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# Bioethanol Production from Spirulina (*Arthrospira platensis*) by Microwave-Assisted Acid Hydrolysis Pretreatment

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## Abstract

The world's high consumption of fossil energy increases carbon dioxide ($CO_2$) emissions. The depletion of fossil fuel sources, combined with rising $CO_2$ emissions, has prompted intensive research into renewable energy sources. Bioethanol is an environmentally friendly energy source that has the potential to reduce reliance on gasoline. Bioethanol is produced through the fermentation of monosaccharides. The first and second generations of bioethanol are derived, respectively, from food crops, agricultural waste, and plantations, while the third generation is derived from algae. However, the third generation bioethanol research is still being conducted intensively to develop an optimal process. Macro/microalgae are low-level plants that have the potential to become raw materials for bioethanol. *Arthrospira platensis,* a spirulina species, is a microalgae with a high carbohydrate content. Apart from that, this type of microalgae is easy to cultivate and grow. This research aims to determine the reducing sugar content which are monosaccharides produced from acid hydrolysis using a microwave at a temperature of 100 °C for 60 – 120 minutes with 0.2 M $H_2SO_4$ as the solvent. The hydrolysate obtained was then fermented anaerobically with *Saccharomyces cerevisiae* in a shaking water bath. The High Performance Liquid Chromatography (HPLC) test was carried out to identify the reducing sugar groups in the hydrolysate. Moreover, the solid content of the biochar remaining from the hydrolysis process was analyzed using Fourier-Transform Infrared Spectrometer (FTIR). From the test results, it was found that the highest concentration of D-glucose (1.19 g/L) occurred at 90 minutes of the hydrolysis. In addition, the hydrolysis of microalgae was also carried out with 0.3 M $H_2SO_4$ solvent for 90 minutes. The hydrolysate was then fermented for 96 hours. From the distillation process, it was obtained a bioethanol yield of 2.89%.

## INTRODUCTION

Indonesia's reliance on energy derived from fossil fuels grows year after year. Several decades ago, Indonesia was one of the world's largest petroleum producing countries, but it is now to be an importer of petroleum because the consumption exceeds production. Indonesian crude oil production fell from 282 million barrels of oil per day (BOPD) in 2018 to 240 million BOPD in 2021, with the transportation sector consuming the most fuel, accounting for 90.3% of total consumption (Suharyati et al., 2022).

In Indonesia, fossil energy continues to dominate the primary energy mix (87.8%), with renewable energy accounting for the rest (Suharyati et al., 2022). The world's high consumption of fossil energy has increased $CO_2$ emissions, which reached 37 Gt in 2017 (Ma'mun et al., 2018; Ma'mun et al., 2019), contributing to the problem of global warming. Meanwhile, biomass-based energy can reduce $CO_2$ emissions based on the supply chain (Röder et al., 2019), even when the Life Cycle Assessment (LCA) shows negative $CO_2$ emissions (Dasan et al., 2019). Some researchers have investigated renewable energy sources that emit less

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$CO_2$ (Chang et al., 2020; Ishaq & Dincer, 2020; Koc et al., 2020; Ong & Wu, 2020; Zhao & Su, 2020; Chaparro-Garnica et al., 2021; Onigbajumo et al., 2021; Zhao et al., 2021). Bioethanol, a renewable energy, has promising prospects to reduce dependence on gasoline. According to the Optimistic (OPT) scenario, Indonesia has targeted to start using biogasoline E5, i.e., 5% of bioethanol in gasoline, in 2030 (Suharyati et al., 2022). Bioethanol is made from biomass conversion through a fermentation process.

Bioethanol is generally produced from the fermentation of food crops (first generation) such as sugar cane, corn, and other agricultural products, and from lignocellulose (second generation) such as residues from agricultural products, forests, and other ligneous cellulosic materials. Commercial use of the first-generation feedstock is cheaper but will cause competition with food sources. Meanwhile, making bioethanol from the second-generation feedstock is more complex than other raw materials due to the presence of lignin. The third generation bioethanol with biomass from microalgae is still being developed. Furthermore, advanced biochemistry techniques and petroleum-like hydroprocessing are used to produce the fourth generation of bioethanol. However, intensive research on the third generation of bioethanol is still being conducted to determine an optimum process (Hwang et al., 2016). This is due to the numerous benefits of microalgae as a fermentation feedstock for bioethanol production. They can overgrow in bodies of water and seas. They do not require agricultural land and thus do not compete with food production, have a very short harvesting cycle (1 – 2 weeks) when compared to other bioethanol feedstock, and produce bioethanol with high productivity, such as *Arthrospira platensis*, *Botryococcus braunii*, *Chlorella vulgaris*, and *Nannochloropsis* (Mussatto et al., 2010; Ma'mun et al., 2022). Moreover, microalgae capture $CO_2$ for photosynthesis, thus lowering greenhouse gas emissions to the atmosphere (Stewart & Hessami, 2005; Choi et al., 2019; Beigbeder et al., 2021; Chen and Xu, 2021; Cheng et al., 2021; Rodas-Zuluaga et al., 2021; Mohapatra et al., 2022; Osat et al., 2023; Yang and Xin, 2023). However, because microalgae have cell walls, additional processing (i.e., pretreatment process) is required to break down the cell walls (Goldemberg & Guardabassi, 2010; Pérez-Pimienta et al., 2016)

Indonesia is a country that has a very wide coastline, making Indonesia a potentially profitable field for algal cultivation. Microalgae have attracted the attention of researchers because of their potential as the third-generation feedstock for making bioethanol (Sadatshojaei et al., 2020). Apart from that, its abundant quantity can provide a high potential for sustainable production. In addition to the benefits mentioned above and high protein content, *Arthrospira platensis*, a biomass of cyanobacteria, has a high carbohydrate content that ranges from 13.6 to 53.85% (Um & Kim, 2009; Tourang et al., 2019; Kusmiyati et al., 2020) when compared to other microalgae (Kusmiyati et al., 2023). The higher the carbohydrate content, the greater the potential for bioethanol production.

Currently, bioethanol synthesis from microalgae does not provide advantages compared to other sources on a large scale, because the process route and energy use are not yet optimal (Mayers et al., 2018). The research currently being carried out is still focused on finding solutions to the problems above by considering the living conditions of microalgae such as pH, temperature, nutrition, and light supply. Apart from that, studies on the pretreatment process and fermentation process are being actively carried out to find optimum conditions. An effective pretreatment should be simple, require fewer chemicals, and use little energy, thus lowering production costs. Supercritical $CO_2$ (Tang et al., 2011), $NH_3$ fiber explosion (Teymouri et al., 2005), ultrasonication (Hwang et al., 2016; Krishnamoorthy et al., 2023), hydrothermal pretreatment (Fu et al., 2021), alkaline pretreatment (Kassim and Bhattacharya, 2016), acid pretreatment (Thangavelu et al. 2019; Yu et al., 2020; Ma'mun et al., 2024), and pulsed microwave pretreatment (Zhang et al., 2022) are some of the pretreatment methods that have been used to produce biofuels from microalgae. Among the pretreatment methods discussed above, a combination of pulsed microwave and acid pretreatment appears to be the best method because it requires less energy, thereby lowering the overall cost. However, the acid concentration used must be low to reduce corrosion. Based on this reason, microwave-assisted acid hydrolysis pretreatment was employed in this study.

The purpose of this study is to determine the content of monosaccharide groups obtained from the hydrolysis with the help of microwaves from *Arthrospira platensis*. The hydrolysis results are

then fermented, and the ethanol content is determined by distillation.

## MATERIALS AND METHOD

### Materials

The materials needed for the research include *Arthrospira platensis* microalgae powder with 100% purity obtained from Beautiful Tropical Life of Bali Ltd. Sulfuric acid ($H_2SO_4$) with min. purity of 98% from Merck was used for the hydrolysis process. *Saccharomyces cerevisiae* culture was used as yeast for fermentation. The other materials used were Whatman filter paper No. 42, nitrogen ($N_2$) 99.99% from AGI Ltd., and deionized water. Meanwhile, the apparatus needed was a microwave reactor equipped with a temperature controller with an accuracy of ±1 °C for the hydrolysis process, a shaking water bath (SWB 30, 20 – 200 rpm) for the fermentation process, and a batch distillation setup to separate ethanol from the fermented liquid.

### Method

#### *Hydrolysis with a microwave reactor*

Hydrolysis is the process of breaking down carbohydrates into simple sugars. The hydrolysis process carried out on microalgae will produce a liquid hydrolysate containing simple sugars such as D-glucose, L-arabinose, D-galactose, D-mannose, L-rhamnose, D-xylose, and solid biochar. The hydrolysis process can be carried out i.e., $H_2SO_4$ as conducted by Yu et al. (2020) and Ma'mun et al. (2024). Apart from that, it can also be used NaOH, $HNO_3$, and HCl which have been done by Markau et al. (2013), Miranda et al. (2012), and Zhou et al. (2012).

A total of 10 g of Arthrospira platensis was added into the reactor containing 100 mL of 0.2 M $H_2SO_4$ solution and was stirred uniformly. The slurry was then put into the microwave and heated up to 100 °C with a heating rate of 8 °C/min. Once the slurry temperature reached 100 ± 1 °C, the hydrolysis process was started for 60 to 120 minutes. In addition, hydrolysis of 10 g of *Arthrospira platensis* was carried out using 0.3 M $H_2SO_4$ as the solvent for 90 minutes. The hydrolysis results are presented in Figure 1. The slurry was first filtered with ordinary filter paper, followed by Whatman 42 filter paper, and finally filtered with Millipore 0.45 μm. Meanwhile, the solid biochar obtained was dried to test its composition using FTIR, while the monosaccharide groups in the hydrolysate were analyzed using HPLC.

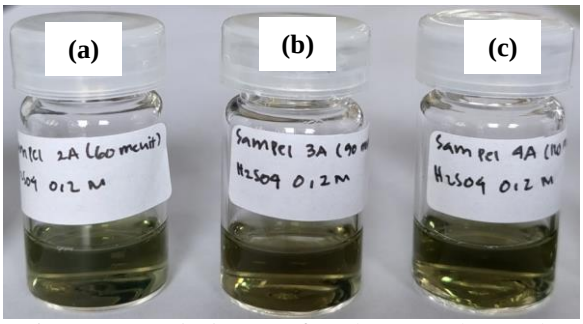
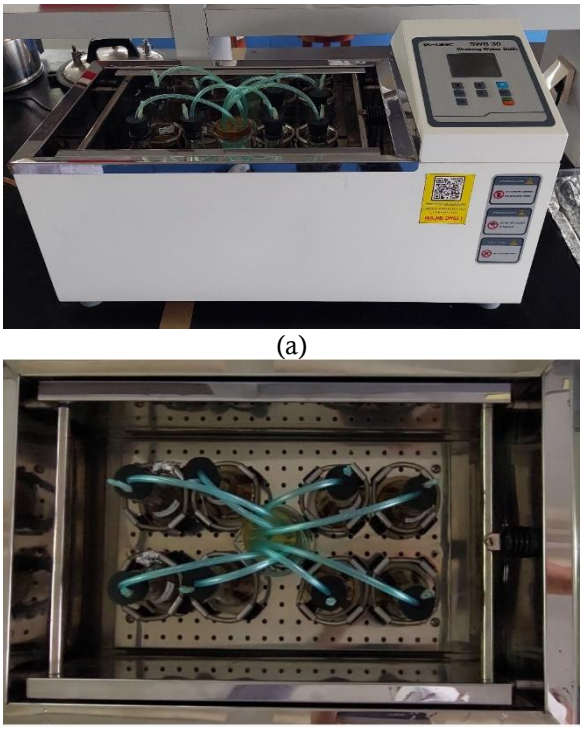



Figure 1. Hydrolysate of *Arthrospira platensis* by microwave-assisted acid hydrolysis at: (a) 60, (b) 90, and (c) 120 minutes.

#### *Fermentation*

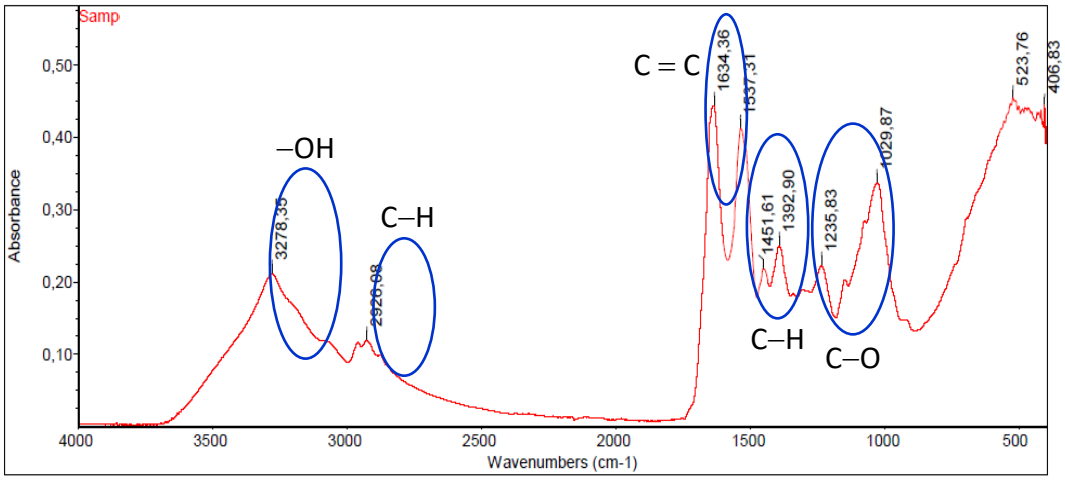
Fermentation was carried out in eight 250-mL Erlenmeyer flasks containing 100 mL of hydrolysate and 5% (w/w) *Saccharomyces cerevisiae* yeast. The flasks were flushed with $N_2$ before fermentation to replace the air. The fermentation was carried out in a shaking water bath for 96 hours at 30 °C in dark and anaerobic conditions, as shown in Figure 2. The fermentation products were then distilled at 95 – 97 °C to separate the ethanol content from the fermented hydrolysate.



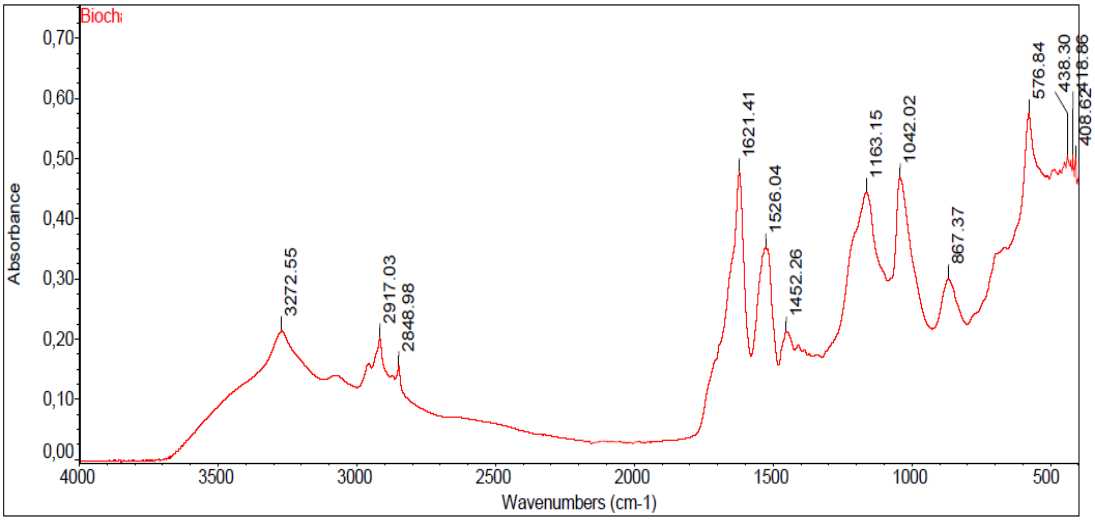
(a)

(b)

Figure 2. Fermentation in a shaking water bath: (a) front view, (b) top view.




(a)




(b)

Figure 3. FTIR analysis results: (a) pure *Arthrospira platensis*, (b) biochar.

## RESULTS AND DISCUSSION

In this study, the hydrolysis process used 0.2 and 0.3 M $H_2SO_4$ as solvents. The hydrolysis process greatly influences the reducing sugar content produced. Moreover, hydrolysis is also influenced by several parameters such as temperature, acid concentration, amount of raw material, time, and carbohydrate content. $H_2SO_4$ concentration of 0.2 M was used for a hydrolysis time between 60 and 120 minutes and 0.3 M for the hydrolysis time of 90 minutes.

The results of hydrolysis are hydrolysate and biochar. The biochar composition was analyzed using FTIR which aims to determine the components contained in the sample. Figure 3 shows the FTIR spectra for biochar obtained from the hydrolysis with 0.3 M $H_2SO_4$. For a comparison, the components of pure *Arthrospira platensis* powder were also analyzed. In addition, the composition of the reducing sugar groups in the hydrolysate was analyzed by HPLC.

The results of FTIR analysis in Figure 3(a) show that the highest peak intensity is in the wavelength of 3278.35 $cm^{-1}$, where this wavelength indicates the hydroxyl – OH stretch functional group which is included in the group of alcohol compounds and phenol. The peak with a wavelength range of 2850 – 2970 $cm^{-1}$ shows the C – H stretch vibrational strain which is a class of alkane compounds, while the peak with strong and sharp absorption in the wavelength range 1610 – 1680 $cm^{-1}$ shows the C = C stretch vibrational strain which is a group of alkene compounds. Then the peak with a wavelength range of 1340 – 1470 $cm^{-1}$ shows the C – H bend vibrational strain which is a class of alkane compounds. The peak in the wavelength range 1050 – 1300 $cm^{-1}$ shows C – O

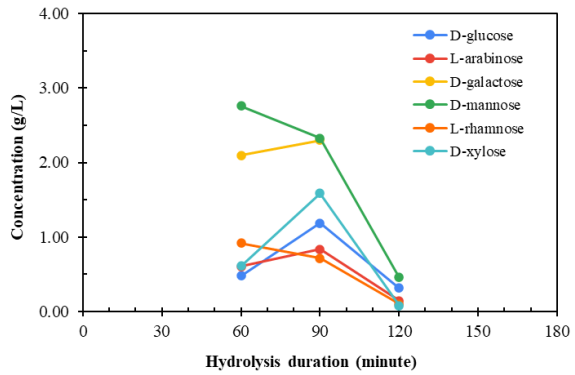



Figure 4. Composition of reducing sugar groups produced from the hydrolysis of *Arthrospira platensis* with 0.2 M $H_2SO_4$ for 60, 90, and 120 minutes.

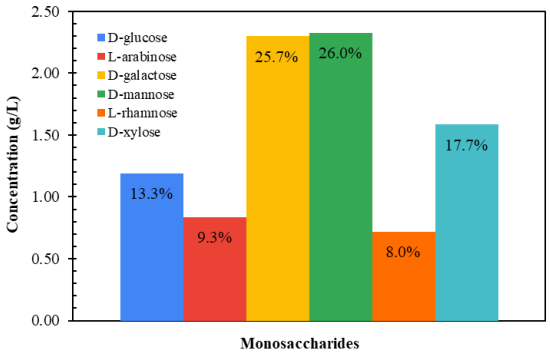



Figure 5. The concentration of reducing sugar groups produced from the hydrolysis of *Arthrospira platensis* with 0.2 M $H_2SO_4$ for 90 minutes.

stretch vibrational which are alcohol, ether, carboxylic acid, and ester compounds (Skoog et al., 2018). As seen in Figure 3(b), the FTIR spectra of the biochar are similar to those of pure *Arthrospira platensis*. This condition indicates that the functional group content is nearly constant.

The effect of hydrolysis time on the concentration of monosaccharide groups was also determined using HPLC in this study. The results are shown in Figure 4. It can be seen that for a hydrolysis time of 90 minutes, the content of reducing sugar groups increases in general, with the exception of D-mannose, whose concentration decreases as the time of hydrolysis increases. Meanwhile, the highest D-glucose concentration was achieved after 90 minutes of hydrolysis, with a concentration of 1.19 g/L. In addition, Figure 5 depicts the composition of reducing sugar groups in *Arthrospira platensis* hydrolysis for 90 minutes. It can be seen that D-galactose and D-mannose account for the majority of the hydrolysate content (51.7 %), with D-glucose accounting for only 13.3 %. The ethanol yield during the fermentation process, on the other hand, is determined by the total reducing sugar concentration.

In addition to the hydrolysis with 0.2 M $H_2SO_4$, *Arthrospira platensis* was also hydrolyzed with 0.3 M $H_2SO_4$ as the solvent. The hydrolysate from this sample was then fermented under the same conditions as the 0.2 M $H_2SO_4$ hydrolysate, i.e., 30 °C for 96 hours. The fermented hydrolysate was distilled, yielding 2.89 % ethanol. This result falls within the range of other biomass sources listed in Table 1.

## CONCLUSION

Based on the experimental results, the optimum hydrolysis time to obtain the highest reducing sugar concentration is 90 minutes. The

Table 1. Yield of ethanol in comparison to those of other researchers

| Biomass | Yeast concentration | Ethanol, % | References |
|---|---|---|---|
| Duckweed | 5, 15, and 25% | 0.45 – 3.80 | Khodijah & Abtokhi (2015) |
| Molases | 0 – 4.26 g/L | 0.14 – 5.43 | Jayus et al. (2016) |
| Corn stalks | 0.1, 0.2, 0.3, and 0.4 g/g sample | 0.26 – 1.25 | Rijal et al. (2019) |
| Giant cassava | 5 and 10% | 0.00 – 2.83 | Candra et al. (2019) |
| Sago pith waste | 5% (v/v) | 15.6 – 30.8 (g/L) | Thangavelu et al. (2019) |
| Arabica coffee skin | 3, 9, and 15 g/25 g sample | 0.35 – 1.46 | Febrina et al. (2020) |
| Industrial Sugar Beet Molasses | 0.2, 0.6, and 1.0 g/L | 12 – 74 (g/L) | Beigbeder et al. (2021) |
| *Arthrospira platensis* | 5% | 2.89 | This study (2024) |

maximum reducing sugar content of D-glucose in 0.2 M $H_2SO_4$ solvent was 1.19 g/L. When the sample was hydrolyzed with 0.3 M $H_2SO_4$, an ethanol concentration of 2.89 % was obtained. It is hoped that the high reducing sugar content of the hydrolysate will increase the ethanol content produced during the fermentation process.

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