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Non-Microplastic Microbeads From Sago Liquid Waste With The Addition Of Chitosan as Antibacterial Function: A Review

S. Silviana*, Fakhri Santo Khoirudin, Ferris Andhika Pratama, Rizky Putri Adelina Harahap, Alfi Hasanah, Queen Ruhmaningrum, Lailatul Khoiriyah, Saskia Vianova, Michelle Nabillarisa Qori Santoso, Yoga Anugra Guslamari, Cantika Aulia Salsabila

Departemen Teknik Kimia, Fakultas Teknik, Universitas Diponegoro,
Jl. Prof. Soedarto, SH, Kampus UNDIP Tembalang, Semarang, Indonesia 5027

Abstract

Plastic has many advantages due to flexibility, unaffordable, transparent, and toughness. Plastics can size into small sizes (microplastics) or large sizes (macroplastics). Microbeads are granules of plastic or fiber that can often be utilized in many personal care products with sizes below 1 mm. These size of microbeads affect to environmental. Microbeads cannot be filtered by the sewage treatment system resulting microbeads go through to end up in water bodies and become a dangerous pollutant. Therefore any efforts must be conducted to replace the use of plastics microbeads. The microbeads can be prepared from with organic materials having easily degradation with stand the same functions. One of the ways can be accomplished through preparation of bacterial cellulose from sago waste since liquid waste can be used to produce bacterial cellulose. Bacterial cellulose is highly potential to be developed into microbeads as it has advantages of high purity, good tissue structure, high degradation ability, mechanical strength, and easy degradability. The utilization of sago liquid waste is very beneficial because it can reduce environmental pollution and production costs. Additionally, antibacterial properties in microbeads can introduce chitosan, eucalyptus filtrate, celery leaf extract, basil, and cinnamon. Chitosan as an additive in the microbeads will reduce the rate of water adsorption, improve mechanical properties, and reduce the moisture in the microbeads that would promote the ability of microbeads to against bacteria. This review article aims to provide information about microbeds products that have good performance so that they can reduce microplastic contamination in waters.

Keywords*:* *microbeads; cellulose; antibacterial; chitosan; sago liquid waste*

1. Introduction

Most people cannot avoid form plastics usage from our daily life (Karuniastuti, 2017; Hayati et al., 2020). Plastics can take from in small sizes (microplastics) and large sizes (macroplastics). Microplastic is a type of plastic granules or frmagments measuring at < 5 mm. Microplastics will accumulate in water and not easily be removed as they are persistent. The amount of microplastics that accumulate is strongly influenced by sources and activity involved as it finds itself becoming pollutant (Bernanetha et al., 2018).

Microplastics are highly persistent and stay long in the marine environment. The very small size of microplastics and their abundance in the oceans make them ubiquitous and bioavailabile for high aquatic organisms (Wulan Cahya, et al, 2019). As for the macroplastics often used in everydaylife, they are large in size and difficult to destroy (Widianarko and Inneke, 2018).

Microbeads, which can also be called microplastics, are pieces of plastic or fiber found in personal care products with sizes almost always smaller than 1 mm (Ivanajayadi, et al. 2019). Microbeads are increasingly being produced (to substitute un-artificial agent in exfoliant shells, such as shells from many seeds) for daily intake in cosmetics and toothpaste (Chang, 2015).

Microbeads are attempted to release through drainage of the wastewater. Unfortunately, the waste water drainage is not equipped to separate the microbeads as microplastic particles. Furthermeaning they will instead be released into the aquatic ecosystems. Due to the fact, it is estimated that the released microbeads into

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*) Corresponding author
E-mail: silviana@che.undip.ac.id

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the aquatic system around 8 trilliun per day (Rochman et al., 2015).

Microbeads cannot be filtered by the sewage treatment system causing these small objects to end up in the water and become the most dangerous pollutant in the water. As inhabitants of marine ecosystems, fish are one of the organisms that are directly affected by microbeads as their small size enables fish to consume them recurrently (Ivanajayadi, et al. 2019). As a result, samples of fishes sold in markets in Indonesia are found to contain plastic flakes and fibers in their digestion system (Rochman, and Chelsea M, 2015).

Since the presence of microbeads give rise to many adverse effects to humans and the environment, their use is thus restricted and prohibited in Indonesia, in large part owing to the policy related to the prohibition of the commercialization of microbeads utilization. The Netherlands as a pioneer country declared its intention to reduce the use of microbeads in cosmetics at the end of 2016, although in that country no official regulations have been passed (The Economist, 2015). However, the Dutch government and local trade organizations have massively reduced the use of microbeads in various industries. The Netherlands is also one of the European countries that initiated a joint statement regarding the ban on the use of microbeads in the EU (Beat the Microbead, 2016).

Participation and support from the public, NGOs, governments, and multinational companies is urgently needed to reduce the use of microbeads. Leading Indonesian companies such as Unilever, The Body Shop, IKEA, Target Corporation, L'Oreal, Colgate/Palmolive, Procter & Gamble, and Johnson & Johnson have pledged to break the chain of using microbeads in their "personal care products", and more than 70 NGOs in 30 countries are seeking legislative action to ban the use of microbeads. (Rochman, et al 2015).

It is apparent that alternative should be made to replace the use of plastic microbeads through the use of natural ingredients that are easily degraded and not difficult to find whilst also maintain the same use and benefits. One of the ways this can be achieved is through making bacterial cellulose from sago liquid waste. For this, Acetobacter bacteria can be used to convert sugar into cellulose gel, commonly called bacterial cellulose (Ahmad et al., 2019). However, biocellulose does not have antibacterial and antioxidant properties, implicating that additives to promote those properties are needed to improve the ability of bioplastics in preventing damage (Ibrahim, et al, 2020).

Among many, chitosan can be used as antibacterial additive. Chitosan can be denoted as a non artificial polymer derived from crustacean shells. Chitosan is an N-acetylglucosamine derivative from chitin which is commonly found in nature. Chitin is the second largest producer of natural chitosan after cellulose (Abdou et al., 2008; Kumar, 2000; Shankar et. Al, 2015). With the presence of cationic primary amine groups, at pH <6.5, chitosan in a solution will be positively charged and become polycationic polymer. As it contains polycation, chitosan is able to suppress bacterial growth (Tsai, et al., 2002). Chitosan would be able to inhibit microbial activity or in other words it posessess antibacterial properties (Winiati, W. et al, 2016).

There are several existing mechanisms that describe the antibacterial properties of chitosan. The first mechanism states that the polycationic nature of chitosan which interacts with anion groups on the surface of the cell, releasing an impermeable layer over the cell, preventing the movement of essential solutions. The second one concerns reluctance of RNA and proteins that are synthesized through penetration into the cell nucleus (Winiati, W et al, 2016).

Liu, N. et al (2006) in Winiati, W et al, (2016) observed oligomeric chitosan with 58 kDa of a molecular weight in E. coli cells in which it showed good antibacterial activity. It also commences another mechanisms for its antibacterial activity where in chitosan acts as an inhibitor of microbial growth by acting as a chelating agent that renders essential metals or nutrients unavailable for normal microbial growth.

This review article aims to provide information regarding the processing of sago liquid waste and the addition of additives in the form of chitosan which will be interpreted into microbeds. The final product in the form of microbeds is expected to have good performance so that it can reduce microplastic contamination in waters.

2. Literature Review

2.1 Microbeads

Microbeads cannot be easily removed from the environment and 85% of them are found to have settled in the sea. Microbeads with particle sizes <5 mm have been detected in many water areas around the world (Eriksen et al., 2013; Barasarathi et al., 2014; and Claessens et al., 2103).

Ekosafitri et al., (2015) in Azizah, et al., (2020) stated that microbeads are categorized into 2 types, namely primary and secondary microbeads. Primary microbeads are made from micro-particles, such as industrial plastic raw materials and from cosmetic scrubs, while secondary microbeads are formed in the marine environment from microplastic waste which fragments into small pieces due to weathering.

Microbeads, which are widely prohibited from being used, are beads that are synthesized from petroleum such as macroplastics in general. Microbeads is an alternative solution obtained using more environmentally friendly materials that have been synthesized by previous researchers. The materials used are composites consisting of synthetic polymers with vegetable oil, starch or other natural materials would undergo accelerated degradation process (Miraj et al, 2019). Table 1 shows the materials, methods, and results from previous research in the manufacture of microbeads. Table 2 represents the characteristics of several kinds of the bacterial cellulose.

As presented in the previous table, the method most widely in creating microbeads refers to the water-in-oil (W/O) method. This method is carried out by mixing two immiscible solutions. The presence of a binder with stirring produces beads in the form of gel. Furthermore, microbead preparation from bacterial cellulose can be developed because of its more transparent color, smooth texture, excellent tissue structure, and high degradability (Piazza et al, 2011., Quang et al, 2011., King et al, 2017., Lee et al, 2017., Brossault et al, 2019).

2.2 Bacterial Cellulose (Biocellulose)

Bacteria Cellulose is cellulose produced by acetic acid bacteria. It possesses several advantages over cellulose derived from plants. These advantages include high purity, excellent tissue structure, high degradation ability, and unique mechanical strength (Takayasu and Fumihiro, 1997 in Rahayu and Rohaeti, 2014). In addition, bacterial cellulose has a high water content (98-99%), a good liquid absorbent, non-allergenic, and safe sterilization without causing a change in its characteristics (Ciechańska, 2004 in Rahayu and Rohaeti, 2014).

As can be gathered from the information above, sago liquid waste may be used to produce bacterial cellulose. Sago liquid waste in common practice is

Table 1. Synthesis of Microbeads

Reference	Method	Materials	Results
Brossault, et al (2019)	Water-in-Oil (W/O) With various emulsifier stirring	Aqueous phase: SiO_2 and water Oil phase: Sunflower oil with or without Span 80.	- The higher the stirring energy the larger the diameter of the beads. - The higher the Span concentration the greater the size and porosity of the beads. - With the addition of Span 80, a rough texture can be given to the beads.
Quang, et al (2011)	Water-in-Oil (W/O)	Sodium Silicate, H_2SO_4, HNO_3, Ag^+ from $AgNO_3$, $NaBH_4$	- The best antibacterial activity was shown by the addition of 0.5% and 1% Ag + ion. - No bacteria were detected after 5 minutes of observation.
Lee, et al (2017)	Emulsification in Microfluidic Capillary	PDA Oil phase (in the capillary): Silica in photocurable resin with PEGPEA. Water phase (outside the capillary): PVA (Poly vinyl alcohol)	- The formed beads were non close-packed. - The color of the beads depends on the size of the silica used. - PEGPEA was added to produce elastic beads. - With the addition of PDA, the color sharpness of beads can be increased
King, et al (2017)	Liquid- Liquid Biphase	Cellulose in chitin, PPG, Microcrystalline Cellulose (MMC).	- Drying with supercritical CO_2 produces beads with a more transparent color, smooth texture but a porous interior.
Piazza, et al (2011)	Water-in-Oil (W/O) Emulsification equipped with Jet Cutter	Matrix: Alginate Filler: Glucose or Olive Oil Hardener: $CaCl_2$	- The best alginate concentration is 1-2% to produce beads that are not crushed due to mixing in the hardener bath. - The best distance between the cutter jet and the hardener tub is 10-15 cm. - The best distance between the cutting jet and the nozzle is 15 cm. - The best $CaCl_2$ concentration is 0.1-1% to produce beads with good mechanical properties.

disposed into the environment. Sago liquid waste comes from the sago pith pulp filtering process (extraction) and starch deposition. In producing 1 kg of sago flour, 20 liters of liquid waste can be produced. Sago liquid waste contains high carbohydrates and acids enabling it to be used as a medium for fermentation of bacteria such as Acetobacter xylinum (acetic acid bacteria). These bacteria can convert sugar into cellulose gel which is commonly called bacterial cellulose (Ahmad et al., 2019).

In the synthesis, the sago liquid waste media that is added with granulated sugar with a concentration of 5-15% is able to support the formation of cellulose pellicles by bacteria. However, sago liquid waste media with a sugar concentration of 20% is not able to form cellulose pellicles (Figure 1). This indicates that the concentration of sugar in the media affects the production of bacterial cellulose. Mohammad et al. (2014) stated that sugar in the production media serves as a carbon source for bacterial growth which also affects bacterial activity in the production of biocellulose (Ahmad et al., 2019).

Biocellulose, however, does not have antibacterial and antioxidant properties, implicating the need for

Table 2. Bacterial Cellulose Characteristics

No	Product	Characteristics	Reference
1.	Nata de seaweed	The best nata de seaweed was obtained in 5% bacterial concentration treatment with a yield value of 16.15g / L, thickness of 0.41 cm, crude fiber of 5.86%, moisture content of 99.21%, 3.83% appearance, 3.17% taste, 4.13% coloe, 3.13% aroma, and 3.67% texture.	Rachmawati et al., 2017.
2.	Nata de Yam	Crude fiber content (5.82%), yield (81.73%), thickness (1.05cm), weight (335.07 g), texture (123.58 N/cm).	Nisa et al., 2020
3.	Nata de soya with lime juice	At the best concentration of 45 g of sucrose and 5 g of urea, the yield value was 7.7 - 14.4%, fiber content was 0.37 - 4.5%, water content was 81.02 - 89%. Organoleptic test was also conducted.	Tamimi et al., 2015
4.	Nata de sweet potato	The nata de sweet potato with the F4 treatment had the best thickness, with an average thickness of 0.58 cm.	Basalamah et al., 2018
5.	Nata de soya	It has physico-chemical parameters yield of 33.23%, thickness of 16.48 mm, moisture content of 87.28%, texture of 3.36 mm/g.se and color brightness (L*) of 4.13.	Wijayanti et al., 2012
6.	Nata de cassava	Nata de cassava obtained on this study had a size of 30 × 22.5 cm and an average thickness of 1.5 cm with a wet yield of 986.25 grams per 1000 mL of media.	Syamsu and Kuryani, 2014
7.	Nata de corn	Yield of 34.07%, thickness of 1.52 cm, texture of 59.3028 Newton (N), water content of 96.3737%, and food fiber of 2.9772%.	Hasanah, 2019
8.	Nata de sago	The thickness of the bacterial cellulose pellicle formed after 14 days with sugar content of 5, 10 and 15% were 15.5; 21.8; and 14.4 mm. The fiber contents were 4.32, 4.55 and 4.26%, respectively.	Ahmad et al., 2019
9.	Nata de milko	The yield was 34.07%, the thickness was 1.52 cm, the texture was 59.3028 Newton (N), the water content was 96.3737%, and the food fiber was 2.9772%.	Ernawati, 2011
10.	Nata de manihot	Nata which has a thickness of 1.4 cm	Rezki et al., 2016
11.	Nata de cucumber	The product with the addition of 10% sucrose had a thickness of 1.1 cm, a texture of 2.57 kgf/cm^2, and a fiber content of 1.11%.	Herawati and Moulina, 2015

antibacterial additives to improve the ability of the bioplastic made to against bacteria.

2.3 Antibacterial

Antibacterial substances are compounds used to control the growth of pathogenic bacteria, aiming at preventing the spread of disease and infection, eradicating microorganisms in infected hosts, and preventing decay and destruction of materials by microorganisms (Sulistyo, 1971). The use of additives such as antibacterial and antioxidant in bioplastic syntheses can give benefits to these bioplastics, improving the ability of bioplastics to prevent damage, especially to food ingredients (Sisnayati et al., 2019). The following are the results of previous researches that have succeeded in making antibacterial material from a variety of ingredients as presented in Table 3.

According to the various studies above, chitosan has the potential to be used as an antibacterial additive on microbeads. Chitosan is an N-acetylglucosamine derivative from chitin which is commonly found in nature as chitin/chitosan is the second largest natural ingredient after cellulose (Winiati et al. 2016). As a natural biopolymer, chitosan has biodegradable properties. Chitosan (Figure 1) is a product of chitin deacetylation through a chemical reaction with stages of deproteinization, demineralization, depigmentation, and deacetylation. Chitin itself is a natural polymer whose abundance is the largest after cellulose and is mostly contained in marine waste, especially the crustacean group such as shrimp, crab, crab, and lobster (Tanasale et al, 2012).

Indonesia has high number of sources to produce chitin and its derivative products considering that Indonesia is a maritime country. However, the production of chitin itself is still low and many of the marine wastes are thus discarded and cause environmental problems (Stanford, 2003). Compared to other antibacterial product, the wach substituent in the combination of cellulose-chitosan complement and cover their respective weaknesses to provide the desired properties. The fixation reaction between chitosan and amines is presented in Figure 3.

Chitosan also has many advantages of being natural, requiring only small amount in its use, detoxifier, bacterial growth inhibitor, easily degraded, and non-toxic (Kaho, 2006; Sarwono, 2010). Chitosan can thus can be added to achieve antibacterial effect in the making of microbeads.

3. Modification of Microbeads from Sago Liquid Waste

3.1 Bacterial cellulose synthesis

The production of bacterial cellulose is carried out using sago liquid waste as a substrate. Filtered coconut water is heated to a boil, then sago liquid waste, sugar, ZA fertilizer as a source of nitrogen are added. This is followed by the addition of glacial acetic acid to adjust the pH of the media to around 4-5 and then left for 5 minutes. The bacterial inoculum of A. xylinum is added to the production medium as a starter. Fermentation is carried out for approximately 14 days using a glass container and covered using a newspaper. The cellulose layer formed after the end of fermentation is removed from the fermentation vessel and washed with water until it is clean. Measurements of bacterial cellulose parameters including thickness, yield, and fiber content are carried out in 3 replications. Bacterial cellulose is pressed to reduce water content with a pressure of 300 psi, then is put an oven at 125 ºC for 10 minutes. Lastly the bacterial cellulose is put in a desiccator for 15 minutes until a constant weight is obtained (Ahmad et al., 2019).

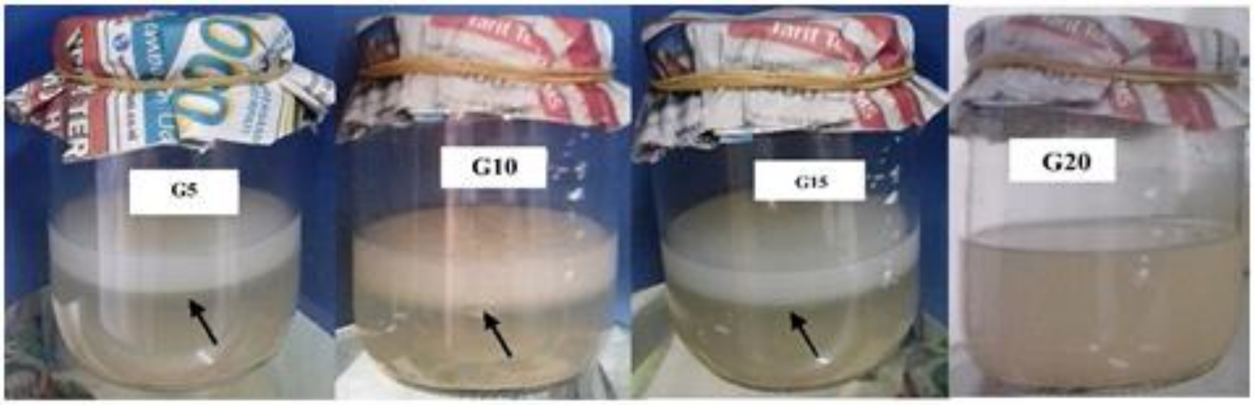

Figure 1. The formation of cellulose pellicle layer on the surface of the sago liquid waste media (Ahmad et al., 2019)

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Tabel 3. Antibacterial Content and Effevtiveness

Material	Content	Effectivity	Reference
Chitosan	Chitosan is the only cationic polysaccharide that exist. All body tissues have a negative charge	It is biocompatible, biodegradable, hydrophilic, with antibacterial property	(Selpiana et al., 2016).
Basil leave	Essential oils, flavonoids, eugenol	The essential oil content in basil leaves contains antibacterial substances that are effective in killing bacteria	(Khalil, 2013)
White Tumeric	Phenols and terpenes	The phenol content in white turmeric can inhibit the growth of E. coli. The terpene compounds have antimicrobial power, especially against gram-positive bacteria.	(Amaliya and Putri, 2013)
Celery	Flavonoids, saponins, tannins	Flavonoids inhibit bacterial nucleic synthesis, saponins will interfere with the surface tension of the cell walls, and tannins can easily enter bacterial cells and coagulate bacterial cell protototoplasm.	(Majidah et al, 2014)
TiO_2 (Titanium dioxide)	The material has a low density, corrosion resistance, high biocompatibility with the body and thus can be used as an implant product.	The material can prevent high relative humidity in packaging, thereby increasing the shelf life of fruits and vegetables	(Wulansari, 2019)
MgO and ZnO	It has thermal conductivity and electrical resistivity at high temperatures as well as a stable cubic structure in a variety of applications.	The antibacterial mechanism in ZnO has the ability to oxidize membrane, and thus damages the membrane structure.	(Farhan,2019)
Aloe Vera	Saponins, flavonoids, terpenoids, tannins and antioxidants	Able to inhibit the growth of staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, and E-coli bacteria.	(Sari and Ferdian, 2017)

3.2 Preparation of chitosan cellulose mixture

The production of biodegradable plastics is carried out using the phase inversion method. The first step involves the solvation of chitosan in 1% acetic acid by determined ratios. The orientation is carried out to chitosan and acetic acid by heating it at 60 °C-70 °C with a hot plate and stirring using a magnetic stirrer with a stirring speed of 1500 rpm for 15 minutes. After mixing, the cellulose is added and stirred for 15 minutes followed by glycerol. The mixture is then heated again for 45 minutes. After the heating time is over, the solution is molded on a glass plate and then dried with free air. The resulting plastic film is then tested with several tests including tensile strength test, elongation test, water resistance test, and biodegradability test, as well as Fourier-transform infrared spectroscopy (FTIR) test (Hayati et al., 2020).

3.3 Microbeads synthesis

Dry chitosan cellulose is hydrolyzed using 5 N HCl (hydrochloric acid) at 55 ºC for 24 hours (Maryam et al., 2019). Microbeads is prepared using an oil-in-water emulsion through a nozzle in a liquid-liquid configuration. The formation of microbeads is carried out in the liquid phase. The emulsion is stirred continuously at room temperature until most of the organic solvent is evaporated to form solid microbeads. The microbeads are collected by centrifugation (Calvo et al., 2006). The microbeads formed are stored in a solution and stirred with a magnetic stirrer for 30 minutes. Lastly, it is filtered

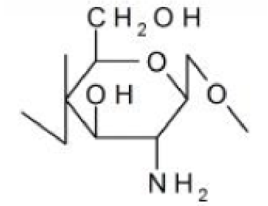

Figure 2. Structure of chitosan repeated monomer unit (Tanasale et al, 2012)

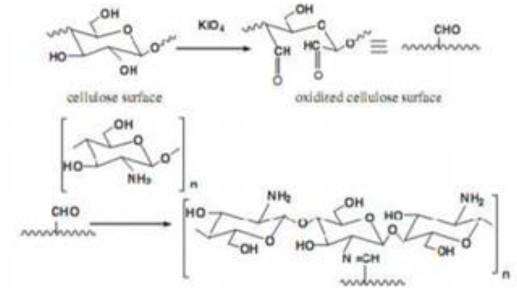

Figure 3. Chitosan fixation reaction on cellulose (Winiati et al, 2016)

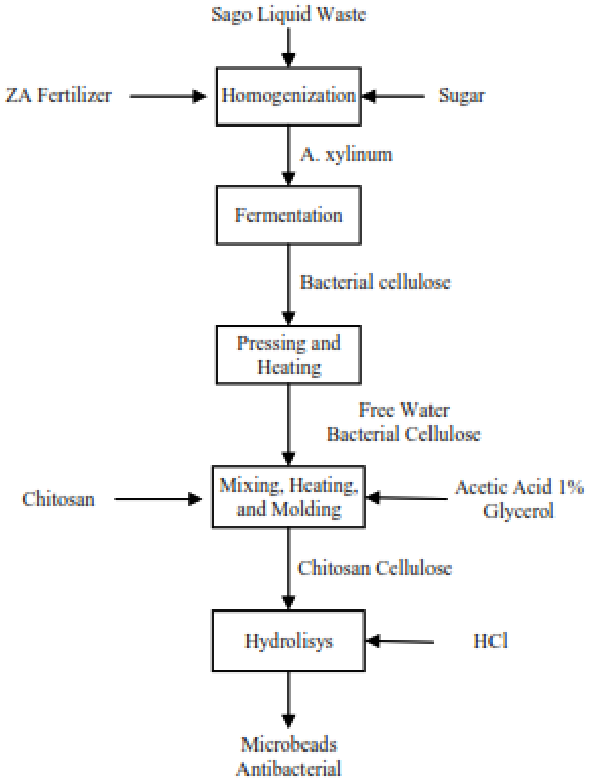

Figure 4. Antibacterial microbead synthesis (Ahmad et

and stored at room temperature for 24 hours (Kuhn et al., 2019).

4. Conclusion

Bacterial cellulose has the potential to be developed into microbeads. Bacterial cellulose has advantages of having more transparent color, smooth texture, excellent tissue structure, high degradability, unique mechanical strength, and easy degradability. Bacterial cellulose can be made through various materials that contain cellulose. Cellulose can be obtained from various materials, one of which is sago liquid waste. Utilization of sago liquid waste is very beneficial because it can reduce environmental pollution and production costs. Antibacterial property in the microbeads can be achieved using chitosan, eucalyptus filtrate, celery leaf extract, basil, and cinnamon. The use of chitosan as an additive in the manufacture of microbeads will reduce the rate of water absorption, improve mechanical properties, and reduce the moisture properties of the microbeads, thus improving the ability of microbeads to kill bacteria. The effect of sago type and variance chitosan concentration needs to be studied further to determine the antibacterial effect and performance of the resulting microbeds.

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