FABRICATION OF BIO COMPOSITE FROM BACTERIAL CELLULOSE/STARCH

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FABRICATION OF BIO COMPOSITE FROM BACTERIAL CELLULOSE/STARCH

SITI NATRAH BINTI ISMAIL

A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JANUARY 2012

SUPERVISOR'S DECLARATION

"I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in term of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)"

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STUDENT'S DECLARATION

"I hereby declare that this thesis entitled "Fabrication of Bio composite from Bacterial Cellulose/Starch" is the result of my own research except as cited references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree".

Signature : .....
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I dedicate this entire work to my family especially to my beloved parents, whose patient, support and companionship have facilitated my study, and made my life enjoyable, to my grateful brothers and my lovely sister. And not forgot to all my friends for their enduring faith and unconditional love in good times and bad.

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Last but not least, there will be errors, inconsistencies and over-simplifications in this thesis and I bear absolute responsibility for the erratic judgments I made. None of the above mentioned people should be held responsible for any errors in this thesis.

ABSTRACT

Bio composite from starch and bacterial cellulose offer good mechanical properties. The objective of this study was to fabricate bio composite from starch, and bacterial cellulose. The research was conducted with 25 samples with difference composition of bacterial cellulose (BC) (0, 7, 14, 21 and 28) wt% and starch (3, 6, 9, 12 and 15) wt%. The bacterial cellulose film produced by *Acetobacter xylinum* was blend and mixed with starch, and glycerol. The film was characterized by using Universal Testing Machine, water absorption, Fourier Transform Infrared (FTIR), Thermo Gravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) and Soil Burial Degradation, biodegradation by using *Aspergillus niger*. When the composition of starch and BC increased, the tensile strength and Tensile modulus was increased and the elongation at break was decreased. The absorption of water was increased proportionate with the increasing the composition of bacterial cellulose and starch. From the FTIR test, the bio composite was showed the present of hydroxyl group, C=O stretching (amide I), and C-O bonding. Besides that, TGA showed the thermal degradation of starch and cellulose occurs when the temperature arise to 280°C, leads to depolymerization and to the formation of 1,6-anhydroglucose-ß-D-glucopyranose. Moreover, the SEM analysis showed a smooth and homogenous structure of the film but when the composition of BC increased, the small mat fragments can be saw and the layered structure becomes clearer. The soil burial test and degradation by using *Aspergillus niger* indicated the degradation rate decreased as the bacterial cellulose content increased. As a conclusion, the film fabricated had a potential application in future to be used as food packaging material because as it had good mechanical properties and biodegradable. In order to improve the properties of bio composite, more research should be done to know the optimum composition of BC and starch should be used to produce the film. Besides that, the study about other alternative material or additive that can be added to the film also can be done to increase the mechanical properties and degradation time.

ABSTRAK

Kanji mempunyai struktur yang lemah sebagai termoplastik, untuk meningkatkan sifat mekanik bio komposit, kajian telah dilakukan dengan menambah dan selulosa bakteria di dalam bio komposit kanji. Tujuan kajian ini adalah untuk menghasilkan bio komposit dari kanji dan Selulosa Bakteria. Penyelidikan dilakukan dengan menggunakan 25 sampel dengan perbezaan komposisi selulosa bakteria (0, 7, 14, 21 dan 28) wt% dan kanji (3, 6, 9, 12 and 15) wt%. Filem selulosa bakteria yang dihasilkan oleh *Acetobacter xylinum* dikisar dan dicampur dengan kanji dan glycerin. Filem tersebut akan dikategorikan dengan menggunakan Universal mesin Testing, kadar penyerapan air, Fourier Transform Infrared (FTIR) Spektroskopi, Thermo Gravimetric Analysis (TGA) dan Mikroskop Elektron (SEM) ujian degradasi tanah, dan ujian degradasi dengan menggunakan *Aspergillus niger*. Apabila komposisi kanji dan BC meningkat, kekuatan tegangan dan kekenyalan tegangan akan meningkat dan pemanjangan pada waktu rehat akan berkurangan. Kadar penyerapan air telah meningkat berkadar dengan peningkatan kandungan selulosa bakteria dan kanji. Daripada ujian FTIR, biocomposite menunjukkan yang hadir kumpulan amide I, ikatan C-O dan ikatan OH. Selain itu, TGA menunjukkan degradasi haba daripada selulosa berlaku apabila suhu meningkat pada 280°C, membawa kepada depolymerization dan pembentukan 1,6-anhydroglucose-ß-D-glucopyranose. Ujian degradasi tanah dan degradasi dengan menggunakan *Aspergillus niger* menunjukkan penurunan kadar penyingkiran apabila kadar selulosa bakteria meningkat. Kesimpulannya, filem telah dibuat berpotensi di masa depan untuk digunakan sebagai pembungkus makanan kerana mempunyai sifat mekanik yang baik, dan terbiodegradasikan. Untuk meningkatkan ciri-ciri bio komposit, lebih banyak kajian perlu dilakukan untuk mengetahui komposisi selulosa bacteria dan kanji yang perlu digunakan untuk menghasilkan filem. Selain itu, kajian mengenai bahan gantian atau tambahan yang boleh di tambah ke dalam filem boleh dilakukan untuk menambah ciri-ciri mekanikal dan masa untuk mengurai.

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LIST OF SYMBOLS

^{o}C	Celsius
$^{o}C/min$	Celsius per minutes
cm	Centimeter
cm^{-1}	Per centimeter
cm^{2}/min	Centimeter square per min
g	Gram
h	Thickness of the film
k	Slope
kN	Kilo Newton
min	Minutes
mL min^{-1}	Milliliter per minutes
mm	Millimeter
MPa	Mega Pascal
Mt	Mass gain in time
$M\infty$	Mass gain at equilibrium (maximum water uptake)
nm	Nanometer
W_o	Initial Mass
W_t	Remaining Mass
w/v	Weight per volume
w/w	Weight per weight
µm	Micrometer

α	Alpha – glycoside link
β	Beta – glycoside link
ε	Elongation at break
%	Percent

LIST OF ABBREVIATIONS

BC	Bacterial cellulose
BCC	Bamboo Cellulosic Crystals
D	Diffusivity
DI	Deionize
FTIR	Fourier Transform Infrared Spectroscopy
IR	Infrared
PS	Plastic Starch
SEM	Scanning Electron Microscope
TC	Terminal Complex
TGA	Thermo Gravimetric Analysis
TPS	Thermoplastic Starch
TM	Tensile Modulus
TS	Tensile Strength

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Petrochemical polymers commonly called as “plastics” have been booming and are by far the most widely used polymers for packaging. Plastic have become very popular with consumers in developing countries, as there are cheap, strong, lightweight, and functional. One of the useful characteristics of plastic is the fact that it is durable. Unfortunately, this is not a positive characteristic when it comes to the environment. The fact that plastic is a durable meaning it cannot be degraded. Over-use of plastic bags extremely causes pollution and damage to the environment. Plastic waste is recognized as one of the most troublesome categories of waste, and disposal of plastic waste has been blamed for shortening the life of landfill sites (Ishigaki et al., 2004). In addition, burning plastic can sometimes result in toxic fumes and risks to the health of the planet.

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The increasing of a waste problem from the usage of petrochemical polymers can be overcome with the use of bio composite. The material component such as natural fiber and biodegradable polymer has been used as the alternative materials in producing the new bio composites. It is safe to be used in food industry as the packaging material and in fact free from toxic. The challenge in producing food packaging is to match the durability of the packaging with the product shelf-life. Liu (2006) list up the factors that can degrade food quality which are the environmental temperature, relative humidity, presence of active bacterial and spoilage microorganisms and ultraviolet exposure. These factors also can influence the rate of degradation of the bio composite material.

There are several of bio-based materials which can be used as innovative applications in food-related packaging. These materials include starch, cellulose, and those derived from microbial fermentation. From all these material, Fama et al., (2009) state that starch is potential to be material for biodegradable plastics because it can form a continuous matrix besides as a renewable and abundant resource. Starch is the major carbohydrate in plant tubes and seed endosperm, where it is found as granules. Each granule contains amylopectin molecules together with a larger number of smaller amylose molecules (Canigueral et al., 2009). Corn, wheat, beets, sugar, potatoes and other plants, as well as vegetable oils are the main materials from which the bio composite is produced. According to Martins et al., (2009), starch can be converted into a thermoplastic material, known as thermoplastic starch (TPS), through the disruption of the molecular chain interactions under specific conditions, in the presence of a plasticizer.

Cellulose is the most abundant biopolymer on earth, recognized as the major component of plant biomass, but also as a representative of microbial extracellular polymers. Such as bacterial cellulose (BC) is synthesized by the *Acetobactor xylinum* bacteria. BC is been used widely in the production of bio composite (Shoda and Sugano, 2005). It is because it has remarkable mechanical properties in both wet and dry states, porosity, water absorbency, moldability, biodegradability and excellent biological affinity.

1.2 PROBLEM STATEMENT

Nowadays, the largest part of all materials used in the packaging industries is produced from fossil fuels and practically non-biodegradable. Plastics have been an environmental trepidation because of the non-degrade behaviour. The chemical constituents of plastic wastes can break down and release toxins that harm the environment, animals and the human being. The amount of plastic waste increases every year, but the exact time needed for its degradation is unknown. Chan-Halbrendt et al. (2009) analyzed the data around the world and conclude that around 500 billion plastic bags were used worldwide every year. Normally, a single plastic can take up to 1000 years, to decay completely. The

world is also running out of landfill space as degradation of plastics requires a long period of time and most of them end up to overburdening the landfill (Xu et al., 2005).

Bio composite is a suitable material that can substitute the usage of conventional plastic. However, the selection of raw materials and low cost and hold quality production is important in bio composite. Hence, this research will investigate the production of biodegradable composite from different composition of bacterial cellulose and starch. Starch composite will cause a weak structure of the bio composite. Thus, the additional of bacterial cellulose will improve the tensile strength of the bio composite.

1.3 OBJECTIVE

The objective of this study is to fabricate the bio composite from bacterial cellulose/starch.

1.4 SCOPE

The scopes of the study are:

1) To fabricate bio composite from bacterial cellulose/starch by using casting method by using different composition of bacterial cellulose (0, 7, 14, 21 and 28) wt% and starch (3, 6, 9, 12 and 15) wt%.

2) To characterize the bio composite film by using, Universal Testing machine, water absorption test, Fourier Transform Infrared (FTIR) Spectroscopy, Thermo Gravimetric Analysis (TGA) Scanning Electron Microscopy (SEM), Soil burial degradation test and biodegradation test by using *Aspergillus niger*.

1.5 RATIONAL AND SIGNIFICANCE

Biodegradable plastics are a new generation of polymers newly emerging in the market. The increasing demand for renewable and bio-based materials has shifts the consumer to prefer an eco-friendly packaging and driving the market for global biodegradable plastics. Among of all the market demand, the starch-based plastics have the largest share in bio composite products. It offers tremendous potential for food packaging, and becomes the largest demand due to the increase of consumer awareness for sustainable packaging. This is because it can degrade faster than the conventional plastics and not produce toxic when degrades in the landfills.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Bacterial cellulose and starch is a potential cheap feedstock for bio composite production. In the past, many literatures and papers had been explored to enhance the reliability and cost effective productivity of bio composite using bacterial cellulose and starch. This chapter will review on the production of bio composite and the main composition of composite which are bacterial cellulose (BC) and starch. Besides that, it also reviews on tests that have been used to study the characteristic of bio composite.

2.2 BACTERIAL CELLULOSE

Cellulose is the most abundant biopolymer on earth and recognized as the major component of plant biomass, but also as a representative of microbial extracellular polymers. Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1-4) linked D-glucose unit. Cellulose is synthesized by bacteria belonging to the genera of *Acetobacter, Rhizobium, Agrobacterium* and *Sarcina* (Jonas and Farah, 1998).

Bacterial cellulose (BC) is synthesized by several bacterial genera, of which *Acetobacter* strains are best known. An overview of BC producers with its structure of cellulose produced is shown in Table 2.1.

Table 2.1: Type of Genus Cellulose and its structure of bacterial cellulose.

Genus Cellulose	Structure of bacterial cellulose
Acetobacter	extracellular pellicle composed of ribbons
Achromobacter	fibrils
Aerobacter	fibrils
Agrobacterium	short fibrils
Alcaligenes	fibrils
Pseudomonas	no distinct fibrils
Rhizobium	short fibrils
Sarcina	amorphous cellulose
Zoogloea	not well defined

Source: Jonas and Farah (1998)

From the research conducted by Retegi et al. (2010), the most efficient producers are a Gram-negative, acetic acid bacterium which is *Acetobacter xylinum*. It has been applied as a model microorganism for basic and applied studies on cellulose and can produce cellulotic bio films. *Acetobacter xylinum* is an obligate aerobe bacterium usually found in vinegar, alcoholic beverages, fruit juices, fruits, and vegetables, and most likely in rotting ones as well (Klemm et al., 2001). BC microfibril have high mechanical properties including tensile strength and modulus high water holding capacity, moldability, crystalline, and biocompatibility (Retegi et al., 2010).

From the properties that have been stated, the production of BC is receiving great attention since they can be used in many fields, including pulp and paper industry (Keshk and Sameshima, 2005). Besides that, Chawla et al. (2009) claims that BC also can be used in various areas including textile industry, food, pharmaceutical, waste treatment, broadcasting, mining and refinery.

2.3 *ACETOBACTER XYLINUM*

Acetobacter xylinum is the most efficient producer of cellulose and has been recently reclassified and included within the novel genus of Gluconacetobacter, as *G. xylinus* together with some other species (*G. hansenii, G. europaeus, G. oboediens, and G. intermedius*). *Acetobacter xylinum* is a type of acetic acid bacteria that can synthesize cellulose when grown in a synthetic and complex medium containing glucose. *Acetobacter xylinum* is a rod shaped, aerobic and gram negative bacteria which has an ability to synthesize high quality of cellulose organized as twisting ribbon or microfibrillar bundle (Setyawati et al., 2007).

Acetobacter xylinum can convert various carbon compounds, such as hexoses, glycerol, dihydroxyacetone, pyruvate, and dicarboxylic acids, into cellulose, usually with about 50% efficiency. It will grow at the optimum temperature of 25-30℃ and pH range from 5.6 to 6.2 (Pourremezan et. al., 2009)

Figure 2.1 illustrated the scheme for the formation of bacterial cellulose from *Acetobacter xylinum*. The synthesis of cellulose in *Acetobacter xylinum* occurs between the outer membrane and cytoplasm membrane by a cellulose-synthesizing complex, which is in association with pores at the surface of the bacterium (Jonas and Farah, 1998). According to Klemm et al. (2001), the formation of cellulose begin when the glucan chain aggregates and consist of approximately 6–8 glucan chains are elongated from the terminal complexes. Then, these sub elementary fibrils are assembled to form micro fibrils and lastly it wills form a ribbon.

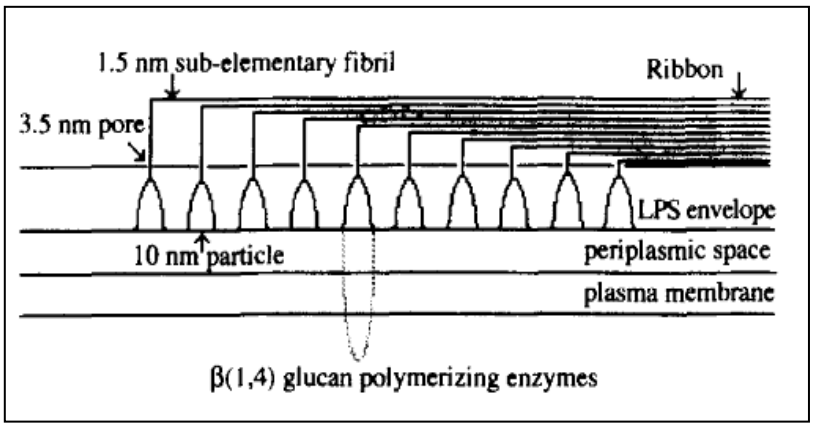

Figure 2.1: Scheme for the Formation of bacterial cellulose from *Acetobacter xylinum.*

Source: Jonas and Farah (1998).

2.4 BIO COMPOSITE

Bio composite is materials that consist of two or more distinct constituent to obtain complex chemical, mechanical and biological properties (Almeida et al., 2010). Biodegradable composites consist of biodegradable polymers as the matrix material and biodegradable fillers. High compatibility occurs between starch matrix and fillers due to the occurrence of intermolecular interactions formed between the different components in the bio composite (Lu et al., 2006). The use of cellulose crystallites as a filler for the preparation of high-performance composite has been explored extensively. When the cellulose crystallites are homogeneously dispersed into polymer matrices, they gave a remarkable reinforcing effect even at a few percent of their concentrations.

From research that conducted by Martins et al. (2009), starch is been used as raw material for development of bio composite. Besides that, incorporating plasticizer agent such as water and glycerol, can make the starch turn into thermoplastic called thermoplastic starch (TPS) or plasticized starch (PS) through destructurization by the introduction of mechanical and heat energy (Carvalho et al., 2003). Owing to hydrophilic attributes of starch, the internal interaction and morphology of starch will be readily changed by water

molecules, which allow starch to be successfully injection moulded to obtain TPS (Lu et al., 2009). On the other hand, the hydrophilicity of starch can be used to improve the degradation rate of some degradable hydrophobic polymers. The mixing of natural fiber and TPS will improve the mechanical properties and give good adhesion between reinforcing fibers and the matrix.

2.5 STARCH

Plants store glucose as the polysaccharide starch. Starch is one of the most promising renewable bio resources due to its versatility, competitiveness in price, and applicability to various industries (Liu et al., 2010). The cereal grains (wheat, rice, corn, oats, and barley) as well as tubers such as potatoes are rich in starch. According to Lu et al. (2009), starch is composed of amylose and amylopectin, which are both polysaccharides made up of α–D–glucopyranosyl units linked by (1-4) and (1-6) linkages. The ratio of amylase and amylopectin varies with the starch source, but it is typically 20:80 amylose to amylopectin. Figure 2.2 illustrated the amylase and amylopectin structure. Amylose is a straight chain polymer with an average of 200 glucose units per molecule while amylopectin consist of 1,000 glucose molecule arranged into a branched chain (Dufresne et al., 2000).

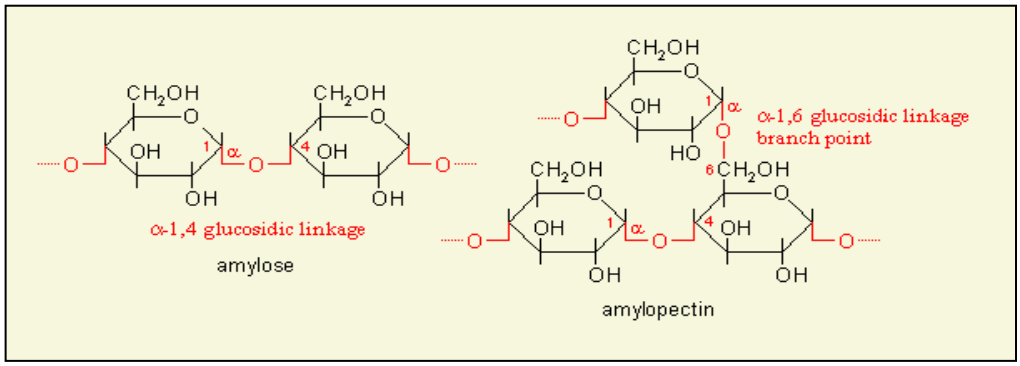

Figure 2.2: Section of a starch molecule (amylose and amylopectin)

Source: Keusch (2003)

Starch is one of the polysaccharides frequently used to develop edible films because it is a natural polymer that capable of forming a continuous matrix and it is a renewable and abundant resource (Bertuzzi et al., 2007). Moreover, starch also inexpensive polysaccharides, as well as biodegradable and nontoxic material. Flieger et al. (2003) draws a conclusion that converted starch to thermoplastic material offers an interesting alternative for synthetic polymers where long-term durability is not needed but rapid degradation is an advantage.

Edible films formulated with starch and glycerol had shown good mechanical properties when the tensile tests were performed (Fama et al., 2009). However, Flores et al. (2007) stated that the starch based films presented higher values of water vapor permeability and smaller elastic modulus than the ones of non-biodegradable packaging materials like polyethylene.

2.6 CHARACTERIZATION OF BIO COMPOSITE

There are several methods for characterization of the biodegradable films which are by using Universal Testing, water absorption test, Fourier Transform Infrared (FTIR), Thermo Gravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) and soil burial degradation test and biodegradation test by using *Aspergillus niger*.

2.6.1 UNIVERSAL TESTING

A Universal Testing Machine also known as a materials testing machine and can be used to test the tensile and compressive properties of materials. This type of machines is called Universal Testing Machine because it can perform all kinds of tests like compression, bending, and tension to examine the material in all mechanical properties. This machine is used for a wide range of industries including materials testers for plastics, elastomers, textiles, adhesives, films, concrete, construction materials, biomaterials, medical devices, ceramic, bone, and metals.

From the previous studies, the tensile strength and tensile modulus are higher for the bacterial cellulose with starch composites compared to those of the unreinforced starch. The tensile strength of bacterial cellulose with starch composites is 2.03 to 2.34 times higher compared to the pure starch when fiber loading is 7.8 wt % to 22 wt %. The tensile modulus increases by 111.7% to 132.4% respectively at 7.8 wt % to 22 wt % fiber loading. Additionally, from research conducted by Liu et al. (2010) it shows that the tensile strength and Young's modulus for starch composite with additional of bamboo cellulosic crystals (BCC) will increase when the content of BCC increased from 0% to 8%. Both increased sharply from 2.5 to 12.8 MPa, and from 20.4 to 210.3 MPa respectively. According to Tongdeesoontorn et al. (2011), the increasing tensile strength attributable to the formation of intermolecular interaction between the hydroxyl group of starch and carboxyl group of cellulose. However, the elongation at break decreased when BCC content increased. This is because the high content of BC fillers might contribute to retarding the intermolecular interaction of the starch films (Wittaya, 2009).

2.6.2 WATER ABSORPTION

In the case of a product packaging whose deterioration is related to its moisture content, the barrier properties of the package relate to the water vapour which will be one of major importance in extending shelf life (Alves et al., 2006). In the BC and starch film, both starch and BC are hydrophilic that can cause the composites become high moisture absorption. However, the chemistry similarity may result in good interface adhesion between the two components. Dufresne et al. (2000) has been supported by state that the presence of cellulose micro fibrils within the starch material can decreases the water sensitivity. The present of cellulose can prevent the moisture absorbance by changing the poor interface adhesion with strong fibre-matrix to resist the diffusion of water molecule along the interface (Wan et al., 2009). Figure 2.3 shows the moisture absorption curves of BC/starch bio composites with different BC contents

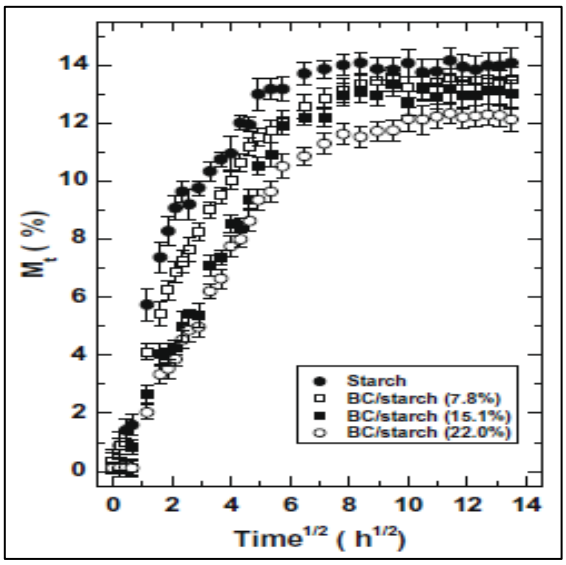

Figure 2.3: Water absorption curves of BC/starch bio composites with different BC contents.

Source: Wan et al. (2009)

From the figure, the water absorption of the composites increases linearly with $t^{1/2}$ in the initial stage of the absorption process. Then, the increasing rate slows down, and finally the water uptake become equilibrium and leads to a plateau at the graph. Moreover, the figure also showed the moisture uptake at equilibrium decreases with increasing BC fibre loading. According to Wan et al. (2009), the water absorption for the BC/starch composites depend on two aspects. Firstly, the starch has lower crystallinity and more bio-susceptible than cellulose. With the present of BC fiber in the starch composite, it absorbs less moisture then the pure starch. Secondly, the water absorption can decrease due to the presence of strong hydrogen bonding interactions between starch and cellulose crystallites and tends to stabilize the starch matrix.

Besides that, the amounts of starch in the composites also affect the percentages of water absorption. Figure 2.4 shows the water absorption of starch based on low density polyethylene blend.

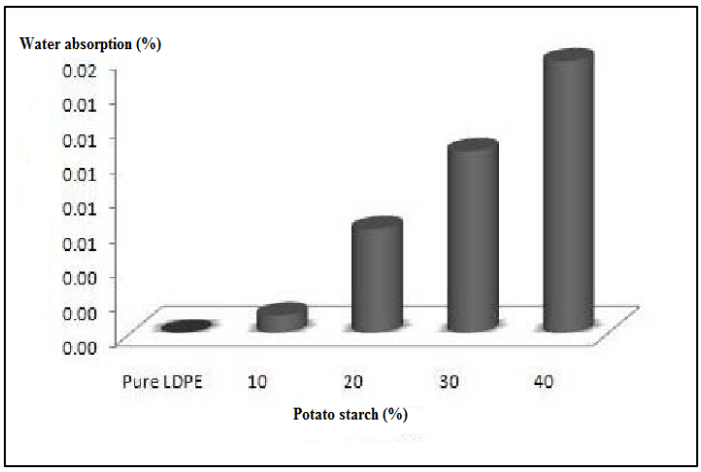

Figure 2.4: Water absorption of starch based low density polyethylene blend.

Source: Khoramnejadian (2011).

From the figure, water absorption increase linearly with the increasing amount of starch. According to Khoramnejadian (2011), starch based compound tends to absorb water because the hydroxyl group in starch makes the hydrogen bond with water molecules and also the water molecules trapped the OH group of starch.

2.6.3 FOURIER TRANSFORM INFRARED (FTIR)

Fourier Transform Infrared (FTIR) is a method where Infrared (IR) radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample, and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint, no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. The FTIR provides specific information about chemical bonding and molecular structures, making it useful for analyzing organic and inorganic materials.

Tables 2.2 illustrate the main wavelength absorption peaks of the Infrared (IR) spectra and the functional group that contain in each material. From this table, it shows the functional groups which have their specific range of IR spectra.

Table 2.2: The IR-Spectra of the functional group and stretching.

IR Spectra	Functional group
3600 cm^{-1}- 3000 cm^{-1}	Hydrogen bonding
3500 cm^{-1}- 3200 cm^{-1}	Hydroxyl group
3400 cm^{-1}- 3300 cm^{-1}	OH stretching, NH streching
2900 cm^{-1}	C-H stretches
1700 cm^{-1} - 1600 cm^{-1}	C=O (amide I)
1600 cm^{-1} - 1300 cm^{-1}	δ O-H bending of water, amide II and amide III
1200 cm^{-1} - 700 cm^{-1}	C-O bonding

Source: Mathew and Abraham (2008) and Bourtoom and Chinnan (2008)

According to Bourtoom and Chinnan, (2008), the FTIR spectra for the starch showed the stretching vibrations of the hydrogen bonding (O-H groups) and C–H stretching while for the bacterial cellulose it showed for hydroxyl groups stretching vibration, C-H stretching, and carbonyl group (C=O group). On the other hand, the research from Mathew and Abraham (2008) stated that the spectra for the chitosan film showed peak at O-H stretching, N-H stretching and C-O bond. In the test of the Bacteria Cellulose-Chitosan, the FTIR spectra ranging from 2800 to 1200 was for amide group, which existed in chitosan molecule. The wave number 1375, 1560 and 1650 cm^{-1} and a 1639 cm^{-1} was attributed to glucose carbonyl of cellulose (Phisalaphong and Jatupaiboon, 2008). Almeida et al. (2010) analyzed the peak near 3380 cm^{-1} was due to the OH-stretching and peaks at 2904–2879 cm^{-1} were assigned to C-H stretching.

2.6.4 THERMAL GRAVIMETRIC ANALYSIS (TGA)

Thermal Gravimetric Analysis (TGA) is a thermal analysis technique that has been used to measure changes in the weight loss of sample by increase the temperature consistently (Villain et al., 2007). According to Araujo et al. (2008), the decomposition of natural fibres occur in two or three stages of weight loss processes under controlled temperature between 25°C to 800°C.

The decomposition of the fibers is characterized by three peaks. The first one is attributed to the evaporation of water and occurs between room temperature and 150 °C. The second step, which corresponds to hemicellulose degradation, starts at about 190 °C; and the third step occurs between 290 and 360 °C, corresponding to the thermal degradation of cellulose. (Rosa et al., 2009). Lignin presents a broad peak throughout the range, degrading between 280 and 500 °C (Tomczac et al., 2007).

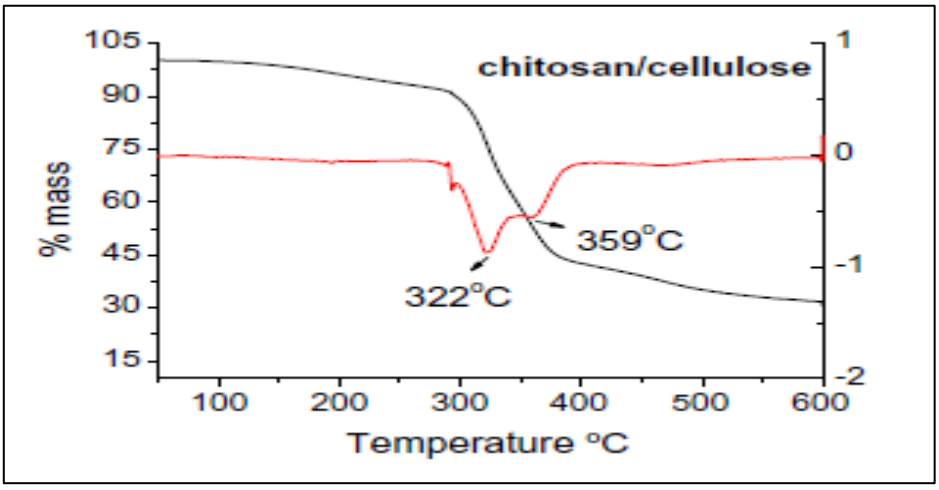

Figure 2.5: Thermal degradation of chitosan/cellulose film.

Source: Almeida et al. (2010)

Figure 2.5 shows the thermal degradation of chitosan/cellulose film. The thermal degradation of cellulose occurs between 250 and 350℃, which leads to depolymerization and the formation of 1,6-anhydroglucose (Almeida et al., 2010). The thermolysis reaction of cellulose occurs by the cleavage of glycoside bonds, C-H, C-O and C-C bonds in the component.

2.6.5 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning Electron Microscopy (SEM) permits the observation and characterization of heterogeneous organic and inorganic materials on a nanometer (nm) to micrometer (μm) scale. SEM uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens.

The layered structure is the characteristic for bacterial cellulose (BC) network that usually observed between the upper surface layer (exposed to air) and the lower layer (exposed to culture medium during cultivation) (Wan et al., 2009). From figure 2.6, shows the SEM images of surface morphology of BC film. As can be seen in the figure, BC pellicles have a networked structure of extremely fine and straight interconnected ribbon-like elements.

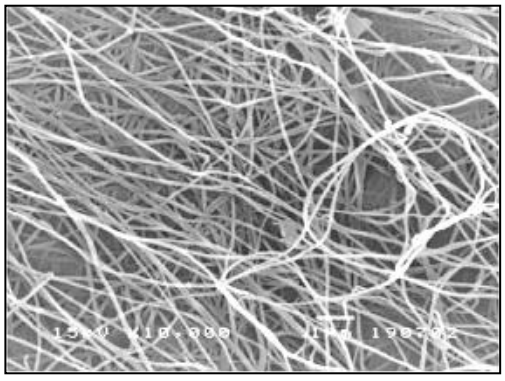

Figure 2.6: SEM images of surface morphology of BC film

Source: Saibuatong and Phisalophong (2010)

The structure of BC can be improved by mixed with starch because it can formed strong interfacial adhesion and homogeneous mixture. This homogeneous matrix of films is a good indicator for their structural integrity, and consequently good mechanical properties (Mali et al., 2002). Figure 2.7 showed the structure of thermoplastic starch/bacterial cellulose composite. From this figure, only small fragment of BC and not the isolated fibrils were observed. It was indeed observed that the characteristic nano-fibril and micro-fibril network of BC was maintained and totally impregnated with TPS (Martins et al., 2009).

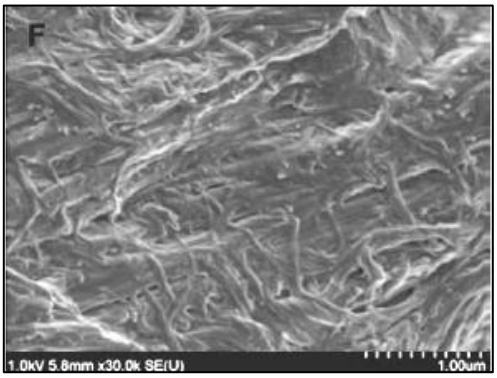

Figure 2.7: Thermoplastic starch (TPS)/bacterial cellulose (BC) composites (5 wt.%).

Source: Martins et al. (2009)

2.6.6 SOIL BURIAL DEGRADATION TEST

Degradation of bio composite in general, is defined as a detrimental change in its appearance, mechanical, physical properties and chemical structure. Any physical or chemical change in polymer as a result of environmental factors, such as light, heat, moisture, chemical condition or biological activity is termed as degradation of plastic. Moreover, biodegradation also can cause by biological activity such as bacteria and fungi.

The biodegradation of plastic proceeds actively under different soil conditions and depend on the structure of the material. From Wan et al. (2009), the weight loss of bio

composite showed an approximately linear relation with the degradation time. The average degradation rate was about 0.9% per day and 1% per day respectively, for the starch with bacterial cellulose composites. According to Mostafa et al. (2010), biodegradation of starch based polymers occurred between the sugars groups leading to a reduction in chain length and the splitting off monosaccharide, disaccharide, and oligosaccharide units as a result of enzymatic attack at the glucosidic linkages.

From the study conducted by Wan et al. (2009), soil burial experiments were performed for the composite containing 15.1 wt. % BC fibres, as well as the unreinforced starch. Figure 2.8 presents the weight loss of BC/starch bio composite (15.1 wt. % BC) and starch as a function of soil burial time

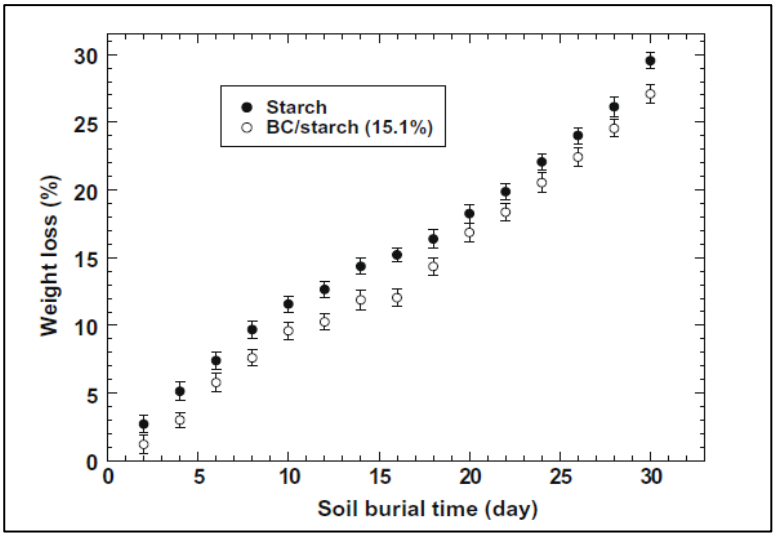

Figure 2.8: Weight loss of BC/starch bio composite (15.1 wt. % BC) and starch as a function of soil burial time.

Source: Wan et al. (2009)

Note that the weight loss showed an approximately linear relation with degradation time for both starch and BC/starch composite. It can be observed that the weight loss of the BC/starch composite were lowered than the starch alone at any given time points. This was

because BC molecules had two regions where one of it was called as ‘crystalline cellulose’ that composed of highly-oriented molecules, and the other one, was called as ‘amorphous cellulose’, that comprises of less-oriented molecules (Wan et al., 2009). Alvarez et al. (2006), claimed that the crystalline regions that formed by bacterial cellulose were difficult to degrade because of the interspaced forming between the fiber of bacterial cellulose and starch.

2.6.7 BIODEGRADATION TEST BY USING *ASPERGILLUS NIGER*

Degradation of waste plastics through microorganism represents one of the alternatives solutions to deal with the environmental and waste problems. Microorganisms such as bacteria and fungi can be used in the degradation of both natural and synthetic plastics. *Aspergillus niger* is a filamentous fungus growing aerobically on organic matter (Schuster et al., 2002). Fungi are able to degrade in a wide variety of polymer through the production of several enzymes such as cellulase and amylase (Geweely and Ouf, 2011). The fungi secrete enzymes that break down the plastic polymer into its molecular building blocks which are utilized as a carbon source for growth. Microbial degradation occur when fungi consuming the material, which leads to increase the porosity, void formation and the loss of integrity of the plastic matrix (Kiatkamjornwong et al., 1997).

The microbial degradation of polymeric materials was carried on by incubating it with *Aspergillus niger* strain which recognized by its ability to grow and degrade in a broad range of substrates. The *Aspergillus niger* showed a potential for breaking down the complex cellulose and rapid conversion to soluble sugars (Iyayi and Losel, 2001). Besides that, it also shows an ability to hydrolyze the starch compounds. Both amorphous and crystalline regions exist in starch. The semicrystalline polymer in starch was tightly packed and less accessible to degradants, which must to diffuse into the regions that need it to be effective (Kiatkamjornwong et al., 1997). Therefore, the amorphous region will degrades first, leaving the remaining more crystalline region.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

In this chapter, it was presented the detail procedure that used to produce bio composite from bacterial cellulose and starch. The film was characterized by using Universal Testing Machine, water absorption, Fourier Transform Infrared (FTIR), Thermo Gravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) and Soil Burial Degradation, biodegradation by using *Aspergillus niger* .

3.2 MATERIAL

The stock culture of *Acetobacter xylinum* was supplied by Malaysia Agricultural Research and Development Institute, Serdang Selangor. The reagent, Starch, Ammonium Sulfate (NH_3SO_4), Sodium Hydroxide (NaOH), Glycerol, Acetic Acid, and ethanol were purchased from Sigma Chemical Co. St. Louis.

3.3 EXPERIMENTAL PROCEDURE

Figure 3.1, showed the overall procedures that had been done to fabricate bio composite film using bacterial cellulose and starch. The first step was the preparation of bacterial cellulose from *Acetobacter xylinum* using coconut water culture medium. In the medium culture, sucrose, ammonium sulfate and acetic acids were added. Then, the production of bio composite was characterization by Universal Testing Machine, water

absorption test, Fourier Transform Infrared (FTIR), Thermo Gravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) and soil burial degradation test and biodegradation test by using *Aspergillus niger*.

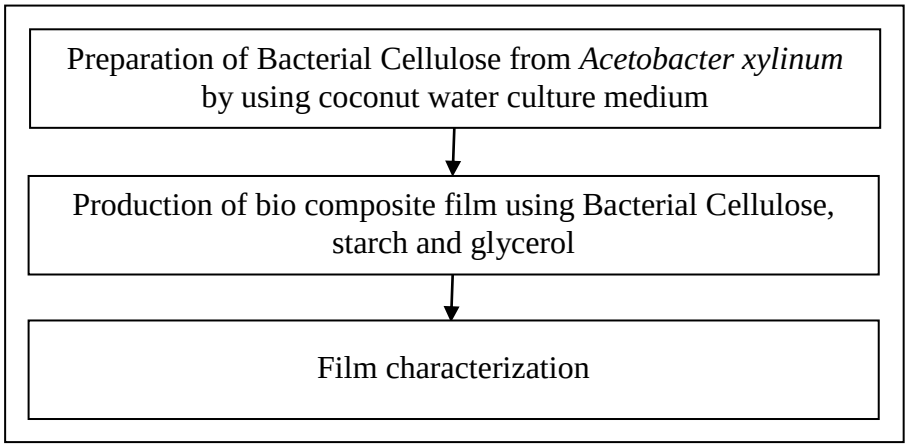

Figure 3.1: Experimental procedure for fabrication of bio composite film

3.3.1 PREPARATION OF BACTERIAL CELLULOSE

Bacterial cellulose was obtained from incubation of *Acetobacter xylinum* in a culture medium. The culture medium for the inoculum was coconut-water containing 8.0% of sucrose, 0.5% of ammonium sulfate ($(NH_4)_2SO_4$) and 1.0% of acetic acid. The medium was sterilized at 121℃ for 20 min. The 100 mL of a stock culture was inoculated into 1000 mL of medium and incubated at 30℃ for 7 days in static culture. After 7 days a white membrane was formed at the medium interface. Then, the membrane was purified by washing with deionize (DI) water and then treated with 1% (w/v) of sodium hydroxide solution (NaOH) at room temperature for 24 hours to remove the bacterial cell. Lastly, it followed by a rinse with deionize (DI) water until the pH of the membrane become neutral (Phisalaphong and Jatupaiboon., 2008). From Figure 3.2, it shows the layer of bacterial cellulose in the medium culture that contains *Acetobacter xylinum*.

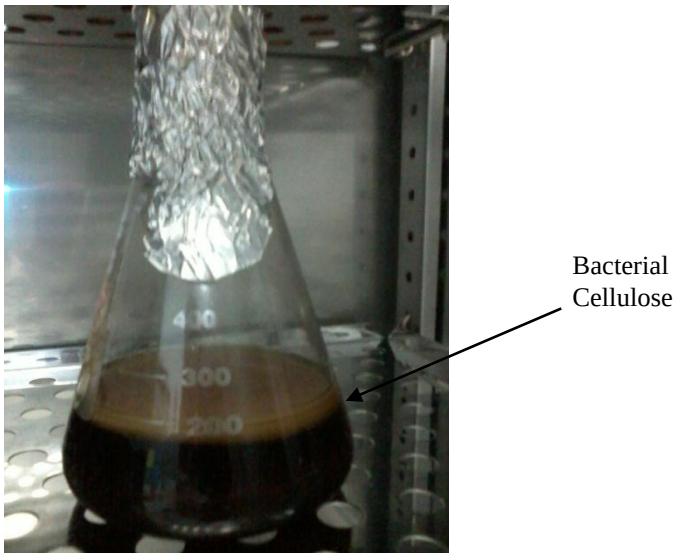

Figure 3.2: *Acetobacter xylinum* culture with bacterial cellulose.

3.3.2 PREPARATION OF BIO COMPOSITE FILM

A series of starch and bacterial cellulose blend was prepared by mixing 100 mL of the starch solution (3, 6, 9, 12, and 15) wt % and blend bacterial cellulose (0, 7, 14, 21 and 28) wt %. The starch and bacterial cellulose were mixed and stirred by using magnetic stirrer for 1 h at temperature 80 ℃ until the solution becomes gelatinized. Glycerol was added about 2.4% (w/w) of the total solid weight in the blend solutions. The resulting solution was put in the Ultrasonic Cleaner at 85% power for one hour to remove bubbles. Then, the solutions was poured into Petri dish and dried at 60℃ for 4 h (Saibuatong and Phisalaphong, 2010). After the film completely dried, the film then been peeled off from the Petri dish.

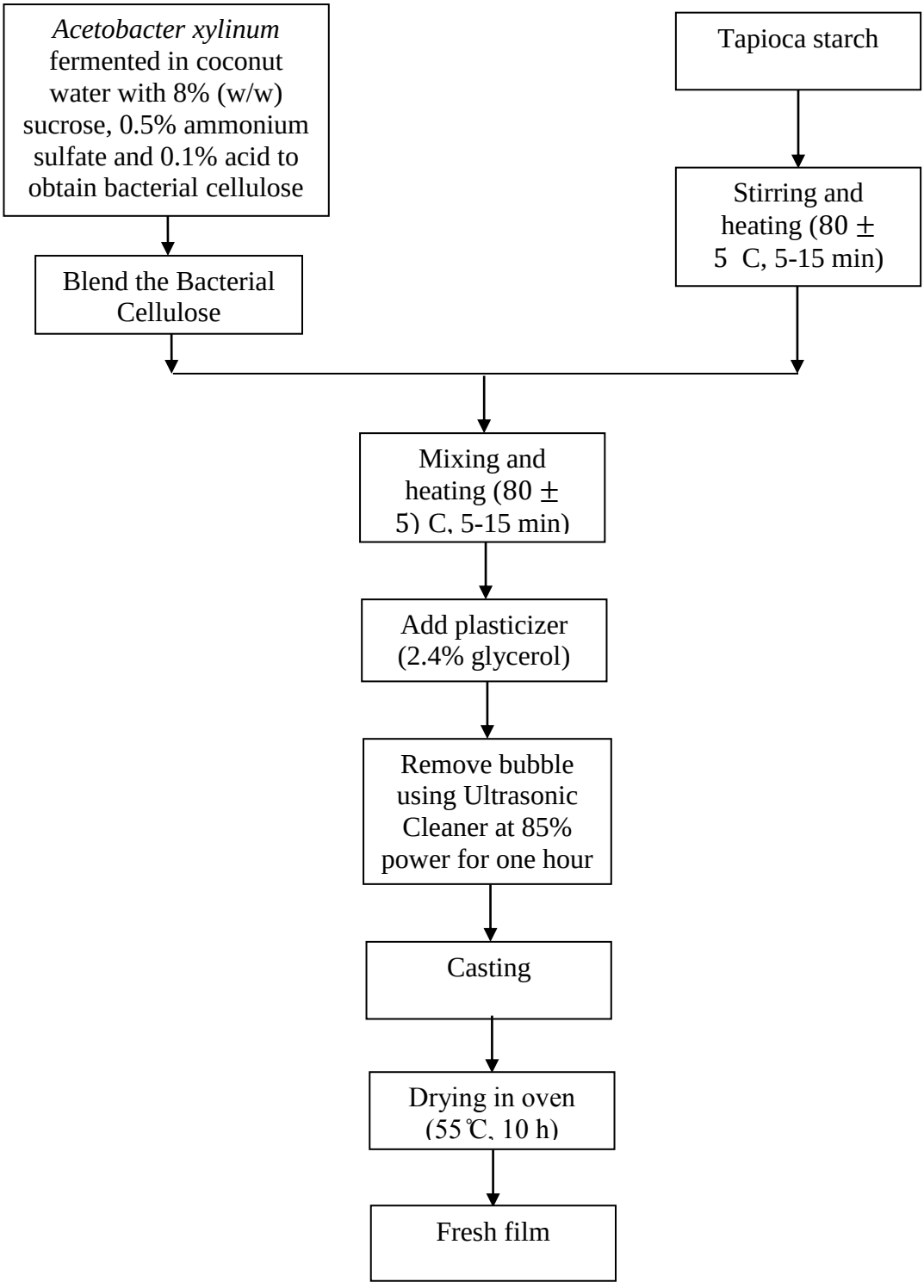

Figure 3.3: The films formation process.

3.3.3 FILM CHARACTERIZATION

3.3.3.1 UNIVERSAL TESTING MACHINE

Universal testing machine was used to test the tensile and compressive properties of materials. The test was performed using a Universal Testing Machine brand Shimadzu according to the standard ASTM D5026-01. The device was equipped with the crosshead 5kN shifting at 50 mm/minimum rate. The sample was cut into rectangular shape with 76 mm long and 13 mm width (ASTM Standard, 2001). The distance between grips is approximately 64 mm.

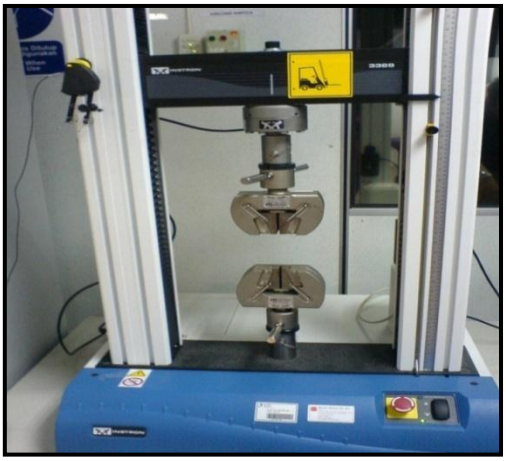

Figure 3.4: The Universal Testing Machine 50kN.

3.3.3.2 WATER ABSORPTION TEST

Water absorption measurements were performed where the dried film was immersed completely in deionize water at the room temperature. The film samples were cut into 3cm x 1cm (Wan et al., 2009). After immersed in the water, the samples were taken out and the excess water on the surface was wiped off with filter paper until reached a constant weight. The weight readings were taken for every 10 minutes interval until the

constant weight obtained. The water absorption was determined by using equation 1 as below:

$$\text{Water absorption} = \frac{W_h - W_o}{W_o} \times 100 \quad (1)$$

Where W_o weight of starch/bacterial cellulose is film at the absorbing equilibrium and W_t is the dry weight of starch/bacterial cellulose film. The water absorption can be described by Fick's second law of diffusion, which is given by using equation 2:

$$\frac{M_t}{M_m} = 1 - \frac{8}{\pi^2} \sum \frac{1}{(2n+1)^2} \exp\left\{ \frac{-D(2n+1)^2 \pi^2 t}{h^2} \right\} \quad (2)$$

Where M_t and M_∞ are the moisture content at time t and at equilibrium, respectively. D is the diffusion coefficient and h is the sample thickness. At the initial absorption $\frac{M_t}{M_\infty} \leq 0.5$ the equation can be reduced to by using equation 3:

$$\frac{M_t}{M_m} = \frac{4}{\pi^{1/2}} \left(\frac{Dt}{h^2} \right)^{1/2} \quad (3)$$

The equation can be re-written as equation 4:

$$M_t = kt^{1/2} \quad (4)$$

Where k is the slope of the M_t vs. $t_{1/2}$ curve and D can be calculated through equation 5:

$$k = \frac{4M_t}{h} \left(\frac{D}{\pi} \right)^{1/2} \quad (5)$$

3.3.3.3 FOURIER TRANSFORM INFRARED (FTIR)

Fourier transform infrared (FTIR) spectroscopy brand Thermo Electron Corporation was used to identify the chemical bonds and specific functional group of the films. The films were cut into strip-shaped specimen 2cm x 2cm in size, and the FTIR spectra of the films were measured in the wave length range from 1000 to 4000 cm-1 (Phisalaphong and Jatupaiboon., 2008). Spectral output was recorded in absorbance as a function of a wave number.

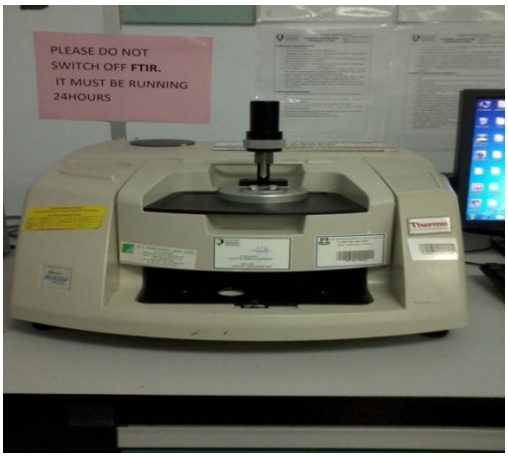

Figure 3.5: Fourier Transform Infrared (FTIR) spectroscopy

3.3.3.4 THERMO GRAVIMETRIC ANALYSIS (TGA)

The analysis was carried out by using Thermo gravimetric analysis (TGA) brand TGA Q500 instrument which approximately 5 mg of sample film was weighed and heated from room temperature to 800℃ at 20℃ min^{-1} under nitrogen flow rate of 20 mL min^{-1}.The data was processed using the powerful Thermo Scientific TGA Thermal Analyst software (Almeida et al., 2010).

Figure 3.7: Thermo Gravimetric Analysis (TGA)

3.3.3.5 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning Electron Microscopy (SEM) brand Zeiss Evo 50 was used to generate high-resolution images of surface and cross section of the film. The films' sample was froze in liquid nitrogen. Immediately, the sample was snapped; vacuum dried and then sputter with gold (Saibuatong and Phisalaphong, 2010). The cross-section of SEM was observed with 100 x resolution for cross section and 250 x resolutions for surface.

Figure 3.8: Scanning Electron Microscopy (SEM)

3.3.3.6 SOIL BURIAL DEGRADATION TEST

Soil burial degradation experiments were carried out at an ambient temperature under moisture controlled conditions. The film was cut into 2 cm x 2 cm, and the specimen was placed in garden poli bag in medium size, which filled with soil. The specimen was burying in the soil at a depth of 5 cm which regularly moistened with distilled water to maintain the humidity. The degradation of the specimen was determined in10 days interval for about 30 days. After that, the sample was removed and carefully washed with distilled water for several times in order to ensure the degradation stopped (Wan et al., 2009). Then, the sample was dried at room temperature and weighted using analytical balance to determine the weight loss and the film also checked by using Fourier Transform Infrared (FTIR) to determine the changes on chemical bonds and specific functional group of the films. The weight loss was determined by using equation 6 as follows:

$$\text{Weight loss} = \frac{W_o - W_t}{W_o} \times 100 \qquad (6)$$

Where W_o was the initial mass and W_t was the remaining mass at any given time, t. The graph was plotted with percentages weight losses of the specimens versus composition of BC to know the effect of weight loss when the composition of BC was changed.

3.3.3.7 BIODEGRADATION TEST BY USING *ASPERGILLUS NIGER*

The microbial degradation of polymeric materials was carried on by incubating with *Aspergillus niger* strain recognized for the ability to grow and degrade a broad range of substrates (Jecu et al., 2008). The *Aspergillus niger* could change the bio composite surface from smoother to rougher and also to disrupt the bio composite structure (Stoica-Guzun et al., 2011). The bio composite film was incubated in the dextrose agar potato medium that contained *Aspergillus niger*. The nutrient agar powder was weighed according to the specification stated on the bottle and dissolved in the distilled water. The medium was prepared by autoclave at 121°C for 20 minutes. After autoclave, the sterile agar medium

was poured into petri dish inside the laminar flow hood and near the flame. The *Aspergillus niger* was subculture by dispersing them using sterile inoculating loop onto the agar medium. Then, the sample was cut into 2cm x 2cm and put in the middle of the disposal petri dish. The microbial was grown in the incubator at temperature 30^{o}C and it was leaved for 5 days. After 5 days, the sample was dried at room temperature and weighted using analytical balance to determine the weight loss of the film and also the change on chemical bond also proved by using Fourier Transform Infrared (FTIR). The weight loss was determined by using equation 7 as below:

$$\text{Weight loss} = \frac{W_o - W_t}{W_o} \times 100 \qquad (7)$$

Where W_o was the initial mass and W_t was the remaining mass at any given time, t. The graph was plotted with percentages weight losses of the specimens versus composition of BC to know the effect of weight loss when the composition of BC was changed.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTRODUCTION

This chapter provides a detail discussion about the results testing and analysis of a bio composite from bacterial cellulose (BC) and starch by using Universal Testing Machine, water absorption test, Fourier Transform Infrared (FTIR), Thermo Gravimetric Analysis (TGA),Scanning Electron Microscopy (SEM) and soil burial degradation test and biodegradation test by using *Aspergillus niger*. The results obtained were compared with the findings from previous researchers

4.2 UNIVERSAL TESTING

The effect of different concentration of starch and bacterial cellulose content in the film was investigated by using universal testing machine. Table 4.1 show the tensile properties of bacterial cellulose (BC) and starch bio composite and the effect of starch and bacterial cellulose (BC) concentration on tensile strength (TS), tensile modulus (TM) and elongation of break (ε) are shown in Figure 4.1, Figure 4.2 and Figure 4.3.

Table 4.1: Tensile properties of bacterial cellulose (BC) and starch bio composites.

Starch	Bacterial Cellulose (BC)	Strength (MPa)	Young's Modulus (MPa)	Elongation (%)
3g	0%	2.1752	10.8752	106.3596
	7%	3.31537	10.1303	48.7199
	14%	4.509	10.4092	46.2196
	21%	5.49902	14.2969	27.0530
	28%	7.29044	17.1184	22.1570
6g	0%	2.47734	11.5576	103.9075
	7%	3.51967	12.263	37.1573
	14%	4.5985	17.5451	31.7612
	21%	5.61311	19.5793	20.0321
	28%	7.42813	48.221	19.1155
9g	0%	3.08694	13.7063	93.5210
	7%	3.72234	13.0996	28.0322
	14%	5.49818	21.6789	27.5322
	21%	5.75623	22.0726	17.4070
	28%	10.2871	67.6269	16.6365
12g	0%	3.5051	15.0516	85.8660
	7%	3.97446	15.7733	24.8657
	14%	6.62599	25.0088	22.2822
	21%	7.08836	29.2578	15.5740
	28%	12.023	73.6311	14.6781
15g	0%	5.55498	17.4465	61.2410
	7%	6.26469	21.3016	21.7405
	14%	7.40901	36.3934	18.1571
	21%	9.40598	38.362	13.2197
	28%	18.5357	81.8168	12.8655

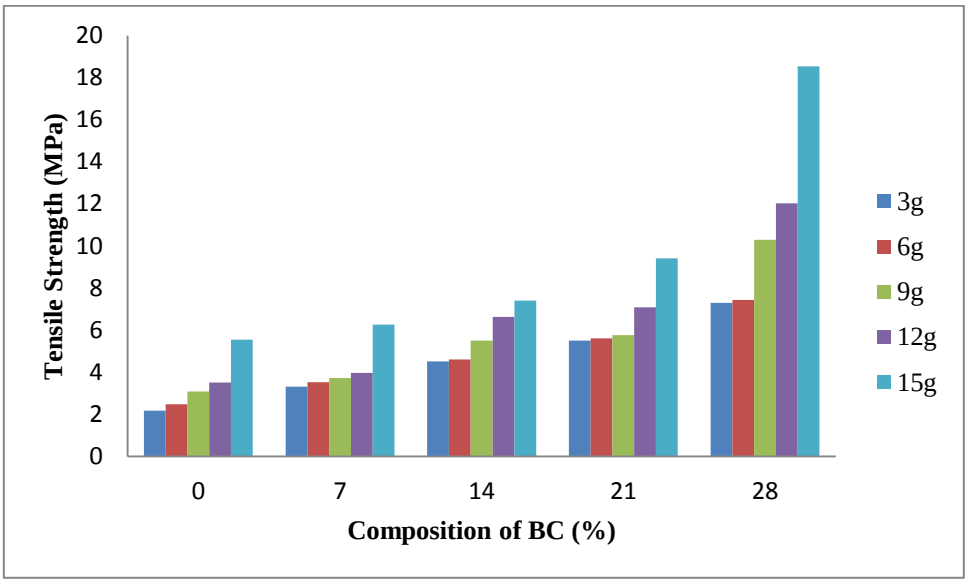

Figure 4.1: The tensile strength (TS) of bio composite with different starch and bacterial cellulose (BC) contents.

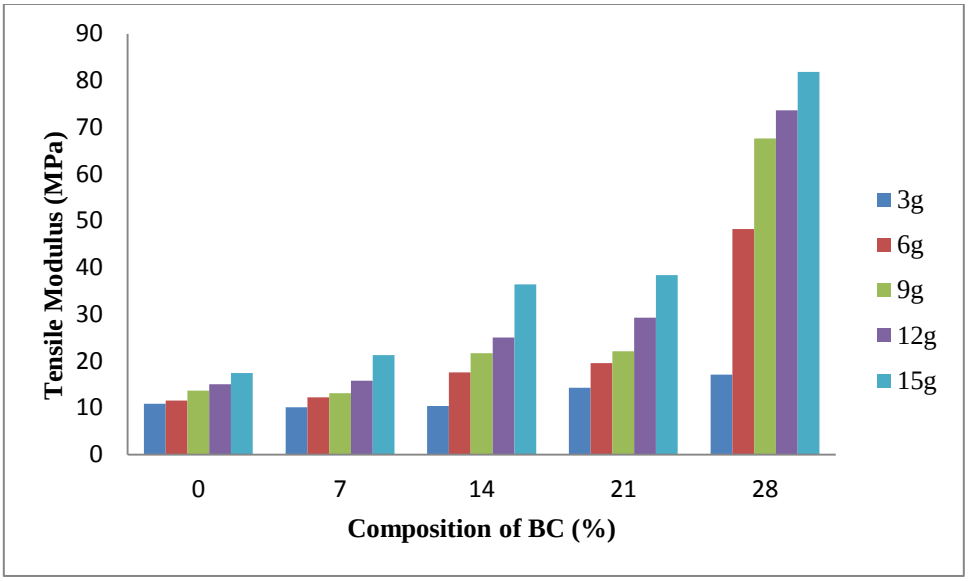

Figure 4.2: The Tensile Modulus (TM) of bio composite with different starch and bacterial cellulose (BC) contents.

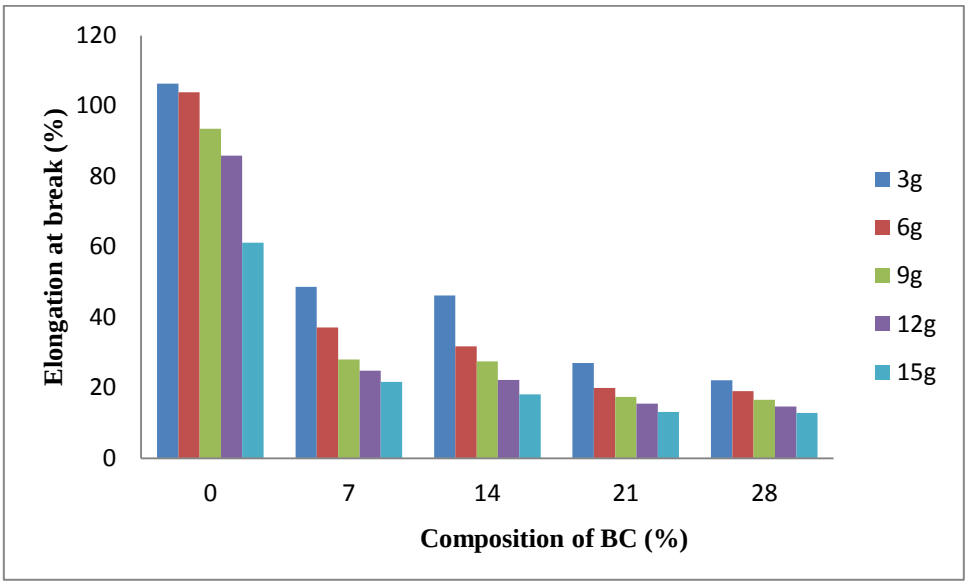

Figure 4.3: The elongation at break (ε) of bio composite with different starch and bacterial cellulose (BC) contents.

According to Wan et al. (2009), the tensile strength (TS) and tensile modulus (TM) are higher for the composites that contain BC compared to starch composite. But, the elongations at break (ε) are decrease when the BC content is increase (Martins et al., 2009). This is because the orientation and degree of interaction between micro fibrils within the film. The BC films showed high modulus value attributed to the uniform, continuous and straight nanoscale network of cellulosic elements in-plane oriented via the compression of BC pellicles (Nakagaito et al. 2005).

Figure 4.1 shows the tensile strength (TS) of the bio composite films for 3g starch increased from 2.1752 MPa to 5.4990 MPa and at 15g starch, the tensile strength (TS) increase from 5.5549 MPa to 18.5357 MPa when increasing the BC content from 0 to 28%. In the other hand, Figure 4.2 shows the tensile modulus (TM) of bio composite film for 3g starch also increase from 10.8752 MPa to 17.1184 MPa and at 15g starch, it increase from 17.4465 MPa to 81.8168 MPa when the BC content increase from 0% to 28%. When the increasing amount of BC content, it indicated the high compatibility occurs between starch matrix and BC fillers and the performances due to hydrogen bonds network formed

between different components (Wittaya, 2009). Besides that, it supported by Tongdeesoontorn et al. (2011) that indicate the increasing tensile strength (TS) also attributable to the formation of intermolecular interaction between the hydroxyl group of starch and carboxyl group of cellulose.

Regarding the elongation at break (ε), Figure 4.3 showed that increases in BC fillers from 0 to 28% for 3g starch provided a decrease in ε from 106.3596% to 22.1570% and at 15g starch, the ε decrease from 61.2410% to 12.8655%. The decreasing in elongation at break (ε) is possibly due to the presence of high content of BC fillers might contribute to retarding the intermolecular interaction of the starch films (Wittaya, 2009).

4.3 WATER ABSORPTION TEST

In water absorption test, the mass of absorbed penetrate is measured as a function of time. The behavior of bacterial cellulose (BC) and starch bio composites was measured and evaluated using the Fick's law equations. Table 4.2 shows the percentage of water absorption and diffusivity for various compositions of BC/starch bio composites and Figure 4.4 show the water absorption curves of BC/starch bio composites with different starch and BC contents.

Table 4.2: Percentage of water absorption and diffusivity for bacterial cellulose (BC) and starch bio composites

Starch	Bacterial Cellulose (BC)	Water absorption (%)	Diffusivity, D x10^6 (mm^2/min)
3g	**0%**	53.88	4.50
	7%	46.21	4.42
	14%	39.6	4.30
	21%	37.25	4.19
	28%	34.34	4.15
6g	**0%**	64.73	4.50
	7%	56.31	4.36
	14%	54.38	4.18
	21%	49.01	4.18
	28%	47.45	4.18
9g	**0%**	84.42	4.99
	7%	74.51	4.81
	14%	68.48	4.61
	21%	63.77	4.39
	28%	61.4	4.38
12g	**0%**	115.79	5.09
	7%	95.17	4.89
	14%	87.23	4.85
	21%	76.26	4.55
	28%	75.5	4.42
15g	**0%**	146.21	5.25
	7%	120.96	5.03
	14%	104.8	4.86
	21%	89.33	4.64
	28%	86.09	4.63

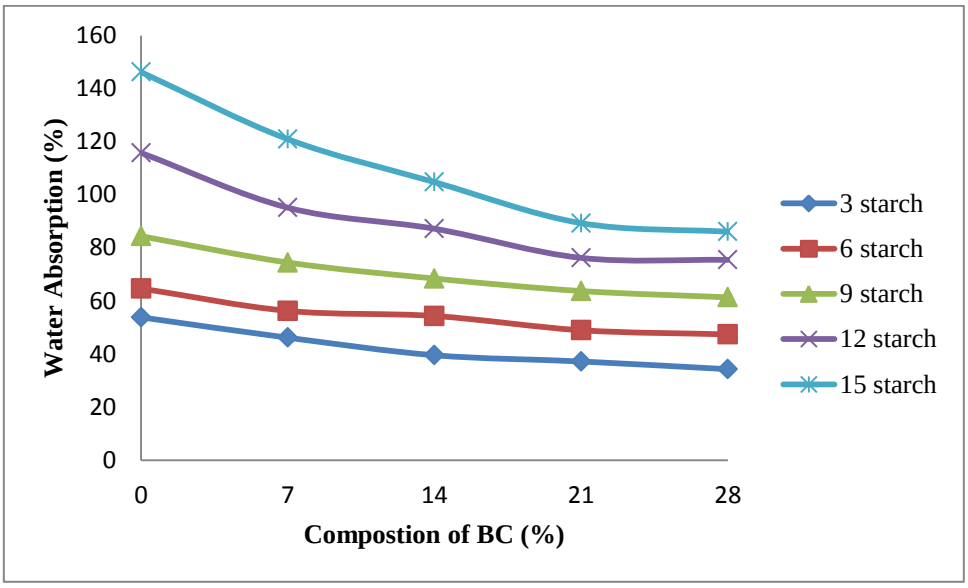

Figure 4.4: Water absorption curves of BC/starch bio composites with different starch and BC contents

Table 4.2 shows the percentage of water absorption and diffusivity (D) of different composition of starch and BC bio composite. From the table, water absorption and diffusivity (D) for all samples were decreased when the composition of BC was increased. The value of diffusivity (D) was high at bio composite with 0% of BC and it will decrease linearly with the increasing of composition of BC from 0% to 28% of BC. From figure 4.4, it shows the percentage of water absorption increased when the composition of starch increased. The figure shows the percentage of water absorption was increased from 53.88% to 146.21% when the composition of starch was increased from 3g to 15g. This is due to the starch based compound tends to absorb water because the hydroxyl group in starch makes the hydrogen bond with water molecules and the water molecules trapped the OH group of starch (Khoramnejadian,2011).

However, from figure 4.4 the percentages of water absorption were decreased when the composition of BC increased. For 3g starch, the percentage of water absorption dropped from 53.88% to 34.34% while at 15 g starch the percentage of water absorption dropped from 146.21% to 86.09% when the composition of BC was increased from 0% to28%.

According to Martin et al. (2009), both of BC and starch were hydrophilic but the starch molecule is more hydrophilic than BC. Due to the hydrophilic properties of both components, the bio composite will absorb more water when the amount of starch and BC were increased. However, the chemistry similarity may result in good interface adhesion between the two components, which can prevent moisture absorbance and strong fibre–matrix adhesion resists diffusion of water molecules along interfaces (Wan et al., 2009).

4.4 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) TEST

Fourier Transform Infrared **(**FTIR) spectroscopy was used to determine the interactions between bacterial cellulose (BC) and starch. FTIR spectroscopy has often been utilized as a useful tool in determining specific functional groups or chemical bonds that exist in a material (Phisalaphong and Jatupaiboon, 2008). The infrared spectrums of films with different BC contents were shown in Figure 4.5 while Figure 4.6 was showed the infrared spectrums films with different starch contents.

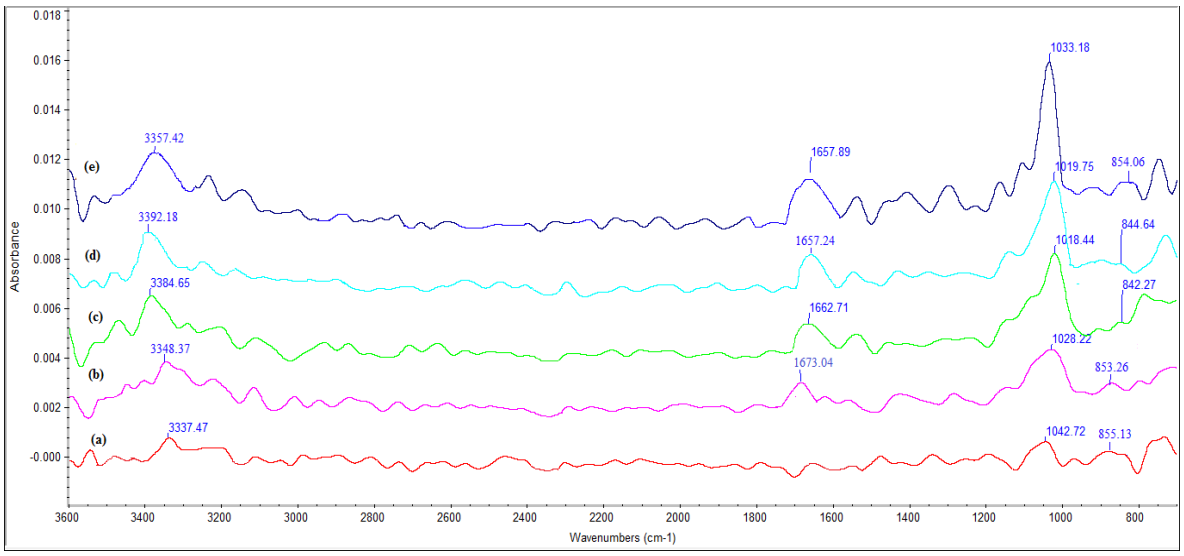

Figure 4.5: Infrared spectra of (a) 9 g starch and 0% BC film; (b) 9 g starch and 7% BC film; (c) 9 g starch and 14% BC film; (d) 9 g starch and 21% BC film; (e) 9 g starch and 28% BC film

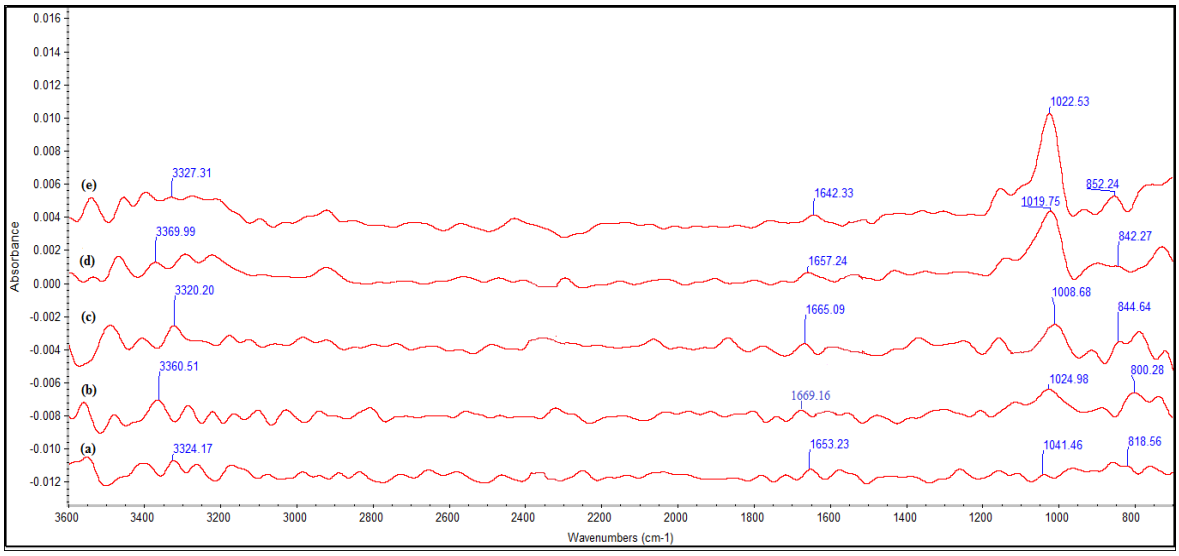

Figure 4.6 : Infrared spectra of (a) 3 g starch and 14% BC film; (b) 6 g starch and 14% BC film; (c) 9 g starch and 14% BC film; (d) 12 g starch and 14% BC film; (e) 15 g starch and 14% BC film

Figure 4.5 and 4.6 shows the FTIR spectra at different sample in the wavelength range of 700 to 3600 cm^{-1}. In the starch film, the band at 3000 to 3600 cm^{-1} corresponds to the hydrogen-bonded hydroxyl groups (Mathew and Abraham, 2008). In this region, the intense absorption in the spectrum of the cellulose was at the band between 1600 until 1700 cm^{-1}, which has been assigned to carbonyl groups (Bourtoom and Chinnan, 2008) and the strong band at 700 until 1200 cm^{-1} was assigned to skeletal vibrations involving the C-O bonding (Almeida et al., 2010).

From figure 4.5 and 4.6, shows the increasing in peak spectrum at 3320 to 3392 cm^{-1} due to the interaction between the OH bonds of cellulose and starch. Besides that, the peak at 818 to 1042 also increase when the content of starch or BC increase attributed to the stretching vibrations of the C–O stretching vibrations in starch (Cao et al., 2008).The characteristic IR band at 1647 cm^{-1} to 1669 cm^{-1} corresponded to the C=O vibration of bacterial cellulose. Figure 4.5 shows the peak for C=O bonding with the increasing amount of BC content from 7% to 28%.

4.5 THERMO GRAVIMETRIC ANALYSIS (TGA)

Thermo gravimetric analysis (TGA) of the composite materials was carried out to assess their thermal stability and degradation profiles. The TGA was conducted in order to assess the effect of different composition of bacterial cellulose (BC) and starch on the thermal properties of bio composites. The thermo gravimetric result for the composites was shown in Figure 4.7, 4.8 and 4.9.

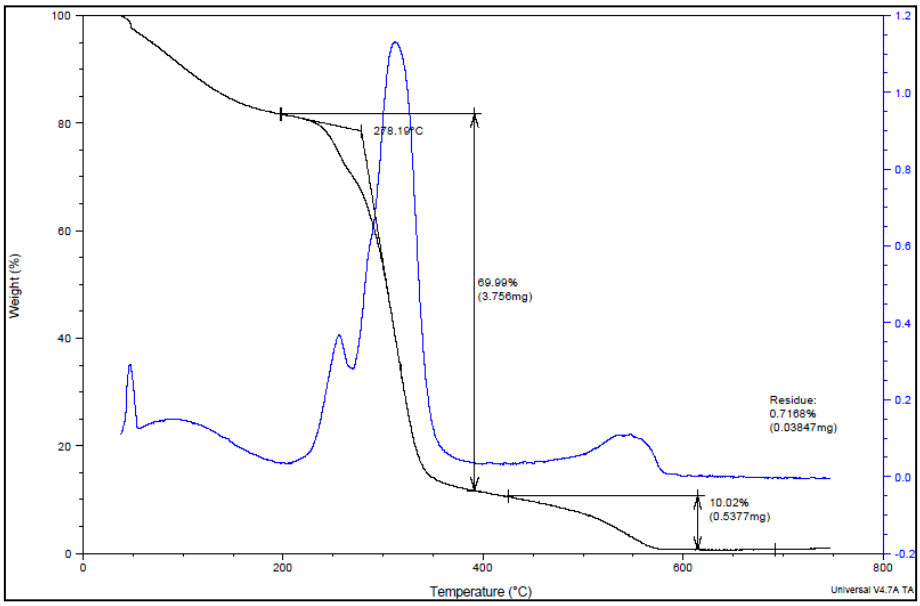

Figure 4.7: Thermo gravimetric curve of film with 9g starch and 0% bacterial cellulose (BC)

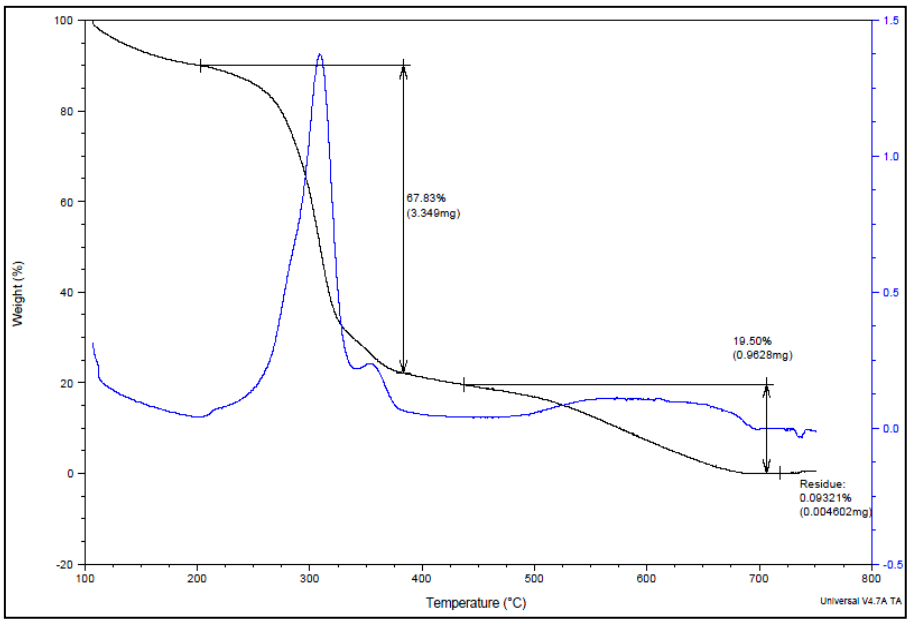

Figure 4.8: Thermo gravimetric curve of film with 9g starch and 14% bacterial cellulose (BC)

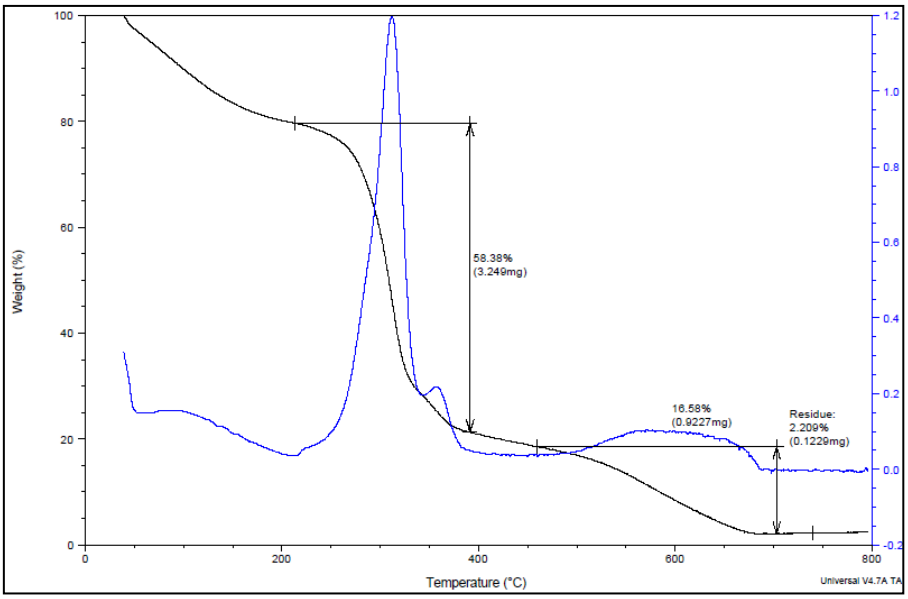

Figure 4.9: Thermo gravimetric curve of film with 6g starch and 14% bacterial cellulose (BC)

From the entire figure, it shows three weights loss processes which are below 280 °C, between 280 and 350°C and above 350 °C. At the temperature below 280°C, the weak loss of weight occur for all sample. According to Martin et al. (2009), the loss was related to the volatilization of water and glycerol in the bio composite. When the temperature keep arise, there are 2 peaks of degradation can be seen. The starch degradation peak in figure 4.7 was because the different degradation rate of amylase and amylopectine (Mano et al., 2003). But, there are slightly different for figure 4.8 and 4.9 because it contains starch and bacterial cellulose (BC). The first peak can be assigned to the decomposition of starch and the second one is related to the decomposition of cellulose (Alvarez & Vazquez., 2004) and it begin when the temperature arise to 280 °C.

From figure 4.7, the weight loss for sample with 9 g starch and 0% BC is 69.99%. This loss was higher than sample with 9g starch and 14% BC and with losses 67.83% and 58.38% for sample that contain 6g starch and 14% BC. The different between the losses was due to the amount of starch that contain in the sample. Besides that, the addition of 14% BC fibers to the starch composite, resulted in a slight increase in the thermal stability of the composites which can be explained by the higher stability of the cellulose substrates and particularly by the excellent compatibility between the two carbohydrate components of the composites (Martin et al., 2009)

For the figure 4.8 and 4.9, as the temperature increase above 350 °C, the thermal degradation rates of cellulose increased. This takes place when the cellulose structure has absorbed enough energy to activate the cleavage of the glycosidic linkage to produce glucose, which is then dehydrated to levoglucosan (1, 6-anhydro-ß-D-glucopyranose) and oligosaccharides (Levan, 1989). As temperature increases to around 680°C, the production of volatile compounds is complete. The continuing weight loss is due to degradation of the remaining char.

4.6 SCANNING ELECTRON MICROSCOPY (SEM)

The surface structures of films were then analyzed by scanning electron microscopy (SEM). The effects of the different treatments on the surface fiber and the adhesion between fibers and matrix were investigated by SEM (Rosa et al., 2009). SEM image for the surface and cross section morphology of films with different composition of bacterial cellulose (BC) and starch was shown in the figure 4.10 and 4.11.

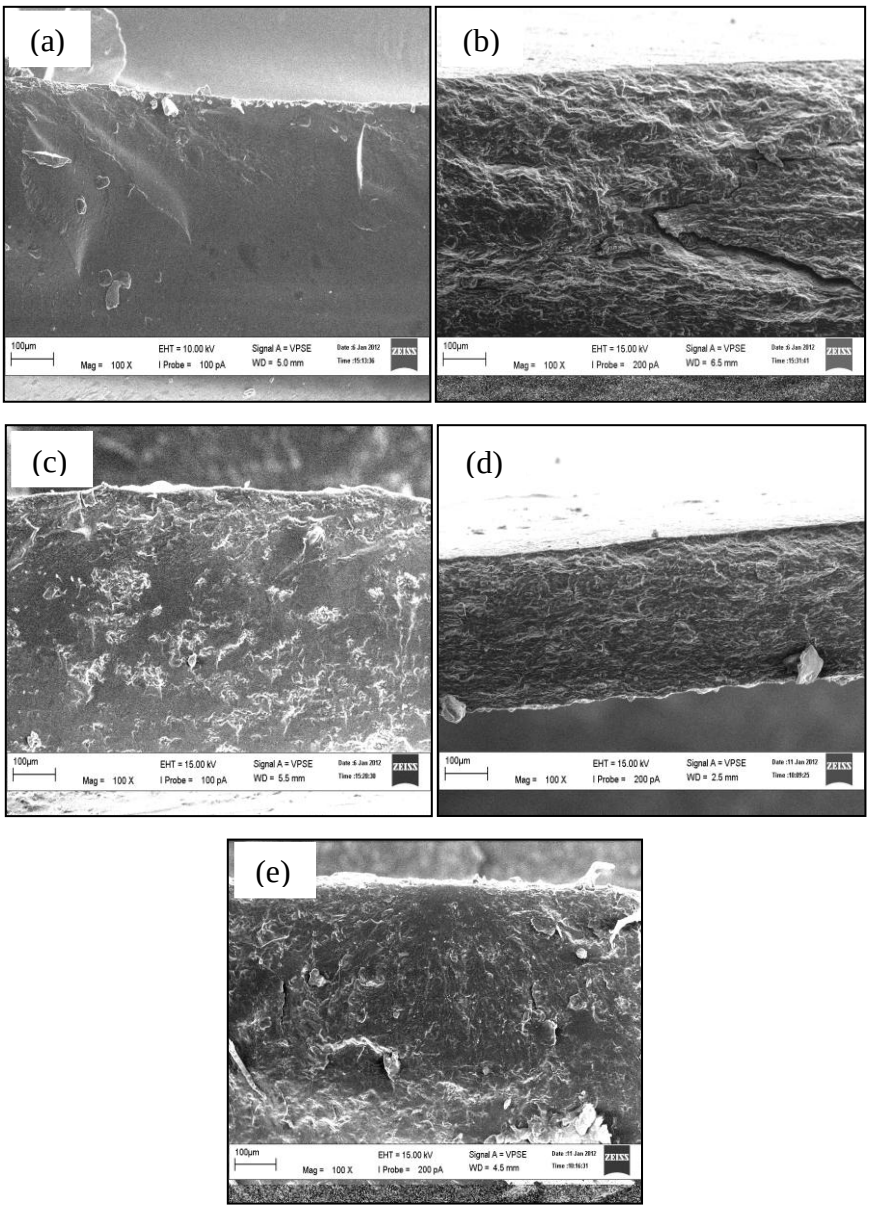

Figure 4.10: Scanning Electron Microscope for cross section (a) 9g starch 0% BC film; (b) 9g starch 14% BC film; (c) 9g starch 28% BC film; (d) 3g starch 14% BC film; (e) 15g starch 14% BC film

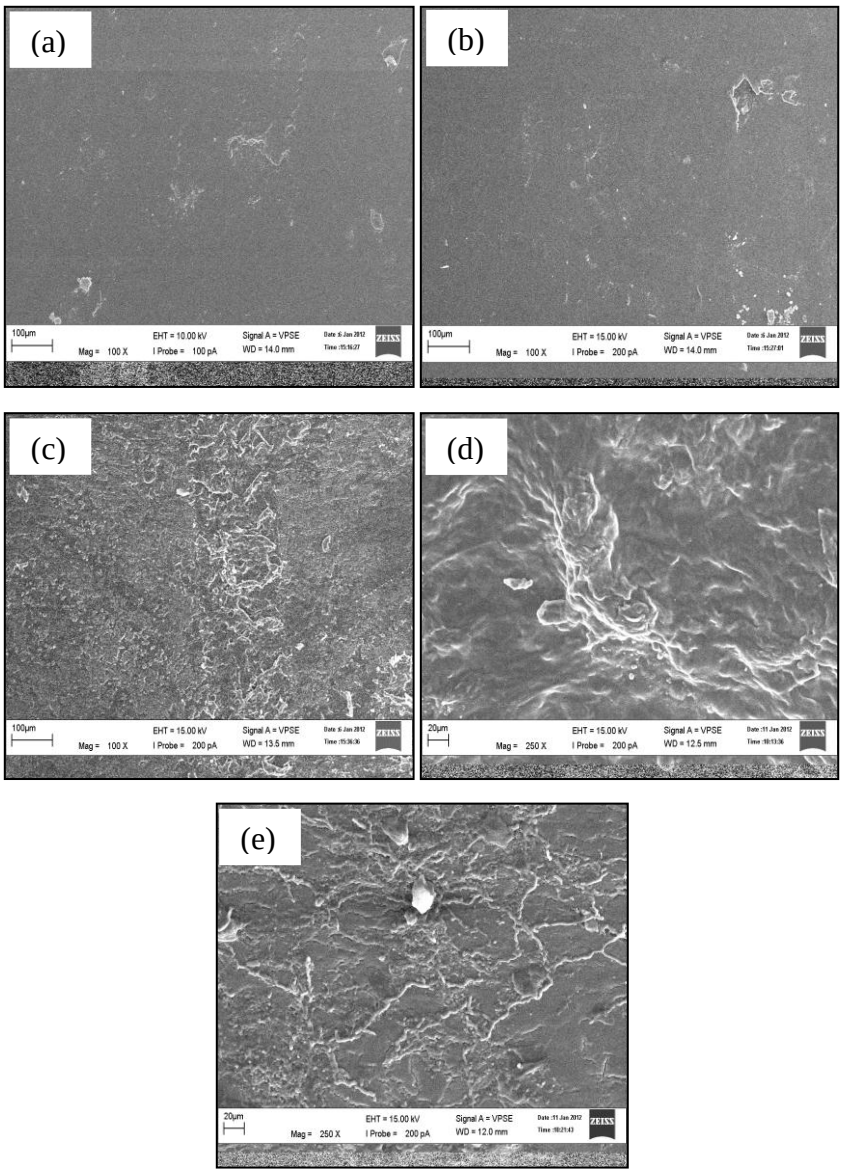

Figure 4.11: Scanning Electron Microscope for surface (a) 9g starch 0% BC film; (b) 9g starch 14% BC film; (c) 9g starch 28% BC film; (d) 3g starch 14% BC film; (e) 15g starch 14% BC film

SEM of the cross section surfaces of the pure starch film, and the blend films between starch and BC are shown in figure 4.10 (a-e) and 4.11 (a-e). The cross section and pure starch film (figure 10. (a)) relatively smooth, homogenous and has continuous matrix without cracks with good structural integrity. It was flat and compact with very sparsely

distributed small particles without any phase separation (Salleh et al., 2009). Figure 4.10 and 4.11 (b and c) show the structure of film when the composition of BC increases. From the figure 4.10 (b), it shows the small mat fragments, and not the isolated fibrils were observed and it became more obvious when the amount of BC increase from 14% to 28%. At figure 4.10 (c), the layered structure in the film becomes clearer. According to Martin et al. (2009), when the composition of BC increases, the strong interfacial adhesion between the cellulose fibers and the starch matrix occur. However, the pores in the composites are obvious and it not indicating a good fibre–matrix bonding (Wan et al., 2009)

Besides that, the structure of film also different when different composition of starch was added. When 3g starch was used, the layered structure can be seen clearly as shown in Figure 4.10 (d) and from figure 4.11 (d) the surface of films rough. However, the structure of film become relatively smooth when the composition of starch was increased to 15g as shown in figure 4.10 and 4.11 (e). This is due to the dispersion of the filler within the matrix was homogeneous (Dufresne et al., 2000). The homogeneous matrix of films is a good indicator of their structural integrity, and consequently good mechanical properties would be expected (Mali et al., 2002).

4.7 SOIL BURIAL DEGRADATION TEST

The studies on degradation behavior of bio composite are important for their application in the environment. The soil burial degradation tests were performed for the composite containing different composition of bacterial cellulose (BC) and starch. The bio composite derived from starch and BC undergoes degradation in the soil from the action of microorganisms such as bacteria, fungi, and algae. Microorganisms are able to consume these materials in their entirety, eventually leaving carbon dioxide and water as by-products (Kolybaba et al., 2003). Table 4.3 show the percentage of weight loss after biodegradation in the soil for 30 days and Figure 4.12 presents the weight loss of bio composite with different composition of starch and BC.

Table 4.3: Percentage of weight loss after biodegradation in the soil after 30 days

Starch	Bacterial Cellulose (BC)	Weight Loss (%)
3 g	**0%**	45.29
	7%	42.07
	14%	41.36
	21%	38.15
	28%	32.51
6 g	**0%**	39.07
	7%	36.68
	14%	34.53
	21%	30.58
	28%	29.70
9 g	**0%**	35.32
	7%	32.20
	14%	29.25
	21%	27.55
	28%	24.74
12 g	**0%**	31.42
	7%	27.17
	14%	25.16
	21%	22.41
	28%	19.76
15 g	**0%**	25.93
	7%	21.35
	14%	19.66
	21%	15.88
	28%	14.32

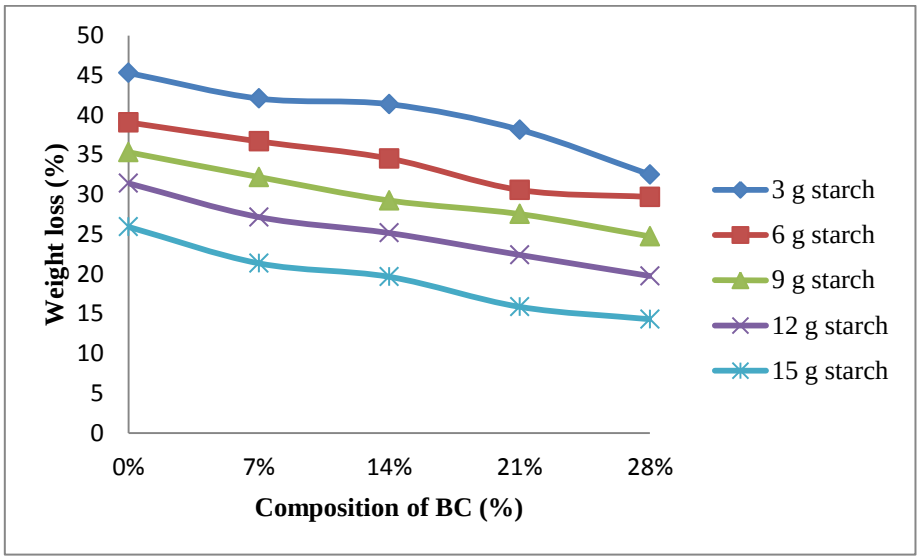

Figure 4.12: Weight loss of bio composite with different composition of starch and bacterial cellulose.

Note that weight loss shows an approximately linear relation with degradation time for all bio composite from BC and starch. From table 4.3, the weight loss for sample with 3 g starch decreased from 45.29% to 32.51% and at 15g starch, the degradation rate decreased from 25.93% to 14.32% when increasing the BC content from 0 to 28%. When the bio composite in contact with soil, the starch was attacked by the microbe until depleted and the matrix begin to degrade by an enzymatic attack. The amylase that secreted by the microbe was used originally to designate enzymes capable of hydrolyzing a-1,4- glucosidic bonds of amylose, amylopectin, glycogen and their degradation products (Aiyer, 2005). The amylase will break the starch into smaller disaccharide, maltose molecules that can then be easily transported into the cell to be used as carbon source. The bio composites were degraded with various degrees, emerging a small number of pores and cracks and their mechanical properties decreased significantly, which may be due to the constant erosion of microorganisms on the starch surface and incision to turn them into fragments (Liu et al., 2010). Each reaction results in the scission of a molecule, slowly reducing the weight of the matrix until the entire material has been digested (Kolybaba et al., 2003)

From figure 4.12, it is observed that the weight loss of the bacterial cellulose (BC)/starch composite is lower than the starch at any given time points and it also shows the degree of degradation was decrease when the increasing amount of BC contain. This is because cellulose made up of repeating of glucose group linked by glucosidic beta linkage allows polymer chain to by crystalline and rigid. This makes the hydroxyl group along polysaccharide chain inaccessible to microorganism. However, biodegradation of starch based polymers occurred between the sugar groups leading to a reduction in chain length and the splitting off of monosaccharide, disaccharide, and oligosaccharide units by a result of enzymatic attack at the glucosidic linkages (Mostafa et al., 2010). When the starch and cellulose mix up together, it will form hydrophilic hydroxyl group along polysaccharide chain which were susceptible to microbial access.

Other than determine the weight loss, the FTIR also used to investigate the changes in chemical bonding of the film after biodegradation process. Figure 4.13 show the infrared spectra of film before and after biodegradation in soil while for every 10 day.

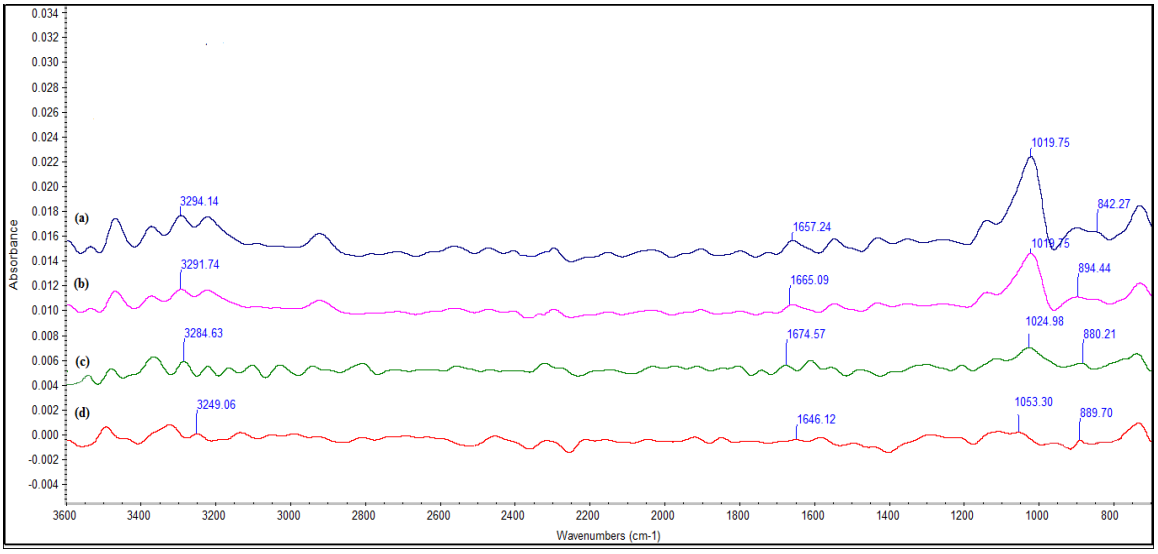

Figure 4.13: Infrared spectra of 9 g starch and 14% BC film (a) Before buried in the soil; (b) After 10 days buried in soil; (c)) After 20 days buried in soil; (d)) After 30 days buried in soil

From the Figure 4.13, it show a reduction on C=O bonding at the region between 1646.12 and 1674.57 cm^{-1}. Highest decreased in spectrum observed at between 1019.75 and 1053.30 cm^{-1} derived from C-O bonding of starch and BC. Besides that, O-H bonding at the region between 3249.42 and 3294.14 cm^{-1} also show the reduction on the peak. According to Geweely and Ouf (2011) stated that during degradation, enzymes from microorganisms break down complex polymers yielding smaller molecules of short chains (oligomers, dimers, and monomers), that are smaller enough to pass the semi-permeable outer microbial membranes, and then to be utilized as carbon and energy sources and the process is called depolymerization.

4.8 BIODEGRADATION TEST BY USING *ASPERGILLUS NIGER*

Microorganism *Aspergillus niger* was used in order to determine the degradation of bio composite. According to Arutchelvi et al. (2008), the biodegradation of polymer occur when the microorganism attaches to the surface of the polymer and the growth of microorganism will utilize the polymer as the carbon source. Table 4.4 shows show the percentage weight loss of bio composite after biodegradation by using *Aspergillus niger* after 5 days and Figure 4.14 presents the percentage weight loss of bio composite with different composition of Bacterial Cellulose (BC) and starch.

Table 4.4: Percentage weight loss of bio composite after biodegradation by using *Aspergillus niger* after 5 days

Starch	Bacterial Cellulose (BC)	Weight Loss (%)
3 g	**0%**	73.39
	7%	66.51
	14%	63.31
	21%	55.32
	28%	41.23
6 g	**0%**	68.15
	7%	62.31
	14%	59.16
	21%	47.02
	28%	38.46
9 g	**0%**	62.27
	7%	58.15
	14%	54.25
	21%	43.20
	28%	31.94
12 g	**0%**	56.49
	7%	52.49
	14%	46.84
	21%	40.47
	28%	28.66
15 g	**0%**	53.35
	7%	47.42
	14%	42.48
	21%	35.00
	28%	26.80

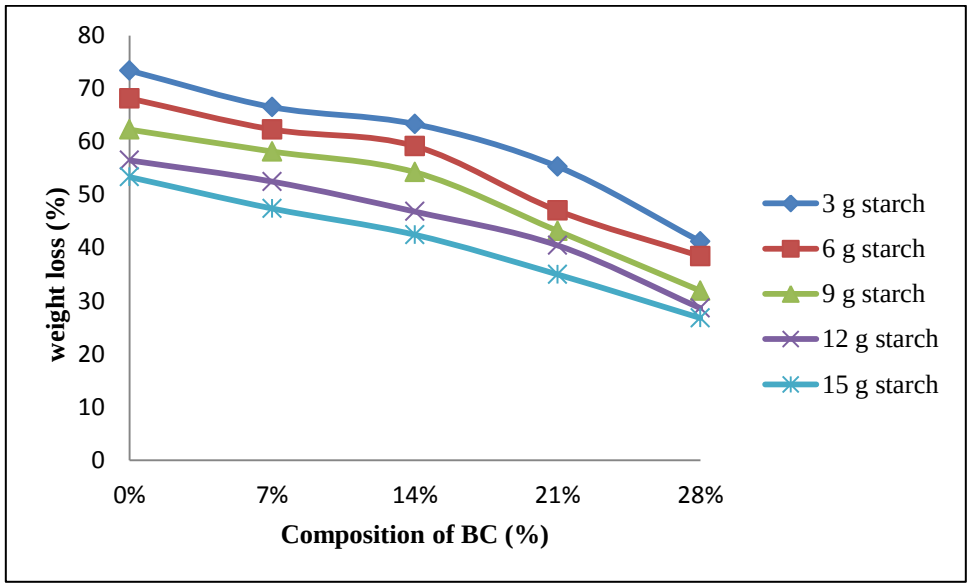

Figure 4.14: Percentage weight loss of bio composite with different composition of starch and bacterial cellulose (BC).

The figure 4.14 shows the percentage of weight loss after biodegradation of bio composite was decrease when the amount of BC and starch in the bio composite was increased. From table 4.4, the weight loss for sample with 3 g starch decreased from 73.40% to 41.23% and at 15g starch, the degradation rate decreased from 53.35% to 26.80% when increasing the BC content from 0 to 28%. . This is due to the existed of both amorphous and crystalline regions in the starch. With the existing of this semi crystalline polymer, the crystalline regions are tightly packed and less accessible to degrade (Kiatkamjornwong et al., 1997). Therefore, the amorphous region degrades first, leaving the more crystalline region.

Furthermore, the weight loss of bio composite because of the capability of *Aspergillus niger* to utilize an enormous variety of substrates for food because of the variety of enzymes they produce (Ja'afaru and Fagade, 2010). The starch is hydrolysis in cultures of *Aspergillus niger* because of the activity of two hydrolytic enzymes, namely, a-amvlase [a-D-(1-4)-glucan glucanohydrolase] or glucoamylase [a-D-(1-4)-glucan

glucohydrolase]. For biodegradation of cellulose, four classes of enzymes are involved. Endoglucanases hydrolyze cellulose to glucooligosaccharides, Cellobiohydrolases release cellobiose from crystalline cellulose, Glucosidases degrade the oligosaccharides to glucose and Exoglucanases release glucose from cellulose and glucooligosaccharides (De Vries and Visser, 2001).

On the other hand, the FTIR also used to get the information about chemical bonding of the film after biodegradation process by using *Aspergillus niger*. Figure 4.8 show the infrared spectra of film before and after biodegradation by using *Aspergillus niger.*

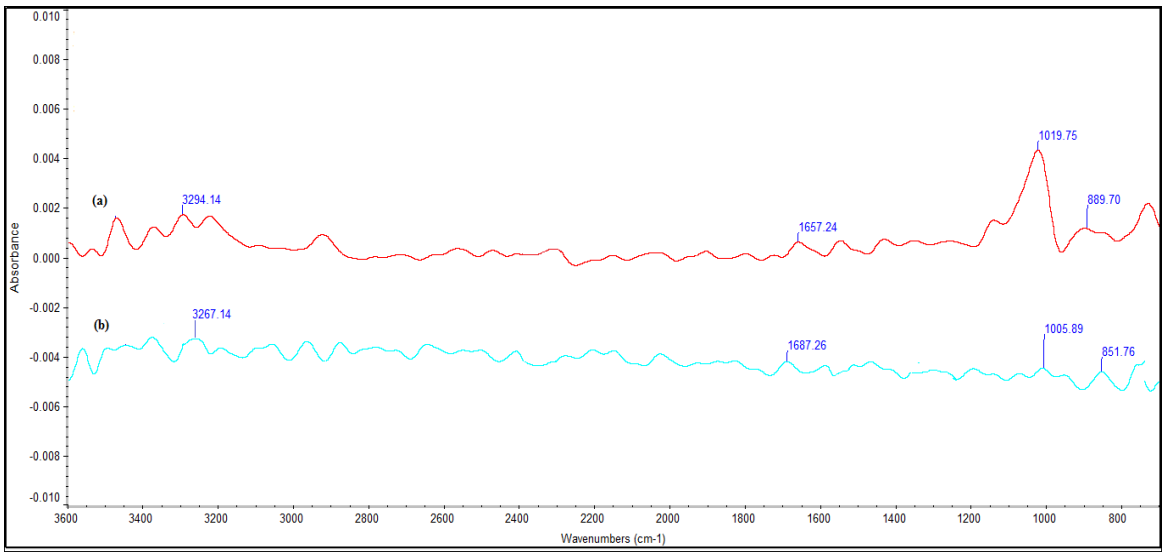

Figure 4.15: Infrared spectra of 9 g starch and 14% BC film (a) Before incubated in the dextrose agar potato medium that contain *Aspergillus niger;* (b) After 5 days incubated in the dextrose agar potato medium that contain *Aspergillus niger*

According to the Stoica-Guzun et al. (2011), the degradation of sample was shown by the reduction of O-H bonding and C-O bonding. From the Figure 4.15, it show high decreased in spectrum at the peak between 1065.19 and 1019.75 cm^{-1} derived from C-O bonding and O-H bonding at the region between 3267.14 and 3294.14 cm^{-1} of starch and

BC. Besides that, it also shows the reduction on the peak. C=O bonding at the region between 1657.42 and 1687.26 cm^{-1}. The reduction of peak occur due to the changes of the structure of film by *Aspergillus niger* activity that degrade the starch based plastic polymer by formation of pits, de-fragmentation and roughening of the surface (Geweely and ouf, 2011).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSION

Bio composite films with different composition of bacterial cellulose (BC) and starch content were fabricated by using casting method. The characteristics of the blend films with different starch composition (3, 6, 9, 12 and 15) wt% and different BC composition (0, 7, 14, 21 and 28) wt% were evaluated using Universal Testing Machine, water absorption test, Fourier Transform Infrared (FTIR), Thermo Gravimetric Analysis (TGA), Scanning Electron Microscopy (SEM) and soil burial degradation test and biodegradation test by using *Aspergillus niger*.

From the universal testing, the BC/starch bio composite films possess much higher tensile strength and tensile modulus than the starch bio composite. However, with the increasing the BC composition, it show lower elongation at break. Among the bio composite fabricated, the films with 9g starch and 28% bacterial cellulose showed good mechanical properties because it high tensile strength, tensile modulus and elongation at break. This film shows the optimum point for all the mechanical properties. Besides that, with the increasing of composition BC the percentage of water absorption and diffusivity of bio composite film will decrease. It also shows strong interfacial adhesion between the cellulose fibers and the starch matrix. The FTIR confirmed that starch and bacterial cellulose were compatible and inter-molecular hydrogen bonds existed between them.

Moreover, the addition of BC fibers to the starch bio composite resulted in a slight increase in the thermal stability of the composites. It was due to the excellent compatibility between the two carbohydrate components of the bio composites because the cellulose can increase the stability of component. In soil burial degradation test, the bio composite derived from BC and starch undergoes degradation in the soil from the action of microorganisms such as bacteria, fungi, and algae and it showed a decrease in the weight of film. Besides, degradation of bio composite also can be done by incubate the film with *Aspergillus niger* strain. From all the test that have been done, it show that the BC/starch bio composite had potential to be used as packaging material because it can enhance the quality of product and had advantageous in term of environmental protection.

5.2 RECOMMENDATION

From this research, it is recommended that more research should be done in bacterial cellulose (BC) and starch bio composite film. It should be done in order to improve the properties of bio composite and to know the optimum composition of BC and starch should be used to produce the film. Besides that, the study about other alternative material or additive that can be added to the film also can be done to increase the mechanical properties and degradation time.

The major problem in this research is to get a lot of BC film because it needs to wait for the fermentation process. In order to overcome this problem, some study need to be done to increase the production of BC with the shorten time for fermentation. In addition, the proper to blend the BC should be introduced in order to get the better amount of BC and reduce the rate of loss during the filtration process.

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APPENDICES

APPENDIX A
FILM PREPARATION

Figure A1: Bacterial Cellulose (BC)

Figure A2: Tapioca Starch

Figure A3: Bio Composite Film

APPENDIX B

TENSILE STRESS CURVE FOR TENSILE TEST

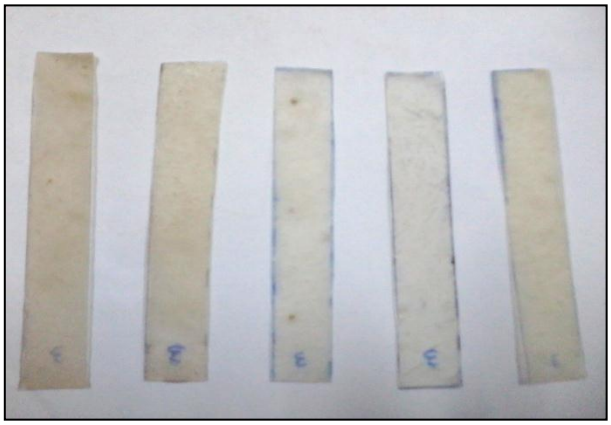

Figure B1: Sample for the tensile test

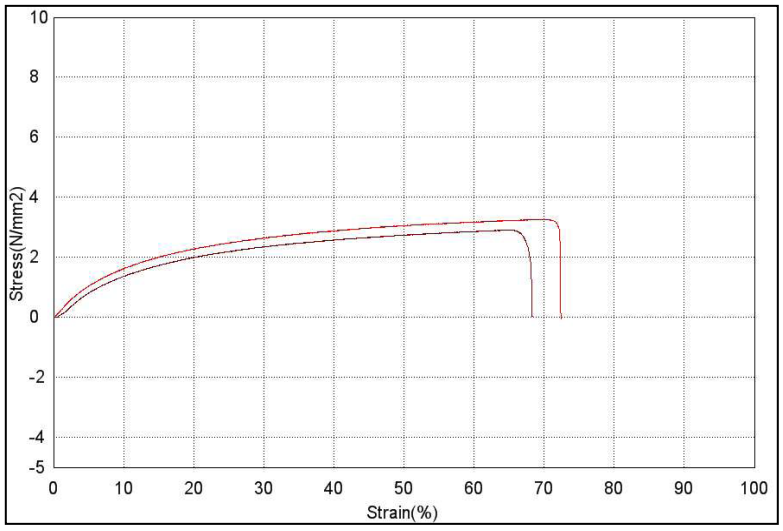

Figure B.2: The stress (N/mm^2) versus strain (%) for 9 g starch and 0% bacterial cellulose (BC) bio composite

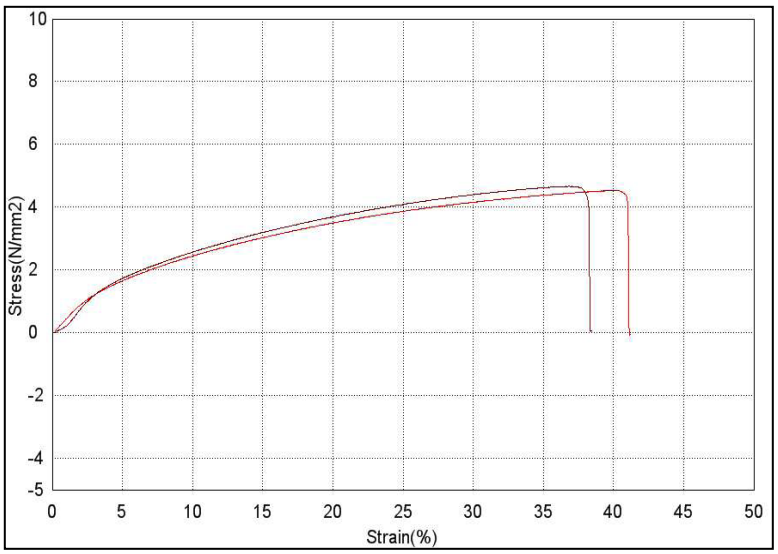

Figure B.3: The stress (N/mm^2) versus strain (%) for 9 g starch and 14% bacterial cellulose (BC) bio composite

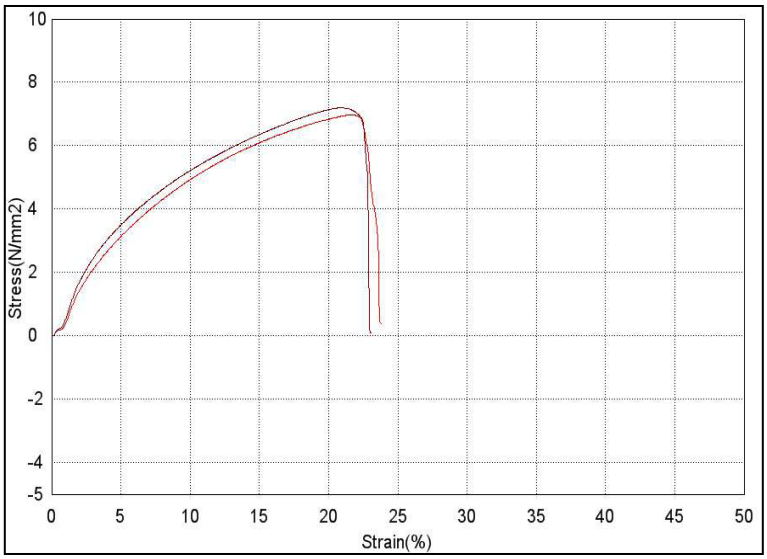

Figure B.4: The stress (N/mm^2) versus strain (%) for 9 g starch and 28% bacterial cellulose (BC) bio composite

APPENDIX C

WATER ABSORPTION CALCULATION

Table C1: The weight of biodegradable film for 3 g starch with different composition of bacterial cellulose (BC)

Weight sample (g)					
Time	**0% BC**	**7% BC**	**14% BC**	**21% BC**	**28% BC**
0	0.1199	0.1201	0.1164	0.1106	0.1188
10	0.1745	0.1709	0.1591	0.1501	0.1591
20	0.1794	0.1735	0.1619	0.1515	0.1595
30	0.1811	0.1743	0.1622	0.1517	0.1596
40	0.1825	0.175	0.1624	0.1518	0.1596
50	0.1838	0.1753	0.1624	0.1518	0.1596
60	0.1842	0.1755	0.1625	0.1518	0.1596
70	0.1843	0.1756	0.1625	0.1518	0.1596
80	0.1844	0.1756	0.1625	0.1518	0.1596
90	0.1844	0.1756	0.1625	0.1518	0.1596
100	0.1845	0.1756	0.1625	0.1518	0.1596
110	0.1845	0.1756	0.1625	0.1518	0.1596
120	0.1845	0.1756	0.1625	0.1518	0.1596

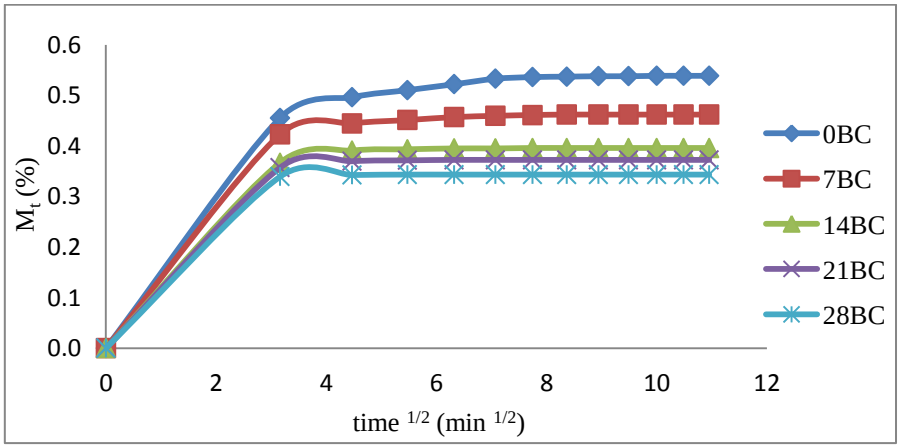

Figure C1: The M_t versus time ^{½} curves for 3 g starch with different bacterial cellulose (BC) contents.

Table C2: The diffusion coefficient for 3 g starch with different composition of bacterial cellulose (BC)

BC	k (min^{-1})	Mm	D (cm^2/min)
0%	0.0629	0.5388	4.50E-06
7%	0.0544	0.4621	4.42E-06
14%	0.0468	0.3960	4.30E-06
21%	0.0441	0.3725	4.19E-06
28%	0.0407	0.3434	4.15E-06

Table C3: The weight of sample for 6 g starch with different composition of bacterial cellulose (BC)

Weight sample (g)					
Time	**0% BC**	**7% BC**	**14% BC**	**21% BC**	**28% BC**
0	0.1185	0.1252	0.1199	0.1259	0.1233
10	0.1859	0.1901	0.1823	0.1853	0.1815
20	0.1899	0.1936	0.1844	0.1871	0.1818
30	0.1918	0.1942	0.1847	0.1875	0.1818
40	0.1931	0.1947	0.1849	0.1876	0.1818
50	0.1938	0.1952	0.1851	0.1876	0.1818
60	0.1946	0.1955	0.1851	0.1876	0.1818
70	0.1949	0.1957	0.1851	0.1876	0.1818
80	0.195	0.1957	0.1851	0.1876	0.1818
90	0.1952	0.1957	0.1851	0.1876	0.1818
100	0.1952	0.1957	0.1851	0.1876	0.1818
110	0.1952	0.1957	0.1851	0.1876	0.1818
120	0.1952	0.1957	0.1851	0.1876	0.1818

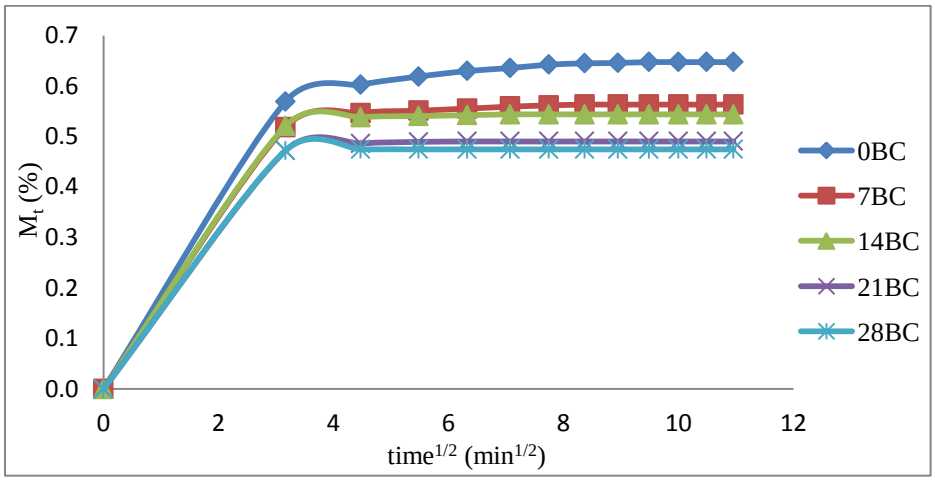

Figure C2: The Mt versus time ½ curves for 6 g starch with different bacterial cellulose (BC) contents.

Table C4: The diffusion coefficient for 6 g starch with different composition of bacterial cellulose (BC)

BC	k (min^{-1})	Mm	D (cm^2/min)
0%	0.0756	0.6473	4.50E-06
7%	0.0663	0.5631	4.36E-06
14%	0.0643	0.5438	4.18E-06
21%	0.0580	0.4901	4.18E-06
28%	0.0563	0.4745	4.18E-06

Table C5: The weight of sample for 9 g starch with different composition of bacterial cellulose (BC)

9 g starch					
Time	**0% BC**	**7% BC**	**14% BC**	**21% BC**	**28% BC**
0	0.1245	0.1228	0.1196	0.1198	0.1259
10	0.2192	0.2059	0.1978	0.1932	0.2012
20	0.2249	0.2119	0.2001	0.1949	0.203
30	0.2265	0.2131	0.2007	0.1959	0.2032
40	0.2273	0.2135	0.201	0.1961	0.2032
50	0.2281	0.2138	0.2013	0.1962	0.2032
60	0.2286	0.214	0.2014	0.1962	0.2032
70	0.2291	0.2142	0.2015	0.1962	0.2032
80	0.2294	0.2142	0.2015	0.1962	0.2032
90	0.2296	0.2143	0.2015	0.1962	0.2032
100	0.2296	0.2143	0.2015	0.1962	0.2032
110	0.2296	0.2143	0.2015	0.1962	0.2032
120	0.2296	0.2143	0.2015	0.1962	0.2032

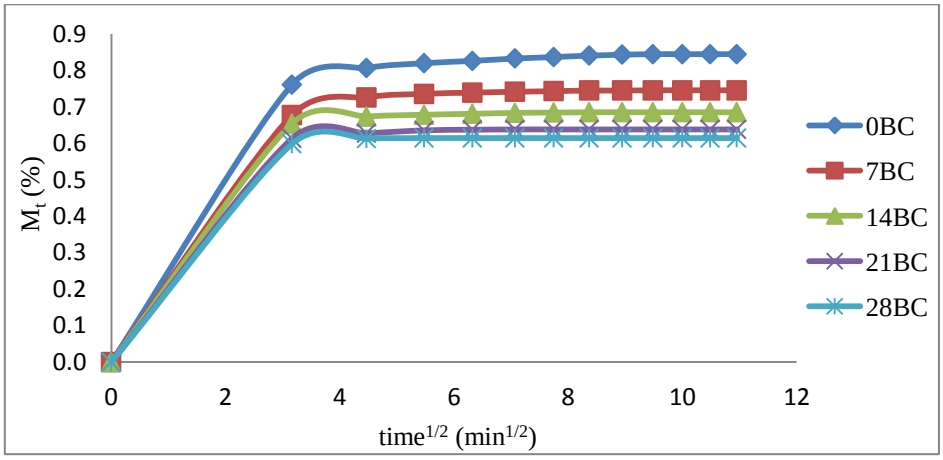

Figure C3: The Mt versus time ½ curves for 9 g starch with different bacterial cellulose (BC) contents.

Table C6: The diffusion coefficient for 9 g starch with different composition of bacterial cellulose (BC)

BC	k (min^{-1})	Mm	D (cm^2/min)
0%	0.099	0.8442	4.99E-06
7%	0.0878	0.7451	4.81E-06
14%	0.0809	0.6848	4.61E-06
21%	0.0754	0.6377	4.39E-06
28%	0.0727	0.6140	4.38E-06

Table C7: The weight of sample for 12 g starch with different composition of bacterial cellulose (BC)

Weight Sample (g)					
Time	**0% BC**	**7% BC**	**14% BC**	**21% BC**	**28% BC**
0	0.1222	0.1242	0.1253	0.1272	0.1241
10	0.2507	0.2337	0.2323	0.2226	0.2171
20	0.2593	0.2383	0.2336	0.2234	0.2176
30	0.2611	0.2397	0.2339	0.2237	0.2178
40	0.2621	0.2409	0.2343	0.224	0.2178
50	0.2626	0.2415	0.2344	0.2242	0.2178
60	0.2631	0.2419	0.2346	0.2242	0.2178
70	0.2634	0.2422	0.2346	0.2242	0.2178
80	0.2636	0.2423	0.2346	0.2242	0.2178
90	0.2636	0.2424	0.2346	0.2242	0.2178
100	0.2637	0.2424	0.2346	0.2242	0.2178
110	0.2637	0.2424	0.2346	0.2242	0.2178
120	0.2637	0.2424	0.2346	0.2242	0.2178

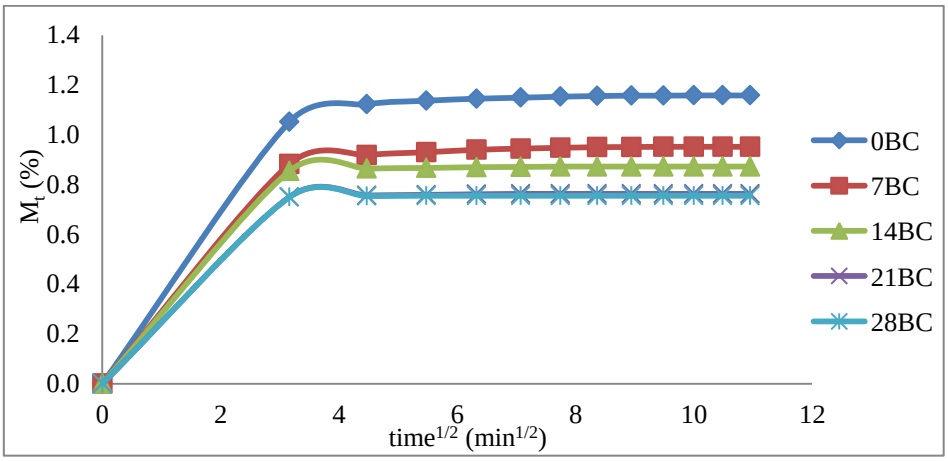

Figure C4: The Mt versus time ½ curves for 12 g starch with different bacterial cellulose (BC) contents.

Table C8: The diffusion coefficient for 12 g starch with different composition of bacterial cellulose (BC)

BC	k (min^{-1})	Mm	D (cm^2/min)
0%	0.1362	1.1579	5.09E-06
7%	0.112	0.9517	4.89E-06
14%	0.1032	0.8723	4.85E-06
21%	0.0895	0.7626	4.55E-06
28%	0.0895	0.7550	4.42E-06

Table C9: The weight of sample for 15 g starch with different composition of bacterial cellulose (BC)

Weight Sample (g)					
Time	**0% BC**	**7% BC**	**14% BC**	**21% BC**	**28% BC**
0	0.1305	0.1312	0.1293	0.1303	0.1316
10	0.3006	0.2759	0.2616	0.2451	0.2437
20	0.3139	0.2839	0.2637	0.2464	0.2446
30	0.3165	0.2864	0.2642	0.2465	0.2449
40	0.3187	0.2881	0.2645	0.2466	0.2449
50	0.3201	0.289	0.2647	0.2467	0.2449
60	0.3209	0.2895	0.2647	0.2467	0.2449
70	0.3211	0.2897	0.2648	0.2467	0.2449
80	0.3212	0.2899	0.2648	0.2467	0.2449
90	0.3212	0.2899	0.2648	0.2467	0.2449
100	0.3213	0.2899	0.2648	0.2467	0.2449
110	0.3213	0.2899	0.2648	0.2467	0.2449
120	0.3213	0.2899	0.2648	0.2467	0.2449

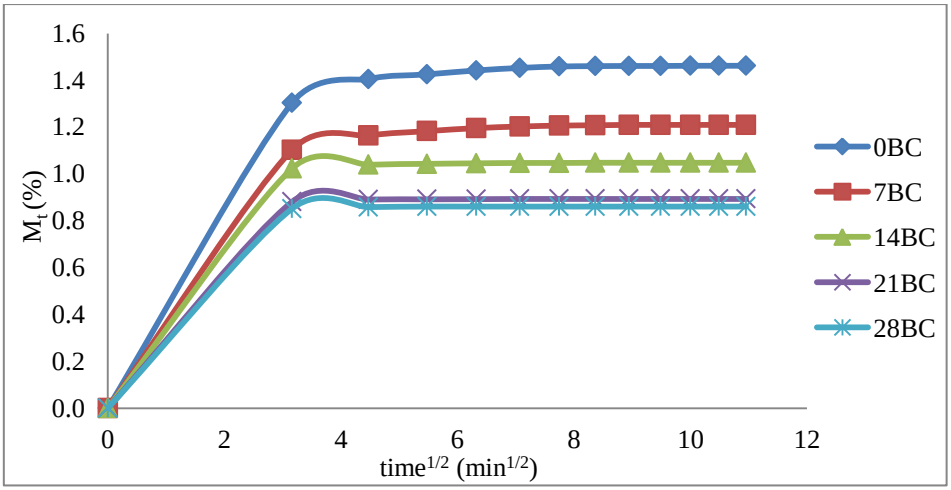

Figure C5: The Mt versus time ½ curves for 15 g starch with different bacterial cellulose (BC) contents.

Table C10: The diffusion coefficient for 15 g starch with different composition of bacterial cellulose (BC)

BC	k (min^{-1})	Mm	D (cm^2/min)
0%	0.1718	1.4621	5.25E-06
7%	0.1423	1.2096	5.03E-06
14%	0.1241	1.0480	4.86E-06
21%	0.1059	0.8933	4.64E-06
28%	0.102	0.8609	4.63E-06

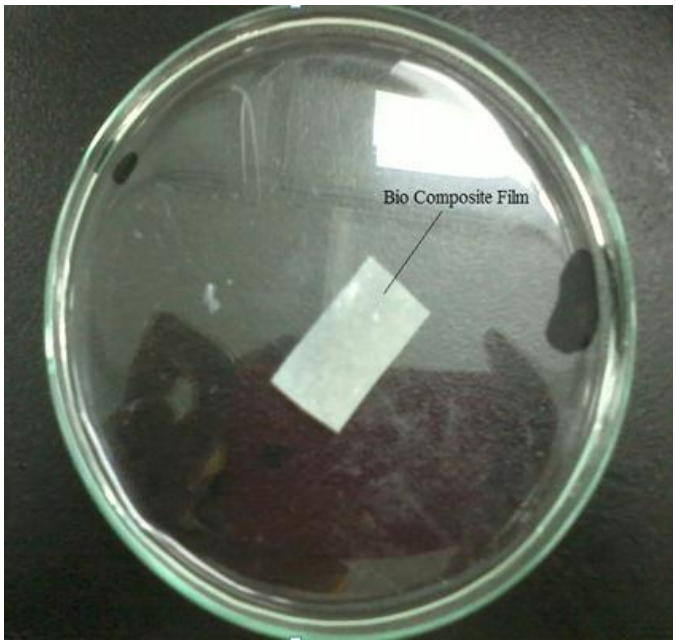

Figure C6: Bio composite film in water during water absorption test

APPENDIX D

SOIL BURIAL DEGRADATION TEST

Table D1: The weight of sample during the degradation in soil test for every 10 days.

Starch	Bacterial Cellulose (BC)	Initial weight (g)	Weight after 10 days (g)	Weight after 20 days (g)	Weight after 30 days (g)
3 g	0%	0.1676	0.1465	0.1265	0.0917
	7%	0.1412	0.1298	0.1021	0.0818
	14%	0.1528	0.1325	0.1087	0.0896
	21%	0.1266	0.1068	0.0923	0.0783
	28%	0.1252	0.1085	0.0924	0.0845
6 g	0%	0.1802	0.1635	0.1365	0.1098
	7%	0.1764	0.1521	0.1384	0.1117
	14%	0.2763	0.246	0.2103	0.1809
	21%	0.2407	0.2087	0.1863	0.1671
	28%	0.1926	0.1757	0.1538	0.1354
9 g	0%	0.265	0.2476	0.2017	0.1714
	7%	0.3084	0.2764	0.2476	0.2091
	14%	0.1812	0.1676	0.1445	0.1282
	21%	0.2461	0.2176	0.1942	0.1783
	28%	0.2454	0.2174	0.1948	0.1847
12 g	0%	0.2155	0.1847	0.1632	0.1478
	7%	0.3294	0.2865	0.2583	0.2399
	14%	0.3362	0.3076	0.2854	0.2516
	21%	0.2454	0.2276	0.2187	0.1904
	28%	0.2935	0.2754	0.2576	0.2355
15 g	0%	0.3297	0.3008	0.2735	0.2442
	7%	0.399	0.3707	0.3475	0.3138
	14%	0.3795	0.3684	0.329	0.3049
	21%	0.3582	0.3396	0.3176	0.3013
	28%	0.4035	0.3884	0.3698	0.3457

APPENDIX E

BIODEGRADATION TEST BY USING *ASPERGILLUS NIGER*

Table E1: The weight of sample during the degradation test by using *Aspergillus niger*

Starch	BC	Initial Weight (g)	Final Weight (g)	Weight Loss (g)	Percentages of weight loss (%)
3 g	**0%**	0.109	0.029	0.080	73.39
	7%	0.152	0.0509	0.1011	66.51
	14%	0.166	0.0609	0.1051	63.31
	21%	0.158	0.0706	0.0874	55.32
	28%	0.155	0.0911	0.0639	41.23
6 g	**0%**	0.162	0.0516	0.1104	68.15
	7%	0.169	0.0637	0.1053	62.31
	14%	0.179	0.0731	0.1059	59.16
	21%	0.181	0.0959	0.0851	47.02
	28%	0.162	0.0997	0.0623	38.46
9 g	**0%**	0.154	0.0581	0.0959	62.27
	7%	0.146	0.0611	0.0849	58.15
	14%	0.362	0.1656	0.1964	54.25
	21%	0.219	0.1244	0.0946	43.20
	28%	0.217	0.1477	0.0693	31.94
12 g	**0%**	0.185	0.0805	0.1045	56.49
	7%	0.189	0.0898	0.0992	52.49
	14%	0.177	0.0941	0.0829	46.84
	21%	0.191	0.1137	0.0773	40.47
	28%	0.179	0.1277	0.0513	28.66
15 g	**0%**	0.209	0.0975	0.1115	53.35
	7%	0.217	0.1141	0.1029	47.42
	14%	0.214	0.1231	0.0909	42.48
	21%	0.246	0.1599	0.0861	35.00
	28%	0.244	0.1786	0.0654	26.8033

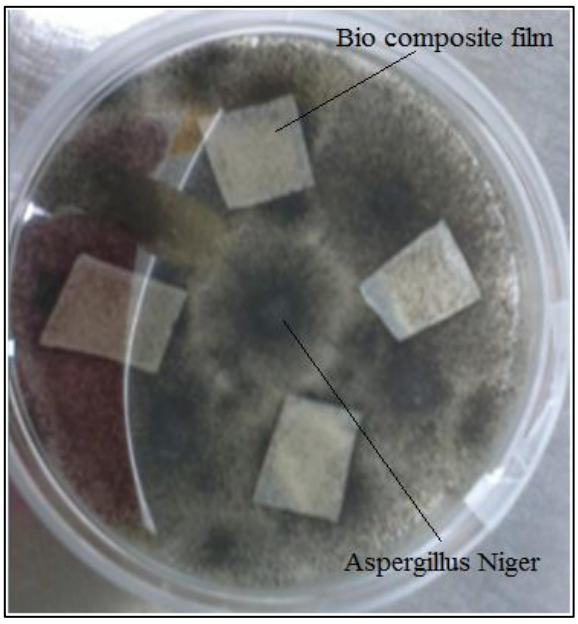

Figure E1: Bio composite film in the culture medium that contain *Aspergillus niger*.

APPENDIX F
EQUATION AND CALCULATION

i. Water absorption test.

By using data from Table C5 (14 g BC and 9 g starch), the diffusivity at t=0(M_t) and t=120min (M_m) were determined by using equation 2.

$$\frac{M_t}{M_m} = 1 - \frac{8}{\pi^2}\sum \frac{1}{(2n+1)^2}\exp\left\{\frac{-D(2n+1)^2\pi^2 t}{h^2}\right\}$$

Equation 3.2 can be reduced to equation 3.

$$\frac{M_t}{M_m} = \frac{4}{\pi^{1/2}}\left(\frac{Dt}{h^2}\right)^{1/2}$$

Diffusivity can be calculated from the slope (k) of M_t vs $t^{1/2}$ curve (Figure C3).

$$D = \left(\frac{kh}{4M_m}\right)^2 \pi$$

$$D = \left(\frac{(0.0809)(0.041)}{4(0.6848)}\right)^2 \pi$$

$$D = 4.61\times10^{-6}\, mm^2/\min$$

ii. Soil burial degradation test

By using data from Appendix D, the percentage of bio composite weight loss was determined by using equation 6.

$$\text{Weight loss (14 g BC and 9 g starch)} = \frac{W_o - W_t}{W_t} \times 100$$

$$= \frac{0.1812 - 0.1282}{0.1282} \times 100$$

$$= 29.25\ \%$$

iii. Biodegradable by using *Aspergillus niger* test.

By using data from Appendix F, the percentage of bio composite weight loss was determined by using equation 7.

$$\text{Weight loss (14 g BC and 9 g starch)} = \frac{W_o - W_t}{W_t} \times 100$$

$$= \frac{0.3620 - 0.1656}{0.1656} \times 100$$

$$= 54.25\ \%$$