Medium modification for the growth of *Rhizopus oligosporus* and *Acetobacter xylinum* as starter cultures in the production of biofoam, environmentally friendly packaging

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Abstract. *Acetobacter xylinum* was injected to reduce the porosity value and water absorption of biofoam which was made using bagasse and *Rhizopus oligosporus*. However, data interpretation of the total counts of both microbes could be misleading. Therefore, the aim of this research was to create a specific medium for the growth of both microbes. The research was conducted in three stages. In the first stage, soybean flour was added into bagasse to produce depithed bagasse at different ratios (1:1 and 1:1.5) for use in the growth of *Rhizopus oligosporus*. In the second stage, urea and bean sprouts extract were used to grow *Acetobacter xylinum*. Finally, modified media was used to grow both starters. The results showed that the total plate counts of *Rhizopus oligosporus* on bagasse medium was quite similar with the depithed bagasse ranging from 4.58 to 4.87 log cfu/gram. The addition of bean sprouts extract had an absorbance yield of 0.162 while the addition of urea had an absorbance value of 0.085. Therefore, bagasse and soybean flour (in the ratio of 1:1.5) and bean sprouts extract are recommended to grow both starters because of the efficiency process and the highest absorbance yield.

1.Introduction

The most widely-used packaging for packing various types of food is Styrofoam. Styrofoam has a negative impact on the environment and humans' bodies because it is so difficult to decompose and is carcinogenic [1]. Over time, people have replaced Styrofoam with biodegradable foam or biofoam. Biofoam is a packaging that is non-carcinogenic and can be decomposed by microorganisms so it is environmentally friendly. Production of biofoam can be done by using bagasse [2] and coconut fibre [10]. Both also used *Rhizopus oligosporus* to bind the matrix.

Rhizopus oligosporus is a mould commonly used in the production of *tempeh*, or fermented soybeans. *Rhizopus oligosporus* has the ability to form good hyphae at 35°C for 36 hours [6]. During the growth of the *Rhizopus oligosporus* mycelium, the hyphae network will branch and unite the

nutrient substrate consisting of biomass and will produce a solid form. This ability can be used as an opportunity in the manufacture of biofoam.

Currently, *Acetobacter xylinum* was injected into the matrix of biofoam. According to [3], *Acetobacter xylinum* is a microorganism that has the ability to produce cellulose sheets. The cellulose sheet produced by *Acetobacter xylinum* is expected to improve the quality of the biofoam by reducing the porosity value and water absorption. Cellulose in large quantities will form strong white fibers and be insoluble in organic solvents. The structure of cellulose consists of amorphous and crystalline parts. The part of the fiber whose molecules are arranged in an orderly manner is called the crystalline part, while the molecular structure of the amorphous fiber is not arranged in an orderly manner. Cellulose has properties that are resistant to strong bases but is easily hydrolyzed by acids into sugars [4].

However, data interpretation in the total counts of both microbes could be misleading during research because there is no specific medium to grow them. In this study, modification of growth media suitable for *Rhizopus oligosporus* and *Acetobacter xylinum* was carried out according to the composition of the ingredients in the manufacture of biofoam. Therefore, the aim of this research was to create a specific medium for the growth of both microbes so it can be used to examine the total counts of both microbes during the production of biofoam, environmentally friendly packaging.

2. Materials and methods

2.1 Materials

The main material in this study was sugarcane bagasse which was collected from waste sugar cane sellers in Rukoh, Darussalam, Banda Aceh. *Rhizopus oligosporus* was sourced from commercial *tempeh* mould (Rapirma) and *Acetobacter xylinum* were purchased from nata de coco makers in South Aceh (War Na Coco). Potato dextrose agar (Oxoid CM0139), microbiological agar (MERCK 1.07324.0500), soybean flour, coconut, bean sprouts, sugar and urea were used as the ingredients of modified medium.

2.2 Formulation and production of modified medium

2.2.1 Modified agar medium for the growth of Rhizopus oligosporus. Modified medium for the growth of *Rhizopus oligosporus* was carried out by adding bagasse with different concentrations of soy flour (1:1 and 1:1.5) into the agar solution. There were two types of bagasse used, namely bagasse and depithed bagasse. All the ingredients (1 gram of bagasse (bagasse or depithed bagasse), 1 or 1.5 grams of soy flour, 1.5 grams of microbiological agar and 100 ml of water) were mixed well and sterilized at 121°C for 15 minutes.

2.2.2 Modified broth medium for the growth of Acetobacter xylinum. Modified medium for the growth of *Acetobacter xylinum* was prepared by adding a nitrogen source. The media was prepared by mixing 100 ml of coconut water, 1 ml of acetic acid, 1 gram of sugar, 2 gram of bagasse, and the nitrogen source (1.5 gram of urea or 10 ml of bean sprouts extract). The mixture was heated until 100°C and was cooled to 20°C.

2.2.3 Modified agar and broth media for the growth of Rhizopus oligosporus and Acetobacter xylinum. The composition of the media used was 1 gram of bagasse, 1.5 grams of soybean flour, 4 grams of microbial agar, 100 ml of coconut water, 10 ml of bean sprouts extract, 10 grams of sugar and 1 ml of acetic acid. After all the ingredients were mixed, they were sterilized using an autoclave at 121°C for 15 minutes. The media is poured into a petri dish that has been sterilized for agar media. Making liquid media has the same composition as agar media, it's just that liquid media doesn't use agar. The liquid media that has been made is put into a jar.

2.3 Growth trials on the modified medium

The first stage of the test was to grow *Rhizopus oligosporus* on modified agar media. At this stage the media that has been planted with *Rhizopus oligosporus* is incubated at 25°C for 48 hours. At this stage of the study, *Rhizopus oligosporus* was also grown on PDA media as a comparison. In the modified broth medium, *Acetobacter xylinum* (10 ml) was added. The incubation process was carried out at 30°C for 48 hours. In the third phase of the study, *Rhizopus oligosporus*, *Acetobacter xylinum* and

Rhizopus oligosporus + *Acetobacter xylinum* were grown on modified agar and liquid media. At this stage of testing, the media that had been added with isolates was incubated at 30°C for 24 hours.

2.4 Sample and Data Analyses

Rhizopus oligosporus grown on the modified agar medium were analysed for total colony counts while *Acetobacter xylinum* grown on the modified broth medium were analysed for absorbance. The thickness of the cellulose produced in the modified media was also measured. In the third stage, observations were made of the growth of *Rhizopus oligosporus* on agar media and calculating the absorbance of the liquid media to which the isolate had been added . The data obtained is averaged and the standard deviation is calculated.

3. Results and discussion

3.1 Growth of Rhizopus oligosporus on the modified agar medium

Growth of *Rhizopus oligosporus* on agar media which was modified using bagasse and soybean flour can be seen in Figure 1. The incubation process was carried out at 25°C. *Rhizopus oligosporus* can grow well on media mixed with bagasse and soybean flour. Treatment with a ratio of depithed bagasse with a ratio of 1:1 had the highest total colony, namely 4.87 lg cfu/g. However, the growth of *Rhizopus oligosporus* on bagasse with a ratio of 1:1.5 did not have a significant difference in total colonies, namely 4.85 log cfu/g. Although the total value of colonies with the addition of depithed bagasse was higher than the addition of bagasse, the depithed bagasse was considered more efficient because it did not require additional treatment to prepare it. The addition of more soybean flour was considered optimal for the growth of *Rhizopus oligosporus* on media added with bagasse. According to [4], one thing that can affect the growth of *Rhizopus oligosporus* is an adequate source of nutrition. Soybean flour is a source of nutrients that can be used by *Rhizopus oligosporus*.

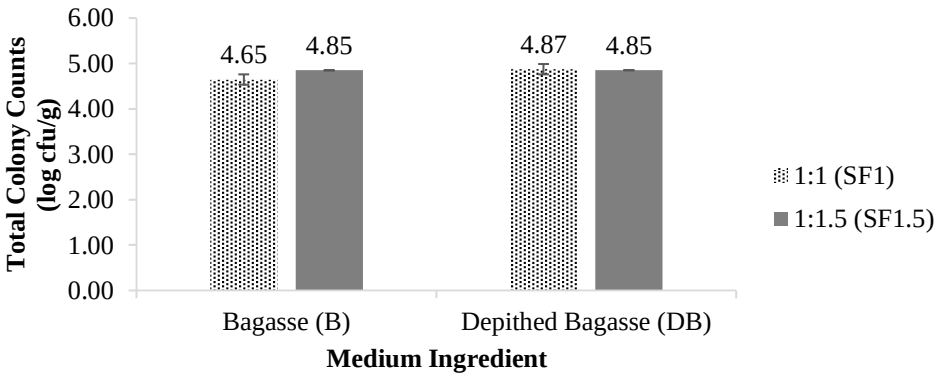

Figure 1. Total colony counts of *Rhizopus oligosporus* on the modified agar medium after two days incubation at 25°C

Figure 2. *Rhizopus oligosporus* on potato dextrose agar (PDA) and the modified medium after two days incubation at 25°C

3.2 Growth of Acetobacter xylinum on the modified broth medium

In this second phase of the study, *Acetobacter xylinum* was grown on media with two different nitrogen sources. The nitrogen sources used are bean sprout extract [8] and urea. The absorbance of the broth medium containing *Acetobacter xylinum* can be seen in Figure 3 while data on the thickness of the cellulose formed can be seen in Table 1.

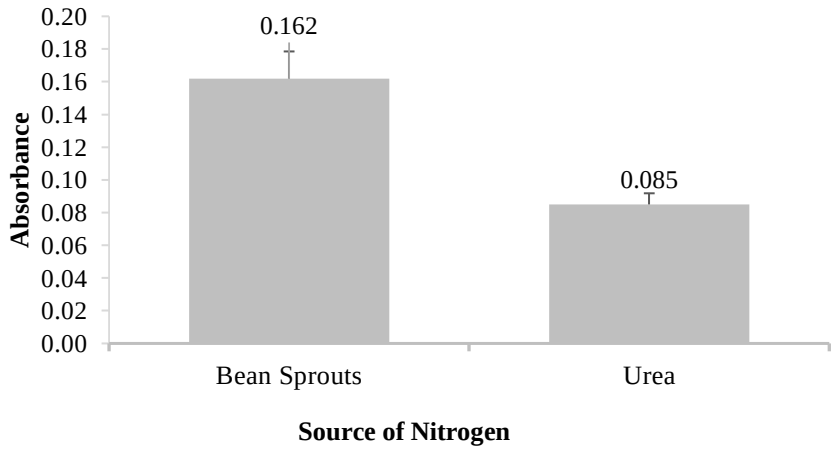

Figure 3. Absorbance of *Acetobacter xylinum* on the modified broth medium

From Figure 3, the growth of *Acetobacter xylinum* in the broth medium with addition of bean sprouts extract has an absorbance of 0.162 ± 0.017 which was higher than that of medium with addition of urea (0.085 ± 0.007). In addition to the higher absorbance value, the use of bean sprouts extract as a nitrogen source for *Acetobacter xylinum* also produces a thicker cellulose layer suggesting the use of bean sprouts extract as a nitrogen source in the production of medium for determining the growth of *Acetobacter xylinum*. This is inversely proportional to previous studies which stated that a urea nitrogen source could produce thicker cellulose compared to using bean sprouts extract as a nitrogen source [5]. This difference was due to the fact that in this study, bagasse was also added. Bagasse contains 47% fiber and 3.33% sugar. The sugar content in bagasse can be used as an additional energy source for the growth of *Acetobacter xylinum* [1].

Table 1. The thickness of the cellulose produced by *Acetobacter xylinum* on the modified broth medium

Media	Thickness (cm)		
	Day 3	Day 6	Day 9
Bean sprouts	0.2	0.7	1.2
Bean sprouts and baggase	0.2	0.8	1.7
Urea	0.2	0.5	1.0
Urea and bagasse	0.1	0.7	1.3

3.3 Growth of Acetobacter xylinum and Rhizopus oligosporus on the modified agar and broth media

The growth of *Rhizopus oligosporus* and *Acetobacter xylinum* on the modified agar and broth medium can be seen in Figure 4 and Figure 5, respectively. In Figure 4 it can be seen that *Acetobacter xylinum*, *Rhizopus oligosporus* and *Acetobacter xylinum* + *Rhizopus oligosporus* can grow on media that has been modified according to the material to be used in the manufacture of biofoam. In Figure 4 it can also be seen that the combination of *Acetobacter xylinum* with *Rhizopus oligosporus* produces a denser mycelium so that the mycelium looks slightly black in color. From Figure 5, the growth of

Rhizopus oligosporus and *Acetobacter xylinum* produced the highest absorbance (0.831 ± 0.067). This shows that *Acetobacter xylinum* and *Rhizopus oligosporus* can exist under modified conditions. The media used for the growth of *Acetobacter xylinum* and *Rhizopus oligosporus* together is different from the media which is used only for the growth of *Rhizopus oligosporus*. The addition of some modified media materials is a source of nutrition for the two types of microorganisms used. In previous studies [6], biofoam production using *Rhizopus oligosporus* only used bagasse, water and soybean flour.

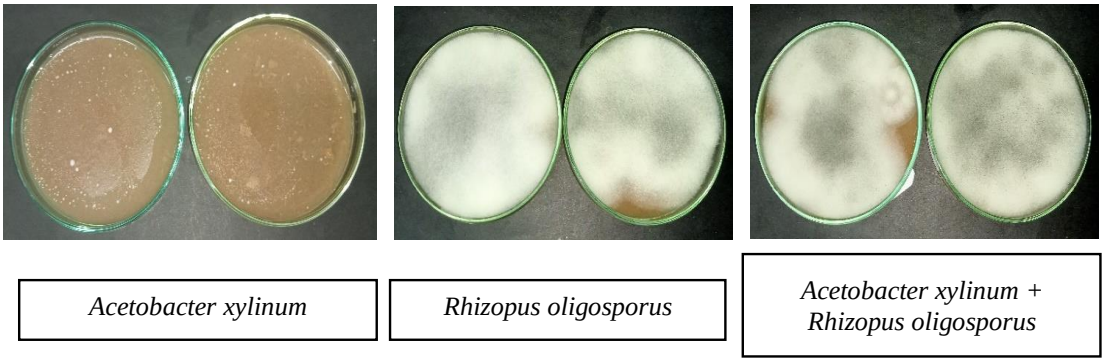

Figure 4. *Rhizopus oligosporus* and *Acetobacter xylinum* on modified medium

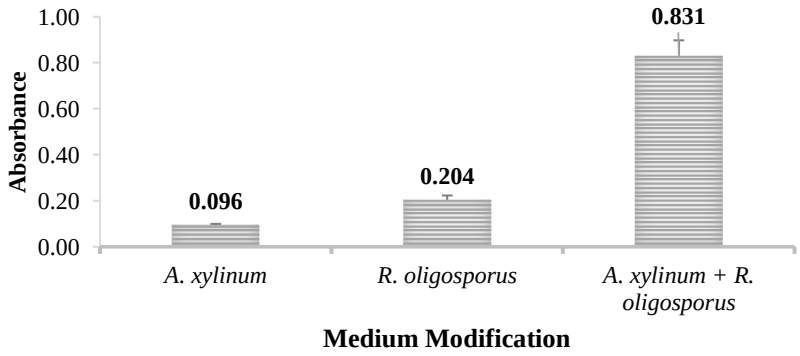

Figure 5. Absorbance of *Acetobacter xylinum* and *Rhizopus oligosporus* on the modified medium

4. Conclusion

In conclusion, the ratio of bagasse to soybean flour used for growth medium is 1:1.5, and the best nitrogen source was bean sprouts extract. Meanwhile, *Rhizopus oligosporus* and *Acetobacter xylinum* can grow together on modified media. The recommended formula for growing these two microorganisms simultaneously is a 1:1.5 ratio of bagasse and soy flour and a 10:1:1:0.1 ratio of coconut water, bean sprouts extract, sugar and vinegar. Media that has been modified according to the needs of the materials is optimal for use in the manufacture of biofoam.

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Acknowledgment
This work was fully funded by Universitas Syiah Kuala under the project “Penelitian Lektor Kepala” with contract number 161/UN11.2.1/PT.01.03/PNBP/2023. The authors thank Sydney Garvis, an Oberlin Shansi fellow at the Language Center of Universitas Syiah Kuala, for proofreading this article.