BIODEGRADATION BEHAVIOR OF BACTERIAL CELLULOSE FIBER REINFORCES WITH STARCH/CHITOSAN BIOCOMPOSITE.

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DATE : 3 DECEMBER 2010

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FARISAH BINTI ABDUL AZIZ

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JUDUL: BIODEGRADATION BEHAVIOR OF BACTERIAL CELLULOSE FIBER REINFORCES WITH STARCH/CHITOSAN BIOCOMPOSITE

SESI PENGAJIAN : 2009/2010

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I dedicate this entire work to my family especially to my beloved parents (Mak and Abah), whose patient, support and companionship have facilitated my study, and made my life enjoyable, to my grateful brothers (Safuan and Shahrullah) and my lovely sister (Aqilah). 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

Biocomposite from starch, bacterial cellulose and chitosan offer A good mechanical properties. The objectiveS of this study are to produce bacterial cellulose fiber reinforce with starch/chitosan biocomposite and to study the characterization of the biocomposite on mechanical, chemical and also biodegradation behavior. The research conducted with 4 samples with difference composition of chitosan (0.5%, 1%, 1.5% and 2% w/w) and 4 samples with difference composition of bacterial cellulose (0%, 7%, 14% and 21%). The bacterial cellulose film produced by *Acetobacter Xylinum* was blend and mixED with chitosan and starch. The film will be analyzed by using Universal Testing Machine, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), Gas Pynometer, Swelling absorption and soil burial degradation method. Among the four samples, the films with 21% of bacterial cellulose, 6% starch and 2% of chitosan composition showed a good mechanical property. It was supported by FTIR results where the tapioca starch, chitosan and bacterial cellulose were in compatible by composition and inter-molecular chemical bonds such as existed in the sample. Moreover, the SEM analysis showed a smooth and homogenous structure of the film. Besides, Gas Pynometer analysis showed the average density more than 1 for the entire sample. The absorption of water was increased proportionate with the composition of bacterial cellulose and chitosan in swelling absorption. The soil burial test indicated the increased degradation rate as the chitosan and bacterial cellulose content was increased. As a conclusion, the film fabricated had a potential application in future to be used as packaging material because as it had good mechanical properties and biodegradable can protect the environment.

ABSTRAK

Kanji mempunyai struktur yang lemah sebagai termoplastik, untuk meningkatkan sifat mekanik biokomposit, kajian lebih lanjut telah dilakukan dengan menambah Selulosa Bakteria di biokomposit Kanji dan Kitosan. Tujuan ekperimen ini adalah untuk menghasilkan serat selulosa bakteria untuk menguatkan dengan Kanji /Kitosan dan mempelajari ciri-ciri biokomposit pada mekanik, kimia dan juga perilaku biodegradasi. Penyelidikan dilakukan dengan 4 sampel dengan perbezaan komposisi kitosan (0.5%, 1%, 1.5% dan 2%) dan 4 sampel dengan perbezaan komposisi selulosa bakteria (0g 7g, 14g dan 21g). Dimana, filem selulosa bakteria dihasilkan oleh Asetobakter xylinum dikisar dan dicampur dengan Kitosan dan Kanji. Filem biokomposit kemudian, dianalisa dengan menggunakan Mesin Pengujian, Spektroskopi Inframerah Transformasi Fourier (FTIR), Mikroskopi Pengimbasan Elektron (SEM), Ujian Ketumpatan, Penyerapan Kelembapan Udara, Penyerapan Air dan uji biodegradasi dengan menggunakan Degradasi Tanah. Di antara sampel, campuran dengan 21g dari selulosa bakteria dengan 2% dari kitosan menunjukkan sifat mekanik yang baik. FTIR ini menegaskan bahawa kanji, kitosan dan selulosa bakteria serasi diantara-molekul ikatan kimia. Selain itu, analisis SEM dengan 21g selulosa bakteria dan 2% kadar kitosan menunjukkan struktur halus dan homogen filem. Dalam ujian ketumpatan, nilai menunjukkan hasil yang lebih dari 1. Untuk penyerapan kelembapan udara dan penyerapan air menunjukkan bahawa peningkatan komposisi kitosan dan selulosa bakteria akan meningkatkan penyerapan air akibat pembentukan ikatan hidrogen. Uji degradasi tanah menunjukkan bahawa kandungan selulosa lebih kitosan dan bakteria meningkatkan laju penyingkiran. Sebagai kesimpulan, filem telah dibuat aplikasi yang berpotensi di masa depan untuk digunakan sebagai bungkusan kerana mempunyai sifat mekanik yang baik dan melindungi alam sekitar.

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LIST OF SYMBOLS / ABBREVIATIONS

BC	-	Bacterial cellulose
^{o}C	-	Celcius
$^{o}C/min$	-	Celcius per minutes
CRT	-	Cathode-ray-tube
cm	-	Centimeter
DP	-	Degree of polymerization
Dt	-	Diffusion Coefficient
FTIR	-	Fourier Transfrom Infrared Spectroscopy
g	-	Gram
GPa	-	Giga pascal
h	-	Hour, thickness of the film
k	-	Slope
M	-	Mole
M^{a}	-	Mass in water
mm	-	Millimeter
mm min^{-1}	-	Millimeter per minute
Mt	-	Mass gain in time
M^{w}	-	Mass in water

M_∞	-	Mass gain at equilibrium (maximum water uptake)
PC	-	Plant Cellulose
SEM	-	Scanning Electron Microscope
SG	-	Specific Gravity
TC	-	Terminal Complex
TPS	-	Thermoplastic Starch

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CHAPTER 1

INTRODUCTION

1.1 Background

Plastic is one of the most important used materials in food packaging and electric appliances. Although plastic is been used in everyday's lives, there are issues arise such as biodegradability and environmental safety. Plastic takes prolong time to degrade after been discarded to the landfill, thus will cause harmful to the environment. In order to overcome this problem, the material components such as natural fiber, and biodegradable polymer has been used as the alternative materials in producing the new biocomposites. Biodegradable composites is consists of biodegradable polymers and biodegradable fillers.

The most abundant natural polymers uses is starch and it can be consider as raw materials in biocomposites. It usually has two major components and appears as a mixture of two glucosidic macromolecules in very different structure and properties: largely linear amylose of molecular weight between one thousand and one million consisting of R-(1f4)-linked D-glucose, and amylopectin, having the same backbone as amylose but with a myriad of R-(1f6)-linked branch points (Vignon *et al.*, 1998) . Through some disruption of the molecule chain interactions under specific conditions, the starch can be converted into thermoplastic material, known as thermoplastic starch (TPS). However, starch is not truly thermoplastic as others synthetic polymers. Thus, to improvise the properties of starch, it can be blend with natural fibers.

The natural fibers are based on plants, animals or minerals. These fibers are used to reinforce thermoplastics to give the good mechanical properties, sustainability and environmental-friendly product. The use of natural fibers as reinforcing elements in composite materials presents important advantages, when compared with their synthetic or inorganic counterparts, namely biodegradability, high availability, low cost, low energy consumption, low density, high specific strength and modulus (with fibers possessing an adequate aspect ratio), high sound attenuation and comparatively easy processing ability due to their flexibility and non-abrasive nature (Dufresne *et al.*,2000). One of the natural fibers uses in biocomposite is bacterial cellulose fibers. Owing to its unique properties, such as high mechanical strength, high crystallinity and a highly pure nanofibrillar network structure, bacterial cellulose, produced by *Acetobacter Xylinum*, is becoming a promising biopolymer for several applications (Martinsa *et al.*,2009).

However, starch and bacterial cellulose blends cannot indicate a good fiber-matrix bonding. Thus, the addition of chitosan will overcome this problem.The amino group (NH_2) in chitosan can be protonated to NH_3^+,and form the hydrogen bonding between OH^- of the starch. This hydrogen bonding will increase the flexibility of obtained biocomposite.

1.2 Problem Statement

In recent years, the problem of environmental pollutant had increase due to the use of synthetic polymer. Synthetic polymer or plastic that been used in our daily life requires prolong time and need high cost to degrade. Hence, the usage of natural polymers such as starch is suitable to become raw material in producing composite. However, starch composite has the weak structure as thermoplastic, so as to improve the mechanical properties of the biocomposite, further research had been made by the addition of bacterial cellulose fiber in starch biocomposite. Nevertheless, the function of bacterial cellulose is just to improve the tensile strength but it gives weak elongation mechanical to the composite hence the addition of chitosan can improve the elongation properties where it can form hydrogen bonding with starch- bacterial cellulose biocomposite.

1.3 Research objective

The objectives of this research are to produce biodegradable biocomposite films and to study the characteristics of bacterial cellulose fiber reinforced with starch/chitosan biocomposite

1.4 Scopes of the study

The scopes of this study are:

a) To produce bacterial cellulose fiber reinforced with starch/chitosan biocomposite.

b) To study the effect of different composition of chitosan from 0.5%, 1%, 1.5%, and 2% (w/w).

c) To study the effect of different composition of bacterial cellulose from 0 g, 7 g, 14 g, and 21 g .

d) To do the characterization of biocomposite using Universal Testing Machine, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), and Gas Pycnometer.

e) To investigate the swelling absorption behavior of biocomposite.

f) To investigate the biodegradation behavior of biocomposite.

1.5 Rational and significance

Polymers that derived from petroleum resources cannot be degraded and decomposed biologically or naturally by bacteria or fungi. Subsequently it will remains in environmental forever and cause harmful to the environment. Thus, the production bacterial cellulose fiber reinforced with starch/chitosan biocomposite can assist to the pollution reducing on earth.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The word of polymer is derived from the Greek words *poly* and *mers* means "many parts". This is applicable with the structure of polymer itself which are large molecules composed of repeated chemical units. Degree of polymerization (DP) or chain length is known as the number of repeat units in chain. As the example, a poly (propylene) chain 5,000 units long would have a DP of 5,000 and an "n" value of 5,000. Due to the chains of varying length of most of the polymer mixture, the chain length is referred to in terms of average chain length or average DP. Polymer chains can be connecting by chemical or physical and this connection call crosslink (Carraher *et al* ,2003).

The degradation of such polymers includes the disintegration into their monomers. Therefore the unstable and hydrolysable linkages are required for chemical, biological or photochemical reactions take place. Most of the biodegradable polymers have been synthesized chemically or by microorganisms and plants. There are four categories of biodegradable polymers depending on the origin have been proposed (i) agro-polymers such as starch or cellulose from agro-resources; (ii) polymers obtained by microbial production (e.g. polyhydroxyalkanoates); (iii) chemically synthesized polymers from monomers derived from agro-resources (e.g. poly (lactic acid)) and (iv) chemically synthesized polymers from monomers obtained conventionally by chemical synthesis (Caniguerala *et al.*,2009). Most of the biodegradable polymers may be divided into

synthetic and natural polymers, where the latter are classified into those of plant and microbial origin (Endres *et al.,* 1995).

Synthetic polymer is the polymer that modified by addition polymers, formed from monomer units directly joined together, or formed from monomer units combining such small molecule such as water. One type of synthetic polymer is thermoplastics. Thermoplastics are the materials that contain little or no crosslink that melt when heated. Thus, this polymer can recycle through heating and reforming. The other type of synthetic polymer is fibers. This polymer requires materials with high tensile strength and high modulus (elongation). Thus, this polymer requires strong forces between the chain and chain that are symmetrical to allow for good crystalline formation. Composite, the other types of synthetic polymer are material that contains strong fibers or reinforcement. However, both polymer and synthetic polymer requires long time and high cost to degrade (Carraher *et al.,* 2003).

There are many natural polymers found in nature. As the example, the human body contains many natural polymers, such as protein and nucleic acids. And, most of natural polymers are condensation polymers, and in the formation from monomers water is a by-product. Thus, there are many research on develop biocomposite using natural polymer. Biocomposite (biodegradable composites) consist of biodegradable polymers as raw material and biodegradable fibres. Since both components are biodegradable, thus the production of biocomposite is also expected to be biodegradable.

2.2 Types of natural polymer

2.2.1 Starch

Among other biopolymers, starch is considered as one of the most promising materials because of its large availability and relatively low price combined with its inherent biodegradability and renewable origin. This is due to the low cost polysaccharide derived from agricultural plants and susceptible to biological attack. Starch is a condensation polymer made up of hundreds of glucose monomers, which remove the water molecule out in chemical reaction as shown in figure 2.1. It also can be referred as polysaccharide because of the monosaccharide glucose. Amylose and amylopectin is the two types of glucose polymers in starch molecules. 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 branched chain (Dufresne *et al.*, 2000).

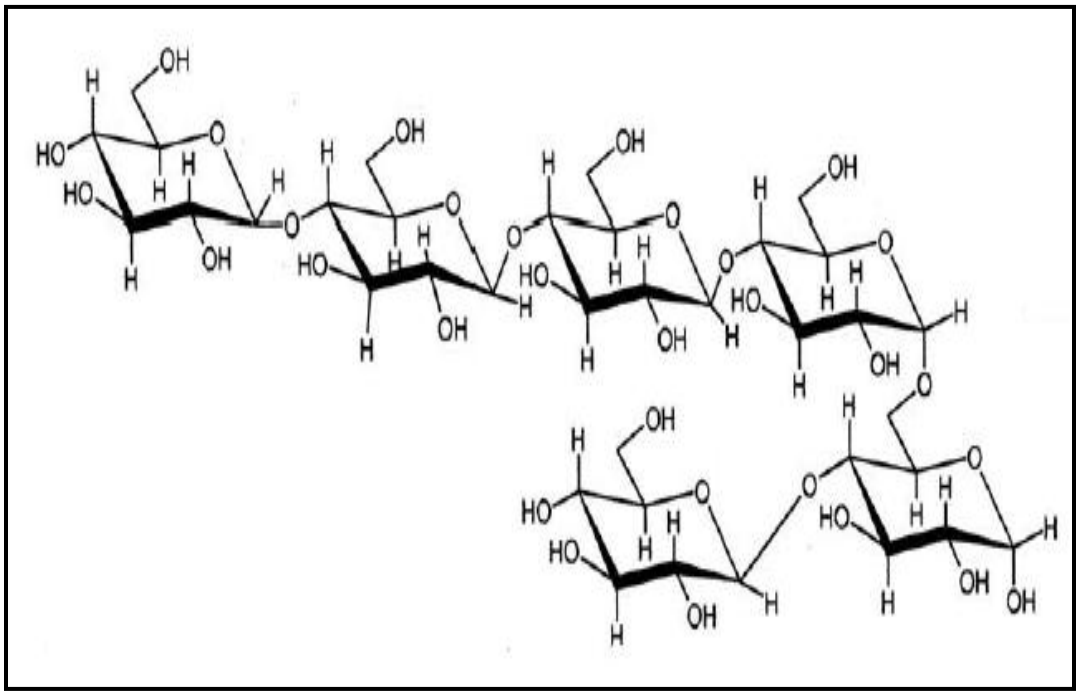

Figure 2.1: The structure of starch (Baran et al, 2004).

The most commercially starch comes from corn, wheat, rice, potatoes, tapioca and peas. TPS known as thermoplastic starch is the converted of starch to the thermoplastic material. However, at high temperature under pressure and, the TPS can be melted. Addition of water and glycerol are widely used as the plasticizers to convert the starch to TPS. But, the TPS stability is strongly decrease by addition of plasticizers. TPS also mostly water soluble, difficult to process and brittle. Despite their interesting mechanical properties, with plasticized starch materials remain highly water sensitive, thus it have poor barrier properties and thus are not fully suitable for packaging applications However, some of properties of starch can be significantly improved by blending with natural fibers (Joesten *et al.,* 1996).

2.2.2 Bacterial cellulose

Cellulose which recognized as major component of plant is the most used biopolymer on earth. The properties of cellulose are hydrophilicity, structure forming potential, and biocompatibility as well as the molecular and supramolecular structure were continually modified by functionalization and regeneration processes. The properties, structure, and shape of the biomaterial could be designed biotechnologically using *Acetobacter xylinum* (*A. xylinum*) (Klemma *et al.*,2001).

The cellulose formation includes five fundamental enzyme mediated step: the transformation of glucose to UDP-glucose via glucose-6-phosphate and glucose-1-phosphate and finally the addition of UDP-glucose to the end of a growing polymer chain by cellulose synthase (Klemma *et al.*,2001). It is used for complicated regulation mechanism, which controls activation and inactivation of the enzyme.Bacterial cellulose (BC) is the specific products of primary metabolism and is mainly a protective coating, while plant cellulose (PC) plays as structural role. The structure of cellulose is an unbranch polymer of -1.4-linked glucopyranose residues while the structure of bacterial cellulose is chemically identical to plant cellulose but differ in macromolecular structure and properties (Bielecki *et al.*,1997)..

Tensile strength and modulus properties are very high for bacterial cellulose. For instance, the BC microfibrils have a density of 1600 kg m^{-3}, Young's modulus of 138 GPa, and tensile strength of at least 2 GPa, which are almost equal to those of aramid fibers (Yano *et al*., 2005). The modulus of BC believed to be several times higher than synthetic polymer. The surface area is more than 200 times greater than isolated softwood cellulose. BC also have the higher ability to holding water, higher crystallinity, higher tensile strength, and a finer web-like network compare from cellulose from plants (PC).

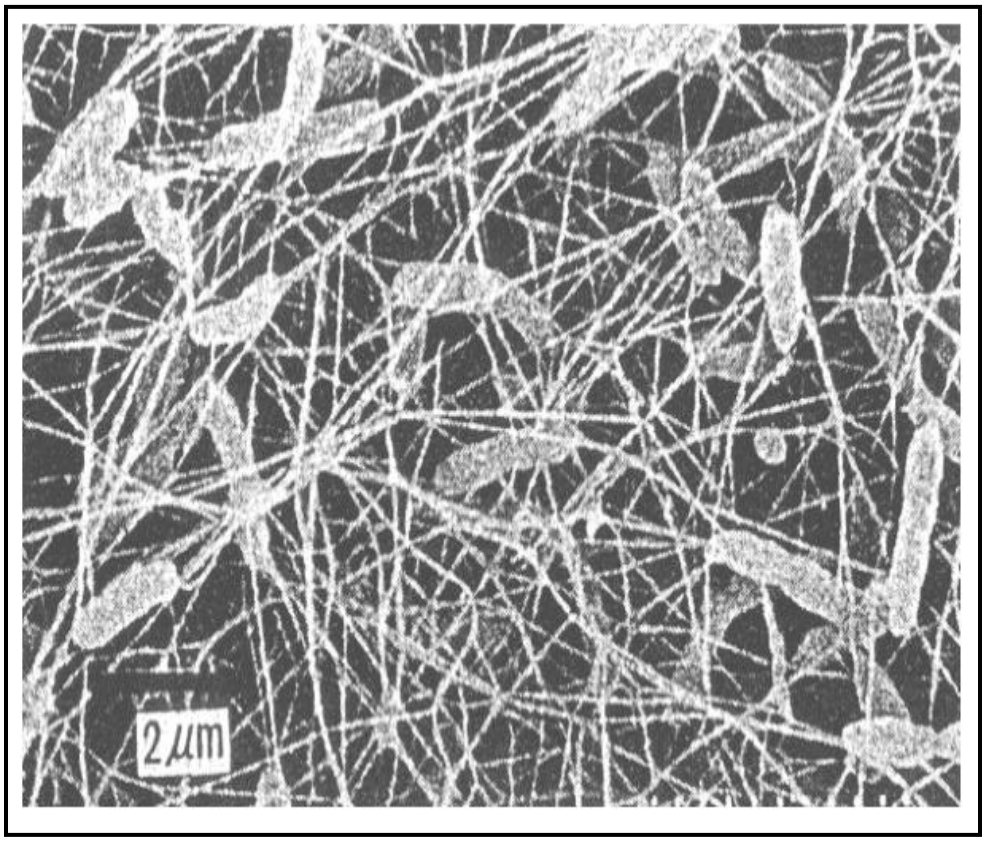

Figure 2.2: Scanning electron micrograph of a bacterial cellulose network including the bacterial cells (Klemma *et al.*,2001).

BC is usually used in foods, in acoustic diaphragms for audio speakers, or making unusually strong paper. Beside that, BC also has medical applications such as healing

effects and the latest study declared that BC also promising material for potential for tissue engineering.

2.2.3 *Acetobacter xylinum*

The bacterium *A. xylinum* was described in 1886 by Brown when he identified a gelatinous mat formed in the vinegar fermentation on the surface of broth that chemically equivalent to cell- wall cellulose. Now, *A. xylinum* serves as a model organism to research cellulose biosynthesis, crystallization processes, and structural properties (Klemma *et al.*,2001). *A. xylinum* is used to synthesis the cellulose in highly aerobic by help the cell to float and reach the oxygen rich surface. It is the gram-negative bacterial with rod-shaped and obligate aerobic bacterium (Setyawatia *et al.*, 2007).

The cellulose was forms by *A.xylinum* between the outer and the cytoplasma membrane. The cellulose was form by synthesizing complexes or terminal complexes (TC) are linearly arranged, and in association with pores at the surface of the bacterium. The first step of cellulose formation, glucan chain aggregates consisting of approximately 6–8 glucan chains are elongated from the complex. Then follow with the second step by these subelementary fibrils are assembled to form microfibrils. In the third steps, this assembly is tight to form a ribbon. Bacterial cellulose membrane or pellicle constitutes by the matrix of the interwoven ribbons (Klemma *et al.*,2001). Figure 2.3 shows the formation of bacterial cellulose.

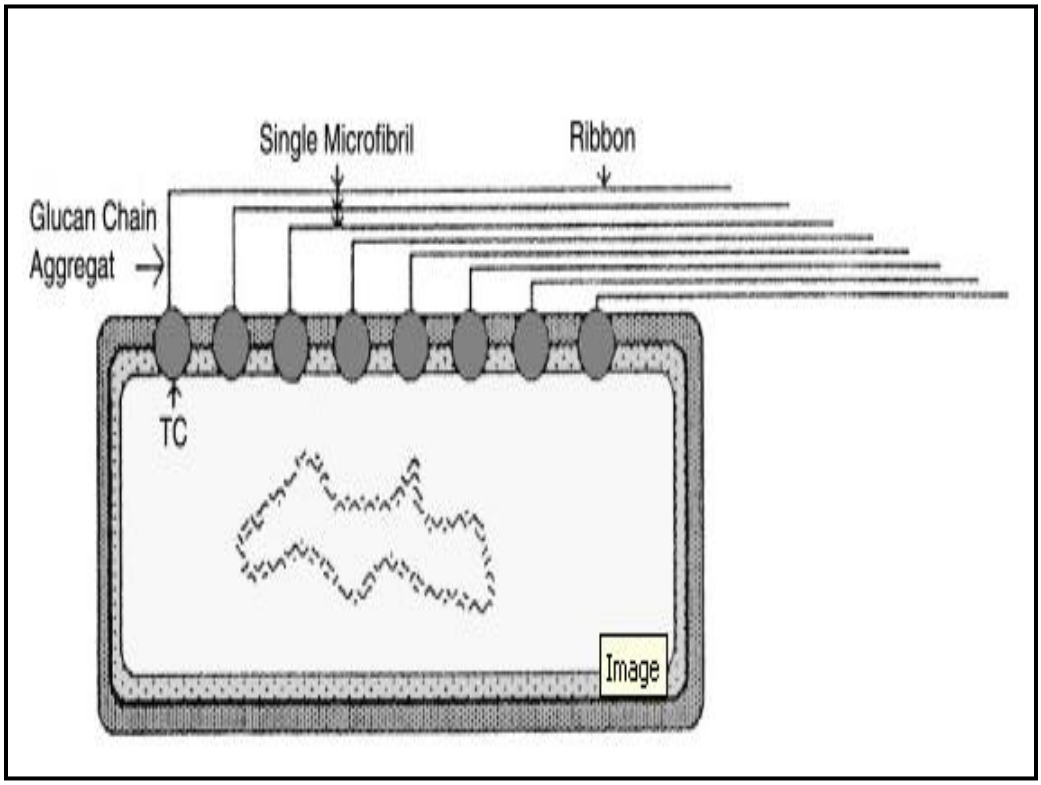

Figure 2.3 Formation of bacterial cellulose (Klemma *et al.*,2001).

2.2.4 Chitosan

Chitosan is a major component of insects and shells. It has a linear high molecule weight of aminopolysaccharide. Normally, chitosan is produced from wastes generated from the crustacean processing such as shrimp and crabs, but it is also possible to obtain from the chitin component of fungal cell walls.

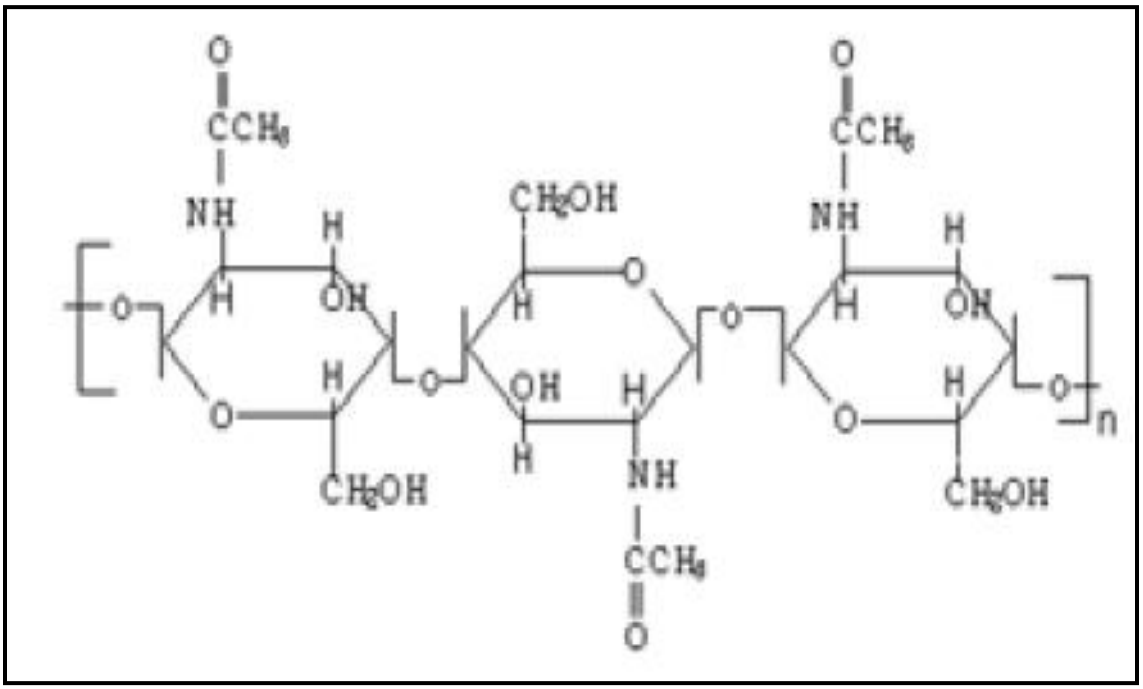

Figure 2.4: The structure of chitosan.

Chitosan have been widely used in packaging application due to it biodegradability and bioactivity, homopolymers and copolymers. Chitosan has attracted considerable used because of its unique properties, abundant commercial supplies and antibacterial properties. The resultant of N-deacetylation of chitin which is second most naturally occurring biopolymer after cellulose is chitosan. The amine group NH_2 can be protonated to NH_3^+ which make chitosan a favorable gel and membrane forming properties (Almeidaa *et al.,* 2009).

Chitosan confers two important characteristics of biopolymer. First, at low ph (<6.3), the chitosan is protonated and resulting cationic polyelectrolyte is water soluble. The water solubility is unusual for β-1,4 linked polysaccharides. Thus, chemical modification such as carboxymethyl or hydroxyethyl groups is required to confer water solubility to the β-1,4-linked polysaccharide cellulose (G. Kumara, 1999).

Nucleophilicity is the second important characteristic of chitosan's amino groups. At high pH (>7), the amino groups are deprotonated and the unshared electron pair can undergo a variety of reactions. Under mild conditions, the reactivity of these amino groups can be exploited to chemically modified (Kumara *et al.*, 1999).

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The chitosan have the poor water barrier characteristics due to it excellent hydrophilic, however have the high impermeable to oxygen. In order to improve the water barrier capability of chitosan, the chitosan is blending with some of hydrophobic materials. (Almeidaa *et al.*, 2009).

2.3 Analysis equipment

2.3.1 Universal Testing Machine

Tensile strength is the stress at which a material breaks or permanently deforms. There are three definitions of tensile strength, yield strength, ultimate strength and breaking strength. Tensile tests are usually carded out on wire; strip or machined samples with either circular or rectangular cross section. Test pieces are screwed into or gripped in jaws and stretched by moving the grips apart at a constant rate while measuring the load and the grip separation. This data is plotted as load versus extension and then converted to engineering stress (load/original area) versus engineering strain (fractional change in length over the test section assuming the deformation is uniform). In special circumstances, the actual stress and strain may be calculated if the true cross section is measured during the test.

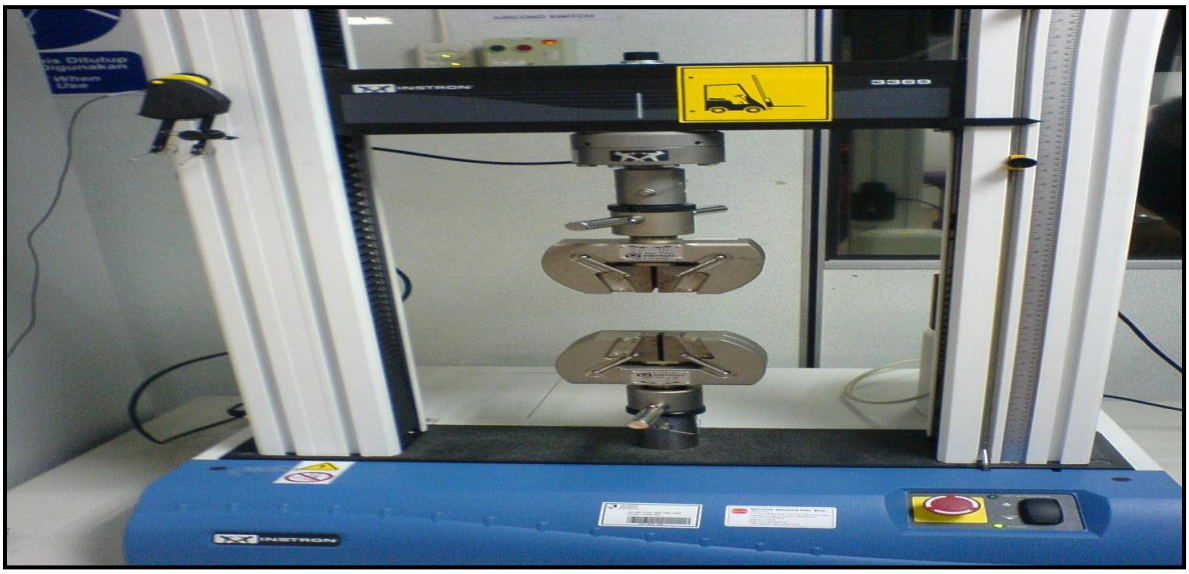

Figure 2.5 : The Universal Testing Machine 50kN.

From the previous studies, the tensile strength and Young's 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. This enhancement indicates the effectiveness of the reinforment (Wan *et al.*,2009).

2.3.2 Fourier transform infrared spectroscopy (FTIR)

FTIR consists of four arms. The first arm contains a source of infrared light, the second arm contains a stationary mirror, the third arm contains a moving mirror, and the fourth arm is open. The beam splitter at the intersection of the four arms is design to transmit half the radiation that impinges upon it, and reflect half of it. As a result, the light transmitted by the beam splitter strikes the fixed mirror and the light transmitted

reflects by beam splitter strike the moving mirror. Then, the two light beams recombine at the beam splitter, and leave the interferometer to interact with sample and strike the detector (Smith *et al.*,1996).

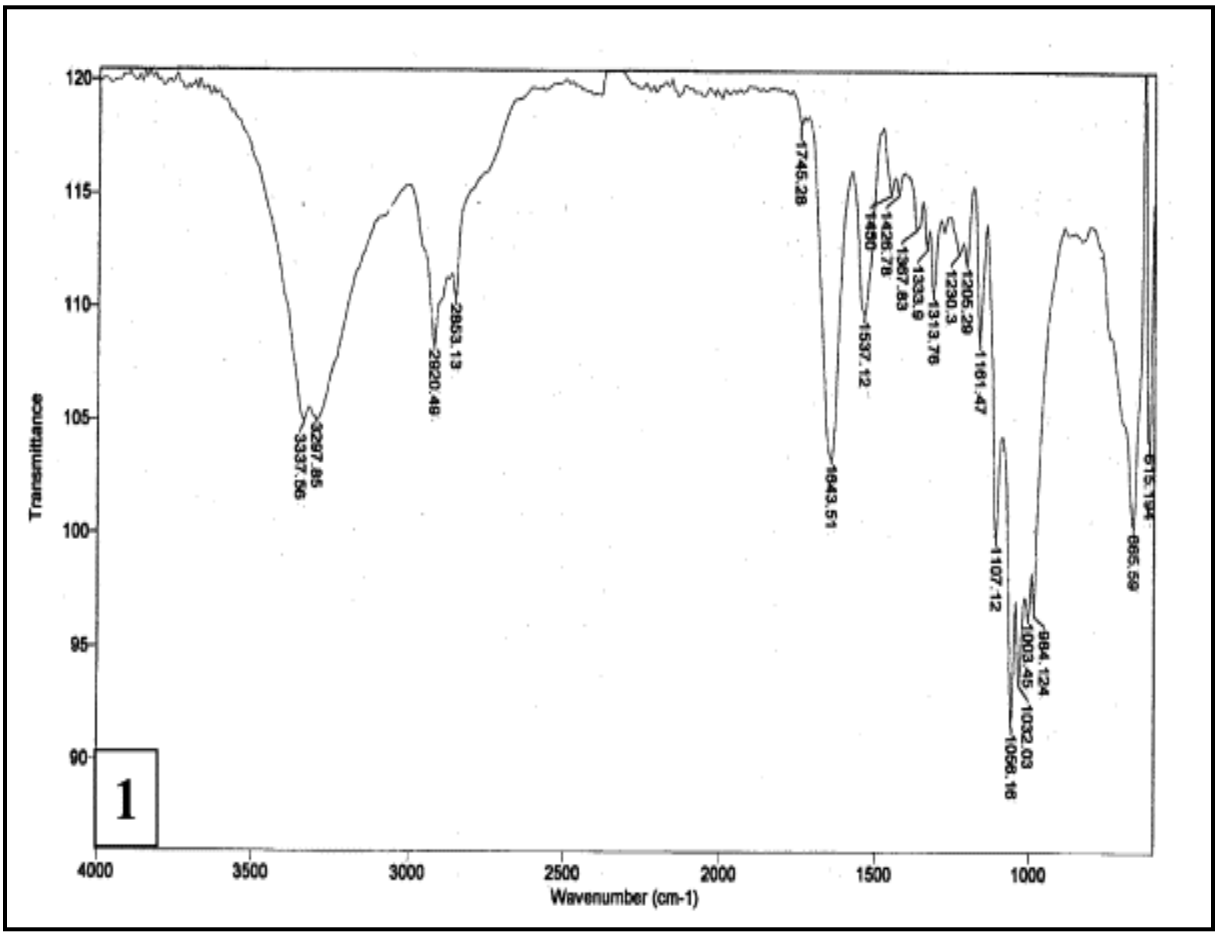

Figure 2.6 FT-IR spectra of bacterial cellulose (Dieter Klemma, 2001).

The advantage of an FTIR instrument is that of acquires the interferogram in less than a second. Thus, it is possible to collect dozens of interferograms of the same sample and accumulate in the memory of a computer. A spectrum with a better signal-to-noise ratio can be plotted when a FTIR is perfume on the sum of the accumulated interferograms. An FTIR is capable of greater speed and greater sensitivity than a dispersion instrument (Pavia *et al.*, 2009).

Table 2.1: The IR-Spectra of the functional group and stretching. (Mathew and Abraham, 2008) ,(Bourtoom and Chinnan, 2007), (Liu *et al*, 2009) and (Sun *et al*, 2009).

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

2.3.3 Scanning electron microscope (SEM)

The most prevailing nano-scale measurement equipment and it is also well known as material analysis equipment is a scanning electron microscope (SEM). The SEM can be categorized into two fold, a thermionic emission and a field emission. The highest resolution SEM has been developed using the field emission but the field emission SEM is extremely expensive and most components dealing with the electrons and need to be placed in extremely higher vacuum state in the range of 10^{-10} Torr, while a thermionic SEM operates in around 10^{-7} Torr. The thermionic SEM still holds a high market share due to its effectiness over the cost (Kima *et al.,* 2008).

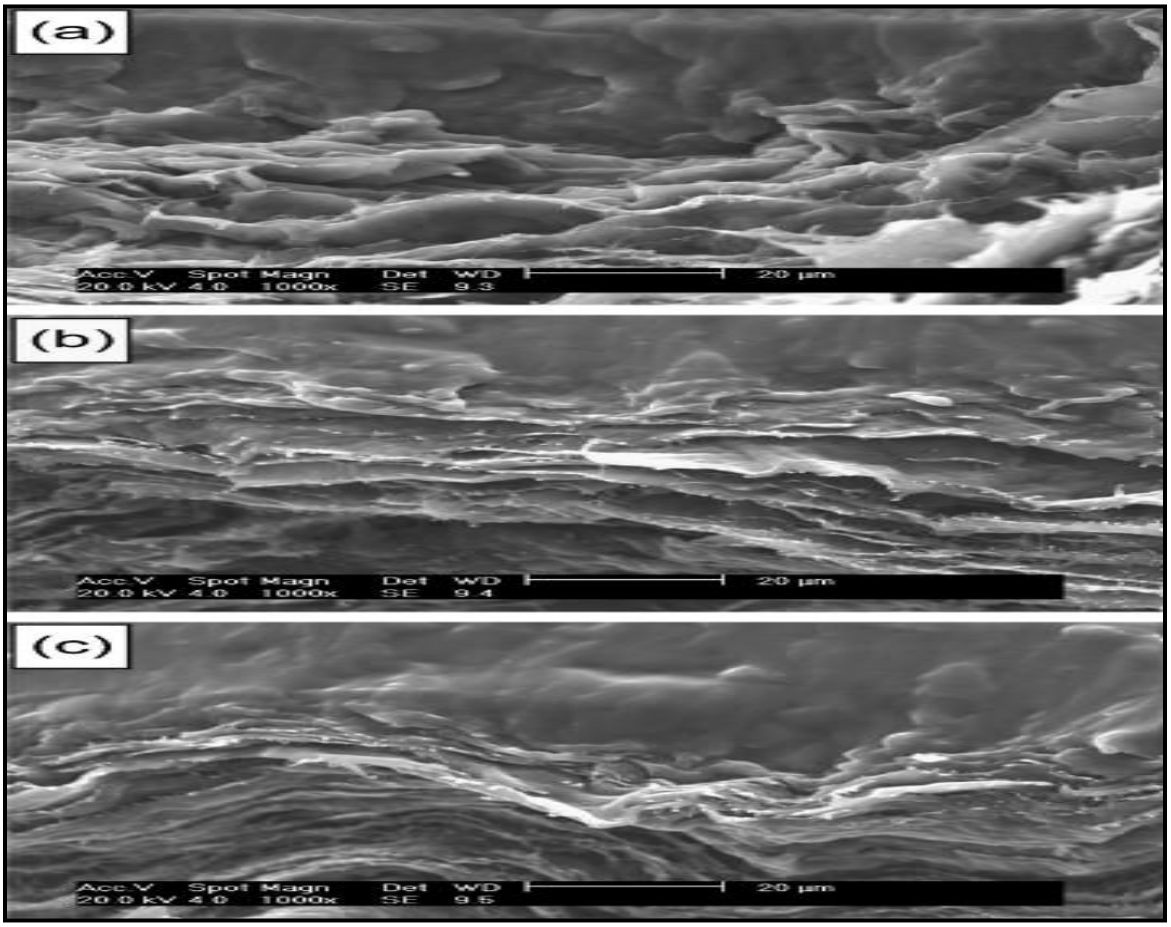

Figure 2.7 Scanning electron micrographs of tensile fracture surfaces of BC/starch biocomposites with different BC contents (a) 7.8 wt.%, (b) 15.1 wt.%, and (c) 22.0 wt.% (Wan *et al.*,2009).

It is seen in Figure 2.7 that the bacterial cellulose with starch composites show a layered structure. The layered structure is the characteristic for bacterial cellulose network as difference in the cellulose network structure is usually observed between the upper surface layer (exposed to air) and the lower layer (exposed to culture medium during cultivation). Note that bacterial cellulose fiber ends are seen in all three photos. However, the fiber pull-out length is not large indicating a good fiber-matrix bonding. Pores in the composites are obvious, thus the suggestion of improvement needed in further investigation (Wan *et al.*,2009).

2.3.4 Gas Pycnometer

Gas pycnometry is based on Boyle-Mariotte's law of volume-pressure relationship (Tamari *et al.*,2004). This is an attractive method to determine the volume of solid particles. The volume of substances that react chemically or physicochemically with water can be determined. It has also been claimed that routine sample-volume determinations with gas pycnometers can be performed in less than twenty minutes and automatically.

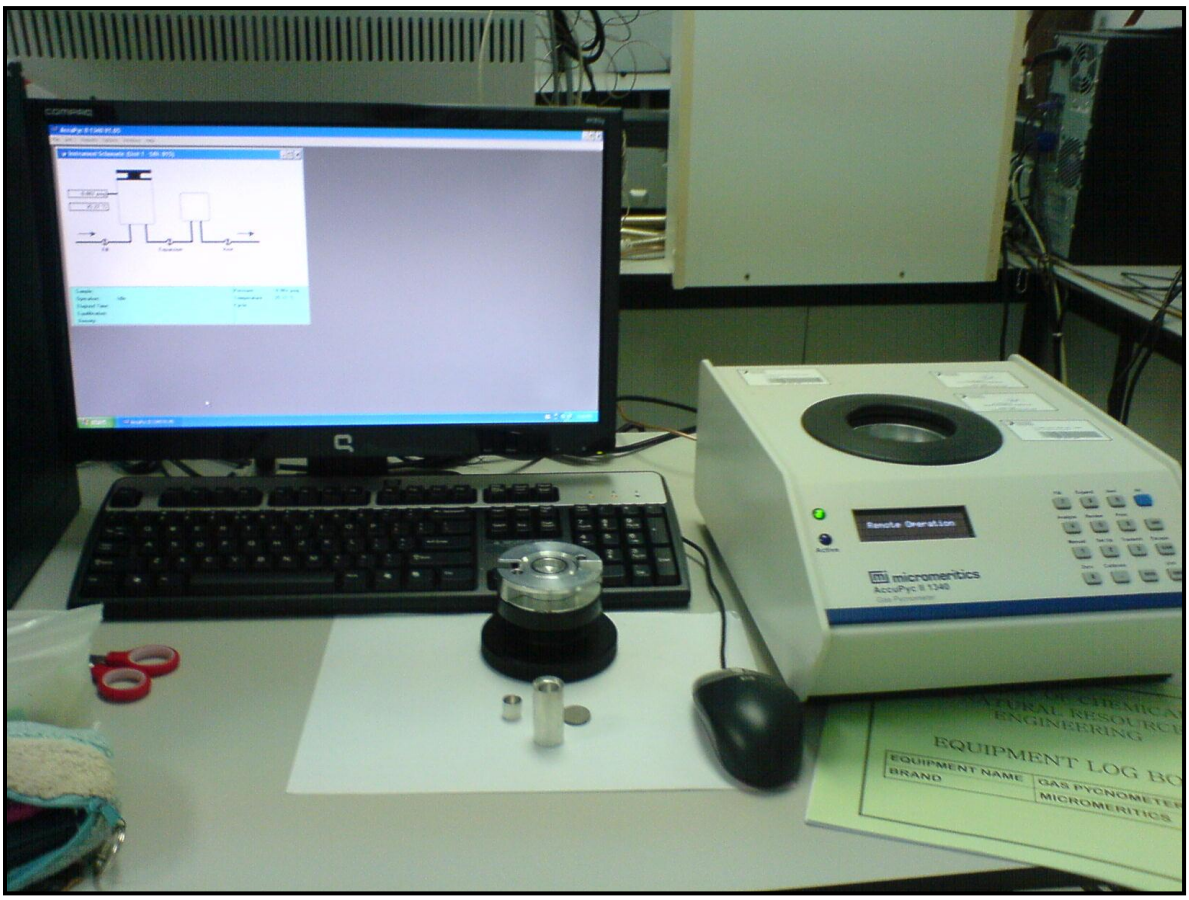

Figure 2.8: The gas pycnometer.

There are three kinds of gas pycnometers which are constant-volume, variable-volume and comparative. Gas pycnometry has been widely used to determine the volume and thus the density of granular, porous or soluble compounds such as rock fragments, soil particles, coal, pigments, ceramic, salts, drugs, aero gels, plastic films, teeth, chocolate, seeds, insects and even living birds (Tamari *et al.*,2004).

A constant-volume gas pycnometer as in Figure 2.8 is composed of a sample chamber with a screw cap, a tank and an absolute pressure transducer. The chamber and the tank can be connected pneumatically through a tube with a coupling valve. The tank is also connected to the pressure transducer and can be connected to a gas supply through a tube with a main coupling valve.

From the previous studies, the true density measurements carried out for reported sample including chitosan. The true density of the compressed expanded graphite including chitosan was found to be close to the density of pure graphite crystal, equal to 2.2 g cm. Filling of pores by chitosan had make more loose material decreased the true density of composite. Higher concentration and cross-linking of chitosan improved compactness of the composite matrix. Probably, the neutralisation process causes strong changes only in the superstucture of the deposited polymer independently of the thickness of it's layer (Krzesinska *et al.*, 2007)

2.3.5 Biodegradation in soil burial

Degradation of plastic and bioplastic in general, is defined as a detrimental change in its appearance, mechanical, physical properties and chemical structure (Grifin, 1994). Biodegradable materials can be integrated directly into the soil where bacterial flora transforms them into carbon dioxide or methane, water and biomass. Natural biodegradable plastics are based primarily on renewable resources. Biodegradation is A degradation caused by biological activity, particularly by enzyme action leading to significant changes in the material's chemical structure.

The biodegradability of plastics is dependent on the chemical structure of the material. The biodegradation of plastics proceeds actively under different soil conditions according to their properties. Biodegradation of starch based polymers occurred between the sugars groups leading to a reduction in chain length and the splitting off of mono-, di-, and oligosaccharide units by a result of enzymatic attack at the glucosidic linkages (Demirbas, 2007).

Chitin, a polysaccharide of animal origin, is obtained from seafood industrial waste material. It occurs to the skeletal material of crustaceans such as crabs, lobsters, shrimps, prawns and crayfish. Chitosan is the deacetylated product formed by treatment of chitin with concentrated (50%) caustic alkali. Thus Chitosan is safe (nontoxic), biocompatible and biodegradable.

From the Wan *et al.,* 2009, noted that weight loss shown an approximately linear relation with degradation time.The average degradation rate is about 1%/day and 0.9%/day respectively, for starch and the starch with bacterial cellulose composites. It is observed that the weight loss of the bacterial cellulose with starch composite is lower than that of the starch at any given time points. As expected, weight loss is accompanied by loss in their mechanical properties.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter presents the detail procedures for the film preparation and production of bacterial cellulose fibre-reinforced starch/chitosan biocomposite. The blend film then characterized by using Universal Testing Machine, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM) and Gas Pynometer, while physical testing using Swelling absorption and biodegradability test using soil burial degradation.

3.2 Materials and Methods

3.2.1 Materials

The tapioca starch with brand Bunga Merah was used and it obtained from local supermarket. The Chitosan with 84% deacetylated was purchased from R&M Chemicals, Essex, U.K and the bacteria *Acetobacter xylinum* used for bacterial cellulose production was purchased from The Department of Microbiology, MARDI, Serdang, Malaysia. The

Glycerol, Acetic Acid, Sucrose, Ammonium Sulfate, Sodium Hydroxide and Acid Nitrate were taken from the storage that used by previous senior. The soils for the soil burial test were taken from the field nearby the university campus.

3.2.2 Methods

3.2.2.1 Films Preparation

Bacterial cellulose (BC) was prepared by ferment the *Acetobacter Xylinum* in coconut water for 3 week in static culture contain 8 % (w/w) sucrose, 0.5 % ammonium sulfate and 0.1 % acid nitrate. The obtained gel-like BC were purified by immersion in a 0.5 M aqueous solution of Sodium Hydroxide for 15 min. The BC pellicle was washed with deionized water several times and blends with concentration of 7, 14, and 21 g. The tapioca starch was dissolved in distilled water at concentration of 6g/ 100 mL by heating the mixtures on hot plate and stirred the mixture until it gelatinized at temperature of 85 ± 2°C for about 5-15 minutes. The chitosan solution was prepared by dispersing at concentration of 0.5, 1, 1.5, and 2 g of chitosan in 100 mL of acetic acid (2% v/v) and stirred overnight until it completely dissolved. A series of starch-chitosan-BC blend were prepared by mixing 100 mL of the starch solution (6 g/ 100 mL) with 100 mL of the chitosan solution (0.5, 1, 1.5, 2 g/100 mL) and blend bacterial cellulose (7, 14 and 21 g). The solutions were mixed with a magnetic stir bar until the solution becomes gelatinized. Glycerol was added as 2.4% (w/w) of the total solid weight in the blend solutions. The resulting solutions were put in the Ultrasonic Cleaner at 85% power for one hour. Then, the solutions were poured about 50mL and casted onto petri dish and leaved it in the oven at 55°C for 10 hours undisturbed. After the film was completely dried, the film then been peeled off from the petri dish.

Table 3.1 Composition of biodegradable films with different amount of chitosan blends.

Blends	**Starch solution (6% w/v) (ml)**	**Chitosan solution (w/v) (ml)**	**Bacterial cellulose (7g)**	**Glycerol (2.4 % v/v) (ml)**
1	100 ml of 6%	100 ml of 0.5%	7 g	100 ml of 2.4%
2	100 ml of 6%	100 ml of 1%	7 g	100 ml of 2.4%
3	100 ml of 6%	100 ml of 1.5%	7 g	100 ml of 2.4%
4	100 ml of 6%	100 ml of 2%	7 g	100 ml of 2.4%

Table 3.2 Composition of biodegradable films with different amount of bacterial cellulose blends.

Blends	**Starch solution (6% w/v) (ml)**	**Chitosan solution (2% w/v) (ml)**	**Bacterial cellulose**	**Glycerol (2.4 % v/v) (ml)**
1	100 ml of 6%	100 ml of 2%	0 g	100 ml of 2.4%
2	100 ml of 6%	100 ml of 2%	7 g	100 ml of 2.4%
3	100 ml of 6%	100 ml of 2%	14 g	100 ml of 2.4%
4	100 ml of 6%	100 ml of 2%	21 g	100 ml of 2.4%

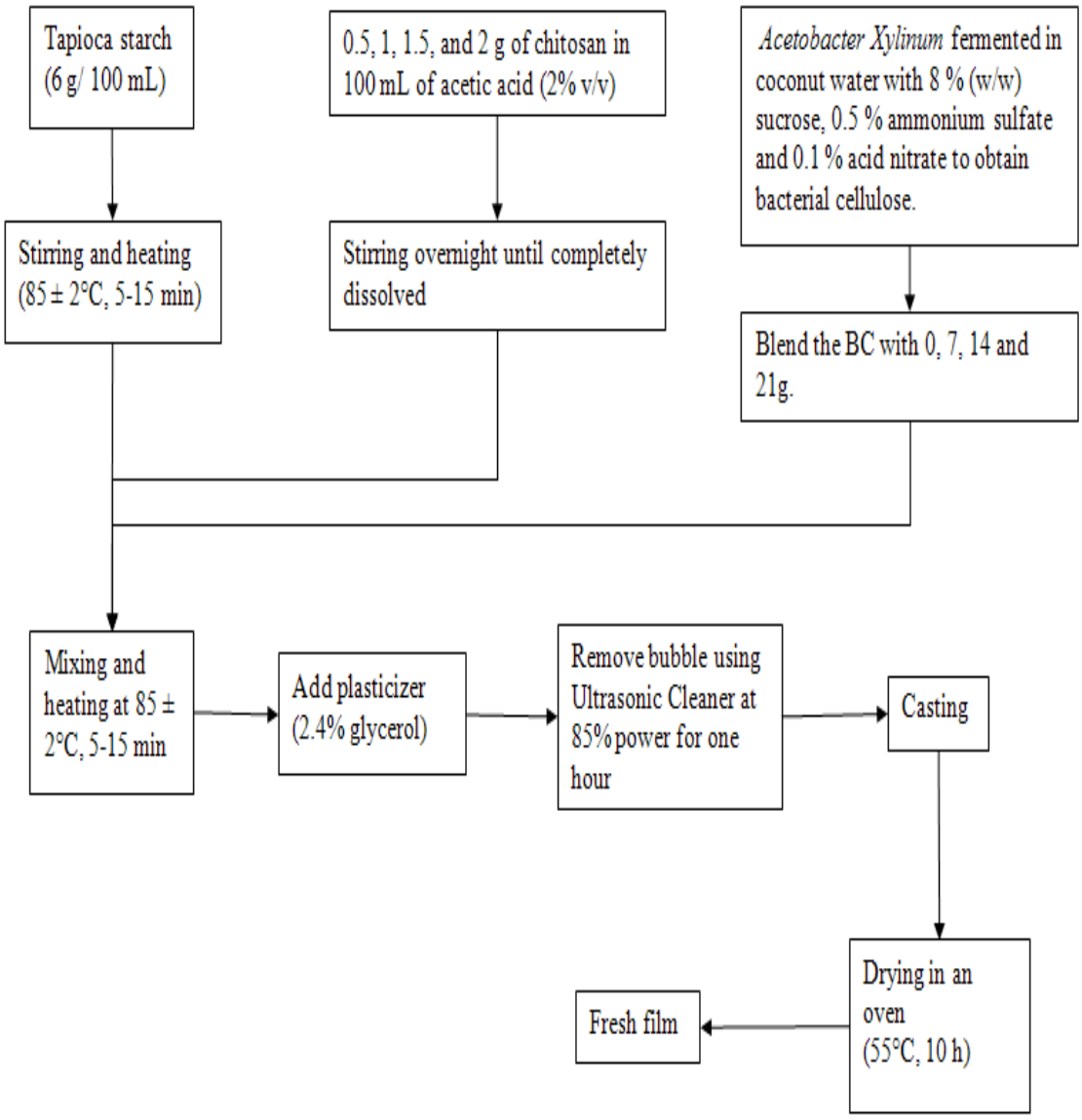

Figure 3.1: The films formation process.

3.2.2.2 Mechanical Properties

The mechanical tests were performed using a Universal Testing Machine brand Shimadzu, fitted with 50kN static load cell. The films were cut into rectangular shape with 20 mm wide and 70 mm long. The tensile properties were measured at a crosshead speed of 5 mm/min and an initial grip separation of 115 mm.

3.2.2.3 FT-IR Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) was performed using FTIR Nicolet Avatar 370 DTGS. The FTIR spectroscopy was used to identify chemical bonds in a molecule by producing an infrared absorption spectrum. The FTIR generates an infrared spectral scan of samples that absorb infrared light. FTIR spectra were recorded between 1100 and 4000 cm-1 with a piece of film 2 cm x 2cm in size. Spectral output was recorded in absorbance as a function of wave number.

3.2.2.3 Scanning Electron Microscopy (SEM)

SEM was performed using SEM EDX Spectrometer EVO 50, operating at an acceleration voltage of 15kV. SEM was used to characterize the microstructure of the samples. The sample of biodegradable blend films was put in the liquid nitrogen and then was coated with white mask under vacuum for SEM observation. The cross section of SEM was observed with 100x and 500x resolution.

3.2.2.4 Density

3.2.2.4.1 Density value from Gas Pycnometer analysis

The true density was measured using a helium gas displacement Pycnometer type 1305 Micromeritics. All the biodegradable blend films with weight of 0.45g were prepared with 3 cycle of replication.

3.2.2.4.2 Manual calculation using specific gravity theory

Manual calculation was performed using the specific gravity theory.

$$SG = M^a / (M^a - M^w) \qquad [1]$$

Where M^a is the mass in air and M^w is the mass in water. All the biodegradable blend films with dimensions of 2 cm by 2 cm were weighed in air and then were suspended in the water. The weights of suspended biodegradable blend films were recorded. The specific gravity was determined using the equation given above, and the density of the biodegradable blend films was determined by multiplied by the density of water.

3.2.2.5 Swelling Absorption

Swelling absorption measurements were performed where the dried film was immersed completely in deionized water for absorbing process. After sufficient swelling, the swollen membranes were taken out from the water 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 rates of water absorption were obtained using the Fick's law of diffusion as follows:

$$Mt/M\infty = (4 / \pi^{½}) (Dt /h^2)^{1/2} \qquad [2]$$

$$Mt = k\, t^{1/2} \qquad [3]$$

$$k = (4M\infty / h) (D/ \pi)^{1/2} \qquad [4]$$

The Mt is the mass gain at time t, M∞ is the mass gain at the equilibrium (maximum water uptake), the h is the thickness of the specimen and D is the diffusion coefficient. From the data plotted as Mt/M∞ against the time, the diffusion k was obtained from the slopes of the straight parts of the plots. From the slopes of the straight parts of the plots, the diffusion coefficient can be calculated.

3.2.2.6 Soil Burial Degradation Test

Soil burial degradation was performed by filling the garden polibag in medium size were filled with soil taken from a field around Universiti Malaysia Pahang (UMP). The biodegradable blend films were cut into 2 cm x 2 cm pieces and buried in the soil at a depth of 5 cm. The polibag were placed in an uncovered placed. The soil was kept moist by sprinkling water at regular time interval to maintain the humidity. The excess water was drained through the hole at the bottom of the polibag. The degradation of the specimen was determined at a regular time interval (3 days) by taking the specimen carefully from the soil, and washed with distilled water several times to stop the degradation, dried at room temperature. Then, the film was weighted to determine the weight loss. Graph plotted with weight losses of the specimens versus time were used to indicate the degradation rate in the soil burial test.

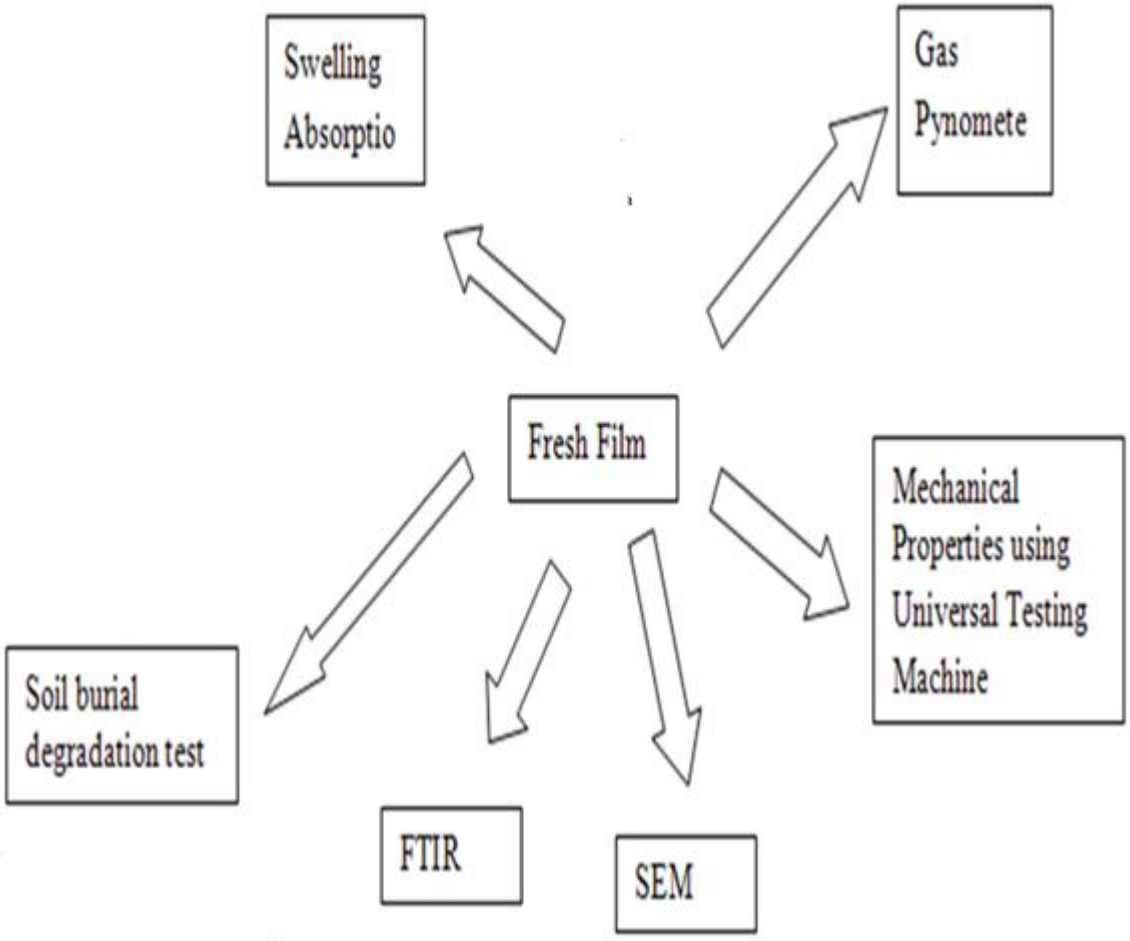

Figure 3.2 The experimental plan of the present study.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter provides a detail discussion about the results analysis of biodegradable biocomposite of bacterial cellulose fiber-reinforced starch/chitosan biocomposite. The results obtained were compared with the findings from previous researchers.

4.2 Mechanical Properties

The tensile strength of biodegradable biocomposite of bacterial cellulose fiber-reinforced starch/chitosan biocomposite with different chitosan contents and different bacterial cellulose content were shown in Figure 4.1 and Figure 4.2. The tensile strength provides a measure of film strength.

From Figure 4.1, the increasing amount of chitosan will increase the tensile strength. The increasing tensile strength value of the biocomposite films were attributed to the formation of inter-molecular hydrogen bonds between NH_{3+} of the chitosan backbone and OH- of the starch (Xu *et al*., 2004).

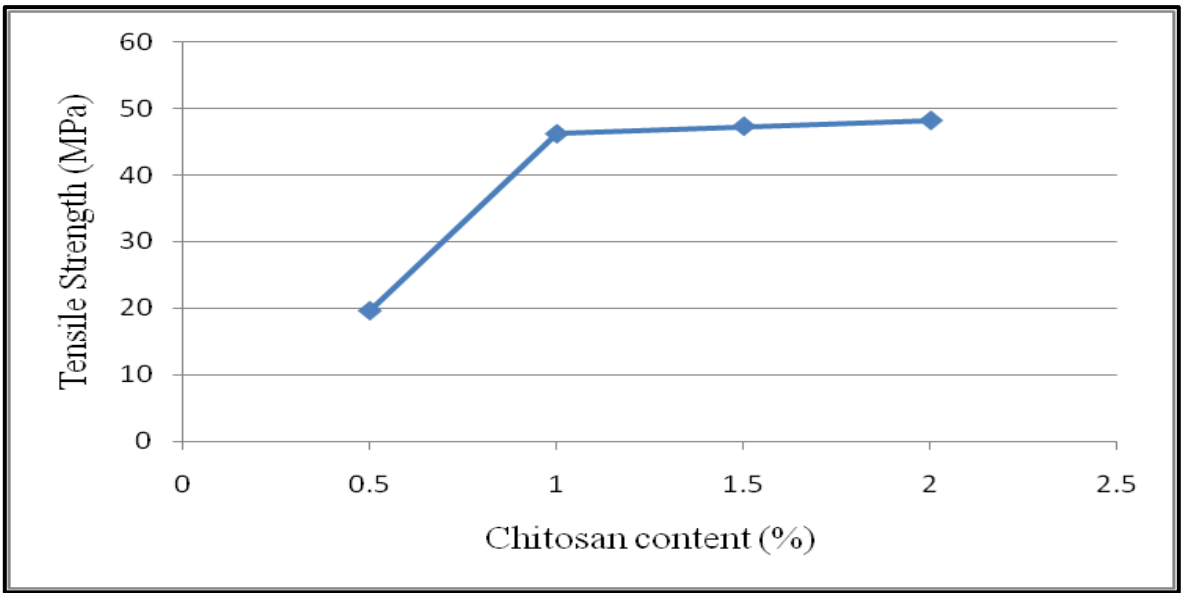

Figure 4.1: The tensile strength of biodegradable blend films with different chitosan contents.

However, the increasing of tensile strength was constant when the amount of chitosan was more than 1%. This is because the limitation of the OH- of the starch to form the inter-molecular hydrogen bonds between NH_{3+} of the chitosan backbone (Xu *et al.*, 2004). Beside that, the constant increasing may occur because of starch intra-molecular hydrogen bond rather than inter-molecular hydrogen bonds were formed, resulting in a phase separation between the two main component.

In addition, the intermolecular arrangement of chitosan in an aqueous solution was influenced by the peculiarity of acid solutions such as ionic strength and the degree of dissociation. The mechanical properties of chitosan films change with the type of acid solvent which might affect both junction density and topological limitations in the film. This may be due to the different interaction between chitosan and acid solution (Park *et al.*, 2002).

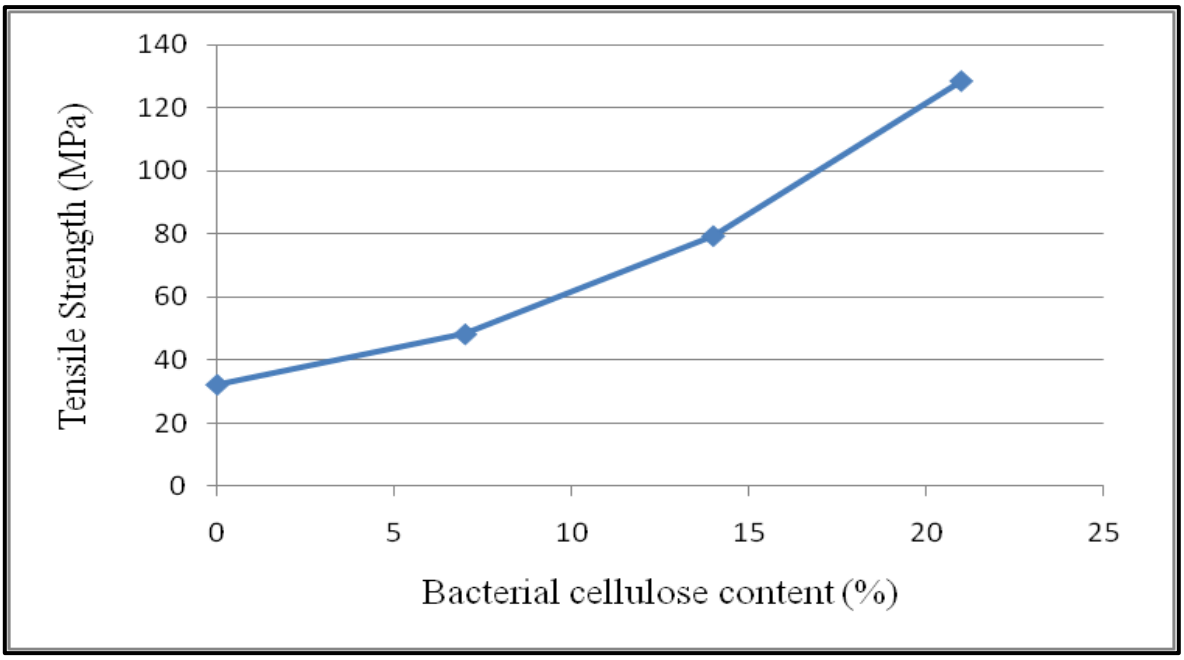

Figure 4.2: The tensile strength of biodegradable blend films with different bacterial cellulose contents.

For samples with composition of bacterial cellulose, the result showed that the increasing of bacterial cellulose will increase the tensile strength. This is because the orientation and degree of interaction between microfibrils within the film. The bacterial cellulose films showed high modulus value attributed to the uniform, continuous and straight nanoscale network of cellulosic elements in-plane oriented via the compression of bacterial cellulose pellicles (Nakagaito *et al.* 2005). It was well accepted that tensile strength and Young's modulus of composites are influenced by nature of matrix and adhesion between fiber and matrix (Wan *et al*, 2009).

4.3 FT-IR Spectroscopy

FTIR spectroscopy was used to determine the interactions between the starch, chitosan and bacterial cellulose in films. The infrared spectrum of films with different chitosan contents were shown in Figure 4.3. While, the infrared spectrum of bacterial cellulose only was showed in Figure 4.4. The infrared spectra films with different bacterial cellulose contents were shown in Figure 4.5.

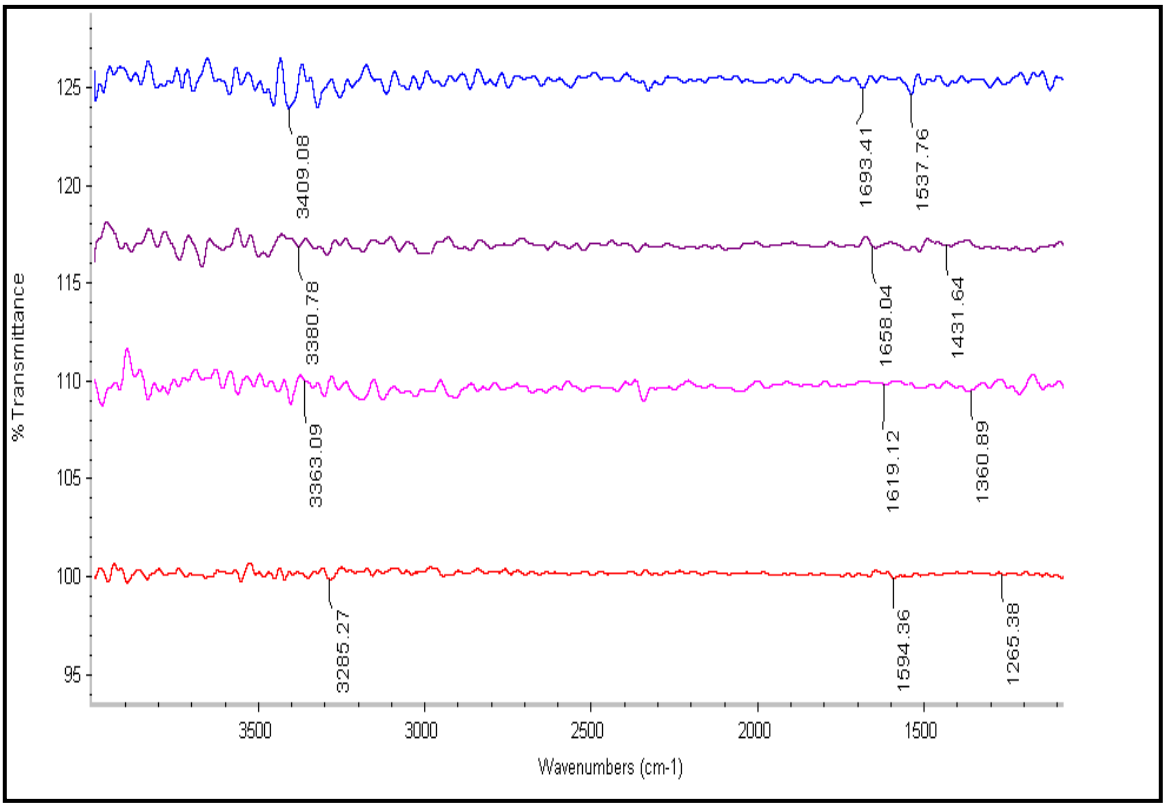

Figure 4.3: The infrared spectra biodegradable blend films with different chitosan contents.

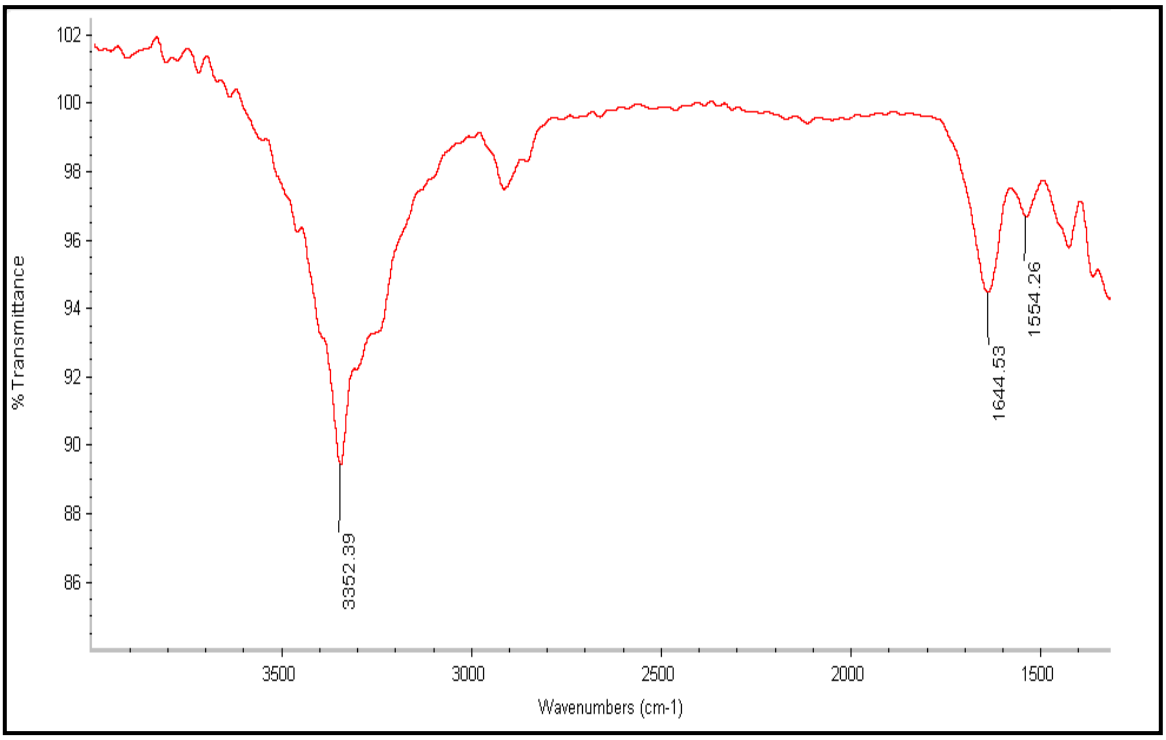

Figure 4.4: The infrared spectra of bacterial cellulose only.

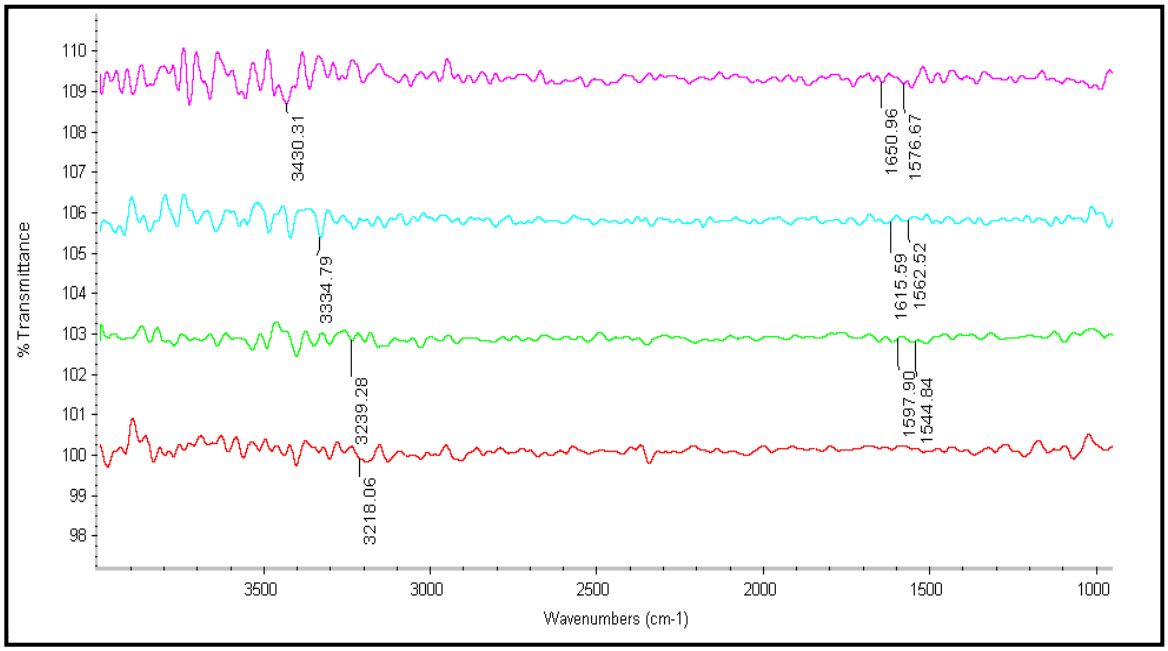

Figure 4.5: The infrared spectra biodegradable blend films with different bacterial cellulose contents.

In spectrum of the both Figure 4.3 and Figure 4.5, the broad band of starch in biodegradable blend films at 3600 cm^{-1} to 3000 $cm^{-1}$was the hydrogen-bonded of hydroxyl groups that contribute to the complex vibrational stretches associated with free inter- and intramolecular bound hydroxyl group. The sharp band at 2900 cm^{-1} is characteristic of C-H stretches associated with the ring methane hydrogen atoms. The bands at 1660 cm^{-1} to 1300 are assigned to the δ O-H bending of water and CH_2 respectively while the band from 700 to 1200 attributed to the C-O bond stretching (Mathew and Abraham, 2008) and (Bourtoom and Chinnan, 2007).

In both Figure 4.3 and Figure 4.5 revealed the IR spectra of chitosan at 3400 to 3300 was the OH stretching, which overlaps the NH stretching in the same region. The small peak at 1700 to 1600 was due to the C=O stretching (amide I), and the peak at 1600 to the 1300 have been reported as amide II and III peaks respectively (Liu *et al*, 2009). When increasing the amount of chitosan, the OH symmetrical deformation mode at 3300 cm^{-1} increased with the increasing in the amount of the chitosan added. This is probably due to the interaction between the OH bonds of chitosan and starch with functional groups contained in acetic acid leading to a covalent bonds and hence cause increasing in peak spectrum (Salleh *et al*, 2009). This situation were also same for the amide I, II, and III where the increasing amount of chitosan will increase the peak of IR spectra.

In turn, the FTIR spectrum showed the occurrence of hydroxyl groups in the region of 3352 cm^{-1} in Figure 4.4. A characteristic IR band at 1650 cm^{-1} to 1578 cm^{-1} corresponded to the O=C vibration of bacterial cellulose (Sun *et al*, 2009). The sharp peak from 1,565 cm^{-1} to 1,540 cm^{-1} could also be attributed to the amide II group characteristic for chitosan (Ciechanska Danuta, 2004). When increasing the amount of bacterial cellulose, all the IR peaks also showed the increasing trend. According to the previous report Yin, et al (1999), reported that when two or more substances are mixed by physical blends, the chemical interactions are reflected by changes in characteristic of spectral peaks.

4.4 Scanning Electron Microscopy (SEM)

The microstructures of the biodegradable blend films with 0g, 7g and 21g bacterial cellulose content were investigated through SEM micrographs and the results were as illustrated in Figure 4.6 to Figure 4.11.

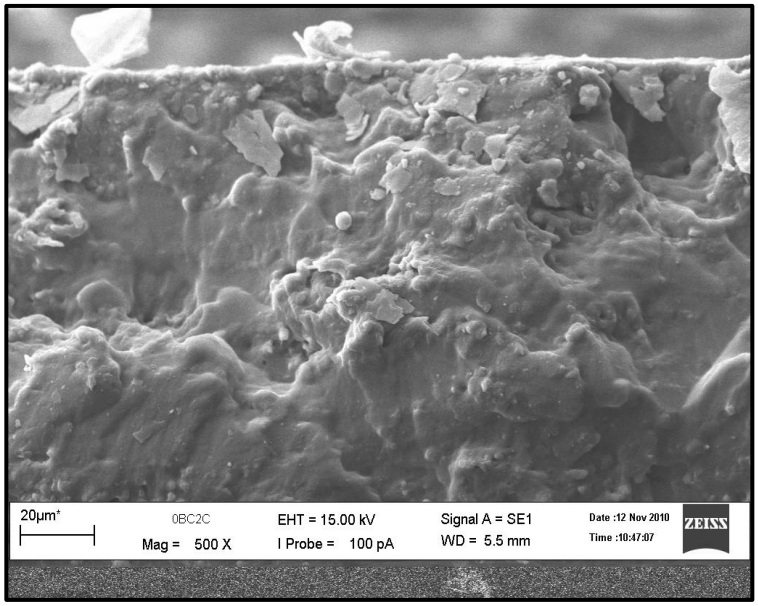

Figure 4.6: SEM micrograph of the cross section of 6% starch, 2%chitosan with 0% bacterial cellulose film.

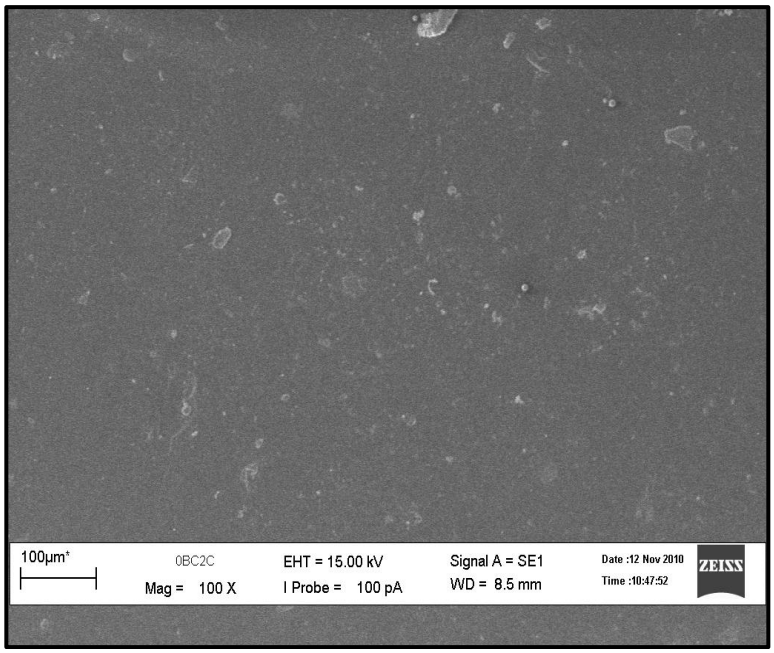

Figure4.7: SEM micrograph of the surface of 6% starch, 2% chitosan with 0% bacterial cellulose film.

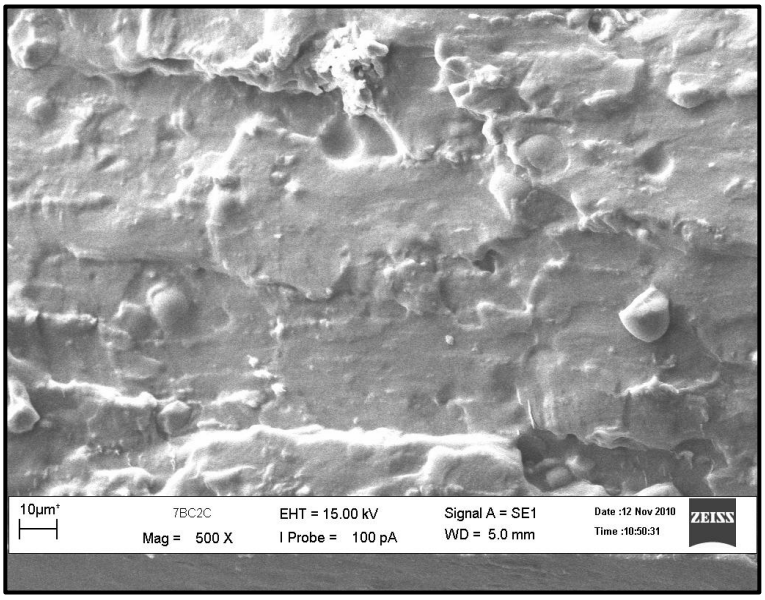

Figure 4.8: SEM micrograph of the cross section of 6% starch, 2% chitosan with 7% bacterial cellulose film.

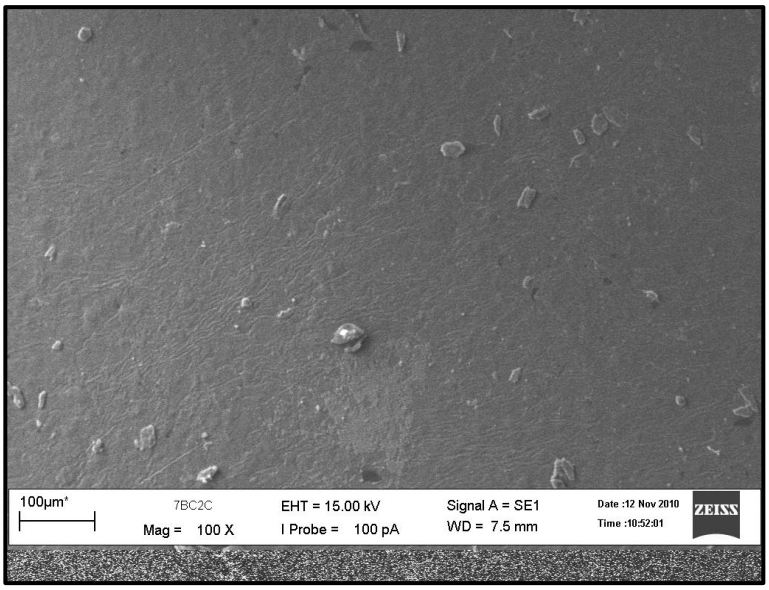

Figure 4.9: SEM micrograph of the surface of 6% starch, 2% chitosan with 7% bacterial cellulose film.

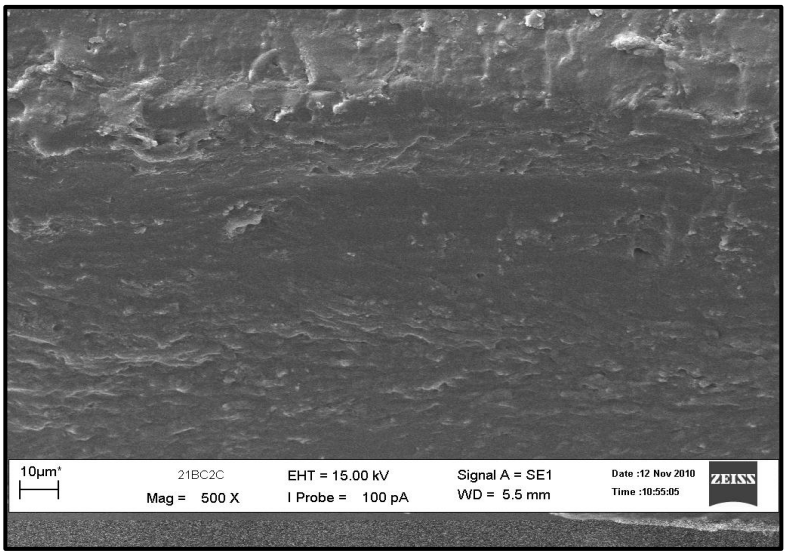

Figure 4.10: SEM micrograph of the cross section of 6% starch, 2% chitosan with 21% bacterial cellulose film.

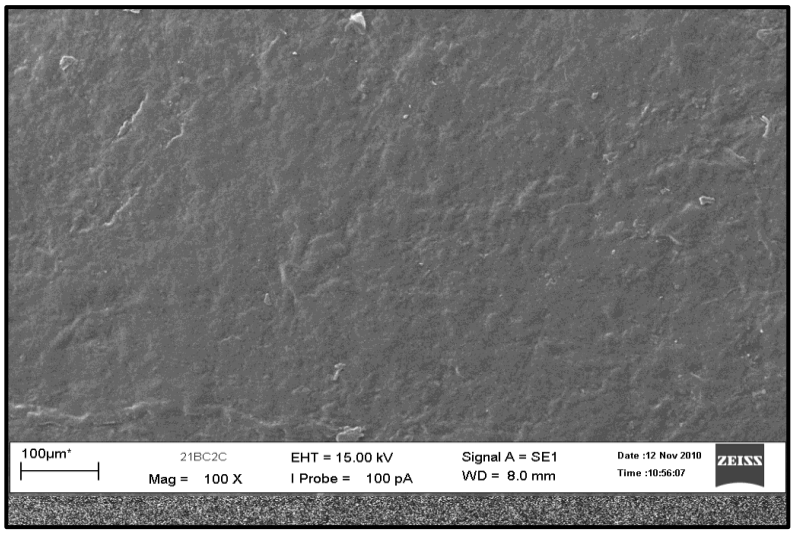

Figure 4.11: SEM micrograph of the surface of 6% starch, 2% chitosan with 21% bacterial cellulose film.

In this study, a soft gel obtained when *Acebacter Xylinum* was treated with a chitosan acetic acid solution and starch solution. At Figure 4.6 of 6% starch, 2% chitosan with 0% of bacterial cellulose content shown a spot of starch not greatly mix with chitosan.. From the Figure 4.6, there were many micro cracks on the cross section view that caused by crinkle of starch linked in the surrounding of abundant chitosan which will decrease the mechanical and permeable properties. These patterns represent the withered ghost granules of starch (Salleh et al, 2009). The surface of the film also does not show a relative smooth morphology. This result was in accordance with those in the previous studies (Liu *et al.*,2008).

Whereas, biodegradable blend film of 6% starch, 2% chitosan with 7g% of bacterial cellulose content in Figure 4.8 shows a less micro cracks compare with the Figure 4.6 of 6% starch, 2% chitosan with 0% of bacterial cellulose content. When the blending and mixing bacterial cellulose gel in chitosan solution the multiple layers of water surrounding polyglucosan chains would be displaced. This will inducing the formation of bonds between cellulose and chitosan, with consequent marked structural modifications. By changing the weight of bacterial cellulose concentration, the surface and cross-sectional of starch/ BC/ chitosan also changes. Chitosan microdomains were dispersed within the starch

matrix in the blend films with relatively good interfacial adhesion between the two components (Li *et al*, 2002).

A porous structure appeared on the surface and cross-sectional morphology especially for low bacterial cellulose content. When the bacterial cellulose content is high such as in Figure 4.10, the porous structure disappeared and a thick layer was formed due to the coverage of BC nanofibrils by chitosan. From the cross- sectional image in Figure4.10, showed that the chitosan molecules penetrated into BC and form BC/chitosan/starch layer composite. The samples show highly fibrous network-like structure consisting of ultrafine cellulose micro fibrils (Retegi *et al*, 2009). The nanofibrous BC and BC/ chitosan/ starch composites have an interconnected porous network structure that has essentially a large surface area. The result was in accordance with those in the previous studies (Chen, 2010). Biodegradable blend film with 21% bacterial cellulose with 2% chitosan and 6% starch content in Figure4.10 shows the relative smooth morphology of film beside increase the structural strength and it is suitable for use as a packaging material.

4.5 Density analysis

Both apparent and true densities were measured to determine the bulk porosity of the samples. Apparent density was calculated from the specific gravity analysis. The true density was measured using a helium gas displacement pycnometer. Table 4.1 and Table 4.2 showed the result of true density compared with manual calculation for different amount of chitosan and different amount of bacterial cellulose.

Table 4.1: The density of biodegradable film with different amount of chitosan.

Sample	Density from Gas Pynometer testing	Density from specific gravity testing (manual)	Percentage of error (%)
0.5% chitosan 7% bacterial cellulose	1.3805 g/cm^3	1.4336 g/cm^3	3.84
1% chitosan 7% bacterial cellulose	1.3459 g/cm^3	1.3896 g/cm^3	3.25
1.5% chitosan 7% bacterial cellulose	1.3364 g/cm^3	1.3687 g/cm^3	2.42
2% chitosan 7% bacterial cellulose	1.3662 g/cm^3	1.3942 g/cm^3	2.05

Table 4.2: The density of biodegradable film with different amount of bacterial cellulose

Sample	Density from Gas Pynometer testing	Density from specific gravity testing (manual)	Percentage of error (%)
0% bacterial cellulose 2% chitosan	1.3583 g/cm^3	1.3621 g/cm^3	0.28
7% bacterial cellulose 2% chitosan	1.3662 g/cm^3	1.3942 g/cm^3	2.04
14% bacterial cellulose 2% chitosan	1.3607 g/cm^3	1.3425 g/cm^3	1.34
21% bacterial cellulose 2% chitosan	1.3150 g/cm^3	1.2500 g/cm^3	4.94

The result showed different value of density because there were totally two difference methods. Both of the method showed error bellow that 5%. Thus the error can be neglected. When increasing the amount of chitosan, the density also decreases. This is because the increasing amount of chitosan will filled the pores inside the biodegradable blend films (Krzesinska *et al*, 2007).

However, at amount of 2% chitosan, the density value of the film suddenly increases. This may occur because of the starch intra-molecular hydrogen bond rather than inter-molecular hydrogen bonds were formed, resulting in a phase separation between the two main component (Xu *et al*., 2004). Thus will affected the value of density.

From Table 4.2, the value of density increasing when the amount of bacterial cellulose was increase, the increasing amount of bacterial cellulose will increases the nanofiber loading which formed the interaction between the starch and chitosan. The natural fiber of bacterial cellulose will improved the compactness of the composite matrix (Wan *et a*l, 2009).However, when the amount of bacterial cellulose reached 21%, the value of density was suddenly decreases. This was because the densities were affected by the porosity of the structure. It is supported by Figure 4.10 of SEM result shown that the micrograph of the surface for 21% bacterial cellulose content no porosity.

4.6 Swelling Absorption

Water sensitivity is another important property for practical application of the film. To analyze the hydrophobic or hydrophilic of the obtained film, the properties diffusion rate were tested and the results were given in Figure 4.12 and Figure 4.13.

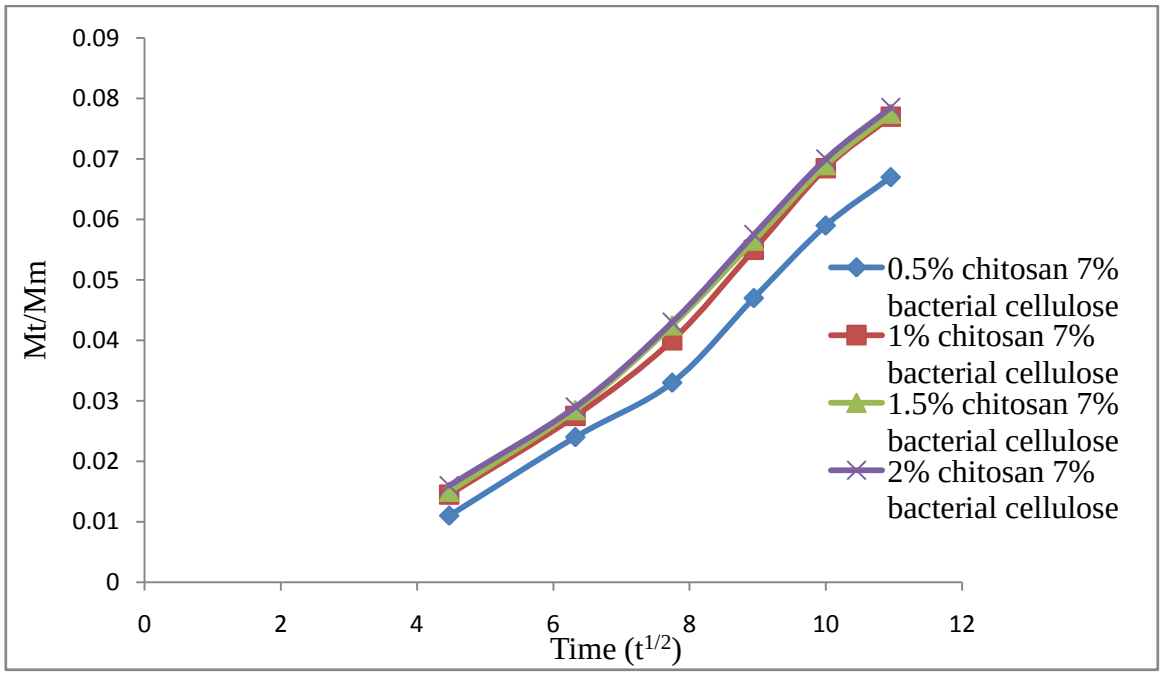

Figure 4.12: The water uptake of biodegradable blend films with different chitosan contents.

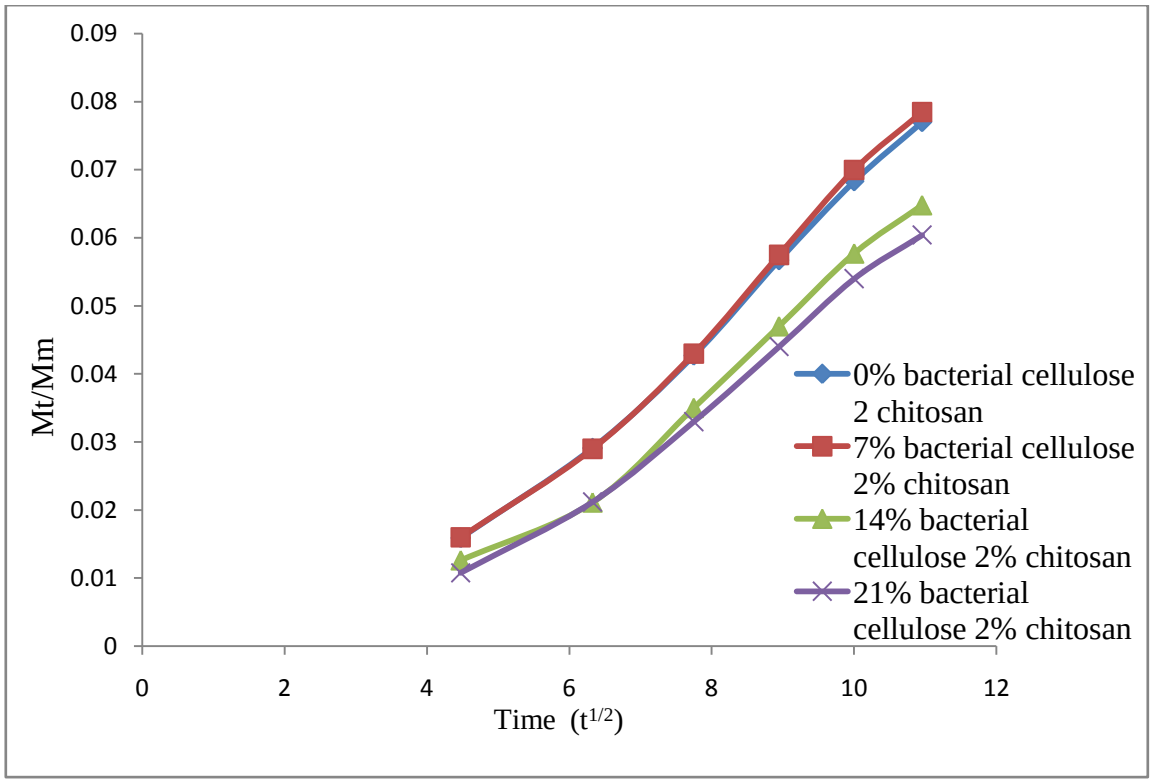

Figure 4.13: The water uptake of biodegradable blend films with different bacterial cellulose contents.

Table 4.3: The diffusion coefficient for different amount of chitosan

Sample	k x 10^{-3} ($s^{-1/2}$)	M ∞	D x 10^{-9} cm^2s^{-1}
0.5 % chitosan 7% bacterial cellulose	8.0501	0.225	1.005
1 % chitosan 7% bacterial cellulose	9.0564	0.200	1.6104
1.5 % chitosan 7% bacterial cellulose	9.2799	0.200	1.6909
2 % chitosan 7% bacterial cellulose	9.2799	0.200	1.6909

Table 4.4: The diffusion coefficient for different amount of bacterial cellulose

Sample	k x 10^{-3} ($s^{-1/2}$)	M ∞	D x 10^{-9} cm^2s^{-1}
0% bacterial cellulose 2% chitosan	9.1234	0.206	1.53605
7% bacterial cellulose 2% chitosan	9.2799	0.200	1.6909
14% bacterial cellulose 2% chitosan	7.6923	0.237	0.8239
21% bacterial cellulose 2% chitosan	7.4240	0.025	0.6926

The value of the diffusion coefficient D increase as of the chitosan content increase as tabulated in Table 4.3. This was due to the hydrophilic behavior of chitosan, starch and bacterial cellulose (Almeidaa *et al.*,2009).

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Noted that the bacterial cellulose, starch and chitosan were hydrophilic which may render the composites high moisture absorption. However, in the Table 4.4 the increasing amount of bacterial cellulose decrease the value of diffusion coefficient. With the constant amount of chitosan and different amount of bacterial cellulose from 0% to 21%, the water uptake was decreased which was probably due to the increasing nanofiber loading between starch, bacterial cellulose and chitosan as evidenced by the SEM micrograph and also been reported in (Wan *et al,* 2009).

The chemistry similarity may result in good interface adhesion between the components, which to some extent, can prevent moisture absorbance and corresponding dimensional changes as poor interface adhesion resists diffusion of water molecules along interfaces. Beside that, the decreased of diffusion coefficient are related to the presence of strong hydrogen bonding interactions between starch, bacterial cellulose and chitosan crystallites since the hydrogen bonding interactions in the composites tends to stabilize the

starch matrix when it was submitted to highly moist atmosphere as reported in the previous reported (Wan *et al*, 2009) and (Liu *et al.*, 2009).

4.7 Soil Burial Degradation Test

The studies on the biodegradation behavior are important for the application of biocomposite in environment. In this testing, the soil burial experiments were performed for the film that contain different amount of chitosan from 0.5% to 2% and film that contain different amount of bacterial cellulose from 0% to 21% as showed in Figure 4.15 and Figure 4.16. The weight loss of the biodegradable film was monitored by means of sample collected from the soil at regular time interval which is 3 day interval.

Figure 4.14 shows the scanned pictures of blend films before and after composting test. The films were eroded significantly and lost their original shape completely after 10 days.

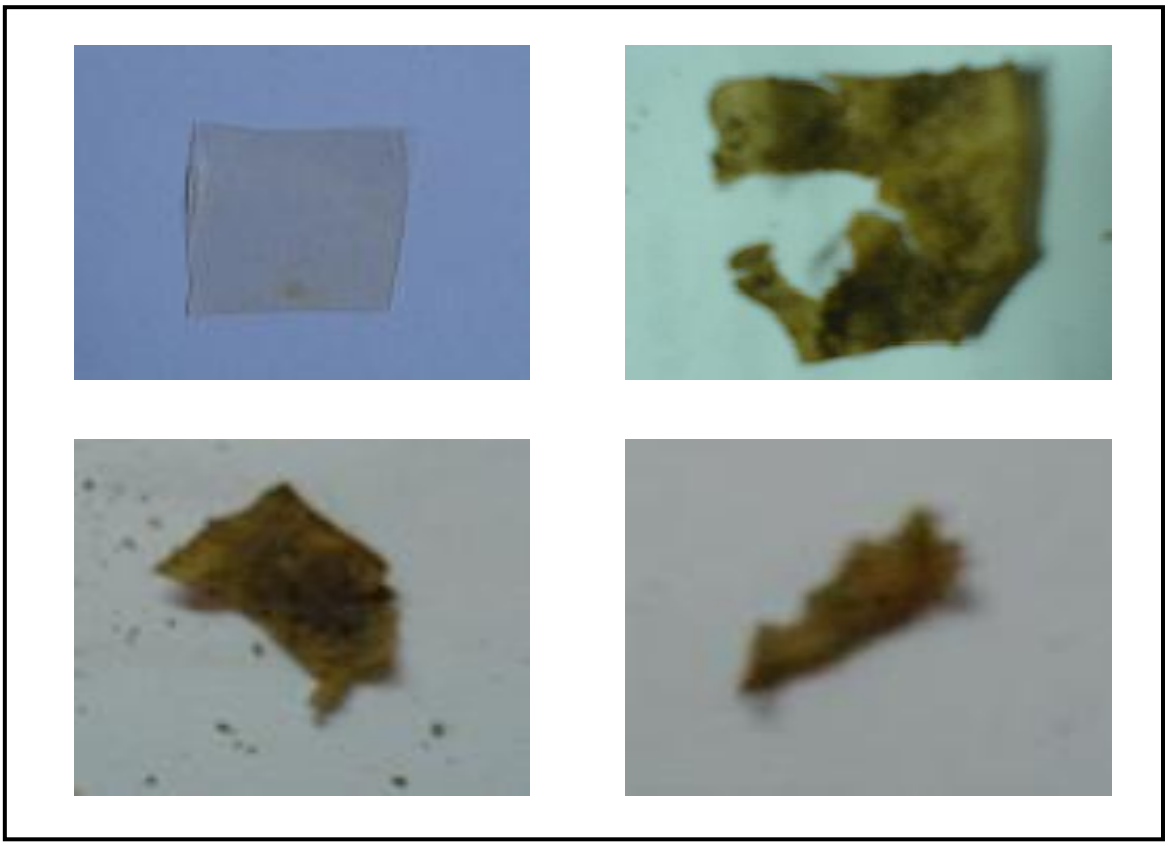

Figure 4.14: Scanned pictures of blend films before and after composting burial test.

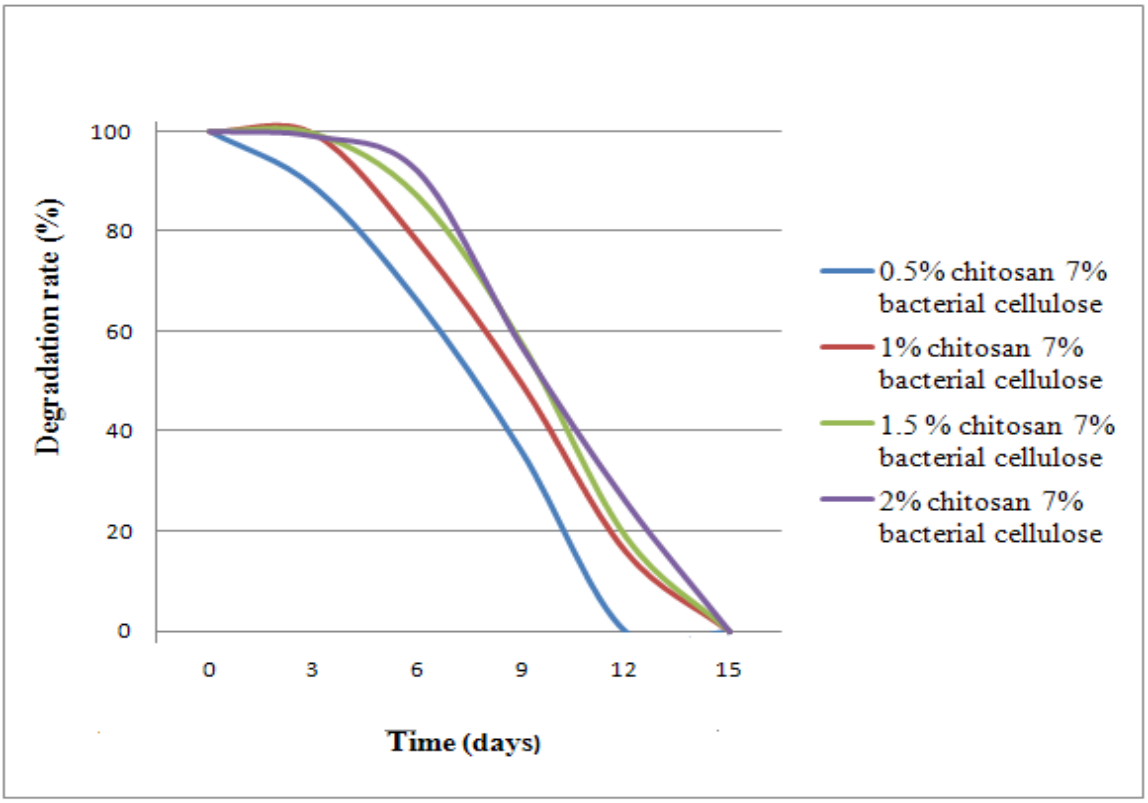

Figure 4.15: The degradation rate of biodegradable blend films with different chitosan contents.

From Figure 4.15 as shown above, with the increasing of degradable time, the compactness of the films will be destroyed. The films had shown a rapid degradation within a period of 5-12 days. More than 50% weight loss of total solids was observed for the films in 10 days. The films were shown to start degrading after 3 days and achieved 100% degradation within 18 days.

When increasing the amount of chitosan, the degradable time also increase. This must be the result of leaching of loosely cross-linked polymeric chains (starch, chitosan and bacterial cellulose). Besides that, the appearance of the chitosan as a natural antimicrobial will inhibited the growth of the fungi. This was due to its polycationic nature and the polymer chain will enhances its antifungal activity. Recent studies had explained the possible effect that chitosan might have on the synthesis of certain fungal enzymes

(Banosa *et al.*, 2005), (Salleh and Muhamad, 2008) and (Rhim *et al.*, 2006). Thus, the increasing amount of chitosan will increase the time to degrade.

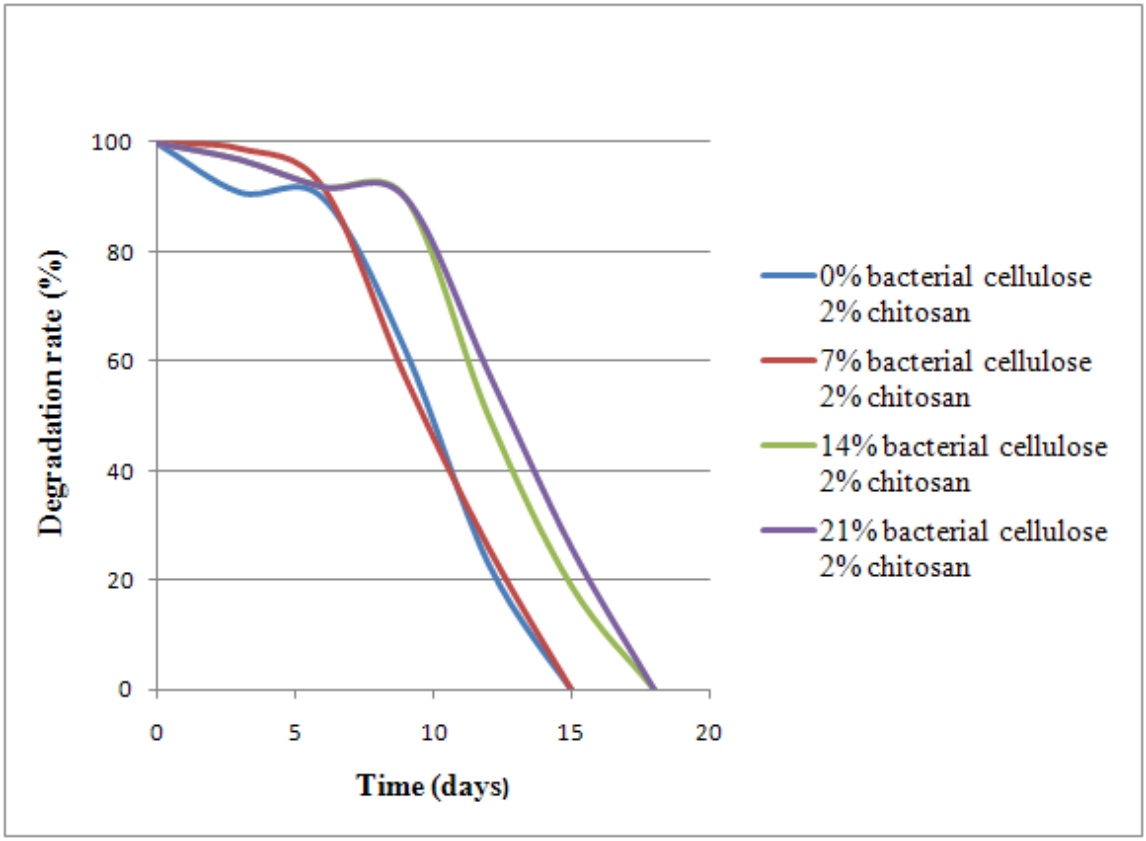

Figure 4.16: The degradation rate of biodegradable blend films with different bacterial cellulose contents.

However, the increasing amount of bacterial cellulose will take prolong degradable time compared to the increasing amount of chitosan. It was because of the interspace forming between the fiber of bacterial cellulose, chitosan and starch. Beside that, the bacterial cellulose formed the crystalline regions that difficult to degrade as mention in previous journal (Alvarez *et al.*, 2006).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Biodegradable blend films with different chitosan and bacterial cellulose content were synthesized by using the mixing process and the casting method. The characteristics of the blend films with different chitosan composition (0.5, 1, 1.5, 2% (w/w) /100 mL) and different chitosan composition (0g, 7g, 14g, and21g) were evaluated using Universal Testing Machine, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM). Beside that, the observation of moisture absorption, density analysis and biodegradable using soil burial degradation test were done throughout samples.

Among the films fabricated, the biodegradable blend films compatibilized with 21g bacterial cellulose with 2% chitosan content showed good mechanical properties and had the smooth surface with no visible pores microstructure. The FTIR confirmed that tapioca starch, chitosan and bacterial cellulose were compatible and inter-molecular hydrogen bonds existed between them.

The biodegrable blend films with 21g bacterial cellulose with 2% chitosan showed the lower diffusion coefficient which contribute to the sensitivity of the film toward the moisture uptake. In density analysis using gas pycnometer and manual calculation showed the lowest value of density. The lowest value of density showed that the film had less porosity or almost non porosity inside the films.

In soil burial test, a rapid degradation occurred for all the films in the initial 10 days, followed by 100% composting within 18 days. The films produced from biodegradable biocomposite starch based films blended with chitosan and bacterial cellulose had potential application to be used as packaging because it can enhance the quality and at the same time protected the environment.

5.2 Recommendation

In order to get the accurate result of Universal Testing Machine, the lower load (2.5kN) must be used in the further studies. Besides, the proper way of blending method of bacterial cellulose should be introduced in order to get the better amount of bacterial cellulose needed.

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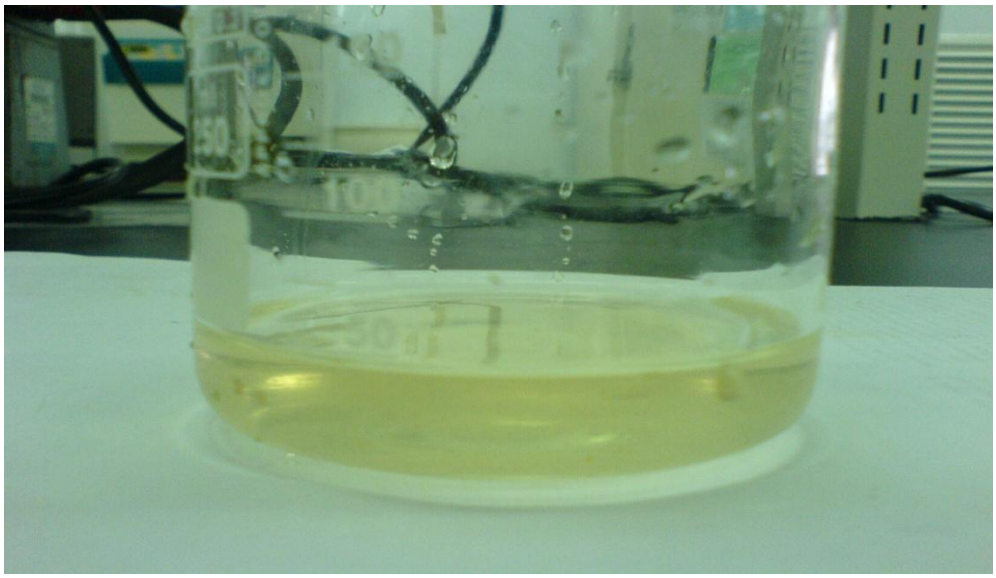
APPENDICES

Appendix A: Films Preparation

Materials preparation



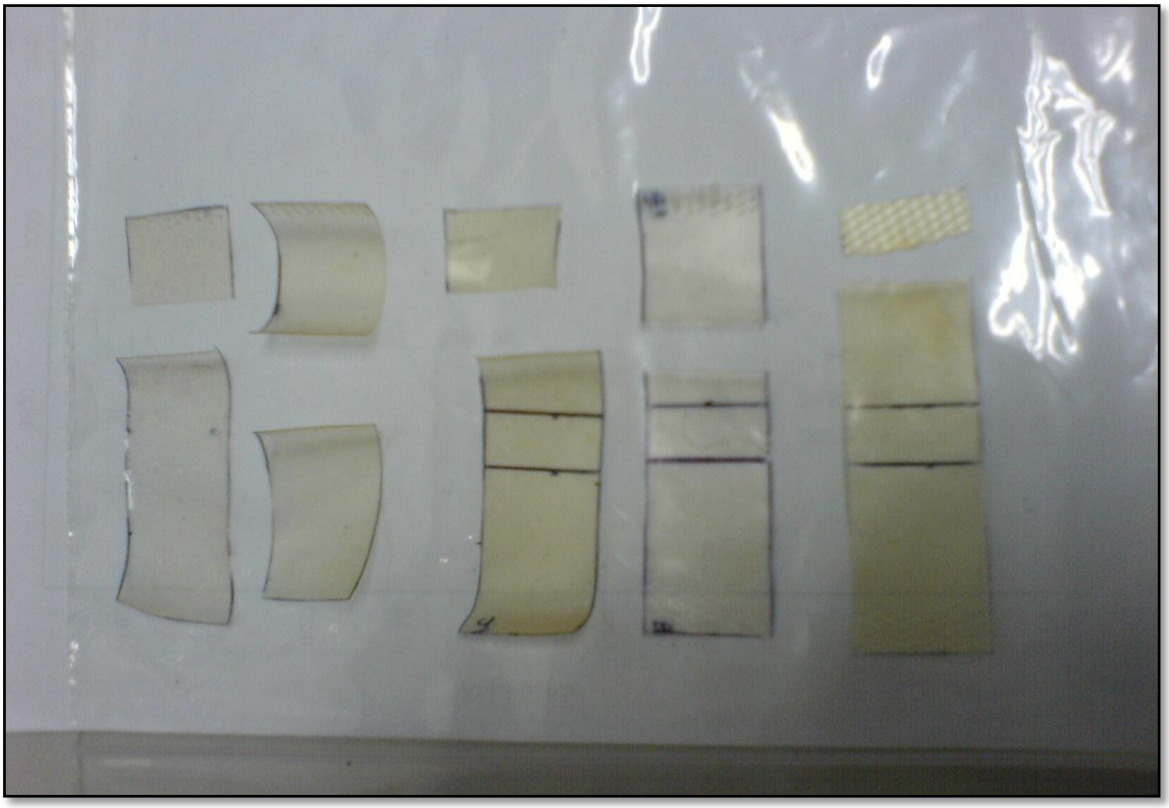
Mixed solution.

Mixed solution in ultrasonic clearner to remove the bubble.

Fresh biodegradable blend films.

Appendix B: Films Testing

i) Mechanical Properties Test

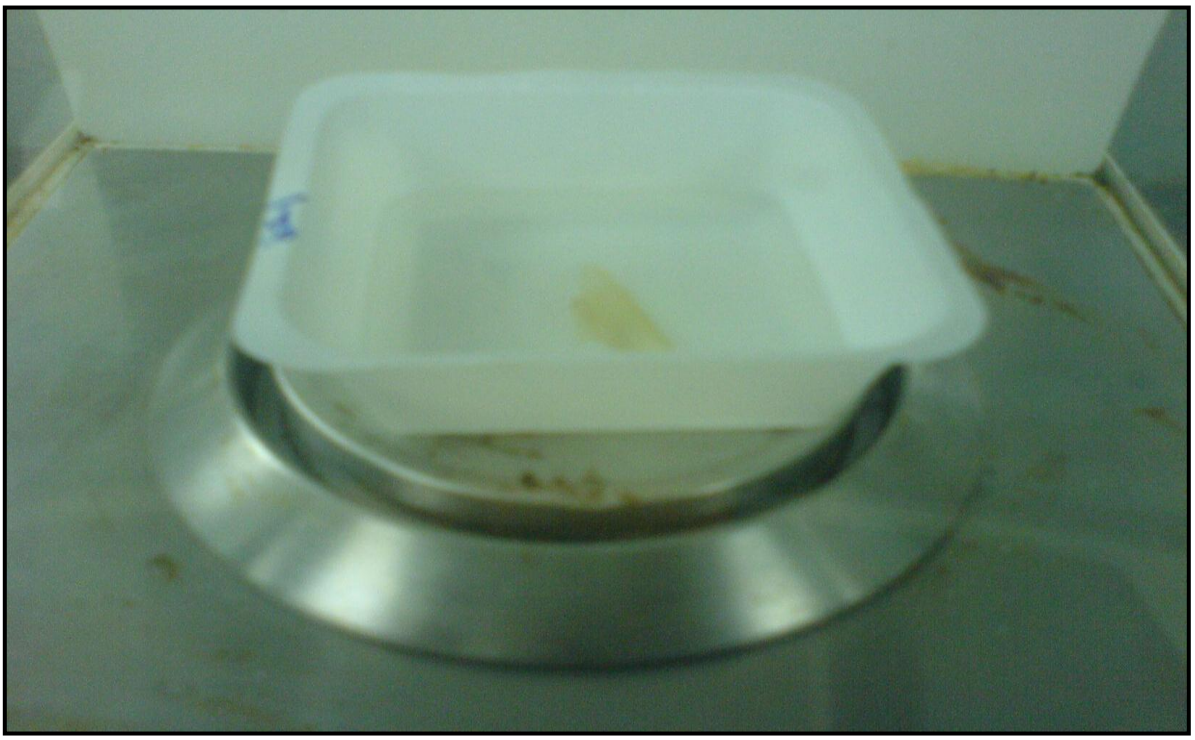


ii) FTIR Spectroscopy

iii) Microstructural Studies

iv) Gas Pycnometer

v) Moisture Absorption.



Appendix C: Tensile Stress Curve for Tensile Test

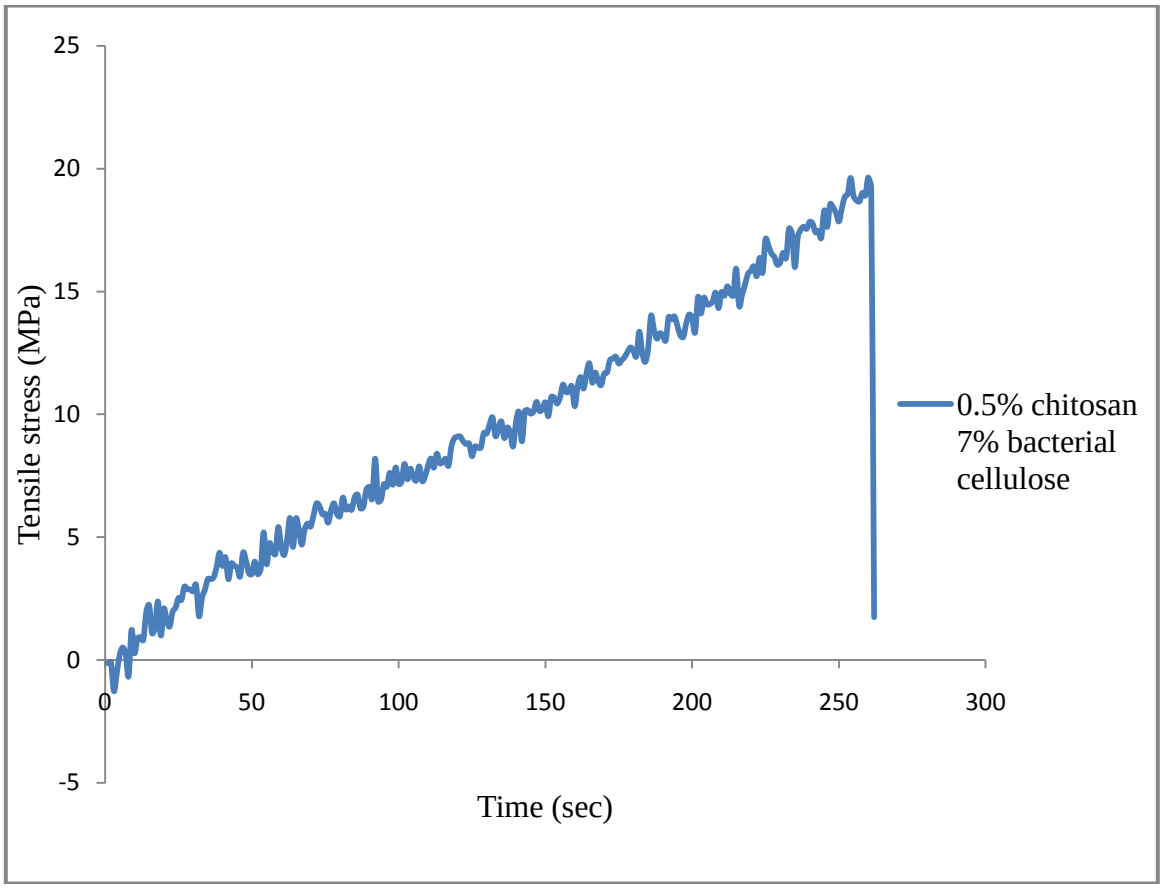


Figure C.1: The tensile stress (MPa) versus time (sec) for 0.5 % chitosan with 7% bacterial cellulose.

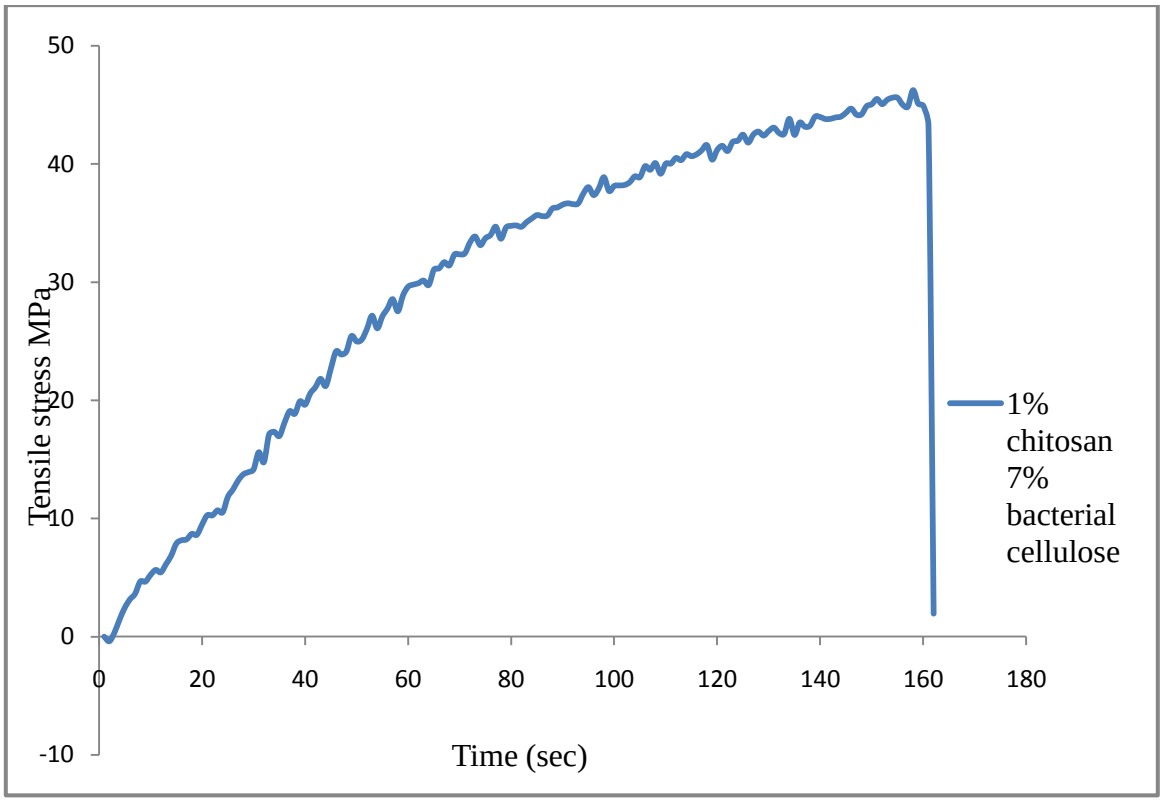


Figure C.2: The tensile stress (MPa) versus time (sec) for 1 % chitosan with 7% bacterial cellulose.

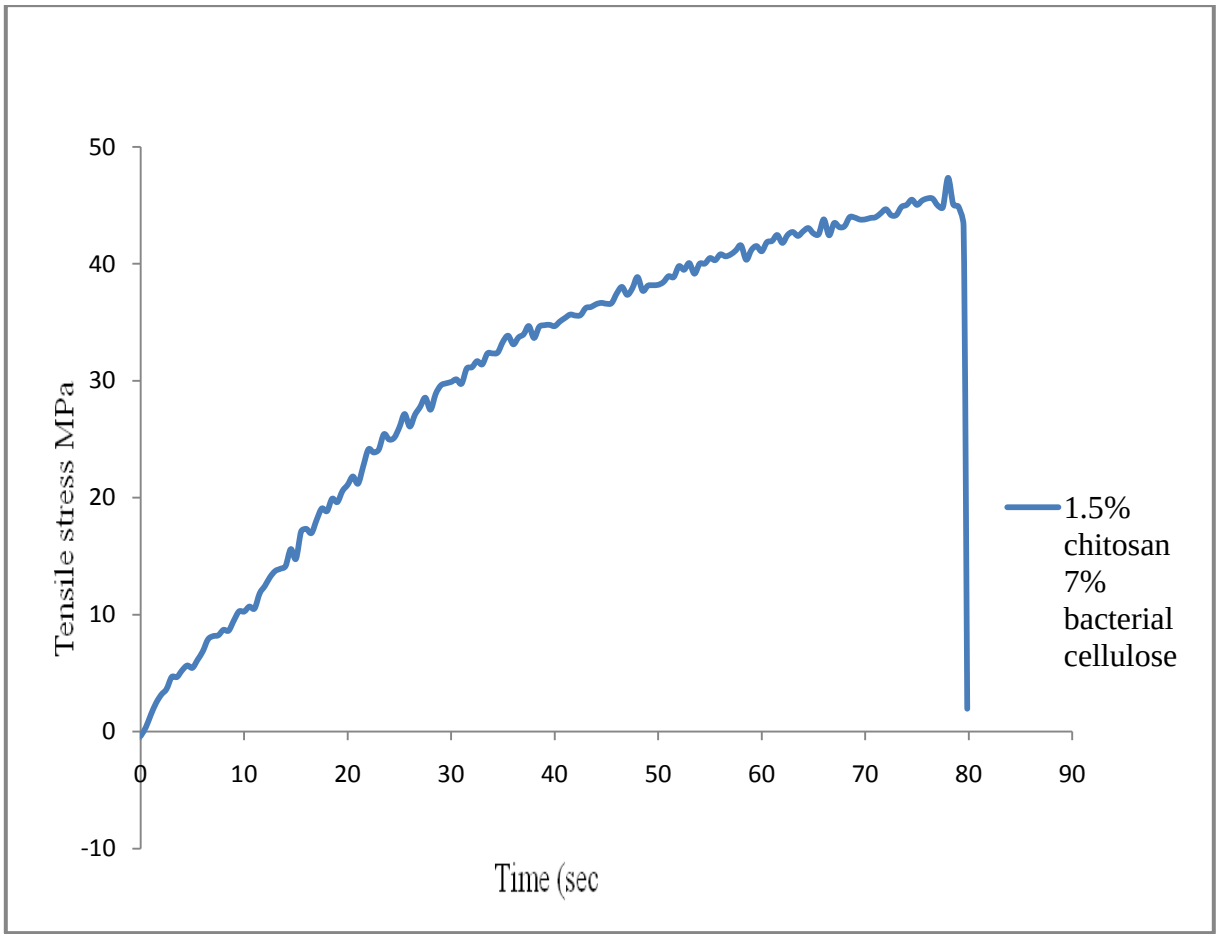


Figure C.3: The tensile stress (MPa) versus time (sec) for 1.5 % chitosan with 7% bacterial cellulose.

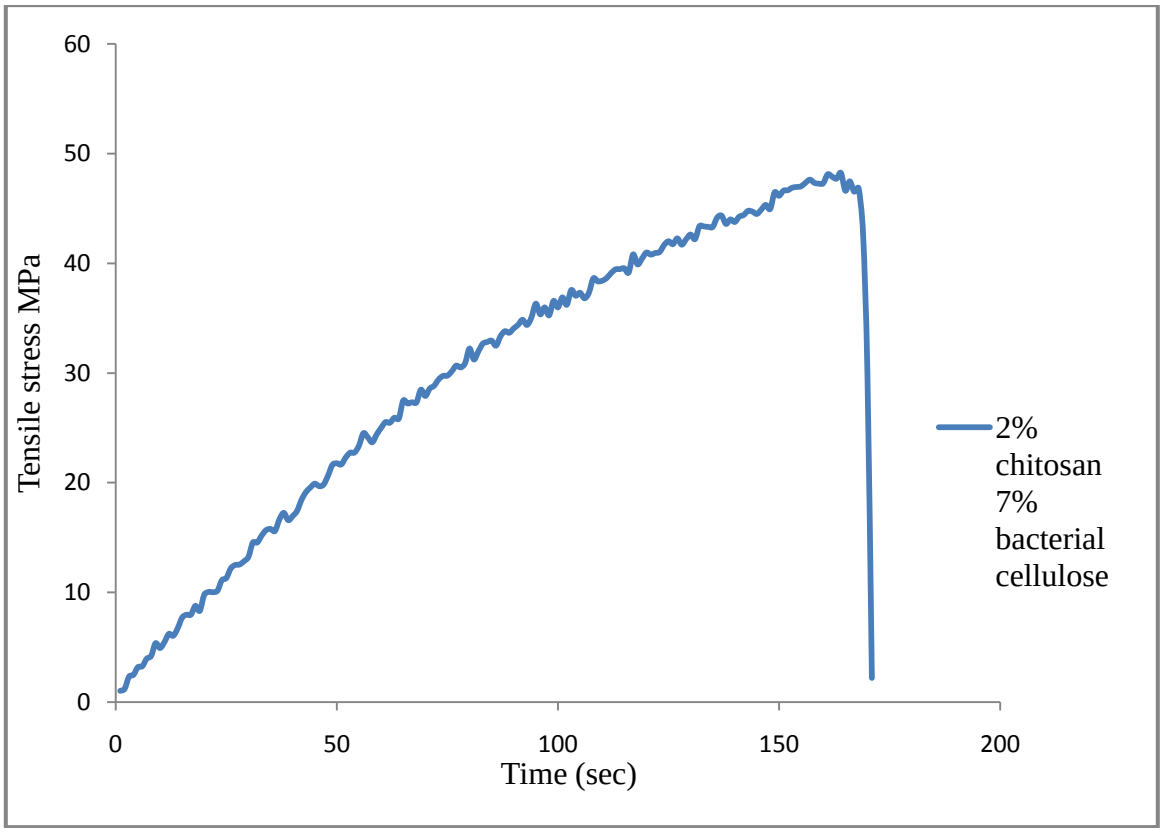


Figure C.4: The tensile stress (MPa) versus time (sec) for 2 % chitosan with 7% bacterial cellulose.

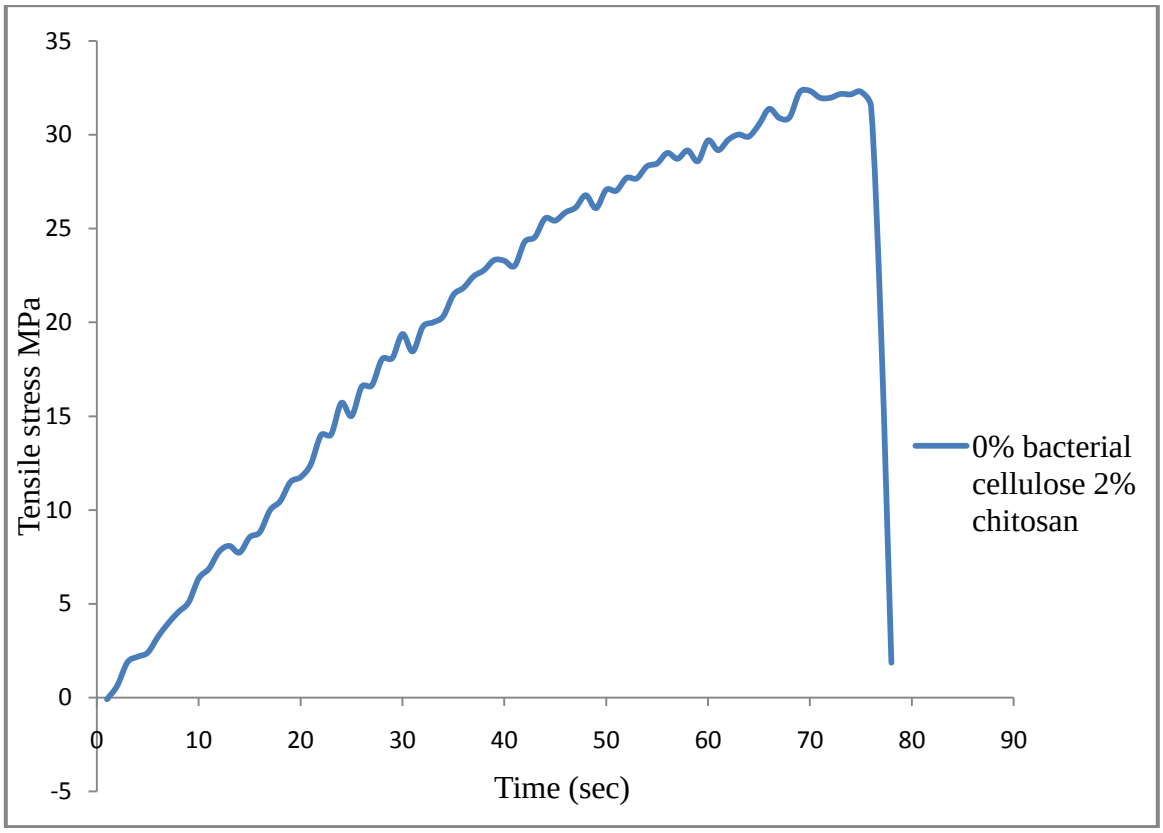


Figure C.5: The tensile stress (MPa) versus time (sec) for 0 % bacterial cellulose with 2% chitosan.

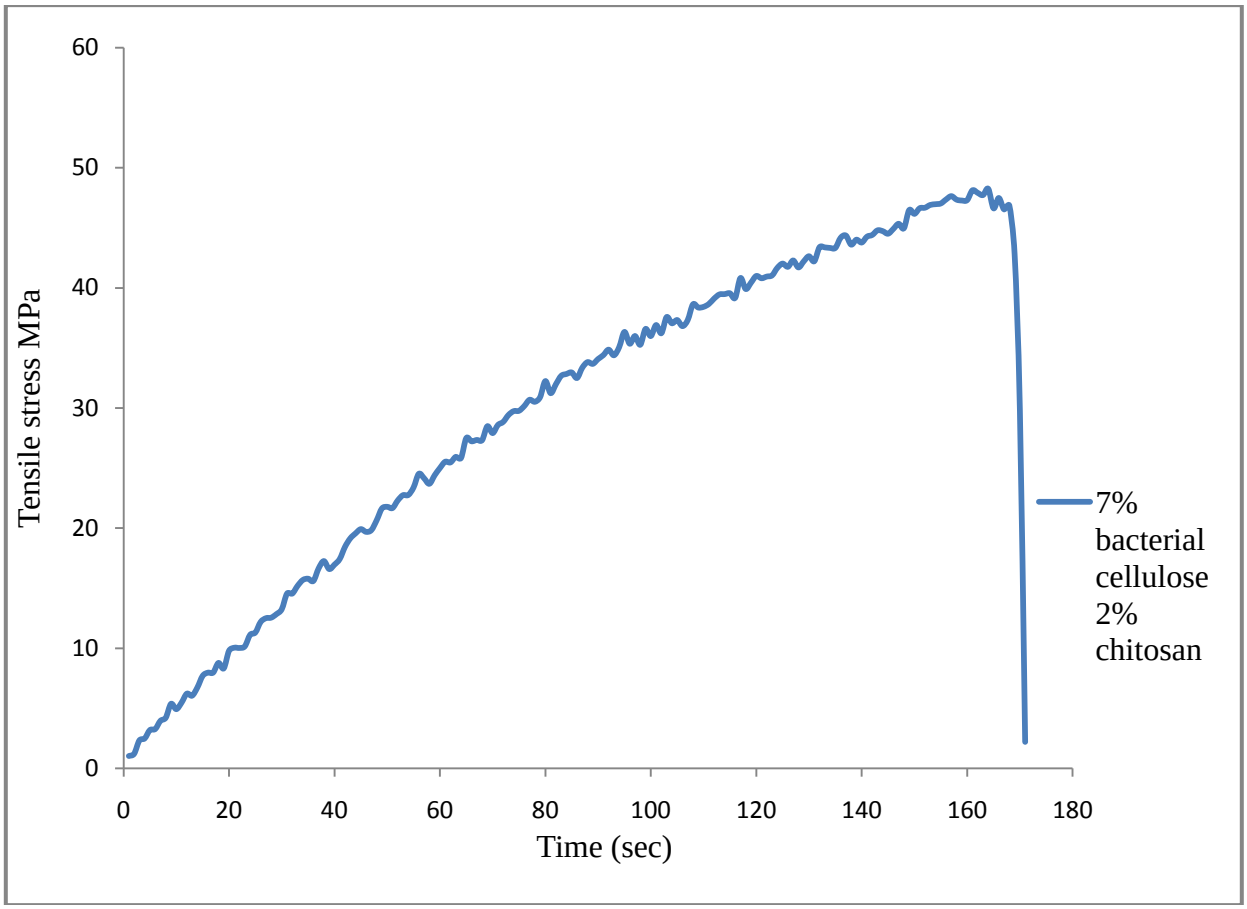


Figure C.6: The tensile stress (MPa) versus time (sec) for 7 % bacterial cellulose with 2% chitosan.

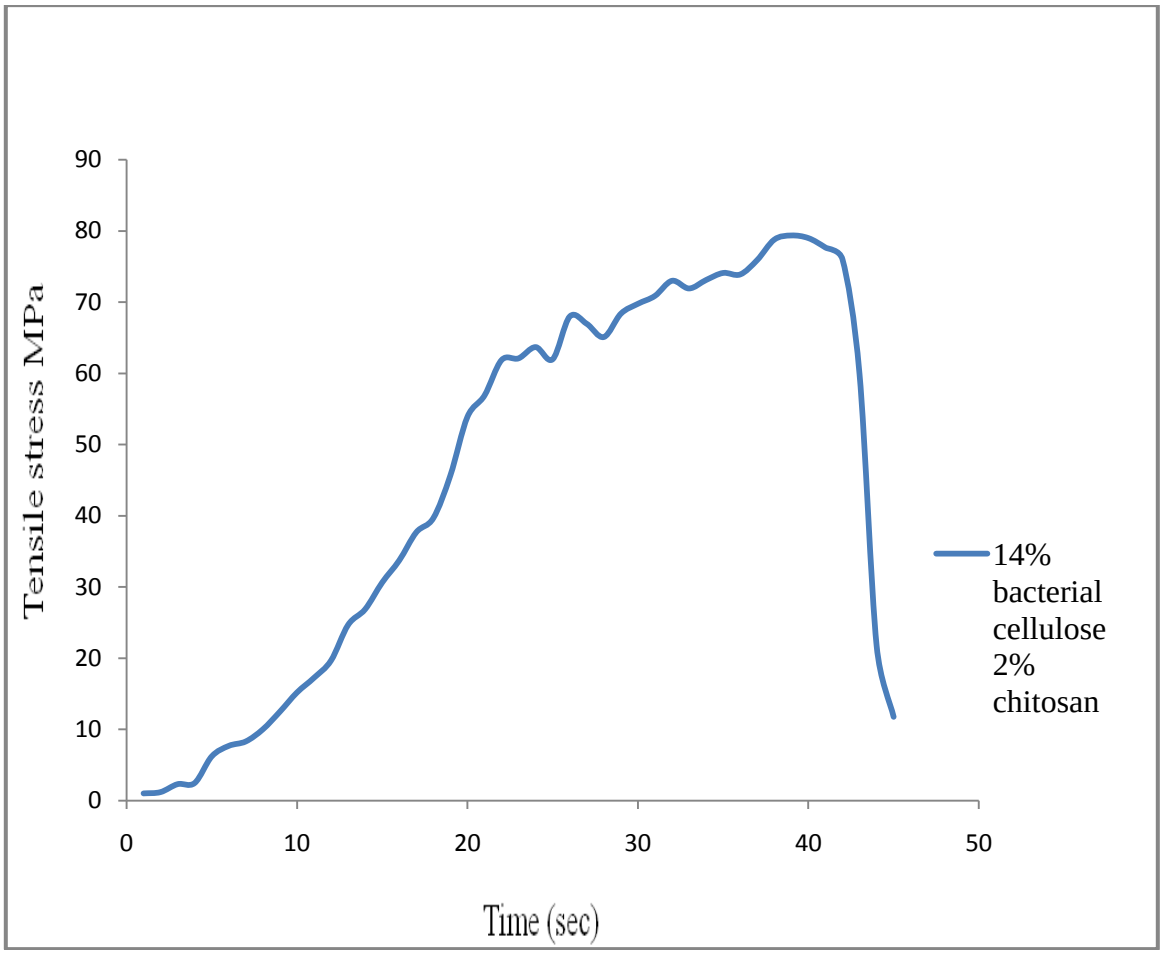


Figure C.7: The tensile stress (MPa) versus time (sec) for 14 % bacterial cellulose with 2% chitosan.

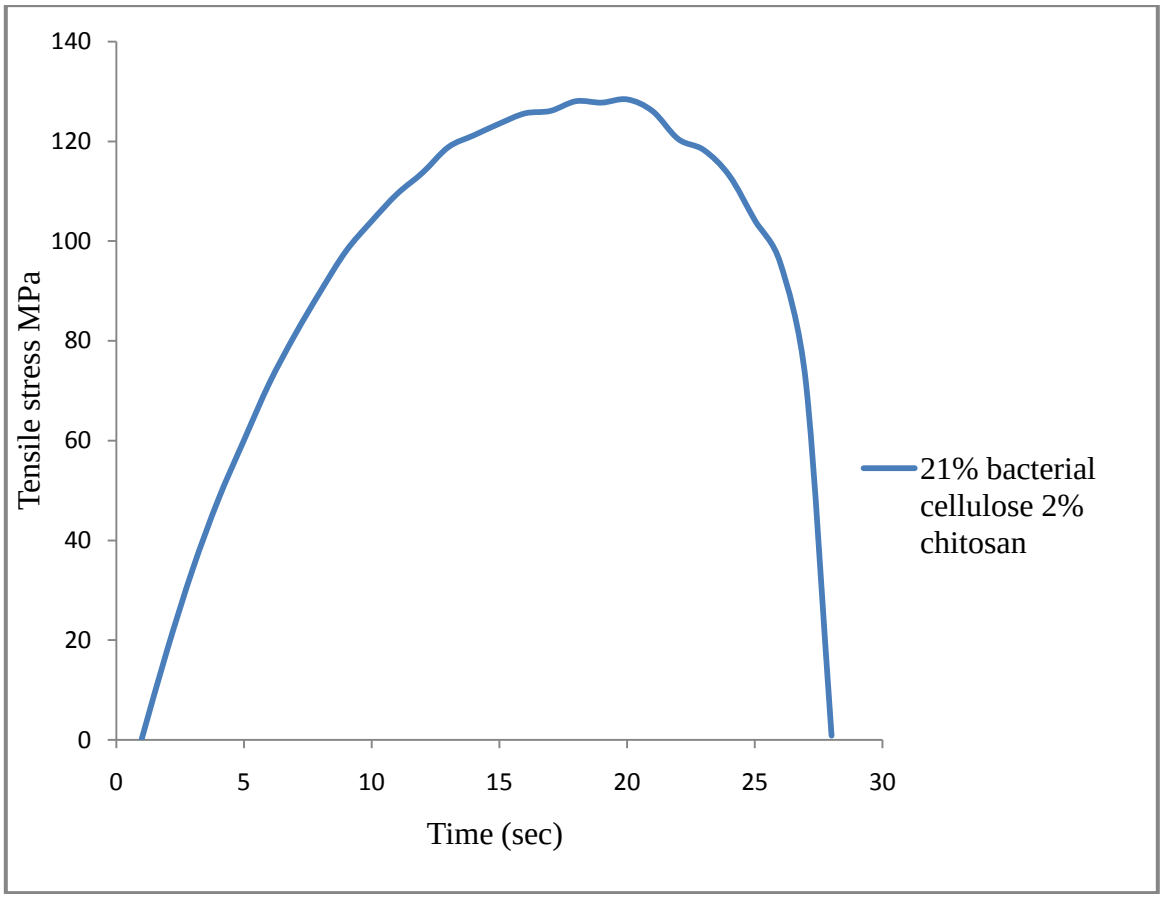


Figure C.8: The tensile stress (MPa) versus time (sec) for 21 % bacterial cellulose with 2% chitosan.

Appendix D: Gas Pynometer

Analysis Gas: Helium
Reported: 11/12/2010 2:24:23PM
Sample Mass: 0.4537 g
Temperature: 25.31 °C
Number of Purges: 3

Analysis Start: 11/12/2010 2:15:48PM
Analysis End: 11/12/2010 2:24:14PM
Equilib. Rate: 0.005 psig/min
Expansion Volume: 9.1684 cm³
Cell Volume: 2.8368 cm³

Combined Report

Summary Report

Sample Volume
Average: 0.3286 cm³
Standard Deviation: 0.0002 cm³

Sample Density
Average: 1.3805 g/cm³
Standard Deviation: 0.0007 g/cm³

Tabular 1

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.083	4.314	0.3289	1.3795	0.2751
2	20.004	4.297	0.3285	1.3810	0.2759
3	20.034	4.303	0.3285	1.3811	0.2759

Summary Data	Average	Standard Deviation
Volume:	0.3286 cm³	0.0002 cm³
Density:	1.3805 g/cm³	0.0007 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Tabular 2

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.083	4.314	0.3289	1.3795	0.2751
2	20.004	4.297	0.3285	1.3810	0.2759
3	20.034	4.303	0.3285	1.3811	0.2759

Summary Data	Average	Standard Deviation
Volume:	0.3286 cm³	0.0002 cm³
Density:	1.3805 g/cm³	0.0007 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Table D.1: The 0.5% chitosan with 7% bacterial cellulose average density.

Analysis Gas: Helium
Reported: 11/12/2010 2:35:21PM
Sample Mass: 0.4540 g
Temperature: 25.32 °C
Number of Purges: 3

Analysis Start: 11/12/2010 2:27:09PM
Analysis End: 11/12/2010 2:35:07PM
Equilib. Rate: 0.005 psig/min
Expansion Volume: 9.1684 cm³
Cell Volume: 2.8368 cm³

Combined Report

Summary Report

Sample Volume
Average: 0.3373 cm³
Standard Deviation: 0.0002 cm³

Sample Density
Average: 1.3459 g/cm³
Standard Deviation: 0.0007 g/cm³

Tabular 1

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	19.943	4.272	0.3375	1.3450	0.2565
2	20.055	4.296	0.3373	1.3459	0.2570
3	19.997	4.284	0.3371	1.3468	0.2575

Summary Data	Average	Standard Deviation
Volume:	0.3373 cm³	0.0002 cm³
Density:	1.3459 g/cm³	0.0007 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Tabular 2

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	19.943	4.272	0.3375	1.3450	0.2565
2	20.055	4.296	0.3373	1.3459	0.2570
3	19.997	4.284	0.3371	1.3468	0.2575

Summary Data	Average	Standard Deviation
Volume:	0.3373 cm³	0.0002 cm³
Density:	1.3459 g/cm³	0.0007 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Table D.2: The 1 % chitosan with 7% bacterial cellulose average density.

Analysis Gas:	Helium	Analysis Start:	11/12/2010 2:38:22PM
Reported:	11/12/2010 2:46:47PM	Analysis End:	11/12/2010 2:46:39PM
Sample Mass:	0.4497 g	Equilib. Rate:	0.005 psig/min
Temperature:	25.34 °C	Expansion Volume:	9.1684 cm³
Number of Purges:	3	Cell Volume:	2.8368 cm³

Combined Report

Summary Report

Sample Volume
Average: 0.3365 cm³
Standard Deviation: 0.0002 cm³

Sample Density
Average: 1.3364 g/cm³
Standard Deviation: 0.0009 g/cm³

Tabular 1

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.092	4.305	0.3368	1.3351	0.2510
2	19.976	4.280	0.3364	1.3369	0.2520
3	20.016	4.289	0.3363	1.3372	0.2521

Summary Data	Average	Standard Deviation
Volume:	0.3365 cm³	0.0002 cm³
Density:	1.3364 g/cm³	0.0009 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Tabular 2

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.092	4.305	0.3368	1.3351	0.2510
2	19.976	4.280	0.3364	1.3369	0.2520
3	20.016	4.289	0.3363	1.3372	0.2521

Summary Data	Average	Standard Deviation
Volume:	0.3365 cm³	0.0002 cm³
Density:	1.3364 g/cm³	0.0009 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Table D.3: The 1.5% chitosan with 7% bacterial cellulose average density.

Analysis Gas:	Helium	Analysis Start:	11/12/2010 2:49:20PM
Reported:	11/12/2010 2:57:33PM	Analysis End:	11/12/2010 2:57:29PM
Sample Mass:	0.4588 g	Equilib. Rate:	0.005 psig/min
Temperature:	25.29 °C	Expansion Volume:	9.1684 cm³
Number of Purges:	3	Cell Volume:	2.8368 cm³

Combined Report

Summary Report

Sample Volume
Average: 0.3358 cm³
Standard Deviation: 0.0005 cm³

Sample Density
Average: 1.3662 g/cm³
Standard Deviation: 0.0019 g/cm³

Tabular 1

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.053	4.297	0.3365	1.3636	0.2666
2	20.077	4.304	0.3354	1.3681	0.2691
3	19.965	4.279	0.3356	1.3669	0.2684

Summary Data	Average	Standard Deviation
Volume:	0.3358 cm³	0.0005 cm³
Density:	1.3662 g/cm³	0.0019 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Tabular 2

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.053	4.297	0.3365	1.3636	0.2666
2	20.077	4.304	0.3354	1.3681	0.2691
3	19.965	4.279	0.3356	1.3669	0.2684

Summary Data	Average	Standard Deviation
Volume:	0.3358 cm³	0.0005 cm³
Density:	1.3662 g/cm³	0.0019 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Table D.4: The 2 % chitosan with 7% bacterial cellulose average density.

Analysis Gas:	Helium	Analysis Start:	11/12/2010 3:01:28PM
Reported:	11/12/2010 3:10:13PM	Analysis End:	11/12/2010 3:10:09PM
Sample Mass:	0.4573 g	Equilib. Rate:	0.005 psig/min
Temperature:	25.34 °C	Expansion Volume:	9.1684 cm³
Number of Purges:	3	Cell Volume:	2.8368 cm³

Combined Report

Summary Report

Sample Volume
Average: 0.3367 cm³
Standard Deviation: 0.0004 cm³

Sample Density
Average: 1.3583 g/cm³
Standard Deviation: 0.0015 g/cm³

Tabular 1

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.066	4.299	0.3371	1.3566	0.2628
2	20.034	4.292	0.3368	1.3579	0.2636
3	20.050	4.297	0.3362	1.3603	0.2649

Summary Data	Average	Standard Deviation
Volume:	0.3367 cm³	0.0004 cm³
Density:	1.3583 g/cm³	0.0015 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Tabular 2

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.066	4.299	0.3371	1.3566	0.2628
2	20.034	4.292	0.3368	1.3579	0.2636
3	20.050	4.297	0.3362	1.3603	0.2649

Summary Data	Average	Standard Deviation
Volume:	0.3367 cm³	0.0004 cm³
Density:	1.3583 g/cm³	0.0015 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Table D.5: The 0% bacterial cellulose with 2% chitosan average density.

Analysis Gas:	Helium	Analysis Start:	11/12/2010 2:49:20PM
Reported:	11/12/2010 2:57:33PM	Analysis End:	11/12/2010 2:57:29PM
Sample Mass:	0.4588 g	Equilib. Rate:	0.005 psig/min
Temperature:	25.29 °C	Expansion Volume:	9.1684 cm³
Number of Purges:	3	Cell Volume:	2.8368 cm³

Combined Report

Summary Report

Sample Volume
Average: 0.3358 cm³
Standard Deviation: 0.0005 cm³

Sample Density
Average: 1.3662 g/cm³
Standard Deviation: 0.0019 g/cm³

Tabular 1

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.053	4.297	0.3365	1.3636	0.2666
2	20.077	4.304	0.3354	1.3681	0.2691
3	19.965	4.279	0.3356	1.3669	0.2684

Summary Data	Average	Standard Deviation
Volume:	0.3358 cm³	0.0005 cm³
Density:	1.3662 g/cm³	0.0019 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Tabular 2

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.053	4.297	0.3365	1.3636	0.2666
2	20.077	4.304	0.3354	1.3681	0.2691
3	19.965	4.279	0.3356	1.3669	0.2684

Summary Data	Average	Standard Deviation
Volume:	0.3358 cm³	0.0005 cm³
Density:	1.3662 g/cm³	0.0019 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Table D.6: The 7 % bacterial cellulose with 2% chitosan average density.

Analysis Gas:	Helium	Analysis Start:	11/12/2010 3:12:45PM
Reported:	11/12/2010 3:21:11PM	Analysis End:	11/12/2010 3:20:58PM
Sample Mass:	0.4515 g	Equilib. Rate:	0.005 psig/min
Temperature:	25.31 °C	Expansion Volume:	9.1684 cm³
Number of Purges:	3	Cell Volume:	2.8368 cm³

Combined Report

Summary Report

Sample Volume
Average: 0.3318 cm³
Standard Deviation: 0.0002 cm³

Sample Density
Average: 1.3607 g/cm³
Standard Deviation: 0.0008 g/cm³

Tabular 1

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	19.972	4.286	0.3320	1.3600	0.2647
2	20.060	4.304	0.3319	1.3602	0.2648
3	19.920	4.275	0.3315	1.3618	0.2657

Summary Data	Average	Standard Deviation
Volume:	0.3318 cm³	0.0002 cm³
Density:	1.3607 g/cm³	0.0008 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Tabular 2

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	19.972	4.286	0.3320	1.3600	0.2647
2	20.060	4.304	0.3319	1.3602	0.2648
3	19.920	4.275	0.3315	1.3618	0.2657

Summary Data	Average	Standard Deviation
Volume:	0.3318 cm³	0.0002 cm³
Density:	1.3607 g/cm³	0.0008 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Table D.7: The 14% bacterial cellulose with 2% chitosan average density.

Analysis Gas:	Helium	Analysis Start:	11/12/2010 3:25:14PM
Reported:	11/12/2010 3:33:36PM	Analysis End:	11/12/2010 3:33:18PM
Sample Mass:	0.4502 g	Equilib. Rate:	0.005 psig/min
Temperature:	25.31 °C	Expansion Volume:	9.1684 cm³
Number of Purges:	3	Cell Volume:	2.8368 cm³

Combined Report

Summary Report

Sample Volume
Average: 0.3424 cm³
Standard Deviation: 0.0002 cm³

Sample Density
Average: 1.3150 g/cm³
Standard Deviation: 0.0007 g/cm³

Tabular 1

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.053	4.289	0.3421	1.3159	0.2400
2	19.989	4.275	0.3426	1.3141	0.2390
3	19.974	4.272	0.3423	1.3151	0.2396

Summary Data	Average	Standard Deviation
Volume:	0.3424 cm³	0.0002 cm³
Density:	1.3150 g/cm³	0.0007 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Tabular 2

Cycle#	P1 Pressure (psig)	P2 Pressure (psig)	Volume (cm³)	Density (g/cm³)	Total Pore Volume (cm³/g)
1	20.053	4.289	0.3421	1.3159	0.2400
2	19.989	4.275	0.3426	1.3141	0.2390
3	19.974	4.272	0.3423	1.3151	0.2396

Summary Data	Average	Standard Deviation
Volume:	0.3424 cm³	0.0002 cm³
Density:	1.3150 g/cm³	0.0007 g/cm³

6202- Total solids concentration is invalid; liquid density is greater-than or equal to solids density.

Table D.8: The 21% bacterial cellulose with 2% chitosan average density.

Appendix E: Swelling Absorption Calculation.

Table E.1 : The moisture water uptake for 6% starch, 0.5% chitosan with 7% bacterial cellulose.

Time (s)	Mt	Mt/ M∞
20	0.0025	0.011
40	0.0053	0.024
60	0.0077	0.033
80	0.0105	0.047
100	0.0132	0.059
120	0.0151	0.067

From the Figure 4.12, the slope k;

k = (0.047 – 0.011) / (8.944- 4.472)

$= 8.0501 \times 10^{-3}$

Using Equation 4,

$k = (4M\infty / h)(D/\pi)^{1/2}$

$M\infty = 0.225$ g/cm^3
h = 0.02 mm

$D = 1.005 \times 10^{-9}$ cm ^{2}s^{-1}