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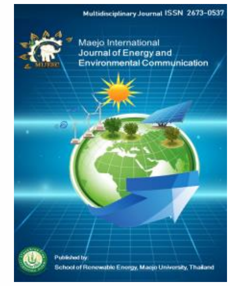

ARTICLE

Pretreatment of barley straw with acid and alkaline solutions to boost the efficiency of fermentable yield enzymatic degradation in the separated fermentation technique for ethanol production

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A B S T R A C T

Barley straw is a lignocellulose agricultural waste material that can be utilized as a raw material for ethanol production since it is easy to find, cheap, and has the potential to produce ethanol yield. This research aims to select the optimum pretreatment conditions to increase fermentable sugar production during enzymatic hydrolysis for bioethanol production. Initially, sulfuric acid at a concentration of 0.5, 1.0, 1.5, and 2.0% (v/v) pre-treated barley straw biomass was applied by autoclaving at 121℃ for 15 lb/in^2 pressure for 15 min. Furthermore, acid-treated barley straw under the alkaline condition with calcium hydroxide concentrations of 1, 2, 3, and 4% (w/v) was autoclaved at 121℃ for 15 lb/in^2 pressure for 15 min. Subsequently, the pretreatment of barley straw in the acetic and alkaline processes was compared. It was found that calcium hydroxide at a concentration of 2% (w/v) gave more sugar concentrations. Finally, combined acid and alkaline pretreatment with 2% of cellulase enzymatic hydrolysis had the highest total sugar concentration of 205.43 g/L and reducing sugar of 134.42 g/L, producing the highest ethanol yield (16.17 g/L) by 24 hours of fermentation.

1. Introduction

Energy is an important component in meeting people's basic needs. It is also essential in the production process in industrial and commercial contexts (Ramaraj et al., 2015; Kumaran et al., 2022). Energy is essential to provide people with the energy required to meet their basic needs. Due to the constant rise in domestic energy consumption has become more dependent on imported fuels to generate energy. The government must provide sufficient energy and high quality, at a reasonable price (Obey et al., 2019). It is to ensure that energy meets the needs of users. This is necessary to ensure that Thailand has enough energy in the future (Pimpimol et al., 2020). It also reduces the risk associated with dependence on foreign fossil fuels. We began to explore ways to create renewable energy from agricultural waste or plants that were easy to obtain, affordable and can be used to their full potential (Ramaraj et al., 2022; Chutturi et al., 2022). They were particularly interested in finding ways to achieve all these goals simultaneously.

Ethanol, a fascinating alternative to oil, is used in many different business sectors. This can be used to reduce oil imports in many different ways. One of these is by blending fuel with ethanol (Pradechboon & Junluthin, 2022). Thailand's main sources of raw materials for ethanol production are sugarcane, cassava, and a

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common crop used in cooking (Sophanodorn et al., 2022a). There is concern that the current supply of raw materials may not be sufficient to produce enough ethanol to meet future energy demands. Global food supplies are also affected by the exploitation and production of ethanol from food crops. This has led to an increase in the barleys of domestic and imported goods. This point has been reached thanks to the development of new technologies (Ramaraj & Unpaprom, 2019a). In order to produce ethanol, it is important that lignocellulosic materials are taken into account. These are substances that remain after plant development has occurred. The high number of these substances is due to Thailand's predominance as an agricultural country (Nguyen et al., 2020) and a large number of tobacco stalks, corn stalks, and barley straw, etc are predominantly available (Sophanodorn et al., 2022b). Farmers often resort to burning to eliminate weeds. They must be used in order to generate ethanol. This is a biological process in which sugars present in cellulose are converted into ethanol (Ramaraj & Unpaprom, 2019b). Before any significant reduction in internal crystallinity can occur, a phase of pretreatment must be performed on the raw materials.

Structure with lignin displacement and hemicellulose increases the amount of cellulose and helps the enzyme work easier. Reconditioned raw materials can reduce costs. Enzymes account for 33% of the total cost of ethanol production (Ibrahim, 2012), whereas for untreated raw materials, the cost of enzymes accounts for 25% of the cost of production. total ethanol (Brodeur et al., 2011). However, the pretreatment process is important to increase the efficiency of ethanol production The pretreatment methods can be divided into four main methods: physical, biological-chemical, and physical-chemical. Each method has strengths and weaknesses in the pretreatment technique that affect the composition of raw materials differently (Agbor et al., 2011; Lai et al., 2018). Optimum pretreatment to reduce the cost of the ethanol production process.

Therefore, this research was to study the chemical and thermal pretreatment of barley straw to determine the appropriate concentration of acidic solutions, namely acetic acid and sulfuric acid and an alkaline solution, including sodium hydroxide sodium chloride and calcium hydroxide to increase the efficiency of barley straw degradation by fungal enzymes by producing ethanol by technique (Rezania et al., 2020). Yeast, Saccharomyces cerevisiae on pre-treated and hydrolysed barley straw using separation hydrolysis and fermentation (SHF) as a guideline or alternative technique for developing commercial ethanol production of Thai fuels in the future. It will also increase the value of barley straw to be used as an alternative raw material instead of using cassava, sugar cane or molasses, and also be a way for the community to apply the local wisdom to create energy sources to use themselves and may contribute to the energy self-reliance of the country. As a result, this study concentrated on the optimal pretreatment concentration and enzymatic hydrolysis for the separation hydrolysis and fermentation (SHF) technique.

2. Materials and Methods

2.1 Feedstock collection and preparation

Barley straw was transferred to the portable solar dryer from the agronomy field zone at the Faculty of Agriculture, Maejo University, Chiang Mai, Thailand, and reduced in size with a grinding machine. It measures roughly 2-3 cm in length.

2.2 Microorganisms culture preparations

The yeast Saccharomyces cerevisiae, which can consume a range of sugars and grows quickly, is the microbe most frequently used in the synthesis of ethanol. S. cerevisiae yeast culture medium for use in yeast fermentation medium (YM) and yeast malt medium (YFM). Malt extract (3g), yeast extract (3g), peptone (5g), and glucose (10g) are all included in the YM, along with 1000 ml of double-distilled water. Additionally, the pH of the YFM, which contains all of the components dissolved in distilled water, is adjusted to 5. Twenty grams of agar for solid food were added, divided into 100 ml flasks, and autoclaved for 15 minutes at 121 ℃ (15 lb/in^2). The cultures were grown on a shaker at 37 ℃ and 150 revolutions per minute for 18 hours. These are going to be used as starter cultures to produce yeast cells for the production of ethanol.

2.3 Acid and alkaline pre-treatments and enzyme hydrolysis

Barley straw soaked with sulfuric acid (H_2SO_4) was pre-treated with plant ratios of 1 g per 20 ml of solution on 1 g of ground barley straw biomass at concentrations of 0.5, 1.0, 2.5, and 3.0 % (v/v) followed by 120 minutes of acclimatization in a hot air oven at 120 ℃. and for 15 minutes in a steam autoclave at 121 ℃, 15 lb/in^2 of pressure. For alkaline pretreatment, $Ca(OH)_2$ solution at concentrations of 1, 2, 3 and 4% (w/v), the biomass ratio was 1 g per 20 ml used. The total and reducing sugar concentrations were then examined using popular methods (Dubois et al., 1956; Miller, 1959). The commercial cellulase enzyme was applied after pretreatment procedures. Then total and reducing sugars were analyzed with the methods mentioned above.

2.4 Ethanol fermentation

The Vu et al. method used for making ethanol through fermentation was used (Vu et al., 2018). ethanol fermentation was done at 35°C for 24 hours with an airlock system to keep air from getting into the flask. This was different from free cell fermentation, which was done at room temperature. Putting away the ethanol fermentation solution. For fermentation, a final volume of 200 mL of hydrolysate solution was fermented with 2% (v/v) of yeast (*Saccharomyces cerevisiae*) and microorganism cells in a 500-mL-working-volume fermenter. This was done to increase the sugar concentration in the solution. The mixture was kept at 35 °C for 3 days while hydrolysis and fermentation took place on their own (SHF). Every day, 50 mL of liquid samples from the fermenter were taken out and put in an ebulliometer to measure the amount of ethanol (alcohol burner, Dujardin-Salleron). Schematic structure of the ethanol production procedure from barley straw is demonstrated in Figure 1.

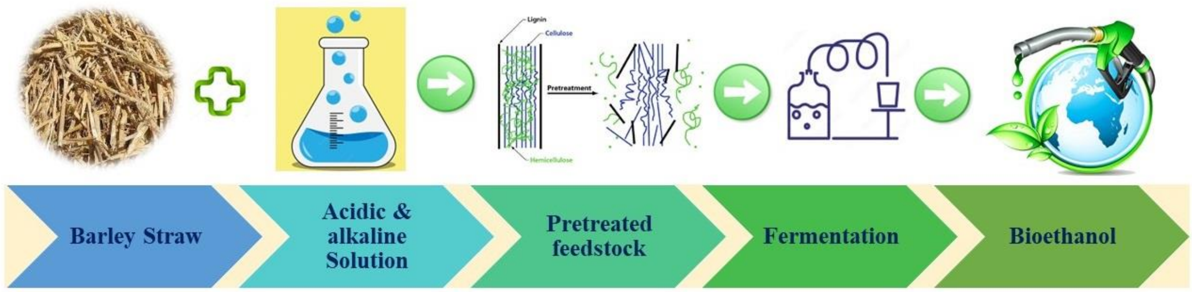

Figure 1 Schematic structure of ethanol production procedure from barley straw

2.5 Statistical data analysis

Three repetitions of each set of experiments were carried out in order to statistically report the experimental data, which were averaged, and the standard deviation given. Use the analysis as well. Using one-way ANOVA, the differences between the experimental sets (ANOVA).

3. Results and discussion

3.1 Barley straw pretreatment in acid conditions

Barley straw is a lignocellulosic biomass. It is made up of cellulose, hemicellulose, lignin, extractives, and ash. On a dry weight basis, it has 37.7% cellulose, 26.7% hemicellulose, 5.5% lignin, 14.4% extractives, and 12.3% ash. Pretreatment methods account for 33% of the overall expenditures associated with the process when looked at from an economic perspective (Moraes et al., 2022). Acid pretreatment is used to separate the different parts of lignocellulosic biomass. It softens the structure of the biomass, breaks the lignin protection and cellulose crystallinity, and lets the sugars dissolve (Trejo et al., 2021). Acid pretreatment is used to separate the different parts of lignocellulosic biomass. This softens the structure of the biomass, breaks the lignin protection and cellulose crystallinity, and lets the sugars dissolve (Whangchai et al., 2021). Due to the use of diluted acid solutions and heating under high-pressure conditions in the pretreatment, it was discovered that autoclave heating had a greater impact on sugar production than the treated barley straw heating with a hot air oven. This combination of chemical and thermal pretreatment was used in this study by bringing barley straw pretreated in sulfuric acid at concentrations of 0.5, 1.0, 1.5, and 2.0 % (v/v). Because of this, cellulose and hemicellulose were able to be extracted from the plant. Sugar will be produced from the end product once it has been refined (Trejo et al., 2021).

The acid concentrations heated by steam pressure or autoclave have a greater effect than sulfuric acid on reducing crystallinity. It may be due to acid treatment under high pressure and temperatures, was resulted in the concentration of acid increasing until there is an excess, therefore, affecting the destruction of the structure of sugar, causing the loss of molecules of sugar and forming other toxic compounds such as furfural, hydroxymethylfurfural and phenolic compounds resulting from the dehydration of sugar (Nguyen et al., 2022). In addition, dilute acid solutions can dissolve hemicellulose. This is because it contains monosaccharides with 5-6 carbon atoms, such as arabinose or xylose. The pretreatment of barley straw in an acidic condition using sulfuric acid results are shown in Figure 2. The optimal condition for acidification was 1.5% (v/v), sulfuric acid. Then, it was autoclaved at 121 °C, pressure 15, lb/in^2, for 15 minutes. The highest total and reducing sugar yield was 22.51 g/L and 10.15 g/L, respectively, which is why sulfuric acid, at a concentration of 1.5% (v/v), had better solubility.

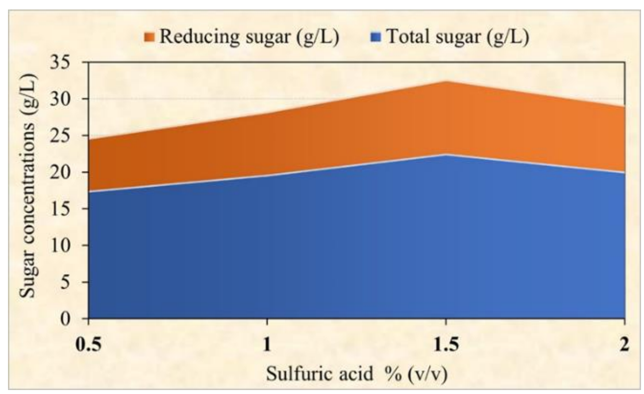

Figure 2 Pretreatment of barley straw in an acidic condition using sulfuric acid

3.2 Barley straw pretreatment in combined acid-alkaline conditions

The combined pretreatment could help solve the problem of how to treat biomass before it is used and give lignocellulosic biorefinery some clear vision (Tarrsini et al., 2021). Several types of lignocellulosic feedstocks, including empty fruit bunch fibers of oil palm, grass, corncob, sugarcane bagasse, and rice straw, have been processed using configurations of the method that involve combined acid and alkaline pretreatment (Guo et al., 2013; Kim et al., 2013; Li et al., 2017; Palamae et al., 2017; Wang et al., 2013). In this study, the results of the acid treatment of barley straw according to the selected conditions were barley straw pretreatment with 1.5% (v/v) sulfuric acid under a temperature of 121 °C and a pressure of 15 lb/in^2 for 15 min in a steam autoclave was treated with an alkaline solution, namely calcium hydroxide at concentrations of 1, 2, 3 and 4% (w/v). The experimental results of the combined pretreatment of acid-alkaline solutions results were presented in Figure 3.

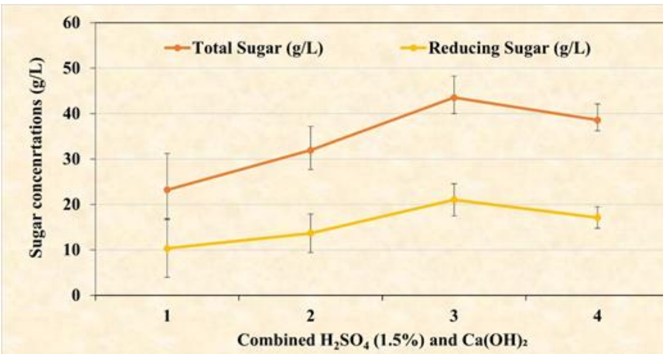

Figure 3. Combined pretreatment of combined acid-alkaline solutions

Total sugar yield of 1.5% (v/v) sulfuric acid combination of calcium hydroxide at concentrations of 1, 2, 3 and 4% (w/v) were 23.21, 31.9, 43.52, and 38.55 (g/L), respectively. In addition, the yield of reducing sugar was, correspondingly, 10.32, 13.65, 21.02, and 17.11 (g/L). The highest total and reducing sugar yield was 43.52 g/L and 21.02 g/L, which is why sulfuric acid, at the concentration of 1.5% (v/v), had better solubility. According to Martínez-Patiño (2017) research, the biomass of olive trees provides a potentially useful source of fermentable sugars. Under the right circumstances, a two-stage pretreatment of olive tree biomass with sulfuric acid, followed by an alkaline-peroxide delignification, led to a significant amount of fermentable sugar being recovered from both the cellulose and the hemicellulose. Similarly, the pretreatment of barley straw with acid and alkaline solutions boosts the efficiency of total and reducing sugars for further enzymatic hydrolysis. As a result, the detoxification of acid prehydrolysate with calcium hydroxide revealed discernible sugar releases in this work.

3.3 Barley straw hydrolysis stage

The hemicellulose in processed biomass stops enzymes from getting to the cellulose. This idea comes from the fact that processed biomass has hemicellulose in it (Manmai et al., 2021). The enzyme with the right amount of cellulase activity can work with other enzymes to go after the parts of cellulose fiber that are typical. This is possible because the enzyme has the right activity for cellulase. For cellulosic ethanol production to be more cost-effective, it needs to produce many sugars. This is done by loading the plant material with a lot of solids. The easiest way to make ethanol is to get as many sugars out as possible and ferment them into ethanol. Therefore, the degradation effect of treated barely straw treated with cellulase enzyme barely straw obtained from pretreatment with acid and base solutions was selected by using 5 g of straw weight, soaking in 100 ml of sodium citrate buffer pH 4.8, and adding cellulase activity, then incubated at 42 °C, shaking rate 200 rpm for 16 h. After combined pretreatment, the surface of rice straw showed cracks at the edge of the surface and disordered organization of the structure. The straw will expand, which will be seen as tearing fibers (Manmai et al., 2020). This is because the lignin on the surface of the straw has been removed, which is good for the function of the enzyme to be able to work more easily. And when the straw is further digested with enzymes, it can be seen that the straw looks like a small fiber stick missing from each other and break out disorderly scattered.

Table 1 Sugar concentrations obtained from barely straw with/without pretreatment and cellulase hydrolysis.

Sugar concentrations	Untreated barely straw	Pretreatment without cellulase digestion	Pretreatment with cellulase digestion
Total Sugar (g/L)	7.11±1.75	43.52± 1.17	205.43±1.22
Reducing Sugar (g/L)	2.99±2.42	21.02±1.96	134.42±0.56

The solution fraction was analyzed. The total sugar and reducing sugar contents were determined and the results are in Table 1. It was found that the sugar concentration obtained from barely straw without pretreatment the total sugar concentration of untreated barely straw was 7.11, and reducing sugar content was 2.99 g/L, while that of pretreated (i.e., selected combined acid-alkaline conditions) barely straw highest total sugar and reducing concentration was 43.52 and 21.02 g/L, respectively. Subsequently, with pretreatment and cellulase hydrolyzed solution released total and reducing sugar concentrations were 43.52 and 21.02 g/L, which can be seen that modified barley straw sugar concentrate over 28 times higher than untreated rice straw due to the pretreatment has a structural change. This resulted in reducing the cellulose's crystallinity and increasing the area for enzymatic digestion, resulting in the enzymes being able to work more efficiently.

3.4 Effects of ethanol fermentation by S. cerevisiae

Ethanol fermentation is generated by hydrolyzing barley straw with cellulase enzyme. The concentration was about 205 g/L, pH was adjusted to 5, and fermentation was carried out with S. cerevisiae yeast cells in barely straw substrate. A batch experiment used yeast to convert sugar to ethanol. This was done in order to increase the efficiency of the fermentation process. Separate hydrolysis and fermentation (SHF), or simultaneous saccharification fermentation (SSF) can be used to produce ethanol adequately. However, the best temperature for yeast is not necessarily the best for enzymes. Therefore, SSF conditions cannot be optimal for yeast and enzymes, leading to lower efficiency and product yields (Lin et al., 2018). The SHF method was chosen for this purpose because it would allow for more efficient ethanol production (Vu et al., 2017). When using SHF processes, it is advisable to use robust yeasts that can ferment glucose to ethanol at higher temperatures (Vu et al., 2018).

These *S. cerevisiae* yeasts are more likely to be active than the cellulolytic complex. It is important to increase the efficiency of fermentation systems for existing and converted fermentable sugars to produce high levels of ethanol. The *S. cerevisiae* yeast cells were then fermented for three days on pretreated and hydrolyzed straw as the following step in the process. The total and reducing sugar concentrations showed a slow but steady decline as the incubation period progressed. This mechanism agreed with previous studies (Moraes et al., 2022; Manmai et al., 2021; Nguyen et al., 2022; Ramaraj et al., 2021; Sophanodorn et al., 2022a; Trejo et al., 2021; Whangchai et al., 2021). Finally, the best ethanol yield of 16.17 g/L was accomplished on the second day of the process.

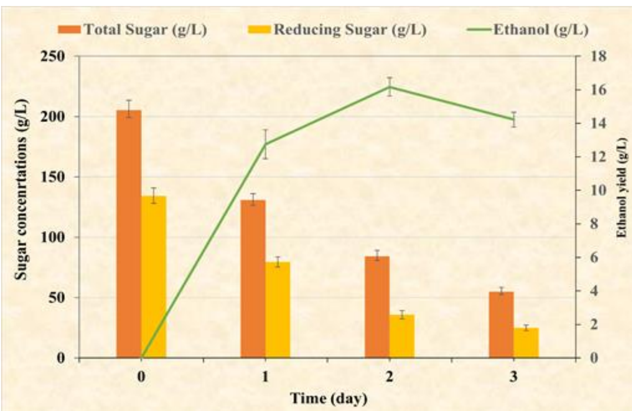

Figure 4 Ethanol production from pretreated and hydrolyzed barley straw

4. Conclusion

In this study, combined acid and alkaline treatment were imitated as the most appropriate pretreatment for barely straw, as high sugar and ethanol yields were gained in enzymatic hydrolysis and fermentation by *S. cerevisiae* yeast cells. The resulting acid and alkaline concentrations are sufficient to allow lignin to break bonds. With combined pretreatment, the total sugar concentration was 43.52 g/L and the reducing sugar was 21.02 g/L. It was revealed that pretreatment with 1.5% of sulphuric acid and alkaline calcium hydroxide (2% w/v), caused the dissolution bonds to dissolve, given the highest sugar concentrations compared to untreated samples. After that, the combined pretreated barley straw, the cellulase activity was used to hydrolyze the enzyme with a total sugar concentration of 205.43 g/L and a reducing sugar of 134.42 g/L was reached. The *S. cerevisiae* yeast cells were next investigated on pretreated and hydrolyzed straw for fermentation for 3 days. On the 2^{nd} day, the highest ethanol yield was achieved at 16.17 g/L. The renewable and alternative energy development strategy, this study result, is yet another intriguing approach that can be utilized and further improved in order to increase yield in ethanol production and develop alternative energy sustainability.

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