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BEAN SPROUT FOR BACTERIAL CELLULOSE PRODUCTION: A REVIEW

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Abstract

Cellulose is an organic material that can be obtained from plants and bacterial fermentation. Cellulose which was produced from bacterial fermentation requires some source of nutrients such as carbon and nitrogen. Several studies have been conducted to produce bacterial cellulose (BC) using various nitrogen sources. This paper reviews the production of bacterial cellulose using bean sprouts as a nitrogen source. From the various studies that have been conducted, it was found that the use of bean sprouts as nitrogen source increased bacterial cellulose production. Higher bean sprouts concentration will result in high bacterial cellulose production and thick layer in bacterial cellulose. However, high concentration of bean sprouts can make lower bacterial cellulose production due to increase in the viscosity of fermentation medium and lack of oxygen supply. Based on the organoleptic test, it was found that transparent white bacterial cellulose tends to be produced from high bean sprouts concentrations and is preferred by consumers.

Keywords: bean sprout, bacterial cellulose, nitrogen

Introduction

Cellulose is water-insoluble organic polysaccharide discovered by Payen in 1838 (Payen, 1838). Cellulose is the main component structure in plant walls (Wang et al, 2019). The constituent components of cellulose were composed from glucose monomers. As part from plants, cellulose can also be produced from bacteria. *Acetobacter xylinum* (*A. xylinum*) is one of the bacterial cellulose (BC) producers reported by Brown in 1886. In addition, BC can also be produced from gram-negative and gram-positive bacteria. However, from all of bacteria that can produce BC, *Acetobacter xylinum* or *G. xylinum* is a bacterium that was used to commercially produce bacterial cellulose with high productivity (Wang et al, 2019).

Bacterial cellulose was characterized as an unbranced long-chain polymer which is composed of many (1-4) β-glycosidic linked glucose units. Glucan chains form nanofibrils with hydrogen bonds which in turn form 3D network bonds. The degree of polymerization of BC is between 16000 and 20000 (Lin et al, 2020). In its application in medical, pharmaceutical, and food industries, BC can be modified into new materials with the addition of other components such as polymers, reinforcing agents and active components (Fernandes et al, 2020).

The production process of BC was influenced by various factors including nutrient, pH, medium density, shear force, viscosity and oxygen supply. Several culture methods such as static, agitation/shaking, and bioreactor culture were selected to adapt the resulting bacterial cellulose in terms of physical, morphological and mechanical characteristics (Wang et al, 2019).

Nutrients for bacterial cellulose production consist of various kinds of raw materials such as carbon sources, nitrogen sources, microelements, and so on (Wang et al, 2019). Carbon sources such as sucrose, glucose, and fructose are commonly used in producing bacterial cellulose (Fernandes et al, 2020). Carbon is used by bacteria as the main material to form cellulose. On the other hand, several alternative carbon materials are widely used to reduce the cost of bacterial cellulose production such as agroforests and food industries. Apart from carbon sources, nitrogen sources are also used by bacteria for cell construction and growth. Some sources of nitrogen to produce bacterial cellulose were peptone, yeast extract, tea extract, sodium glutamate, hydrolyzed casein, ammonium sulfate, and glycine (Yim et al, 2016).

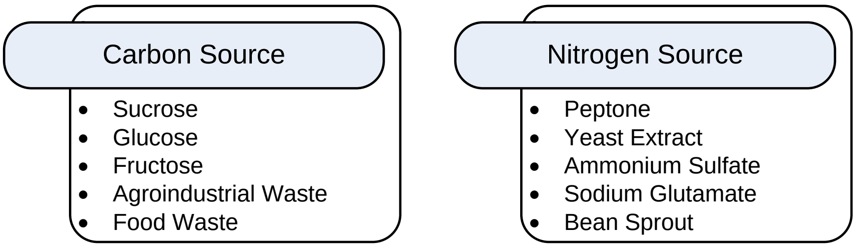

Figure 1. Carbon and Nitrogen Source for Bacterial Cellulose Production

Bean sprouts is a source of nitrogen that can be used to produce bacterial cellulose. Several studies have examined the production of BC with bean sprouts as a nitrogen source. Setiani et al (2019) studied the interaction of bean sprouts (5%, 10%, and 15%) and sucrose (5%, 10%, and 15%) to produce bacterial cellulose from guava (*Syzygium samarangense*). Hardianti et al (2018) also examined the effect of *Acetobacter xylinum* (no. 1, 2, and 3) and soybean sprouts (500, 750, and 1000 gr / L) addition on BC production from dragon fruit skin (*Hylocereus polyrhizus*). Putranto and Taofik (2017) examined the effect of bean sprouts extract (0%, 2%, 4%, 6%, 8%, and 10%) addition on nata production from coconut water. Ngan et al (2019) produce BC by replacing coconut water with bean sprouts.

This paper will review bean sprouts as raw material for bacterial cellulose production. Bean sprouts as source of nitrogen in BC production will affect the resulting BC products such as nata yield, nata thickness, water content, fiber content and organoleptic evaluation.

Bacterial cellulose production using bean sprouts as nitrogen source

The first stage of bacterial cellulose production starts with the preparation raw material. If the raw materials for making BC come from food waste (banana peels, papaya peels, pineapple peels, etc.), special preparations will be made such as washing, size reduction, adding water, and filtration to extract the raw material solution. After that the raw materials can be mixed with sugar, bean sprouts, and adjusted the pH using acids. Then, the mixture can be boiled for 5 - 15 minutes and let it cooled into room temperature. The *Acetobacter xylinum* bacteria was then added to the mixture and allowed to stand for 5 - 14 days until bacterial cellulose was formed.

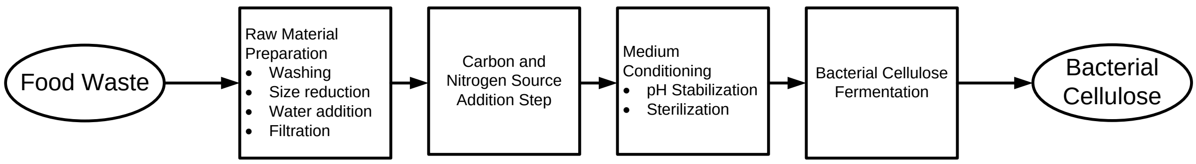

Figure 2. General Steps for Bacterial Cellulose Production from Food Waste

Some analyzes for bacterial cellulose include:

- Yield nata
- Texture analysis
- Water content analysis
- Organoleptic analysis
- Fiber content analysis
- Analysis of the effect of pH
- Analysis of the effect of ethanol
- Inorganic content analysis

Yield of bacterial cellulose with bean sprouts as nitrogen source

Setiani et al. (2019) studied the interaction of bean sprouts (5%, 10%, and 15%) and sucrose (5%, 10%, and 15%) concentration to produce BC from guava (*Syzygium samarangense*). From their research, it was found that bean sprouts and sucrose affected BC production where the highest yield was obtained from the medium composition of 5% bean sprouts and 5% sucrose. While the lowest yield was obtained from the medium composition of 15% bean sprouts and 15% sucrose. At high concentration of bean sprouts, the yield of BC was low because bean sprouts as a nitrogen source make the viscosity of fermentation medium increased which results in a lack of oxygen supply. Lack of oxygen reduce BC production due to aerobic characteristics of *Acetobacter xylinum*.

Hardianti et al. (2018) also examined the effect of *Acetobacter xylinum* concentration (number 1, 2, and 3) and the effect of adding soybean sprouts (500, 750, and 1000 gr/L) on bacterial cellulose production from dragon fruit skin (*Hylocereus polyrhizus*). From their research, it was found that higher concentration of *Acetobacter xylinum* bacteria produced harder BC. This variation in bacterial concentrations made the number of bacteria unbalanced. The highest weight of BC was obtained from the medium composition of *Acetobacter xylinum* McFarland no 2 with 750 gr/L soybean sprout cooking water.

Putranto and Taofik (2017) obtained the same results that higher concentration of bean sprouts extract produced higher yield of bacterial cellulose. The higher bean sprout extract will make the fermentation medium rich in nutrients such as macronutrients, mesonutrients and micronutrients.

The thickness of bacterial cellulose with bean sprouts as a nitrogen source

Hardianti et al. (2018) produced the highest thickness of BC from *Acetobacter xylinum* McFarland no 2 with 750 gr/L soybean sprout cooking water for 14 days of fermentation. This was due to the addition of soybean sprout cooking water as a source of protein in the growth of *Acetobacter xylinum* bacteria. Kornmann et al (2003) have stated that the nitrogen source in *Acetobacter xylinum* can stimulate organisms to synthesize stronger cellulose and more cellulose bonds, so that it can affect the thickness of the nata.

The water content of the bacterial cellulose with bean sprouts as a nitrogen source

Based on the research of Setiani et al. (2019), it was found that bacterial cellulose with the highest water content of around 98.68% was BC which came from medium composition of 15% bean sprouts and 10% sucrose. However, their research also showed that there was no relationship/interaction between the water content and the medium composition. The water content with medium composition of 10% bean sprouts and 15% sucrose was 97.78%. The water content in BC has more effect on the texture of BC that was produced. According to Ratnawati et al. (2007), it was known that BC generally had 95% - 98% water content and 2% - 5% cellulose.

A different result was found in Putranto and Taofik (2017) which stated that the addition of bean sprouts affected the BC water content. In their research, it was found that higher concentration of bean sprouts made lower water content in BC. Thin BC has a tighter structure, so that it has a lower water content. Meanwhile, thick BC has a looser structure which the water content was higher. The highest water content of BC was 95.8% obtained from nata with 10% bean sprouts extract.

Organoleptic Tests

Organoleptic tests was tests related to consumer acceptance of a product. The parameters that were usually assessed on this test include color, taste and texture (Setiani et al, 2019). From Setiani et al. (2019), the medium composition of 15% bean sprouts and 5% sucrose was the composition of BC that was preferred by consumers in terms of color (transparent color). Meanwhile, BC color in the medium composition of 5% bean sprouts and 5% sucrose was not preferred by consumers because of the brown transparent color. According to Indonesian Standards (SNI 01-4317-1996), the good BC has a transparent white color. Besides that, in terms of taste, according to Setiani et al. (2019), the most preferred taste of BC was the taste from medium composition of 10% bean sprouts and 5% sucrose.

Putranto and Taofik (2017) also tested the level of preference for the color, chewiness and taste of BC with variations in the addition of bean sprouts. Based on their research, the color of BC was influenced by the thickness of BC and the color of fermentation medium. It was found that BC color with 10% bean sprouts extract was the most preferred transparent white, while nata with 0% bean sprouts extract was the least preferred. The most preferred level of chewy in BC was in the medium of 4% bean sprouts extract with 8 days of fermentation which produce soft BC that contain lots of water and breakable fibers. The elasticity of BC can also be affected by several physical treatments after BC production such as heating/boiling. Heating can make the BC fibers break more easily. Meanwhile, the level of preference for taste has relationship with water content and chewiness. BC product from Putranto and Taofik (2017) showed that the taste in the medium composition of 10% bean sprouts extract concentration was the most preferred.

Effect of medium viscosity on oxygen supply in bacterial cellulose production

Setiani et al. (2019) was found that a high concentration of bean sprouts made the yield of bacterial cellulose low because bean sprouts as a nitrogen source made the viscosity of the fermentation medium increase which resulted in a lack of oxygen supply. Therefore, the effect of media viscosity is an interesting matter to be discussed in the production of bacterial cellulose.

Tantratian et al. (2005) investigated the effect of adding carboxymethyl cellulose (CMC) into the bacterial cellulose medium. From the results of their research, it was found that the addition of CMC makes the medium run out of oxygen faster than the medium without CMC addition. The addition of CMC was carried out with various concentrations such as 0.1, 0.2, and 0.3% into coconut water media which increased the viscosity to 1.58, 1.82, and 2.06 mPa.s. CMC was added because CMC could increase glucose polymerization in bacterial cellulose production by Acetobacter xylinum. CMC concentrations 0.1 and 0.2% which added to the medium showed high BC production, but after 48 hours of fermentation the dissolved oxygen level was detected very low. This lack of oxygen causes a lack of energy for bacteria to grow and produce bacterial cellulose.

The effect of medium viscosity on oxygen volumetric transfer coefficient (kLa) had also been investigated by Kim et al. (2012). Based on their research, it was known that the higher viscosity of the medium, the lower oxygen volumetric transfer coefficient (kLa). In addition, the oxygen volumetric transfer coefficient (kLa) was also compared to the BC concentration, and it was found that the oxygen volumetric transfer coefficient (kLa) decreased with increasing BC concentration. However, the effect of BC concentration is not too significant compared to the effect of medium viscosity.

Further discussion on the effect of BC concentration on oxygen availability in fermentation had been discussed by Hornung (2010). Based on his research, it was known that in the BC production process, the BC layer is divided into aerobic zone (0 – 1000 μm) and lack of oxygen zone or anaerobic zone (1000 – 1800 μm). The division of this layer occurs because the formation of cellulose will always occur at the top layer of the bacterial cellulose. Bacterial cellulose production in the aerobic zone will further press the previously formed cellulose to the bottom layer which the anaerobic zone. The nature of bacteria in the aerobic zone will be active in forming cellulose while in the anaerobic zone the bacteria will be inactive and cannot produce bacterial cellulose. Therefore, it is important to know the number of bacteria present in the aerobic zone.

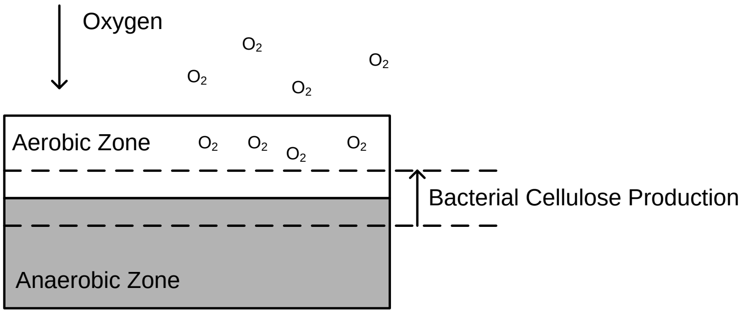

Figure 3. Aerobic and Anaerobic Zone of Bacterial Cellulose Production

Diffusion of oxygen into the BC layer is one of the key to increase BC production so that the aerobic zone becomes wider and bacterial cellulose becomes thicker. Tantratian et al. (2005) showed the effect of impeller speed around 50, 100, and 150 rpm to increase dissolved oxygen BC production. It was found that the maximum BC production was at 100 rpm. The speed of 150 rpm resulted in lower BC compared to 100 rpm because the large dissolved oxygen at 150 rpm caused oxygen to act as a proton acceptor so that it tends to oxidize glucose become gluconic acid. Meanwhile, at 50 rpm, the dissolved oxygen was low so that the available oxygen was not used for cell activity and BC production.

Conclusion

This study reviewed the production of bacterial cellulose using bean sprouts as a nitrogen source. From various studies that have been done, it was found that higher bean sprouts concentration will produce higher bacterial cellulose. However, a very high concentration of bean sprouts can make the production of bacterial cellulose lower because high concentration of bean sprouts increased the viscosity of fermentation medium and lack of oxygen supply in bacterial cellulose production. In addition, higher bean sprouts concentration can make thicker BC structure. Transparent white BC tended to be produced using high concentration of bean sprouts and preferred by consumers.

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