The Pattern of Extraction Factor by the Delignification Agent of *Gracilaria Sp*. Seaweed for Bioethanol

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Abstract. Absorption of carbon dioxide gas through the increased production of macroalgae in the future is the answer to one of the increasing temperature problems in global warming. Carbon absorption can be implemented by producing biomass for the food sector from non-residue and residue to renewable energy; biofuel of bioethanol by engineering the extraction of glucose molecules. This research was conducted to study patterns of lignin depolymerization effect on water content for a residual utilization basis in subsequent saccharification and ethanol fermentation. Neutralization of the hydrogen peroxide oxidizer requires capturing oxygen atoms by the changing principle of the functional group of organic compounds. The resulting pattern of delignification/ depolymerization of lignin using an oxidizer provides an increased water content. The residue handling process for effective saccharification based on dry residual biomass conditions.

Keywords: Oxidizer, sodium hypochlorite, hydrogen peroxide, sodium hydroxide, water content.

1 Introduction

In the short term, until 2025, the Indonesian government's target for the energy transition is expected to reach 23%, but currently, the energy mix reaches 12% [1], and bioethanol is needed. Saling of Pertamax-green 95 fuel type Research Octane Number (RON)-92, namely a composition of 5% ethanol compound (E5) and Pertamax-green 92 (RON-90) 7% ethanol (E7) are the implementation. Ethanol production in the scope of renewable energy divides liquid fuels other than gasoline (petroleum) and gasoline-methanol-ethanol (GME), and the GME A20 type has a composition of 15% methanol, 5% ethanol and 80% 88 octane gasoline.

Ethanol, called bioethanol, is the result of fermentation by the single-cell fungus *Saccharomyces cerevisiae* and requires a substrate raw material with an absolute component of glucose molecules. As a carbon source in cell metabolism during fermentation, glucose is the number of 6 carbon atoms (hexose) in traditional substrates that are natural fruit ingredients and hydrolysates from starch with various plant sources. Another alternative is hydrolysate from the fibre, which is a hexose component of cellulose and has a beta-bonded polymer. The type of hexose source category as cheap comes from organic waste, namely molasses. The need for

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molasses for 1 liter of bioethanol is 4.075 kg, and the efficiency of the fermentation process according to chemical reactions is 0.51 kg of ethanol and 88 kg of CO_2 gas per kg of glucose and various types of biomass sources [2].

Gracilaria sp. macro-algae is a plant that has excellent potential to produce gelatine products and materials for the medical sector and by-product waste that has plant residue components. Besides that, macroalgae that live naturally in the water of coastal areas and estuaries can absorb carbon emissions that dissolve around the surface. That plant has been searching for this potential to reduce temperatures due to global warming since the end of the 20th century. Recently, on beach zones for economic purposes and absorbing greenhouse gases and their effects, it has been cultivated. Therefore, the absorption of carbon dioxide gas through increasing the production of macro-algae in the future is the answer to one of the problems of increasing air temperatures. Absorbing the carbon can be implemented by cultivating macro-algae and biomass for producing energy or using renewable energy. The biomass is a residual component and is a by-product in the form of waste after extracting the agar component (non-residue).

The increase in macro-algae production can supply the need for gelatine in the food sector, and solid waste is a quantity of plant residue for raw materials of renewable energy. The residual waste of *Gracilaria sp*. in Indonesia is 29,088 metric tons/year, and production by-products are 3,509 metric tons per year [3]. The residue has the potential for conversion to glucose using saccharification techniques. Recently, it continues to be studied in various research, especially the saccharification process aimed at ethanol fermentation activity. The industrial need for types of grass and algae cultivation is 204,078.38 metric tons [4], with a production output of 30,000 carrageenan and 10,000 tons of agar [5].

Increasing the production of macroalgal biomass can be linked to the primary living medium in seawater, which can absorb carbon content for its body. Carbon in the surface zone amounts to 5,1011 metric tons in the form of CO_2 gas, CO_3^{-2}, and HCO_3- ions, which dissolve and come from the air, while in the ocean, it amounts to 3,45,1013 metric tons [6]. The current problem is the rapid increase in carbon dioxide gas in the air, the cause of global warming, while the absorption of terrestrial areas by the amount of vegetation continues to decrease. Therefore, *Gracilaria sp*. and other macro-algae can be cultivated in coastal areas and have great potential for indirect carbon absorption in the air. Carbon absorption into the macro algae's body can take place with the help of sunlight.

The formation of carbohydrate compound types resulting from photosynthesis are cell wall components in the form of fibre; glucose polymer forms cellulose. The delignification techniques were available from previous studies on cell walls of woody plant species from terrestrial environments for pulp. The knowledge that has yet to be studied much is about what processes and applying agents to the body of *Gracilaria sp*. Therefore, at the start of the non-residue extraction treatment, the proposed question is: Are processes and agents appropriate for opening the low-lignin encapsulating cellulose?

In concept and laboratory practice, the cellulose fibres (residues) can be converted to hexoses through acid hydrolysis by engineering the lignin envelope, the open-crack. Previous studies on hydrolysates resulting from acidic agents using *Gracilaria sp.* residue showed a typical ethanol fermentation substrate using sodium hypochlorite oxidizer with ion facility engineering without non-residue extract [7]. The method category is separate hydrolysis and fermentation (SHF)-

batch. Engineering at a 17-26% lignin content implemented the ethanol fermentation process using the simultaneous saccharification and fermentation (SSF) method, including the source from the non-food category [2]. In contrast, the lignin content in macro-algae fibre is relatively low.

The study of the utilization of agar production waste, such as the growth of *Saccharomyces cerevisiae* cells for 48 hours, was obtained in *Gracilaria sp*. agar waste using acid and fermenting for 120 hours to produce 2.93% ethanol [8]. Meanwhile, the concept of using 3% NaOH to influence the lignin or dietary fibre content in *G verrucosa* soaked for 0.5, 1, and 1.5 hours and followed by boiling at 100℃ for 2.5 hours [9]. One hour at the same temperature, gracilaria material from the inshore cultivation method obtained the highest agar extract content (20.67 ± 0.27%) while wild or control was between 13.95-19.39% [10]. The author's assessment focuses on cellulose hydrolysis with pretreatment for delignification, which means opening lignin is an analogy to woody plants. In opening lignin for *Gracilaria sp*., this research is related to non-residue extracts in pretreatment because they are helpful in the food and medical fields.

In the SHF-batch method without conducting non-residue extracts on *Gracilaria sp*., hydrolysis of cellulose with the HCl agent and its neutralization is saccharification of residues, and ethanol is reached to a safe limit for the environment of *S cerevisiae* [7]. The lignin content between land and aquatic plants is base for comparison. The implementation concentration of the agent can be less than 1%. The lignin depolymerization agent with high reactivity is nascent oxygen, and the reaction product without excess chlorine is peroxide. This oxygen affects the OH^- group, forming $H_2O + HO^\circ 2$ [11], [12].

The results of the action of peroxide as an oxidizing agent similar to bleach [13] show the release of O_2, and if the predicted $2\ HO^\circ 2$ releases oxygen bubbles, then lignin degradation occurs. Water release means the formation of water so that at a drying temperature higher than 100℃, much water is released into the air. A proposed hypothesis: There is depolymerization of lignin by oxidizing chemical agents at specific concentrations with the release stage of non-residue *Gracilaria sp*.; the residue has a higher water content.

2 Materials and methods

2.1 Materials

The research material is *Gracilaria sp*. inshore sun-dried; cultivation on the coast of the Pasuruan area of East Java, technical Sodium hydroxide, Hydrogen peroxide 3% from the Malang City chemical distributor, Hydrogen peroxide indicator strips 0-750 ppm (Hydrion), Sodium hypochlorite 5.25% (scJohnson), source water (ground) Malang Regency, 1 L volume stainless steel container, filter cloth, 50 L Autoclave, high-pressure gas stove, plastic filter, 350 Watt thermostat-drying cupboard, plastic bag, 200 mL Beaker glass.

2.2 Methods

The research uses a descriptive-quasi-experiment method regarding the results of a process using temperature and pressure in the hydrothermal process during extraction. The method for obtaining solid residues is to follow the modified producing agar [9] using sodium hypochlorite [7] and sodium hydroxide for agar waste [14].

A quasi-experiment consists of the factors:

- A : Water and materials (control)
- AA : 0.5 permille NaClO solution (NaOH and $O_{nascent}$ oxidizer)
- B : 3 permille NaClO solution (NaOH and $O_{nascent}$ oxidizer)
- C : 1.5 permille H_2O_2 ($O_{nascent}$ oxidizer)
- D : 3 permille H_2O_2 ($O_{nascent}$ oxidizer)
- X : 1 permille NaOH (without $O_{nascent}$ oxidizer)
- Y : 2 permille NaOH (without $O_{nascent}$ oxidizer)
- Z : 3 permille NaOH (without $O_{nascent}$ oxidizer)

Gracilaria sp. plants dry, soaked for 12 hours (overnight), and washed several times until clean. Dry in the sun as stock I for experiments. 500 g of material is soaked in 5% lime water for 30 minutes and washed several times until it is free of lime. Dry in the sun as stock II for further experiments.

Residue generation: Weighed 25 g of stock II dry material and put it into the various soaking treatment solutions; depolymerization factor in the container until it meets 20% of the algae material and soaking for 3 hours. Neutralizing and washing repeatedly, blending for 1 minute and placing in a stainless-steel container. Covered with two layers: first, cotton cloth (soy-tofu filter), and second, cover the container with holes. Steeming the experimental unit for each treatment with the same agent at various levels (independent variable) in a 50 L autoclave. Next, analyze the water content (dependent variable) in duplicate. Hydrothermal extraction process 115-119℃, pressure at 0.07 bar and left for 15 minutes, then cooled to around 45℃.

The residue is washed many times with boiling and cold water. The residue is dried in a hot air dryer at a temperature of 33-35℃ until it is easily separated with a filter cloth and collected, and determined the water content of each experimental unit sample.

Determination of Water Content: Samples of dry residue for each factor were determined in duplicate for water content using the water evaporation method at 105℃ to obtain a constant dry weight. Comparing the amount of water evaporated (*b*) g from the sample (*a*) g. The percentage of water content is

$$\% \text{ Water content (WC)} = b/a.100 \quad (1)$$

3 Results and discussion

The tradition of physically releasing agar molecules is chemical-heating by engineering the cellulose barrier; lignin and macro-algae are the dietary fibre category. Preliminary research shows that the lignin content can change with heating, namely boiling for more than 2 hours, and increasing the pressure can reduce the time. Pre-experiment with a temperature of 115-119℃ for 15 minutes obtained a non-residue fraction of 14.16% from the results of cultivation and inshore drying, no different from the reference [10]. Studies on cracking lignin by chemical means, namely the effect of NaOH and nascent oxygen (On) on lignin depolymerization, show the results of growth and ethanol fermentation by Saccharomyces cerevisiae. Both substances are derived from bleach compounds but have the problem of chlorine residue, which pollutes the surrounding environment, especially in water. The composition of bleaching compounds in

which NaOH and chlorine (Cl^-) are suitable for terrestrial plants with high lignin shows that only a concentration of 1% lignin depolymerization occurs so that cellulose (including hemicellulose) is easily hydrolyzed by other agents [15].

WC reflects the portion of water content, and high and low values indicate the organic material content in the body of Gracilaria sp. High WC means lower organic matter changes and vice versa after depolymerized lignin. This performance is related to oxidants, which release oxygen ions (nascent oxygen). It is also related to its effect on the release of non-residues, which can be measured using the specific gravity of the agar components. This water is not biased toward the water bound in the gel (agar), so the non-residue is released first. Parameters related to depolymerization are in Table 1.

Table 1. WC of residue and Specific Gravity of Pre-experiment non-residue fraction

Samples Code	WC mean of Residue (%)	The Specific Gravity of non-residue (.10)	pH	Chlorine Residue (ppm)
A	9,395	7,95	7,25	
AA	9,391	9,64	9,12	>1,7
B	11,223	9,87	12,95	
X	9,947	9,61	8,42	
Y	10,188	9,56	8,42	-
Z	9,882	9,43	6.69	
C	9,989	10,06	8,14	-
D	12,533	8,58	8,38	

The highest non-residue release performance was the hydrogen peroxide factor at a concentration of 1.5 permille. The pattern showed that the factors for lignin degradation provided all higher WC than the control (A). The part of the cell wall containing lignin, and its damage gives a higher specific gravity of non-residue fraction. Sodium hypochlorite and sodium hydroxide have the effect of opening lignin polymers. Adding the oxidizing factor sodium hypochlorite provides a higher pH due to the presence of NaOH compounds for oxidizing compound conditions (code B). Hydrogen peroxide (code D) has a different effect on pH, so hydrogen peroxide is lower even though the concentration is the same at three permille. Lignin degradation influenced the WC pattern (**Figure 1**), which is higher than the control (A).The higher water content is no different from the flour of Gracilaria sp. on the effect of 10,000 ppm NaClO with a soaking time of 30 minutes; (10.167 ± 0.315) % compared to the control (8.703 ± 0.188) % [16].

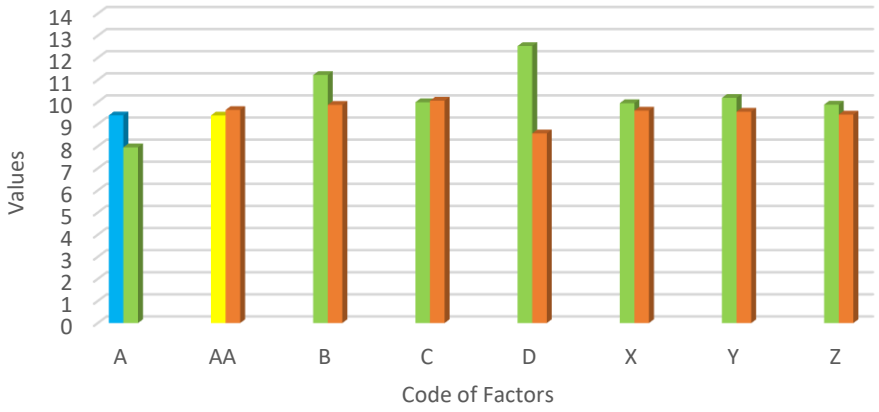


Fig. 1. WC of residue and non-residue specific gravity values (multiples of 10) in various Lignin *Gracilaria sp*. open-crack treatment factors: Higher water content, the non-residue fraction was higher, relatively the same as the control, the control.

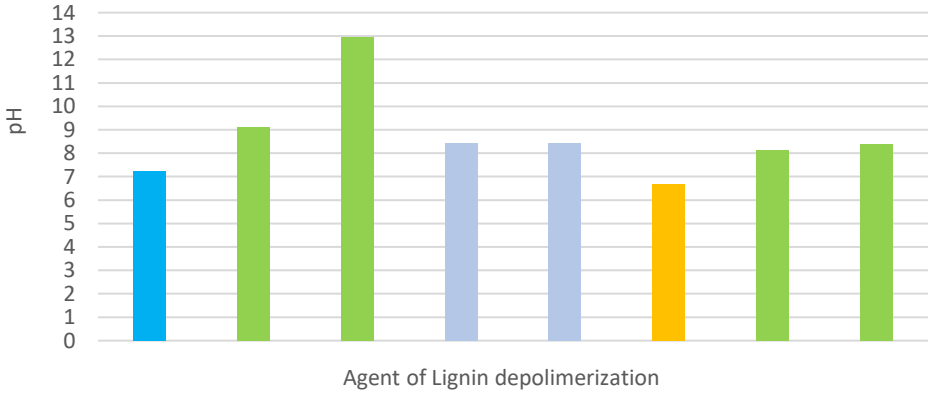
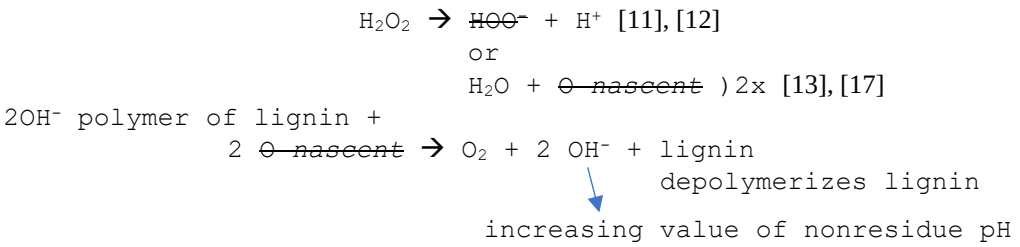


Fig. 2. Ion concentration conditions after the action of the lignin depolymerization agent are expressed by the pH value (-log [ion]): increase in OH^- ions (AA, B, C, D), the control (A) and (X, Y), the presence of H^+ ions (Z).

The effect of the peroxide oxidizer, which has a higher pH than the control (**Figure 2**), can be explained by the mechanism of oxygen formation during the soaking process of *Gracilaria* sp. The oxidizer produces nascent oxygen (O_n) and performs chemical reactions with the alcohol groups in the lignin polymer. Referring to [11], [12], each of these two groups, which has changed into a HOO^- ion, respectively, can release O_2 so that depolymerization occurs. Literature studies show that hydrogen peroxide has a working method that does not include hydrogen atoms and electron transfer [17]. The action of the oxidizer on the functional groups and the breakdown of the lignin polymer result in more hydroxide ions, causing the pH value to

be higher than the control. The reaction that occurs between the action of the oxidizer and the sheath (lignin) that encloses cellulose can be structured as follows:



The higher pH value in the non-residue results is due to the higher concentration of hydroxy ions compared to the control. The non-reducing extract has a lower pH than the control that occurs with the NaOH agent, resulting in the final effect of the NaOH compound without an oxidizer during the hydrothermal process, causing a higher hydrogen ion concentration. The non-residue fraction (agar) had a pH below the control pH value for the three permille of NaOH treatment without oxidizer, indicating that physical desulfication occurred without gel formation; sulfate ions occur, dissolve, and a negative correlation between alkali and gel strength is shown by the lack of sulfate [18].

4 Conclusion

Depolymerization of low-lignin by the oxidizing chemical agent of hydrogen peroxide three permille with the first stage releasing non-residue Gracilaria sp., the residue has the highest water content compared to the others and a concentration of 1.5 permille provides a non-residue extract with the most increased specific gravity. Lignin depolymerization influences the pattern of water content; the influence of the peroxide oxidizer has a higher pH than the control. Obtaining characteristics of non-residual substances of Gracilaria sp. is a source of substances useful for implementation in gel form. Using NaOH without oxidizer at three permille shows that desulfication occurs physically without gel formation.

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