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Synthesis of Single Cell Protein from *Lactobacillus sp.* and *Saccharomyces cerevisiae* by Fermentation using Corn Cob

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A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Bio Industrial Technology) with Honours

Faculty of Bioengineering and Technology

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DECLARATION

I hereby declare that this final year project entitled "Synthesis of Single Cell Protein from *Lactobacillus sp*. and *Saccharomyces cerevisiae* by Fermentation using Corn Cob" is the result of my own research except as cited in the references. This report has not been submitted for a higher degree to any universities or institutions.

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LIST OF ABBREVIATIONS AND SYMBOLS

SCP	Single cell protein
FTIR	Fourier Transform Infrared spectroscopy
BSA	Bovine Serum Albumin
Man, Rogosa and Sharpe	MRS
Yeast Extract Peptone Dextrose	YPD
v/v	Volume per volume
w/v	Weight per volume
g	Gram
mg	Milligram
ml	Millilitre
mg/ml	Milligram per millilitre
L	Litre
mm	Millimetre

Synthesis of Single Cell Protein from *Lactobacillus sp.* and *Saccharomyces cerevisiae* Using Corn Cob

ABSTRACT

Single cell protein (SCP) is a type of protein that produced from microorganisms such as bacteria, yeast and algae. It can be used as protein source for human and animals. In this study, two types of microorganisms; bacteria (*Lactobacillus sp.*) and yeast (*Saccharomyces cerevisiae*) were used in the SCP production. Cellulose was extracted from corn cob using maceration process, bleaching and alkali treatment and then converted into monosaccharides by acid hydrolysis for carbon source in fermentation process. Fourier-Transform Infrared Spectroscopy (FTIR) was used to determine functional groups and peak region of cellulose and monosaccharides. Two parameters were used for the optimization of SCP production which are type of microorganism and volume of inoculum in the fermentation process. *Lactobacillus sp.* resulted in higher protein yield with 8.5% compared to 7.36% of protein yield from *Saccharomyces cerevisiae*, respectively. Volume of inoculum were then varied (2, 4, 6 and 8 ml) for the next fermentation using *Lactobacillus sp.* 8 ml of inoculum shows the highest yield of protein which is 5.33% compared to 3.32%, 4.09% and 5.17% of protein content for 2, 4 and 6 ml of inoculum, respectively. From this study, it can be concluded that corn cob can be used as carbon source in the SCP production and agriculture waste can be served as cheap raw materials which acts as potential substrate for SCP production.

Keywords: Single cell protein, bacteria, yeast, fermentation, corn cob, cellulose, monosaccharides, FTIR.

Sintesis Protein Sel Tunggal daripada *Lactobacillus sp.* dan *Saccharomyces cerevisiae* Menggunakan Batang Jagung

ABSTRAK

Protein sel tunggal (PST) adalah sejenis protein yang dihasilkan daripada mikroorganisma seperti bakteria, yis dan alga. Ia boleh digunakan sebagai sumber protein untuk manusia dan haiwan. Dalam penyelidikan ini, dua jenis mikroorganisma; bakteria (*Lactobacillus sp.*) dan yis (*Saccharomyces cerevisiae*) telah digunakan dalam penghasilan PST. Selulosa diekstrak daripada batang jagung menggunakan proses maserasi, rawatan pelunturan dan rawatan alkali, kemudiannya ditukarkan kepada monosakarida oleh asid hidrolisis sebagai sumber karbon dalam proses fermentasi. Spektroskopi Inframerah Ubah-Fourier (FTIR) digunakan untuk menentukan kumpulan berfungsi dan kawasan puncak selulosa dan monosakarida. Dua parameter telah digunakan bagi mengoptimumkan penghasilan PST termasuk jenis mikroorganisma dan isipadu inokulum dalam proses fermentasi. *Lactobacillus sp.* menghasilkan kandungan protein yang lebih tinggi dengan 8.5% berbanding 7.36% yang dihasilkan daripada *Saccharomyces cerevisiae*. Isipadu inokulum bagi *Lactobacillus sp.* yang ditetapkan adalah berbeza (2, 4, 6 dan 8 ml) telah digunakan dalam proses fermentasi yang seterusnya. Inokulum sejumlah 8 ml menunjukkan hasil protein paling tinggi iaitu 5.33% berbanding 3.32%, 4.09% dan 5.17% daripada penggunaan 2, 4 dan 6 ml inokulum. Daripada penyelidikan ini, dapat disimpulkan bahawa batang jagung boleh digunakan sebagai sumber karbon dan sisa pertanian boleh dijadikan sebagai bahan mentah murah yang mempunyai potensi sebagai substrat dalam penghasilan PST.

Katakunci: Protein sel tunggal, bakteria, yis, fermentasi, batang jagung, selulosa, monosakarida, FTIR.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Single cell protein (SCP) is protein that extracted from microbial culture which can be used as protein source for human and animals (Suman *et al.,* 2015). It can be considered as eminent source of food as most of its production generally give yield in the range of 43 - 85% of protein. The content of amino acids including methionine and lysine are highly found in SCP. SCP is produced through fermentation process (Suman *et al.*, 2015) and not dependent on climate or environmental factor thus can palliate problems in the production.

Microorganisms such as bacteria, yeast (e.g. *Saccharomyces cerevisiae*), algae and fungi are the potential candidates in the production of SCP. They are allowed to grow and multiply in fermenters, as they utilize inexpensive feedstock and waste such as fruits peels, vegetables waste, wood shavings and paddy straw as carbon source. Bacteria is well known in the fermentation of single cell protein. They grow rapidly as they have short generation time, therefore they could duplicate cell mass in the range of 20 – 120 minutes. Moreover, they have better growth on the substrates compared to yeast thus they

can supply higher protein content than yeast (Adedayo *et al.*, 2011). Furthermore, the culturing and fermentation conditions of bacteria is simple. They are proficient to grow on wide range of raw materials such as food processing waste, agriculture waste and alcohol production residues. These wastes can be used as their carbon source in the fermentation process. Besides bacteria, yeast is also a good choice in producing SCP. The advantages of using yeast are they are easy to harvest as they are larger in size than bacteria, can grow at acidic pH and have high lysine content (Srividya *et al.*, 2012).

Microorganism is proven to have potential in upgrading low protein of organic material into food that contains high protein. Therefore, industry has exploited the production of SCP from microorganisms. For example, German has employed SCP in the World War I and yeast *Saccharomyces cerevisiae* was utilized for consumption. In addition, *Candida utilis* and *Candida arborea* were used in the World War II and this method can replace around 60% of country's food source during the war (Adedayo *et al.*, 2011).

1.2 Problem Statement

Protein source is necessary to human because it can repair cells and tissues, and also to produce new ones. If human body lacks of protein, growth and normal body functions will begin to shut down. For this reason, scientists made some efforts to explore new and unconventional protein. This includes the use of microorganism such as bacteria, yeast and fungi that are proven can produce SCP through fermentation process with the help of fruit and vegetable wastes as the carbon source. SCP can replace the conventional protein

(e.g. poultry, soybean and green peas) as it has the same function with the conventional protein, which can repair body tissues and cells.

Agricultural waste such as corn cob is one of natural sources that has great potential for carbon source in fermentation process as it causes high production of monosaccharide including xylose and glucose. According to Arumugam & Anandakumar (2016), the utilization of corn cob in producing monosaccharide resulted in less time and cost of production. On that note, a study by Asad *et al.* (2000) had used corn cob as the substrate in the production of SCP from *Arachniotus sp.* and only 11.20% of protein was obtained from four days of fermentation. The yield of protein can be considered low and it may give trouble in the further processing of SCP including purification method.

In related to that, the use of fungi (*Arachniotus sp.*) is believed the factor of low protein yield, as stated by Subramaniyam *et al.* (2015) that fungi could be easily harvested but their productivity is low due to the slow growth rate and protein content. Other than that, other factor such as inadequate fermentation time used which is 4 days could also be the reason for the low protein yield in SCP. Therefore, this study was conducted with the use of bacteria (*Lactobacillus sp.*) and yeast (*Saccharomyces cerevisiae*) in the production of SCP and fermentation time was set constant for every batch of fermentation to optimize the fermentation process. Apart of that, another study by Malav *et al.* (2017) reported that SCP production conducted was unsuccessful as corn cob did not undergo pre-treatment thus it cannot be utilized by microorganism in the fermentation process. Therefore, in this study, pre-treatment of corn cob was conducted before being used as substrate in the fermentation process.

1.3 Objectives

1. To extract monosaccharides from corn cob as a substrate for SCP production.

2. To compare the efficiency of *Lactobacillus sp.* and *Saccharomyces cerevisiae* in the bioconversion of single cell protein.

3. To optimize the production of single cell protein using different volume of inoculum.

1.4 Scope of Study

This study focused on producing single cell protein using two types of microorganism which are bacteria (*Lactobacillus sp.*) and yeast (*Saccharomyces cerevisiae*). The use of *Lactobacillus sp.* and *Saccharomyces cerevisiae* are to scrutinize the capabilities of these microorganism to produce SCP. In addition, this study is conducted to evaluate the capabilities of corn cob as the carbon source in bioconversion process. The yield of SCP is optimized using bioconversion process based on type of microorganism and volume of inoculum in the fermentation process respectively.

1.5 Significant of Study

This study is predicted to educate the public on the advantages of microorganism in daily life as not all microorganism is harmful. For instance, there are several microorganisms that can produce single cell protein, which can replace conventional protein that might decrease in the future. Single cell protein is produced with the purpose

of using them as a substitute for protein source to human as well as to animals, if there is inadequate supply of protein. Other than that, the application of bioconversion in this study may reduce environmental pollution because bioconversion can convert animal and plant waste by biological process into utilizable products.

CHAPTER 2

LITERATURE REVIEW

2.1 Single Cell Protein

Single cell protein (SCP) is microbial protein that can be used as protein rich food for human and animal. Several natural products such as paddy straw, sugarcane bagasse and agricultural residue (e.g. sawdust and corn cob) were investigated in the production of microbial protein. In cultivating microorganism, the use of industrial waste and natural cheap substrates is a common trend (Suman *et al.*, 2015). Fruits and vegetable wastes are potentially used in the SCP production. The benefits of using fruit and vegetable waste have been identified as such they are abundantly available and can minimize environmental pollution. The examples of natural cheap substrates that often be used for SCP production are the waste from fruits such as pomegranate, banana, orange and watermelon (Uchakalwar & Chandak, 2014).

There are several benefits and drawbacks of using microorganism for the SCP production. Bacteria generally have high protein content and grow rapidly. However, it is small in size and has low density which give effect in harvesting of biomass from the fermented medium, therefore operation cost will increase. Bacteria also have high content

of nucleic acid that have a tendency to elevate level of uric acid in human blood by the consumption of SCP. Contrary to bacteria, yeast is larger in size hence harvesting is easier. Additionally, yeast has high content of lysine, lower content of nucleic acid and has the ability to grow in acidic condition. However, growth rate of yeast is low and it has low protein content and methionine compared to bacteria. On the other hand, algae have cell wall that made up of cellulose which human have difficulty to digest with (Subramaniyam *et al.*, 2015). On top of that, the primary characteristic of SCP contrast to animal and plant based protein is the time required for SCP to double their cells is small (Israelidis, 1988). Table 2.1 shows the difference of mass doubling time for different organisms that supply protein.

Table 2.1: Mass doubling time

Organism	**Mass doubling time**
Yeast and bacteria	10 to 120 minutes
Algae and mould	2 to 6 hours
Plants and grass	1 to 2 weeks
Chicken	2 to 4 weeks
Pig	4 to 6 weeks
Cattles	1 to 2 months

Source: Israelidis (1988)

Table 2.1 indicates that microorganism has highest efficiency of doubling time in the production of protein and Table 2.2 shows that bacteria has the largest amount of protein that can be produced within 24 hours compared to other protein source organisms.

Table 2.2: Protein production efficiency of particular protein sources within 24 hours

Organisms (1000 kg)	Amount of protein
Cattle's beef	1.0 kg
Soybeans	10.0 kg
Yeasts	100.0 tonnes
Bacteria	100 x 10000000 tonnes

Source: Israelidis (1988)

Moreover, SCP has more benefits than conventional protein sources as it can be genetically controlled, and its production causes less environmental pollution.

2.2 Production of Single Cell Protein

According to Srividya *et al.,* (2012), the production of SCP from any microorganism or substrate would have seven steps. The first step is the provision of carbon source. The carbon sources used in the fermentation may need physical or chemical treatment. Next, carbon sources such as nitrogen or phosphorus need to be added to help the growth of desired microorganism. Then, contamination during fermentation is prevented by maintaining sterile circumstance. This condition is crucial in the streaking technique to obtain a single colony of microorganism. In addition, inoculation of pure state microorganism is required and adequate aeration must be provided during fermentation as SCP processes are highly aerobic. The next step is microbial biomass should recover out of the medium and lastly, it must be further processed to enhance the usefulness.

Besides that, the main process in the production of single cell protein is fermentation process. The initial step in fermentation process is selection strains of microorganisms, which will be then multiplied on raw materials in fermentation process. The fermentation process administered the growth of cell culture and then is followed by a process of separation. In addition, process development starts with screening of microorganism where acceptable microbial strain is obtained from water, oil or soil sample. Technical conditions in fermentation process to optimize strains must be applied and determination of metabolic pathways is conducted. Then, for a ready production where it can be used on large scale, process of engineering and technology of apparatus should adjust well for the best technical performance during the operation (Nasseri *et al.*, 2011).

In addition, microorganism can use inorganic compounds such as carbon and ammonium salts, to generate energy for the metabolism and growth during the fermentation process. Waste of inorganic resources can be converted into protein biomass via rapid growth of microorganism on the substrate. Abundantly available and inexpensive waste from industrial and agricultural such as wood residues, molasses, pineapple waste and papaya peel can be used for SCP production. There are two available processes in the SCP production which are submerged and solid-state fermentation. Submerged fermentation employs liquid substrates including broths and molasses while solid-state fermentation employs solid substrate, for example bran (Bharti, Pandey, & Koushlesh, 2014).

Moreover, Adedayo *et al.* (2011) stated that SCP production can be described according to Figure 2.1.

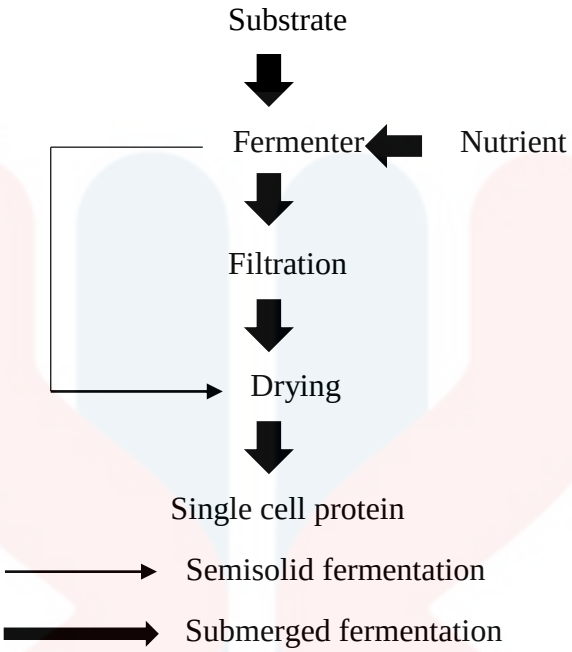

Figure 2.1: Flowchart of SCP production (Adedayo *et al.*, 2011)

Other than that, there are seven criteria in the production of SCP. Firstly, physical or chemical pre-treatments of substrates that may be necessary depends on the carbon source condition. For instance, polysaccharides have to be broken down into simple sugar first before fermentation while monosaccharides can be directly fermented along the desired microorganism. Secondly, in maintaining optimal growth of microorganism, other additional nutrients such as nitrogen and phosphorus are required. Thirdly, it is compulsory to maintain aseptic and sterile conditions by avoiding contamination. Medium components and equipment must be sterilized before fermentation process begins. Fourthly, the desired microorganisms have to be inoculated in pure form. Next, in the fermentation process, sufficient aeration should be supplied because the production of SCP involves highly aerobic condition. Lastly, the recovery of microbial biomass is important to apply in order to enhance its storage capacity and usage (Ali *et al.*, 2017).

According to Bajpai (2017), the production of SCP consists of six steps which are screening of microorganism, raw material selection, process of engineering and

optimization, technology development, economic consideration or process feasibility and safety concerns. Throughout the fermentation process, selected microorganism must be in a pure culture which is in correct physiological state, and sterilized growth medium (fermenter) should be in steady state. In addition, during technology development, separation of cell and cell free supernatant would be collected. Then, purification of single cell protein will be done and lastly, the treatment of effluent will be applied.

2.3 Factors Affecting Production of Single Cell Protein

Irfan *et al.*, (2011) stated that various factors for instance nitrogen and carbon sources, growth medium pH, potassium and phosphorus mainly influenced the fermentation process. As the fermentation process is affected, the production of SCP will be also affected as fermentation is the main process in producing SCP. They also stated that effective SCP production from yeast can be achieved by optimizing some parameters such as initial pH of media, inoculum size, fermentation period, incubation temperature, different inducers such as pyridoxin, biotin, calcium pentathionate and different molasses concentration.

In addition, according to Mensah & Twumasi (2017), substrate concentration effects the production of SCP as the growth rate of yeast cells is highest in 60% v/v concentration with a doubling time of 19 minutes, compared to substrate concentration of 80%, 70%, 50%, 40% are 24, 21, 15 and 12 minutes respectively. Besides that, Taran & Bakhtiyari (2012) indicates that temperature, type of nitrogen source, nitrogen source concentration and carbon source concentration are relevant factors in the production of SCP. Apart of that, type of phosphorus source and phosphorus source concentration using

glucose as carbon source using *Haloarcula sp.* IRU1 by Taguchi experimental design are also applicable factors. Result shows that 8% of glucose (w/v), 0.8% of tryptone (w/v), 0.016% of monosodium dihydrogen orthophosphate (NaH_2PO_4), and temperature of 55 ℃ are the optimal conditions for SCP production. Since Taguchi approach is mainly on using *Haloarcula sp.* IRU1, the study revealed that *Haloarcula sp.* IRU1 could be used satisfactorily on SCP production in different conditions such as high salt and high temperature.

From this study, it shows that different concentration of glucose as the carbon source (1, 2, 4 and 8%) affected the SCP production as the highest level of SCP production was obtained by growing the microorganism with 8% of glucose (w/v). Other than that, the use of nitrogen source is one of the important factors during synthesis of SCP by microorganism, due to the structural properties of protein. The best nitrogen sources for *Haloarcula sp.* IRU1 are tryptone and yeast extract. The choice of phosphorus source also give effect to SCP production. Dipotassium phosphate (K_2HPO_4) and NaH_2PO_4 are the best phosphorus sources compared to trisodium phosphate (Na_3PO_4) and KH_2PO_4. This study also proved that increasing concentration in source of the phosphorus resulted in higher SCP production as 0.016% of phosphorus give the highest production of SCP than using 0.001%, 0.004% and 0.008% of phosphorus.

In addition, factors such as fermentation period, inoculum size, initial pH of medium, and temperature have been supported to effect SCP production from a study by Munawar *et al.*, (2010). This study showed that maximum production of SCP extracted from *Candida utilis* is achieved after 72 hours of fermentation. Besides that, rising the inoculum size increases SCP production, and the optimum initial pH of medium for maximum yield of biomass is 6.0 which having 2.61 g over 100 ml of protein, the highest quantity of protein compared to 4,5,7 and 8 pH were applied in the media. In term of

temperature during fermentation process, *Candida utilis* biomass obtained at the maximum rate at the temperature of 30 ℃. The yield of *Candida utilis* biomass increases from temperature of 20 to 30 ℃ but began to decrease at the temperature of 35 and 40 ℃.

Moreover, Adoki (2008) made a study to determine factors that can influence biomass production of *Candida sp.* by using wastes of fruit citrus. He stated that temperature, pH, type of nitrogen source and presence of phosphorus are the factors which can affect the single cell protein production. Apart from that, several factors such as extraction buffer, fermentation time, initial moisture content of substrate, bioreactor temperature, bioreactor relative humidity and substrate pre-treatment give effect to the yield of SCP production (Samadi *et al.*, 2016). The result revealed that the optimum fermentation condition achieved when the initial moisture content of substrate is 70% with 72 hours of fermentation time, 85% of relative humidity, the temperature of bioreactor is 35 ℃, carbonate-bicarbonate buffer is used for protein extraction and using 2% of NaOH solution as the pre-treatment for substrate.

2.4 Bioconversion

2.4.1 Bioconversion of Agricultural Waste

Bioconversion is the conversion of organic materials into usable products or energy sources by microorganism using fermentation process. Desired microbial strains is multiplied on acceptable raw materials in bioconversion (Ali *et al.*, 2017). The use of agricultural waste is effective in the bioconversion of SCP as the fruit wastes utilization

for microbial protein production could elicit protein sources extracted from microorganism (Munawar *et al.*, 2010). For example, Mensah & Twumasi (2017) showed that pineapple waste generated in the food processing industry serves as good substrates for *Saccharomyces cerevisiae* in the SCP production as 100% (v/v) substrate concentration produced at least SCP yield of 2.5 x 10^{-2} kg/m^3.

Besides that, Mondal & Sengupta (2012) showed that agricultural waste like cucumber peel and orange peel generates crude protein with 53.4% and 30.5% respectively per 100 g substrate used. In addition, papaya extracts were utilized as substrate for production of SCP with the used of *Saccharomyces cerevisiae* has resulted in 30.4% protein yield (Maragatham and Panneerselvam, 2011). A study by Umesh *et al*. (2017), stated that carica papaya waste is the ideal substrate for SCP production because of its high carbohydrate content. Other than that, 35.5% of protein has successfully obtained from a study by Ojokoh & Uzeh (2005) when papaya extracts were used as the substrate in the production of SCP using *Saccharomyces cerevisiae*.

Haddish, (2015) stated that belles fruit peels generated 27% crude protein per 100 gm of substrate used, in the production of SCP from *Saccharomyces cerevisiae* by submerged fermentation. Other than that, 35.7% of protein was obtained from *Cellulomonas sp.* and *Bacillus subtilis* using different granulometric fractions of corn cob as the carbohydrate source (Perotti & Molina, 1988). In addition, a study by Gervasi *et al.* (2018), showed that 40% of protein is found using residual biomass which is wheat-bran medium in the fermentation of *Aspergillus niger* and *Aspergillus terreus*. Profile of amino acid from the biomass showed that all of the essential amino acid are present except methionine.

2.4.2 Submerged Fermentation Process

Submerged fermentation is a fermentation process that employs flowing liquid as substrates. Bioactive compounds are released into fermentation media. The substrates need to be constantly supplemented and replaced with nutrients as they are utilized rapidly. Microorganisms including bacteria that needs high level of moisture content is best suited for this fermentation technique (Subramaniyam & Vimala, 2012). Submerged fermentation gives advantage as the purification of product is easier. In addition, this fermentation has lower total investment costs, lower labour costs, simple operations, reduce floor space requirements and easier maintenance of aseptic conditions.

In addition, substrate for submerged fermentation must be in the liquid state which consists of nutrients required for growth. Fermenter that consists of substrate is continuously run and product of biomass is harvested continuously from fermenter. Then, the product will be filtered, centrifuged and followed by drying. The important operation in cultivation is aeration, as heat is produced during the cultivation then it is eliminated using cooling device. Various methods could be used to harvest microbial biomass. Centrifugation is commonly used to recover single cell organisms such as bacteria and yeast, while filtration is used to recover filamentous fungi. It's important to recover water as much as possible during final drying, under a hygienic state (Suman *et al.*, 2015).

Besides that, Haddish (2015), Azam *et al.* (2014), Mondal & Sengupta, (2012) used submerged fermentation in SCP production and resulted in 32.5%, 11.9%, and 53.4% of protein content respectively. Moreover, 49% of total crude protein is estimated obtained from the submerged fermentation of *Candida utilis* (Munawar *et al.,* 2010). This percentage of total crude protein indicates that it has the potential for animal feed

supplement. This means that using submerged fermentation is the best possible way in the bioconversion to produce SCP.

2.4.3 Microorganism as Agent in Bioconversion

Bacteria, yeast, fungi and algae have the potential as agents in bioconversion process to produce single cell protein. For instance, *Lactobacillus sp.* is gram-positive bacteria, and is non-pathogenic. Therefore, *lactobacillus sp*. is suitable to use in the bioconversion process to produce SCP as it does not cause harm, disease, and death to other organism. *Lactobacillus sp.* is widely known to be used in bioconversion process as it can digest monosaccharides like glucose, amylose and maltose that are used as substrates in SCP production (Bhalla *et al.,* 2007). Besides that, yeast also have been used in the production of SCP. A study by Gervasi *et al.* (2018) stated that 39.8% of protein percentage is obtained by using *Saccharomyces cerevisiae* as the agent in the fermentation process of SCP production.

Other than that, 41.02% of crude protein was yielded from the fermentation of *C. utilis* and *R. oligosporus* by Yunus *et al.* (2015). According to Swaminathan *et al.* (1989), paper mill wastes disposition using bioconversion process is a great approach to convert cellulosic wastes into fungal biomass directly. A cellulotytic microorganism, *Myrothecium verrucaria* was used in protein production and resulted in 0.375 g yield from 50-60% of solid waste usage. Besides that, Suman *et al.* (2015) stated that using bacteria to produce single cell protein is a common practise as bacteria contain more than 80% protein although they have small quantity of sulphur containing amino acids.

On the other hand, the effectiveness of yeast in producing single cell protein is proven by a study from Shahzad & Rajoka, (2011) as 43.7% of crude protein and 26.60% of pure protein obtained from the biomass chemical evaluation. The outcome of this study revealed that *Aspergillus terreus* could produce microbial biomass that can replace up to 30% of protein supply from the soybean meal without permit any detrimental effects on the growing broiler chick.

2.5 Biochemical Analysis of Cellulose

2.5.1 Cellulose

Cellulose is the primary building material for plants. It is an organic compound that makes up most of plants' cell walls. Cellulose can be found abundantly on the earth as it is made by all plants. It has many uses according to how it is treated, for instance, it can be used to make paper, film, and plastics. Cellulose is emerged as highly potential substrate for production of SCP, as its sources from agricultural and forestry constituents that can be found plentifully (Nasseri *et al.*, 2011). Volume of cellulosic waste in the world is sufficiently enough for additional protein supply on continuous basis because cellulose is one of the major renewable resources (Bellamy, 1974). The example of cellulosic waste is lignocellulose biomass such as sugarcane bagasse, paddy straw, corn stover, herbaceous crop and forestry residues.

2.5.2 Extraction of Cellulose

Extraction is a process of separating components from solid or liquid mixture by the action of solvent. Nn (2015) stated extraction is also use to separate any medicinally active part of plant by using particular solvents along with standard procedures. Separating plants soluble metabolites and leaving the insoluble residues are the aim of extraction. In order to extract cellulose, two methods of extraction process can be applied which are chemical and mechanical. Chemical method usually involves the use of chemicals such as acid (hydrochloric acid, sulphuric acid) and bases (sodium hydroxide). Maceration and alkali treatment are some of examples of chemical extraction method.

Maceration process is a process where plants cell wall is soften and broken in order for soluble phytochemicals (various biologically active compound found in plants) to release out (Hariani *et al.*, 2016). The choice of solvent in maceration process is crucial as it will enhance extraction process by reducing the required volume (Nn, 2015). Besides that, alkali treatment can be used to extract a complex of hemicellulose-lignin, because hemicellulose-lignin is soluble in an alkaline solution. It is proven that samples which undergo alkali treatment in extraction process has high purity of cellulose (Aboody, 2013).

In contrary, mechanical method can also be used in extraction of cellulose. It involves the use of machine or apparatus in extraction process such as Soxhlet extraction, ultrasound extraction and microwave-assisted extraction. Soxhlet extraction was invented by Franz von Soxhlet (Derksen *et al.*, 1879) and it was initially designed for lipid extraction from solid materials. Nowadays, it is generally use when the solubility of necessary compound is limited in solvent and the presence of impurities are insoluble in

the solvent. Next, ultrasound extraction is a process where extraction is assisted by using ultrasound which can induce mechanical stress on biomass cells (Segneanu *et al*., 2013). Microwave-assisted extraction is efficient for solid sample extraction which is only relevant to compound that is thermally stable (Hartati *et al.*, 2016). In accordance to two methods of extraction, there are four steps involved in extracting cellulose which are firstly removal of sugars, phenolic compound, and starch, secondly removal of pectic polysaccharides, thirdly removal of hemicellulose, and fourthly removal of lignin (Szyma *et al*., 2017).

2.5.3 Acid Hydrolysis of Cellulose

Acid hydrolysis is a process of using acid to catalyse chemical bond cleavage through reaction of nucleophilic substitution with addition of water elements. It can be used as pre-treatment in order to break beta 1,4-glycosidic bonds which present in cellulose to release cellulose as fermentable or simple sugar. Amiri & Karimi (2013) undergo pre-treatment process using phosphoric acid (H_3PO_4), sodium hydroxide (NaOH) and N-methylmorpholine-N-oxide (NMMO) to evaluate the improvement of dilute-acid hydrolysis of cotton fibre that has high difficulty in breaking down cellulose. The result from this study showed that H_3PO_4 pre-treatment obtained maximum glucose yield and has the least formation of by-product compared to other methods that have been tested. This proves that acid hydrolysis has improved the yield formation of glucose, by using H_3PO_4 pre-treatment.

Other than that, Yah *et al.* (2010) conducted acid hydrolysis method for extraction of sugar from corn cobs and result showed that the amount of glucose and xylose obtained

from acid hydrolysis is higher than enzymatic hydrolysis method. Moreover, a study by Gonzales *et al*., (2016) stated that complex interaction of polysaccharide-lignin will be broken, thus simple fermentable sugar could be released during the pre-treatment process. In accordance to that, fermentative microorganism will utilize the simple fermentable sugar easily during fermentation. Dilute acid pre-treatment is the most universal performed method used for lignocelluloses' biomass.

2.5.4 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is used for determination of cellulose and monosaccharide functional group and peak region in order to compare with the standard cellulose and monosaccharides from the previous study. It is a type of analytical technique that is applied in organic materials and inorganic materials identification. FTIR works by measuring infrared radiation (IR) absorption of sample versus wavelength. FTIR results in spectra can be used to identify the sample, since molecules exhibit specific IR fingerprints. Molecular structures and components are identified by the bands of IR radiation. When IR irradiated the sample materials, IR radiation will excite molecules to high vibrational state. Wavelength of the absorbed light by molecules involves the difference of energy between excited state of vibration and at rest. Sample's wavelength that has absorbed are used to characterize molecular structure. Therefore, FTIR spectroscopy can be extensively applied for quantitative and qualitative analysis in almost every field of science. It creates many advantages and applications as compared to dispersive intra red technology (Sawant *et al*., 2011).

2.6 Protein Content Determination by Bradford Assay

Determination of exact protein quantity in a particular solution is necessary in biochemical principle. Bradford assay is a common way in determining protein content due to its relative sensitivity (can measure small amount of protein that range from 5 µg to 100 µg), convenient and has a rapid protocol. 595 nm of absorbance measurement is used in UV-vis spectrophotometer as it is the maximum absorbance when Coomassie brilliant blue dye that present in Bradford reagent binds to the protein. In addition, the presence of ionic and hydrophobic interactions will stabilize the dye anionic form thus generating a visible change of colour. Simple and rapid quantification for the justification of biochemical measurement of protein cellular fractions, recombinant protein samples or cell lysates can be achieved by the use of this assay (Ernst & Zor, 2010).

CHAPTER 3

MATERIALS AND METHOD

The main material used was corn cob, and below is the flowchart of method involved as shown in Figure 3.1.

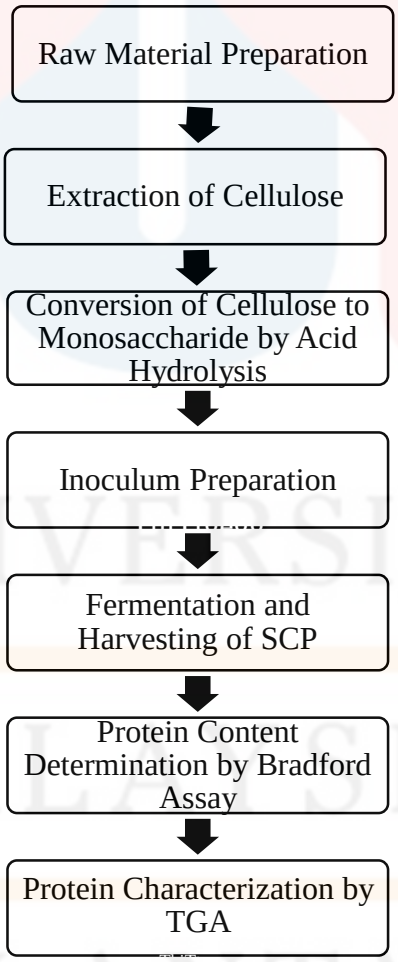

Figure 3.1: Flowchart of SCP production from *Lactobacillus sp.* and *Saccharomyces cerevisiae* using corn cob

3.1 Raw Material Preparation

10.73 kg of corn cobs were collected from a local market at Tanah Merah, Kelantan. The corn cobs were washed using distilled water several times and were cut in small sizes. Initially, corn cobs were oven dried for pre-extraction method. Moisture content of corn cobs was calculated using the formula:

$$\text{Moisture content (\%)} = \frac{weight\ before\ (g) - weight\ after\ (g)}{weight\ before\ (g)} \text{ x } 100\%$$

Moisture content was removed by thermal energy so phytochemicals can be preserved. The corncobs were oven dried at 80 ℃ for 6 hours and crushed into 0.55 mm using wood grinder. Crushing of sample before undergo extraction process is crucial to increase the extraction efficiency as lowering particle size of sample can increase the surface contact between sample and extraction solvent (Nn, 2015). The lower the sample size, the higher the surface area of sample and the quicker the extraction process. Then, 1883.05 g of corn cob powder obtained was transferred in zipper bags and stored in desiccator until further use.

3.2 Extraction of Cellulose

Extraction is separation of a specifically portion of plant using suitable solvent. One of the methods of extraction is maceration. In this study, maceration process is used because it is convenient, inexpensive and more applicable for small scale operation than other extraction methods. Maceration process involved soaking course or powdered plant materials with solvent in stoppered bottle and is allowed to stand at room temperature for

3 days or above (Nn, 2015). 5.60 L of ethanol (100%) was added into 1883.05 g of corncob powder by using (3:1 ratio) and was left for 5 days. Ethanol was used as extraction solvent because it is a very polar molecule due to the hydroxyl group (OH), with high electronegativity of oxygen. This allowed hydrogen bonding to take place with other molecules. Ethanol also has an ethyl group that is non-polar, thus it can dissolve polar and non-polar substances. Then, sodium hypochlorite (NaOCl) was used in bleaching treatment and 4.7 L of 6% NaOCl solution was added into the sample. The solution was stirred and heated at 80 ℃ on stirring hot plate for 2 hours. Next, sodium hydroxide (NaOH) was used in alkali treatment and 5.6 L of 4% w/v of NaOH was added into the sample and stirred at 60 ℃ for 3 hours for hemicellulose removal. The solution was let to cool, filtered and washed with distilled water. Then, 3.8 L of hydrogen peroxide (H_2O_2) (10%) was added to the precipitate, stirred for an hour at 70 ℃, filtered and washed with distilled water several times until pH neutral. The precipitated was dried for 24 hours at 40 ℃. Weight of cellulose was recorded and the yield was calculated using the formula:

$$\text{Yield of cellulose (\%)} = \frac{weight\ of\ cellulose\ (g)}{weight\ of\ corncob\ powder\ (g)} \text{ x } 100\%$$

3.2.1 Characterization of Cellulose by FTIR

Cellulose was identified using FTIR to determine the peak region and its functional groups.

3.3 Acid Hydrolysis of Cellulose

413.17 g of cellulose was treated with 516.00 ml of sulphuric acid (H_2SO_4)(10%). The solution was placed in a water bath at 75 ℃ for 1 hour. After allowed to cool, the sample was then filtered using vacuum pump and oven dried for storage. Weight of fermentable sugar (monosaccharide) was recorded and the yield was calculated using the formula:

$$\text{Yield of monosaccharide (\%)} = \frac{\text{weight of monosaccharide (g)}}{\text{weight of cellulose (g)}} \text{ x } 100\%$$

Monosaccharide was diluted with sterile distilled water. The concentration was adjusted to 0.25 g/ml (refer Appendix A) using formula:

$$\text{Molarity} = \frac{mass\ (g)}{volume\ (ml)}$$

1084 ml of monosaccharide (refer Appendix A) was autoclaved at 121 ℃ for 15 minutes. The diluted monosaccharide was used as carbon source for SCP production.

3.3.1 Characterization of Monosaccharide by FTIR

Monosaccharide formed was identified using FTIR to determine the peak region and its functional groups.

3.4 Inoculum Preparation

3.4.1 Man, Rogosa and Sharpe (MRS) Agar and Yeast Extract Peptone Dextrose (YPD) Agar Preparation

31 g of MRS agar and 32.5 g of YPD agar were separately added into 500 ml of distilled water. Both of the mixture were mixed thoroughly and heated with frequent agitation for 5 minutes to completely dissolve the powder. Initial pH was recorded. The mixture was autoclaved at 121℃ for 15 minutes. Both of the mixture were allowed to cool, poured into sterile petri dishes to solidify and were kept in refrigerator at 4℃ until further use.

3.4.2 Man, Rogosa and Sharpe (MRS) Broth and Yeast Extract Peptone Dextrose (YPD) Broth Preparation

27.58 g of MRS broth was added into 500 ml of distilled water. Next, 5 g of yeast extract, 10 g of peptone and 10 g of glucose were added into 500 ml of distilled water. Both of the solution was stirred and heated respectively until it was fully dissolved. Both of the solution was autoclave at 121 ℃ for 15 minutes. The broth solution were kept in refrigerator at 4 ℃ until further use.

3.4.3 Subculture of Microorganism

Streak plate technique was used in subculture method. *Lactobacillus sp.* from glycerol stock was streaked on MRS agar and incubated at 30 ℃ for 48 hours. Next, 11 g of baker's yeast was added to 50 ml of sterile distilled water. Baker's yeast (*Saccharomyces cerevisiae*) was streaked on YPD agar and incubated at 30 ℃ for 48 hours. This technique thins out microorganism as inoculating loop was flamed after streaking for each quadrant. Aseptic techniques had been followed correctly. Some possible contamination sources like nutrient media (MRS agar and YPD agar), equipment used during operation, operator and environment of the laboratory were handled properly. Nutrient media were autoclaved first before being used, to kill microorganism and spores existed. High temperature and pressure used during disinfection indicates no harmful organism can survive in the condition.

The operations had been done in the laminar flow and UV light inside the chambers are switched on for 10 minutes before using it. Laminar flow consists of high efficiency particulate air (HEPA) filter which can trap pollutants and help to bring allergy relief. It works by forcing air through a fine mesh that traps harmful particles such as dust mites, pollen, tobacco smoke and pet dancer (WebMD, 2016).

3.4.4 Preparation of Starter Culture (inoculum) in Fermentation

Inoculum acts as a starter culture in fermentation process that provide adequate amount of active cells to the fermenter. Single colony from *Lactobacillus sp.* and

Saccharomyces cerevisiae was inoculated in 5 ml of MRS broth and YPD broth respectively. Both of liquid cultures were shaken in orbital shaker at 30 ℃ and 120 rpm for 7 hours. Shaking the liquid cultures ensure oxygen availability. Aeration increase mixing of oxygen present in flask thus help the culture to grow. In addition, shaking the culture avoid bacterial settlement at the bottom of the flask, which could result in lack of nutrient for the cells culture and may lead to cell death. After that, the optical density (OD) of microorganism (bacteria and yeast) culture was measured. OD was used to measure microorganism concentration in suspension. It is measured using UV-Vis Spectrophotometer at 600 nm until it reached 0.6, as 0.6 is in logarithmic phase where the cell culture doubles (Kobayashi *et al.*, 2006). Cells that are in log phase of growth was used as they are actively growing. OD at 600 nm was measured because the cells are not killed under controllable UV light at this wavelength.

3.5 Fermentation and Harvesting of SCP

3.5.1 Submerged Fermentation

Fermentation is a bioconversion technique of complex substrates into simpler compounds with the help of microorganism such as yeast, bacteria and fungi. Submerged fermentation is a process involving microorganism development in liquid broth. This process was done by taking specific volume of inoculum from microorganism and were placed in a conical flask containing rich nutrient including carbon source. *Lactobacillus*

sp. and *Saccharomyces cerevisiae* were used along with monosaccharide as the carbon source. Carbon source was diluted as submerged fermentation involves the use of liquid broth. High volume of oxygen is required in the fermentation. Oxygen was supplied into fermentation flask by plugging sterile cotton wool loosely into the mouth of conical flask. Cotton wool was ensured to be free from any moisture as it was also used to prevent airborne microorganism from getting into fermentation media.

50 ml of 0.25 g/ml monosaccharide was added into each of 250 ml conical flask used for fermentation. Fermentation was carried out in 250 ml of conical flask. All of the conical flasks were duplicated. Volume of monosaccharide used in each fermentation was set constant which is 50 ml, because as the volume of medium in conical flask decreases, the oxygen transport rate (OTR) efficiency in fermentation will increase. The preferable volume in fermentation is 50 ml in a 250 ml conical flask. This volume should allow the best OTR thus the best result could be obtained (Taborsky, 1992).

3.5.2 Batch Fermentation

Carbon source (50 ml of 0.25 g/ml monosaccharide) was added only at the initial phase of fermentation. 2 ml inoculum of *Lactobacillus sp.* and *Saccharomyces cerevisiae* were added for the first run of fermentation respectively (Table 3.1). Next, the best yield of SCP from a particular microorganism was used in the next run of fermentation. Volume of inoculum was set differently (Table 3.2). Every batch fermentation was carried out in orbital shaker at 120 rpm and 30 °C for 6 days. Aseptic techniques were carefully applied before fermentation. All apparatus including conical flask, beaker, measuring cylinder,

pipette tips and inoculating loop were sterilized using 95% ethanol before used. Transfer of substrate and inoculation was carried out in laminar flow.

Table 3.1: Different microorganism

Type of microorganism	**Control variable**		
	Volume of monosaccharide (ml)	Volume of inoculum (ml)	Fermentation time (day)
Lactobacillus sp.	50	2	6
Saccharomyces cerevisiae	50	2	6

Table 3.2: Different volume of inoculum

Volume of inoculum (ml)	**Control variable**		
	Volume of monosaccharide (ml)	Type of microorganism	Fermentation time (day)
2	50	Best microorganism	6
4	50	Best microorganism	6
6	50	Best microorganism	6
8	50	Best microorganism	6

3.5.3 Harvesting of SCP by Centrifugation

SCP was harvested by centrifugation, a technique that separate suspended solid in liquid of different density by rotating sample at a high speed. High speed rotation ensures denser component of sample to settle at the bottom of the tube and least dense component is at the above. High gravity force is generated by centrifugation technique, allowing precipitates and suspended cells separation much efficient compared to conventional filtration. The mixture was placed in 50 ml of Falcon tube and centrifuged at 3500 rpm for 10 minutes at 30°C. Supernatant was removed and cell free supernatant obtained was used to determine protein content.

3.6 Protein Content Determination by Bradford assay

Protein content was measured using Bradford assay. Concentration of SCP was set to 200 mg/ml. Standard curve was prepared with different concentration of Bovine serum albumin (BSA). A linear graph was plotted. 500 µl of sample was added into 2.4 ml of phosphate buffer and 2.5 ml of Bradford reagent. The mixture was vortex and absorbance value at 595 nm was recorded after 5 minutes. Protein content was calculated based on the standard curve. Data for standard curve and sample can be found in Appendix A.

Bradford assay was used to measure the concentration of total protein in a sample. The advantages of using Bradford assay are it is very simple, sensitive, fast (sample can be retested in shorter time), and fairly accurate. Bovine serum albumin (BSA) is a serum albumin protein derived from cows. It is commonly used as protein concentration standard in experiments. The amount of protein in SCP was determined using Bradford

reagent. A linear graph for protein standard was drawn using Bovine serum albumin (BSA) as the standard. The measured absorbance of each BSA concentration (Appendix A) together with the equation for the line generated in the BSA standard curve is shown in Figure 3.2. Based on the graph, the equation of standard curve is:

$$y = 0.8208x - 0.8129, \text{ where:}$$

y is absorbance reading

x represents protein concentration

Correlation coefficient (R^2) in Figure 3.2 indicates that there is a strong relationship between concentration of BSA and absorbance reading as R^2 close to 1.0 indicates the strength between two variables (Mukaka, 2012). It can be concluded that absorbance reading at 595 nm increases with the increase of BSA concentration.

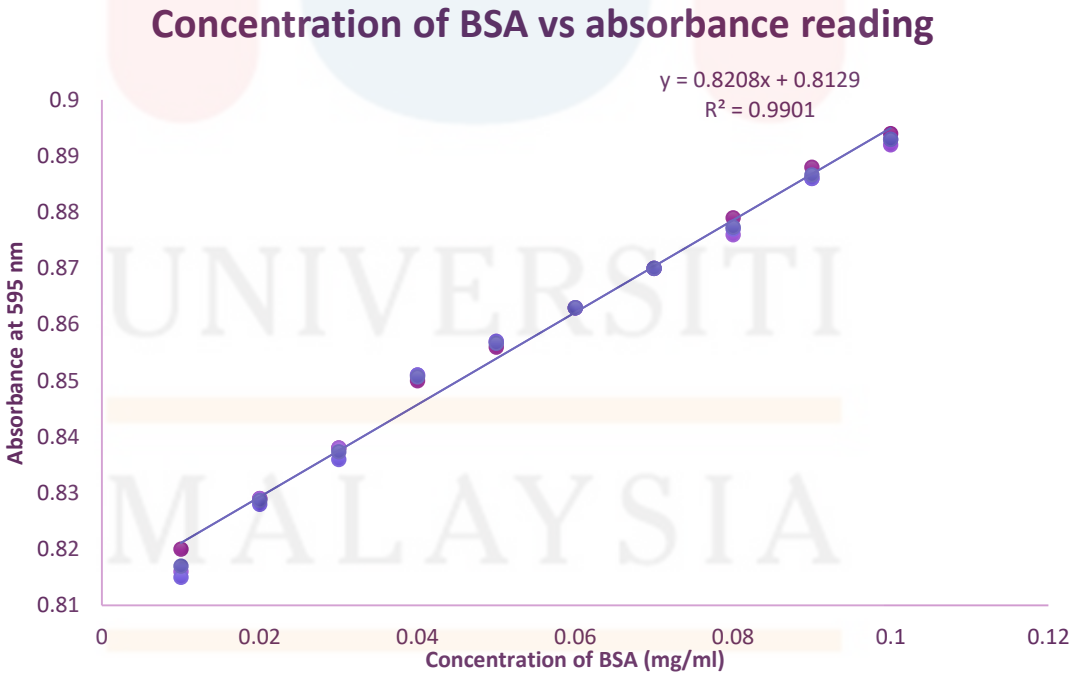

Figure 3.2: Graph of Bovine Serum Albumin (BSA) Standard Calibration Curve

Protein content for each sample was determined using the average of absorbance reading (refer Appendix A). Higher absorbance reading indicates higher protein content presence. This is because the brown Bradford reagent changes to blue colour due to presence of protein. The amount of protein was indicated by the intensity of blue colour and the optical density (OD) obtained from the spectrophotometer. The absorbance wavelength used was 595 nm. The more intense the blue colour, the large amount of protein present, the higher the absorbance reading.

3.7 Protein Characterization by TGA

Thermogravimetric analysis (TGA) was used to analyse the mass changed of SCP over time under controlled temperature. TGA was conducted by heating SCP from 25 °C to 130 °C at 20 °C/minute of heating rate. Generally, sample is pyrolysed in the absence of oxygen but nitrogen is required to study the kinetic analysis of sample during pyrolysis (Cai *et al*., 2018). In this study, nitrogen was used for the atmosphere with 100 ml/minute flow.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physico-chemical Properties of Corn Cob

Chemical analysis of corn cob was recorded in Table 4.1. Result shows that pH of corn cob is 6.7 which indicate that corn cob is neutral, it has an equal molar concentration of hydrogen ion and hydroxide ion. 6.7 pH of corn cob is best for chemical adsorption as according to Laufenberg & Schulze (2009), they have proven experimentally that vanillin was best to absorbed by corn cob at a pH of 7. Moreover, Ali *et al.* (2009) stated that corn cob is best used at pH 5 - 6 in the fermentation to produce lactic acid. Hence, corn cob with neutral pH is potentially used as a raw material in any application of industrial biotechnology process.

Temperature of corn cob which is 24 ℃ shows that room temperature is a secure place for corn cob's storage. Apart from that, moisture content of corn cob was measured (Table 4.1). The result of 49.25% is quite different with a study by Danish *et al.* (2015) which obtained lower value (11.74%) as well as from a study by Danje (2011) which is 4.6%. The difference in these result is because of different sources of corn cob including

its size and type such as dent corn, flint corn and sweet corn. Other than that, factor such as different temperature used during oven drying would also affect the moisture content.

Table 4.1: Chemical analysis of corn cob

Property	Value
pH	6.7
Temperature (℃)	24
Moisture content (%)	49.25

Based on the table above, 49.25% moisture content of corn cob indicates that its texture and appearance was in a good state as moisture content of corn cob generally range between 20 to 55% (Gusman & Campo, 2014). Temperature and duration used in oven drying of corn cob which is 80 ℃ for 6 hours can be considered accurate. Applying correct temperature in the determination of moisture content is crucial as excess moisture content of product or agricultural waste can affect the stability for storage of sample (Appoldt & Raihani, 2017).

4.2 Analysis of Cellulose

4.2.1 Yield of Cellulose

Table 4.2 shows 21.93% yield of cellulose (calculated in Appendix A) extracted from 1883.05 g of corn cob powder which comes originally from 10729.70 g of corn cob. Based on a study by Ainaa *et. al* (2017), 40% of cellulose was extracted from oil palm fronds. Contrary with their study, the low yield of cellulose obtained in this study is believed because the used of different raw materials. Oil palm frands consists of 58% of

cellulose, 24% of hemicellulose and 5% of hemicellulose (Ainaa *et al.*, 2017) meanwhile 27.71% of cellulose, 38.78% of hemicellulose and 9.4% of lignin were found in corn cob (Kapoor *et al*., 2016). This is supported by Sulaiman *et al.* (2012) that stated cellulose content in oil palm fronds is higher than other wastes from other part of plants.

Besides that, the small yield of cellulose is because sample had undergo various steps in the extraction process such as maceration, bleaching (removal of lignin) and alkali treatment (removal of hemicellulose) were applied during extraction process to eliminate the unneeded composition. Therefore, the yield was lost during the transfer of sample from one step to another. Transfer of sample in the steps of extraction process took up a lot of actual sample weight. Some of the sample got stuck and suspended at the bottom and side of the apparatus (beaker and aluminium foil). Besides that, the loss of yield was unavoidable during the filtration process using vacuum pump, as sample often got trapped on the filter paper. However, the yield of cellulose obtained (21.93%) from corn cob can be considered an accepted value as Ditzel *et al.* (2017) obtained 23.5% cellulose extracted from corn cob, which is quite similar with the value in this study.

Table 4.2: Yield of cellulose (%) extracted from corn cob

Weight of corn cob (g)	10729.70
Weight of corn cob powder (g)	1883.05
Yield of cellulose (%)	21.93

4.3 Yield of Monosaccharide from Acid Hydrolysis of Cellulose

Table 4.3 shows 65.62% of monosaccharide (calculated in Appendix A) was converted from cellulose by the acid hydrolysis using sulphuric acid. Based on the previous studies, Fan & Zhang (2014) obtained 25% of xylose (classified as

monosaccharide) that extracted from corn cob by acid hydrolysis using sulphuric acid. They conducted acid hydrolysis using different chemicals including sulphuric acid, oxalic acid and acetic acid. They found that among those acids used, the highest value of monosaccharide obtained was from the used of sulphuric acid. This conclude that even though the yield of monosaccharides extracted was relatively low, the used of sulphuric acid in the acid hydrolysis is still the best method as it gives the highest yield compared to other acids used.

In addition, Monroe (1919) also obtained small yield of monosaccharide from the extraction of corn cob by acid hydrolysis, which is 8 – 10% of xylose. The low yield of monosaccharides obtained compared to this study (65.62%) is believed because the absence of cellulose extraction method from corn cob before acid hydrolysis. This study conducted extraction process of cellulose from corn cob to obtain pure yield of cellulose before it was converted into monosaccharide. On top of that, 65.62% yield of monosaccharide that converted from cellulose shows that the loss of sample throughout acid hydrolysis was controllable because the sample only undergo a single process, compared to extraction process that consists of several steps including maceration, bleaching and alkali treatment. Thus, the yield of monosaccharide is higher than cellulose in the previous method.

Table 4.3: Yield of monosaccharide (%) converted from cellulose

Weight of cellulose (g)	413.00
Weight of monosaccharide (g)	271.00
Volume of diluted monosaccharide (ml)	1084.00
Yield of monosaccharide (%)	65.62

4.4 Effect of Different Types of Microorganism

4.4.1 Subculture of Microorganism

Single colony of *Lactobacillus sp*. and *Saccharomyces cerevisiae* were successfully obtained using streak plate technique as shown in Figure 4.1 (a) and (b). Streak plate technique was done to obtain pure bacteria culture. Obtaining pure bacteria culture is the primary step in bacterial identification to study morphology of any bacteria strain (Eleonor, 2014). Therefore, gram-staining was carried out to differentiate between gram-positive and gram-negative bacteria. Figure 4.2 indicates that *Lactobacillus sp*. is gram-positive bacteria as morphology under the microscope shows long and slender rods. This statement can be reinforced by Goldstein *et al.* (2015) that stated the morphology of *Lactobacillus sp.* can be vary such as slender or plump rods, long, short, in chains or palisades.

Figure 4.3 shows the comparison between broken yeast cells and normal yeast cells under microscope. 4.3 (a) shows that *Saccharomyces cerevisiae* used in this study is in red stained, which indicate that it is a gram-negative. However, theoretically, *Saccharomyces cerevisiae* is a gram-positive that supposedly appeared in purple or violet colour from gram-staining as shown in 4.3 (b). This error was identified and it was proven that yeast cells are disrupted, as Bianchi, (1965) stated that intact yeast cells are gram-positive but broken or disrupted cells are gram-negative. Morphology of *Saccharomyces cerevisiae* had also deformed from the actual yeast shape which is in elliptical shape (Bullerman, 2003) as shown in 4.3 (b).

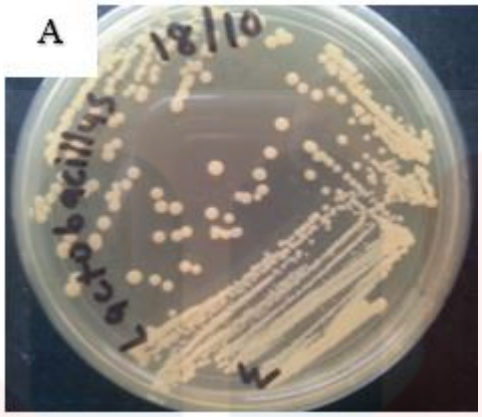

Figure 4.1 (a): Single colony of *Lactobacillus sp.*

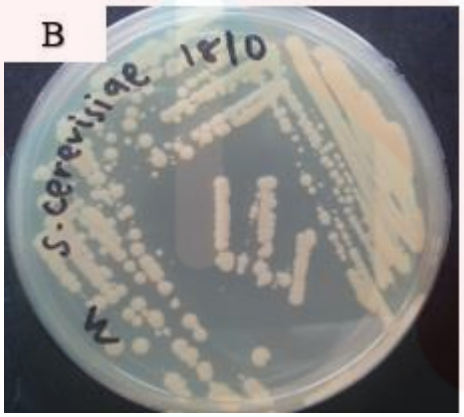

Figure 4.1 (b): Single colony of *Saccharomyces cerevisiae*

Figure 4.2: *Lactobacillus sp.* under microscope (100x magnification)

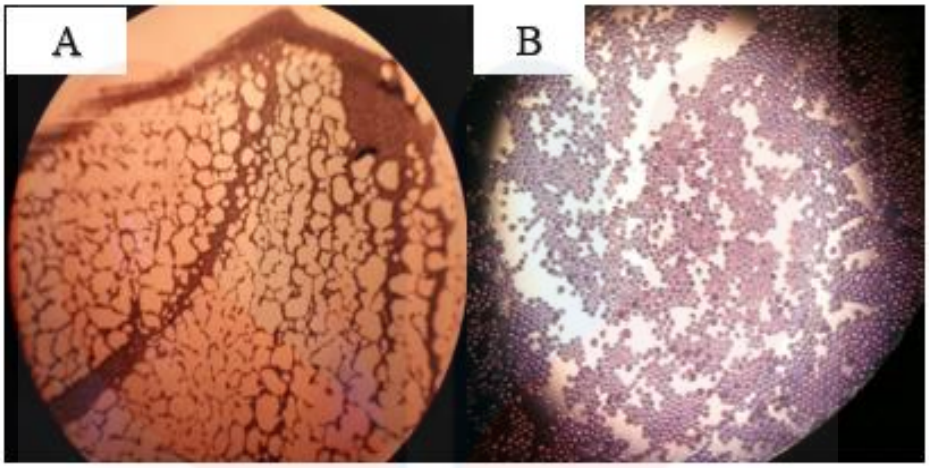

Figure 4.3: (a) Broken cells of *Saccharomyces cerevisiae* and (b) Actual cells of *Saccharomyces cerevisiae* under microscope (100x magnification)

4.4.2 Yield of SCP from Different Types of Microorganism

Table 4.4 shows that the weight of crude protein was measured before and after dry. Data in Table 4.4 was used to calculate moisture content of SCP (refer Appendix A). According to Nasseri *et al*., (2011), SCP must be dried to 10% moisture or it can be denatured and condensed for the spoilage prevention. In Appendix A, *Lactobacillus sp.* shows 97.21% of moisture content while *Saccharomyces cerevisiae* shows 98.31%. This result indicates that SCP produced can be safely stored for any further processing.

Besides that, based on Table 4.5, the result shows that both *Lactobacillus sp.* and *Saccharomyces cerevisiae* had successfully utilized the carbon source (monosaccharides) which originally extracted from corn cob, to produce SCP. Corn cob is proven as a potential raw material which can be converted into monosaccharides and can be used as carbon source in the SCP production. This can be supported by Asad *et al.* (2000), which

stated that utilization of organic matter such as residues from agricultural waste can act as an energy source for the growth of microorganisms. In order for cell biomass to be synthesised, carbon compound is mandatory as microorganism has capability to convert inorganic nitrogen into their body proteins (Khan, 1992).

Table 4.5 shows that SCP produced from *Lactobacillus sp.* with 50 ml of monosaccharide resulted in higher yield of SCP compared to SCP produced from *Saccharomyces cerevisiae* with the same volume of monosaccharide used. *Lactobacillus sp.* shows 8.50% of SCP yield from 0.36 g of crude protein obtained while *Saccharomyces cerevisiae* shows 7.36% of SCP yield from 0.11 g of crude protein obtained after fermentation. *Saccharomyces cerevisiae* has lower yield compared to *Lactobacillus sp.* because the cells of *Saccharomyces cerevisiae* was proven has disrupted, as shown in Figure 4.3 (a). *Saccharomyces cerevisiae* is a sturdy yeast that has rapid growth, great fermentation efficiency and effective sugar use (Reis *et al.*, 2013). However, the cell disruption that occur would give effect to the capabilities mentioned. Despite that, the lower yield obtained from yeast probably because it already has low concentration of amino acids (Goldberg, 1988). Besides, higher yield shown by *Lactobacillus sp*. proves a claim made by Goldberg (1988) which stated that bacteria generally have greater cellular yield from carbon source, higher growth rate and protein content than other microorganisms. According to the result in Table 4.5, further analysis was conducted using *Lactobacillus sp.* as it gives higher yield compared to *Saccharomyces cerevisiae*.

Table 4.4: Weight of crude protein (g) from different types of microorganism (duplicated)

Weight of crude protein from different types of microorganism						
	1		2		Average weight of crude protein before dry (g)	Average weight of crude protein after dry (g)
Type of microorganism	Before dry (g)	After dry (g)	Before dry (g)	After dry (g)		
Saccharomyces cerevisiae	6.53	0.12	6.51	0.09	6.52	0.11
Lactobacillus sp.	13.10	0.44	12.70	0.28	12.90	0.36

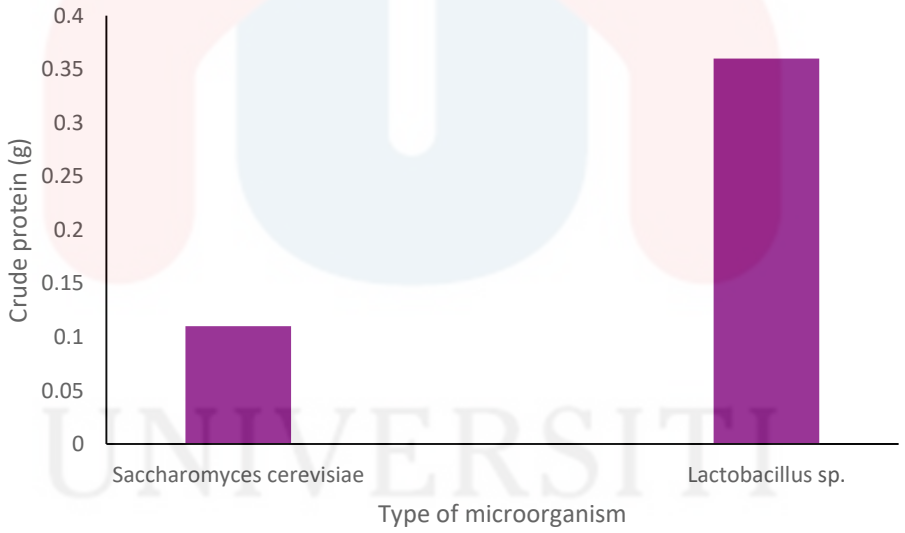

Figure 4.4: Graph of crude protein (g) vs type of microorganism

Table 4.5: Yield of SCP (%) from different types of microorganism

Type of microorganism	Concentration of SCP (mg/ml)	Yield of SCP (%)
Lactobacillus sp.	0.0306	8.50
Saccharomyces cerevisiae	0.0081	7.36

4.5 Effect of Acid Hydrolysis

Table 4.6 shows the weight of crude product from *Lactobacillus sp.* and *Saccharomyces cerevisiae* without acid hydrolysis of cellulose. The weight of crude product in Table 4.6 is higher compared to the weight of crude protein from Table 4.4, which is by the use of acid hydrolysis of cellulose. This is believed because microorganism cannot utilize the polysaccharides (cellulose), thus the crude product obtained cannot be considered as protein, in fact it is only the cellulose remained after fermentation. This can be further explained by the result from Table 4.7.

Table 4.7 shows the comparison between concentration of SCP with acid hydrolysis and without acid hydrolysis of cellulose. SCP concentration when cellulose was treated with acid hydrolysis is higher than without acid hydrolysis. The use of cellulose in fermentation without the treatment of acid hydrolysis resulted in negative concentration thus the yield of SCP cannot be calculated. Negative result is categorized as invalid result, and this proves that *Lactobacillus sp.* and *Saccharomyces cerevisiae* cannot break down cellulose which is in large molecules form (polysaccharides). This result can be supported by Nasseri *et al*. (2011), where it is stated that cellulose has a large potential in the production of SCP, however it is in a complex form where starch, hemicellulose and lignin also found along with the cellulose. Thus, if cellulose was chosen as substrate, pre-treatment has to be done to release cellulose as fermentable sugars, either by the use of enzyme (cellulase) or the use of chemical (acid hydrolysis).

Acid hydrolysis is one of the pre-treatment methods that functions to break down polysaccharides into monosaccharides. It was used to break beta 1,4-glycosidic bonds

that present in cellulose to release cellulose as simple sugar. This method is important because bacteria do not have the enzyme system that hydrolyses polysaccharides into monosaccharides. The unsuccessful result obtained from the absence of acid hydrolysis method can be contended by Kshirsagar *et al.* (2015), that stated cellulose always associated with hemicellulose and lignin, thus it does not occur in the pure form. Naturally occurring celluloses do not susceptible to the microbial attack. As a consequence, cellulose must undergo pre-treatment by either chemical or physical methods in order to facilitate the growth of microorganism.

Table 4.6: Weight of crude product (g) without acid hydrolysis of cellulose (duplicated)

Weight of crude product from different types of microorganism without acid hydrolysis of cellulose						
	1		2		Average weight of crude product before dry (g)	Average weight of crude product after dry (g)
Type of microorganism	Before dry (g)	After dry (g)	Before dry (g)	After dry (g)		
Saccharomyces cerevisiae	18.80	1.01	15.40	0.62	17.10	0.82
Lactobacillus sp.	11.84	1.44	17.31	1.65	14.58	1.55

Table 4.7: Comparison of SCP concentration (mg/ml) between with acid hydrolysis of cellulose (Table 4.5) and without acid hydrolysis of cellulose from different types of microorganism

Type of microorganism	**Concentration of SCP (mg/ml)**	
	With acid hydrolysis	**Without acid hydrolysis**
Lactobacillus sp.	0.0306	- 0.1254
Saccharomyces cerevisiae	0.0081	- 0.1473

4.6 Effect of Inoculum Volume

Table 4.8 shows the data for weight of crude protein from different inoculum volume used and Figure 4.5 indicates the comparison of crude protein obtained from each inoculum volume used, respectively. The weight of crude protein obtained from 8 ml of inoculum volume which is 9.40 g is the highest than the use of 2, 4 and 6 ml of inoculum which resulted in 7.38 g, 8.59 g and 9.28 g respectively. The data was further calculated in Appendix A to obtain the yield of SCP. Table 4.9 shows the yield of SCP from the use of 2 ml, 4 ml, 6 ml and 8 ml of inoculum (*Lactobacillus sp*.) with 50 ml of monosaccharide in fermentation is 3.32%, 4.09%, 5.17% and 5.33% respectively. This shows that SCP produced from 8 ml inoculum of *Lactobacillus sp.* resulted in the highest yield of SCP compared to 2 ml, 4 ml and 6 ml of inoculum used. According to Wardani *et al.* (2017), initial inoculum size is an important factor that can massively influence productivity rate and yield of fermentation product. From this study, it can be concluded that increasing volume of inoculum in fermentation would increase the yield of SCP.

In placing more emphasis, a study by Chen *et al.* (2016) resulted in increasing yield of SCP aligned with the increasing of inoculum size. SCP has the highest yield at the inoculum size of 15% compared to 2, 5, 10, and 20%. Except for 20%, the trend for Chen's study is the same as this study where increasing inoculum volume or size will increase the production of SCP. 20% of inoculum size did not obtain the highest yield can be explained by when the concentration of bacteria is increased, the demand for nitrogen and carbon source also increased. Thus, nitrogen and carbon content in fermentation media decreased and microorganism experienced nutrient deficiency. Growth rate became slower and microorganism declined as time passed and effecting the fermentation

rate, making protein yield became less. Contrary with this study, yield of SCP is constantly increasing with the increase of inoculum volume. This can be said that the volume of inoculum used (2, 4, 6 and 8 ml of *Lactobacillus sp.*) were not exceeding the log phase of *Lactobacillus sp.* cells culture. This is due to the limited cell culture (inoculum volume) and over supplied of carbon source in fermentation media.

Table 4.8: Weight of crude protein (g) from different inoculum volume of *Lactobacillus sp.* (duplicated)

Weight of crude protein from different volume of inoculum of *Lactobacillus sp.*						
	1		2		Average weight of crude protein before dry (g)	Average weight of crude protein after dry (g)
Volume of inoculum (ml)	Before dry (g)	After dry (g)	Before dry (g)	After dry (g)		
2	28.50	6.99	27.50	7.76	28.00	7.38
4	35.50	8.50	34.50	8.68	35.00	8.59
6	43.50	9.40	40.50	9.16	42.00	9.28
8	46.75	9.53	42.45	9.27	44.60	9.40

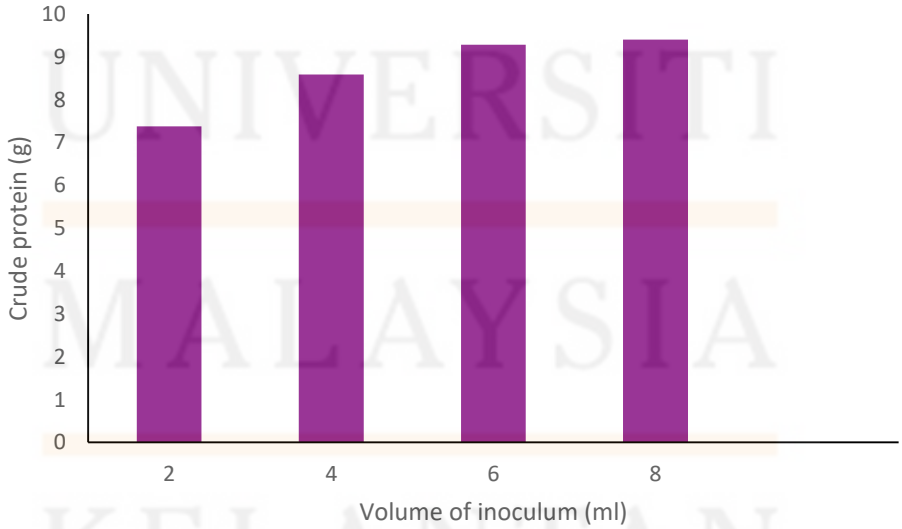

Figure 4.5: Graph of crude protein (g) vs inoculum volume of *Lactobacillus sp.* (ml)

Table 4.9: Yield of SCP (%) from different volume of inoculum

Volume of inoculum (ml)	Concentration of SCP (mg/ml)	Yield of SCP (%)
2	0.2450	3.32
4	0.3510	4.09
6	0.4801	5.17
8	0.5009	5.33

4.7 Characterization and Chemical Analysis

4.7.1 FTIR of Cellulose

Figure 4.6 below shows the FTIR graph of cellulose extracted from corn cob. FTIR was performed in the range of 400 – 4000 cm^{-1} with a resolution of 4 cm^{-1}. FTIR was used to analyse the spectra of cellulose. The peak region of 3326 cm^{-1} which is related to hydroxyl group indicates the presence of O-H stretching as it is in the range of 3300 – 3400 cm^{-1} and the peak region of 1635 cm^{-1} represents bending of O-H of absorbed water, as it is in the range of 1610 – 1639 cm^{-1} (Sci *et al*., 2015). Besides that, 1615 cm^{-1} peak region was related to O-H groups, while 2897 cm^{-1} peak region indicates C-H groups (Khalil *et al.*, 2001). The peak region of 1317 cm^{-1} could related to C-H and C-O bonds of bending vibration in aromatic ring of polysaccharides after the treatment of alkali and bleaching (Nacos *et al*., 2006). In the graph, the peak region of 1028 cm^{-1} which is considered near to 1060 cm^{-1} represents the conformation of cellulose structure, because the presence of C-O and C-H stretching vibration (Sci *et al.*, 2015). Overall, the peak

regions that appear from extracted cellulose from corn cob which are 3326, 1635, 1456, 1028 and 895 cm^{-1} can be considered similar to pure cellulose that extracted from orange peel at 3426, 1631, 1434, 1031 and 895cm^{-1} (Bicu & Mustata, 2011).

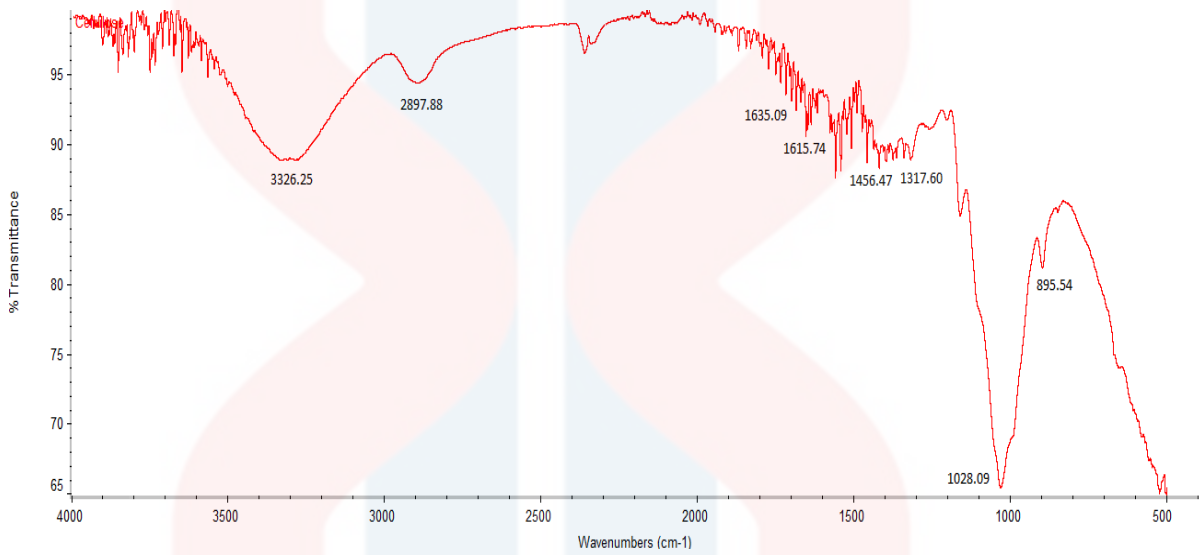

Figure 4.6: FTIR graph of cellulose extracted from corn cob

4.7.2 FTIR of Monosaccharide

Figure 4.7 below shows the FTIR graph of fermentable sugar (monosaccharide) that had been converted from cellulose. Based on a study by Wang *et al.*, (2010), most of characteristic absorption bands that relevant to the major sugars such as glucose, fructose, maltose and sucrose represent by the spectral region between 1500 and 800 cm^{-1} . In the graph, the peak region of 1316, 1160 and 896 cm^{-1} lies within the range, thus it can be concluded that there is presence of monosaccharide. Besides that, the peak region of 1160 and 1033 cm^{-1} also indicate the presence of monosaccharide as 1192 – 958 cm^{-1} are the area of monosaccharides peak (Delle *et al.*, 2011). Glucose, sucrose and fructose show

characteristic bands in the peak region of 1250 – 900 cm^{-1} (Duarte *et al.*, 2002). Based on Figure 4.7, 1160 and 1033 cm^{-1} lies within that range. In addition, the peak region of 1033 cm^{-1} was considered as a key peak of fructose that corresponds to C-OH stretching and C-O bending, as it was similar to fructose in honey where 1053 cm^{-1} is the key peak (Wang *et al.*, 2010).

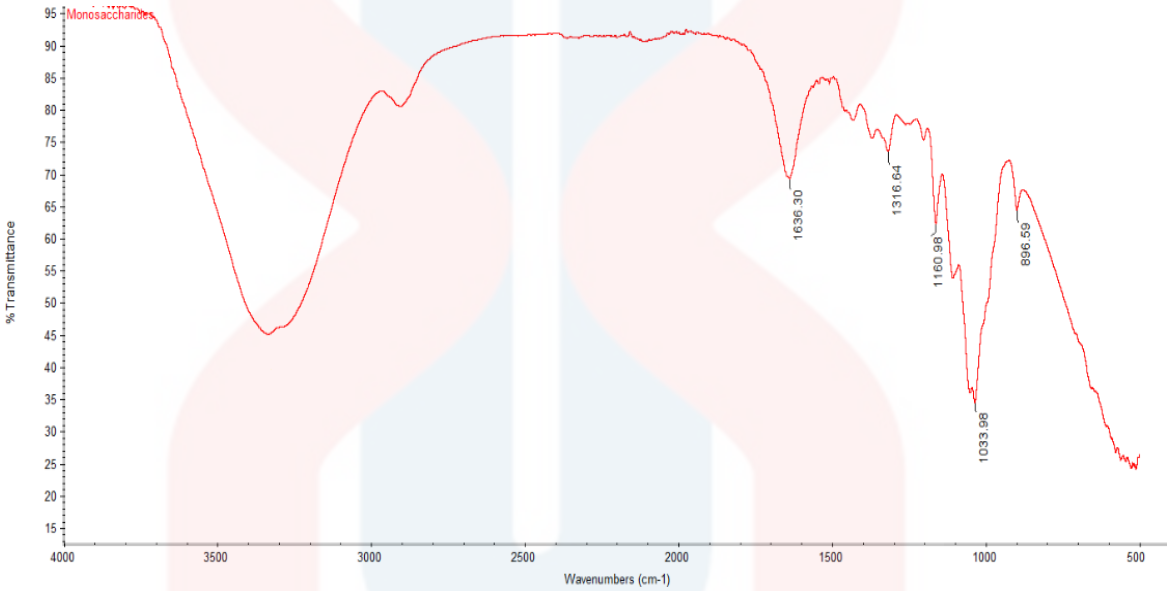

Figure 4.7: FTIR graph of monosaccharide converted from cellulose

4.7.3 Protein Characterization by TGA

Figure 4.8 below shows that the weight of SCP is decreasing from 100% to the range of 70-60% at 140-160 °C. Denaturation temperature was considered the peak temperature, and protein usually denatured at a temperature below 100 °C (Guimarães *et al.*, 2012). However, SCP exceeds the temperature for denaturation as it is synthesised with the help of substrate such as monosaccharides, and it is proven experimentally that the addition of sugar allowed the heat-denaturation temperature to raise, as the thermal

stability is enhanced (Oshima & Kinoshita, 2013). The graph from Figure 4.8 then shows that the weight of SCP is decreased from 60 to approximately 40% at 260 °C. The peaks in the graph shifted to higher temperature with the decreasing of water content in the sample (Kitabatake *et al.*, 1990). Next, the peak of graph shows a slight decrease and constant state at 260 to 340 °C. This can relate to the stability state of SCP as amino acids (basic unit of protein) stable in the range between 200 to 300 °C (Weiss *et al*., 2018). In related to that, SCP produced by *Lactobacillus sp*. with monosaccharides extracted from corncob as the sole carbon source, and peptone and glucose as nitrogen source have synthesised a thermostable protein as it maintains its state accordingly with the temperature.

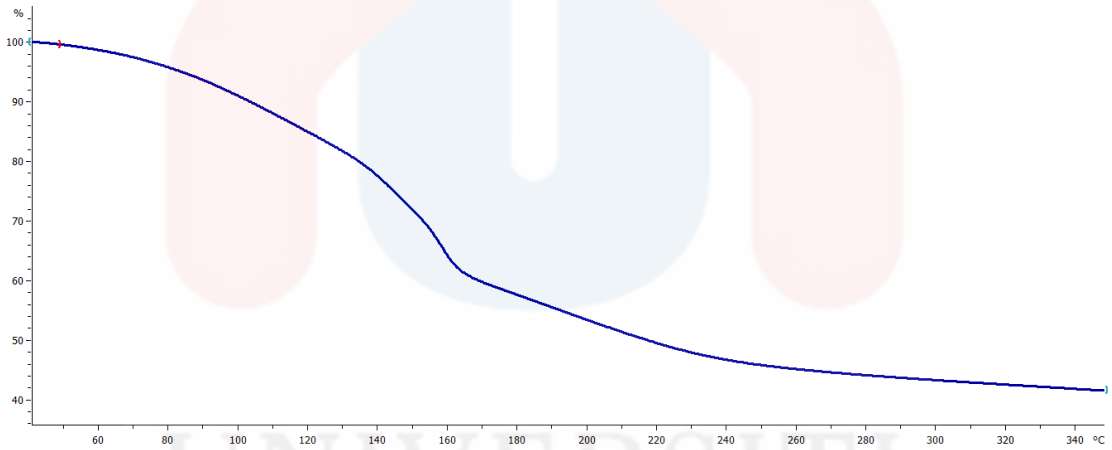

Figure 4.8: TGA graph of SCP

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From this study, the ability of corn cob as carbon source for *Lactobacillus sp.* and baker's yeast (*Saccharomyces cerevisiae*) in synthesis of single cell protein was examined. Corn cob is able to use as carbon source for both bacteria and yeast, which are *Lactobacillus sp.* and *Saccharomyces cerevisiae* because monosaccharides was successfully extracted from corn cob, and was used as a substrate for SCP production. *Lactobacillus sp.* is more efficient than *Saccharomyces cerevisiae* as it obtains 8.50% of SCP yield from 0.36 g of crude protein obtained while *Saccharomyces cerevisiae* shows 7.36% of SCP yield from 0.11 g of crude protein obtained after fermentation. Next, the production of SCP using different volume of inoculum was optimized. The highest volume of inoculum which is 8 ml resulted in higher yield of SCP which is 5.33%.

5.2 Recommendation

There are a few recommendations for the further study of this project. Firstly, to get a higher yield of SCP, volume of inoculum used in the fermentation should be increased because protein source itself comes from inoculum that acts as a starter culture in fermentation. Secondly, pH of fermentation media, different of substrate concentration and co-culturing of microorganism can be added as parameters. Thirdly, the choice of microorganism must be screened and studied thoroughly from the previous study before it is chosen. Other than that, raw materials have to be collected in a large amount in order to improve the yield of substrate for the cells. Lastly, this study requires a further work including liberation of cell proteins by destruction of indigestible cell walls and reduction of nucleic acid content for the effective use of microbial protein for human consumption.

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APPENDIX A

Table A.1: Sample (SCP) set up for Bradford Assay

Volume of phosphate buffer (ml)	Volume of Bradford reagent (ml)	Volume of sample (µl)
2.4	2.5	500

Table A.2: Set up of Bradford Assay for Standard Curve

[BSA] (mg)	Volume of BSA (ml)	Volume of phosphate buffer (ml)	Volume of Bradford reagent (ml)
0.01	0.05	2.45	2.5
0.02	0.10	2.40	2.5
0.03	0.15	2.35	2.5
0.04	0.20	2.30	2.5
0.05	0.25	2.25	2.5
0.06	0.30	2.20	2.5
0.07	0.35	2.15	2.5
0.08	0.40	2.10	2.5
0.09	0.45	2.05	2.5
0.10	0.50	2.00	2.5

Table A.3: First absorbance reading of standard curve

[BSA] (mg)	First reading	Second reading	Third reading	Average 1
0.01	0.817	0.816	0.814	0.816
0.02	0.829	0.829	0.828	0.829
0.03	0.838	0.837	0.836	0.837
0.04	0.855	0.855	0.853	0.854
0.05	0.856	0.856	0.856	0.856
0.06	0.861	0.852	0.864	0.859
0.07	0.870	0.870	0.870	0.870
0.08	0.880	0.879	0.877	0.879
0.09	0.883	0.886	0.887	0.885
0.10	0.894	0.892	0.893	0.893

Table A.4: Second absorbance reading of standard curve

[BSA] (mg)	First reading	Second reading	Third reading	Average 2
0.01	0.820	0.816	0.815	0.818
0.02	0.829	0.829	0.828	0.828
0.03	0.838	0.838	0.836	0.837
0.04	0.850	0.851	0.851	0.848
0.05	0.856	0.857	0.857	0.857
0.06	0.863	0.863	0.863	0.866
0.07	0.870	0.870	0.870	0.869
0.08	0.879	0.876	0.877	0.875
0.09	0.888	0.886	0.886	0.888
0.10	0.894	0.892	0.893	0.892

Table A.5: Final absorbance reading of standard curve

[BSA] (mg)	Average 1	Average 2	Final average
0.01	0.816	0.818	0.817
0.02	0.829	0.828	0.829
0.03	0.837	0.837	0.837
0.04	0.854	0.848	0.851
0.05	0.856	0.857	0.857
0.06	0.859	0.866	0.863
0.07	0.870	0.869	0.870
0.08	0.879	0.875	0.877
0.09	0.885	0.888	0.887
0.10	0.893	0.892	0.893

Table A.6: Average absorbance reading of SCP from different types of microorganism

Type of microorganism	First reading	Second reading	Third reading	Average
Lactobacillus sp.	0.839	0.837	0.838	0.838
Saccharomyces cerevisiae	0.821	0.822	0.821	0.821

Table A.7: Average absorbance reading of crude product from different types of microorganism (without acid hydrolysis of cellulose)

Type of microorganism	First reading	Second reading	Third reading	Average
Lactobacillus sp.	0.709	0.710	0.710	0.710
Saccharomyces cerevisiae	0.690	0.695	0.691	0.692

Table A.8: Average absorbance reading of SCP from different inoculum volume of *Lactobacillus sp.*

Volume of inoculum (ml)	**First reading**	**Second reading**	**Third reading**	**Average**
2	1.014	1.013	1.014	1.014
4	1.101	1.101	1.100	1.101
6	1.207	1.207	1.206	1.207
8	1.223	1.223	1.225	1.224

Calculation A.1:

$$\text{Yield of cellulose (\%)} = \frac{weight\ of\ cellulose\ (g)}{weight\ of\ corn\ cob\ powder\ (g)} \text{ x } 100\%$$

$$= \frac{413.17\ g}{1883.05\ g} \text{ x } 100\%$$

$$= 21.93\%$$

Calculation A.2:

$$\text{Moisture content of cellulose (\%)} = \frac{weight\ before\ (g) - weight\ after\ (g)}{weight\ before\ (g)} \text{ x } 100\%$$

$$= \frac{830.60\ g - 413.17\ g}{830.60\ g} \text{ x } 100\%$$

$$= 50.26\%$$

Calculation A.3:

$$\text{\% yield of monosaccharide} = \frac{weight\ of\ monosaccharide\ (g)}{weight\ of\ cellulose\ (g)} \text{ x } 100\%$$

$$= \frac{271\ g}{413\ g} \text{ x } 100\%$$

$$= 65.62\%$$

Calculation A.4:

Concentration of monosaccharide (g/ml):

$$\text{Molarity} = \frac{mass\ (g)}{volume\ (ml)}$$

$$= \frac{1\ g}{4\ ml}$$

$$= 0.25 \text{ g/ml}$$

Calculation A.5:

Volume of diluted monosaccharide (ml):

$$\text{Molarity} = \frac{mass\ (g)}{volume\ (ml)}$$

$$0.25 \text{ g/ml} = \frac{271\ g}{x}$$

$$x = 1084 \text{ ml}$$

Calculation A.6:

Moisture content of SCP:

$$\text{Moisture content (\%)} = \frac{weight\ before\ (g) - weight\ after\ (g)}{weight\ before\ (g)} \text{ x } 100\%$$

Lactobacillus sp.:

$$\frac{12.90\ g - 0.36\ g}{12.90\ g} \text{ x } 100\% = 97.21\%$$

Saccharomyces cerevisiae:

$$\frac{6.52\ g - 0.11\ g}{6.52\ g} \text{ x } 100\% = 98.31\%$$

Calculation A.7:

Concentration of SCP from different type of microorganism:

Based on the formula y=mx + c from standard curve (refer Figure 3.2),

y = absorbance reading of sample (refer Table A.6), x = concentration of sample

SCP from *Lactobacillus sp.*:

y = 0.8208x + 0.8129

0.838 = 0.8208x + 0.8129

0.8208x = 0.838 – 0.8129

x = 0.0306 mg/ml

SCP from *Saccharomyces cerevisiae*:

y = 0.8208x + 0.8129

0.821 = 0.8208x + 0.8129

x = 0.0081 mg/ml

Calculation A.8:

Yield of SCP (%) from different type of microorganism:

$$\frac{Concentration\ of\ SCP\ (\frac{mg}{ml})}{Crude\ protein\ (g)} \text{ x } 100\%$$

SCP from *Lactobacillus sp.*:

$$= \frac{0.0306\ mg/ml}{0.36\ g} \text{ x } 100\%$$

$= \frac{0.00306\%}{0.036\%}$ x 100%

= 8.50%

SCP from *Saccharomyces cerevisiae*:

$= \frac{0.0081\ mg/ml}{0.11\ g}$ x 100%

$= \frac{0.00081\%}{0.011\%}$ x 100%

= 7.36%

Calculation A.9:

Concentration of SCP from different type of microorganism (without acid hydrolysis of cellulose):

Based on the formula y=mx + c from standard curve (refer Figure 3.2),

y = absorbance reading of sample (refer Table A.7), x = concentration of sample

SCP from *Lactobacillus sp.*:

y = 0.8208x + 0.8129

0.710 = 0.8208x + 0.8129

0.8208x = 0.710 – 0.8129

x = - 0.1254 mg/ml

SCP from *Saccharomyces cerevisiae*:

y = 0.8208x + 0.8129

0.692 = 0.8208x + 0.8129

0.8208x = 0.692 – 0.8129

x = - 0.1473 mg/ml

Calculation A.10:

Concentration of SCP from different volume of inoculum:

Based on the formula y=mx + c from standard curve (refer Figure 3.2),

y = absorbance reading of sample (refer Table A.8), x = concentration of sample

2 ml inoculum of *Lactobacillus sp.:*

y = 0.8208x + 0.8129

1.014 = 0.8208x + 0.8129

0.8208x = 0.2011

x = 0.2450 mg/ml

4 ml inoculum of *Lactobacillus sp.*:

y = 0.8208x + 0.8129

1.101 = 0.8208x + 0.8129

0.8208x = 0.2881

x = 0.3510 mg/ml

6 ml inoculum of *Lactobacillus sp.*:

y = 0.8208x + 0.8129

1.207 = 0.8208x + 0.8129

0.8208x = 0.3941

x = 0.4801 mg/ml

8 ml inoculum of *Lactobacillus sp.*:

y = 0.8208x + 0.8129

1.224 = 0.8208x + 0.8129

0.8208x = 0.4111

x = 0.5009 mg/ml

Calculation A.11:

Yield of SCP (%) from different volume of inoculum:

$$\frac{Concentration\ of\ SCP\ (\frac{mg}{ml})}{Crude\ protein\ (g)} \text{ x } 100\%$$

2 ml inoculum of *Lactobacillus sp.*:

$= \frac{0.2450\ mg/ml}{7.38\ g}$ x 100%

$= \frac{0.02450\%}{0.738\%}$ x 100%

= 3.32%

4 ml inoculum of *Lactobacillus sp.*:

$= \frac{0.3510\ mg/ml}{8.59\ g}$ x 100%

= $\frac{0.03510\%}{0.859\%}$ x 100%

= 4.09%

6 ml inoculum of *Lactobacillus sp.*:

= $\frac{0.4801\ mg/ml}{9.28\ g}$ x 100%

= $\frac{0.04801\%}{0.928\%}$ x 100%

= 5.17%

8 ml inoculum of *Lactobacillus sp.*:

= $\frac{0.5009\ mg/ml}{9.40\ g}$ x 100%

= $\frac{0.05009\%}{0.940\%}$ x 100%

= 5.33%

APPENDIX B

Figure B.1: Cellulose extracted from corn cob

Figure B.2: Baker's yeast used in fermentation

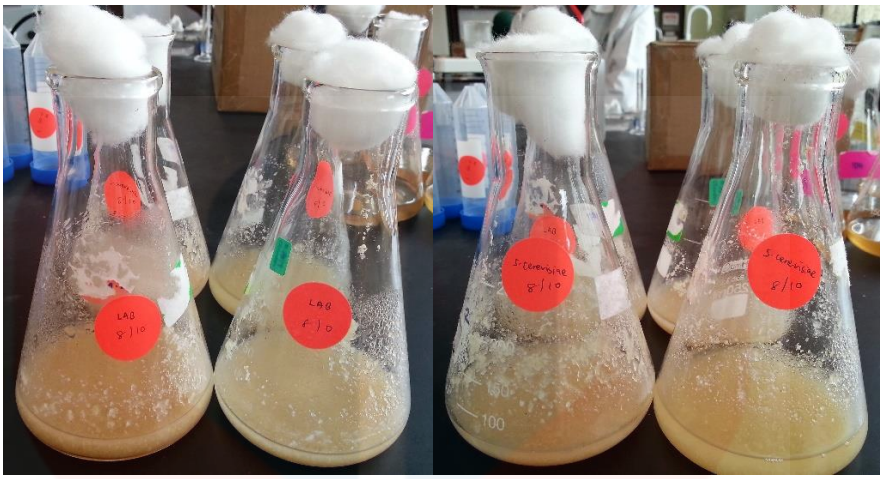

Figure B.3: Preparation for first run of fermentation (different types of microorganism)

Figure B.4: Preparation for second run of fermentation (different volume of inoculum)

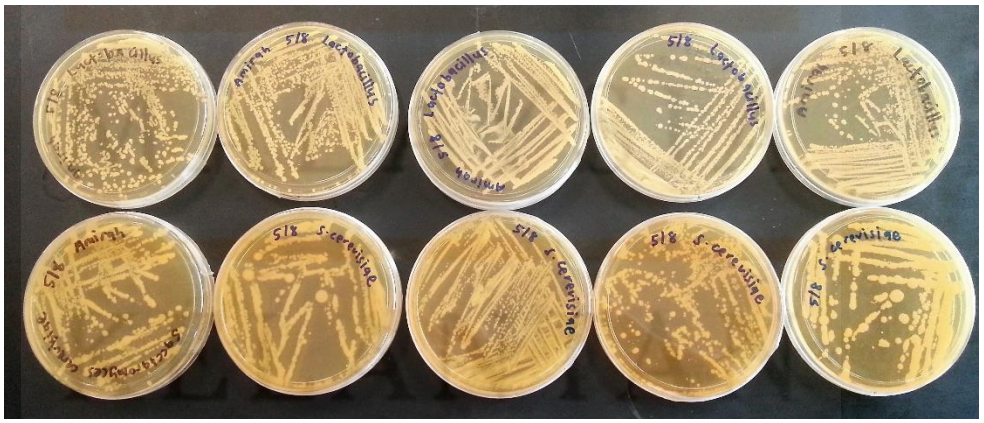

Figure B.5: Single colony of *Lactobacillus sp.* and *Saccharomyces cerevisiae*