to produce bioethanol utilizing the SHF (separate hydrolysis and fermentation) technique from corn stove lignocellulose. Pretreatment with sodium hydroxide at various concentrations was also studied. The influence of enzymatic saccharification, fermentation time, and substrate concentration on sugar yield and, eventually, ethanol production was investigated. Fermentation was carried out by using the enzymatically saccharified hydrolysate and monoculture of Saccharomyces cerevisiae. The results reveal that pretreatment with 2% NaOH followed by 48 hours of hydrolysis produced the maximum bioethanol production (30.21 ±0.13 g/L). This study findings indicated that alkali-pretreated corn stove might be used as a feedstock for bioethanol production, reducing reliance on fossil fuels.

1. Introduction

Increasing interest in biomass fuel has grown in recent decades as the price of petroleum-derived gasoline has risen. Moreover, lignocellulosic biomass is one of the best replacement energy sources for addressing energy security issues (Bautista et al., 2019; Khunchit et al., 2020). Second-generation biofuels come from non-food crops (Manmai et al., 2020a, b). Included are the use of lignocellulosic resources such as crop residues and lignocellulosic crops. The phrase 2nd generation is used loosely in the scientific literature to refer to feedstocks, conversion pathways, and products (Mejica et al., 2021; Trejo et al., 2021). Lignocellulosic biomass appears to be an appealing alternative for inexhaustible biofuel supplies, reducing reliance on fossil fuel resources (Ramaraj et al., 2015; Manmai et al., 2019). In addition, the lignocellulosic biomass feedstock is abundant, recyclable, inexpensive, and spread uniformly throughout nature (Nong et al., 2020; Saengsawang et al., 2020). Lignocellulose is a chemical compound found in plants that are made up of polysaccharides that are tightly linked by lignin. Thus, the raw material for biorefinery products is lignocellulosic material (Dussadee et al., 2014; 2016; 2017; Bhuyar et al., 2021).

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Because of its abundance and low cost, using lignocellulosic biomass as an alternative transportation fuel is an appealing idea (Khammee et al., 2019; 2021). As a result, future fossil fuel consumption reductions will be based on a comprehensive approach that includes nuclear, solar, hydrogen, wind, and, in particular, biofuels, which several countries have already begun by pursuing research and development projects (Vu et al., 2017; 2018; Ramaraj et al., 2021). In addition, bioethanol has become a popular renewable energy source because of its high-octane number, heat of vaporization, and compatibility with current automobiles. Bioethanol can be made from lignocellulosic biomass using two distinct methods (i.e., biochemical or thermochemical conversion). Both procedures entail lignocellulose's resistant cell wall structure is broken down into lignin, hemicellulose, and cellulose fragments. Then, each polysaccharide is hydrolyzed into sugars, which are then turned into bioethanol before being purified.

Carbohydrates (i.e., hemicelluloses and cellulose) and lignin are the major components of lignocellulosic plant biomasses such as forest and agricultural wastes (Chuanchai and Ramaraj, 2018). The proportion of these basic polymer blocks changes depending on the biomass's origin and kind. Agricultural residues are thus the most promising cellulose feedstocks obtained from plant residues around the world (Manmai et al., 2017a, b). The three primary components of lignocellulosic material are cellulose (30–50 %), hemicellulose (15–35 %), and lignin (10–20 %). Cellulose and hemicelluloses account for over 70% of the total biomass and are closely attached to the lignin component via covalent and hydrogenic interactions, resulting in a highly durable structure and resistance to treatment. For lignocelluloses to be converted to ethanol, their polysaccharides must first be broken down into monosaccharides, which are then fermented to ethanol by microbes (Ramaraj and Unpaprom, 2019a, b).

When discussing enzymatic or acidic degradation of the lignocellulosic structure, it is vital to remember that d-xylose is the second most crucial sugar, accounting for one-third of the sugars in the lignocellulosic feedstock and forming the hemicellulosic component of the plant cell wall., The most common industrial yeast used in bioethanol production, *Saccharomyces cerevisiae*, can only metabolize hexose carbohydrates like glucose. Thus, pretreatment, hydrolysis, fermentation, and distillation are the four phases in manufacturing lignocellulosic-based ethanol (Nguyen et al., 2020a, b, c; Whangchai et al., 2021). Over the last few decades, significant breakthroughs in genetic and enzymatic technology have helped to optimize these ethanol production processes and expand *S. cerevisiae*'s ability to ferment many sugars simultaneously. Biofuels are being researched as potential replacements for present high-polluting fuels derived from traditional sources, making discarded corn Stover appealing. In addition, using lignocellulose materials in bioethanol synthesis, such as waste maize corn Stover, offers an advantage over sugar and starch since it reduces the conflict between using land for food cultivation and energy feedstock production. Therefore, this study aims to the transformation of lignocellulose from corn stoves for bioethanol production.

2. Materials and Methods

2.1. Material preparation

After harvest yields of the farm of Program in Agronomy, Maejo University, Chiang Mai, Thailand (18° 8′98″ N99°0′13″E), corn stove was collected. The expenditure of tubing water eliminated dust and perceptible contaminants from biomass. This raw material has been dried for three days in the initial preparation using a solar hot air oven used for sun rays and ambient temperature. The dried biomass was then reduced in size by an animal feed cutting machine as the next step in raw material processing. In the second drying stage, the corn stove was milled again until a size of 1 mm was achieved using a high-speed blender (Otto BE-127). In the last phase, the biomass was dried in a hot air oven at 60 °C for 48 h before being stored in polyethylene bags without air at room temperature until further use.

2.2. Sugar production from alkaline pretreatment

Dried corn stove biomass (5 g) was placed in a beaker, and 15 ml of NaOH was added, with a solid to liquid ratio of 1:3 at various concentrations. (1, 2, and 3 percent (w/v)) were added to these beakers and covered with aluminum foil, and extraction durations were set points (1, 2 and 3 days) at Chiang Mai ambient temperature (30°C) as specified in the experimental designs. After the extraction procedures were completed, 20 mL distilled water was added to the beakers, and the sugar solution was squeezed and filtered from the solid to determine total sugar yield using the phenol–sulfuric acid technique.

2.3. Alkaline pretreatment and enzymatic degradation

Both 2% (w/v) of NaOH and the 100 g biomass were processed. The combination of water and solid substrate was allowed (i.e., addition solid to liquid ratio of 1:3 was conducted at room temperature for 3 days). Enzymatic hydrolysis was applied by the Union Science Company, Chiang Mai, Thailand, to depolymerize lignocellulose in sugar samples. The commercial cellulase enzyme was utilized to depolymerize lignocellulose in sugar samples. The pretreated sample was adjusted pH by 2% (v/v) of cellulose enzyme to pH 5 by 0.5 M of hydrochloric acid. Ambient temperature (30°C) digestion of the combination for 72 hours; all 24 hours samples have been corrected. Then the samples were evaporated water until 200 mL as the last final volume before fermentation. In order to measure the total sugar and reducing sugar according to these procedures each time the liquid hydrolyzed sample was collected.

2.4. Yeast preparation

This investigation was carried out by the Biotechnology Program, Faculty of Science, Maejo University, Chiang Mai, Thailand, sponsored by *S. cerevisiae* TISTR5020. On plates of 10 g/L, yeast extract (Himedia laboratories, Telangana, India), 20 g/L of peceptone (Himedia Laboratories, Telangana, India), 20 g/L of glucose, and 20 g/L (Union Science Co., Ltd, Chiang Mai, Thailand), YPD agar were inoculated in 35 °C for 24 h., the yeast (one loop) was passed on to the YPD broth. Medium broth composition of YPD; yeast extract (10g/L), peptone (20g/L)

(Himedia Laboratories, Telangana, India), Glucose (20g/L) (Union Science Co., Ltd, Chiang Mai, Thailand) The diluted NaOH is pH 5.5. At 121°C and 15 psi in autoclaves for 15 min, the medium was sterilized. The fermentation inoculum, yeast was placed in a 150-rpm shaker for 24 hours at 35°C. Yeast biomass was produced without centrifuges and utilized as inoculum by centrifugation at 7,000 rpm for 10 minutes at 4°C.

2.5. SHF technique for bioethanol fermentation

In the fermentation phase, 2% (w/v) of yeast (*S. cerevisiae* TISTR 5020), introduced microorganism cells into the fermenter, has been fermented for the last 200 ml of fermentable liquid after vaporized water. Each mixer was incubated at 35°C for 96 hours of solution mixture. The fermented solution was collected every 48 hours, the concentration of ethanol using the Ebulliometer has been measured by collecting 50 ml liquid from samples of the fermenter.

2.6. Analytical techniques

1 mL of each sample with 2 mL of 3,5-dinitrosalicylic acid (DNS) (Sigma Aldrich, Missouri, USA) reagent were distributed in clean test tubes and combined by a vortex for reducing sugar measurement. The mixture was heated for 15 minutes at 90°C in a boiling water bath. It was then allowed to cool before adding 4 mL of distilled water. A UV-Spectrophotometer DV-8000 (Drawell, Osaka, Japan) was used to measure the absorbance at 540 nm, using a blank as a control. 0.5 ml of sample. 0.5 ml of 5% (w/v) phenol (Qrec, Selangor, New Zealand) (Miller, 1959; Mariano et al., 2020), and 2.5 ml of 98% H_2SO_4 in a vortex mixed for total sugar determination (RCI Lab Scan, Bangkok, Thailand). The solution was allowed for 10 minutes before being measured at 490 nm with a Spectrophotometer model DV-8000 (Drawell, Osaka, Japan) (Dubois et al., 1956). An Ebulliometer was used to calculate the ethanol content (Laboratoires Dujardin-Salleron, Noizay, France) (Sophanodorn et al., 2020a, b, c).

3. Results and Discussion

3.1. Characterization of raw material

Previously, corn stalk was reported proximate analysis; moisture 3.41%, ash 5.87%, volatile matter 80.77% and FC 11.95%, ultimate analysis; C 43.34%, H 6.12%, O 48.42%, N 2.12%. For lignocellulose of corn stalk consists of cellulose 38.40%, hemicellulose 35.73% and lignin 5.78% (Fan et al., 2020).

3.2. Sugar from alkaline pretreatment

Pretreatment generally decreases the physical obstacles to mass movement by breaking down the macroscopic stiffness of the biomass. Table 1 compares total sugar concentrations from three different percentages of NaOH and times for pretreatments. The control parameter as DI water. The lowest quantity of total sugar was 9.213 ± 0.022 g/L found from the control at Day 1, while the maximum amount was 27.164 ± 0.021 g/L from 2% NaOH of pretreatment for 3 days. This implies that NaOH had a significant impact on the material's structure and caused it to release more sugar. Total sugar concentrations from 2 and 3 % NaOH and 2 and 3 days. They were similar concentrations of total sugar as presented in Table 1.

3.3. Sugar from cellulase enzyme hydrolysis

During the enzymatic hydrolysis period, the total sugar (g/L) and reducing sugar (g/L) concentrations of corn stove. The total sugar and reducing sugar concentrations were determined using the sulfuric-phenol and DNS methods, in which all of the oligosaccharides in the sample was completely hydrolyzed into polysaccharide. The best results of this raw material are digestion by cellulase enzyme for 24 h, total sugar and reducing sugar concentrations; corn stove of 195.215 ± 1.253 and 45.712 ± 0.515 g/L, individually. All data are presented as means standard deviations (n = 3) (Figure 1).

3.4. Bioethanol fermentation

In the process of biomass conversion to bioethanol, the initial fermentable sugar content, total sugar, and reducing sugar concentration (g/L) of corn stove, 165.154 ± 1.643 and 35.213 ± 1.514, respectively. To liberate water, it was evaporated by boiling. The bioethanol concentration throughout fermentation (0–96 h). Bioethanol concentration increased significantly from 0 to 48 h, with corn stove of 30.213 ± 0.570 g/L. Due to the yeast has reached the death phase, the bioethanol content in both samples has dropped after 48 to 96 h (Figure 2).

Table 1 Total sugar concentration from alkaline pretreatment.

Material	Pretreatment	Total sugar (g/L)					
		Day 1		Day 2		Day 3	
		Content	SD	Content	SD	Content	SD
Corn stove	1% NaOH	12.643	0.021	18.251	0.012	19.324	0.011
	2% NaOH	15.377	0.018	26.316	0.014	27.164	0.021
	3% NaOH	17.213	0.025	25.526	0.017	26.153	0.019
	DI water (Control)	9.213	0.022	11.895	0.023	12.791	0.026

* 3 replications

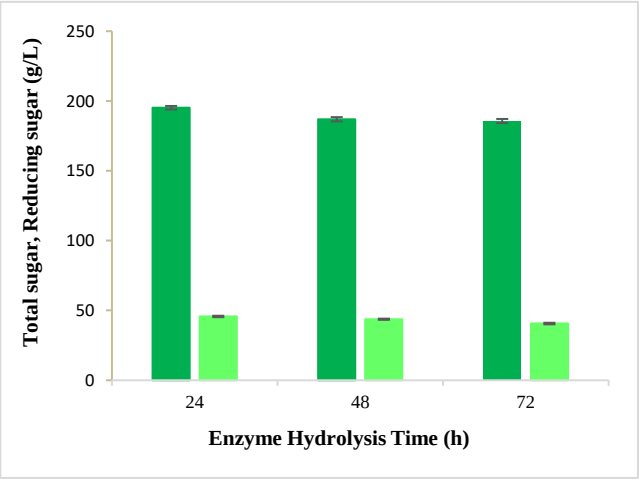

Figure 1 Total sugar and reducing sugar concentrations from enzymatic hydrolysis.

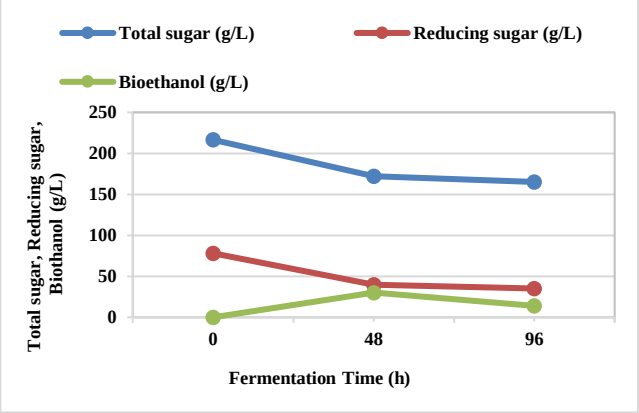

Figure 2 Bioethanol concentration from corn stove.

4. Conclusion

The impact of NaOH concentrations and time on total sugar and reducing sugar of chemical pretreatment of corn stove biomass has been studied. The maximum sugar concentration was reached at the pretreating time, 2% NaOH and ambient pretreating temperature. In terms of the enzymatic hydrolysis stage with 2% cellulase enzyme, the time that was achieved at 24 h of hydrolysis efficiency. The increased interest in corn stove as a possible feedstock for bioethanol production in this study. However, further study is needed to increase digestion and fermentation efficiency by utilizing various enzymes and microbes. Thailand's economic plants after harvesting biomass waste. Then agricultural wastes are abundant on farms, and they represent a sustainable and low-cost source of biomass for biofuel.

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