Comparative studies on the alcohol types presence in *Gracilaria sp.* and rice fermentation using Sasad

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Abstract. Alternative fuel sources such as biofuels are needed in order to overcome environmental problem caused by fossil fuel consumption. Currently, most biofuel are produced from land based crops and there is a possibility that marine biomass such as macroalgae can be an alternative source for biofuel production. The carbohydrate in macroalgae can be broken down into simple sugar through thermo-chemical hydrolysis and enzymatic hydrolysis. Dilute-acid hydrolysis was believed to be the most available and affordable method. However, the process may release inhibitors which would affect alcohol yield from fermentation. Thus, this work was aimed at investigating if it is possible to avoid this critical pre-treatment step in macroalgae fermentation process by using Sasad, a local Sabahan fermentation agent and to compare the yield with rice wine fermentation. This work hoped to determine and compare the alcohol content from *Gracilaria sp.* and rice fermentation with Sasad. Rice fermentation was found containing ethanol and 2 – methyl – 1 – propanol. Fermentation of *Gracilaria sp.* had shown the positive presence of 3 – methyl – 1 – butanol. It was found that Sasad can be used as a fermentation agent for bioalcohol production from *Gracilaria sp.* without the need for a pretreatment step. However further investigations are needed to determine if pre-treatment would increase the yield of alcohol.

1. Introduction

Alternative fuel is needed to reduce the dependency on the energy based on fossil fuel. Biofuels, as an alternative fuel are known as “carbon neutral fuels” because carbon dioxide emitted from vehicles is adsorbed by means of photosynthesis during the growth of plant material [10]. Currently, biofuel is mostly produced from land based crops but the near future could see the alternative usage of marine biomass as a source for biofuel production [1]. Macroalgae biomass has been identified as a potential fermentation substrate for third generation biofuel processes due to its high carbohydrate content. The...
macroalgae criteria of containing no lignin, also another advantage for it to be suitable biofuel candidates as it can be converted into alcohol without any expensive lignin removal process [12].

Current researcher mostly utilized single culture fermentation for macroalgae fermentation. Saccharomyces cerevisiae or yeast is the common fermenting agent used in the work, but there are also other attempt was done by using other types of microbe. Researchers have obtained 2.33% of ethanol from the fermentation of Gelidium elegans by using yeast strain, Saccharomyces cerevisiae IAM 4178 [14]. In addition, researchers also have obtained 86% theoretical yield of ethanol from the fermentation of Gracilaria verrucosa using Saccharomyces cerevisiae [7]. As for other type of microbes used in fermentation, a research demonstrated the used of recombinant Escherichia coli KO11 in Laminaria japonica fermentation with ethanol production of 0.40 g ethanol/g of carbohydrate [6]. Other than that, modified B. castersii (KCTC18154P) has also been used and achieved higher yield of ethanol in a continuous reactor of 30°C and 39 hours which was 38.2% from 72.2 g/L of glucose and galactose content [12].

Single culture macroalgae fermentation is happening when the microbes consume fermentable sugars in macroalgae to produce bioethanol anaerobically [9]. Bioethanol is basically produced from biomass by fermentation process of available carbohydrates into bioethanol and carbon dioxide as described in equation below [5].

\[ C_n H_{2n} O_n (sugar) \xrightarrow{\text{yields}} n \frac{2}{3} C_2 H_5 OH + n \frac{2}{3} CO_2 + \text{Heat} \] (1)

However, the idea of macroalgae fermentation using traditional fermentation method was still undiscovered. Traditional fermentation method promotes the significance of microbial coexistence in any substrates. A study shows that in traditional fermentation using mixed culture as agent; molds, yeasts, Lactic Acid Bacteria (LAB) and Acetic Acid Bacteria (AAB) can cooperate with each other in any substrates such as rice and dropped ripe fruit hence producing fermented foods [4]. Yeasts and LAB are also noticeable in brewed rice with Sasad, a local Sabahan brewing agent [2]. Hence, this unique criterion of Sasad may help in macroalgae fermentation for biofuel as it may eliminate the hydrolysis stage which was initially conducted as a saccharification step for macroalgae. Thus, it is a critical step in the fermentation step as it is not a normal substrate for common ethanol producing agent such as Saccharomyces cerevisiae to undergo a successful alcohol metabolic pathway [13].

Thus, this research is a work to determine and compare the alcohol types presence produced in Gracilaria sp. and rice fermentation using Sasad through a traditional fermentation method.

2. Materials and Methods

2.1. Raw materials preparation
Raw materials used in this research are Gracilaria sp., Sasad and rice. Gracilaria sp. was purchased from Sg. Petani, Kedah Malaysia. Gracilaria sp. samples were washed and soaked in tap water to remove sand, salts and other foreign matters. Samples were then oven dried at 50°C until constant weight. Dried Gracilaria sp. was then size reduced using a kitchen blender, producing smaller particle size of dried seaweed. Dried seaweed was then kept in an airtight container for further usage.

2.2. Starter culture preparation
Sasad, a local Borneo fermenting agent which was originated from Kg.Bulu Silou, Keningau Sabah, Malaysia was used as fermenting agent in this research. It was sized reduced using a manual mortar and then sieve to obtain powder. Then it was stored in a dry container and kept in a fridge for further usage.

2.3. Fermentation
Using modified method of tapai or rice wine making production as in [2], fermentation of Gracilaria sp. and rice were conducted. 300 g of dry Gracilaria sp. and rice were cooked using distilled water.
After cooking, the samples were then allowed to cool down to room temperature before they were inoculated with 75 g of Sasad. After cooling, Sasad was added into the samples by sprinkling all over them and mixed thoroughly using a wooden spoon. Inoculated samples were then transferred into containers. The containers were left open for 1 day before they were sealed. Fermentation was done for 7 days for all samples at room temperature.

2.4. Distillation
The fermented extract was filtered using vacuum filter before distilled so as to separate solid and liquid content. Then, the product was distilled with simple distillation unit as in figure below. Distillate is collected and filtered using syringe filtration unit and kept in the fridge for further analysis.

2.5. Alcohol analysis
Alcohol presence analysis was conducted using GCMS and FTIR analysis. After distillation, samples were added with sodium sulphate anhydrous, Na₂SO₄·H₂O as drying agent to remove water from samples prior for alcohol GC-MS analysis. Samples were syringed through a 0.45 µm Durapore (PVDF) syringe-driven filter unit (Millex-HV, Millipore, Tokyo, Japan) into 0.5 mL glass vials, sealed with a crimp cap and stored at 5°C to 8°C prior to gas chromatography mass spectrophotometry (GCMS) and FTIR analysis. Alcohol analysis was done by using gas chromatography (Agilent Model 6890 N, USA) with a capillary column (30 m L x 0.25 ID x 0.25 µm film thickness). The automatic injection volume was 1 µL with an inlet split ratio of 200:1. The initial and maximum oven temperatures were 40°C and 100°C, respectively.

3. Results and Discussion

3.1. FT-IR analysis
Figure 1 and 2 shows the FT-IR spectrum of the fermentation product. Rice fermentation, T₁ shows a very interesting spectrum as it differs from Gracilaria sp. sample. There are four major peaks can be found from rice fermentation FT-IR spectrum as in figure 2 below. The peaks are OH peak (3389.02 cm⁻¹), C – H peak (2979.36 cm⁻¹), C = C peak (1647.26 cm⁻¹) and C – O peak (1045.87 cm⁻¹). Major peaks from FT-IR analysis shows that several functional groups can be found from the sample. In rice fermentation, T₁ peak results, it shows that alcohol group is most probably presence in the sample. This is due to the significant presence of OH and C – H peak from the analyzed sample.

As for Gracilaria sp. fermentation, S₁ shows that three major peaks can be found. The peaks are OH peak (3389.0 cm⁻¹), C = C peak (1640.43 cm⁻¹) and C – O – C peak (1095.06 cm⁻¹). Those peaks represent the possibility of hydroxyl groups, alkene group and ether group presence in the sample.
3.2. GC-MS analysis

Figure 3 and 4 shows the GC-MS analysis for all samples. Table 1 shows the compound composition for major component detected from the samples at certain retention time and peak. Based on the result analysis, rice fermentation sample, T1 shows that there is possibility of ethyl alcohol or ethanol existed in the compound. Other than that, there is high percentage of 1-propanol 2-methyl detected which is 87% and 91% at 3.45 minutes and 3.49 minutes respectively. Another component of 1-butanol 3-methyl formates might also exist as it was 53% detected at 4.876 minutes. This result can be related to the FT-IR result, as the peak of OH and C–H might exist due to the presence of ethyl alcohol component in the sample. The result is comparable with the finding whereby ethanol and 2–methyl propanol were produced from the rice fermentation using Sasad [2].

Interestingly, Gracilaria sp. samples show different compound in the GC–MS analysis result. Sample S1 represent that there is possibility of 1–Butanol, 3–methyl presence in the samples by 86% of detection. The hydroxyl peak presence in the FT-IR spectrum for Gracilaria sp. sample might due to the 1–Butanol, 3–methyl component which is most possibly presence in the samples. Moreover, there is other component such as 1–Pentene detected from T1 samples however it was in a smaller percentage of only 38%. This result however can be related to the C=C peak as detected in the FT-IR.
result. As for *Gracilaria sp.* sample, 2 – Pentene was 37% detected from S1 sample which can be related to the FT-IR result.

This research work might be comparable to other research work that utilized mixed culture as bioagent for macroalgae fermentation [8]. Brown seaweed was fermented using mixed culture known as *Nuruk*, which contain yeast, bacteria and fungi and yield ethanol as the major product produced. *Sasad*, on the other hand which holds same characteristic as *Nuruk* however yield 1 – butanol, 3 – methyl as major product. This component usually identified in small quantities in yeast fermentation however research works demonstrated that 1 – butanol, 3 – methyl can also be produced by other microbes such as *E. coli* through conserved and native amino acid biosynthesis pathways [3]. Hence, there is possibility for microorganisms in *Sasad* may help in the production of bioalcohol through macroalgae fermentation.

### Table 1. Compound composition from GC-MS analysis

| Sample | Retention Time (min) | Main Compound                  | Kovats Index (KI) | Percentage (%) |
|--------|---------------------|--------------------------------|-------------------|----------------|
| T1     | 3.144               | Ethyl Alcohol                  | 46                | 50             |
|        | 3.453               | 1 – Propano, 2 – Methyl        | 74                | 87             |
|        | 3.494               | 1 – Propano, 2 – Methyl        | 74                | 91             |
|        | 4.876               | 1 – Butanol, 3 – methyl        | 116               | 53             |
| S1     | 3.868               | 1 – Butanol, 3 – methyl        | 88                | 86             |

**Figure 3.** GC-MS Spectrum *Gracilaria sp.* direct fermentation, S1.  

**Figure 4.** GC-MS Spectrum for Rice Fermentation, T1.

### 4. Conclusion

Results demonstrated that the direct fermentation of rice and seaweed shows different type of alcohol yield. Rice fermentation product analyzed shows two types of alcohol which are ethanol and propanol. While, traditional fermentation of *Gracilaria sp.* with *Sasad* had shown the presence of butanol. On
the basis of results, Sasad is feasible to be utilized as fermentation agent of Gracilaria sp. for bioalcohol production. In conclusion, this research work has demonstrated that there is possibility in fermenting macroalgae biomass using other fermentation agent instead of using the common fermentation agent, Saccharomyces cerevisiae or baker’s yeast. The procedure that was developed and used for the bioalcohol production however requires further development, specifically in the pre-treatment and fermentation part.

5. References

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