EFFECT OF *Acetobacter aceti* CONCENTRATION AND FERMENTATION TIME ON ACETIC ACID CONTENT PRODUCED FROM SEAWEED *Gracilaria sp.*

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Abstract: This study evaluates the conversion of *Gracilaria sp.* seaweed into acetic acid using the bacterium *Acetobacter aceti*. Acid hydrolysis breaks down the carbohydrates in *Gracilaria sp*. into glucose, which is then fermented by *Acetobacter aceti* to produce acetic acid. Assessed the impact of varying concentrations of Acetobacter aceti and different fermentation durations on acetic acid yield. Results indicated that *Gracilaria sp*. produced bioethanol at approximately 7.108% using bread yeast and 1.572% with tapai yeast. The bioethanol from bread yeast was used for fermentation. FTIR analysis revealed specific absorption peaks for hydroxyl (OH) at 3248 cm^{-1}, carbonyl (C=O) at 1635.64 cm^{-1}, and carbon-oxygen (C-O) at 1249 cm^{-1}. Acetic acid concentrations were influenced by the concentration of *Acetobacter aceti* (5%, 10%, 15%) and fermentation time (7, 10, 13 days), with the highest concentration of 0.380% achieved after 10 days at 10% *Acetobacter aceti*.
Keywords: *Gracilaria sp*; Bioethanol; Acetic acid; Fermentation; Yeast; *Acetobacter aceti*

Abstrak: Penelitian ini mengevaluasi perubahan rumput laut *Gracilaria sp.* menjadi asam asetat menggunakan bakteri *Acetobacter aceti*. Hidrolisis asam memecah karbohidrat dalam *Gracilaria sp.* menjadi glukosa, yang kemudian difermentasi menggunakan *Acetobacter aceti* untuk menghasilkan asam asetat. Pengaruh dari berbagai konsentrasi *Acetobacter aceti* dan durasi fermentasi yang berbeda pada hasil asam asetat. Hasil penelitian menunjukkan bahwa bioetanol *Gracilaria sp.* diproduksi sekitar 7,108% menggunakan ragi roti dan 1,572% dengan ragi tapai. Bioetanol dari ragi roti digunakan untuk fermentasi. Analisis FTIR mengungkapkan puncak serapan spesifik untuk hidroksil (OH) pada 3248 cm^{-1}, karbonil (C=O) pada 1635,64 cm^{-1}, dan karbon-oksigen (C-O) pada 1249 cm^{-1}. Konsentrasi asam asetat dipengaruhi oleh konsentrasi *Acetobacter aceti* (5%, 10%, 15%) dan lama fermentasi (7, 10, 13 hari), dengan konsentrasi tertinggi sebesar 0,380% dicapai setelah 10 hari pada 10% *Acetobacter aceti*.
Kata kunci: *Gracilaria sp*; Bioetanol; Asam Asetat; Fermentasi; Ragi; *Acetobacter aceti*;

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Introduction

Seaweed is an important national commodity in the fisheries sector. It is viewed as a primary product to enhance the country's foreign exchange earnings. Seaweed has many advantages, including a short cultivation period of about 45 days and high productivity. Its carbohydrate content also makes it a valuable source of food and energy (Haslianti et al., 2016). In recent years, Indonesia has seen a significant increase in seaweed production. The Ministry of Maritime Affairs and Fisheries (KKP) aims for national seaweed production to grow by 2.92% annually between 2020 and 2024, targeting 10.99 million tons for 2020.

Gracilaria sp. is a type of seaweed commonly found in Indonesia, especially in Aceh Province, which has extensive coastal areas ideal for cultivation. The total cultivation area in Aceh Province is approximately 62,568 hectares (Central Statistics Agency, 2019). This seaweed biomass can be used as an alternative source of renewable energy, including biofuels like bioethanol, biodiesel, and biogas. Additionally, *Gracilaria sp.* is extensively used in agar production (Sa'diyah & Puryantoro, 2018).

The carbohydrate content in seaweed varies based on the species and cultivation method. Research by Sandi et al. (2016) shows that *Gracilaria sp.* from Maukawini Beach has a carbohydrate content of 73.66%. Other studies, such as those by Adini et al. (2015), demonstrate that byproducts from agar production, like agar waste, have significant carbohydrate content as well. The total carbohydrate content in agar waste is 72.17%, while the original seaweed contains 52.87%. Given its high carbohydrate levels, *Gracilaria sp.* also holds promise as a source of renewable energy in the form of biogas (Kawaroe et al., 2016).

Carbohydrates are the dominant chemical component in *Gracilaria sp.*, making it essential to develop effective processing technologies for this resource. Currently, coastal communities in Aceh mainly use seaweed as vegetables and animal feed (Gazali et al., 2018). One innovative approach to improving *Gracilaria sp*. utilization is converting it into acetic acid. While acetic acid is typically produced through chemical synthesis methods, exploring its production from natural sources like seaweed is crucial for safety. This study focuses on producing bioethanol from *Gracilaria sp*. and converting it into acetic acid by varying the concentration of *Acetobacter aceti* and fermentation time. The bioethanol levels will be analyzed using gas chromatography (GC), and the resulting acetic acid will be characterized using Fourier transform infrared spectroscopy (FTIR).

Research methods

Material

This research used seaweed *Gracilaria sp.* from Neuhen Village ponds, aquadest, sodium hydroxide (NaOH) at 0.01 M, 10%, and 0.1 N, 1% hydrochloric acid (HCl), fermipan brand baker's yeast, tapai yeast, granulated sugar, urea, NPK fertilizer, phenolphthalein (PP) indicator, Nutrient Broth (NB), and *Acetobacter aceti* FNCC 0016 culture from UGM PSPG Lab.

Method

Sample Preparation

Three kilograms of *Gracilaria sp*. seaweed were collected from the Neuhen Village pond. The seaweed was cleaned with water to remove surface dirt and then dried in the sun for 2 to 3 days. After drying, it was cut into 1-2 cm pieces and ground in a blender to obtain a coarse powder. This coarse powder was oven-dried at 60 °C for 4 hours. It was then blended again to create a fine powder and sifted through a 50-mesh sieve. The powder that passed through the sieve was used for further treatment (Habibah, 2015). A solution of 0.01 M NaOH was added to the seaweed powder to fully submerge it, and the mixture was soaked for 24 hours. After soaking, the residue was filtered and washed with hot water until it reached a neutral pH. The washed residue was dried in an oven at 105 °C. Once dried, the residue was crushed in a porcelain cup and sieved through a 100-mesh sieve. This residue, with a particle size of 100 mesh, was ready for hydrolysis (Habibah, 2015). Thirty grams of seaweed flour were mixed with 300 mL of 1% HCl and hydrolyzed on a hot plate at 121 °C for 60 minutes. After hydrolysis, the pH of the mixture was adjusted to 5 by adding 10% NaOH, which also detoxified any harmful compounds produced during the process (Ngamput, 2018). Bread yeast (Fermipan brand) and tapai yeast were used as starters. The growth medium was prepared using 100 mL of sterile distilled water and 10 grams of granulated sugar in a 125 mL Erlenmeyer flask. Nutrients were added: 0.4 g/L of urea and 0.5 g/L of NPK. The mixture was homogenized with a magnetic stirrer for 10 minutes and then sterilized in an autoclave at 121 °C for 15 minutes. Once cooled to 30-33 °C, 5% (w/v) of both bread yeast and tapai yeast were added. The mixture was incubated at 30 °C for 24 hours (Ngamput, 2018). Next, 10% (v/v) of the bread yeast and tapai yeast starters were aseptically added to the hydrolyzed seaweed slurry and stirred for 10 minutes using a shaker. The Erlenmeyer flask was connected to a rubber hose submerged in water to prevent air contact. The mixture was left to ferment at room temperature for three days. After fermentation, the mixture was filtered, and the filtrate was collected for distillation. The fermentation product was placed in a round-bottom flask and distilled at 78-80 °C until all liquid evaporated. The resulting distillate was collected in bottles for gas chromatography (GC) analysis (Ngamput, 2018). The gas chromatograph with an FID detector was set to a column temperature of 170 °C, an injector temperature of 210 °C, and a detector temperature of 250 °C. Nitrogen was used as the carrier gas at a flow rate of 0.5 bar, and hydrogen flowed at 0.65 bar. A standard series of ethanol was prepared by diluting with distilled

water to concentrations of 0.2%, 0.4%, 1%, and 2%. Each standard solution was analyzed by injecting 1 µL into the gas chromatograph to obtain area data. A sample of 1 µL was injected into the GC column through a heated injection port. The analysis generated a chromatogram showing retention time and area. A calibration curve was created by plotting the area against standard ethanol concentrations. Finally, the bioethanol content of the sample was calculated using the equation y=ax+b, where y is the area and x is the sample content.

Acetobacter aceti Inoculum

Production of Activation Liquid Media

Dissolve 1.3 grams of nutrient broth (NB media) in 100 mL of hot distilled water. Autoclave the solution at 121 °C for 15 minutes to sterilize it (Nurismanto et al., 2014). Inoculate pure cultures of *Acetobacter aceti* into 10 mL of nutrient broth (NB) medium aseptically. Incubate for 48 hours at 37 °C. Then, aseptically add 10 mL of the NB culture into an Erlenmeyer flask containing 100 mL of NB medium and incubate for an additional 48 hours at 37 °C (Nurismanto et al., 2014). Add bioethanol with *Acetobacter aceti* inocula at concentrations of 5%, 10%, and 15%. Conduct fermentation for 7, 10, and 13 days, respectively. Analyze the acid levels and pH after fermentation. Place the sample in the plate holder of the FTIR tool. The FTIR analysis identifies samples within the 400–4000 cm^{-1} wave number range. The scanning process produces a wave image of the resulting spectrum. FTIR analysis aims to determine the functional groups present in the sample. The seaweed, *Gracilaria sp*., has fermented in an acetic acid solution. Place in a 100-mL measuring flask. Add distilled water until the volume is 100 mL, then put it in an Erlenmeyer flask. In another Erlenmeyer flask, pipette 25 mL of the test sample. Add 2-3 drops of PP indicator solution (phenolphthalein), then titrate the sample with 0.1 N NaOH solution. Note the volume of NaOH used until the color changes to pink. A pH meter measured the acetic acid that the seaweed *Gracilaria sp.* produced.

Result And Discussion

Results

The following table shows the yield value for bioethanol based on the distillation purification results.

Table 1. Bioethanol yield *of Gracilaria sp.*

Sample	Bioethanol Yield (%)
Bioethanol *Gracilaria sp.* with baker's yeast	37.5
Bioethanol *Gracilaria sp.* with yeast tapai	29.23

The standard measurement results for ethanol are 0.2, 0.4, 1, and 2% using GC-FID data obtained as shown in the following table.

Table 2. Peak Area Standard Ethanol (0.2–2%) uses GC-FID.

Sample (%)	*Peak Areas*
0.2	587196
0.4	1088467
1	2673848
2	6666151

Measured the bioethanol sample, *Gracilaria sp*. The GC-FID method yields the data presented in the following table:

Table 3. Peak Area Bioethanol *Gracilaria sp.* using GC-FID

Sample	RT(mins)	*Peak Areas*	Ethanol (%)
Bioethanol *Gracilaria sp.* with baker's yeast	4,599	23799687	7,108
Bioethanol *Gracilaria sp.* with tapai yeast	4,563	5032531	1,572

The following table presents the results of FTIR (Fourier Transform Infrared) characterization of acetic acid from the seaweed *Gracilaria sp*.

Table 4. Acetic Acid FTIR Results

Functional groups	Wavenumber (cm^{-1})	
	Results	(Silverstein *et al*, 2005)
Bond CO	1249	1290.38
Stretch C=O	1635.64	1730.15
Stretch OH	3248.13	3589.53

The effect of *Acetobacter aceti* concentration and fermentation time on the acetic acid content of bioethanol from seaweed *Gracilaria sp.* with baker's yeast is investigated. The following data is obtained:

Table 5. Results of analysis of acetic acid levels and pH

Acetobacter aceti concentration (%)	Fermentation Time (days)	CH_3COOH content (%)	pH
5	7	0.032	6.4
5	10	0.074	6.3
5	13	-	-
10	7	0.223	6.1
10	10	0.380	5.5
10	13	-	-
15	7	0.181	6.2
15	10	0.199	6.1
15	13	-	-

Discussion

In a heating chamber set to 60 ºC for 4 hours, the granular material loses water. Higher drying temperatures result in more evaporation, making the final

sample lighter and containing less moisture. However, if the drying temperature is excessively high, it can cause the breakdown of the chemicals in the seaweed sample, which is undesirable. Once the seaweed is completely dry, it is ground into a finer powder. This powder is then passed through a 50-mesh screen to achieve a smoother consistency, which can enhance the reaction response. As Aiman (2016) suggested, reducing particle size increases the available surface area, thereby speeding up the reaction rate.

Delignification is the first step in the process, aimed at removing lignin compounds to facilitate hydrolysis. Lignin is part of lignocellulose, a polysaccharide component made up of cellulose, hemicellulose, and lignin. Lignin can inhibit enzyme activity by binding to cellulose, preventing effective hydrolysis (Zhao et al., 2020). To initiate delignification, the seaweed powder is soaked for 24 hours in a 0.01 M sodium hydroxide (NaOH) solution. Lignin dissolves in this alkaline solvent, and NaOH disrupts the structure of cellulose, affecting forms such as beta and gamma cellulose. However, alpha-cellulose remains unaffected as it does not dissolve in NaOH.

Initially, the NaOH solution has a pH of 12. After soaking the seaweed powder, the pH decreases to 8. The powder is then washed with hot distilled water, bringing the pH to 7 (neutral). This washing step removes residual solvent and helps decompose lignin, consistent with findings by Visca et al. (2020), which indicate that high temperatures can promote lignin decomposition from cellulose. The resulting residue, now at a neutral pH, is dried in an oven at 105 ºC. Once dry, it is ground using a porcelain cup and sifted through a 100-mesh sieve to produce seaweed flour, which will be used in the hydrolysis process.

Seaweed flour that has undergone delignification is hydrolyzed using 300 mL of 1% hydrochloric acid (HCl) solution. This hydrolysis process is conducted on a hot plate at a temperature of 121ºC for 60 minutes. The principle of hydrolysis involves breaking down polymer chains into simpler monomers with the aid of a catalyst, in this case, the HCl solution. The targeted polymer chains are cellulose and hemicellulose, which are converted into monosaccharides to serve as substrates for fermentation into ethanol.

During hydrolysis, HCl dissociates into hydrogen ions (H^+) and chloride ions (Cl^-). The hydrogen ions combine with water (H_2O) to form hydronium ions (H_3O^+), which facilitate the cleavage of glycosidic bonds in cellulose and hemicellulose. As a result, simple sugar monomers are generated. The mechanism begins with protons from the acid rapidly interacting with glycosidic bonds in the glucose units, forming a conjugate acid. This conjugate acid has an unstable conformation, leading to the breaking of carbon-oxygen (C-O) bonds and the release of the conjugate acid.

The presence of water in the reaction system allows hydroxide ions (OH^-) from the water to bind with carbonium ions, ultimately freeing glucose and protons. The released protons further interact with the glycosidic bonds in other glucose

units, continuing the process until the cellulose molecules are completely hydrolyzed into glucose (Balat et al., 2008). The acid hydrolysis reaction mechanism is illustrated in **Figure 1**.

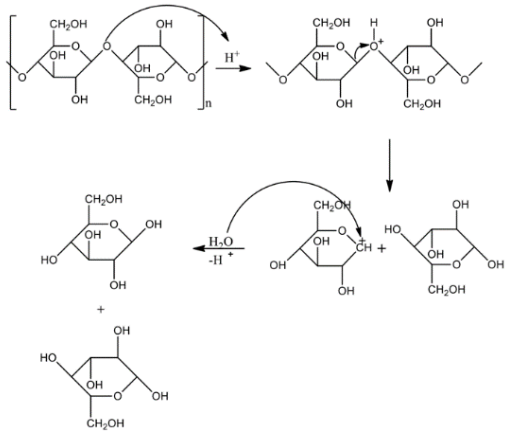

Figure 1. Acid Hydrolysis Reaction Mechanism

This study used a dilute acid with low concentration because it has higher water content, leading to more glucose production. In contrast, a high acid concentration reduces water content, which lowers glucose yield. Ahmad et al. (2020) reported that more concentrated acid results in lower glucose levels due to less available hydroxide ions (OH^-) for binding free radicals during hydrolysis. Therefore, selecting the right acid concentration is crucial for optimizing glucose yield.

The acid hydrolysis results, known as acid hydrolyzate, produced a light brown solution, indicating incomplete degradation of hemicellulose and cellulose into glucose. Erna et al. (2016) noted that complete cellulose hydrolysis is marked by a dark brown filtrate. The acid hydrolyzate has a low pH of 1, making it unsuitable for fermentation. This acidity negatively impacts the growth of Saccharomyces cerevisiae, which prefers a pH of 3-6. To prepare for fermentation, the pH is adjusted to 5 by gradually adding a 10% NaOH solution, which also detoxifies the hydrolysis products, reducing toxic compounds. Fuadi et al. (2015) emphasized that detoxification is important for increasing pH and eliminating fermentation inhibitors.

Before fermentation, bread yeast, and tapai yeast are prepared as starters. These starters are grown in a sterile medium made of distilled water, granulated sugar, urea, and NPK fertilizer. Urea and NPK provide essential nutrients for microbial growth. The mixture is sterilized in an autoclave at 121ºC for 15 minutes to eliminate microorganisms. Subsequently, 5% (w/v) bread yeast and 5% (w/v) tapai yeast are added to the medium and incubated at 30ºC for 24 hours. This controlled environment supports optimal growth by maintaining suitable temperature and humidity.

During fermentation, bacteria progress through four phases: lag, log, stationary, and death. In the lag phase, bacteria adapt to their environment. The log phase is characterized by rapid growth as cells divide. The stationary phase occurs when growth and death rates stabilize the population size. Finally, in the death phase, the death rate exceeds the growth rate. Factors such as temperature, humidity, nutrients, and light influence bacterial growth. Limited nutrients can lead to residual sugar, reducing fermentation efficiency. Umam (2018) noted that insufficient nutrients can slow or stop fermentation. Saccharomyces cerevisiae thrives at 30-35°C, where growth is most vigorous. Outside this range, bacterial reproduction continues but at a slower rate.

The hydrolyzed macroalgae slurry was treated with both bread yeast and tapai yeast starters and incubated for 24 hours. Fermentation occurred at room temperature for 3 days under anaerobic conditions. The Erlenmeyer flask containing the yeast starters was connected to a hose, with the other end submerged in water to release CO_2, indicated by bubbling. Bubbles were observed on the second day of fermentation, confirming the process was proceeding well.

After 3 days, two layers formed: the fermentation solution on top and seaweed sediment at the bottom. Filtration was necessary to separate these layers. The next step was distillation, which utilizes differences in boiling points. Distillation was conducted at 78-80ºC since ethanol boils at 78.4ºC. This temperature range is critical for effective distillation. The process lasted 8 hours, resulting in a colorless solution with a characteristic alcoholic odor. The yield of bioethanol from baker's yeast was 37.5%, while that from tapai yeast was 29.23%.

The resulting distillate was then quantitatively analyzed using gas chromatography with a Flame Ionization Detector (FID). An ethanol standard calibration curve was prepared using concentrations of 0.2%, 0.4%, 1%, and 2%, as illustrated in **Figure 2**.

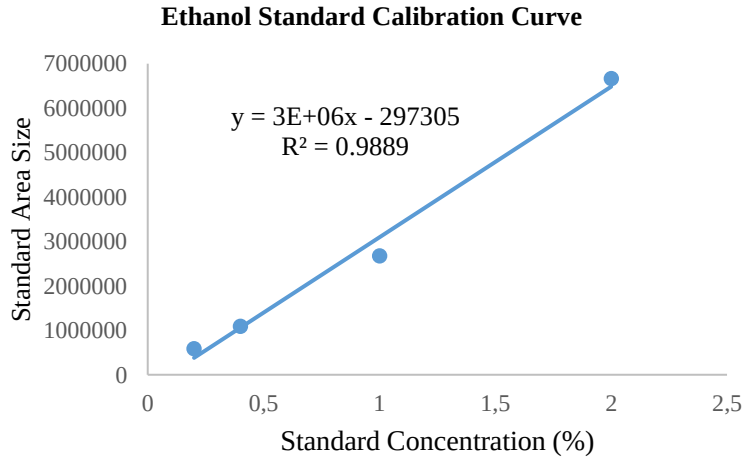

Figure 2. Ethanol Standard Calibration Curve

The calibration curve represents the relationship between instrument response and known sample concentration. This curve allows for the determination of a substance's concentration in an unknown sample by comparing it to a set of standard samples with known concentrations (Rohman, 2014). Quantitative analysis using gas chromatography (GC) produces chromatograms, which display peaks based on retention time and area. The sample area, when close to the standard area, indicates the presence of ethanol. The standard area ranges from 587,196 to 6,666,151, while the sample area varies from 5,032,531 to 2,379,968. Based on these areas, the ethanol content was found to be 1.572% for bioethanol produced using tapai yeast and 7.108% for bioethanol from baker's yeast. These values are lower than those reported by Ngamput (2018), who achieved an ethanol content of 13.17% using both yeasts to ferment *Ulva lactuca* green seaweed. The lower ethanol yield in this study is likely due to incomplete acid hydrolysis and the simultaneous use of both yeasts.

Baker's yeast produced a higher bioethanol content compared to tapai yeast, which yielded only 1.572%. This difference is attributed to the distinct characteristics of the two yeasts. Baker's yeast, *Saccharomyces cerevisiae*, is highly effective at fermenting sugars due to its selective breeding and mutations. In contrast, tapai yeast contains a mixture of genera, including *Aspergillus*, *Candida*, *Hansenula*, and *Acetobacter*, which may inhibit optimal fermentation. Walker and Stewart (2016) noted that *S. cerevisiae* efficiently ferments various sugars, including glucose and sucrose. Additionally, Maryana et al. (2020) reported that baker's yeast is best suited for short-term fermentation.

Mukti and Aryani (2016) found that baker's yeast produced 2.51% bioethanol from cherry fruit, while tapai yeast yielded 1.69%. The ethanol content is influenced by the levels of reducing sugars produced during fermentation. Reducing sugar levels decreases as *S. cerevisiae* cells utilize them for growth and ethanol production; thus, higher sugar consumption results in higher ethanol concentrations (Putri et al., 2016). Fermentation time also plays a crucial role; this study employed a 3-day fermentation period, while Adini et al. (2015) conducted a 5-day fermentation. Extended fermentation can push the process beyond the exponential growth phase, characterized by rapid cell division. After this phase, nutrient depletion occurs, leading to reduced ethanol production. The exponential phase for *S. cerevisiae* typically lasts 36-72 hours, making the 5-day duration from Adini et al. less effective in producing ethanol. Given the higher ethanol content from baker's yeast, it was selected for further treatment in this study.

In acetic acid fermentation, bioethanol with a 7% concentration is used as the substrate. The alcohol concentration significantly impacts the acetic acid yield. High alcohol levels inhibit *Acetobacter aceti* activity, preventing acetic acid formation, as noted by Patel and Pandya (2015), who found that excessive alcohol concentrations lead to increased bacterial cell death. Conversely, low alcohol concentrations do not allow for complete acetic acid formation, as the substrate is

instead oxidized to H_2O and CO_2. The optimal alcohol concentration for acetic acid production is between 10-13%.

Acetobacter aceti inoculum is added to a bioethanol substrate. The inoculum is prepared by dissolving Nutrient Broth (NB) in distilled water to create an activation medium, allowing pure *Acetobacter aceti* cultures to grow. The NB medium is sterilized for 15 minutes, after which a pure culture of *Acetobacter aceti* is added to 10 mL of NB medium and incubated at 37ºC for 48 hours. Following this, the culture is transferred into 100 mL of NB medium and incubated for another 48 hours at 37ºC, forming the final inoculum.

This inoculum is then added to bioethanol at concentrations of 5%, 10%, and 15%, with each concentration undergoing fermentation for 7, 10, and 13 days. After fermentation, acetic acid is tested qualitatively using FTIR to identify its functional groups. The FTIR spectrum for the acetic acid sample, recorded at wavelengths from 4000 cm^{-1} to 400 cm^{-1}, is shown in **Figure 3**.

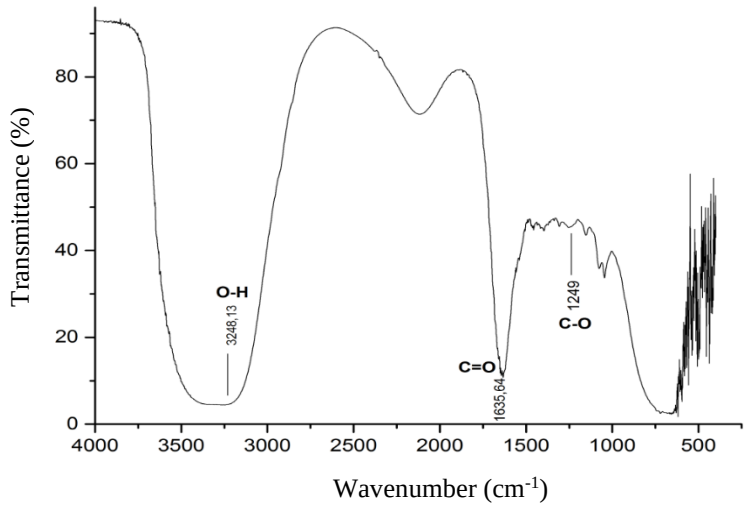

Figure 3. FTIR Spectra of Acetic Acid from Seaweed *Gracilaria sp.*

Tests on acetic acid samples from *Gracilaria* sp. seaweed revealed the presence of an OH functional group, indicated by a broad absorption band in the 3000-3500 cm^{-1} range, specifically at 3248.13 cm^{-1}, confirming the hydroxyl group. Additionally, the 1635.64 cm^{-1} region showed a C=O stretching vibration, while a CO stretch was detected at 1249 cm^{-1}, within the 1320-1210 cm^{-1} range. These groups -OH, C=O, and CO confirm the formation of acetic acid in the sample. The acetic acid content was determined through acid-base titration, a method for measuring the concentration of an acid or base using a solution of known concentration. In this study, acetic acid was titrated with a 0.1 N NaOH solution. The results of acetic acid content and fermentation time for optimal conditions are shown in **Figure 4**.

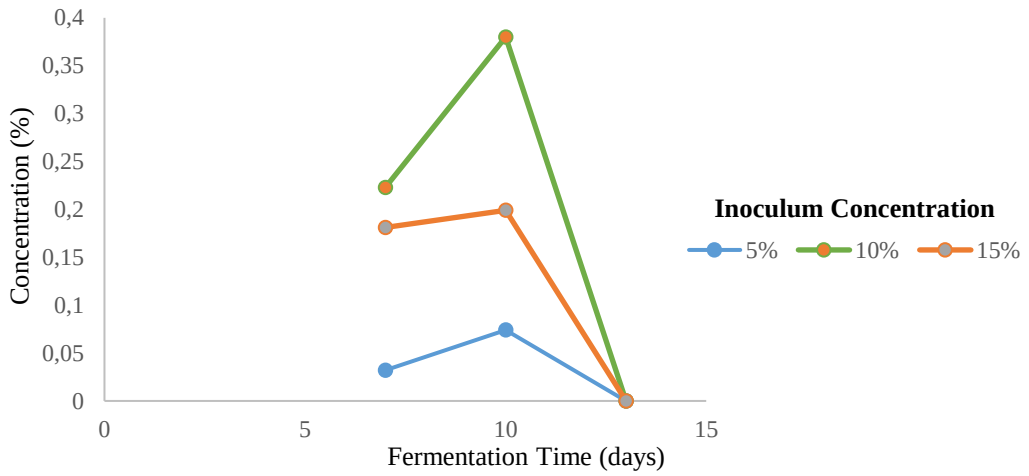

Figure 4. Relationship between inoculum concentration and fermentation time on acetic acid levels

Acetic acid levels in seaweed fermentation ranged from 0.032% to 0.380%. As shown in Figure 4, acetic acid levels increased between days 7 and 10. At a 5% concentration, acetic acid reached 0.032% on day 7 and 0.074% on day 10. Similar increases were observed at 10% and 15% concentrations. However, by day 13, acetic acid was no longer produced. This lack of production was likely due to black mold growth and the depletion of alcohol, indicating that *Acetobacter aceti* could no longer convert alcohol into acetic acid. The prolonged fermentation required more nutrients, emphasizing the need for an adequate substrate supply for sustained bacterial activity. Research by Rachmawati et al. (2019) on cherry vinegar fermentation demonstrated that the highest acetic acid levels occurred within 10 days, with minimal over-oxidation to CO_2 and H_2O. Once the nutrients in the fermentation media were depleted, *Acetobacter aceti* began oxidizing acetic acid itself. This aligns with findings by Kanchanarach et al. (2010), showing that *Acetobacter* not only converts ethanol to acetic acid but can also oxidize acetic acid in a process known as acetate over-oxidation, a common issue in acetic acid fermentation.

The concentration of inoculum affects the production of acetic acid. During fermentation, on day 7, acetic acid levels increased with 5% and 10% inoculum concentrations but decreased at 15%. This pattern also appeared on day 10. High acetic acid levels inhibit *Acetobacter aceti* from effectively converting alcohol to acid. The reduction in acetic acid at the 15% concentration is likely due to nutrient limitations in the substrate, which hinder optimal bacterial activity. The amount of good inoculum must be proportional to the amount of substrate (Palimbong, 2017). Nutrition plays a crucial role in the growth of bacterial cells. When nutrition is limited, bacterial cells begin to die, leading to a reduction in cell numbers and, consequently, a decrease in acetic acid bacteria. This reduction limits the oxidation

of alcohol to acetic acid. Additionally, extended oxidation treatments further reduce the acetic acid concentration (Kusmawati, 2017).

The next step is to determine the pH of acetic acid using a pH meter. The results of pH measurements are presented in **Figure 5**.

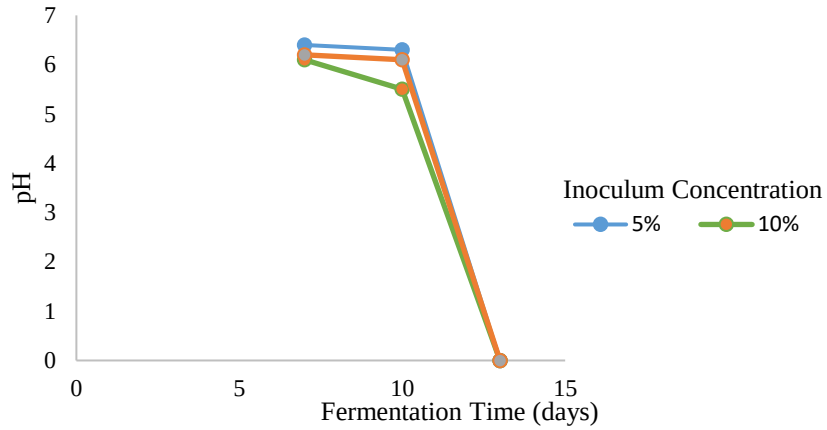

Figure 5. Results of pH measurements

The pH results of acetic acid from seaweed vary from 5.5-6.4. The pH value is inversely proportional to the acetic acid level. The higher the acetic acid level, the lower the pH of the acetic acid. On the other hand, the lower the acetic acid level, the higher the pH of the acetic acid. It can be proven that 0.380% acetic acid has a pH of 5.5, while 0.223% acetic acid has a pH of 6.1. This is confirmed by research (Andayani *et al* ., 2019), pH decreases with increasing levels of acetic acid. The pH value decreased presumably because the concentration of acetic acid increased during the fermentation process. The higher the level of dissolved acetic acid, the faster it will dissociate to release free protons so that the pH will decrease (Nendissa *et al* ., 2015).

Conclusion

Gracilaria sp. seaweed, rich in carbohydrates, can be converted into acetic acid through fermentation using *Acetobacter aceti*. The study found that both the concentration of *A. aceti* inoculum and fermentation duration significantly impacted acetic acid levels, with observed fluctuations over time. By the 13th day, no acetic acid was present across all inoculum concentrations due to further oxidation, resulting in CO_2 and H_2O.

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References

Adini, S., Kusdiyantini, E., & Budiharjo, A. (2015). Produksi Bioetanol Dari Rumput Laut dan Limbah Agar *Gracilaria sp*. dengan Metode Sakarifikasi Yang Berbeda. BIOMA, 16(2), 65– 75.

Ahmad, A., Muria, S. R., & Rahani. (2020). Pengaruh Konsentrasi Asam Klorida (HCl) Pada Hidrolisis dan Waktu Fermentasi Terhadap Limbah Padat Sagu Menjadi Bioetanol. Prosiding Seminar Nasional Teknik Kimia "Kejuangan" Pengembangan Teknologi Kimia Untuk Pengolahan Sumber Daya Alam Indonesia, 1–7.

Aiman, S. (2016). Pengaruh Ukuran Partikel Biomasa Lignoselulosa pada Pembuatan Bioetanol dan Biobutanol: Tinjauan. Jurnal Kimia Terapan Indonesia, 18(1), 11–25.

Andayani, N., Nurhayati, D., & Saing, M. D. (2019). Optimalisasi Lama Fermentasi Dengan Penambahan Konsentrasi *Acetobacter Aceti* Pada Pembuatan Cuka Buah Apel Rhome Beauty Menggunakan Alat Fermentor. Seminar Nasional Hasil Pengabdian Masyarakat Dan Penelitian Pranata, 313–320.

Balat, M., Balat, H., & Öz, C. (2008). Progress In Bioethanol Processing. Progress in Energy and Combustion Science, 34(5), 551–573.

Badan Pusat Statistik Provinsi Aceh. (2019). Provinsi Aceh Dalam Angka 2019. ISBN : 2088-8910. Seri 46. BPS Provinsi Aceh, Aceh.

Erna, Said, I., & Abram, P. H. (2016). Bioetanol dari Limbah Kulit

Singkong (Manihot Esculenta Crantz) Melalui Proses Fermentasi. Jurnal Akademika Kimia, 5(3), 121–126.

Fuadi, A., Harismah, K., & Setiawan, A. (2015). Pengaruh Suhu Dan Ph Terhadap Banyaknya Yield (Kadar Glukosa) Yang Dihasilkan Pada Proses Hidrolisis Enzimatis Dari Limbah Kertas. Simposium Nasional, 179–185.

Gazali, M., Nurjanah, & Zamani, N. P. (2018). Eksplorasi Senyawa Bioaktif Alga Cokelat Sargassum sp. Agardh sebagai Antioksidan dari Pesisir Barat Aceh. Jurnal Pengolahan Hasil Perikanan Indonesia, 21(1), 167–178.

Habibah, F., (2015). Produksi Substrat Fermentasi Bioetanol Dari Alga Merah *Gracilaria verrucosa* Melalui Hidrolisis Enzimatik Dan Kimiawi. Universitas Negeri Semarang, Semarang,

Haslianti, Purnama, M. F., & Piliana, W. O. (2016). Potensi Industri Pengolahan Rumput Laut menjadi Bioetanol. Jurnal Bisnis Perikanan FPIK UHO, 3(1), 89–96.

Kanchanarach, W., Theeragoolz, G., Inoue, T., Yakushi, T., Adachi, O., & Matsushita, K. (2010). Acetic Acid Fermentation of *Acetobacter pasteurianus*: Relationship between Acetic Acid Resistance and Pellicle Pokysaccharide Formation. Bioscience, Biotechnology, and Biochemistry, 74(8), 1591–1597.

Kasari, N., Iryani, & Bahrizal. (2012). Konversi Bioetanol Hasil Fermentasi dari Ubi Jalar Putih (Ipomoea batatas LAM.) Menjadi Asam Asetat menggunakan Acetobakter aceti. Periodic, 1(2), 39–41.

Kawaroe, M., Hasanudin, U., & Krisye. (2016). Pencernaan Anaerobik Makroalga *Gracilaria sp.* Pada Sistem Batch Untuk Memproduksi Bio-Metana. Jurnal Ilmu Dan Teknologi Kelautan Tropis, 8(2), 595–603.

Kementerian Kelautan dan Perikanan., "Genjot Nilai Ekspor, KKP Targetkan Produksi 10,99 Juta Ton Rumput Laut Di 2020," Artikel KKP,2020. [Online]. Available: https://kkp.go.id/artikel/16505-genjot-nilai-ekspor-kkp-targetkan-produksi-10-99-juta-ton-rumput-laut-di-2020. [Accessed: 12-Des-2020].

Kusmawati, W. (2017). Analisis Kadar Asam Asetat Dalam Media Limbah Fermentasi Biji Kakao Akibat Penambahan Konsentrasi *Acetobacter aceti* Dan Waktu Inkubasi. Jurnal Filsafat, Sains, Teknologi, Dan Sosial Budaya, 23(1), 67–72.

Maryana, T., Silsia, D., & Budiyanto. (2020). Pengaruh Konsentrasi Dan Jenis Ragi Pada Produksi Bioetanol Dari Ampas Tebu. Jurnal Agroindustri, 10(1), 47–56.

Masriatini, R. (2016). Penambahan Induk Cuka Pada Pembuatan Asam Asetat Dari Bonggol Pisang Uli (Musa X Paradisiacal Triploid Aab). Jurnal Redoks, 1(1), 65–72.

Mukti, N. L., & Aryani, W. (2016). Pengaruh Waktu Fermentasi Dan Jumlah Ragi Terhadap Persentase Hasil Dalam Pembuatan Bioetanol Dari Buah Talok (Kersen) Menggunakan Ragi Tapai Dan Ragi Roti (Saccharomyces cerevisiae). Jurnal Inovasi Proses, 1(1), 18–27.

Nendissa, S. J., Breemer, R., & Melamas, N. (2015). Pengaruh Konsentrasi Ragi Saccharomyces cerevisiae Dan Lama Fermentasi Terhadap Kualitas Cuka Tomi-Tomi (Flacourtia inermis). AGRITEKNO: Jurnal Teknologi Pertanian, 4(2), 50– 55.

Ngamput, & Amelia, H. M. (2018). Pengaruh waktu hidrolisis asam terhadap kadar etanol yang dihasilkan dalam fermentasi Ulva lactuca. Skripsi thesis, Sanata Dharma University.

Nurismanto, R., Mulyani, T., & Tias, D. I. N. (2014). Pembuatan Asam Cuka Pisang Kepok (Musaparadisiaca L.) Dengan Kajian Lama Fermentasi Dan Konsentrasi Inokulum (*Acetobacter aceti*). Jurnal Rekapangan, 8(2), 149–155.

Palimbong, S. (2017). Pengaruh Konsentrasi *Acetobacter aceti* Dan Lama Fermentasi Terhadap Total Asam Cairan Fermentasi Pepaya Burung (Carica papaya, L.). Jurnal Sains Dan Teknologi Pangan, 2(2), 478–485.

Patel, R., & Pandya, H. N. (2015). Production of acetic acid from molasses by fermentation process. IJARIIE, 1(2), 58–60.

Putri, S. A., Restuhadi, F., & Rahmayuni. (2016). Hubungan Antara Kadar Gula Reduksi, Jumlah Sel Mikrob Dan Etanol Dalam

Produksi Bioetanol Dari Fermentasi Air Kelapa Dengan Penambahan Urea. Jurnal Online Mahasiswa Fakultas Pertanian, 3(2), 1–8.

Rachmawati, N., Nurlaily, F. A., & Wijatniko, B. D. (2019). Pengaruh Waktu Fermentasi dan Penambahan Konsentrasi Inokulum (*Acetobacter aceti*) terhadap Kualitas Asam Cuka dari Buah Kersen (Muntingia calabura L). (IJHS) Indonesian Journal of Halal Science, 1(1), 12–17.

Rizwan, M., Diah, A. W. M., & Ratman. (2018). Pengaruh Konsentrasi Ragi Tapai (Saccharomyces Cerevisiae) Terhadap Kadar Bioetanol Padaproses Fermentasi Biji Alpukat (Persea americana Mill). Jurnal Akademika Kimia, 7(4), 173–178.

Rohman, A. (2014). Validasi dan Penjaminan Mutu Metode Analisis Kimia (Pertama). Gadjah Mada University Press.

Sa'diyah, A., & Puryantoro, D. A. S. (2018). Potensi Rumput Laut *Gracilaria sp.* Sebagai Alternatif Biomassa Studi Kasus Di Kawasan Tambak Tanjungsari, Kecamatan Jabon, Sidoarjo. Prosiding Seminar Nasional Inovasi Dan Aplikasi Teknologi Di Industri 2018, 279–284.

Sandi, Y. A., Rita, W. S., & Ciawi, Y. (2016). Hidrolisis Rumput Laut (Glacilaria Sp.) Menggunakan Katalis Enzim Dan Asam Untuk Pembuatan Bioetanol. Jurnal Kimia, 10(1), 7–14.

Umam, M. S. (2018). Pengaruh Konsentrasi Ragi Roti (Saccharomyces Cerevisiae) Dan Waktu Fermentasi Terhadap Kadar Bioetanol Nira Siwalan (Borassus flabellifer L.). Universitas Islam Negeri Maulana Malik Ibrahim.

Visca, R., Nurjanah, S., & Yuliana, N. (2020). Kajian Karakterisasi SEM pada Mikrokristalin Selulosa Kulit Sukun (Artocarpus astilis) Melalui Proses Hidrolisa Rinette. Jurnal Teknologi, 8(1), 11–21.

Walker, G. M., & Stewart, G. G. (2016). Saccharomyces cerevisiae in the Production of Fermented Beverages. Beverages, 2(30), 1–12.

Yasminto, H. M., Chairul, & Utami, S. P. (2019). Pengaruh Volume Inokulum *Acetobacter aceti* Dan Waktu Fermentasi Terhadap Fermentasi Asam Asetat Dari Nira Aren (Arenga pinnata). Jom FTEKNIK, 6, 1–6.

Yeni, L. F., Hidayat, A., & Marlina, R. (2011). Isolasi dan Aktivitas Fermentasi Bakteri Asam Asetat pada Nira Nipah (Nypa fruticans). Jurnal Pendidikan Matematika Dan IPA, 2(1), 1–10.

Yoneda, N., Kusano, S., Yasui, M., Pujado, P., & Wilcher, S. (2001). Recent advances in processes and catalysts for the production of acetic acid. Applied Catalysis A: General, 221(1), 253–265.

Fadilah, U., Wijaya, I., & Antara, N. (2018). Studi Pengaruh pH Awal Media Dan Lama Fermentasi Pada Proses Produksi Etanol Dari Hidrolisat Tepung Biji Nangka Dengan Menggunakan S*accharomyces cerevisiae*. jurnal rekayasa

dan manajemen agroindustri, *6*(2),92-102. doi:10.24843/JRMA.2018.v06.i02.p01

Zhao, C., Qiao, X., Shao, Q., Hassan, M., & Ma, Z. (2020). Evolution of the Lignin Chemical Structure during the Bioethanol Production Process and Its Inhibition to Enzymatic Hydrolysis. Energy and Fuels, 34(5), 5938–5949