SYNTHESIS OF BACTERIAL CELLULOSE BY *Acetobacter xylinum* sp. USING PINEAPPLE PITH FOR BIOCOMPOSITE APPLICATION

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ABSTRACT

Bacterial cellulose produced by *Acetobacter xylinum* sp. was a new type of biopolymer. It was far superior to its counterpart from plants because of its exceptional purity, ultra fine network structure, high biodegradability and unique mechanical strength. Bacterial cellulose was expected to be used for many industrial applications as a high-strength construction material, food additive and a component of biodegradable products and paper. The objectives of this study were to investigate the production of bacterial cellulose in shaken culture condition and in various value of pH using pineapple pith juice as the substrate for the fermentation process. The shaken culture method had been carried out at 100 rpm, 110 rpm, 120 rpm, 130 rpm and 140 rpm while the pH value was investigated in the range of 4.0 to 8.0. The parameter conditions were fixed at the temperature of 30 ˚C and glucose concentration of 9 g/L for all the experiments in this research. From this research, the bacterial cellulose production in shaken culture at 120 rpm and pH of 6.0 were the most suitable conditions for culture medium because it formed the highest yield that was 14.1860 g. The qualitative analysis with Fourier Transform Infrared (FTIR) shows that the compound of cellulose was detected. As a conclusion, the objectives in this study were achieved by investigated the effect of shaken speed of the stackable incubator shaker and the pH of substrate towards the production of bacterial cellulose using *Acetobacter xylinum* sp. in the medium of pineapple pith juice.

ABSTRAK

Selulosa bakteria yang dihasilkan oleh *Acetobacter xylinum* sp. merupakan sejenis biopolimer yang terbaru. Ianya jauh lebih baik berbanding selulosa daripada tumbuhan kerana keasliannya, struktur rangkaiannya yang amat halus, kebolehan biodegradasi yang tinggi dan kekuatan mekanikal yang unik. Selulosa bakteria dapat digunakan dalam pelbagai aplikasi industri sebagai bahan binaan yang amat kuat, aditif makanan dan komponen produk biodegradasi dan kertas. Projek penyelidikan ini bertujuan untuk menyiasat pengeluaran selulosa bakteria dalam keadaan keadaan goncangan dan kepelbagaian nilai pH menggunakan jus empulur nenas sebagai substrat untuk proses penapaian. Kaedah goncangan ini dijalankan pada kelajuan 100 rpm, 110 rpm, 120 rpm, 130 rpm dan 140 rpm serta nilai pH di antara 4.0 hingga 8.0. Keadaan optimum telah ditetapkan pada suhu 30 ˚C dan kepekatan glukosa pada 9 g/L dalam semua ujikaji yang dijalankan. Daripada kajian ini, pengeluaran selulosa bakteria adalah paling tinggi pada kultur goncangan 120 rpm dan pH 6.0 dimana pengeluarannya adalah sebanyak 14.1860 g. Kualitatif analisis dengan *Fourier Transform Infrared* (*FTIR*) menunjukkan bahawa glukosa telah dikesan. Sebagai kesimpulannya, objektif di dalam kajian ini diperoleh dengan meneliti kesan kelajuan goncangan daripada inkubator goncangan dan pH substrat terhadap pengeluaran selulosa menggunakan bakteria *Acetobacter xylinum* sp. di dalam jus empulur nenas.

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˚C	-	Degree Celcius
rpm	-	Round per minute
BC	-	Bacterial Cellulose
W	-	Weight
V	-	Volume
min	-	Minute
hr	-	Hour
mL	-	Milliliter
L	-	Liter
sp.	-	Species
g	-	Gram
HCL	-	Hydrochloric Acid
NH_4^+	-	Ammonium ion
NAOH	-	Sodium Hydroxide
$MgSO_4.7H_2O$	-	Magnesium Sulfate Heptahydrate
Na_2HPO_4	-	Disodium hydrogen phosphate
DNS	-	Dinitrosalicyclic acid
FTIR	-	Fourier Transform Infrared Spectroscopy
UV-VIS	-	Ultra Violet Visible
OD	-	Optical Density
C	-	Carbon
O	-	Oxygen
H	-	Hydrogen
MARDI	-	Malaysian Agricultural Research and Development Institute

CHAPTER 1

INTRODUCTION

1.1 Research Background

New functional materials obtained from renewable resources have gained much attention in the last decades due to the global increasing demand for alternatives to fossil resource. For example, composites based on thermoplastic polymers and natural fibers are very attractive materials because of their good mechanical properties, sustainability and environmental-friendly connotation. These materials have been widely used in the automotive and packaging industries and their applications in other areas are being actively sought.

The use of natural fibers as reinforcing elements in composite materials presents important advantages, when compared with their synthetic or inorganic counterparts which are biodegradable, high availability, low cost, low energy consumption, low density, and high specific strength and modulus. In addition, if the natural fibers are combined with biodegradable matrices, the obtained composite materials are expected to be fully biodegradable. The development of biocomposites gained particular relevance with the increasing availability and diversity of biodegradable polymers.

The best known renewable resources capable of making biodegradable plastics are starch and cellulose. Starch is one of the least expensive biodegradable materials available in the world market today. It is a versatile polymer with immense potential for use in non-food industries.

Cellulose is the most abundant organic polymer in nature, where it plays a crucial role in the integrity of plant cell walls (Delmer and Amor, 1995). Plant-derived cellulose is being used extensively by the paper and textile industries, leading to a significant demand on wood biomass.

It is known that some Acetobacter strains produce cellulose. This cellulose is called bacterial cellulose (Ross *et. al*., 1991). Bacterial cellulose is a form of cellulose that is produced by an acetic acid-producing bacterium, *Acetobacter xylinum* sp. Bacteria from the other species such as *Aerobacter*, *Acetobacter*, *Achromobacter*, *Agrobacterium*, *Alacaligenes*, *Azotobacter*, *Psedomonas*, *Rhizobium* and *Sarcina* also synthesize cellulose but only the *Acetobacter* species produce enough cellulose to justify commercial interest. Bacterial cellulose is devoid of lignin and hemicelluloses. It is extremely hydrophilic, and has an excellent shape and strength retention. It has superior properties such as an ultra fine network structure, high biodegradability and unique mechanical strength as compared with green plant cellulose (Tsuchida T. and Yoshinaga F., 1997). Bacterial cellulose is extremely pure, and exhibits a higher degree of polymerization and crystallinity than the fibrous polymer obtained from the plant sources in which the cellulose fibrils are embedded with lignin, hemicelluloses and waxy aromatic substances (Ross *et. al*., 1991). Because of its high tensile strength and water hold capacity, BC has been used as a raw material for producing high fidelity acoustic speakers, high quality paper and diet and dessert foods. Moreover, the bacterial cellulose has been used in the production of artificial skin, ultrafiltration membrane a cover membrane for glucose biosensors and in binders for powders and thickeners for paint, ink and adhesive (Iguchi *et. al*., 2000). Therefore, it is expected to be a new biodegradable biopolymer.

The process for the production of BC is by using static cultivation methods, with pellicles of BC being formed on the surface of static culture. However, this requires a large area in which to place the culture vessel and is impractical for large scale BC production (Okiyama *et. al*., 1992). Therefore, an economical mass production system based on shaking culture is necessary in order to synthesis more cellulose.

In general, a nutritionally rich medium containing yeast extract and peptone supports good BC production by *Acetobacter* strains. Thus, Fontana *et. al*, (1997) and Hestrin and Schramm (1954) have reported that the production of BC from *Acetobacter* sp. is in a complex medium which is contained of 2 % glucose, 0.5 % yeast extract, 0.5 % polypeptone, 0.675 % $Na_2HPO_4.12H_2O$ and 0.115 % citric acid monohydrate.

1.2 Problem Statement

Bio-composite is a material formed by a matrix of starch or resin and reinforced by a natural fiber usually derived from cellulose. Bio-composite is used because it is environmental friendly product that would help to reduce the pollution. In order to fabricate and enhance the properties of the bio-composite, cellulose is used because of its fiber structure and the biodegradable characteristic. Bacterial cellulose is one of the cellulose that is produced from *Acetobacter xylinum* sp. It is more favorable in the biocomposite application due to its properties such as low density, high purity and biodegradability compared with the plant cellulose that is difficult to purify and limited source which could decrease the forest area.

In order to produce BC, waste from pineapple processing such as pineapple pith is a potential alternative to be used as a carbon source for bacterial growth to produce bacterial cellulose. Pineapple pith is used in this research as a raw material so as to reduce the bulk of wastes produced from food and beverage industry.

1.3 Statement of Objective

The objective for this research is to investigate the effect of agitation speed of rotary shaker and the pH of substrate towards the production of bacterial cellulose using *Acetobacter xylinum* sp. in the medium of pineapple pith.

1.4 Research Scope

The scopes of the research are included as below:

i. To analyze the bacterial cellulose detection using Fourier Transform Infrared Spectroscopy (FTIR)

ii. To investigate the effects of agitation speed of rotary shaker at 100 rpm, 110 rpm, 120 rpm, 130 rpm and 140 rpm.

iii. To examine the effects of differences pH substrate at 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0.

CHAPTER 2

LITERATURE REVIEW

2.1 Bacterial Cellulose

Bacterial cellulose is a form of cellulose that produced by acetic acid bacteria from the genera *Aerobacter, Acetobacter, Achromobacter, Agrobacterium, Alacaligenes, Azotobacter, Pseudomonas, Rhizobium and Sarcina*. However, only the *Acetobacter* species can produce enough cellulose to justify commercial interest. The most extensively studied member of the *Acetobacter* species is *A. xylinus*, formerly known as *A. xylinum*.

Bacterial cellulose produced by acetic acid bacteria is far superior to its counterpart from plants. It is because of its exceptional purity, ultra fine network structure, high biodegradability and unique mechanical strength. Bacterial cellulose is expected to be used for many industrial applications as a high-strength construction material, food additive and a component of biodegradable products and paper. One of the problems that hinder the industrial application of bacterial cellulose is its low yield from static culture systems. Therefore, an economical mass production system based on agitated culture is necessary (Kouda *et al*., 1997).

2.2 Characteristic of *Acetobacter xylinum* sp.

Bacteria belonging to the genus *Acetobacter* are aerobic microorganisms (respiratory metabolism), gram negative, sometimes variable, ellipse- or rod-shaped, and catalase positive. There are usually a single cell or sometimes have a link to another cell. The main characteristic of these bacteria is their capability of oxidizing ethanol to acetic acid. This bacteria will formed acid from glucose, ethyl alcohol (ethanol), propyl alcohol and glycol which its oxidized the acetic acid into CO_2 and water. The *Acetobacter xylinum* sp. has the specific nature that its ability to form a thick coating on the surface of the liquid fermentation which it is a component with cellulose Most *Acetobacter* are capable of using as a carbon source different substrates, in addition to ethanol which are glucose, lactate, acetate, and mannitol. *Acetobacter* bacteria are microorganisms which can be found on fruit, flowers, alcoholic drinks, and they have never shown a pathogenic activity towards humans, on the contrary they are present in human intestine in physiological conditions.

Acetic acid bacteria have a temperature optimum around 30°C, whereas the optimum pH ranges from 5 to 6. A further, important characteristic of acetic acid bacteria is their capability of reproducing even in the presence of antibiotics. Moreover, *Acetobacter* can multiply in the intestine thanks to their capability of surviving in the presence of small amounts of O_2. (Andriolli *et. al*., 2002)

2.3 Basic Nutrition for *Acetobacter xylinum* sp.

2.3.1 Carbon Sources

Acetobacter xylinum sp. is an organism that requires carbon and nitrogen, most often in the form of organic compounds. According to Cruz *et al.* (2002), the mutual interaction of carbon and nitrogen has an important role in the metabolism of living organisms. Carbohydrates are the most readily utilizable form of carbon in both oxidative and anoxidative way. Among the basic monosaccharides of hexoses, D-glucose, D-fructose and D-mannose which are used by all *Acetobacter*, while sucrose is the dissaccharide most easily utilized by *Acetobacter* cells as reported by Lee and Kim, (2001). However, it is noted that while glucose is routinely added to laboratory culture media for growing bacteria, glucose is not freely available in natural *Acetobacter* habitats or in many industrial fermentation substrates (maltose, sucrose, fructose, xylose and lactose are the more common sugars). Indeed, glucose generally exhibits a repressive and inhibitory effect on the assimilation of other sugars by the *Acetobacter*. Other saccharides, polyols, polysaccharides such as soluble starch, pectin; and alcohols such as ethanol, methanol, glycerol; as well as organic acids and other substances might be used by these bacteria as a carbon sources. In the cellulose fermentation, it usually used glucose and ethanol as the carbon sources to enhance the production of bacteria cellulose.

2.3.2 Nitrogen Source

The source of nitrogen for the fermentation by the *Acetobacter xylinum* sp. is usually provided by organic compounds where some natural and semi natural media are based on peptone, yeast extract and others. Smith *et al.*, (1975) found that nitrogen is the main stimulatory factor in yeast extract as it encourages biostimulation on microbial growth. However, yeast extract in media contributes to a major cost in fermentation process. Minimum yeast extract supplementation or replacing yeast extract with a less expensive nitrogen source in order to develop an economically viable industrial process. The improvement of fermentation production has been studied under the control of various factors and media components (Arasaratnam *et al.*, 1996; Kim *et al.*, 2005) and industrial valorizations of agricultural sub product like date juice, pineapple cannery effluent represents a useful research topic (Pessoa *et al.*, 1996; Nigam, 1999; Nancib *et al.*, 2001). Yeast extract consists primarily of amino acids to promote bacteria growth (Chae *et al.*, 2001) that may not only serve as nitrogen but also carbon source. Cruz *et al.*, (2002) suggested that when free amino acids are incorporated directly without modification into proteins or degraded by the cell; the nitrogen is then used for the synthesis of other nitrogenous cell constituents and the amino acid derivative keto-acids may be used by the cell for synthesis purposes.

Ammonium salts (sulphate, phosphate, nitrate) are common inexpensive nitrogen supplements used in the fermentation (Rajoka *et al.*, 2006). Ammonium salts of organic acids are better utilized than salts from inorganic acids as the decomposition produces weak acids that can serve as an additional carbon source (Briggs *et al.*, 2004). Strong organic acids however, change the pH levels and have an inhibitory effect on cells. An exception is ammonium phosphate as phosphorus is a principal biogenic element and phosphoric acid acts a good buffer system. Sims and Ferguson (1974) investigated the metabolism of ammonium ion (NH_4^+), suggested that 75% of NH_4^+ were incorporated into glutamate while the remainder into the amide group of glutamine. Glutamines or other amino acids can also be produced from glutamate via transamination. Therefore, glutamate is a central compound in the metabolism of nitrogen substances.

2.3.3 Phosphorus and Sulphur Sources

One of the important elements in nutrient media for *Acetobacter* is phosphorus. Phosphorus is crucial in the synthesis of substances such as phosphoproteins, phospholipids, nucleoproteins, nucleic acids, phophorylated polysaccharides and is also present in cells as inorganic orthophosphate, pyrophosphate, metaphosphate and polyphosphate (Walker, 1998; Briggs *et al.*, 2004). Phosphate is added to nutrient media in the form of potassium, ammonium or sometimes sodium phosphates. Molasses or other media with a low level of assimilatable nitrogen are usually supplied with ammonium phosphates. Medium requires sulphur principally for the biosynthesis of sulphur-containing amino acids (Walker, 1998).

2.4 Agricultural Wastes

Agriculture has played a vital role in the development of modern Malaysia and continues to be a major contributor to the Malaysian economy. Agriculture is designated to be the third engine of growth under the Eighth Malaysia Plan (2001–2005) (Agriculture in Malaysia, 2006). A strategic agricultural development master plan, The Third National Agricultural Policy (NAP3) was also formulated for years 1998–2010. This agro-food policy is directed towards increasing exports and reducing imports of agricultural commodities (Anonymous, 2004). Table 2.1 showing the extrapolated agricultural production of the primary commodities in Malaysia for the year 2008 indicated an increase from the actual results in year 2005. It has resulted in the increase of agricultural land use from 5.7 million hectares to more than 6.0 million hectares in year 2008 (Crops statistic, 2008). However, the expansion of quantity of the land used for agriculture has increased the number of agriculture related environmental pollution. Traditional methods that were applied to manage crop residues through open-burning were prohibited as haze in South-East Asia reached critical levels (Ahmed *et al.*, 2004). Thus, one of the objectives of NAP3 is to conserve and utilize natural resources on a sustainable basis through developing industrial ecosystems where wastes from one economic activity are used as the inputs of other useful products.

Table 2.1: Agricultural Production (metric tonnes) from 2000 to 2008 (Crops Statistic, 2008)

Commodity	2000	2005	2008[e]
Paddy	2,140,904	2,314,378	2,384,143
Fruits[1]	993,497	1,648,607	1,886,680
Pineapple[2]	-	355,937	323,747
Vegetable	404,671	580,738	816,244
Herbs	-	522	988
Spices	21,503	35,535	51,839
Flowers	120,353,234	135,145,712	16,595,290
Coconut	469,662	536,807	496,974
Coffee	77,241	35,408	28,685
Sugarcane	-	749,979	733,500
Tea	-	3,880	5,570

[e] Estimated production.
[1] Refers to commercial cultivation.
[2] Refers to subunit from fruits production

In Malaysia, agricultural wastes such as rice straws and husks, empty oil palm fruit bunches, saw dust, animal droppings, POME has been successfully recycled (Faridah, 2001). Bio-fuels are very desirable in view of serious concerns over the rising levels of greenhouse gases such as carbon dioxide, global warming and dwindling reserves of fossil fuels. Systematic programs have been introduced to optimize the use of resources on a sustainable basis including the recycling the food production waste. Recycling of agricultural wastes also presents a great opportunity in supporting sustainable development by the production of bio-fuels and single cell proteins with yeast fermentation and, as reported by Nigam (1999) ethanol may also be obtained from pineapple cannery waste.

2.4.1 Pineapple Waste

Pineapple waste is one of the potential wastes, as it is one of the main commodities in Malaysia for either domestic or export markets. Pineapple, *Ananas comosus* from Bromeliaceae family, originated from South America. It is a fruit rich in vitamins, fibers as well as many other nutrients and antioxidants (Morton, 1987). It can be found in plantations in Thailand, Philippines, Africa and other tropical countries. Over the past century, it has become one of the leading commercial fruit crops of the tropics.

In Malaysia, pineapple plantations are found in Johor, Selangor, Kelantan and Penang with a total land use of 14,716 hectares and a yearly production of 323,747 million tons in year 2008 (Crops statistic, 2008). Malaysia exported most of its canned pineapple products to Europe between the 1960s to the 1990s. However, in the past decade, Malaysia's export of pineapple to Europe was declining while demands were increasing in East Asia, United States, Singapore and Japan. In 2006, fresh pineapple contributed RM 13.439 million of Malaysia's total export.

In the process of canning the pineapple fruit, the outer peel and the central core are discarded. The waste, called pineapple bran, accounts for about 50% of the total pineapple weight, about 10 tons of fresh bran or one ton of dry bran per hectare. Pineapple bran, either fresh or dried, may be used as feed for ruminants and is usually combined with grass to form the pellet of the diet (Palafox and Reid, 1961). However, it is not an attractive use in animal feed as it contains, on a dry matter basis, high fiber content and soluble carbohydrates.

2.4.2 Carbon Sources in Pineapple Wastes

Several studies have been carried out in Malaysia focused on pineapple solid and liquid waste, For example, a study on recycling the pineapple leaves to obtain sustainable potassium sources in land for agricultural use were carried out by Ahmed *et al.* (2004) while Norzita *et al.* (2005) obtained high yield of lactic acid production from both solid and liquid pineapple waste. Pineapple waste was also used in silage activities to ensure feed availability during periods of shortage (Chin, 2001). However, pineapple waste is still an underutilized agricultural waste in Malaysia and it is often used as fertilizer for other agricultural crops.

Based on the chemical composition shown in Table 2.2, pineapple cannery waste is a potential source of sugars, protein, vitamins and other growth factors. Pineapple waste is favorable for bacteria growth as reported by Nigam (1999) because it is able to yield large amounts of reducing sugars such as glucose and fructose that are most easily utilized by the bacteria cell. Thus, pineapple cannery waste can be used as a substrate for ethanol production, and may reduce the costs of waste disposal in waste treatments before disposal in order to reduce the organic load. In addition, pineapple waste was used as substrate in solid substrate fermentation (SSF) for the production of citric acid by *Yarrowia lipolytica* (Imandi *et al.*, 2008) or phenolic antioxidant by *Rhizopus oligosporus* (Correia *et al.*, 2004). However, pineapple contains relative low nitrogen content compared to the 0.5-1% (w/w) of nitrogen sources required for bacteria growth (Lee and Kim, 2001).

Table 2.2: Mean chemical composition of pineapple cannery waste[a] (Nigam, 1999)

Chemical Component	Concentration (g/L)
Total Sugar	82.53 ± 0.78
Reducing Sugar	39.46 ± 0.60
Glucose	22.70 ± 0.85
Sucrose	38.70 ± 1.12
Fructose	15.81 ± 0.83
Raffinose	2.62 ± 0.27
Galactose	2.85 ± 0.33
Protein	6.40 ± 0.33
Fat	1.20 ± 0.17
Kjedahl Nitrogen	2.32 ± 0.15
Total Solid	50 - 60*
Microbial Count	10^2-10^4 mL^{-1} *
pH	4.0 ± 0.08

[a] Each value corresponds to the mean of five experiments ± SD (Standard Deviation).
[*] In the case of total solids and microbial count, SD is not estimated.

2.5 Application of Bacterial Cellulose

The unique properties of bacterial cellulose have gain vast applications in various field such as in the food industry for applications in low-calorie desserts, salads, and fabricated foods. It has also been used in the paper manufacturing industry to enhance paper strength, the electronics industry in acoustic diaphragms for audio speakers, the pharmaceutical industry as filtration membranes, and in the medical field as wound dressing and artificial skin material.

2.5.1 Bacterial Cellulose in Food Application

Chemically pure cellulose can be used in processed foods as thickening and stabilizing agent. The first use of microbial cellulose in the food industries was in nata de coco in the Philippines. The gel-like properties of microbial cellulose, combined with its complete indigestibility in the human intestinal tract, made this an attractive food base. Nata, a bacterial cellulose product which is prepared from *Acetobacter xylinum* sp., is a popular snack in the Philippines and other countries. It is also widely used in food processing because of its distinctly soft texture and high fiber content. Stephens S. R.(1990) has studied a method of using bacterial cellulose as a dietary fiber component where microbial cellulose was introduced into diet drinks in Japan. *Acetobacter* was grown along with yeast in the tea extract and sugar. This is consumed as a kombucha, or Manchurian tea for improved health needs.

2.5.2 Bacterial Cellulose in Pharmaceutical and Medical Application

Bacterial cellulose has high tensile strength, high porosity and microfibrillar structure. Chronic wounds such as venous leg ulcers, bedsores, and diabetic ulcers are difficult to heal, and they represent a significant clinical challenge both for the patients and for the healthcare professionals. The treatment of chronic wounds involves the application of various materials (hydrocolloids, hydrogels, biological or synthetic membranes) that provide a moist wound healing environment, which is necessary for optimal healing. According to the modern approaches in the field of wound healing, an ideal wound dressing system must display similarity to artificial skin, both structurally and functionally. The characteristics of modern wound dressing materials are nontoxic, nonpyrogenic and biocompatible, ability to provide barrier against infection, ability to control fluid loss, ability to reduce pain during treatment, ability to create and maintain a moist environment in the wound, provide easy and close wound coverage, enable introduction or transfer of medicines into the wound, ability to absorb exudates during inflammatory phase, display high mechanical strength, elasticity and conformability, display an optional shape and surface area, and allow for easy and painless healing of the wound.

As microbial cellulose is a highly porous material, it allows the potential transfer of antibiotics or other medicines into the wound, while at the same time serving as an efficient physical barrier against any external infection. It satisfies the requirements of modern wound dressing material. Bacterial cellulose has high water holding capacity and the size of fibrils is about 100 times smaller than that of plant cellulose. A Brazilian company at Biofill investigated its unique properties for wound healing. A bacterial cellulose preparation, Prima CelTM has produced by Xylos Corp. (USA) and has been applied in clinical tests to heal ulcers and wounds. Tissue engineered blood vessels (TEBV) represent an attractive approach for overcoming reconstructive problems associated with vascular diseases by providing small caliber vascular grafts, and bacterial cellulose exhibits properties that are promising for use as a scaffold for tissue engineered blood vessels (Backdahl *et. al.*, 2006).

Among new commercial applications, bacterial cellulose has been shown to be very beneficial in the treatment of secondary and third degree burns. Bielecki S. (1996) has performed a clinical study on 34 patients. The bacterial cellulose wound dressing materials were directly applied on the fresh burn covering up to 9-18% of the body surface. It appears to be one of the best materials to promote wound healing from burns. Factors for this success include but are not limited to a moist environment for tissue regeneration, significant pain reduction, specific cellulose nano-morphology which promotes cell interaction and tissue re-growth, significant reduction of scar tissue formation and easy and safe release of wound care materials from the burn site during treatment. Microbial cellulose promises to have many new applications in wound care that extend beyond burn applications including the surgical wounds, bedsores, ulcers, tissue and organ engineering.

2.5.3 Bacterial Cellulose in Other Applications

The unique physical and mechanical properties of microbial cellulose such as high reflectivity, flexibility, light mass and ease of portability, wide viewing angles, and its purity and uniformity determine the applications in the electronic paper display (Shah *et. al*., 2005). From the other journal, Iguchi M.(2000) has stated that fragmented bacterial cellulose has promising prospects in papermaking, so test pieces of flexure durable papers and high filler content papers, which are ideal for banknote paper. Microbial cellulose also has applications in mineral and oil recovery. There is a patented invention related to the use of bacterial cellulose in hydraulic fracturing of geological formations at selected levels of wells drilled for recovery of hydrocarbons. Addition of relatively small quantities of bacterial cellulose to hydraulic fracturing fluids improves their rheological properties and the friction through well casings is significantly reduced, resulting in lower pumping energy requirements. Addition of cellulose micro fibrils obtained by acid hydrolysis of cellulose fibers at low concentrations to polymer gels and films as reinforcing agents showed significant changes in tensile strength and mechanical properties (Orts *et. al*., 2005). It is because the micro fibril can supply multiple adhesion sites in the medium. Therefore, it is able to enhance cellulose synthesis with the result of the development of an oxygen-limiting bio film around these particles. Based on the tensile strength, low oxygen transmission (barrier property) rate and its hydrophilic nature, the processed cellulose membrane appears to be of great relevance for its application as packaging material in food packaging, where continuous moisture removal and minimal oxygen transmission properties play an important role. The unique dimensional stability of microbial cellulose gives rise to a sound transducing membrane which maintains high sonic velocity over a wide frequency ranges, thus being the best material to meet the rigid requirements for optimal sound transduction. Sony Corporation (Japan) in conjunction with Ajinimoto (Japan) developed the first audio speaker diaphragms using microbial cellulose. However, the production of the speaker membrane by using bacterial cellulose is not justifiable for the market because of the high costs (Iguchi *et. al*., 1988).

2.6 Production of Bacterial Cellulose

An extensive study was performed detailing the method to produce BC including by Masaoka *et. al*., (1993). Many strains of organisms belonging to the genus *Acetobacter* and *Agrobacterium* were screened to find the best cellulose producer. Off the 41 strains tested, only four show significant cellulose formation, and these were all strains of *Acetobacter xylinum*. The best of these four was used for the remainder of the study. The cellulose production was found to be proportional to the surface area of the culture when volume was held constant. Similar experiments found that culture volume and depth had no effect on production rate. A simple experiment show that only cells close to the surface of the medium could produce cellulose, supporting the general hypothesis that growth is at the air/liquid interface. The optimal pH range for cellulose formation is between 4 and 6, where glucose, fructose, and glycerol were the best substrates for growth. Gluconic acid was formed in glucose cultures, and the amount increased with increasing glucose concentration. While this caused a decrease in the yield of cellulose on glucose, it did not have much of an effect on the cellulose production rate (Pourramezan, *et. al*., 2009). Besides the pH, shaken speed or aeration also give the effect towards the cellulose production. In the journal of Ch'ng, *et. al*. (2009), it was proved that at 120 rpm, it yield the highest amount of bacterial cellulose but that research experiment was studied based on shaking speed at 80 rpm, 120 rpm and 160 rpm for 4 days fermentation in an incubator shaker at 28 ˚C. The authors stated that when a medium culture is shaken, *Acetobacter xylinum* sp. grows more rapidly but do not form in well-organized pellicles like what produced under static condition.

2.7 Media Studies

The effective culture method to produce bacterial cellulose is from fruit juice by *Acetobacter xylinum* sp. Bacterial cellulose production from various fruit juices including pineapple, orange, Japanese pear and grape were investigated and the possibility of producing bacterial cellulose was suggested. The yield of bacterial cellulose was increased by addition of the nitrogen source to the fruit juice. The