Properties Activity of Yeast Against Seaweed Bioethanol Fermentation Time and Its Functional Group

V D Loupatty*, M S Y Radiena
Institute of Research and Standardization Industry of Ambon Kebun Cengkeh Street, Ambon, Moluccas, Indonesia

* E-mail: voulda_loupatty@yahoo.co.id

Abstract. The activity of yeast in relationship with fermentation time of seaweed bioethanol and functional groups formed. The bioethanol fermentation process begins with the liquefaction process which is the cooking stage with the addition of water as much as 50 times the weight of seaweed, cooked until it boils. Saccharification is the step of adding bean sprouts to seaweed filtrate at a temperature ± 90°C. For the breakdown of polysaccharides into glucose. Fermentation, carried out at a temperature ± 30°C, by adding about 0.02% yeast. Furthermore, anaerobic fermented for second days; thirth days; fourth days; fifth days and sixth days. The bioethanol obtained were then analyzed with spectroscopic IR. The results showed that the functional group formed was –OH in absorption 3600–3200 cm⁻¹. Reinforced of absorption C–O, 1200–1000 cm⁻¹, shows the presence of the alcohol compound, consist of tertiary alcohol and secondary alcohol. Primary alcohol, only appears on 2nd day and 4th day. Thus it can be said that the activity of yeast (Saccharomyces cerevisiae) can break the primary alcohol bond on 3rd day and up.

1. Introduction
Seaweed grows and is spread almost in all Indonesian waters, including macroalgae and microalgae. One type of seaweed that is cultivated in Indonesia is Eucheuma sp. The uniqueness of physical properties and chemical composition of seaweed provides many possibilities for a wider new application, seaweed as a bioethanol feedstock that can be used for bioenergy or pharmaceuticals. Utilization of seaweed as a raw material for bioethanol is very suitable to be applied because it is supported by a wide area of available cultivation in all Indonesian waters and is supported by a short production cycle, around 6 weeks.

Bioethanol is processed from plant biomass which contains a lot of carbohydrates. Seaweed is generally a source of carbohydrates. Red seaweed (Rhodophyceae), produces hydrocolloids called agar, carrageenan, and fulcellaran. One type of red seaweed is Eucheuma cottonii. Polysaccharides found in seaweed are mostly in the form of carrageenan as a component of cell walls. Carrageenan is a linear polysaccharide, its main structure consists of α (1-3) -D-galactose-4-sulfate and β (1,4) - 3,6-anhydro-D-galactose [1,2]. The carbohydrate content of Eucheuma spp. 56.2% D-galactose and 43.8% 3.6-anhydro-galactose [3]. Analysis of the chemical composition of seaweed Eucheuma cottonii types are as follows: water 31.09%; 4.36% protein; ash 32.86%; fat 0.58%; carbohydrate 29.04% [4].

Water is the main carrageenan solvent. All carrageenan dissolves in hot water at temperatures over 70 °C. Kappa carrageenan is stable in a gel state and hydrolyzed when heated to a neutral or alkaline PH. Kappa carrageenan dissolves at temperatures above 60 °C and dissolves in the concentrated sugar
solution under hot conditions, readily dissolves in water, forms a thick, hydrated solution quickly at low PH [1].

In the process of converting carbohydrates into sugars (glucose) water soluble is done by the addition of water and enzymes; then the fermentation process or fermentation of sugar into ethanol by adding yeast or yeast. The process of breeding yeast to get alcohol is called fermentation. High ethanol concentrations will be toxic to yeast. The most tolerant type of yeast can only survive in an environment of 15% by volume ethanol. This research aims to study the effect of Saccharomyces cerevisiae on the fermentation time of seaweed bioethanol and the functional groups formed.

2. Experimental

2.1 Materials
Materials used in the bioethanol production process are: Eucheuma cottonii seaweed, auxiliary materials: bean sprouts, sugar, yeast (Saccharomyces cerevisiae). The equipment used is: one unit of liquification equipment, one unit of storage container, one unit of fermentation equipment, one unit of distillation equipment and others.

2.2 Work procedures
The bioethanol production process by the fermentation method, as follows: Liquification is the cooking stage with the addition of water to seaweed as much as 50 times the weight of seaweed, then cooked until it boils. Saccharification is the stage of adding bean sprouts (enzymes) to seaweed extract at a temperature of around 90\(^0\) C. For the breakdown of polysaccharides into glucose. Fermentation, carried out at a temperature of about 30\(^0\) C, by adding about 0.02% yeast. Furthermore, anaerobic fermented for 2\(^{nd}\) day; 3\(^{rd}\) day; 4\(^{th}\) day; 5\(^{th}\) day and 6\(^{th}\) day.

2.3 Testing
Tests were carried out on bioethanol obtained on fermentation 2\(^{nd}\) day, 3\(^{rd}\) day, 4\(^{th}\) day, 5\(^{th}\) day and 6\(^{th}\) day, using FTIR, to determine the functional groups formed.

3. Results and Discussion

3.1 Bioethanol production process technology
Liquification process was cooking process, with the addition of water as much as 50 times the weight of seaweed used. Cooking is done until it boils, where seaweed dissolves into seaweed pulp. The purpose of adding 50 times the weight of seaweed used is to help break down the polysaccharides contained in seaweed into other monosaccharides. The polysaccharide is an amalgamation or condensation polymer produced from monosaccharides and composed of many monosaccharide molecules that bind to each other, by releasing a water molecule for each bond formed [5,6].

\[
(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6
\]

polysaccharide water glucose

Saccharification process was addition of bean sprouts extract has a dual function as an enzyme to break down carbohydrates into monosaccharides and also to condition a comfortable atmosphere for the proliferation of Sacharomyces cerevisiae during the fermentation process. Furthermore, the addition of sugar, as much as 1/100 of the volume of seaweed juice. In the fermentation process, the process of breaking down simple sugars into ethanol involves Sacharomyces cerevisiae. In this process sugar will be converted into ethanol and carbon dioxide gas. The fermentation process lasts for 2 - 6 days. At this stage CO\(_2\) gas is produced.
Saccharomyces cerevisiae is suitable for the fermentation of E. cottonii seaweed hydrolyzate and is capable of producing an alcohol content of 4.6% after 5-6 days of fermentation at room temperature [7].

3.2 Relationship between bioethanol fermentation time and formed functional groups
Yeast used in the fermentation stage is Saccharomyces cerevisiae. This yeast has a high fermentation power against glucose, fructose, galactose, maltose and has resistance in an environment with relatively high alcohol content and is resistant to other microbes [8]. During the fermentation process anaerobic decomposition of simple sugars becomes ethanol and CO₂.

- Second Day Fermentation
The results of bioethanol test on second day fermentation, showed the widening and typical absorption of -OH at wave numbers 3600 - 3200 cm⁻¹, reinforced C-O absorption at wave numbers 1050 - 1200 cm⁻¹, this indicates the presence of alcohol in samples that were tested.

Figure 1. FTIR analysis results, seaweed bioethanol second day fermentation

Table 1. Results of functional groups identification of seaweed bioethanol, second day fermentation

| Frequency (Cm⁻¹) | Absorption (Cm⁻¹) | Appearance | Function Group | Compound Class | Reference |
|------------------|-------------------|------------|----------------|----------------|-----------|
| 3700 – 3584      | 3600.2            | Medium     | O              | –H Alcohol     | [9-11]    |
| 3550 – 3200      | 3418.88           | Sharp      | Stretching     | Alcohol        |           |
|                  | 3264.58           | Strong, Broad | O              | –H             |           |
| 1400 – 1000      | 1399.38           | Medium     | O – H Bending  | Alcohol        | [9-11]    |
| 1205 – 1124      | 1182.38           | Strong     | C – O          | Tertiary Alcohol|          |
| 1124 – 1087      | 1125.48           | Strong     | Stretching     | Secondary      |           |
1085 – 1050 1065.69 Strong C – O alcohol
Stretching
Primary Alcohol

2100 – 2200 2169.96 – Weak C ≡ C Alkyne [9-10]
2073.51

1600 – 1670 1652.06 – Medium C = C Alkene [9-10]
1623.13 Stretching

Day 3 Fermentation
The results of seaweed bioethanol fermentation on the third day of fermentation can be seen in Fig. 2 and Table 2.

When compared with Fig. 1 (second day fermentation), then in Fig. 2 (third day fermentation), we see that there is still a wide-absorption and typical absorption at 3600 - 3200 cm⁻¹ and reinforced with CO groups in the absorption area of 1180.46 - 1093.66 cm⁻¹, this indicates the presence of alcohol compounds. The results of the identification of the functional groups produced on third day fermentation can be seen in Table 2.

| Frequensi Range (Cm⁻¹) | Absorption (Cm⁻¹) | Appearance | Function Group | Compound Class | References |
|-------------------------|------------------|------------|----------------|----------------|------------|
| 3700 – 3584             | 3610.81          | Medium, Sharp | O –H Stretching | Alcohol    | [9-11]    |
| 3550 – 3200             | 3468.07 – 3287.72 | Strong, Broad | O –H Stretching | Alcohol    | [9-11]    |
| 1205 – 1124             | 1180.46          | Strong      | C – O Stretching | Tertiary Alcohol | [9-11] |
| 1124 – 1087             | 1114.87 – 1093.66 | Strong      | C – O Stretching | Secondary alcohols | [9-11] |
| 2400 – 2000             | 2171.88 – 2073.51 | Weak        | C ≡ C             | Alkyne    | [9-10]    |
| 1600 – 1670             | 1653.99 – 1636.63 | Medium      | C = C Stretching | Alkene    | [9-10]    |
| 1000 – 650              | 991.43           | Strong      | C = C Bending     | Alkene    | [9-10]    |
absorption area of 1200 - 1000 cm\(^{-1}\), shows that the sample tested contained alcohol compounds. C-O function groups in the absorption area of 1180.46 cm\(^{-1}\), showing tertiary alcohol and in the absorption area of 1114.87 - 1093.66 cm\(^{-1}\), showing secondary alcohol. While the primary alcohol is gone. Furthermore, the C≡C functional group is an alkyne compound, and the C = C functional group, an alkene compound, is still detected. That means the functional group that was lost on third day fermentation, is the -OH group in the absorption area of 1400-1000 cm\(^{-1}\), and the functional group C-OH (primary alcohol) in the absorption area of 1085-1050cm\(^{-1}\).

Fourth day fermentation results
The results of seaweed bioethanol fermentation on the third day of fermentation can be seen in Fig. 3 and Table 3.

![Figure 3. FTIR analysis results, seaweed bioethanol fourth day fermentation](image)

When compared with Fig. 1 (second day fermentation), then in Fig. 3 (fourth day fermentation), it appears that there is still a wide-absorption and typical absorption at 3600 - 3200 cm\(^{-1}\) and reinforced with C-O groups in the absorption area 1156.27 - 1074.37 cm\(^{-1}\), this indicates the presence of alcohol compounds. The results of the identification of the functional groups produced on fourth day fermentation can be seen in Table 3.

| Frequency Range (Cm\(^{-1}\)) | Absorption (Cm\(^{-1}\)) | Appearance | Function Group | Compound Class | References |
|-----------------------------|--------------------------|------------|----------------|----------------|------------|
| 3700 – 3584                 | 3616.59 – 3605.02        | Medium, Sharp | O –H Stretching | Alcohol        | [9-11]     |
|                             | 3271.33 – 3264.58        | Strong, Broad | O –H Stretching | Alcohol        |            |
| 1205 – 1124                 | 1156.27 – 1141.88        | Strong       | C – O Stretching | Tertiary Alcohol | [9-11]     |
| 1085 – 1050                 | 1099.44 – 1074.37        | Strong       | C – O Stretching | Secondary alcohol |            |
|                             |                          |             | C – O Stretching | Primary Alcohol |            |
| 2400 – 2000                 | 2168.03 – 2073.51        | Weak        | C ≡ C             | Alkyne         | [9-10]     |
| 1600 – 1670                 | 1652.06 – 1634.70        | Medium      | C = C Stretching  | Alkene         | [9-10]     |
| 1000 – 650                  | 991.43                    | Strong      | C = C Bending     | Alkene         | [9-10]     |
When compared to Table 2 (second day fermentation), then in Table 3 (fourth day fermentation), the results of identification of seaweed bioethanol functional groups containing hydroxy function groups (-OH) on absorption of 3600 – 3200 cm\(^{-1}\), reinforced with groups C-O function in the absorption area of 1200-1000 cm\(^{-1}\), indicates that the sample tested contained alcohol compounds. The C-O function group in the absorption area 1156.27 - 1141.88 cm\(^{-1}\), shows a tertiary alcohol, in the absorption area 1099.44 cm\(^{-1}\), shows secondary alcohol. Whereas the absorption area of 1074.37 cm\(^{-1}\) indicates primary alcohol. Furthermore, the C≡C functional group is an alkyne compound, and the C=C functional group, an alkene compound, is still detected.

- **Fifth day fermentation**

The results of seaweed bioethanol fermentation on the third day fermentation can be seen in Fig. 4 and Table 4. When compared with Fig. 1 (second day fermentation), then in Fig. 4 (fifth day fermentation), it appears that there is still a wide absorption and typical absorption at 3600 - 3200 cm\(^{-1}\) and reinforced with CO groups in the absorption area of 1194.92 - 1116.80 cm\(^{-1}\), this indicates the presence of alcohol compounds. The results of the identification of the functional groups produced on the 5th day fermentation can be seen in Table 4.

![Figure 4. Analysis of FTIR, seaweed bioethanol fifth day fermentation](image)

When compared to Table 2 (second day fermentation), then in table 4 (fifth day fermentation), we can see the identification of bioethanol functional groups of seaweed containing hydroxy function groups (-OH) on absorption of 3600 - 3200 cm\(^{-1}\), reinforced by groups C-O function in the absorption area of 1200 - 1000 cm\(^{-1}\), shows that the sample tested contained alcohol compounds. The C-O function group in the absorption area of 1194.92 cm\(^{-1}\), showing tertiary alcohol and in the absorption area of 1116.80 cm\(^{-1}\), showing secondary alcohol. While the primary alcohol is gone. Furthermore, the C≡C functional group is an alkyne compound, and the C=C functional group, an alkene compound, is still detected. That means the functional group lost on fermentation day 5, is the functional group C-O (primary alcohol) in the absorption region 1085 - 1050 cm\(^{-1}\).

**Table 4. Results of functional groups identification of seaweed bioethanol fifth day fermentation**

| Frequensi Range (Cm\(^{-1}\)) | Absorption (Cm\(^{-1}\)) | Appearance | Function Group | Compound Class | References |
|-------------------------------|--------------------------|------------|----------------|----------------|------------|
| 3700 – 3584                   | 3623.34                  | Medium, Sharp | O – H Stretching | Alcohol | [9-11]     |
| 3550 – 3200                   | 3454.57                  | –          | O – H Stretching | Alcohol |            |
|                               | 3252.04                  |            |                |                |            |
| 1420 – 1330                   | 1404.20                  | Medium     | O – H Bending  | Alcohol | [9-11]     |
| 1205 – 1124                   | 1194.92                  | Strong     | C – O Stretching | Tertiary |            |
| 1124 – 1087                   | 1116.80                  | Strong     | C – O Stretching | Alcohol |            |
Sixth day fermentation
The results of seaweed bioethanol fermentation on the sixth day fermentation can be seen in Fig. 5 and Table 5.

**Figure 5.** Analysis of FTIR, seaweed bioethanol sixth day fermentation

When compared with Fig. 1 (second day fermentation), then in Fig. 5 (sixth day fermentation), it appears that there is still a wide-absorption and typical absorption at 3600 - 3200 cm\(^{-1}\) and reinforced with CO groups in the absorption area of 1180.46 - 1101.37 cm\(^{-1}\), this indicates the presence of alcohol compounds. The results of the identification of the functional groups produced on sixth day fermentation can be seen in Table 5.

**Table 5.** Results of functional groups identification of seaweed bioethanol, sixth day fermentation

| Frequency Range (cm\(^{-1}\)) | Absorption (cm\(^{-1}\)) | Appearance | Function Group | Compound Class | References |
|-------------------------------|--------------------------|------------|----------------|----------------|------------|
| 3700 – 3584                   | 3610.81                  | Medium,    | O – H          | Alcohol        | [9-11]     |
| 3550 – 3200                   | 3478.68                  | Sharp      | Stretching     | Alcohol        | [9-11]     |
|                              | 3264.58                  | Strong, Broad | O – H          | Alcohol        | [9-11]     |
| 1205 – 1124                   | 1180.46                  | Strong     | C – O          | Tertiary Alcohol | [9-11]   |
| 1142 – 1087                   | 1132.23                  | Strong     | Stretching     | Secondary alcohol | [9-11]   |
|                              | 1101.37                  |             | C – O          |                |            |
|                              |                          |             | Stretching     |                |            |
| 2400 – 2000                   | 2168.99                  | Weak       | C ≡ C          | Alkyne         | [9-10]     |
|                              | 2073.51                  |             |                |                |            |
| 1670 – 1600                   | 1664.80                  | Medium     | C = C          | Alkene         | [9-10]     |
|                              | 1626.98                  |             | Stretching     |                |            |
| 1000 – 650                    | 990.46                   | Strong     | C = C Bending  | Alkene         | [9-10]     |
When compared to Table 2 (second day fermentation), then in table 6 (sixth day fermentation), the results of identification of bioethanol functional groups of seaweed contained hydroxy function groups (-OH) on absorption of 3600 – 3200 cm⁻¹, reinforced by groups C-O function in the absorption area of 1200 - 1000 cm⁻¹, shows that the sample tested contained alcohol compounds. The C-O function group in the absorption area of 1180.46 cm⁻¹, showing tertiary alcohol and in the absorption area of 1132.23 - 1101.37 cm⁻¹, showing secondary alcohol. While the primary alcohol is gone. Furthermore, the C≡C functional group is an alkyne compound, and the C = C functional group, an alkene compound, is still detected. That means the functional group lost on fifth day fermentation, is the functional group C-O (primary alcohol) in the absorption region 1085 - 1050cm⁻¹.

Based on the description above it can be seen that the functional groups that appear on fermentation 2nd day, 3rd day, 4th day, 5th day and 6th day were function groups of hydroxide (-OH) in the absorption of 3600 - 3200 cm⁻¹, reinforced by the C-O functional groups in absorption area 1200-1000 cm⁻¹, indicating that the sample tested contained alcohol compounds. C-O function groups in the absorption region 1205 - 1124 cm⁻¹, showing tertiary alcohol and in the absorption area 1124 - 1087 cm⁻¹, showing secondary alcohol. Furthermore, the C≡C functional group is an alkyne compound, and the C = C functional group, is an alkene compound. Whereas the C-O functional group (primary alcohol) in the absorption area 1085 - 1050cm⁻¹, only appears on fermentation 2nd days and 4th day. Thus it can be said that the activity of yeast (Saccharomyces cerevisiae) can break the bond of primary alcohol on days 3 and so on. The presence of alcohol compounds was shown to have a widening absorption (very typical) at 3500 - 3300 cm⁻¹ and was strengthened with C-O uptake at around 1300 - 1000 cm⁻¹ [11]. Alcohol is an organic compound that contains a hydroxy function group (-OH).

4. Conclusions

Based on the results of the study it can be concluded that:
- Functional group formed was -OH in absorbtion 3600 - 3200 cm⁻¹,Reinforced of absorbtion C-O, 1200-1000 cm⁻¹, C-O function group in absorption area 1205-1124 cm⁻¹, shows the presence of alcohol compound, consist of tertiaray alcohol and secondary alcohol.
- Primary alcohol, only appears on 2nd day and 4th day. Thus it can be said that the activity of yeast (Saccharomyces cerevisiae) can break the primery alcohol bond on 3rd day and up.

Acknowledgments

Thank you for Mr. Prof. DR. H J Sohilait MS and Mr. Jhoe L Wairata SSi MSc, who assisted in testing using FTIR and Mr. DR. E. Dompeipen MSi who helped interpret the functional groups.

References

[1] Winarno F G 1990 Seaweed processing technology Pustaka Sinar Harapan Jakarta.

[2] Ellis A, Jacquier J C 2009 Manufacture of food grade k-carrageenan microspheres J. Food Eng. 94 16-20.

[3] Lin L, Tako M, Hongo F 2000 Isolation and characterization of I-carrageenan from Eucheuma serra (Togekirinsai) J. Applied Glycoscience. 47 03-10.
[4] Loupatty V D 2014 Utilization of *Eucheuma cottonii* seaweed as bioethanol raw material *Proseding Seminar Nasional Basic Science VI F-MIPA Unpatti* 307-314.

[5] Sastrohamidjojo H 2009 Organic chemistry: stereochemistry carbohydrates, fats, and proteins *Gadjah Mada University Press*.

[6] Gaman P M and K B Sherrington 1994 Food Science: Introduction to food science nutrition and microbiology *Gadjah Mada Universitas Press*.

[7] Candra K P, Sarwono, Sarinah 2011 Study on bioethanol production using red seaweed *eucheuma cottonii* from bontang sea water *J. Coastal Development* 15 45-50

[8] Putra and Amran 2009 Manufacture of bioethanol from nira siwalan by fermentation liquid phase using fermipan *Skripsi* Jurusan Teknik Kimia Universitas Diponegoro

[9] Dacriyanus 2004 Structure analysis of organic compounds: *Institute for Information and Communication Technology Development Universitas Andalas Padang publishing*

[10] Chemistry 2013 Infrared Spectroscopy Absorption Table [https://chem.libretexts.org](https://chem.libretexts.org)

[11] Fessenden R J and J S Fessenden 2010 Basics of Organic Chemistry *Binarupa Aksara Publisher, Tangerang*. 