Isolation of Bacteria from Kombucha and Environment-friendly Superabsorbent Polymer (SAP) Production

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Abstract – With e-commerce market growth as well as the COVID-19 pandemic, there is an increase in the number of households ordering fresh produce and frozen food through online grocery shopping rather than in-store grocery shopping. This change in consumer behavior has resulted in an increase in ice packs usage, raising environmental concerns; a large amount of superabsorbent polymer (SAP), used in ice gel packs as well as diapers, are being disposed of in the landfill. A rapidly aging population from increased life expectancy brings environmental concerns as adult incontinence products cause more waste problems than baby diapers.

Cellulose powder was obtained from a naturally fermented tea, Kombucha, through isolation and bacterial fermentation process. A novel method for synthesis of a pH-responsive, smart superabsorbent hydrogel from cellulose powder was suggested. The jelly-like white pellicles obtained by cultivating acetobacter bacteria obtained by separation of bacteria from Kombucha were dried and mortar-ground, which was then dissolved in an aqueous solution of sodium hydroxide and urea. Acrylamide and various crosslinking agents were added to this aqueous solution, which was then synthesized using a household microwave to yield the SAP. The swelling ratio of the synthesized SAP was measured using different pH solutions as swelling media; the swelling ratio of the SAP decreased as the pH of the solution decreased. Through this experiment, it is expected to develop pH-sensitive SAP from natural, fermented drinks, which then can be utilized in targeted drug delivery systems as well as selective release of a specific drug.

Keywords: Kombucha, microwave-assisted hydrogel, pH-sensitive hydrogel, smart hydrogel, superabsorbent polymer

1. Introduction

Environmental pollution has become a serious problem, and one of the main causes comes from plastic pollution from landfill waste. A large amount of superabsorbent polymers (SAPs) used in ice gel packs and baby diapers are being disposed of in the landfill, posing a serious environmental damage because it takes more than 500 years to decompose naturally.

There have been a lot of negative views on SAP's natural destructive characteristics, and research on eco-friendly SAP has begun. In fact, some studies use fruit scraps and coconut jelly as the main materials to produce eco-friendly SAP. Reflecting this trend, this experiment uses a method of producing SAP through bacteria found in Kombucha.

2. Background

Kombucha, a fermented tea with a symbiotic culture of bacteria and yeast, produces a bacterial cellulose (BC). BC is a compound produced by Acetobacter species, a gram-negative bacterium which goes through an aerobic process to produce a cellulose on the liquid-air interface [1]. BC is formed only on the interface because the bacteria need oxygen for growth.

Producing SAP from BC has a lot of advantages, more specifically environmentally. As opposed to plant-derived cellulose produced from cotton or wood, BC is the most abundant natural biopolymer on Earth. The most abundant, economically feasible, and readily available biopolymer, BC also has a high water retention due to its structure. It can absorb over 200 times its own weight in water as it consists of a three-dimensional network with high porosity [2]. It also has high mechanical strength. BC is biodegradable with non-toxicity. It is inert and biocompatible, which makes it possible to implement BC in tissue engineering, wound dressings, and drug delivery.

This experiment involves synthesis of SAP from bacterial cellulose with a smart-swelling behavior; smart hydrogels respond to various external stimuli such as pH, temperature, and light [3]. Among smart hydrogels, pH-responsive hydrogels are gaining attention in the research area because they are suitable for targeting a specific organ by releasing the drug at the desired pH of the target organ [4-6]. In this experiment, SAP produced from BC exhibited different swelling ratios as the pH of the solution changes by manifesting a foundation for further research and development for targeted delivery of drug.

3. Materials and Methods

Experimental methods in synthesizing pH-responsive superabsorbent polymer (SAP) from a natural, fermented tea, Kombucha, are shown in **Figure 1.**

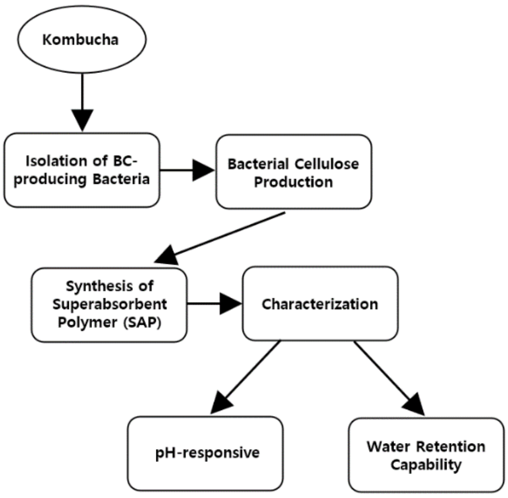

Figure 1. Flowchart of pH-responsive Superabsorbent Polymer (SAP) Synthesis

3.1 Isolation of Bacteria from Kombucha

Kombucha was purchased from a local store. The Kombucha juice was diluted to 10^{-3} with distilled water to isolate the Acetobacter bacteria, as shown in **Figure 2**. The diluted juice was spread on Acetobacter xylinum (AX) agar media, and each colony with a different shape and size was isolated by taking a loop and scraping on the new AX agar. The isolated bacteria was identified by Macrogen, a biotechnology company, to be Acetobacter species bacteria.

Figure 2. Cultivation of bacteria in Kombucha

3.2 Bacterial Cellulose Production

Upon identification of the Acetobacter bacteria, it was cultivated in a liquid media, Acetobacter xylinum (AX) broth; a 34.2 g of ready-made broth powder and a 1 L of distilled water were placed in wide-mouthed laboratory flask, as shown in **Figure 3**. The mixture was heated to dissolve the powder completely until boiling. Once it started to boil, the broth was sterilized using the high-pressure cooker in a reagent bottle with its lid closed tightly. After the broth cooled to a room temperature, AX bacteria was inoculated into the broth, which was then incubated at 28 ◦C for 14 days in order to produce jelly-like white pellicle on the top of the liquid cultivation media.

Figure 3. Manufacturing process of AX broth

3.3 Synthesis of Superabsorbent Polymer (SAP)

Upon identification of the Acetobacter bacteria, it was cultivated in a liquid media, Acetobacter xylinum (AX) broth; a 34.2 g of ready-made broth

In order to use the BC as one of the reactants in SAP production, a method to dissolve the BC in a solvent had to be devised. By making solutions with different concentrations of sodium hydroxide and urea, a solvent with 10 w/v% NaOH and 5 w/v% urea was found to dissolve the BC at a very low temperature [7]. The BC obtained from cultivation was ground with a food blender, and the ground BC was dried for 14 days to obtain the BC powder. A 2.5 g of BC powder was dissolved in a NaOH/urea solvent, in 10 w/v% and 5 w/v% respectively, in an ice bath overnight on a stirring plate. The BC solution showed a transparent color after stirring for at least 12 hours, presented in **Figure. 4**.

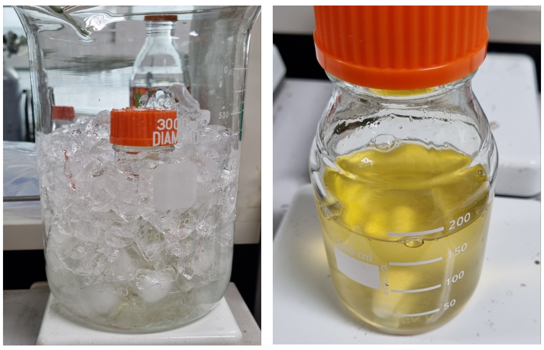

Figure 4. Laboratory setup for BC solution making

A 20 mL of BC solution, which contains 0.5 g of BC, was placed in a 50 mL vial. 20 g of acrylamide and 0.14 g of N,N-methylene-bis-acrylamide (BIS) were added and stirred for at least 1 hour. Once BIS, the cross-linking agent, is dissolved completely, 0.14 g of potassium persulfate (KPS) was added. A 0.5 g of the reactant solution was transferred to a silicone mold to produce a hydrogel that can be easily removed after microwave-assisted synthesis. The solution was microwaved for 5 seconds for three consecutive times under the defrosting setting. The SAP was removed from the silicone mold and dried for further characterization. Experimental setup is shown in **Figure 5**.

Figure 5. SAP produced from microwave-assisted synthesis

4. Experimental Result

The water retention capability of the SAP produced from BC was characterized. Buffer solutions with pH's of 2, 4, 7, and 10 were prepared. The water retention ratios of the SAP in relation to the pH of the solution are graphically depicted **Figure 6** and its laboratory setup is shown in **Figure 7.**

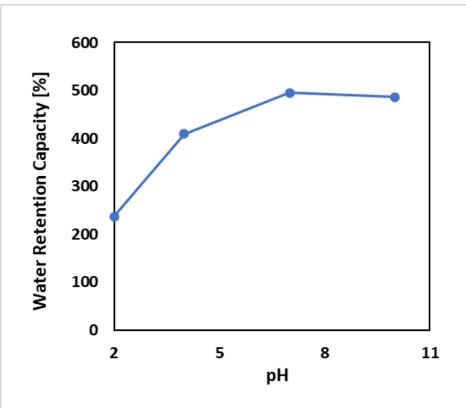

Figure 6. Smart swelling behavior of the SAP

Figure 7. Laboratory setup for measuring pH response

5. Conclusion

In order to diminish environmental destruction caused by humans, a superabsorbent polymer (SAP) was produced by isolating a cellulose-producing bacteria, Acetobacter, from Kombucha and employing the microwave-assisted synthesis method. The SAP produced from BC showed a smart-swelling behavior; the swelling ratio of the SAP decreased as the pH of the solution decreased. This experiment poses a potential development for applications in targeted drug delivery.

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