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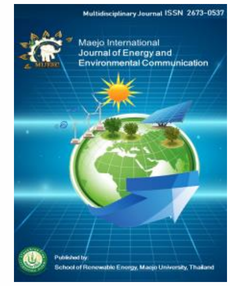

ARTICLE

Bioethanol production from low-grade konjac powder via combination of alkaline and thermal pretreatments

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ABSTRACT

Combining alkaline and thermal pre-treatments for the conversion of low-grade konjac powder to fermentable sugars was studied. After pretreatment, an enzyme hydrolysis procedure was applied with cellulase. The pretreatment and hydrolyzed liquids were used with Saccharomyces cerevisiae for the fermentation process in the batch system. Analysis with a spectrometer revealed that the fundamental matter of low-quality konjac powder was severely degraded in order to enhance the fermentable sugars. Our investigation found that the alkaline combination thermal pretreatment produced the most reducing sugars. The impacts of several operational factors were examined. The highest alcohol content (3.551g/L), heating value (3570.27Cal/g) and energy values (17.15 MJ/kg) were achieved, this might be a very promising method of low-grade konjac powder use. Pretreatment of low-grade konjac powder for bioethanol production is highly advised due to its extremely low cost and high yield of reducing sugars and bioethanol.

1. Introduction

Energy availability is a precondition for human life since it is a fundamental component. Increasing their energy independence while also cutting their carbon dioxide emissions is a primary motivation for many nations worldwide, which is why they are examining their possibilities in renewable energy right now (Chuanchai & Ramaraj, 2018). In recent years, there has been a significant shift in the quantity of energy generated from fossil fuels like coal and oil. This is a significant factor adding to the planet's overall warming trend (Dussadee et al., 2016). A combination of factors, such as an increase in global population and falling food production, has led to a decline in the world's food supply (Unpaprom et al., 2021).

More living space is used in regions with limited energy resources, like sugarcane, maize, cassava, starch, and maize. These places are home to a smaller number of people. Both are food crops, and they are used in food production on a global level (Khunchit et al. 2020). The energy crops used to produce ethanol can be classified into three groups: The first includes sugar crops such as sugarcane, sweet radish, and sorghum; the second includes food in the form of corn, wheat and rice; and the third is the lignocellulose category, which includes agricultural wastes and plants (Manmai et al. 2021a). However, humans have a growing need for lignocellulose. Each year, there is an increase in the population. As a result, many countries are working hard to find alternative energy sources, such as natural gas, bioethanol, wind, and sun.

Bioethanol is the principal fuel used as an alternative to gasoline in cars used for road transportation (Nguyen et al., 2020).

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The fermentation of sugar is the primary production method for bioethanol fuels (Manmai et al. 2021b). Fuel or energy crops are the primary sources of sugar for ethanol production. These crops are grown solely to produce energy. They include maize crops, wheat, waste straw, willow and popular trees, sawdust, canary sedge, hay, Jerusalem artichoke, miscanthus, konjac, and sorghum well as other types of community solid waste for the production of ethanol fuel (Bhuyar et al., 2022).

Konjac is also known as elephant-yam. It is a native Asian plant. The Konjac plant scientific title is Amorphophallus Konjac. It is a tuberous perennial. Corm, also known as starchy bulb root, is another name. Konjac, an indigenous crop in Thailand, is found in the northern regions. Buk Nuea Si, also known by Buk Khai due to its high glucomannan level in its corms, is highly valued in the food industry and considered highly valued (Bhuyar et al., 2022).
Therefore, this particular species is becoming a precious commercial species. The Konjac plant lives through several seasons despite being a biennial. The trunk can be thought of as a subterranean version of the head. Lowlands, areas with water nearby, or areas along the water's edge are ideal locations for its growth.

A current can lead to a fresh start if one follows it. The leaves are individual leaves that are fashioned like hearts and have long petioles that are succulent. A color between purple and green may be seen at the surface. There is sticky latex that has a murky appearance. It may cause itching if it comes in contact with the skin and may be found across Thailand in every location despite being a nuisance plant. The tuber of the konjac plant was the source of the lignocellulose that was investigated in this research. Fresh tubers contain about 80-90% water, the solid part (Bhuyar et al., 2022). It is a carbohydrate nutrient consisting of glucomannan, composed of manose and glucose in a ratio of 3: 2, connected by a glycosidic bond at the beta-1,4. The konjac tree is classified as a herbaceous plant that has many years of life. The trunk thrusts up from the tuber. The height of the plant is about 50-150 cm, and the tuber is large. The appearance of head is somewhat round and slightly flat (Bhuyar et al., 2022). The lactic material used for this research was konjac because it is a common weed. These materials were studied in order to increase alternatives for konjac powder used to produce sugar to produce bioethanol.

Research is being done on these materials to find new routes that will improve the value of the material itself. This conductivity is required in order to produce bioethanol from sugar. In addition, the purpose of this research was to evaluate the pretreatment of konjac powder using sodium hydroxide (NaOH), which was selected as the pretreatment method in order to guarantee accurate findings. Therefore, the research was carried out to obtain information from relevant theories, whether the theory of pretreatment or lignocellulose materials, bioethanol production fermentation process and conduct relevant research studies, the demand for energy on a global scale has increased, necessitating increased use of fossil fuels.

2. Material and Methods

The low-graded konjac tubers originated from the northwest provinces of Thailand. The tubers were collected from the forests where they were found and then brought to the laboratory. The Konjac tubers first had to be washed with enough tap water to remove any germs or dirt. The Konjac tubers were then chopped and dried. They were then ground up and kept at room temperature. Once the tubers had been dried, they were ground into a fine powder and then put through further steps to prepare them for hydrolysis. A schematic illustration of the research pretreatment strategy used for this investigation is shown in Figure 1. The experiments were accomplished at SRSE-LAB (Sustainable Resources and Sustainable Engineering Research Lab), Maejo University, Chiang Mai, Thailand.

In order to do this, a sample of 10 g of Konjac tuber powder was weighed, mixed with 100 mL of various sodium hydroxide solutions, and then heated in a water bath at three different temperatures. Finally, a sample was taken to assess the amount of total sugar and reducing sugar after heat treatment. For starch-rich feedstocks, alkaline pretreatment, including NaOH, is an efficient way to raise the amount of sugar in the raw materials used in bioethanol production.

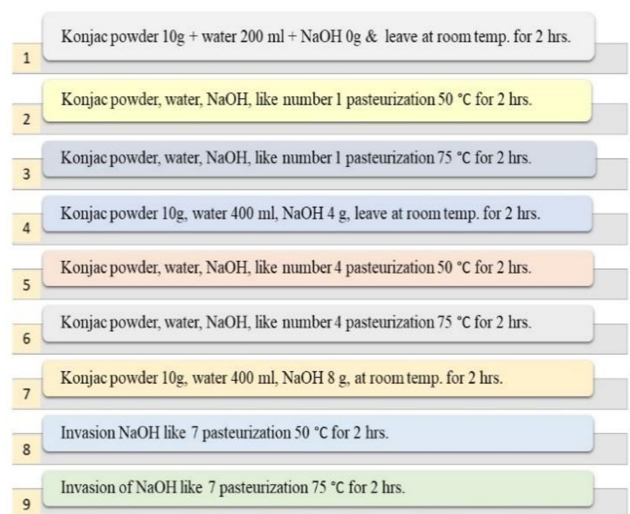

Figure 1 Alkaline thermal pretreatment procedure for low-graded konjac tubers

After undergoing thermal treatment, followed by enzymatic hydrolysis, the pH of the pretreated mixture was first measured using a potentiometer and adjusted to a range of 5 to 5.5 with a NaOH solution at [5 N] to execute the hydrolysis process. A 1% cellulose enzyme concentration was added to the mixture before the hydrolysis process began. The mixture was kept in an incubator at a constant 35 °C. In order to measure total sugar and the rate of decline in sugar, a sample was obtained after 24 hours.

All stages of the experiment, including pretreatment, hydrolysis, and fermentation, were assessed by total sugar and reducing sugars. Each experiment was conducted in triplicate. Measurements of total sugar and reducing sugars were made using spectrophotometry. When calculating total sugar content, the phenol sulfuric acid method was utilized (Dubois et al., 1956). Dinitrosalicylic acid (DNS) was used to ascertain the reducing sugar (Miller, 1959).

Before fermentation, the sample was allowed to check the pH and adjust the pH to be between 5.0 -5.50. dividing the data into 3 ratios of water to caustic soda, 1:0, 1:5 and 1:10, respectively, and selecting the best experimental data to enter into the yeast fermentation process. A basic fermentation procedure is adopted by Vu et al. (2018). In the fermentation process for ethanol production, 8g of konjac powder, 400 mL of water, and 2% sodium hydroxide were used twice in the experiment. *S. cerevisiae* yeast, at a concentration of 1%, was used during the fermentation stage in bioethanol production. Fermented for 14 days and alcohol was collected and checked for alcohol content twice a day. Knowing the best alcoholic fermentation conditions allowed for the distillation process. Take the selected samples from the fermentation process into the ethanol distillation process. Then bring the distilled alcohol into the testing process to determine the energy value and the resulting calorific value by bomb calorimeter.

3. Results and discussion

3.1 Alkaline and thermal pretreatment

Bioethanol is sugar-and-starch-rich plant biomass fermented by microbes. Future fuel. Bioethanol may be a future fuel due to its high output, low consumption and potential to meet future energy needs (Dev et al., 2019). Rising food costs are being blamed on first-generation biofuel. Although biofuels might increase food prices slightly, this argument ignores the environmental and energy security benefits they offer. Tuber and root crops provide food for people, animals, and industries. Globally, 45 % of root- and tuber crop yields are consumed. The rest is used to make animal feed, bioethanol, or other agricultural products (Thatoi et al., 2014). The consumption of root and tuber crops is lower in industrialized than in the poor. Extra tuber crops could be used to make food, but not for fuel. From 1980 to 2012, root crop production grew dramatically due to increased production, better technology, and more planting resources. Thailand produces cassava, sweet potato, and konjac tubers.

Low-grade tubers can be used in industrial processes like bioethanol production but cannot be eaten by humans or animals. Farmers may be able to reap the economic benefits of low-grade materials. Pretreatment alters the substrate properties to prepare them for enzymatic degradation. Tuber crops contain starch, which is a long-chain sugar molecule (Thatoi et al., 2014). Pre-treating starchy substrates convert starch molecules to glucose molecules. This allows fermentation. Pretreatment alters the substrate properties to prepare them for enzymatic degradation. Mechanical milling of whole tuber flour is required for starchy substrate (Dev et al., 2019). Mechanical milling reduces dried tubers into small enough particles that they can be passed through a mesh screen. The flour can then be used for substrate hydrolysis. Tuber crops can be chemically pretreated (Bhuyar et al., 2022). A thick gel is produced by water-heated starch. Gelatinization. Enzymatic hydrolysis requires starch gelatinization.

In this study, a pretreatment study with sodium hydroxide, the ratios of konjac powder were divided as follows: 1:0, 1:5 and 1:10, with different thermal pretreatment times used with an autoclave. From Figure 2, the total sugars and reducing sugars are different at different temperatures of pasteurization, indicating that the ratio of 1:5 was used for pasteurization at 75°C. The highest total sugar yield was 12.716 g/L and the ratio of 1:10 at room temperature, and the reducing sugar yield was 2.370 g/L, which had the maximum sugar value.

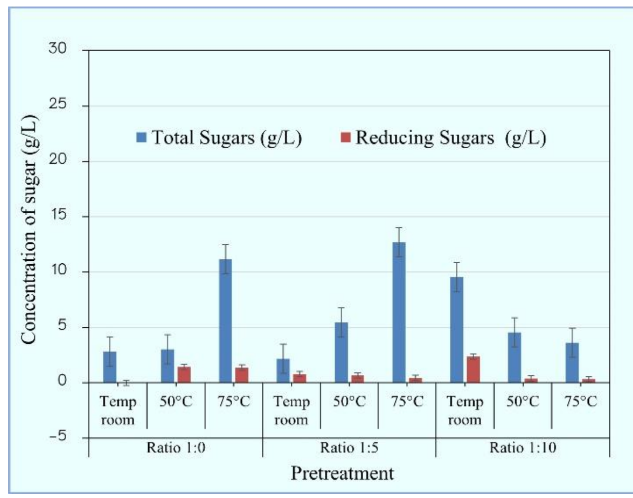

Figure 2 Sugar yield of Konjac tuber pretreatment with different ratios

3.2 Hydrolysis process and sugar yield

Following pretreatment, the cellulose is subjected to enzymatic hydrolysis to produce glucose, which is then converted by microorganisms to ethanol (Ebabhi et al., 2018; Zabed et al. 2017). High levels of carbohydrates in biomass make hydrolysis easier. This increases the sugar yield per gram of biomass which, in turn, increases overall bioethanol yield and productivity. These parameters were very close to the ones reported in the original study.

This small variation in composition could be due to the analysis method, location or accuracy (Behera et al., 2019; Suriya et al. 2016). After pretreatment with sodium hydroxide, 1% cellulase was added to undergo hydrolysis. The result will be shown in the following graph. From Figure 3, the highest total sugars and reducing sugars were 1:5 ratio, using pasteurization at 50 °C to 12.716 °g/L, which was the best value for fermentation.

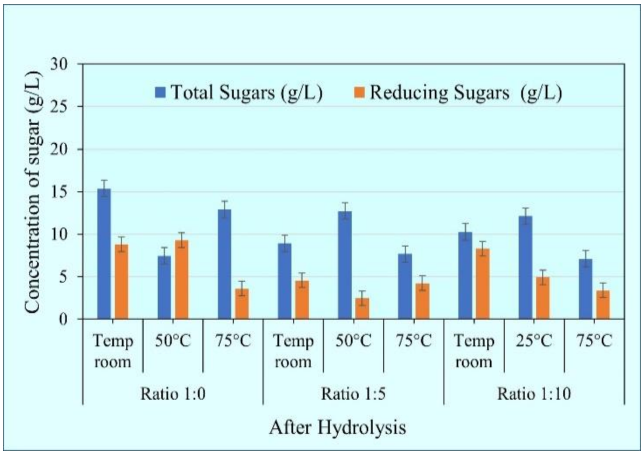

Figure 3 Sugar yield of Konjac tuber after hydrolysis with different ratios

3.3 Results of ethanol fermentation

The metabolic conversion of monosaccharides into bioethanol or other byproducts is referred to as fermentation (Pradechboon & Junluthin, 2022). This process takes place in circumstances that are supportive, such as temperature and pH (Ramaraj & Unpaprom, 2019a). The presence of yeast or bacteria might result in the process of fermentation occurring (Ramaraj & Unpaprom, 2019b). Separate hydrolysis and fermentation, also known as SHF, was chosen over simultaneous saccharification and fermentation, also known as SSF, in order to achieve the highest possible rate of starch conversion to glucose (Sree et al., 1999; Szambelan 2018). Put the results from the hydrolysis process into the ethanol fermentation process with yeast for 14 days as shown in the following graph.

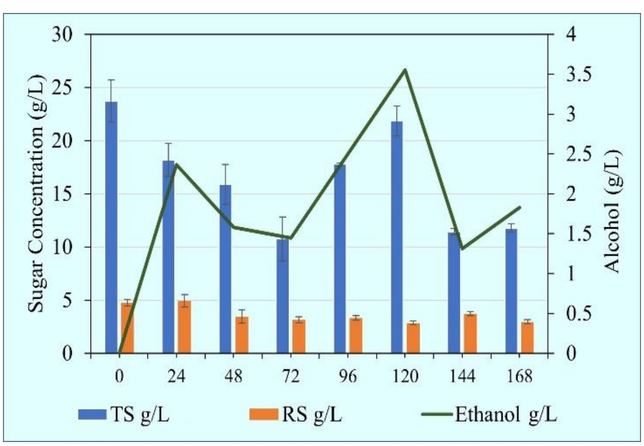

Figure 4 Amount of sugar utilization during the ethanol production period of 14 days

Figure 4 shows that the amount of total sugars and reducing sugars has been progressively increasing and decreasing from the beginning of fermentation. Total sugars began at 21.844 g/L and continued to drop until day 14, when they reached 11.733 g/L. Meanwhile, reducing sugars began with ethanol at 3.551 g/L and began to decline on the 12th day or 144 hours with the ethanol content at 1.315 g/L. The highest amount was at 4.399 g/L, and it continued to decrease until the 14th day, when it reached 2.967 g/L. g/L, and it continued to decrease until the 14th day, when it reached 2.967 g/L.This study acknowledged the fact that dilute alkaline pretreatment could be a promising alternative method replacing acid pretreatment to enhance reducing sugar production through enzymatic saccharification of microalgal biomass. This conclusion was reached after looking at the overall results of the study.

4. Conclusion

This experimental study shows that the ratio of low-graded konjac powder to sodium hydroxide is 1:15. This ratio is ideal for yeast fermentation as it contains the highest total sugars at 12.72 g/L and the lowest reducing sugars during hydrolysis. This fermentation time is required to make ethanol. The alcohol content was 3.551g/L. *S. cerevisiae* yeast, at a concentration of 1%, was used during the fermentation stage in bioethanol production. The hydrolyzate step saw sugars converted to ethanol at a concentration of 2.483g/L. The distilled alcohol was applied to a bomb calorimeter to determine its energy content. The process was repeated twice, with the first running lasting 25 minutes. The final temperature reached 28.9°C, and the energy content at 14.95 MJ/kg. The second heat of 3570.27Cal/g reached. The energy value was 17.15 MJ/kg. It takes 26 minutes to complete the second heat of 3955.75 Cal/g. The energy content is 15.71 MJ/kg, and the calories are 3753.11 Cal/g. As a result, Low-grade konjac powder can be used as a raw material for bioethanol production and may also be used as an alternative energy source.

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