BACTERIAL CELLULOSE PRODUCTION FROM FOOD WASTE

CHUA GEK KEE @ YEO GEK KEE

RESEARCH VOTE NO: RDU170329

Faculty of Chemical and Process Engineering Technology

UNIVERSITI MALAYSIA PAHANG

2020

ACKNOWLEGMENT

The authors gratefully acknowledge financial support received from Universiti Malaysia Pahang RDU170329.

ABSTRACT

The objectives of this project is to investigate the feasibility of using food wastes for bacterial cellulose production, where the best pretreatment methods were identified and the effect of carbon and nitrogen sources variation together with the operating conditions were examined. Food wastes were collected from the café inside the Gambang Campus of Universiti Malaysia Pahang. After screening and grinding, food wastes were subjected to pretreatment in order to hydrolyze the nutrients. Three methods were employed, they are hydrothermal pretreatment, alkaline pretreatment and enzymatic pretreatment. The performance of the pretreatment was monitored using Bradford assay (protein content), phenol-sulphuric acid assay (carbohydrate content) and Bligh & Dyer method (lipid content). Food wastes hydrolysate (FWH) obtained after hydrothermal pretreatment was used as fermentation medium to produce bacterial cellulose by *Gluconacetobacter xylinus*. The effect of carbon and nitrogen content in the FWH was studied by varying the composition of the food wastes collected before pretreatment. While the operating conditions studied were inoculum density, temperature, pH and stirring speed. The yield of bacterial cellulose (BC) produced was monitored together with its ability to hold water. The physical property of BC was characterized with FTIR and morphological property was observed using SEM. Hydrothermal and enzymatic pretreatments were found to solubilize more nutrients into the solution. Increase in carbon content will increase BC yield provided that nitrogen content is not a limiting factor. The optimal operating conditions to produce BC were found to be at 30°C, pH 5.5, 10% inoculum density and 150 rpm stirring speed. BC produced using FWH has a similar physical property as that of Nata de Coco. From the SEM results, BC from FWH has a similar microfibril networks as the BC produced from pineapple juice. Nevertheless, it is denser with some impurities trapped in the network. Thus, further pretreatment may be required if the quality of BC is to be increased.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cellulose is the most abundant, inexpensive and readily available carbohydrate polymer in the world that extracted majorly from plants. However, various bacteria are able to produce cellulose through fermentation process as an alternative source (Faezah & Masrinda, 2014) and hence industry tends to replace the use of plant cellulose with bacterial cellulose. In comparison with the bacterial cellulose, plant cellulose needs further purification in order to remove the content of hemicellulose and lignin material (Klemma, Schumannb, Udhardta, & Marsch, 2011) which is complicated.

Bacterial cellulose production is getting attention from various industries because it offered huge significances through wide applications in various fields such as medicine, pulp and paper industry, and food industry (Shia, Zhang, Phillips, & Yang, 2014). Bacterial cellulose is traditionally used in the food industry to make nata de coco which is an indigenous dietary fiber that served as a gelatinous cube which has no cholesterol, low in fat and low calories. Besides, packaging plays an important role to protect and preserve the food. It is also being identified as good material in the packaging industry due to its biodegradable property (Lin et al., 2013).

Despite its potential in various applications, its production in industrial scale is limited due to the low productivity and time-consuming production. In this case, researchers from all over the world tried improving the fermentation process from many aspects such as the components of media, the technique of cultivation and also other parameters as the yield is not high enough (Żywicka et al., 2018). In the fermentation

process, one of the factors that bacterial cellulose production depends heavily is the growth medium. Carbon sources and nitrogen sources in the media play a significant role in bacterial cellulose production, and at the same time cost for bacterial cellulose production need to be considered too. Inexpensive components such as food waste can be used in medium preparation without chemical additives in order to minimize the cost.

In Malaysia, there are 15,000 tonnes of food waste produced daily from the public and most of them cannot be marketed anymore due to hygiene factor (Malaysians waste 15,000 tonnes of food daily, 2016). However, they are rich in nutrients such as carbon source and nitrogen source (Lehman, 2017). If the food waste is exposed to the environment without proper management, issues such as toxic leachate, emission of greenhouse gases or vermin infestation may happen (Azlina, Ahmad, Mahamad, & Nik, 2012). Hence, utilizing food wastes as fermentation media could not only improve the cost-competitiveness of bacterial cellulose production, but also eliminate the negative impact on to the environment.

Food waste is a complex organic matter that is rich in nutrients content and is a valuable source of energy (Chua et al., 2019; Antonopoulou et al., 2020). Hence, it is an excellent source of value added products, which can be generated via biological processes. Most common way of exploiting the remaining value of the food waste is by anaerobic digestion to produce methane and hydrogen. In addition to this, food waste can also be used to produce bioactive compounds, biopolymer, enzymes, organic acids etc. (Sindhu et al., 2019). Though some of these products could be produced by using other methods such as chemical treatment, microwave extraction, thermal heating, and enzyme hydrolysis, most of the high-value products are produce via microbiological fermentation. Fermentation using complex organic matters like food waste, which is heterogeneous in nature, typically take longer period of time as the complex molecules need to be broken down by the microorganism before new molecules synthesizing process could begin. Thus, pretreatment of food waste become necessary.

The aim of any pretreatment is to break down the available nutrients in the wastes from its complex form into a simple structure that is more accessible to the microorganisms, which will then speed up the fermentation process that follows. Common pretreatment methods used for organic matter like agriculture, municipal and food wastes are chemical hydrolysis using acid or alkaline, physical pretreatment such as thermal, microwave and ultrasonic, and enzymatic hydrolysis using various enzyme mixtures (Perez-Pimienta et al., 2017; Patel et al., 2019; Shen et al., 2019). Physical pretreatment is normally energy intensive, while chemical method, especially acid hydrolysis, was reported to generate toxic compound. Enzymatic hydrolysis, on the other hand, is a costly process. As a consequence, the most suitable pretreatment method would highly depend on the type and nature of the waste to be treated.

1.2 Problem Statement

Since bacterial cellulose is typically produced by coconut water or pineapple juice, or even from other agricultural juice. Report of using food wastes as the fermentation medium is rarely found. When a new medium was developed for a product, its composition may affect the growth characteristics of the microorganisms, which in turn affect the operating conditions for optimal production. Thus, the effect of medium composition and operating conditions in the production of bacterial cellulose using food wastes hydrolysate need to be investigated in depth.

1.3 Objectives

The objectives of this research are:

1. To determine the most effective pretreatment method of food wastes to obtain high nutrients content food waste hydrolysate.
2. To investigate the effect of carbon and nitrogen sources variation on the production of bacterial cellulose by using food waste hydrolysate.
3. To examine the effect of the operating conditions in the production of high quality bacterial cellulose by using food waste hydrolysate.

1.4 Scopes of Study

1) Collection of food waste from the cafeteria of Universiti Malaysia Pahang.
2) Pre-treatment of food wastes using hydrothermal, alkaline or enzymatic treatments.
3) Characterization on the sample before and after pretreatment with its moisture content, ash content, pH, total solid (TS), volatile solid (VS), amount of carbohydrate and protein.
4) Use YGC medium as inoculum medium for *Gluconacetobacter xylinus*.
5) Study the effect of C content and N content of food wastes by manipulating the wastes compositions.
6) Study the effect of inoculum density, pH, temperature and stirring speed on the production of bacterial cellulose.
7) Analyze the product on water holding capacity, yield, physical structures and morphological structures.

CHAPTER 2

LITERATURE REVIEW

2.1 Bacterial Cellulose

Cellulose is a water-insoluble polysaccharide that mostly produced by vascular plants (Brigham, 2018). It is the main constituent of cotton and wood. Both cotton and wood together are the major resources for all cellulose products such as paper, textiles and construction materials. Most of cellulose from land and forest is produced in the living plant cell from photosynthesis. In the oceans, most cellulose is produced by unicellular plankton or algae using the same type of carbon dioxide fixation found in photosynthesis of land plants (Keshk, 2014).

One of the ways to reduce the demand for cellulose from plants is the production of cellulose using microbial systems. Bacterial cellulose (BC) was first reported by Adrian Brown, who observed that sometimes a not-expected solid mass was formed at the surface of the fermentation medium (Moumita, 2019). The compound was then known as cellulose and the author proposed the name Bacterial xylinum for the microorganism and now the bacterium is known as *Gluconacetobacter xylinum*. BC does not require additional processing to remove undesirable impurities and contaminations. Therefore, it can retain a greater degree of polymerization. BC also demonstrates unique properties, including a high degree of crystallinity, water retention value, tensile strength, and moldability (Khairuddin, 2013).

Other than that, despite the fact that plant cellulose shares the same molecular formula $(C_6H_{10}O_5)n$ with bacterial cellulose, their physicochemical properties are different. Bacterial cellulose is characterized by higher purity, due to the fact that it does not contain any hemicellulose or lignin, higher water holding capacity, hydrophilicity,

degree of polymerization, mechanical strength, crystallinity, porosity, and a highly pure fiber network structure, compared to plant cellulose (Phisalaphong & Jatupaiboon, 2008). The enhanced mechanical properties of bacterial cellulose occur due to its uniform, continuous and nano-scalar network of cellulosic fibers. These properties are affected by various factors, such as the culture conditions, the microorganism and the fermentation media employed. For instance, bacterial cellulose is produced in either static or agitation conditions. From the research, thick pellicle was reported under static condition while under agitated condition produced irregular shape spherical cellulose particle (Tsouko, Kourmentza, Ladakis, & Kopsahelis, 2015). Under static condition aeration and carbon source forms the important parameters to enhance yield. Pellicle grows downward indicating the maximum of production.

2.2 Application of BC

Bacterial cellulose has been efficiently used in various areas including the textile industry, paper, food, pharmaceutical, cosmetics and tissue engineering due to its versatile properties.

2.2.1 Cosmetics Industry

Stabilization of oil-in-water (O/W) emulsion without the addition of any of surfactant can be done by applying cellulose fibrils in cosmetics. Such formulations may not be irritant to sensitive skin due to the absence of any surfactant (Hasan, Biak, & Kamarudin, 2012). BC has also been reported to be an exceptional non-allergenic biopolymer for use in cosmetics.

One of the applications of BC in the cosmetics industry is facial masks. BC facial masks are great interest as cosmetic devices to treat dry skin due to its biodegradability, low toxicity and ability to hydrate the skin (Amnuaikit et al., 2011). The BC masks augmented the moisture contents of the skin significantly than normal moist towels upon

a single treatment. In a study, the BC facial masks rated around 4 out of 5 on the satisfaction rating scale (Amnuaikit et al., 2011). The BC mask can be used for increasing the moisture content of the skin. In a patent, BC facial mask was fabricated with holes for eyes, mouth and nose (Lee, Hsu, Cho, Kim, & Han, 2013). The author claimed that such mask may be suitable for repeated or prolonged use for skin beautifying purpose, skin nutrition, and moisturizing and cosmetic effects (Lee et al., 2013).

BC is also playing a role in the fabrication of contact lenses due to the properties such as transparency, light transmittance, and permeability to liquid and gases. Bacterial cellulose-based contact lens was prepared by high viscosity solution which remained intact in its shape and transparency for about 42 days (Coelho et al., 2019).

2.2.2 Medical Industry

Based on Brown (1999), BC contains high tensile strength, high porosity and microfibrillar structure. For example, chronic wounds such as venous and diabetic ulcer are very difficult to heal. The treatment for chronic wounds involves a lot of materials. As bacterial cellulose contains highly porous material, it allows the potential transfer of antibiotics or other medicine into the wound (Takagi, 1993). Also, the high mechanical strength of BC in the wet state, substantial permeability for liquids and gases and low irritation of skin indicated that the gelatinous membrane of bacterial cellulose was usable as an artificial skin for temporary covering of wounds. The products of bacterial cellulose in skin therapy with wide applications in surgery and dental implants and realities in the human health-care sector. Cases of second and third degree of burns, ulcers and others could be treated successfully with skin therapy products as a temporary substitute for human skin (Fontana et al., 1990).

2.2.3 Food Industry

Other than the typical tradition desserts, vegetarian meat may be prepared by using BC in combination with *Monascus* extract obtained from a natural red pigmented mould

(Purwadaria, 2010). The composite is stable against changes in color and morphology, and its flavour is much like natural meat (Wong & Kong, 2010). The vegetarian meat adds a cholesterol-lowering effect to the other advantages of BC dietary fibers (Ng & Shyu, 2004). Moreover, due to the non-animal origin, this meat could be a suitable substitute to animal-based products for certain consumers with dietary restriction.

2.2.4 Paper Industry

In the paper industry, bacterial cellulose has been investigated as a binder in papers. This is because it consists of enormously small clusters of micro fibrils and this greatly adds to strength and durability of pulp when being integrated into paper. For example, Ajinomoto Co. along with Mitsubishi Paper Mills in Japan are currently active in developing microbial cellulose for paper products (Kawecki et al., 2004).

2.3 Types of Pre-treatment

Methods of pre-treatment are divided into three main groups which are physical (mechanical and thermal pre-treatment), chemical and biological. The choice of a suitable pre-treatment depends on the biomass properties, optimal pre-treatment condition, financial and environmental issues (Gea et al., 2011). The pre-treatment that chosen should increase the surface area of the substrate to make the carbohydrates more accessible for enzymes, minimize the loss of carbohydrates during the pre-treatment, increase the recovery of lignin degradation into useful products, limit the formation of inhibitors compounds and reduces energy demand (Hendriks & Zeeman, 2009).

2.3.1 Mechanical Pre-treatment

Mechanical pre-treatment is a physical process that used to reduce the size of the sample by chipping, milling or grinding. The crystallinity of the materials and degree of polymerization greatly decreased during the size reduction process and increased the surface area for enzyme degradation. Irradiation such as gamma rays, microwaves and

electron beams are also known as mechanical pre-treatment which will increase the surface area and pore size of the material and decreases the crystalline structure (Taherzadeh & Karimi, 2008). The main disadvantage of this method is the high energy demand but the formation of inhibitory is low (Biernacki et al., 2013).

2.3.2 Thermal Pre-treatment

Thermal pre-treatment is a process of heating lignocellulosic materials to destroy the structure under certain temperature and pressure. The examples of technique that involved in this method are steam, liquid hot water, autohydrolysis, and aquasolv pre-treatments (Kostas & Michael, 2013) which enhance biodegradability of the substrates (Taherzadeh & Karimi, 2008). The main limitation of this technology is the formation of inhibitors such as soluble phenolic compounds and furfural, which inhibit the production of methane (Hendriks & Zeeman, 2009). The formation of inhibitors during liquid hot water pre-treatment is relative low due to high water input (Zheng et al., 2014). Addition of alkali might be needed to keep the substrate at the pH around 4 to 7 to minimize the formation of these inhibitors (Hendriks & Zeeman, 2009).

2.3.3 Chemical Pre-treatment

Acid and alkaline pre-treatment are considered as chemical process used for food waste. Acid pre-treatment can be classified into dilute or concentrated acid under low or high temperature. The examples of acid under this pre-treatment are nitric acid, sulphuric acid, phosphoric acid and hydrochloric acid. This pre-treatment is mainly used to remove hemicelluloses and solubilize lignin effectively. On the other hand, the reagents used in alkaline pre-treatment are sodium hydroxide, calcium hydroxide, potassium hydroxide, aqueous ammonia and ammonium hydroxide to increase cellulose accessibility by solubilizing lignin and hemicellulose (Taherzadeh & Karimi, 2008). The effect of chemical pre-treatment is highly depending on the types of method used and the characteristics of substrate. Biodegradable substrates containing high amounts of carbohydrate is not suitable for this kind of pre-treatment due to their accelerated

degradation and subsequent accumulation of volatile fatty acid (VFA), which leads to failure of the methanogenesis step in anaerobic digestion (Dwyer et al., 2008) while this kind of method is more suitable to apply on the substrate that rich in lignin (Li & Noike, 1992).

2.3.4 Biological Pre-treatment

Microorganisms viz, brown, white and soft rot-fungi are used in biological pre-treatment to degrade lignin and hemicellulose so that the biodegradability of organic matter can be enhanced. The lignin is more resistance than cellulose and hemicellulose, thus the efficiency of microorganism to degrade lignin is slower (Taherzadeh & Karimi, 2008). This technique required longer time due to low biological hydrolysing rate but is economic and low in energy demand (Hendriks & Zeeman, 2009).

2.4 Effect of medium composition

The common carbon sources used in bacterial cellulose production is glucose and sucrose. Initial glucose concentration acts an important role in the cellulose production as there is the formation of gluconic acid caused by nitrogen source that will decline the pH of the culture and as a result, cellulose production decreases. The addition of polyphenol compounds or antioxidants which are costly tends to inhibit gluconic acid production (Dahman, Jayasuriya, & Kalis, 2010). To reduce the cost of production of bacterial cellulose, many studies have been carried out using waste as a potential carbon source and nitrogen sources which are much cheaper. Some of them used as carbon source for the production are waste beer yeast, thin stillage, maple syrup and sugarcane molasses.

Based on existing study, the effect of various carbon and nitrogen sources on cellulose membrane production by *Acetobacter xylinum* was evaluated (Ramana, Tomar, & Singh, 2000). In the study, among the carbon sources, sucrose, glucose and mannitol were found to be suitable for optimum levels of cellulose production. By calculating the

composition of carbon in those carbon sources, the composition of carbon sources in sucrose (42.1%), glucose (40%) and mannitol (39.5%) were 40.5% in average.

Besides, in the study of bacterial cellulose production by *Gluconacetobacter xylinus* using carob and haricot bean, the production is high (Bilgi, Bayira, Sendemir-Urkmezab, & Hames, 2016). It is shown that carob contains over 50% sugar its dry weight with around 75% of those sugars is sucrose and the rest are fructose, maltose and glucose.

In terms of nitrogen sources, based on the study from Kadir *et al.* (2013), the best nitrogen in BC production is ammonium nitrate (34%N), potassium nitrate (14% N), yeast extract (10% N) and malt extract (1.5% N) with average of 14.88%N. The author in this study concluded that the biomass production decreased with lower supply of N, and complete absence of nitrogen stopped growth altogether. Thus, if more carbon were to be applied, nitrogen may become a growth-limiting factor

2.5 Culture Condition

The BC production by fermentation is conducted in static, stirred or agitated conditions. Various forms of cellulose will be resulted under these conditions. In static condition, a three dimensional interconnected reticular pellicle was observed meanwhile, an irregular shaped cellulose particle were observed (Esa et al., 2014). Production in static medium is regulated by air supply from surface of the medium while the yield depends on concentration of the carbon source (Budhiono et al., 1999). Majority of BC production at commercial level will be produced through agitated condition due to lower yield obtained under static condition. However, the quality of the cellulose in static condition is higher compared to other conditions (Shi et al., 2013). Thus in this study, static condition will be used to produce a higher quality BC. Medium condition plays a major role in BC production. BC production using *G. xylinus* requires temperature of 30 ℃ and pH of 6.5 to ensure growth of bacteria which results in the highest dry weight of BC (Pourramezan *et al*., 2009). Previous research mostly studied on the effect of incubation temperature and

pH involving conventional medium but in this research the incubation pH and temperature involving food waste as culture medium was studied as parameter.

Temperature is a crucial parameter as the growth and production of BC is influenced by temperature. Majority studies concludes that the maximum cellulose production was obtained at temperature between 28 and 30 ℃ (Keshk et al., 2006; Chawla et al., 2009). Based on previous study, during fermentation process the medium pH varies throughout the process due to the production of by product as glucose is converted into cellulose. The accumulation of these by products will affect the condition of the culture medium thus decrease the BC production (Chawla et al., 2009). From the similar study, incubation temperature of 5 ℃ and 40 ℃ shows no growth of bacteria due to denaturation of the cell components such as nucleic acids and proteins (Son et al., 2001). A study conducted by Hameed (2012) showed that the maximum yield of BC (8.5g/L) was obtained at temperature of 30 ℃. Results reported by Al-Shmary (2009) also had the similar result however a study from Son et al., (2001) stated that there was no significant difference in the production of BC using *G. xylinus* at incubation temperature ranging from 25 ℃ to 30 ℃. The similar study also stated that there was a decrease in BC production when the temperature is raised above 35 ℃. Therefore, in this experiment, the range of temperature was selected from 26 ℃ to 34 ℃ with an interval of 2.

The optimum pH of the culture medium for BC production was observed at range of 4.0 to 6.0 meanwhile; below pH of 4.0 there will be a gradual decrease in the yield of BC (Masoka et al., 1993). This situation can be justified as the accumulation of gluconic, acetic or lactic acid throughout the fermentation process, which will drop the pH value. A study from Hameed (2012) shows that the maximum yield (8.5g/L) was obtained when the medium pH was adjusted at pH of 6.5. The similar study also obtained an approximate similar value at pH of 7. Panesar et al., (2009) and Al-Shmary (2009) also obtained a similar result in where the maximum yield of BC was obtained at pH ranging 6.5 to 7.0. However, a study conducted by Jozala et al., (2004), a higher production of BC was obtained at pH of 4 to 5. Thus in this experiment, the pH range studied was from 4.5 to 6.5 with interval of 0.5.

Besides, a study from Ruka et al (2012) showed that difference in inoculum volume does not affect the cellulose yield. In their study, inoculum volume of 100 and 200 ml were inoculated with 1.0, 0.5 and 0.25 % (v/v) of *Gluconacetobacter xylinus* ATCC 53524 stock each. The results also concluded that the number of cells introduced into the culture has no impact on the cellulose production. Moreover, the inoculation time also affects the BC production. A study by Jozala et al. (2004) observed that there were no significant difference between inoculation time of 72 and 96 hours. Using rotten fruits as substrate, the highest yield of BC was obtained after 96 hours of inoculation in HS medium.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Chemicals

3.1.1 Materials

Viscozyme® L has an enzyme activity of 100 Fungal Beta-Glucanase Units per gram (FBGU/g), together with bovine serum albumin, they were obtained from Sigma-Aldrich, USA. Sodium hydroxide, potassium hydroxide, calcium hydroxide, ethanol, methanol, phosphoric acid and phenol were bought from Merck, Germany. Whereas sulphuric acid, chloroform and glucose were purchased from Fisher Scientific, USA. Coomassie Brilliant Blue G-250 was obtained from R & M Chemicals, Malaysia.

3.1.2 Sample collection & characterization

Food waste were collected from restaurants and night market around Kuantan, Malaysia. They were segregated manually into edible or inedible waste, where the waste such as plastic or non-biodegradable materials will be discarded before weighed. Then, the food waste was homogenized using a lab blender (Oster Heritage Blend 400, Oster, United States). Food waste samples were divided into 50 g size and stored separately in a -20 ℃ freezer prior to experiment. Characterization for the elemental composition, the amount of carbohydrate, protein and lipid were performed on the food waste collected. Initial nutrients content detected for the raw food waste were taken as a control to all the pretreatment methods.

3.2 Pretreatment of food wastes

3.2.1 Hydrothermal pretreatment

Fifty gram thawed food waste sample was heated at 80 ℃ for 60 minutes in an oven (UT6, Heraeus, United Kingdom) without addition of water, this is taken as food waste to water ratio of 1:0. The sample was cooled to room temperature (23 ±2 ℃) before centrifuged at 15 ℃ and 3214g for 10 minutes (5810R, Eppendorf, Germany). The liquid part is termed as food waste hydrolysate (FWH) and was stored at -20 ℃ prior to further analysis. The experiment was then continued with the food to water ratio from 1:1 to 1:4. The best food to water ratio was used in the study of temperature effect from 60 to 140 ℃ and heated for 60 min. In the study of holding time (30 to 75 min), the best temperature and food to water ratio from the previous study were used. Similar centrifugation process was performed to all samples at the end of each experiment. All experiments were run in triplicate and the samples were analyzed for the lipid content, and its dissolved amount of protein and carbohydrate.

3.2.2 Alkaline pretreatment

Three types of alkaline were used in pretreating 50 g food waste sample, they are 10 M of sodium hydroxide, potassium hydroxide and calcium hydroxide. The treatment process was run at 30 ℃ and stirring at 300 rpm for 1 hr in a shaking incubator (Ecotron, Infors HT, Switzerland). A small part of sample was taken for lipid analysis and the rest of the samples were then centrifuged for 10 minutes at 15 ℃ and 3214g. FWH was separated and stored for protein and carbohydrate analyses. The best alkaline determined was used in the following studied, where the concentration of the alkaline was adjusted between 8 to 12 M and incubated with the food waste sample at 30 ℃ with a stirring speed of 300 rpm for 1 hr. With the similar stirring speed and incubation time, study was also performed using the best alkaline in its best concentration determined previously to pretreat the food waste sample at various temperature from 30 to 70 ℃. All experiments were run in duplicate with duplicate measurements and similar analyses were performed to all samples as described previously.

3.2.3 Enzymatic pretreatment

Viscozyme L was used in this study at the enzyme activity of 5 to 15 FBGU to treat 50 g of food waste sample. The reaction was performed at 40 ℃, pH 6.5 for 1 hr with stirring speed of 300 rpm in a shaking incubator. After the optimal enzyme activity was determined, it is used to determine the optimal reaction temperature from 30 to 60 ℃. Similarly, the optimal reaction time from 0.5 to 2 hr and optimal reaction pH from 4.5 up to 6.5 were determined using the optimal conditions obtained previously. Enzyme activity was stopped by immersing in the boiling water bath for 10 min before centrifugation. FWH was separated by centrifugation at 15 ℃ and 3214g for 10 minutes. All experiments were performed in duplicate with duplicate sample measurement. Lipid, carbohydrate and protein content were determined for all samples as described before.

3.3 Carbon and nitrogen composition variation

In this study, food wastes were prepared following different composition as per Table 3.1 below before pretreatment was done as per description in the Section 3.2.1 and culture using the best conditions obtained from Section 3.4.

Table 3.1: The mass ratio of food sources used

Category	Sample	Carbon Sources	Nitrogen Sources	Other Sources
1	C1-1	5	1	1
	C1-2	5	2	1
	C1-3	5	3	1
	C1-4	5	4	1
	C1-5	5	5	1
2	C2-1	3	1	1
	C2-2	4	1	1
	C2-3	5	1	1
	C2-4	6	1	1
	C2-5	7	1	1

3.4 Effect of operating conditions

3.4.1 Inoculum Preparation

The ATCC medium: 459 YGC medium was produced by mixing 1 L distilled water with 50 g/L glucose, 5 g/L yeast extract and 12.5 g/L calcium carbonate and the medium pH was set to pH 5 by adding 2 M glacial acetic acid. The medium was sterilized at a temperature of 121 ℃ for 15 minutes. 1% (v/v) glycerol stock of *Gluconacetobacter xylinus* was added into 99 mL YGC broth and was incubated statically at a temperature of 28 ℃ for 3 days (Czaja, Romanovicz, Brown and Jr, 2004).

3.4.2 Effect of Inoculum density

75 mL food waste or pineapple peel juices were used in all experiments and the experiment was run in triplicate. *Gluconacetobacter xylinus* at a concentration of 0.05 g/mL were prepared from the inoculum. Different inoculum density of 2% to 20% (v/v) was added into the fermentation medium from this preparation. The culture was then incubated statically at a temperature of 30 ℃ for 7 days (Biyik and Coban, 2011).

3.4.3 Effect of stirring speed

The best inoculum density that was able to produce the highest bacterial cellulose yield was used to study the effect of different stirring speed in the production of bacterial cellulose. The culture was incubated at a temperature of 30 ℃ for 7 days at the stirring speeds ranging from 0 rpm up to 250 rpm (Zywicka, Peitler, Rakoczy, Konopacki, Kordas and Fijalkowski, 2015).

3.4.4 Effect of Incubation pH

The best inoculum density and static condition were used in this study. Different incubation pH of 4.5, 5.0, 5.5, 6.0 and 6.5 at 30 ℃ were studied.

3.4.5 Effect of Incubation Temperature

From the above experiment, the best incubation pH was applied in this section. The experiment was repeated using different incubation temperature of 26 ℃, 28 ℃, 30 ℃, 32 ℃ and 34 ℃.

3.4.6 Purification of bacteria cellulose

The bacterial cellulose produced was filtered by using Whatman no.1 filter paper to isolate the bacterial cellulose from the fermentation medium. This was followed by several washing with distilled water. Then, the bacterial cellulose was soaked in 1 N sodium hydroxide and heated in the oven at a temperature of 80 ℃ for 15 minutes. After that, it was washed again with distilled water and soaked in 4% glacial acetic acid for 5 minutes at room temperature. When the pH of the solution became neutral, the bacterial cellulose was taken out and final rinse with distilled water.

3.5 Analyses

3.5.1 Carbohydrate determination

Phenol-sulphuric method (Dubois et al., 1956) was used to determine the amount of carbohydrate present in the food waste. In brief, 1 mL of sample (or standard) was added with 0.5 mL of 5% (w/w) phenol solution and 2.5 mL of concentrated sulphuric acid rapidly, vortexed and incubated in a water bath at 30 ℃ for 30 minutes. The absorbance was measured by UV-Vis spectrophotometer (Cary 50 Bio, Varian, Australia) at 490 nm for hexoses. For blank, distilled water was used to replace the sample. The amount of carbohydrate in the unknown sample was determined from the calibration curve with glucose as a standard.

3.5.2 Total protein amount

Protein amount was quantified by Bradford assay (Bradford, 1976). A 5 times concentrated protein reagent was prepared by dissolving 0.1 g of Coomassie Brilliant Blue G-250 in 50 mL of 95% (v/v) ethanol, mixing with 100 mL of 85% (w/v) phosphoric acid and diluting to a final volume of 200 mL with distilled water. The reagent needed to be

filtered by Whatman no.1 filter paper and stored at 4 ℃. The distilled water will be used as blank and the standard solution used was bovine serum albumin (BSA) for the standard curve. To run the assay, 100 µL of sample was added to 5 mL of 1× Bradford reagent and vortexed. After 10 min incubation at room temperature, the absorbance reading at 595 nm was measured with a cuvette against a reagent blank. The amount of protein in the unknown sample was determined by referring to the calibration curve.

3.5.3 Lipid content

The determination of lipid was done according to Bligh & Dyer method (1959). Briefly, 4 g of sample was added with the 10 mL of chloroform and 20 mL of methanol and stirred. Then, another 10 mL of chloroform was added and mixed well. This was followed by 10 mL of distilled water. After that the solution was filtered by using Whatman no.1 filter paper. The liquid part was then transferred to the graduated cylinder for phase separation (methanol phase and chloroform phase). The volume of chloroform was recorded, and the methanol phase was removed by pipette. Then, 5 mL of chloroform layer was transferred into aluminum dish. The aluminum dish was placed in a drying oven (UT 6, Heraeus, United Kingdom) at 105 ℃ for 15 minutes. Lastly, the dish was cooled in a desiccator and weighed to calculate the lipid content. To calculate the mass of lipid content in the sample:

$$W_C = W_B - W_A$$

$$Mass\ of\ lipid\ (g) = \frac{W_C \times V_{Chloroform}}{V_{Aliquot}}$$

where

W_A = Mass of aluminium dish (g)

W_B = Mass of aluminium dish and dried residue (g)

W_C = Mass of lipid in aliquot (g)

$V_{Chloroform}$ = Volume of chloroform (mL)

$V_{Aliquot}$ = Volume of aliquot (mL)

3.5.4 Elemental analysis

Elemental analysis of the food samples before and after pretreatments were performed by CHNOS analyzer (Vario Macro cube, Elementar, Germany).

3.5.5 Relative difference & percentage of composition change

Relative difference in each analysis for each sample was calculated based on the formula below.

$$Relative\ difference = \frac{amount\ of\ nutrient\ detected\ AFTER\ pretreatment\ at\ a\ specific\ condition\ (\frac{mg}{g})}{amount\ of\ nutrient\ detected\ BEFORE\ pretreatment\ (\frac{mg}{g})}$$

Elemental analysis was performed on the sample of suboptimal conditions from each pretreatment method. The percentage change in composition as compared with the control (initial) food waste was calculated as follows.

$$Percentage\ change\ in\ composition = \frac{\%\ composition\ AFTER\ pretreatment - \%\ composition\ of\ control}{\%\ composition\ of\ control} \times 100\%$$

3.5.6 Moisture Content

California Test 226 was done to determine the moisture content in the food waste. Firstly, the crucible was heated at 102 ℃ for 15 minutes and weighed. Next, 5g of food waste was put in the crucible and transferred into the oven (UF100, Memmert, Germany) at 110 ℃ for at least 16 hours. Constant mass can be achieved when less than 0.1 % of the test sample wet mass is lost during an additional exposure to the drying process. Subsequent drying periods to verify constant mass shall be of at least one hour duration. To calculate the moisture content in the food waste:

$$\text{Moisture content}, \% = \frac{(W_B - W_A) - (W_C - W_A)}{W_C - W_A} \times 100$$

Where

W_A = Mass of crucible (g)

W_B = Mass of wet sample and crucible (g)

W_C = Mass of dried sample and crucible (g)

3.5.7 Ash Content

The ash content can be measured using dry ashing procedures (Analysis of Ash and Minerals, 2003). The first step is to weigh the crucible with dried food waste from section 3.5.1. Then, transferred it into a muffle furnace (MF140, Nabertherm, Germany) with temperature of 550 ℃ for 8 hours. The food waste was weighed after ashing. To calculate the percentage of ash content in the food waste:

$$\text{Ash content}, \% = \frac{W_C - W_A}{W_B - W_A} \times 100$$

Where

W_A = Mass of crucible (g)

W_B = Mass of dried sample and crucible (g)

W_C = Mass of ashed sample and crucible (g)

3.5.8 Total Solid (TS)

The total solid was measured according to the Standard Method (2340 Hardness, 2012). Firstly, aluminum or ceramic dishes was placed into a 550 ℃ muffle furnace (MF140, Nabertherm, Germany) for one hour to dry. Then, the dishes need to be cooled in a desiccator before usage. 5g of sample was put in the dish and weighed. Sample was dried at 103 ℃ in a drying oven (UF100, Memmert, Germany) for an hour. The heating, desiccating and weighing procedures were repeated until the change in mass remained

within 4%. A duplicate sample measurement was done, and the average results should be within 5% difference. To calculate the percentage of total solid in the sample:

$$\% \text{ Total solid} = \frac{W_C - W_A}{W_B - W_A} \times 100$$

Where

W_A= Mass of evaporating dish (g)

W_B= Mass of wet sample and evaporating dish (g)

W_C= Mass of dried residue and evaporating dish (g)

3.5.9 Volatile Solid (VS)

Standard method was also used to measure volatile solid. The dry residues from the total solid analysis was transferred into a furnace. The furnace need to be heated to 550 ℃ and the residue is ignited for 30 minutes. The sample was cooled in a desiccator and weighed. The steps were repeated by igniting for 30 minutes, cooling, desiccating and weighing until the mass change is less than 4%. A dry residue from total solid testing will used for duplicate measurement and the average results should be within 5% difference. To calculate the percentage of volatile solid in the sample:

$$\% \text{ Volatile solid} = \frac{W_C - W_D}{W_B - W_A} \times 100$$

Where

W_A= Mass of evaporating dish (g)

W_B= Mass of wet sample and evaporating dish (g)

W_C= Mass of dried residue and evaporating dish (g)

W_D= Mass of residue and evaporating dish after ignition (g)

3.5.10 Determination of bacterial cellulose water holding capacity

The bacterial cellulose wet mass was measured by using an analytical balance. Bacterial cellulose was wrapped by using filter paper and centrifuged at a speed of 5000 rpm for 10 minutes. The amount of water released during the centrifugation process was recorded by measuring the weight of bacterial cellulose. The water holding capacity of bacterial cellulose is a ratio of moisture content after centrifugation to the initial moisture content. The percentage was measured by multiply the answer with 100% (Jagannath et al., 2009).

3.5.11 Determination of bacterial cellulose yield

The percentage of bacterial cellulose yield is a ratio of dried mass to the volume of the fermentation medium and multiply with 100%.

$$yield = (Dried\ mass(mg))/(initial\ volume\ of\ culture\ medium\ \ (mL))$$

where dried mass is the mass of BC after drying.

3.5.12 Determination of bacterial cellulose physical structures by FTIR test

The dried BC was analyzed to get information about functional group contained in it by using laboratory Fourier transform infrared spectrometer (IS50, Thermoscientific, USA) in the spectral range of 4000 to 400cm^{-1} (Phisalaphong & Jatupaiboon, 2008).

3.5.13 Determination of bacterial cellulose morphological structures by SEM test

Scanning electron microscopy (TM3030 plus, Hitachi, Japan) was conducted to observe BC morphology and microstructure image.

CHAPTER 4

RESULTS AND DISCUSSION

4.0 Distribution of wastes type in the food wastes sample collected

Table 4.1 shows the distribution of wastes type collected from the café. Rice, noodles or roti canai, vegetables and fruits were categorized as carbon source as further pretreatment could solubilized and broken it down to simple sugars. Egg shells, prawn shell etc. would give minerals after processing, while bone, meat & seafood contribute to the nitrogen contents.

Table 4.1: Waste types distribution in the food wastes sample collected

Wastes type	University's Café (%w/w)
Rice, mee or roti canai	52.0
Vegetables	11.0
Fruits	22.0
Egg shells, prawn shell etc.	1.0
Bone, meat & seafood	14.0

4.1 Study of different pretreatment methods

4.1.1 Hydrothermal pretreatment

Thermal pre-treatment is considered as a promising method to improve the food waste properties because it provides enhancement to the solubilization of a complex particulate organic substrate (Wang et al., 2010). Figure 4.1(a) shows the results obtained when the food waste was mixed with different water ratio and heated at 80 ℃ for 1 hr. It can be seen that with water added, more nutrients will be solubilized. Thermal pre-

treatment will increase the rate of degradation and surface area of the substrate by broken down complex cellulose, hemicellulose or lignin causes more carbohydrate can be accessed and dissolved (Perez-Pimienta et al., 2017). Water will then act as a solvent to dissolve soluble carbohydrate from the food waste. Similarly, water plays an important role for dissolution of protein from the food waste. Water molecules interact with the surface of protein that was detached from its complex counterpart, reorienting both of themselves and the surface groups of the proteins, whereas other water molecules link these to the bulk in an ordered manner. Thus, the solubilized proteins would possess a more stable conformational flexibility at this state. As a result, increment of food waste to water ratio (in volume basis) increased the carbohydrate and protein detected in the solution. However, at a food to water ratio above 1:2, insignificant increment of both carbohydrate and protein solubilization were observed (Figure 4.1(a)). This is due to additional amount of water will only dilute the nutrients dissolved and will not further dissolved those nutrients that are still trapped in the food wastes. On the other hand, lipid content was reduced at the food to water ratio above 1:2 as depicted in Figure 4.1(a). This is possibly due to the separation of lipid from the solid into the liquid parts when a big amount of water was added; thus, a dilution effect. Karki et al. (2015) have suggested that the ratio between solids and water should be in the range of 1:1 to 1:2 for a proper solubilization of organic materials. Since this is in agreement with the results, food waste to water ratio of 1:2 has been chosen in the following experiments.

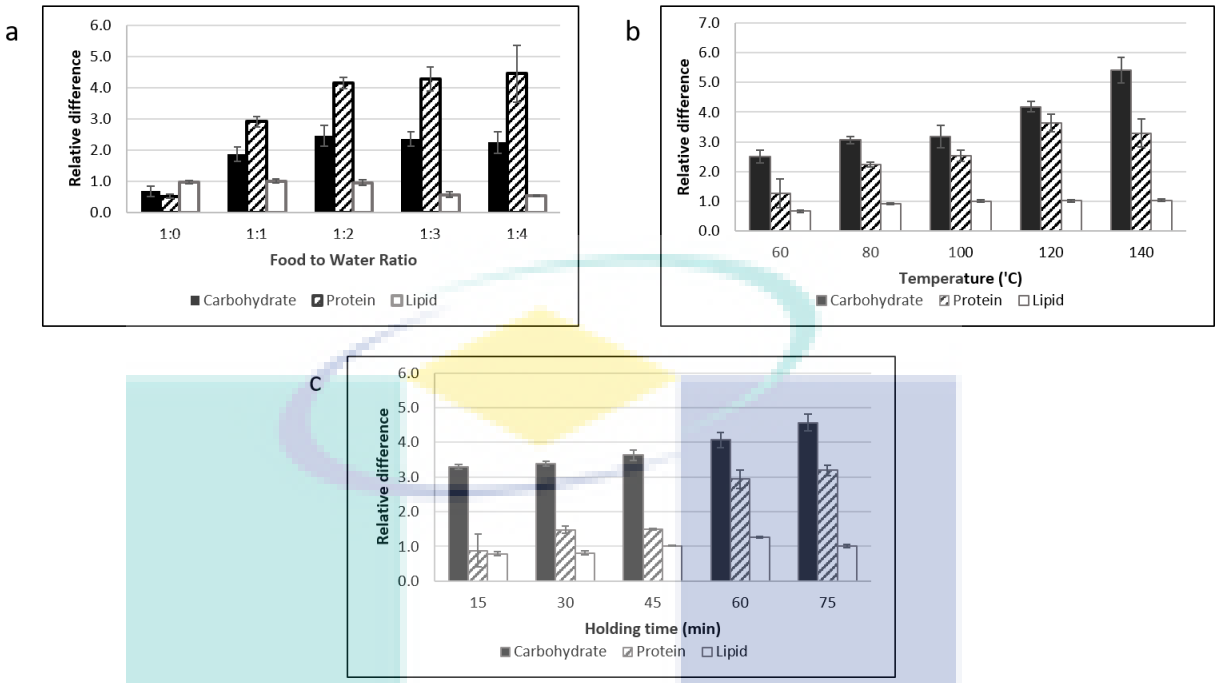


Figure 4.1: Relative difference of the nutrients obtained during hydrothermal pretreatment at different (a) food to water ratio; (b) temperature; and (c) holding time.

A low temperature treatment region was selected in this study based on the finding of Li et al. (2016), where low nutrient loss was observed when the kitchen waste was treated at a low temperature of 55-120 ℃ than that of the high temperature range. This phenomenon was observed for the protein as in Figure 4.1(b) of this study. Generally, all nutrients content increase when the temperature was increased from 60 to 140 ℃. Though carbohydrate content doubled at this temperature range, protein content showed a slight decrease when temperature of treatment exceeds 120 ℃. Lipid content, on the other hand, shows no obvious changes above 100 ℃. According to Liu et al. (2012), thermal pretreatment can disrupt the complex structure of food waste and release intracellular compounds into the soluble phase. Nevertheless, the amount of protein was decreased at 140 ℃ in this study, which may be due to denaturation and degradation of protein at higher temperature. In addition, the decrement may be attributable to the formation of Amadori compounds or melanoidins, a product between the reaction of soluble carbohydrate and soluble protein, at temperature exceeded 120 ℃, which is undesired (Li and Jin, 2015). Carbohydrate is the main source of energy for living organisms. In

fermentation, microbes required energy source sorted from their environment to produce adenosine triphosphate (ATP). It is required for biosynthesis process where bacteria use for their maintenance and reproduction. Besides, protein as the source of nitrogen also plays an important role for the growth and metabolism of microbes (Paritosh et al., 2017). Microbes use nitrogen to build their cell walls and produce enzyme for the use of fermentation. Thus, amount of carbohydrate and protein are needed to be considered rather than lipid. Therefore, the optimal duration was examined next using 120 ℃.

Figure 1(c) shows the relative difference of all nutrients at different hydrothermal holding time. Both soluble carbohydrate and soluble protein were increased with increasing holding time. About 4.5-fold increment of carbohydrate content and 3.2-fold increment of protein were observed at 75 min of hydrothermal treatment. Lipid content shows insignificant changes throughout the change in holding time. When the holding time is increased, more time for the heat energy to transfer into the internal part of the food wastes and thoroughly broken down the bonds to make available nutrients. Carbohydrates are commonly in the form of polysaccharides and crude fibre (Liu et al., 2012). With increasing of thermal pretreatment period, the carbohydrate in the food waste splits into short-chain fragments that are better suited for biological digestion by microorganisms (Scheidat et al., 1999). Likewise, more proteins are released from the protein complex and dissolved into the solution. Therefore, increasing amount of these nutrients are detected during longer period of hydrothermal pretreatment. Since changes of protein content at 60 and 75 min holding time has no substantial difference, 75 min was taken as the optimal treatment time.

In short, hydrothermal pretreatment of food waste at a food waste to water ratio of 1:2 for 75 min at 120 ℃ would increase the carbohydrate and protein contents of a FWH for 4.5- and 3.2-folds, respectively.

4.1.2 Alkaline pretreatment

According to Salihu and Alam (2016), chemical pretreatment is an easy pretreatment method which requires no or minimal energy demand. They are effective in breaking down the organic constituents. Alkaline pretreatment was chosen here based on its high efficiency and easy operation. Common alkaline such as sodium hydroxide, potassium hydroxide and calcium hydroxide have been chosen in this study. Figure 4.2(a) shows the results of using 10 M of these alkaline in treating the food waste at 30 ℃ for 1 hr and shaking at 300 rpm. It can be seen that sodium hydroxide is good in extracting protein from the food waste (1.4-fold increment), while potassium hydroxide is good in extracting carbohydrate (about 1.8-fold increment). These are in line with most of the research involved protein solubilization, where the alkaline used is sodium hydroxide (Salihu and Alam, 2016; Ghaly et al., 2013; Ovissipour et al., 2012). On the other hand, treatment of wheat straw was found to be better using potassium hydroxide (Shen et al., 2019) in breaking down lignin and cellulose into monosaccharide before anaerobic digestion. Thus, in agreement with current study.

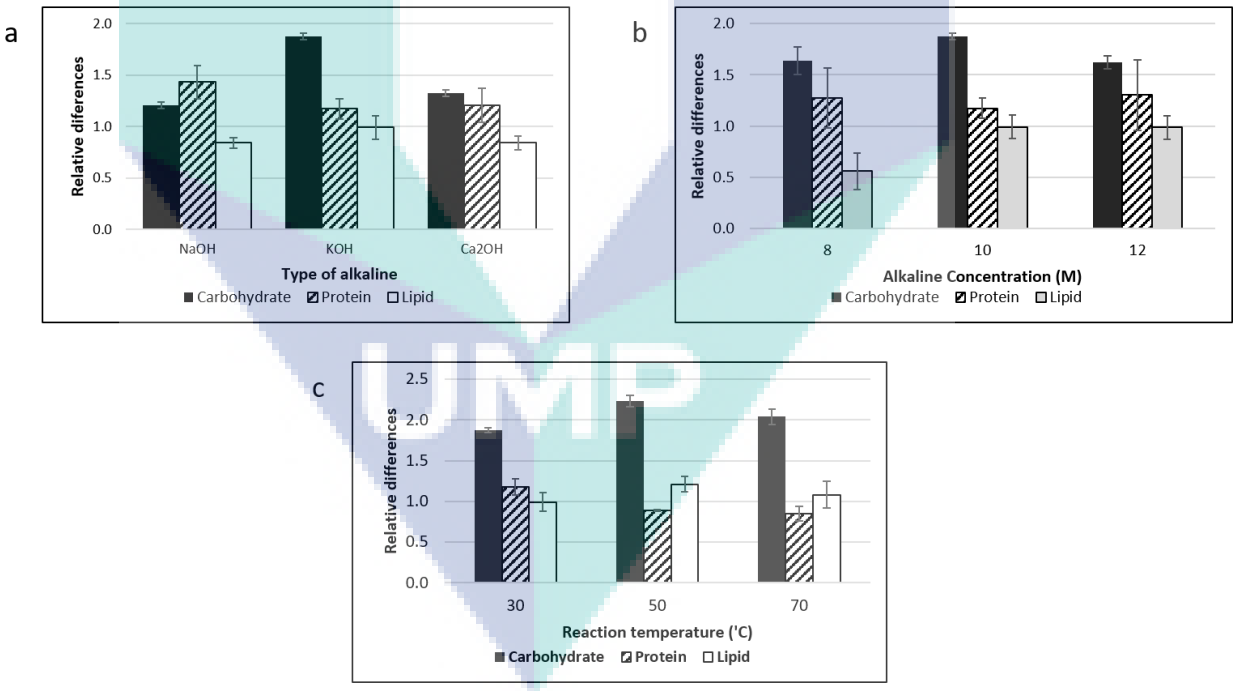


Figure 4.2: Relative difference of the nutrients obtained during alkaline pretreatment at different (a) type of alkaline; (b) alkaline concentration, and (c) reaction temperature.

Based on the results obtained above, potassium hydroxide has been chosen to be the alkaline used for the food waste pretreatment. This is because the aim of this study is to obtain FWH as the potential replacement of fermentation medium, which focuses more on the carbon source. As such, potassium hydroxide was chosen instead of sodium hydroxide. Potassium hydroxide was then adjusted for its molarity within the range of 8 to 12 M and added into food waste, shaking at 300 rpm and 30 ℃ for 1 hr. Figure 4.2(b) depicts that no significant change in the protein content at the end of the pretreatment when the concentration was changed from 8 to 12 M, while the carbohydrate content was the highest at 10 M (1.8-fold as compared to the control). Therefore, 10 M potassium hydroxide is kind of optimal for this pretreatment.

Following this, pretreatment temperature was varied from 30 to 70 ℃ using 10 M potassium hydroxide and mixing speed of 300 rpm for 1 hr. Temperature of 50 ℃ has enhanced the carbohydrate solubilization and even lipid extraction. Nevertheless, protein solubilization seems to reduce, which may cause by the cleavage of large water-soluble protein into peptide fractions at high temperature and alkaline condition (Kristinsson and Rasco, 2000) as can be seen from the relative differences that was below 1.0 in Figure 4.2(c).

In brief, about 2.2-fold carbohydrate content in FWH could be obtained if alkaline pretreatment of food waste were run at 50 ℃ for 1 hr using 10 M potassium hydroxide mixing at 300 rpm. Nonetheless, protein content would decrease to 0.9-fold as compared to the control at this operating conditions.

4.1.3 Enzymatic pretreatment

Enzymatic pretreatment is a mild process that is less energy intensive, which can be carried out at low temperature without addition of any harsh chemicals. In this study, Viscozyme® L, producing by *Aspergillus aculeatus*, was used as the enzyme in treating the food wastes. It is a multi-enzyme complex which containing a wide range of carbohydrases. Figure 4.3(a) shows the changes of nutrients obtained in FWH when

enzyme amount was changed from 5 to 15 FPG at the reaction temperature of 40 ℃ for 1 hr at pH of 6.5. It can be seen that when 10 FPG of enzyme was used in the pretreatment, carbohydrate, protein and lipid content were increased 4.2-fold, 3.6-fold and 1.5-fold, respectively. To our surprise, when the amount of enzyme was increased to 15 FPG, all nutrients content in the FWH were decreased. The reason behind is not known and worth further investigation.

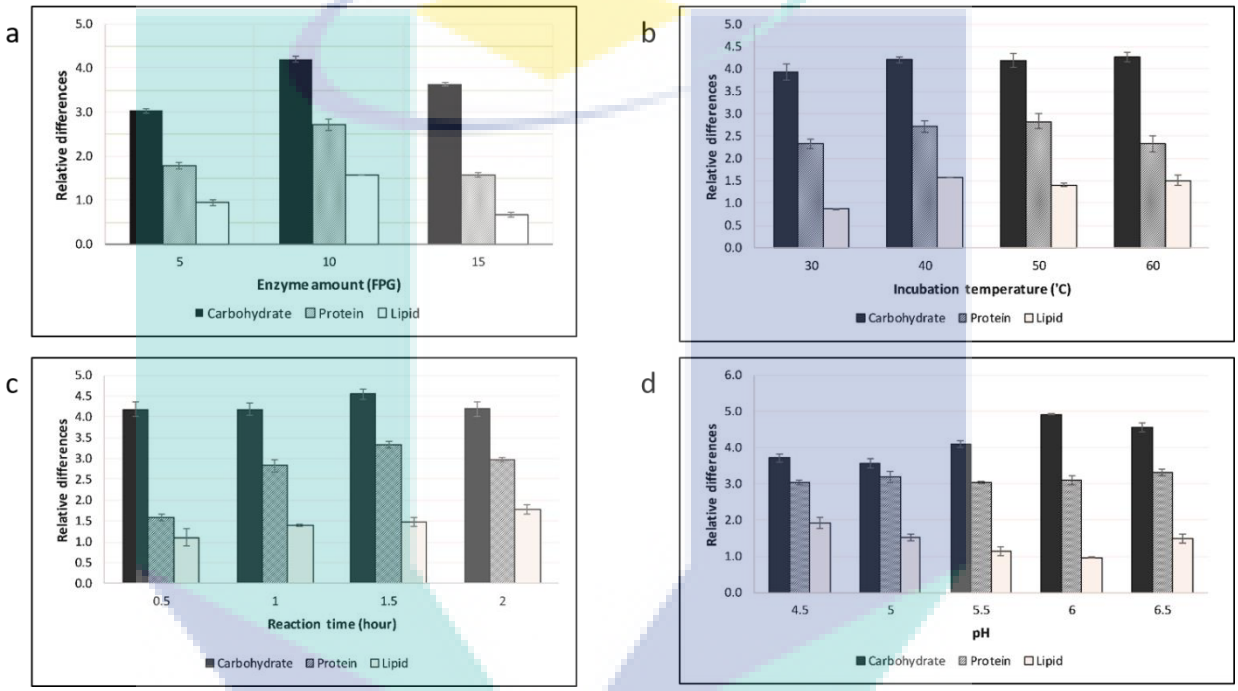


Figure 4.3: Relative difference of the nutrients obtained during enzymatic pretreatment at different (a) enzyme amount; (b) incubation temperature; (c) reaction time; and (d) pH.

Reaction temperature was examined next between 30 to 60 ℃ by using 10 FPG of Viscozyme® L, incubating for 1 hr at pH 6.5 together with the food wastes. As shown in Figure 4.3(b), increasing reaction temperature from 30 to 40 ℃ would increase the nutrients solubilized. Above 40 ℃, the changes are insignificant for carbohydrate and lipid. However, protein was slightly higher at 50 ℃ and decreased above that. Decrement of protein content above 60 ℃ may be due to the denaturation at high temperature as some

proteins are highly temperature sensitive. It can be concluded from this result that Viscozyme® L has a wide reaction temperature range from 40 to 60 ℃.

The optimal incubation time was determined subsequently from 0.5 to 2 hrs using 10 FPG enzyme at pH 6.5 and incubated at 50 ℃. Reaction time of 1.5 hr was found to be the best where carbohydrate and protein contents solubilized in the FWH were the highest, 4.5- and 3.3-folds respectively (Figure 4.3(c)). Amount of nutrients detected in the FWH increased with incubation time because greater amount of macromolecules could be hydrolyzed into simple molecules at longer reaction time. Reaction time longer than 1.5 hr causing decrement in both the nutrients.

To obtain the most effective pH for Viscozyme® L reaction, the reaction pH was varied from pH 4.5 to 6.5 at 50 ℃ with an enzyme concentration of 10 FPG and reacted for 1.5 hr. Figure 4.3(d) shows that reaction at pH 6 hydrolyzed the most carbohydrate from food waste into FWH as compared to the control (~ 4.9-fold). Above or lower this pH value, the amount of carbohydrate detected in the FWH was lower. pH seems to give no effect to the hydrolysis of protein. On the other hand, lipid content detected was the lowest in FWH at pH 6.

Briefly, the optimal operating conditions for enzymatic pretreatment of food waste using Viscozyme® L are 10 FPG react with 50 g food waste at 50 ℃, pH 6 for 1.5 hr at 300 rpm. The carbohydrate content solubilized was 4.9-fold, while the protein content solubilized was 3.1-fold as compared to control.

4.1.4 Comparison between pretreatment methods

Figure 4.4 shows the comparison between the elemental compositions of the FWH using different pretreatment methods. It can be seen that all elements are reduced in composition when alkaline pretreatment was used. Hydrothermal pretreatment of food waste greatly increased the carbon content of the FWH and a small increment of nitrogen content was also observed. Similarly, enzymatic pretreatment also increased both the

carbon and nitrogen content of the FWH. In contrast, hydrogen composition was decreased after all pretreatments were performed.

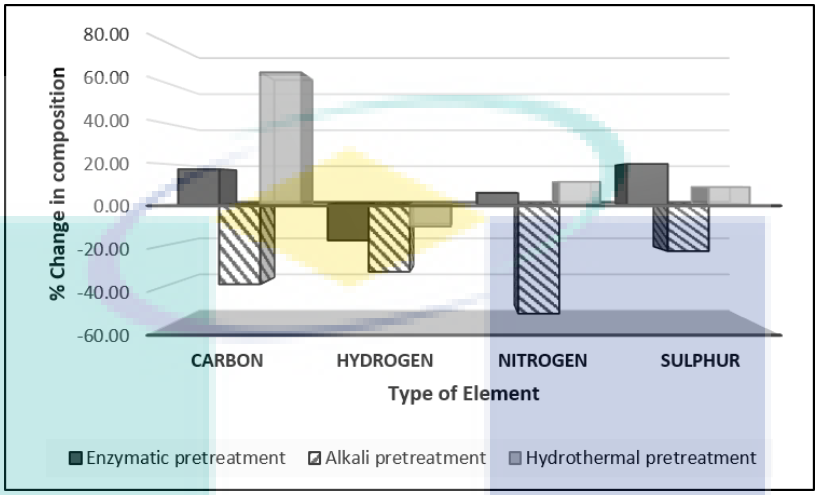


Figure 4.4: Percentage change in the composition of the elements after different pretreatment methods analyzed with CHNS analyzer.

Using phenol-sulphuric assay, Bradford assay and Bligh and Dyer method, on the other hand, showed quite a similar outcome obtained from the elemental analysis. As shown in Figure 4.5, enzymatic pretreatment resulted in the highest amount of carbohydrate solubilized from the food waste as compared to hydrothermal and alkaline pretreatments. Nevertheless, the performance of hydrolyzing protein is similar in both hydrothermal and enzymatic pretreatment methods. Conversely, alkaline pretreatment would reduce the amount of protein solubilized in FWH and release certain amount of lipid into the FWH. As reported by Ovissipour et al. (2012), alkaline hydrolysis normally recover lower amount of protein as compared to other hydrolysis methods. This is owing to substrate properties that are believed to affect protein recovery using chemical method. In general, alkaline hydrolysis of protein yield products with poor functionality and reduced nutritional qualities as the process is difficult to control and leads to variable chemical composition and functional properties (Kristinsson and Rasco, 2000).

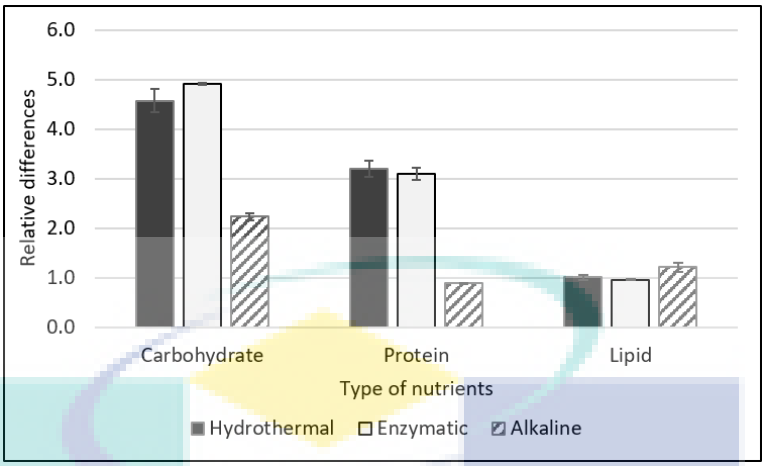


Figure 4.5: Relative differences for the amount of nutrients obtained with different pretreatment methods.

4.2 Effect of carbon and nitrogen sources variation

The food waste collected were used to examine for 10 samples. The mass of food waste collected for each sample is 300g and was blended with water with ratio of 1:2. The ratio of carbon (C) sources, nitrogen (N) sources and others in each sample before blending are summarized in Table 4.2. The sample in category 1 was with different nitrogen sources ratio but same ratio in carbon sources and other sources; whereas for category 2 was with different carbon sources ratio but same ratio in nitrogen sources and other sources. Theses samples were subjected to hydrothermal pretreatment and the food wastes hydrolysate produced was used as the fermentation medium.

Table 4.2: The mass ratio of food sources used

Category	Sample	Carbon Sources	Nitrogen Sources	Other Sources
1	C1-1	5	1	1
	C1-2	5	2	1
	C1-3	5	3	1
	C1-4	5	4	1
	C1-5	5	5	1
2	C2-1	3	1	1
	C2-2	4	1	1

	C2-3	5	1	1
	C2-4	6	1	1
	C2-5	7	1	1

4.2.1 Moisture Content

The moisture content of each sample is summarized in Table 4.3. The typical value for moisture content of food waste was 70% (Peavy, Rowe, & Tchobanoglous, 1985). Also, the moisture content of food waste ranges from 74% to 90% depending on its composition (Zhang et al., 2007). The moisture content of the food waste collected is between the range of 70.10% and 76.84% which were within the typical range. The higher the moisture content, the more effective the microbial growth (Chen, Hsu, & Wang, 2018). Hence, the food waste was suitable to use for bacterial cellulose production.

Table 4.3: Moisture content of each sample

Sample	Moisture Content (%)
C1-1	76.84 ± 0.06
C1-2	72.45 ± 0.14
C1-3	76.24 ± 1.22
C1-4	72.73 ± 1.96
C1-5	70.10 ± 0.18
C2-1	77.32 ± 1.09
C2-2	75.00 ± 0.51
C2-3	76.29 ± 0.45
C2-4	74.19 ± 1.38
C2-5	73.27 ± 2.81

4.2.2 Ash Content

Analysis of ash content helps determine the amount of minerals in food. It is important because the high amount of minerals can retard the growth of microorganisms (Arizona, 2010). The ash content of each sample is summarized in Table 4.4. Based on the research, the typical value of ash content in food waste is 5% (Peavy, Rowe, & Tchobanoglous, 1985). The ash content of the food waste collected was between the range of 21.48% and 26.74%, which was higher than typical value. The higher ash content may

slow down the microbial growth. The difference of ash content might be due to the sample obtained from different geographical origins and different composition.

Table 4.4: Ash content of each sample

Sample	Ash Content (%)
C1-1	23.96 ± 0.21
C1-2	25.69 ± 0.70
C1-3	24.46 ± 2.02
C1-4	26.74 ± 2.47
C1-5	25.09 ± 0.48
C2-1	21.48 ± 1.14
C2-2	22.29 ± 0.45
C2-3	23.16 ± 1.00
C2-4	21.89 ± 0.14
C2-5	22.06 ± 0.05

4.2.3 Total Solid and Volatile Solid

The total solid (TS) also known as the removal of the moisture content. Although the moisture content can be useful for some applications but it is a variable component of the sample that changes with time, storage or manipulation (Peces, Astals, & Mata-Alvarez, 2014). In contrast, TS remains constant and can be used for the correcting or normalizing other characteristic. On the other hand, the determination of volatile solid (VS) can give an estimation of the organic matter present in the waste (Peces et al., 2014).

The previous study that demonstrated the effect of TS content on microbial fermentation reported that the optimum TS content was 28% (Motte et al., 2013). Lower value of VS than TS can give better performances. (Yi, Dong, Jin, & Dai, 2014). The total solid and volatile solid of each sample are summarized in Table 4.5. The TS content was between the ranges of 24.20% and 30.14% which indicated that the food waste collected can be used for fermentation. Meanwhile, the VS content was between the ranges of 23.86% and 29.71% which were lower than TS.

Table 4.5: Total solid and volatile solid of each sample

Sample	Total Solid (%)	Volatile Solid (%)
C1-1	26.48 ± 0.07	26.62 ± 0.02
C1-2	29.05 ±0.42	28.53 ± 0.35
C1-3	27.55 ± 1.41	27.17 ± 1.21
C1-4	30.13 ± 2.64	29.70 ± 1.10
C1-5	28.32 ± 1.98	27.88 ± 1.89
C2-1	24.20 ± 0.18	23.86 ± 0.27
C2-2	25.29 ± 2.03	24.77 ± 1.01
C2-3	26.15 ± 0.76	25.73 ± 0.39
C2-4	24.77 ± 1.30	24.32 ± 0.86
C2-5	27.13 ± 0.19	24.51 ± 1.22

4.2.4 Carbohydrate

Carbohydrates are the most readily utilizable form of carbon sources (Staples, 2018). The carbohydrate content increased with the increased of carbon sources in the food waste. The carbohydrate content in each sample was illustrated in Figure 4.6 and Figure 4.7. Based on Figure 4.6, it showed that the carbohydrate content in category 1 was decreasing from C1-1 to C1-5, which was from 346.5945 mg C/g FW to 341.0930 mg C/g FW. The sample with highest ratio of carbon sources in category 1 had the highest carbon carbohydrate content which was 346.5945 mg C/g FW.

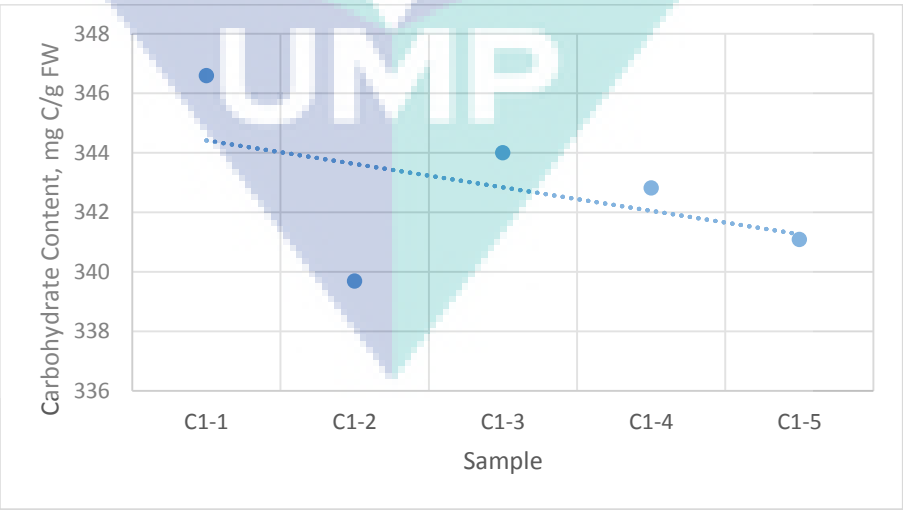


Figure 4.6: Carbohydrate content of each sample in category 1

From Figure 4.7, in category 2, the carbohydrate content was increasing from C2-1 to C2-5, which was from 341.0930 mg C/g FW to 379.0641 mg C/g FW. The sample with highest carbohydrate content of 379.0641 mg C/g FW was C2-5 which had the highest carbon sources ratio compared to other samples in category 2.

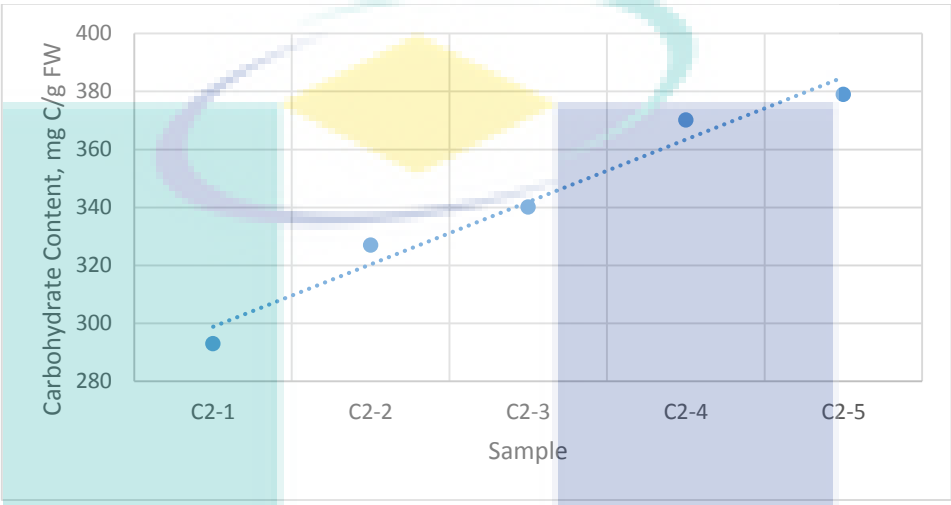


Figure 4.7: Carbohydrate content of each sample in category 2

Based on the results obtained, the carbohydrate content in each sample increased when the ratio of carbon sources increased. This indicated there were different amount of carbon sources in each sample for the production of bacterial cellulose.

4.2.5 Protein Content

Protein naturally contains nitrogen. A good measurement of nitrogen in food or food group is the determination of protein content (Litherland, 2018). The protein content in each sample was illustrated in Figure 4.8 and Figure 4.9. Based on Figure 4.8, it showed that the protein content in category 1 was increasing from C1-1 to C1-5, which was from 49.2810 mg N/g FW to 65.4387 mg N/g FW. The sample with highest mass ratio of nitrogen sources in category 1 had the highest protein content which was 65.4387 mg N/g FW.

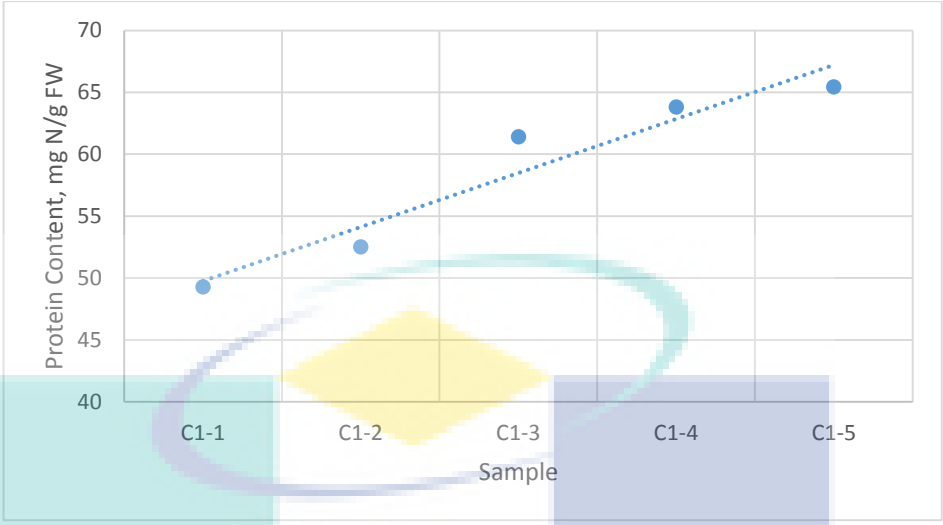


Figure 4.8: Protein content of each sample in category 1

From Figure 4.9, in category 2, the protein content was decreasing from C2-1 to C2-5, which was from 66.2466 mg N/g FW to 7.2710 mg N/g FW. The sample with highest carbohydrate content of 66.2466 mg N/g FW was C2-1 which had the highest nitrogen sources mass ratio compared to other samples in category 2. Based on the results, the protein content in each sample increased when the mass ratio of nitrogen sources increased. This indicated there were different amount of nitrogen sources in each sample for the production of bacterial cellulose.

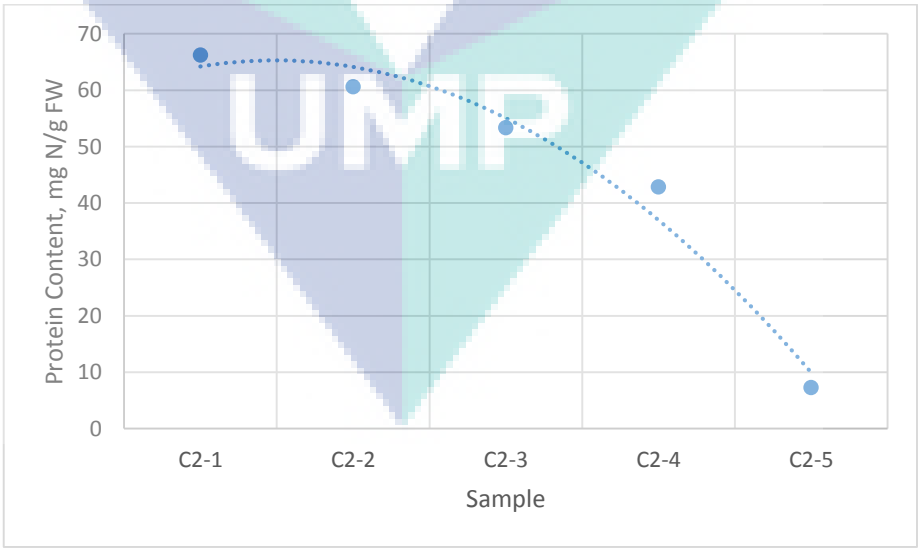


Figure 4.9: Protein content of sample in category 2

Acetobacter xylinum is an organism that requires carbon and nitrogen. However, high amount of carbon concentration could inhibit cell growth and cellulose production due to the accumulation of keto that lead to lower pH, a suitable ratio of C:N needed for optimum production (Vandamme et al., 1998). In other words, if more carbon were to be applied resulting in a high C:N ratio, nitrogen may become a growth limiting factor (Kadir et al., 2013). Therefore, growth can be increased by higher supply of carbon source only within limits, depending on nitrogen being sufficient.

4.3 Effect of operating conditions

4.3.1 Inoculum density

By referring to Figure 4.10, the graph shows the yield of bacterial cellulose production from food waste hydrolysate at different inoculum density in a static culture. The yield of the food waste bacterial cellulose is increasing with the increasing of inoculum density up to 10%, it then decreased with the increasing of inoculum density. This means inoculum density of 10% is an optimum to produce a high yield of bacterial cellulose by using food waste hydrolysate as a medium. *G. xylinus* cells that were inoculated into the fermentation medium will consume all the nutrients provided by the medium when supported by good aeration to produce cellulose. The high amount of *G. xylinus* in the fermentation medium will contribute to the high yield of cellulose production. This is due to the high amount of cells that are involved in the production of cellulose, thus increases the amount produced and the yield obtained.

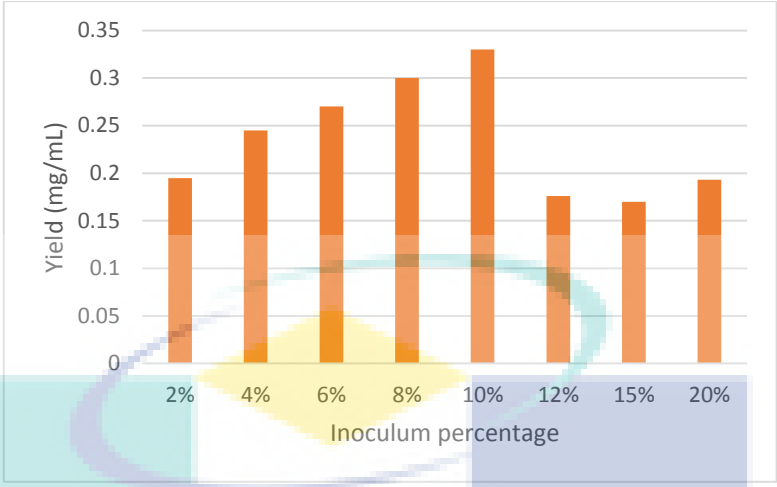


Figure 4.10: The yield of food waste BC against inoculum density

At 12% to 20% of inoculum density, the yield of bacterial cellulose has no significant changes. A very high amount of *G. xylinus* cells will consume quickly the nutrients provided by the substrate. All the nutrients were completely consumed before the end of the fermentation period. To continue living, *G. xylinus* cells will degrade the cellulose they produced to get the required carbon source. To be clear, the structure of cellulose molecules is a repeating chain of ß-D-glucopyranose with 1,4-glycosidic linkages, cellulose is first converted to cellobiose by enzyme cellulose and then split into glucose by enzyme cellobiase, and finally assimilated by *G. xylinus* (Grassmann, 1993). When the fermentation period is extended, the cellulose will completely degrade and *G. xylinus* species will die because they no longer have carbon and nitrogen sources in the substrate. However, the result of present study was contradicted with the previous study. Omojasola and others (2008) investigated the effect of 1% to 10% inoculum size on the production of BC by using pineapple peel medium. Fungi *Trichoderma longibrachiatum* species in 3% concentration of pineapple peel media, had produced optimal BC with 10% inoculum size at 7 days fermentation period. Fungi *Aspergillus niger* species were producing optimal BC in 2% concentration of substrate, with 6% inoculum size at 5 days fermentation period while at days 3, an optimal BC production was produced from fermentation of fungi *Saccharomyces cerevisiae* species in 3% concentration of pineapple peel media with 6% inoculum size. On the other hand, fermentation of *G. xylinus* in Hestrin-Schramm media produced an optimal BC with 5% inoculum size at days 7

(Rangaswamy, 2015). It proves that, the optimal BC produced was dependent on the bacteria or fungi species involved, the media prepared, the nutrients content and the fermentation period.

Figure 4.11 shows the moisture content of food waste bacterial cellulose at different inoculum density. The moisture content of food waste bacterial cellulose is well above 98% for all inoculum density tested. The moisture content for pineapple peel bacterial cellulose is slightly decreased with the increasing of inoculum density from 2% to 10%, but the change is not significant. The moisture content was maintained above 95% at the inoculum density greater than 10%. Moisture content is the amount of water contained in cellulose. Cellulose is a prominent scaffolding polysaccharide found in plants as microfibrils and the bound water is associated with the surface of the cellulose. When the surface of the cellulose is clear and clean, the moisture content will increase because of the water is easily bound with microfibrils through the narrow spaces in the cellulose (Chaplin, 2018).

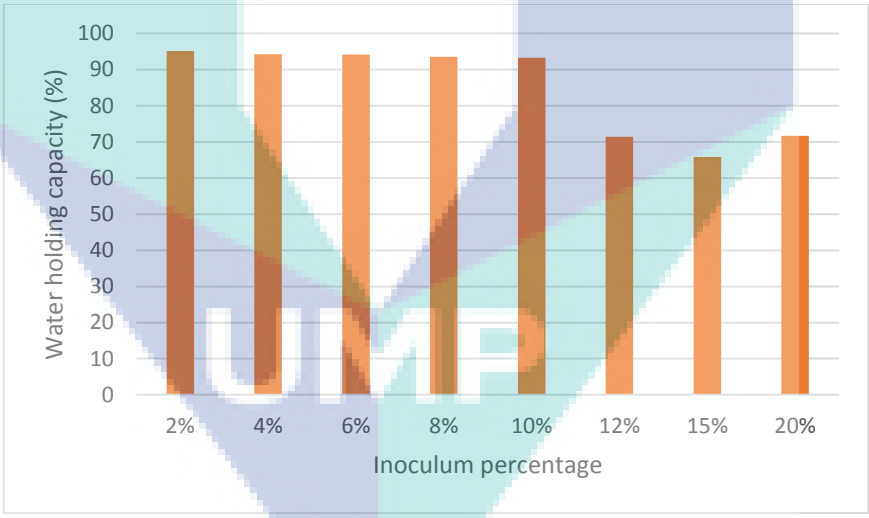


Figure 4.11: The moisture content of food waste BC against inoculum density

Water holding capacity is the tendency of the cellulose to absorb water and it is relating with the surface area and porosity of BC. The bigger surface area of BC will have larger pore size (Guo and Catchmark, 2012). The water holding capacity of food waste cellulose is ranging from 71% to 96% (Figure 4.12). At lower inoculum density (≤10%), the percentage of water holding capacity was higher and at higher inoculum density, the

percentage of water holding capacity was low. It is because when the yield of BC was low, the fibrils were arranged as loose as possible and emptier space occur between them. Therefore, it is able to absorb more water compare to the BC with high yield because of the pack arrangement of microfibrils with low number of pores (UI-Islam, Khan, Park, 2012). Investigation have been done in this particular scope by Gunduz and others (2018), BC with high yield has higher water holding capacity and BC with high yield, have higher water holding capacity.

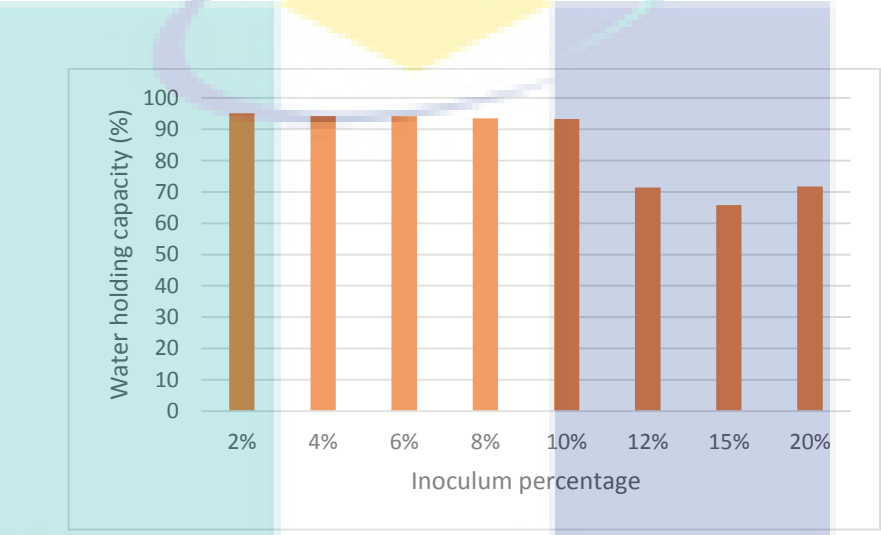


Figure 4.12: The water holding capacity of food waste BC against inoculum density

4.3.2 Static and stirred culture

Shaken culture give a good aeration to supply enough oxygen to the *G. xylinus* species to grow and synthesis the microfibrils. The increasing of the shaken speed will provide a good aeration and increase the oxygen dissolved in the fermentation medium. The presence of oxygen and glucose promoting bacteria to synthesize cellulose (Zahan et al., 2015). *G. xylinus* species able to swim freely in the medium and consume enough nutrients. Bacterial cellulose produce in an agitated culture was irregular in shape. At lower shaken speed, the aeration is not good, similar to that of the static culture. *G. xylinus* species gather at the surface of the medium to get enough oxygen and produce bacterial cellulose with regular shape followed the shape of conical flask. The increasing of the cellulose's thickness was blocking the aeration, making the *G. xylinus* species move to the surface of the medium and let the cellulose drop to the bottom of the flask. New cellulose

was produced, make it becomes a twin layers. In shaken culture, yield varied with different stirring speed. By referring to Figure 4.13, the optimum stirring speed was at 200 rpm, where 2.0599 mg/ml food waste bacterial cellulose was produced at 7 days fermentation period. The increasing of stirring speed will increase the yield of bacterial cellulose up to optimum speed, thereafter the yield was reduced. BC produced from agitated culture can be in the formed of fibrous suspensions, pellets and irregular shape (Watanabe et al., 1998). Zywicka (2015) reported that BC with pellicles shape was produced at the speed of 100-150 rpm, while irregular shape was formed at a speed of 300 rpm in Herstin-Schramm medium. According to Chawla (2009) and Krystynowicz et al. (2002), the optimal rotation speed of rotating discs in fermenter was 15 rpm and 4 rpm respectively. However, the optimal speed was found at 140-150 rpm by using orbital shaker (Attariansah, 2003; Zywicka, 2015). The different equipment uses will give different result at particular speed.

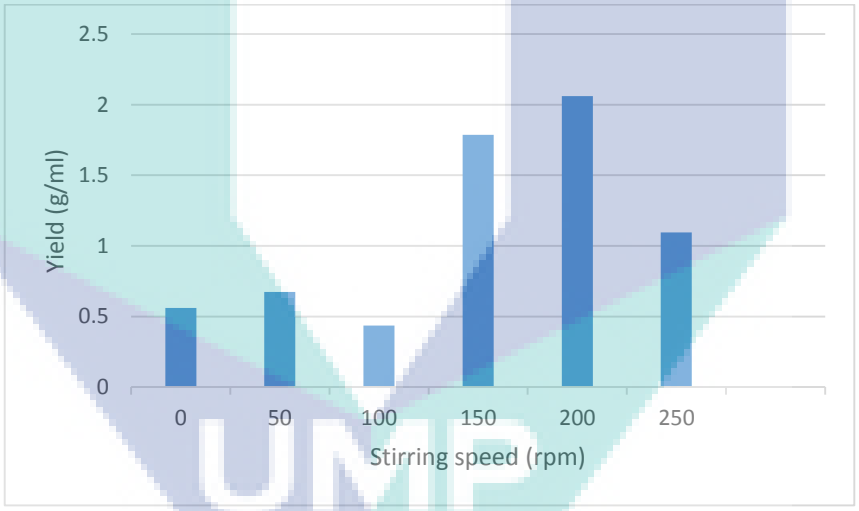


Figure 4.13: The yield of food waste BC at different stirring speed

The concentration of inoculum and good aeration at high speed has enhanced the production of cellulose. At higher speed of 250 rpm, the microfibrils synthesis is fast and nutrients contained in the medium was completely consumed in a short period. However, the yield was reduced because of the cellulose degradation that occurred.

Figure 4.14 shows that the speed of shaking didn't affect the amount of moisture content in the BC. In shaking culture, microfibril forms in a shorter length and the pellicle form has a structure like cotton which able to absorb more water.

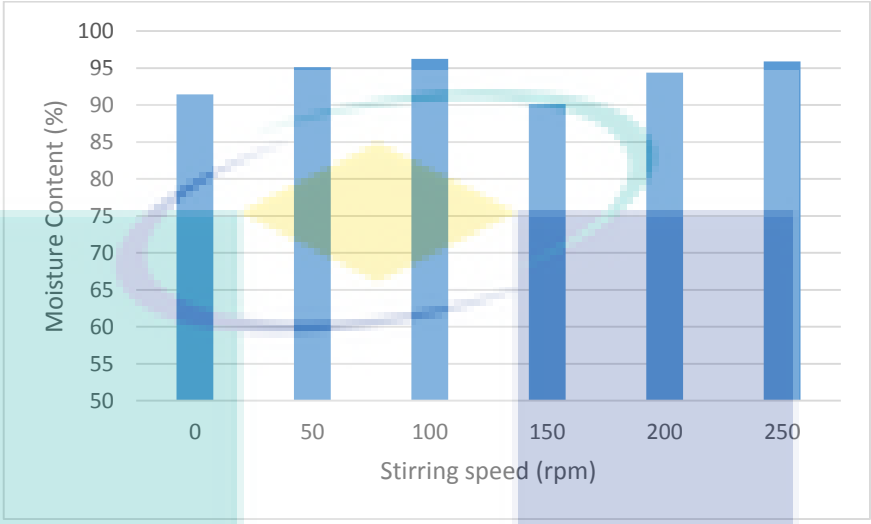


Figure 4.14: The moisture content of food waste BC at different stirring speed

The water holding capacity of food waste bacterial cellulose is shown in Figure 4.15. It can be seen that stirring speed do not have significant effect on the water holding capacity of the BC. Nevertheless, stirring has increased the water holding capacity for the BC obtained from both media. Similar to the moisture content that BC can withhold, it is due to the short microfibril of BC formed that increase its space between the structures.

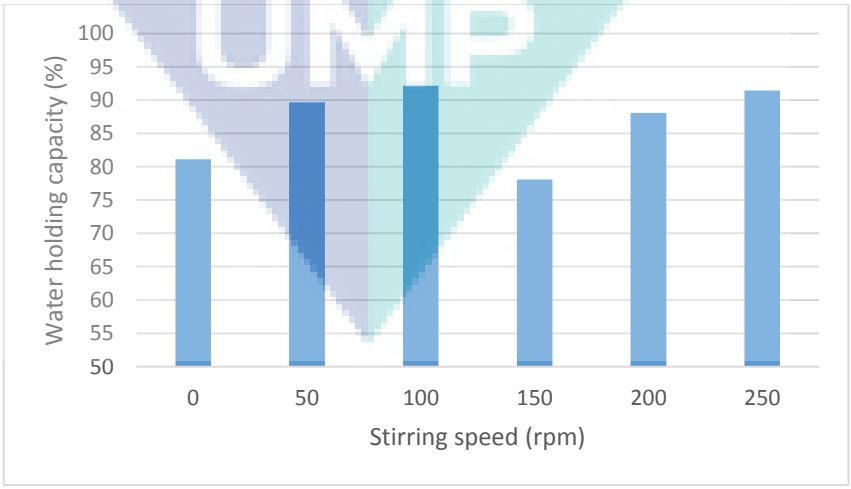


Figure 4.15: The water holding capacity of food waste BC against stirring speed

4.3.3 pH effect

The relationship between the yield of BC against pH is shown in Figure 4.16. At pH 5.5 the maximum yield of BC was obtained. At pH above 5.5, there were no significant change in the results as the data obtained at pH 6.0 and 6.5 was approximately close to each other. Based on study from Chawla et al. (2009), the optimum yield for BC production is in the range of 5.0 to 6.0 and the yield will decrease below pH of 4.0. The pH will decrease throughout the fermentation process due to the accumulation of gluconic acid and acetic acid in the medium (Kongruang, 2007). This decrease lowers the suboptimal levels for the cell viability and synthesis of cellulose (Klemm et al., 2005).

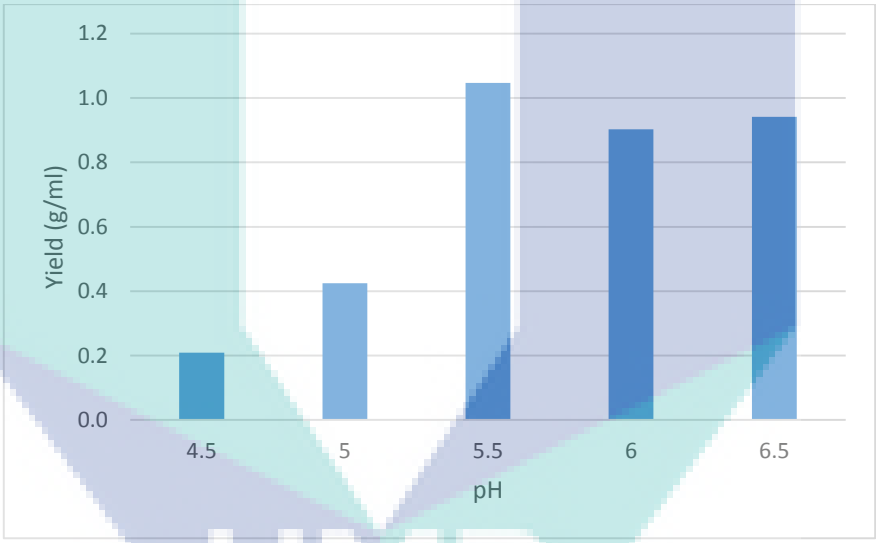


Figure 4.16: The yield of BC against pH

Figure 4.17 shows the relationship of WHC with pH. The porosity and surface area of each BC can be related with the difference between the WHC obtained. A greater surface area and pore size will result in a larger value of WHC as a larger amount of water can penetrate and trapped in the BC matrix. Based on Figure 4.17, it can be concluded that the changes in pH has no significant effect on the BC produced. However, this observation contradicts to the study from Yunoki et al. (2007) who observed a rapid decrease in water content and ability of the BC to absorb water after 11 days due to increase in density of BC and secretion of fibril networks. The amount of water that escapes from the matrix of

BC to the environment relies on the cellulose microfibrils arrangement. The closer the arrangement, the more the WHC of the BC. This is because, closely packed microfibrils tend to bind the water molecules more efficiently due to presence of stronger hydrogen bonds interactions compared to loosely packed microfibrils.

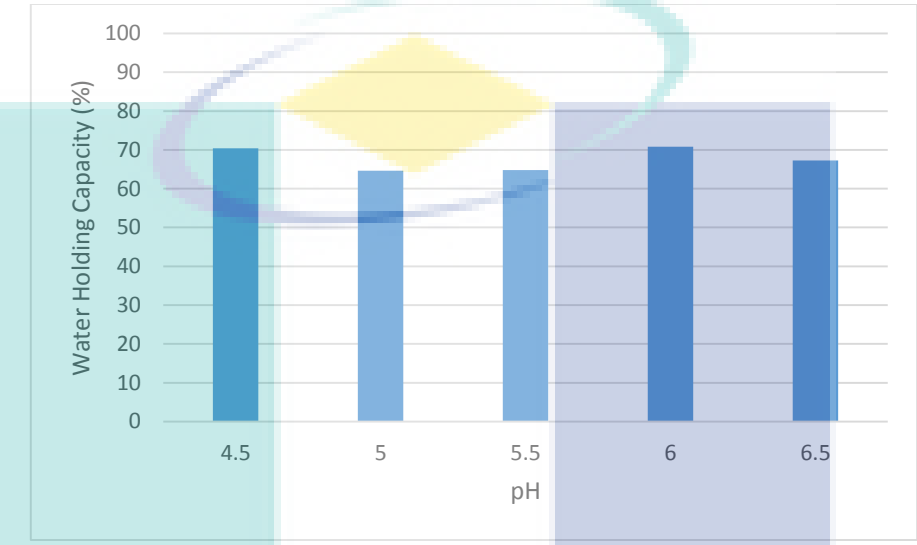


Figure 4.17: The water holding capacity of BC against pH

4.3.4 Temperature effect

From Figure 4.18, at temperature below 26 ℃ the yield of BC produced was low. From 26 ℃ to 30 ℃, there was a slight increase in the yield. At temperature of 34 ℃, also there is a significant decrease in the yield of BC. Based on the trend, it can be concluded that at temperature below 28 ℃ and above 32 ℃ the BC yield obtained is very low whereas the optimum temperature for BC production is in the range of 28 ℃ to 32 ℃. However, the maximum yield was obtained at 30 ℃. The optimum temperature is very important to be considered during fermentation because it is associated with energy supplied for the cell growth and biosynthetic pathway of cellulose that involves the conversion of glucose into bacteria cellulose (Shoda and Sugano, 2005). A study from Al-Shmary (2009) concluded that the optimal temperature for BC production by *G. xylinus* was in the range of 28 ℃ to 30 ℃ which was similar to the results obtained. The same study also observed that at temperature above 35 ℃ there was a decrease in the productivity of BC.

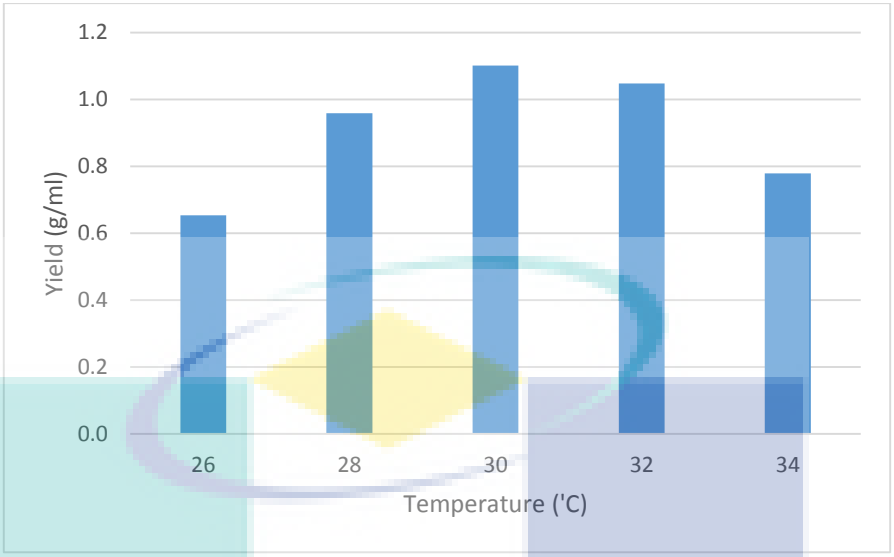


Figure 4.18: The yield of BC against Temperature

Figure 4.19 shows the WHC of BC obtained at various temperature. Similar to the effect of pH, the changes of WHC were insignificant.

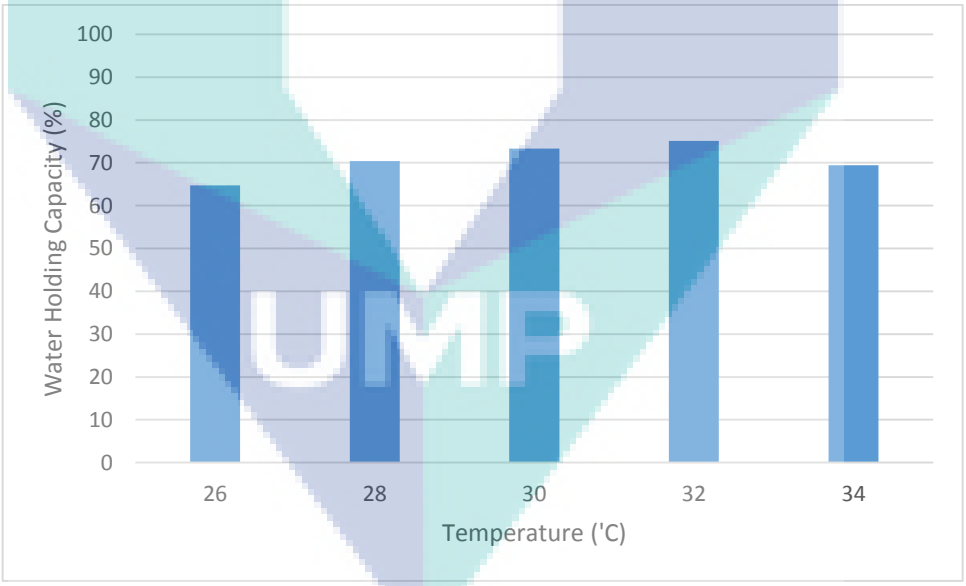


Figure 4.19: The WHC of BC against Temperature

4.4 Physical Structure

The physical structure of bacteria cellulose produced was determined by Fourier Transform Infrared Spectrometer (FTIR) test and was compared with nata de coco. The cellulose and nata de coco were dried in an oven at the temperature of 60 ˚C for a day. Figure 4.20 shows the FTIR spectra for nata de coco and bacteria cellulose, the spectra of nata de coco was plotted with 0.6 unit higher than BC. The FTIR spectra of all samples were detecting at wavenumbers ranging from 4000 to 400 cm^{-1}.

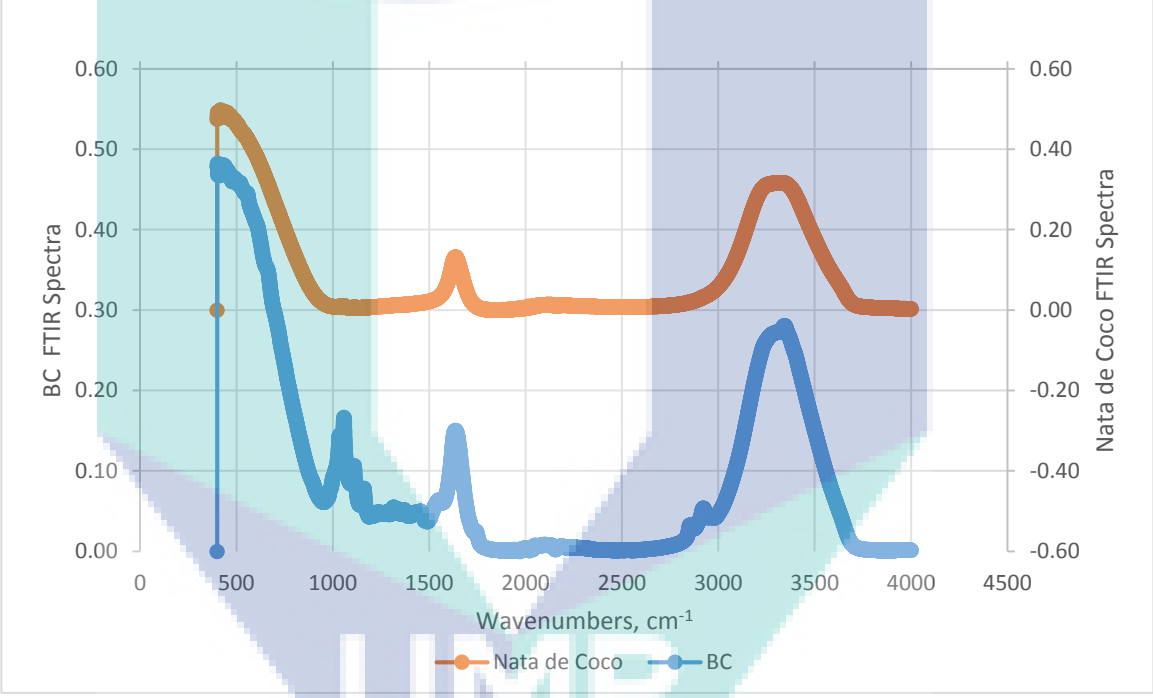


Figure 4.20: FTIR spectra of nata de coco and BC

From Figure 4.20, it clearly showed that the spectra in nata de coco existed in the spectra of bacteria cellulose. However, the number of peak of spectra showed in bacteria cellulose were more than in nata de coco. Based on the result obtained from the FTIR analysis, the absorbance peak at 3320.12 cm^{-1} and 3344.21 cm^{-1} was originated from the O-H bonding. This is proven from the previous study by Parmjit et al. (2011) where the peaks that appear in range of 3853 -3256 cm^{-1} was hydroxyl functional group. Also, the peak of wavenumber at 1635 cm^{-1} was attributed to the bending mode of the absorbed

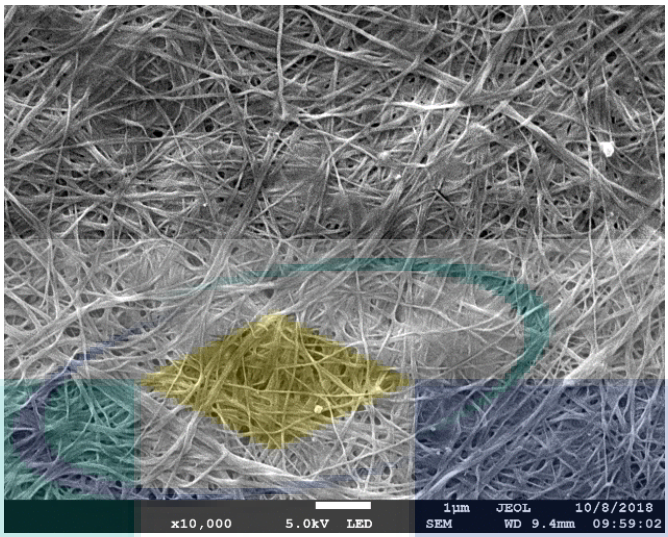
water (Cao et. al., 2008). The highest peak showed in both Figure 4.5 and Figure 4.6 that fell between ranges of 402.56 cm^{-1} to 437.17 cm^{-1} were due to C-O-C bonding at b-glycosidic linkage (Sun et. al., 2008).

There were some other peaks showed in spectra of bacteria cellulose. The peak with wavenumber of 1455.02 cm^{-1} was due to plane bending vibration of CH_2, CH and OH group (Sun et al., 2008). An addition of absorbance peak at 1160.93 cm^{-1} was due to C-O-C asymmetric stretching (Cao et al., 2008). According to Sun et al. (2008), the spectra of 1057.94 cm^{-1} and 1034.05 cm^{-1} were due to C-O bonding and C-C bonding respectively.

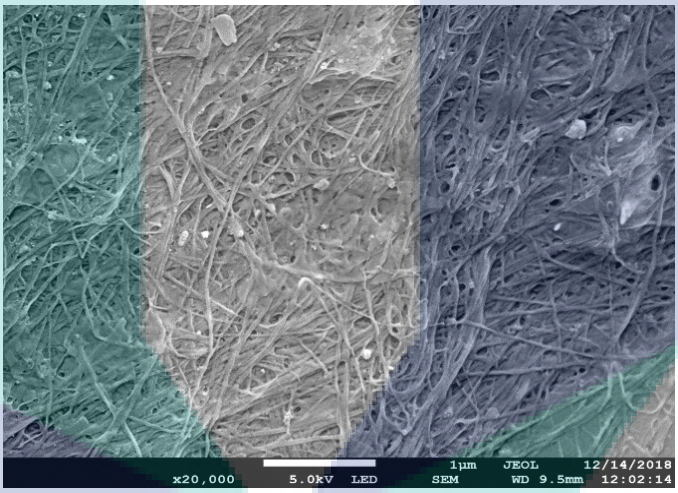
Although there were some impurities in BC produced compared to nata de coco used, the wavenumber did exist in others studies of physical structure of BC (Sun et al., 2008; Cao et al., 2008). Hence, the physical structure of BC produced were similar with the common BC.

4.5 Morphological Structure

The morphological structures of bacterial cellulose obtained was analyzed by Scanning Electron Microscopy (SEM). The morphology of bacterial cellulose strictly depends on culture condition (Watanabe et al., 1998). It can be seen from the Figure 4.21(b) the surface of bacterial cellulose produced using food waste hydrolysate was denser and the microfibrils were closely packed as compared to the BC produced using pineapple juice as the medium (Figure 4.21(a)). Besides, there were more impurities that were captured in between the fibrils network. It was suspected that this impurity might be in part due to the oil content that was carried over from the food wastes. Extra pretreatment step might be required to remove this oil for better quality of the BC obtained.



(a)



(b)

Figure 4.21: Scanning Electron Microscopy (a) BC from pineapple juice (10,000× magnification) and (b) BC from food wastes hydrolysate (20,000× magnification)

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Optimal operating conditions of food wastes pretreatment employing different methods are as follows: hydrothermal pretreatment at a food waste to water ratio of 1:2 for 75 min at 120 ℃; reaction at 50 ℃ for 1 hr using 10 M potassium hydroxide mixing at 300 rpm for alkaline pretreatment; and using 10 FPG Viscozyme® L with 50 g food waste at 50 ℃, pH 6 for 1.5 hr at 300 rpm. Among these methods, the results of elementary analysis and nutrients assay of the FWH revealed that hydrothermal and enzymatic pretreatment have better performance than alkaline pretreatment as more nutrients could be extracted from the food wastes with no obvious denaturation.

When the mass composition of carbon sources increased, the production yield of bacterial cellulose decreased unless there is sufficient nitrogen sources. Also, when the mass composition of nitrogen sources increased, the production yield of bacterial cellulose increased.

Optimum operating conditions in producing BC were 30°C, pH 5.5, stirring at 200 rpm and the inoculum density of 10%. These conditions produced the highest yield of BC with compatible water holding capacity. The physical structure of the BC was confirmed by using Fourier Transform Infrared (FTIR). Nevertheless, the morphology observed under Scanning Electron Microscope (SEM) showed that there were impurities trapped in the microfibril network of BC and affected its density.

5.2 Recommendations

Further characterization should be done to confirm the compatibility of BC produced using food wastes hydrolysate to that of common media. In addition, to improve the morphology and performance of the BC, extra pretreatment step to remove oil content from the food wastes collected should be done. On top of that, fed-batch culture should be studied in order to increase the yield of the BC. Design of the apparatus for easy handling and space saving production of BC at static conditions is also required as static culture take up space, but producing better quality BC.

REFERENCES

2340 Hardness. (2012). Retrieved from Standard Methods.

350–356.

Al-Shamary, Elham & Khalaf, Amir. (2013). Influence of Fermentation Condition and Alkali Treatment on the Porosity and Thickness of Bacterial Cellulose Membranes.

Al-Shmary, E. I., Muhyaddin, M. O., & El-Dahhan, A. H. (2009). Production Of Sodium Carboxy Methyl Cellulose From Bacterial Cellulose. Iraq Journal Of Agriculture, 14(4).

Amnuaikit, Chusuit, Boonme, P., & Raknam. (2011). Effects of a cellulose mask synthesized by a bacterium on facial skin characteristics and user satisfaction. Medical Devices: Evidence and Research, 77.

Analysis of Ash and Minerals. (2003). Retrieved from Mc Clements: http://people.umass.edu/~mcclemen/581Ash&Minerals.html

Antonopoulou G, Alexandropoulou M, Ntaikou I, Lyberatos G (2020) From waste to fuel: Energy recovery from household food waste via its bioconversion to energy carriers based on microbiological processes. Science of the Total Environment, 732: 139230.

Arizona. (2010, June 30). Moisture, Ash Testing in Food Processing. Retrieved from Dairy Foods: https://www.dairyfoods.com/articles/85312-moisture-ash-testing-in-food-processing

Azlina, H., Ahmad, A., Mahamad, I., & Nik, R. N. (2012). Food Waste Management in Malaysia- Current situation and future. *Journal of Industrial Research & Technology*, 36-39.

Biernacki, P., Steinigeweg, S., Borchert, A., & Uhlenhut, F. (2013). Application of Anaerobic Digestion Model No. 1 for describing anaerobic digestion of grass, maize, green weed silage, and industrial glycerine. Bioresource Technology, 127, 188–194.

Bilgi, E., Bayir, E., Sendemir-Urkmez, A., & Hames, E. E. (2016). Optimization of bacterial cellulose production by Gluconacetobacter xylinus using carob and haricot bean. International journal of biological macromolecules, 90, 2-10.

Biyik, Halil & Çoban, Esin. (2011). Evaluation of different pH and temperatures for bacterial cellulose production in HS (Hestrin-Scharmm) medium and beet molasses medium. African Journal of Microbiology Research, 5(9), 1037-1045.

Bligh, E., & Dyer, W. (1959). Canadian Journal of Biochemistry and Physiology. Canadian Journal of Biochemistry and Physiology, 37.

Bradford, MM. (1976). A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-254.

Brigham, C. (2018). Chapter 3.22 - Biopolymers: Biodegradable Alternatives to Traditional Plastics. *Green Chemistry*, 364(1526), 753-770.

Brown R.M. Jr. (1999). Thermal stability of the cellulose synthase complex of *Acetobacter xylinum*. Cellulose 6, 137-152.

Budhiono, A., Rosidi, B., Taher, H., & Iguchi, M. (1999). Kinetic aspects of bacterial cellulose formation in nata-de-coco culture system. Carbohydrate Polymers, 40(2), 137-143.

Cao, X., Chen, Y., Chang, P.R., Muir, A.D. & Falk, G. (2008). Starch-based Nanocomposites Reinforced with Flax cellulose nanocrystals. eXPRESS Polymer Letters 2(7), 502–510.

Chawla P.R., B. I. (2009). Microbial cellulose: fermentative and applications. FoodTechnology and Biotechnology, 47(2), 107-124.

Chawla, PR, Bajaj, IB, Survase, SA, and Singhal, RS (2009). Production of cellulose by microorganisms and its application. Food Technology and Biotechnology, 47 (2), 107-124.

Chen, Y.-C., Hsu, Y.-C., & Wang, C.-T. (2018). Effects of storage environment on the moisture content and microbial growth of food waste. Journal of Environmental Management, 214, 192–196. doi:10.1016/j.jenvman.2018.03.009

Chua GK, Tan FHY, Chew FN, Mohd-Hairul AR (2019) Nutrients content of food wastes from different sources and its pretreatment. AIP Conference Proceedings, 2124(1): 020031.

Coelho, F., Vale Braido, G. V., Cavicchioli, M., Mendes, L. S., Specian, S. S., Franchi, L. P.,O. & Capote, T. S. (2019). Toxicity of therapeutic contact lenses based on bacterial cellulose with coatings to provide transparency. Contact Lens and Anterior Eye, 42, 512-519. doi:10.1016/j.clae.2019.03.006

Czaja, W., Romanovicz, D., & Brown Jr, R. M. (2004). Structural investigations of microbial cellulose produced in stationary and agitated culture. Cellulose, 11(3/4), 403–411.

Dahman, Y., Jayasuriya, K. E., & Kalis, M. (2010). Potential of Biocellulose Nanofibers Production from Agricultural Renewable Resources: Preliminary Study. Applied Biochemistry and Biotechnology, 162(6), 1647–1659.

Dubois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith, F.(1956). Colorimetric method

Esa, F., Tasirin, S. M., & Rahman, N. A. (2014). Overview of bacterial cellulose production and application. Agriculture and Agricultural Science Procedia, 2, 113-119.

Faezah, & Masrinda. (2014). Overview of Bacterial Cellulose Production and Application. Agriculture and Agricultural Science Procedia, 2, 113–119.

Fontana, J. D., De Souza, A. M., Fontana, C. K., Torriani, I. L., Moreschi, J. C., Gallotti, B. J., … Farah, L. F. X. (1990). Acetobacter cellulose pellicle as a temporary skin substitute. Applied Biochemistry and Biotechnology, 24-25(1), 253–264.

for determination of sugars and related substances. Analytical Chemistry, 28(3),

Gea, S., Reynolds, C. T., Rooh, N., Wirjosentono, B., Soykeabkaew, N., Bilotti, E., & Hendriks, A. T. W. M., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource technology, 100(1), 10-18.

Ghaly AE, Ramakrishnan VV, Brooks MS, Budge SM, Dave D (2013) Fish processing wastes as a potential source of proteins, amino acids and oils: A critical review. Microbial & Biochemical Technology, 5(4), 107-129.

Gunduz G., K. E. (2018, January 24). production of bacterial cellulose fibers in the presence of effective microorganisms. Journal of Natural Fibers, 1-8.

Guo J., Catchmark J. (2012). Surface area and porosity of acid hydrolyzed cellulose nanowhiskers and cellulose produced by Gluconacetobacre xylinus. Carbohydrates polymers 87, 37-1026.

H.G. Kristinsson, B.A. Rasco. Crit. Rev. Food Sci. Nutr. 40 (2000) 43-81.

Hasan, N., Biak, D. R., & Kamarudin, S. (2012). Application of Bacterial Cellulose (BC) in Natural Facial Scrub. *International Journal on Advanced Science, Engineering and Information Technology*, *2*.

Jagannath, A., Manjunatha, S. S., Ravi, N., & Raju, P. S. (2009). The Effect of Different Substrates And Processing Conditions On The Textural Characteristics of Bacterial Cellulose (Nata) Produced By Acetobacter Xylinum. Journal Of Food Process Engineering, 34(3), 593–608.

Jozala, A. F., de Lencastre-Novaes, L. C., Lopes, A. M., de Carvalho Santos-Ebinuma, V., Mazzola, P. G., Pessoa-Jr, A., ... & Chaud, M. V. (2016). Bacterial nanocellulose

production and application: a 10-year overview. Applied microbiology and biotechnology, 100(5), 2063-2072.

Kadir, Hailmi, & Nazihah, B. (2013). Effects of Carbon and Nitrogen Sources and Carbon-to-Nitrogen Ratio on Production of Exserohilum longirostratum. Journal Of Agrobiotechnology. 4. 56-66.

Karki AB, Nakarmi AM, Dhital RP, Sharma I, Kumar P (2015) Biogas as renewable source of energy in Nepal: Theory and development, 3rd Edition, Alternative Energy Promotion Center, Khumaltar Heights, Lalitpur Sub Metropolitan City, Nepal.

Kawecki, Krystynowicz, Wysota, Czaja, & Sakiel. (2004). Bacterial cellulose biosynthesis, properties and applications. *International Review Conference Biotechnology*, 14-18.

Keshk, S. M. (2014). Bacterial Cellulose Production and its Industrial Applications. Journal of Bioprocessing & Biotechniques, 04(02).

Keshk, S. M. A. S., & Sameshima, K. (2006). Influence of lignosulfonate on crystal structure and productivity of bacterial cellulose in a static culture. Enzyme and Microbial Technology, 40(1), 4-8.

Khairuddin, N. (2013). Effect of Different Drying Methods on the Morphology, Crystallinity, Swelling Ability and Tensile Properties of Nata De Coco. Sains Malaysiana. 43. 767-773.

Klemm, D., Heublein, B., Fink, H.-P., & Bohn, A. (2005). Cellulose: Fascinating Biopolymer and Sustainable Raw Material. Angewandte Chemie International Edition, 44(22), 3358–3393.

Klemma, D., Schumann, D., Udhardt, U., & Marsch, S. (2011). Bacterial synthesized cellulose — artificial blood vessels for microsurgery. Progress in Polymer Science, 26(9), 1561–1603.

Kongruang, S. (2007). Bacterial cellulose production by Acetobacter xylinum strains from agricultural waste products. In Biotechnology for Fuels and Chemicals (pp. 763-774). Humana Press.

Kostas Triantafyllidis, Michael Stöcker, A. L. (2013). The Role of Catalysis for the Sustainable Production of Bio-fuels and Bio-chemicals. Oslo, Norway.

Krystynowicz, A., Czaja, W., Wiktorowska-Jezierska, A., Gonçalves-Mi kiewicz, M., Turkiewicz, M., & Bielecki, S. (2002). Factors affecting the yield and properties of bacterial cellulose. Journal of Industrial Microbiology & Biotechnology, 29(4), 189–195.

Lee, C.K.; Hsu, K.C.; Cho, J.C.; Kim, Y.J. & Han, S.H. (2013). Cosmetic Bio-Cellulose Mask Pack Sheet and Method for Manufacturing Same. U.S. Patent 2013/0244977A1.

Lehman, S. (2017, May 31). *Wasted food adds up to wasted nutrients*. Retrieved from Reuters: https://www.reuters.com/article/us-health-food-waste-nutrients-idUSKBN18R21G

Li Y, Jin Y (2015) Effects of thermal pretreatment on acidification phase during two-phase batch anaerobic digestion of kitchen waste. Renewable Energy, 77, 550–557.

Li Y, Jin Y, Li J, Li H, Yu Z (2016) Effects of thermal pretreatment on the biomethane yield and hydrolysis rate of kitchen waste. Applied Energy, 172(1020), 47–58.

Lin SP., Loira-Calvar, Catchmark, & Chen K. (2013). Biosynthesis, production and applications of bacterial cellulose. Springer Science+Business Media Dordrecht, 2191-2219.

Litherland, N. (2018). What Foods Are High in Nitrogen? Retrieved from LEAFtv: https://www.leaf.tv/articles/what-foods-are-high-in-nitrogen/

Liu X, Wang W, Gao X, Zhou Y, Shen R (2012) Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste. Waste Management, 32(2), 249–255.

Malaysians waste 15,000 tonnes of food daily. (2016, May 24). Retrieved from The Lehman Star Online: https://www.thestar.com.my/news/nation/2016/05/24/malaysians-waste-15000-tonnes-of-food-daily/

Motte, J. C., Trably, E., Escudié, R., Hamelin, J., Steyer, J. P., Bernet, N., & Dumas, C. (2013). Total solids content: a key parameter of metabolic pathways in dry anaerobic digestion. Biotechnology for biofuels, 6(1), 164.

Moumita K. (2019). Curcumin entrapped gelatin/ionically modified bacterial cellulose based self-healable hydrogel film: An eco-friendly sustainable synthesis method of wound healing patch. *International Journal of Biological Macromolecules*, 940-953.

Ng, C.-C., & Shyu, Y.-T. (2004). Development and production of cholesterol-lowering Monascus-nata complex. World Journal of Microbiology & Biotechnology, 20(9), 875–879.

Ovissipour M, Safari R, Motamedzadegan A, Shabanpour B (2012) Chemical and biochemical hydrolysis of Persian Sturgeon (Acipenser persicus) visceral protein. Food and Bioprocess Technology, 5(2), 460-465.

Panesar, P. S., Chavan, Y. V., Bera, M. B., Chand, O., & Kumar, H. (2009). Evaluation of Acetobacter strain for the production of microbial cellulose. Asian Journal of Chemistry, 21(10), S099-102.

Paritosh K, Kushwaha SK, Yadav M, Pareek N, Chawade A, Vivekanand V (2017) Food Waste to Energy: An Overview of Sustainable Approaches for Food Waste Management and Nutrient Recycling. Biomed Research International, 2017: 2370927, 1-19.

Parmjit S.P., Yogita Chavan., Harish, K., Chopra & John F. Kennedy. (2011). Production of microbial cellulose: Response surface methodology approach. Carbohydrate Polymer, 19, 312-319.

Patel A, Hrůzová K, Rova U, Christakopoulos P, Matsakas L (2019) Sustainable biorefinery concept for biofuel production through holistic valorization of food waste. Bioresource Technology, 294: 122247.

Peavy, H., Rowe, D., & Tchobanoglous, G. (1985). Everonmental Engineering. McGraw-Hill Inc.

Peces, M., Astals, S., & Mata-Alvarez, J. (2014). Assessing total and volatile solids in municipal solid waste samples. Environmental Technology, 35(24), 3041–3046.

Perez-Pimienta JA, Sathitsuksanoh N, Thompson VS, Tran K, Ponce-Noyola T, Stavila V, Singh S, Simmons BA (2017) Ternary ionic liquid–water pretreatment systems of an agave bagasse and municipal solid waste blend. Biotechnology for Biofuels, 10(1), 72.

Phisalaphong, M., & Jatupaiboon, N. (2008). Biosynthesis and characterization of bacteria cellulose–chitosan film. Carbohydrate Polymers, 74(3), 482–488.

Pourramezan, G. Z., Roayaei, A. M., & Qezelbash, Q. R. (2009). Production by Acetobacter sp. 4B—2. Biotechnology, 8(1), 150-154.

Purwadaria, T. (2010). The production of nata colored by Monascus purpureus J1 pigments as functional foods. . *Microbiol Indones* , 62(5), 6-10.

Ramana, K.V. & Tomar, A. & Singh, Lokendra. (2000). Effect of various carbon and nitrogen sources on cellulose synthesis by Acetobacter xylinum. World Journal of Microbiology and Biotechnology. 16. 245-248.

Rangaswamy B.E., V. K. (2013, July 31). Microbial cellulose production from Bacteria isolated from rotten fruit. International journal of polymer science, 2015, 1-8.

Ruka, D. R., Simon, G. P., & Dean, K. M. (2012). Altering the growth conditions of Gluconacetobacter xylinus to maximize the yield of bacterial cellulose. Carbohydrate polymers, 89(2), 613-622.

Salihu A, Alam MZ (2016) Pretreatment methods of organic wastes for biogas production. Journal of Applied Science, 16: 124-137.

Scheidat B, Kasche V, Sekoulov I (1999) Thermal hydrolysis (THD) as a pretreatment method for the digestion of organic waste. In: Proceedings of the Second International Symposium on Anaerobic Digestion of Solid Wastes. Barcelona, 1, 169–174.

Shen J, Zheng Q, Zhang R, Chen C, Liu G (2019) Co-pretreatment of wheat straw by potassium hydroxide and calcium hydroxide: Methane production, economics, and energy potential analysis. Journal of Environmental Management, 236, 720-726.

Shi, Q. S., Feng, J., Li, W. R., Zhou, G., Chen, A. M., Ouyang, Y. S., & Chen, Y. B. (2013). Effect of different conditions on the average degree of polymerization of bacterial cellulose produced by Gluconacetobacter intermedius BC-41. Cell. Chem. Technol, 47, 503-508.

Shia Z.J., Zhang Y., Phillips O., & Yang G.. (2014). Utilization of bacterial cellulose in food. Food Hydrocolloids, 35, 539-545.

Shoda, M., & Sugano, Y. (2005). Recent advances in bacterial cellulose production. Biotechnology and Bioprocess Engineering, 10(1), 1.

Sindhu R, Gnansounou E, Rebello S, Binod P, Varjani S, Thakur IS, Nair RB, Pandey A (2019) Conversion of food and kitchen waste to value-added products. Journal of Environmental Management, 241: 619-630.

Son, H. J., Heo, M. S., Kim, Y. G., & Lee, S. J. (2001). Optimization of fermentation conditions for the production of bacterial cellulose by a newly isolated Acetobacter. Biotechnology and applied biochemistry, 33(1), 1-5.

Staples, P. (2018). Carbon-Based Foods That Humans Eat. Retrieved from LEAFtv: https://www.leaf.tv/articles/carbon-based-foods-that-humans-eat

Sun, Y., Lin, L., Deng, H., Li, J., He, B., Sun, R. & Ouyang, P. (2008), Structure of Bamboo in Formic Acid, Bioresources, 3(2), 297-315.

Taherzadeh, M. J., & Karimi, K. (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. International Journal of Molecular Sciences 9(9), 1621-1651.

Tsouko, E., Kourmentza, C., Ladakis, D., Kopsahelis, N., Mandala, I., Papanikolaou, S., & Koutinas, A. (2015). Bacterial Cellulose Production from Industrial Waste and

by-Product Streams. International Journal of Molecular Sciences, 16(12), 14832–14849.

UI-Islam, M., Khana, T., Parka, J.K. (2012). Water holding and release properties of bacterial cellulose obtained by in situ and ex situ modification. Carbohydrate Polymers. 88, 596-203.

Vandamme EJ, Baets S De, Vanbaelen A, Joris K, & Wulf P De (1998) Improved production of bacterial cellulose and its applications potential. Polymer Degradation and Stability 59:93-99.

Wang W, Hou H, Hu S, Gao X (2010) Performance and stability improvements in anaerobic digestion of thermally hydrolyzed municipal biowaste by a biofilm system. Bioresource Technology, 101(6), 1715–1721.

Watanabe, K., Tabuchi, M., Moringa, Y., & Yoshinaga, F. (1998). Structural features and properties of bacterial cellulose produced in agitated culture. Cellulose. 5(3): 187-200.

Wong A., & Kong R.. (2010). Red bacterial cellulose production by fermentation of Monascus purpureus. 2010 International Conference on Chemistry and Chemical Engineering. Yamanaka, S., & Sugiyama, J. (2000). Structural modification of bacterial cellulose. Cellulose. 7(3): 213-225.

Yi, J., Dong, B., Jin, J., & Dai, X. (2014). Effect of increasing total solids contents on anaerobic digestion of food waste under mesophilic conditions: performance and microbial characteristics analysis. PloS one, 9(7), e102548.

Yunoki, S., Osada, Y., Kono, H., & Takai, M. (2007). Role of ethanol in improvement of bacterial cellulose production: analysis using 13C-labeled carbon sources. Food science and technology research, 10(3), 307-313.

Zahan, K. A., Pa'e, N., & Muhamad, I. I. (2015). Monitoring the Effect of pH on Bacterial Cellulose Production and Acetobacter xylinum 0416 Growth in a Rotary Discs Reactor. Arabian Journal for Science and Engineering, 40(7), 1881–1885.

Zhang, R., Elmashad, H., Hartman, K., Wang, F., Liu, G., Choate, C., & Gamble, P. (2007). Characterization of food waste as feedstock for anaerobic digestion. Bioresource Technology, 98(4), 929–935.

Zheng, Y., Zhao, J., Xu, F., & Li, Y. (2014). Pretreatment of lignocellulosic biomass for enhanced biogas production. Progress in Energy and Combustion Science. 42(1), 35-53.

Żywicka, A., Junka, A. F., Szymczyk, P., Chodaczek, G., Grzesiak, J., Sedghizadeh, P. P., & Fijałkowski, K. (2018). Bacterial cellulose yield increased over 500% by

supplementation of medium with vegetable oil. Carbohydrate Polymers, 199, 294–303.