CHAPTER 1

INTRODUCTION

1.1. Background

Bacterial cellulose (BC) is an extracellular polysaccharide produced by various species of bacteria (Chawla et al., 2008; Huang et al., 2014). The basic structure of BC is micro fibrils that are build out of glucan chains with intermolecular hydrogen bonds. Brown first found BC in 1886; he observed the development of a soft transparent layer on the surface of *Mycoderma aceti* culture (mother of vinegar) that turned out to be pure cellulose produced by the bacteria. Further characterization and identification lead to a discovery of the organism responsible for the layer manifestation, which he named *Acetobacter xylinum* (Brown, 1886). The organism is now reclassified as *Komagataeibacter xylinus*. Extensive studies have shown that the organism has the capability to produce cellulose in the form of a gelatinous membrane that could reach a thickness of 25 mm with a strong and tough structure as well as high liquid-resistant (Lee et al., 2014).

Researchers have mentioned BC as the best substitute for plant cellulose since they have the same molecular formula, which is $(C_6H_{10}O_5)_n$. Nevertheless, in contrast to plant cellulose, BC possesses better chemical and physical properties such as higher purity, polymerization, crystallization index, tensile strength, porosity, and water holding capacity (WHC) despite of the micro fibril size of BC that is multiple times slender than plant cellulose (Chawla et al., 2008). Due to this superior quality, BC can be applied in various fields. Recent studies have explored its application in the pharmaceutical and medical industry as a wound healing agent, in the food industry as a stabilizing and a thickening agent, in the waste management industry as a biosorbent, and in many other areas (Chawla et al., 2008; Huang et al., 2014; Lee et al., 2014). These together are the main reason why researchers are captivated to conduct more extensive study on BC synthesis in bacteria and to further optimize the yield production.

In industrial scale, BC is produced in a bioreactor using a pure or mixed bacteria culture. Pure sugar such as glucose, sucrose, mannitol, fructose, and arabitol are often used as the fermentation medium (Çakar et al., 2014). This has become one of the limiting factors in industrial BC production since the medium alone contributes up to 30% the total production cost (Çakar et al., 2014). Thus, a new alternative medium that allows high production and is economically feasible is needed.

BC can be produced through a home-scale fermentation called kombucha fermentation that involves fermentation of sugar-rich black tea using tea fungus or also known as a symbiotic consortium of bacteria and yeast (SCOBY) (Jayabalan et al., 2014; Chakravorty et al., 2016). In kombucha fermentation, *G. xylinus* in tea fungus relies on caffeine compound inside the black tea to stimulate the production of BC. Adopting this fermentation principle could dramatically lower the BC production cost at an industrial scale. Furthermore, replacing sweetened tea with agricultural waste such as molasses could further reduce the cost.

According to recent studies, molasses has the potential to be the candidate for the BC fermentation medium. It is a thick, dark brown syrup that is obtained as a byproduct of sugarcane and sugar beet final processing step (Premjet, Premjet, Ohtani, 2007; Çakar et al., 2014). Inside the molasses are sugars, carbohydrates, minerals, vitamins, nitrogenous compounds, nucleic acids, and non-nitrogenous acids (Premjet, Premjet, Ohtani, 2007). The composition of molasses might vary, but it could support the growth of many microorganism strains (Çakar et al., 2014). In Indonesia, molasses is a readily available resource. It is usually used as raw material to produce ethanol biofuel, acetic acid, or monosodium glutamate. The availability of molasses reached 499,050 tons/year in East Java, 96,378 tons/year in Central Java, 56,689 tons/year in West Java, 40,789 tons/year in Lampung, 29,686 tons/year in North Sumatra, 29,596 tons/year in South Sumatra, 24,302 ton/year in South Sulawesi, 21,044 tons/year in North Sulawesi (Jaya & Mahendra, 2008). The abundance of molasses can be utilized to replace the expensive carbon source generally used in BC production. This approach could reduce the total cost as well as increase the value of molasses.

This study aims to produce BC using kombucha culture from a cost-effective medium that was prepared from molasses, pure caffeine, and acetate buffer. An agroindustrial waste molasses was utilized to replace pure sucrose in order to reduce the production cost, while bacteria used caffeine to enhance the BC production. Acetate buffer was used, replacing water as the solvent, to maintain pH during the fermentation. In this study, there are three independent variables, which include pH of acetate buffer, molasses, and caffeine concentration. The variables were varied at three levels. On the other hand, this study has three dependent variables: BC yield, BC mechanical properties (tensile strength, elongation at break, and Young's modulus), and BC water holding capacity (WHC). The effect of the variation of each independent variable on dependent variables are studied.

1.2. Problem Formulation

Several problems are addressed in this study:

1. What are the optimum pH of acetate buffer, molasses, and caffeine concentration to produce BC at high yield?
2. What is the effect pH of acetate buffer, molasses, and caffeine concentration on the yield, the mechanical properties, and the WHC of BC biofilms?

1.3. Objectives

The objectives of this study are:

1. To know the optimum pH of acetate buffer, molasses, and caffeine concentration to produce BC at high yield.
2. To evaluate the effect of pH of acetate buffer, sugar and caffeine concentration on the yield the mechanical properties and the WHC of BC biofilms.

1.4. Scope

The scope and limitations of this study are:

1. Swedish kombucha tea is adapted to molasses medium in three steps procedure: first in sugared tea medium, second in molasses-sugared tea medium, and last in acetate buffered molasses medium.
2. BC biofilms are produced through fermentation using Swedish kombucha that has been adapted to molasses medium.
3. Three level variations on three independent variables including molasses concentration, caffeine concentration, and pH of 200 mM acetate buffer are involved in the experimental design.
4. The yield, the mechanical properties, and the WHC of the BC samples produced at different fermentation condition are evaluated.