การพัฒนาเมมเบรนที่มีโครงสร้างระดับนาโนจากแบคทีเรียเซลลูโลสที่ผ่านการรีเจนเนอเรท

นาย ฐาปนา สุวรรณมาโจ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต
สาขาวิชาวิศวกรรมเคมี ภาควิชาวิศวกรรมเคมี
คณะวิศวกรรมศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2549

ISBN 974-14-2016-1

DEVELOPMENT OF NANOSTRUCTURE MEMBRANE FROM REGENERATED BACTERIAL CELLULOSE

Mr. Thapanar Suwanmajo

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering in Chemical Engineering
Department of Chemical Engineering
Faculty of Engineering
Chulalongkorn University
Academic Year 2006
ISBN 974-14-2016-1

Thesis Title	DEVELOPMENT OF NANOSTRUCTURE MEMBRANE FROM REGENERATED BACTERIAL CELLULOSE
By	Mr. Thapanar Suwanmajo
Field of Study	Chemical Engineering
Thesis Advisor	Assistant Professor Muenduen Phisalaphong, Ph.D.

Accepted by the Faculty of Engineering, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of Faculty of Engineering
(Professor Direk Lavansiri, Ph.D.)

THESIS COMMITTEE

..... Chairman
(Assistant Professor Vichitra Chongvisal, Ph.D.)

.....Thesis Advisor
(Assisiant Professor Muenduen Phisalaphong, Ph.D.)

.....Member
(Assistant Professor Joongjai Panpranot, Ph.D.)

..... Member
(Soorathep Kheawhom, Ph.D.)

..... Member
(Sopee Sanguandekul, Ph.D.)

ฐาปนา สุวรรณมาโจ: การพัฒนาเมมเบรนที่มีโครงสร้างระดับนาโนจากแบคทีเรียเซลลูโลสที่ผ่านการรีเจนเนอเรท (DEVELOPMENT OF NANOSTRUCTURE MEMBRANE FROM REGENERATED BACTERIAL CELLULOSE)
อ. ที่ปรึกษา: ผศ. ดร. เหมือนเดือน พิศาลพงศ์, 94 หน้า. ISBN 974-14-2016-1.

ในการศึกษาวิจัยนี้แผ่นเยื่อกรองเซลลูโลสที่ผ่านการรีเจนเนอเรทแบบใหม่ได้ถูกเตรียมขึ้นด้วยการขึ้นรูปโดยใช้แบคทีเรียเซลลูโลสและอัลจีเนตเป็นวัตถุดิบ ขั้นตอนการเตรียมที่เป็นมิตรต่อสิ่งแวดล้อมได้ถูกพัฒนาขึ้นในการทำรีเจนเนอเรทแบคทีเรียเซลลูโลส โดยสภาวะที่เหมาะสมที่สุดคือ 4 % wt NaOH/3 % wt urea ตามด้วยกระบวนการแช่แข็งแล้วปล่อยให้ละลาย (Freeze thaw process) จากนั้นนำมาผสมกับอัลจีเนตในอัตราส่วนที่แตกต่างกัน กระบวนการทำแห้งด้วยการใช้คาร์บอนไดออกไซด์วิกฤตยิ่งยวด (Supercritical CO_2 drying method) ถูกนำมาใช้ในการสร้างรูพรุนของแผ่นเยื่อกรอง จากนั้นนำเมมเบรนที่ขึ้นรูปได้จะถูกนำไปวิเคราะห์เพื่อศึกษาโครงสร้างระดับนาโนและคุณสมบัติทางกายภาพของเมมเบรน จากการศึกษาพบว่าแบคทีเรียเซลลูโลสเมมเบรนมีคุณสมบัติเชิงกลที่ดีกว่ากว่าแผ่นเมมเบรนที่ได้จากการผสมของแบคทีเรียเซลลูโลส และอัลจีเนต แต่ค่าการซึมผ่านของไอน้ำ (Water vapor transmission rate) และ ค่าการดูดซึมน้ำ (Water absorption) ของแบคทีเรียเซลลูโลสเมมเบรนมีค่าต่ำกว่า ค่าคุณสมบัติการต้านทานของแรงดึง (Tensile strength) ค่าอัตราการเปลี่ยนแปลงความยาวต่อความยาวเดิมที่จุดขาด (Elongation at break) และค่าร้อยละการดูดซึมน้ำ (Percent water absorption) ของแบคทีเรียเซลลูโลสเมมเบรนคือ 4.32 MPa, 35.20% และ 49.67 % ตามลำดับ อัตราการซึมผ่านไอน้ำของเยื่อแผ่นแบคทีเรียเซลลูโลสที่ผ่านการรีเจนเนอเรท (RBC) คือ 2,504 g/m^2 day และขนาดเส้นผ่าศูนย์กลางรูพรุน คือ1.26 nm และพบว่าเมมเบรนที่ผสมอัลจีเนตในอัตราส่วนร้อยละ 20 โดยน้ำหนักแสดงโครงสร้างพื้นผิวที่มีการจัดเรียงของเส้นใยเป็นระเบียบมากที่สุด ค่าคุณสมบัติการต้านทานของแรงดึง ค่าอัตราการเปลี่ยนแปลงความยาวต่อความยาวเดิมที่จุดขาด และ ค่าร้อยละการดูดซึมน้ำของแบคทีเรียเซลลูโลสเมมเบรนที่ผสมกับอัลจีเนตในอัตราส่วนร้อยละ 20 โดยน้ำหนัก คือ 3.38 MPa, 31.60% และ 52.25% ตามลำดับ อัตราการซึมผ่านไอน้ำคือ 5,609 g/m^2 day และขนาดเส้นผ่าศูนย์กลางรูพรุน คือ1.06 nm

ภาควิชา.....วิศวกรรมเคมี..... ลายมือชื่อนิสิต.....ฐาปนา สุวรรณมาโจ.....

สาขาวิชา.....วิศวกรรมเคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2549.....

4670291421: MAJOR CHEMICAL ENGINEERING

KEY WORD: CELLULOSE/ BACTERIAL CELLULOSE / CELLULOSE DISSOLUTION/ REGENERATED CELLULOSE / MEMBRANE SEPARATION

THAPANAR SUWANMAJO: DEVELOPMENT OF NANOSTRUCTURE MEMBRANE FROM REGENERATED BACTERIAL CELLULOSE
THESIS ADVISOR:ASSIST.PROF.MUENDUEN PHISALAPHONG, Ph.D., 94 pp. ISBN 974-14-2016-1

In this research study, bacterial cellulose (BC) and alginate were used as raw materials for the frabrication of a new regenerated cellulose membrane. The environmental friendly procedure for regenerated bacterial cellulose (RBC) was developed. The optimum conditions involved swelling BC in 4% wt NaOH/ 3% wt urea solution, followed by freeze thaw process and blending with alginate at various ratios. Supercritical CO_2 drying method was then applied for porous preparation of the membrane. Afterward, the frabricated membranes were characterized for nanostructure and physical properties. The mechanical properties of the pure RBC membrane were better than the blend membranes. However, the water vapor permeability and the percent water absorption of the pure RBC membrane were lower. The tensile strength, the elongation at break and the percent water absorption of the pure RBC membrane were 4.32 MPa, 35.20% and 49.67%, respectively. The water vapor transmission rate of the RBC was 2,504 g/m^2 day and its pore size was 1.26 nm. The RBC blend membrane with 20% wt alginate exhibited the most orderly alignment of fibers on its surface with the tensile strength, the elongation at break and the percent water absorption at 3.38 MPa, 31.6% and 52.25%, respectively. The water vapor transmission rate was 5,609 g/m^2 day and the pore size was 1.06 nm.

Department.....Chemical Engineering..... Student's signature.....Thapanar S.

Field of study.....Chemical Engineering..... Advisor's signature.....

Academic year.....2006.....

ACKNOWLEDGEMENTS

I have learnt a great deal from those whom I had the pleasure of working with. I would like to take this opportunity to thank those who have made this thesis possible. First, I will extend a thank you to Dr. Muenduen Phisalaphong as mentor and advisor on this project. With her support and advice not only has she made this project possible, but has also been a great support throughout my rough times. I would like to thank her for her patience and understanding when progress was slow.

Thanks also go out to Mr. Preecha Sangtheerapitikul. He provided a lot of the knowledge and inspiration that kept the project progressing. In addition, I would also grateful to Assistant Professor Vichitra Chongvisal, as the chairman, Assistant Professor Joongiai Panpranot, Dr. Soorathep Kheawhom and Dr. Sopee Sanguandekul, member of thesis committee.

Many thanks for Mr. Nirun Jatupaiboon and many best friends in Chemical Engineering department. Each of them has helped me in their own way.

Last, but definitely not least, I would like to thank my parents for their great faith in me and for their continuous support, unlimited patience and encouragement.

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CHAPTER I

INTRODUCTION

Nowadays, polymers derived from renewable resources have become incredibly attractive as potential raw materials according to the limitation of petroleum resources and gradually more environmental concerns. It is well known that cellulose, the most prevalent polymer in nature, is environmentally friendly and non-toxic. Cellulose is one of the most important structural elements in plants as the main components of cell walls. While cellulose from plant is considered unpurified cellulose containing many kinds of complex carbohydrates, cellulose produced from bacteria or fungi is nearly-purified cellulose. Nata de Coco is Bacterial Cellulose (BC) synthesized in a culture of *Acetobacter xylinum* with coconut water being used as liquid medium.

BC presents significant advantages over plant-derived cellulose with its structural and mechanical properties. Unlike cellulose from plants, BC is chemically pure, free of lignin and hemicellulose, has a high crystallinity and a high degree of polymerization that distinguishes it from other forms of cellulose. Due to these unique properties, BC can be applied for a wide range of utilizations such as a food matrix, diet food or high performance acoustic diaphragms for audio speakers (Yamanaka *et al.*, 1989). For medical uses, BC has been applied as an artificial skin for patients with burns and ulcers (Farah, 1990 and Fontana *et al.*, 1990), temporary substitute for animal skin (Jonas and Farah ,1998) and artificial blood vessels for microsurgery (Klemm, 2001). Other applications may include the use of BC as a matrix of palladium-BC membrane to produce experimental fuel cells for the generation of electricity (Evans, 2003). Furthermore, BC flim was tested for its performance as a

dialysis membrane. BC film showed superior mechanical strength to that of a dialysis-grade regenerated cellulose membrane, allowing the use of a thinner membrane than the latter. As a result, the bacterial membrane gave higher permeation rates (Shibazaki *et al*., 1993).

In the recent years, regenerated cellulose membranes from plant have been widely utilized in membrane separation technique such as dialysis, ultrafiltration and reverse osmosis. Many extensive regenerated cellulose membrane productions have been studied; however, they are mostly centered on the cellulose from plant. It has been known that the strong inter-chain and intra-chain hydrogen bonding involved in the crystalline regions make reactions and dissolution of cellulose difficult. The current industrial routes for dissolving of cellulose usually require a strong alkali condition and also have pollution problem of noxious gas emission. Consequently, environmental concerns are driving the efforts to find new solvent to dissolve cellulose. In addition, studies of plant-derived cellulose blended with natural polymer have been carried on to improve or modify the structure and properties of membranes.

Plant-derived cellulose and BC have the same chemical composition, but different chemical and physical properties. BC has mechanical properties that are superior to plant cellulose and many synthetic fibers. Young's modulus of BC is approximately 4 times greater than organic fibers (Yamanaka *et al.*, 1989). Compared with cellulose from plants, BC possesses higher water holding capacity, higher crystallinity, higher tensile strength, and a finer web-like network. BC can be produced within a few days in a simple and inexpensive procedure, so it is expected to become a potential biodegradable biopolymer.

Studies of regenerated cellulose from plant-derived cellulose have been carried out by many researchers (Filho and de Assuncão, 1993; Okajima *et al*.,1996 ; Isagai *et al*., 1998; Zhou *et al*., 2000; Yang *et al*., 2000; Zhou *et al*., 2001; Yang *et al*., 2002), however, only few, if any, has been done on using BC as a source of cellulose.

Due to its unique properties, this study is attempted to develop a new regenerated cellulose membrane from BC. The structure, pore morphology, tensile strength, components and water vapor permeability of the developed membrane are then investigated and compared with others for further separation approaches. The overview of content of this study is shown below:

Overview

This present work is organized as follows :

Chapter I presents an introduction of this study.

Chapter II contains background theory of cellulose, feature and application of BC, dissolution of cellulose and separation and transport of membrane.

The literature reviews of BC and the development of cellulose membrane process are presented in chapter III.

The details of the experimental procedures and techniques of this research are described in Chapter IV.

Chapter V presents the experimental results of the characterization of regenerated cellulose membrane from BC. The pore morphology, structure, tensile strength and water vapor permeability of the developed membranes are reported and discussed.

Chapter VI contains the overall conclusion obtained from this research. Future work and recommendations are stated.

Finally, the additional data of the experiments which had derived from this study are included in appendices at the end of this thesis.

CHAPTER II

BACKGROUND THEORY

2.1 Cellulose

Cellulose is the earth's major biopolymer and is of colossal economic importance globally. It is certainly one of the most important structural components in plants and other living systems. In the oceans, plankton or algae can produce this biopolymer by using the same type of carbon dioxide fixation found in photosynthesis of land plants. Besides, fungi and bacteria can assemble cellulose as well.

Cellulose is a linear polymer consisting of D-anhydroglucopyranose units (15-40000 glucose units) joined together by ß-1,4-glycosidic bonds as shown in figure 2.1. Its Molecular formula is $(C_6H_{10}O_5)_n$ and its molecular weight relies on the raw material which is approximately 1,500,000 daltons (Brown, 1983).

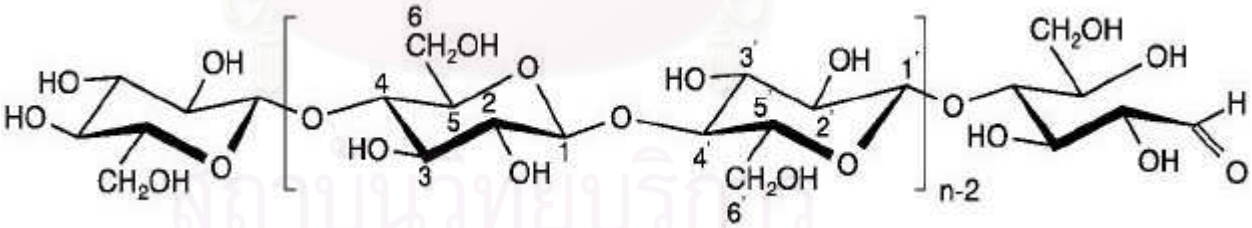

Figure 2.1 Molecular structure of cellulose (Klemm *et al.*, 2002).

When a cellulose molecule is completely extended, its chain resembles a flat ribbon, with hydroxyl groups extending laterally from the edges. This structure is capable of forming inter- and intra-molecular hydrogen bonds. The hydrogen atoms oriented above and below the plane of the ribbon and are thus hydrophobic. This structure allows for Van der Waals interactions between the hydrogen atoms (Klimentov *et al.,* 1991). These two characteristics result in a supramolecular structure (figure 2.2) composed of crystalline regions, where the chains are arranged in highly ordered three-dimensional lattices, and amorphous regions, where there is little or no order among the chains (Krässig, 1993). The overall structure is of aggregated particles with extensive pores capable of holding relatively large amounts of water by capillarity.

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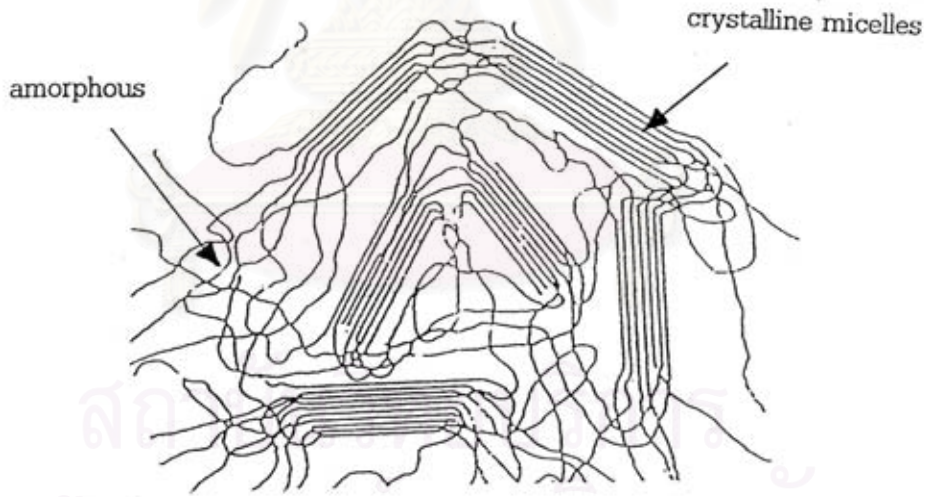

Figure 2.2 The supramolecular structure of cellulose (Mark, 1940)

It has been concluded from H-NMR spectroscopy that the ß-D-glucopyranose adopts the $^4C_1$ chain conformation, the lowest free energy conformation of the molecule (Krassig, 1993). As a result, the hydrogen atoms are positioned in the vertical axial, whereas the hydroxyl groups are placed in the ring plane. Cellulose holds three hydroxyl groups at the C-2, C-3, and C-6 atom positions, which are, in general, accessible to the typical conversions of primary and secondary alcoholic OH groups. Based on the molecular structures, i.e. the tacticity and the uniform distribution of the hydroxyl groups, ordered hydrogen bond system forms several types of supramolecular structures.

The hierarchical structure of cellulose is formed by both intra- and intermolecular hydrogen bond network between hydroxyl groups. The existence of intramolecular hydrogen bonds is of high relevance with respect to the single-chain conformation and stiffness. The presence of hydrogen bonds between O-3-H and O-5' of the adjacent glucopyranose unit and O-2-H and O-6' in native crystalline cellulose (cellulose type I, Figure 2.3) can be summarized from XRD and NMR- and IR spectroscopical data (Liang and Marchessault, 1959; Marchessault and Liang, 1960; Gardner and Blackwell, 1974; Sarko and Muggli, 1974).

In type II cellulose , the hydrogen bonds are basically the same as those proposed for type I cellulose, considering the O-3-H and O-5' hydrogen bond. The conformation of the C-6 hydroxymethyl group dissimilars in each chain because the chains are oriented anti-parallel in the unit cell. One of the chains holds one intramolecular hydrogen bond per anhydroglucose unit while the other chain has two, therefore a more complex hydrogen-bonding network takes place (Stipanovic and Sarko, 1976).

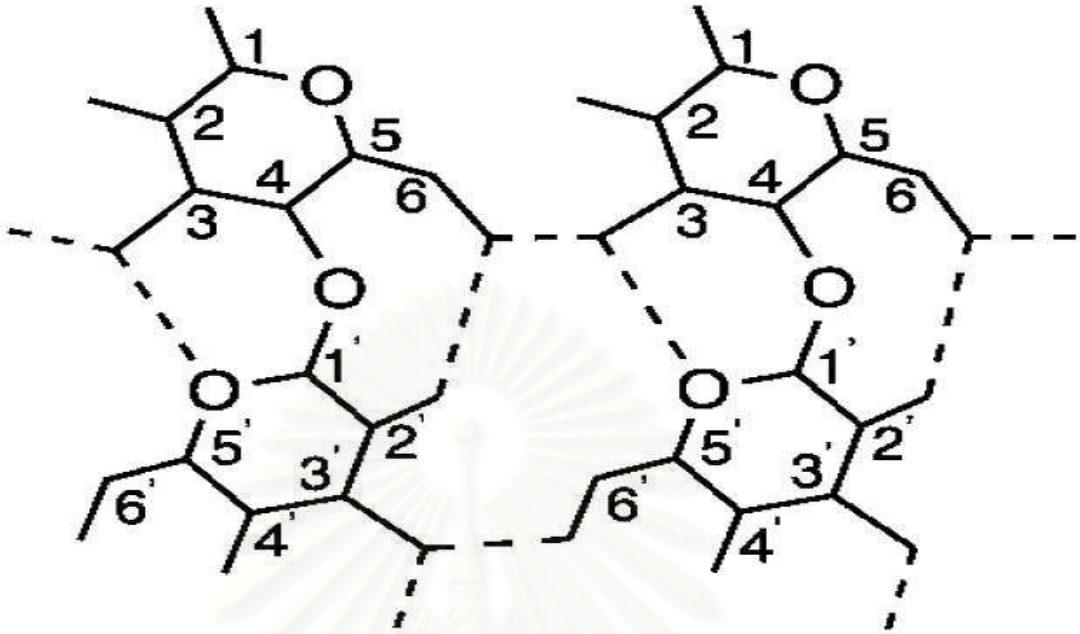

Figure 2.3 Hydrogen bond system of cellulose type I.

The intermolecular hydrogen bonding in cellulose is considerable for the sheet-like nature of the native polymer. It is assumed that intermolecular hydrogen bonding between the hydroxyl group at the C-3' and C-6' positions of cellulose molecules adjacently occurred along the same lattice planes (Blackwell *et al*., 1977).

Compared to that of cellulose I, cellulose II's intermolecular hydrogen bonding is significantly more complex. The anti-parallel chain model enables the formation of both interchain and interplane hydrogen bonds. The most widely approved representation of the bonding situation in cellulose has been proposed by Kolpak and Blackwell (1976), as shown in figure 2.4.

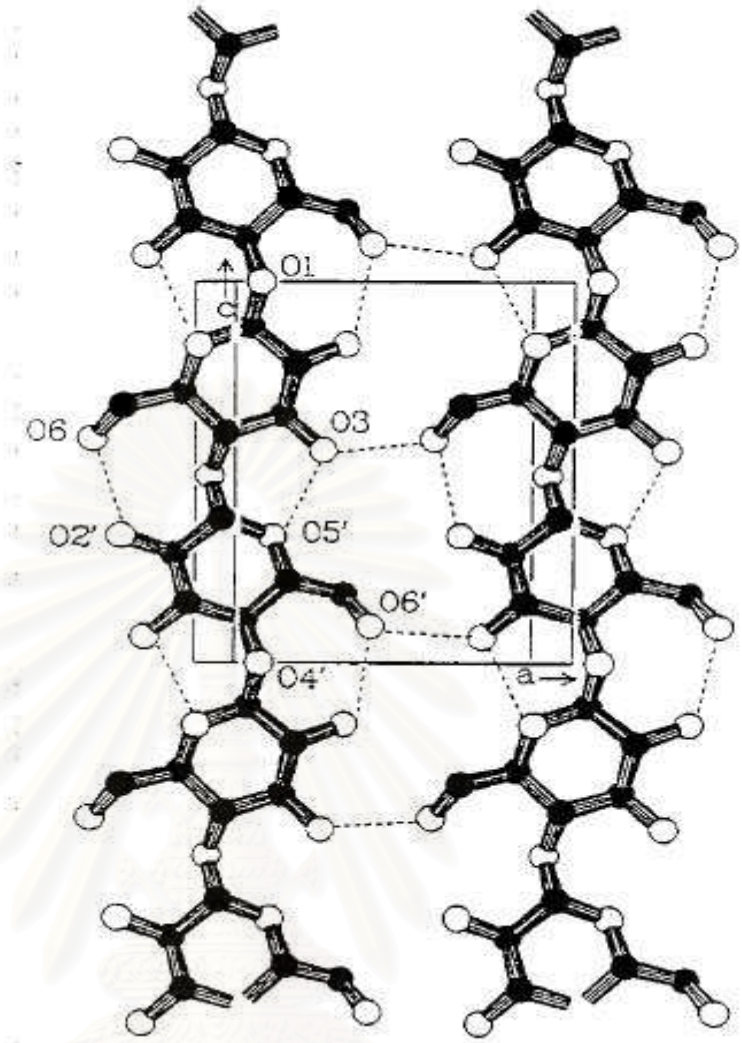

Figure 2.4 Most probable bond pattern of cellulose. (Kolpak and Blackwell, 1976)

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Cellulose fibrils are highly inelastic and insoluble (Ross *et al.*, 1991). The ß-1,4 linkage results in a stiff, ribbon-like molecule that is ideally suited for forming fibrils via hydrogen bonding. Bacterial cellulose has the same chemical constituency as cellulose from plants. It is obtained from the polymerization process into long microfibrills outside the cell wall. Its crystallographic form of the microfibrils containing parallel extended glucan chains is designed as cellulose I. It has been recognized that cellulose II is obtained by treating native cellulose with a concentrated alkaline swelling agent and subsequence precipitation/regeneration as is done in the

formation of fiber and film. Because cellulose II is found to form adjacent antiparallel glucan chains as shown in figure 2.5, thus cellulose II are more thermodynamically stable (Nevell, 1985).

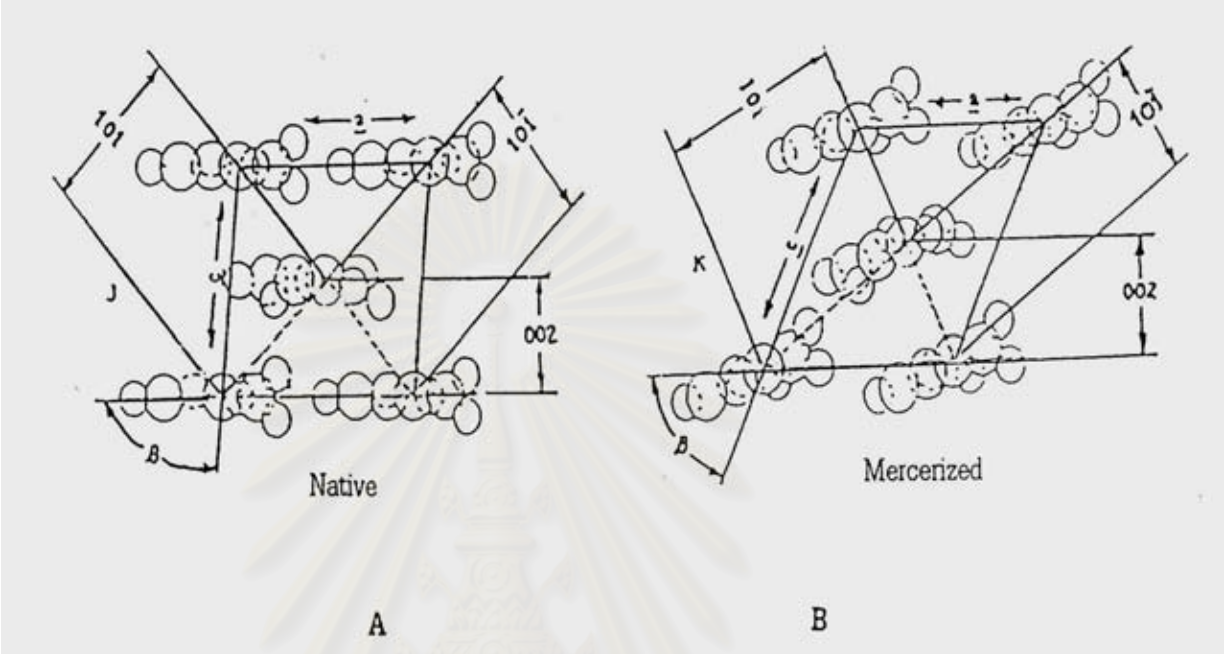


Figure 2.5 The structure of cellulose unit (Krassig, 1985).

A : Maryer-Mark-Misch' model.

B : Cellulose II, Andress's model.

In the field of the electron microscope, it has been known that the microfibril term has been denoted the thinnest fibril structures visible. In 1991, Ross *et al* described that microfibrils' cellulose I occured in a spectrum of dimensions ranging from 1 to 25 nm width (corresponding to 10-250 chains) and from 1 to 9 □m in length containing 2,000 to 18,000 glucose residues. The function and location of the microfibrils are markedly different in each living system. It has been reported that the cellulose fibril from plant is an integral part of the complex polysaccharide cell wall matrix while bacterial cellulose fibril is a metabolically inert, highly pure extracellular deposit for some ecological uses (Cannon and Anderson, 1991).

2.2 Feature of bacterial cellulose

Bacterial cellulose, produced by *A. xylinum*, is markedly different from cellulose derived from plants with many advantages that can be commercially useful. Bacterial cellulose, highly pure network structure, is devoid of lignin, hemicellulose and other complex carbohydrate. It can be produced from many different substrates such as Nata de pina and Nata de coco, synthesized by using *Acetobacter xylinum* with pineapple water and coconut water as medium, respectively.

According to the unpurified-structure of plant cellulose, plant cellulose must endure a many stage pulping process to remove lignin, hemicellulose and other compounds. This procedure is costly for the delignification step which is necessary for paper manufacturing. Besides, this process requires energy and chemicals that are often harmful to the environment.

In the era of declining forests, global climate changes and continuing expansion of industrialization with increased population, it is thus reasonable to consider the importance of an alternative source of cellulose. Bacterial cellulose is pure and does not require harsh processing. Due to the fewer steps required to process bacterial cellulose, less money is spent and less waste is generated. Moreover, bacterial cellulose fiber remains stronger and retains attractive properties than cellulose from plant.

Bacterial cellulose displays unique properties, including high mechanical strength, high water absorption capacity, high crystallinity, and has an ultra-fine and highly pure fibre network structure. It is extremely hydrophilic, absorbing 60 to 700 times its weight in water. Plant-derived cellulose, wood or cotton, must be physically disintegrated to make them hydrophilic (Brown, 1991). Bacterial cellulose will retain

its long fibrils and exceptional strength because it is formed in a hydrophilic matrix and needs no treatment.

Cellulose is a linear β-(1,4)-D-glucan polymer synthesized by plants or bacteria . Bacterial cellulose is chemically identical to plant-derived cellulose, but both macromolecular structure and properties dissimilar from the latter one as shown in figure 2.6. The subfibrils of bacterial cellulose are crystallized into microfibrils (Jonas and Farah, 1998), these into bundles, and the latter into ribbons (Yamanaka *et al.*, 2000).

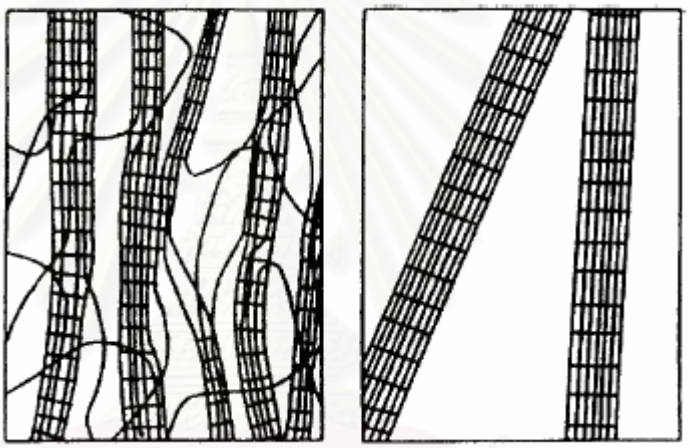


Figure 2.6 Schematic models of bacterial cellulose microfibrils (right) drawn in comparison with the `fringed micelles' of plant cellulose fibrils (Iguchi *et al*., 2000).

The diameter of bacterial cellulose fibril is about 1/100 of that of plant cellulose as shown in figure 2.7 and Young's modulus of bacterial cellulose is approximately 4 times greater than any organic fiber (Yamanaka *et al.,* 1989). Compared with cellulose from plants, bacterial cellulose possesses higher water holding capacity, higher crystallinity, higher tensile strength, and a finer web-like

network. Bacteria produce bacterial cellulose in a few days so it is expected to be a new biodegradable biopolymer and the replacement for cellulose from plants.

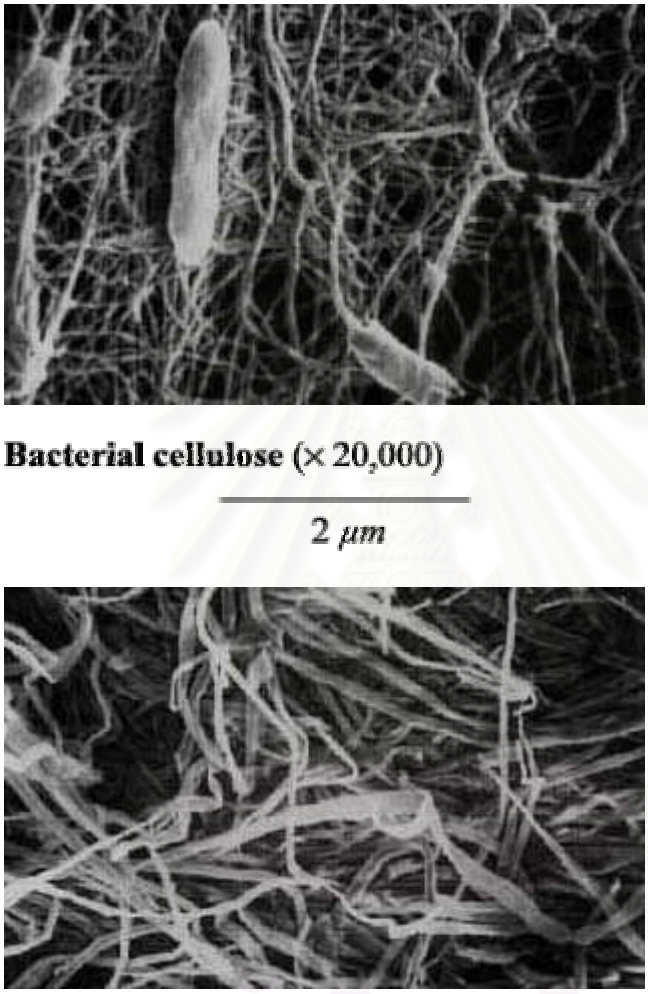


Figure 2.7 Bacterial cellulose and plant-derived cellulose (http://www.res.titech.ac.jp)

Bacterial cellulose can be produced with a variety of substrates, with different strains showing preference for certain ones. This is important for commercial applications as inexpensive substrates lower production costs.

2.3 Applications of Bacterial Cellulose

Due to the outstanding properties of bacterial cellulose, there have been several applications of the material in various fields. The properties of bacterial cellulose are quite different from those of plant-derived celluloses. By virtue of its mode of production, physical properties such as hydrophilicity, tensile strength, and permeability, the bacterial cellulose can be altered to suit many potential commercial applications. It has been known that many researches on the production of bacterial cellulose have been studied in many years but applications of the material are mostly current. This is because it could be a change in mentality towards the value of biodegradable materials.

One of the first commercially available products of bacterial cellulose is Nata de coco, a food product of the Philippines. It was only in the last ten years that other possible commercial application of bacterial cellulose were widely investigated

It is well known that the potential and current applications of bacterial cellulose are used in many fields. The first listed application is acoustic speaker diaphragms. Sony Corporation worked with Ajinomoto and Japanese Textile Research Institute to develop the first audio speaker diaphragms using bacterial cellulose (Yamanaka *et al.,* 1994). The unique characteristic of bacterial cellulose paper is used to create a sound transducing membrane which is about the best material available to meet the strict requirements for optimal sound reproduction. In the field of paper, it is found that adding disintegrated bacterial cellulose to paper pulp was able to create a stronger paper (Yamanaka *et al.,* 1994).

The specific application of bacterial cellulose as a dialysis membrane was examined by Shibazaki *et al.* (1993). Bacterial cellulose film showed a significantly

higher permeation rate and a greater molecular weight cut-off when compared to a commercial dialysis membrane (regenerated cellulose membrane). Also, the additional benefit of the bacterial cellulose film compared to the regenerated cellulose membrane was that the added mechanical property allowed the use of a thinner material.

In the field of paper, it is well known that bacterial cellulose has several advantages over synthetic paper: (a) unique nanostructure, (b) complete purity, (c) higher dimensional stability, (d) greater mechanical strength and (e) greater capacity to hold water (Brown, 1989). It is synthesized in a wet state from culture and is never dried during synthesis. In its dry state, bacterial cellulose has extraordinary absorptivity, which leads to many uses in fields such as wound care (Brown,1989) and fuel cell membranes (Evans *et al*, 2003), where it can be manipulated to absorb external materials to impart functionalities.

There are studies on the production and efficiency of bacterial cellulose as a temporary skin substitute called Biofill. This artificial skin for burn and skin injuries treatment shows dramatic clinical results (Fontana *et al*., 1990). Positive indications are immediate pain relief, diminished post-surgery discomfort, faster healing, reduced infection rate and reduced treatment time and cost. These products of bacterial cellulose that now have wide applications in surgery and dental implants. Besides, the bacterial cellulose can also be applied for temporary substitute for animal skin (Jonas and Farah, 1998) and artificial blood vessels for microsurgery (Klemm, 2001).

In a series of papers published in by Okiyama *et al*. (1992) dealt with applications of bacterial cellulose in the food industry. It has been known that, in its native state, bacterial cellulose is too tough to bite. When treated with ethanol and

calcium chloride, it becomes softer and has a texture similar to fruit and other foods. Applications of bacterial cellulose as food products are such as low-calorie desserts, salads and fabricated foods. Also, this material is used as a food additive for a chocolate drink in place of xanthan gum (Okiyama *et al*., 1993). Besides it can be used as a food thickener and coater.

Yoshino and coworker (1990) were prepared a graphite film by pyrolysis of bacterial cellulose. It has been concluded that the membranes made from bacterial cellulose were stable 6 to 7 times longer than those that were made from wood. Also, bacterial cellulose has also been used as a substrate for mammalian cell cultures (Watanabe *et al.*, 1993).

The study recently performed by Svensson *et al*. (2005) declared that bacterial cellulose was also a promising material for a potential scaffold for tissue engineering of cartilage. However, there was a concern when it was used as bone substitute material as it might not bond directly to bone as happened with plant cellulose (Martson *et al.*, 1998).

Figure 2.8 Bacterial cellulose from Nata de Coco (Evans *et al*., 2003).

2.4 Alginate

Alginates are the salts of alginic acid extracted from marine brown algae (Phaeophyceae) which comprising up to 40% of the dry matter. This biopolymers is a linear binary copolymer containing ß-(1→4)-linked D-mannuronic acid (M) and α-(1→4)-linked L-guluronic acid (G) monomers of varying composition and sequence as shown in figure 2.9 (a and b). These two monomers occur in homogenous regions of M and G-blocks, interspersed with region of alternating structure (MG-blocks) as shown in figure 2.9 (c). The polymannuronic acid is flatted, ribbon-liked while the shape of polyguluronic acid is buckled, ribbon-liked.

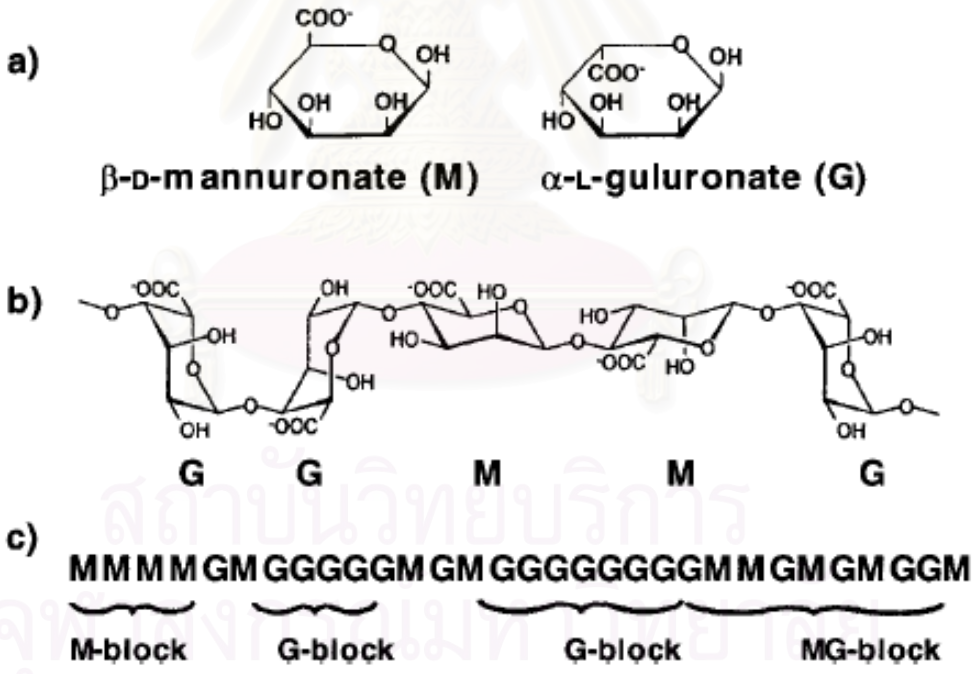


Figure 2.9 Structural characteristics of alginate: (a) monomers, (b) chain conformation and (c) block distribution (Dragett *et al.*, 1999).

Since alginic acid is insoluble, to convert to a water-soluble form, alginic acid is transformed into commercial alginate through the incorporation of different salts (Table 2.1). The most widely used alginic acid salt is sodium alginate (Na-alginate) which is promptly soluble in cold or hot water (Imeson, 1992)

Salts	Commercial alginates
Na_2CO_3	Na-alginate
K_2CO_3	K-alginate
NH_4OH	NH_4-alginate
$Mg(OH)_2$	Mg-alginate
Ca_2Cl_2	Ca-alginate
Propylene oxide	Propylene glycol-alginate

Table 2.1 Production of commercial alginates by incorporation different salts into alginic acids (Imersion, 1992).

2.4.1 Application

Alginate absorbs water quickly, which makes it useful as an additive in dehydrated products such as slimming aids, and in the manufacture of paper and textiles. It is also used for fireproofing fabrics, for thickening drinks, ice cream and cosmetics, and as a detoxifier that can absorb poisonous metals from the blood. The most alginates applications are utilized in the food industry. Dressings, sauces, and beverages are important propylene glycol alginate applications. For sodium alginate,

the application include cheese sauces, fruit fillings, instant flans and mousses, icings and glazes, and restructured foods such as onion rings, pimentos, and meats. The important and useful property of alginates in the food industry is the ability to form eatable gels by cross linking with calcium salt. Alginate gel is of interest since alginate is able to use for viscosity tuning. The gel can be formed without any heating and the gel network will remain through freeze and thaw cycles. Its gel is thermally stable and therefore continue to provide functionality even when the food is heated. Also, it is also effective at both highly acidic and neutral pH levels and it is not thermoreversible as gelatin.

One of interesting properties of soluble alginate solution found useful in the food industry is film formation. Alginate film is prepared by drying a thin layer of soluable alginate solution or by treating a soluable film with di- or trivalent metal (Glicksman, 1985). Calcium has found to be the most favorable as the divalent ion for gel formation because its salts are cheap, readily available and non-toxic. Calcium ions are reported to be more effective than magnesium, alumina, manganese, ferrous and a ferric ions, in bridging alginate chains together ionic interactions followed by interchain hydrogen bonding (Gannadios, 1997). Stronger alginate coatings will be obtained from calcium chloride rather than calcium gluconate, nitrate and propionate. Besides, there are the applications of alginate film in membrane separation process such as pervaporation. Alginate is also most widely used as thickener for the reactive dyes used for cellulose fibers. The great advantage of alginate is that it does not react with the dye or the fiber, so it results in clear colors and soft fabrics.

Owing to alginate's biocompatibility and simple gelation with divalent cations, it is utilized for medical applications, such as encapsulation of cells. One example of this application is the use of alginate for the encapsulation of insulin-producing cells

for better treatment of patients suffering from diabetes (Gimmestad *et al*., 2003). Alginate is used extensively as a mold-making material in dentistry and prosthetics. Besides, calcium alginate is also used in burn dressings to promote healing and can be removed painlessly.

2.5 Polymer blends

Polymer blend is derived from the blending of two or more polymers. It has become an important technique for improving the cost-performance ratio of commercial polymer. Generally, polymer blend is categorized into three types by its miscibility as shown in figure 2.10.

2.5.1 Miscible blend.

In miscible blends, the polymer-polymer mixture can be homogenously combined in any circumstances. This polymer blend has unique properties at specific proportion of mixture. However, there are very few of this type of polymer blend.

2.5.2 Immiscible blend.

Immiscible blend is a polymer blend that is non-homogeneous mixture under any circumstances. This polymer blend has several phases - some phase may contain either one of the polymers or both of the polymers in different proportions. The morphology of each phase has a direct relation to the property of polymer blend. Usually, the morphology of polymer blend can be varied due to several factors such as laboratory technique, equipment and temperature applied during mixing process.

2.5.3 Partially miscible blend.

Partially miscible blend is a polymer blend that can be circumstantially blended under certain conditions where temperature and proportions are influential. The model of miscibility and phase separation of polymer are always displayed in the form of phase diagram between temperature and proportion of polymer mixture.

Figure 2.10 The miscibility of each polymer blends.

2.6 Dissolution cellulose in NaOH/urea aqueous solution

Due to the strong hydrogen bonds linking cellulose molecules, cellulose cannot be easily dissolved or melted in ordinary solvents. However, it has been shown that there are some solution systems that have a strong power to dissolve cellulose. The viscose and cuprammonium are currently applied by most of the regenerated cellulose industries to dissolve cellulose due to its unique abilities, such as chemical derivatization and modification in cellulose dissolution. Unfortunately, these processes usually require a strong alkali condition which unavoidable cause environmentally hazardous problem. Consequently, there have been intensive search of various methods for cellulose dissolution to solve this problem.

The NaOH/urea aqueous solution system is eligible since the reaction between cellulose and urea gives a derivative (cellulose carbamate) which can be dissolved in dilute sodium hydroxide:

$$Cellulose - OH + NH_2 - CO - NH_2 \rightarrow Cellulose - O - NH_2 + NH_3$$

This solution system has a strong power to dissolve cellulose from plant and presents significant advantages including simple, inexpensive, non-pollution, and safe (Zhou *et al*., 2000). Urea is used as a substitute for the toxic CS_2 and the advantage of this process is that the cellulose carbamate is relatively stable at room temperature, which permits storage times for more than a year without loss of quality (Klemm *et al*., 2005). Therefore, this process can be carried out on a large scale in a central location, from which products can then be shipped to decentralized facilities for processing.

2.7 Membrane separation

Membrane separation processes have become one of the emerging technologies which undergo a rapid growth during the past few decades. It has drawn the world attention especially in the separation technology field, one of the chemical engineers' specialties with its distinguish performance compared to the conventional separation technology. Membrane separation is a technique that permits concentration and separation without the use of heat. A driving force such as a gradient in pressure, concentration, temperature, electrical field is applied and has to be maintained over the membrane. Permeate designates the liquid passing through the membrane, and retentate (concentrate) designates the fraction not passing through the membrane as shown in figure 2.11.

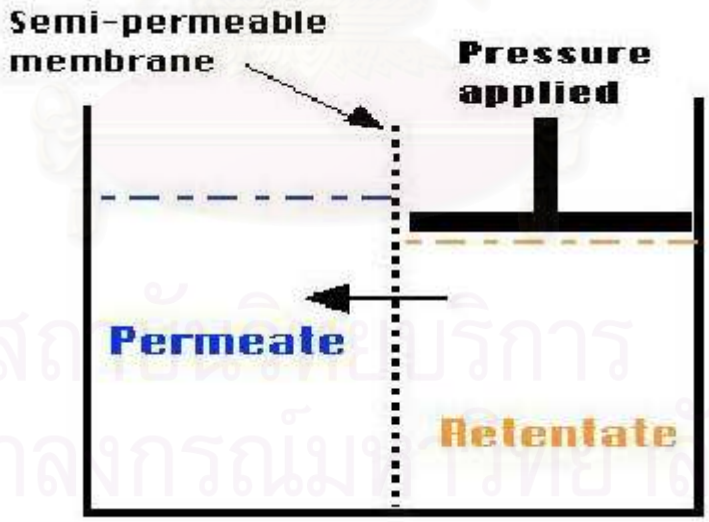


Figure 2.11 The simple membrane processing.

The membrane can be defined essentially as a barrier, which separates two phases and restricts transport of various chemicals in a selective manner. Transport through a membrane can be effect by convection or by diffusion of individual molecules, induced by the driving forces. Under the influence of the driving force components from the mixture held at a higher chemical potential, migrate through the membrane to the side of the lower chemical potential. Separation between different components is effected by the difference in transport rates. The gradient in the chemical potential has to be maintained by continuous removal of the migrating components from the side of the lower chemical potential. If this is not done, equilibrium would be reached and no separation future occurs. The membrane can be homogenous or heterogeneous, symmetric or asymmetric in structure, solid or liquid and can carry a positive or negative charge or be neutral or bipolar. The types of membrane are shown in figure 2.12.

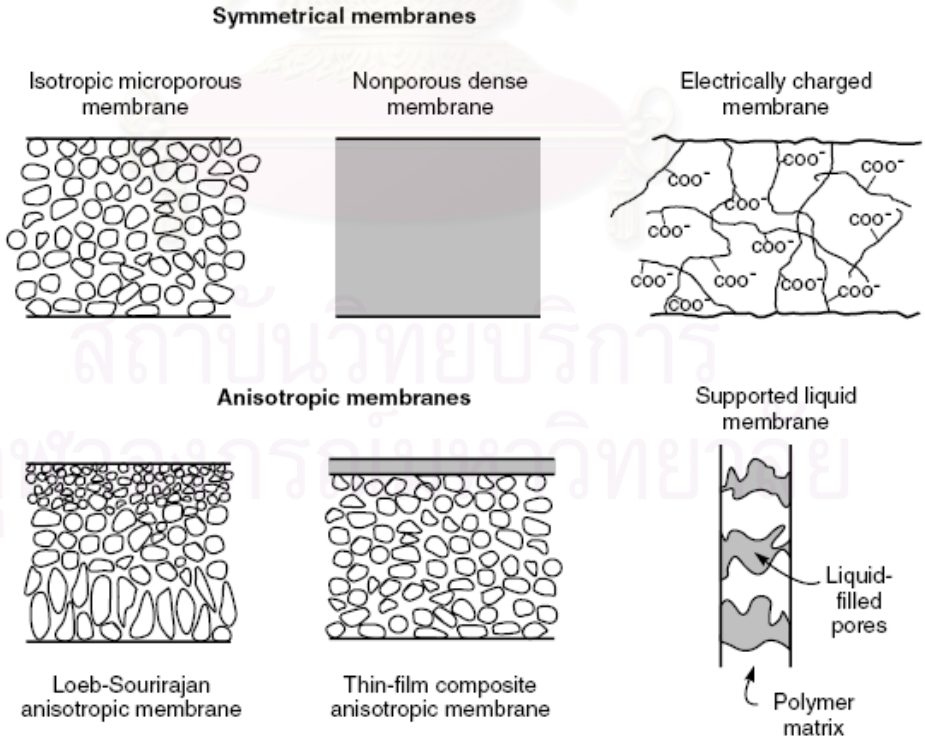


Figure 2.12 Schematic diagrams of the principal types of membranes (Baker, 2000).

2.8 Transport of substances across membrane

.

Transport through a membrane can be induced by driving force between two phases caused by the difference of concentration, pressure, temperature and electrical potential. It has been known that the differences in mass transfer rate of each substance cause separation. Ordinary separation presently used is based on the differences in concentration in each phase at equilibrium state. The velocity of mass transfer of a substance across a permeable membrane is shown in the term of " flux ". Flux is defined as the rate of mass transfer that flows through a unit area. Besides, the term that describes the diffusion of particles through membranes is denoted as "permeability". Permeability is defined as flux per unit driving force (Baker, 2000). The value of flux depends on driving force and motion of substances. The motion of substances depends on many factors such as the structure of membrane and substances, size of substances, interaction between substance and membrane (Baker, 2000).

The rate of mass transfer of a substance across a permeable membrane can be illustrated by Fick's law.

$$\text{Flux} = \text{Diffusivity} \times \text{driving force}$$

2.9 The advantages of membrane separation

1. This process could be operated at room temperature; therefore, it's proper to separate substances that could be destroyed by heat.
2. There are less waste as it can differentiate products exactly required. Generally, both permeate and retentate can be utilized.
3. Only (relatively) small amount of energy is required for this process.
4. This process can be easily scaled up from phototype to industrial scale.
5. Both batch and continuous processes can be used in this process. Also, this process can be easily automated.
6. The equipment does not take up space, and its size is relatively compact. Complex instrumentation is not required.
7. The basic concept is simple to understand and it requires no specific chemical knowledge.

2.10 The disadvantages of membrane separation.

Now membrane separations have been widely utilized due to the advantages described as the above. Unfortunately, this process also has limitation and disadvantages as follows:

1. Concentration polarization (CP) is the accumulation of excess particles in a thin layer adjacent to the membrane surface as shown in figure 2.13. This phenomena increases resistance to solvent flow and thus reduces the permeate flux. To minimize concentration polarization, it is necessary to maintain the cross flow velocity to reduce the film thickness and to scour deposits such as two pump system as shown in figure 2.14.

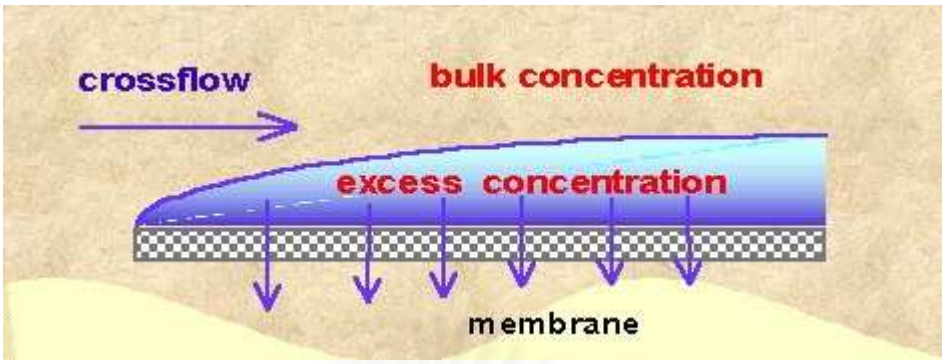


Figure 2.13 Concentration polarization phenomena.

2. Fouling is the process that results in a decrease in performance of a membrane, caused by the deposition of suspended or dissolved solids on the external membrane surface, on the membrane pores, or within the membrane pores. It causes a higher energy use, a higher cleaning frequency and a shorter life span of the membrane.
3. The limitation of the stability of membrane and its cost.

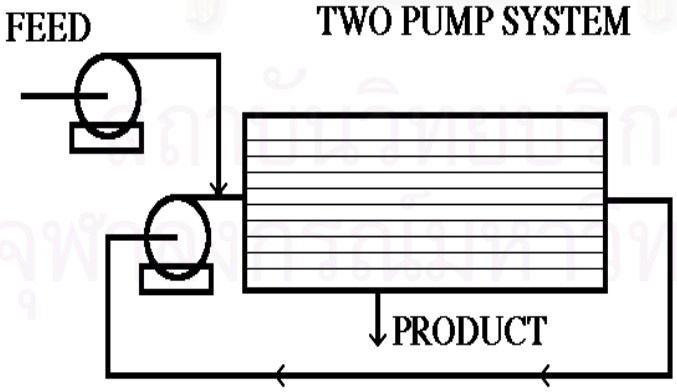


Figure 2.14 The systems for minimizing the concentration polarization.

2.10 Membrane process

The membrane technologies and membrane processes primarily based on species size are shown in table 2.2 and figure 2.15 respectively.

Category	Process	Status
Developed industrial membrane separation technologies.	Microfiltration. Ultrafiltration. Reverse osmosis. Electrodialysis.	Well-established unit operations. No major breakthroughs seem imminent.
Developed industrial membrane separation technologies.	Gas separation. Pervaporation.	A number of plants have been installed. Market size and number of applications served are expanding.
To-be-developed industrial membrane separation technologies.	Carrier facilitated transport. Membrane contactors Piezodialysis, etc.	Major problems remain to be solved before industrial systems will be installed on a large scale.
Medical applications of membranes.	Artificial kidneys. Artificial lungs. Controlled drug delivery.	Well-established processes. Still the focus of research to improve performance, for example, improving biocompatibility

Table 2.2 Membrane technology (Baker, 2000)

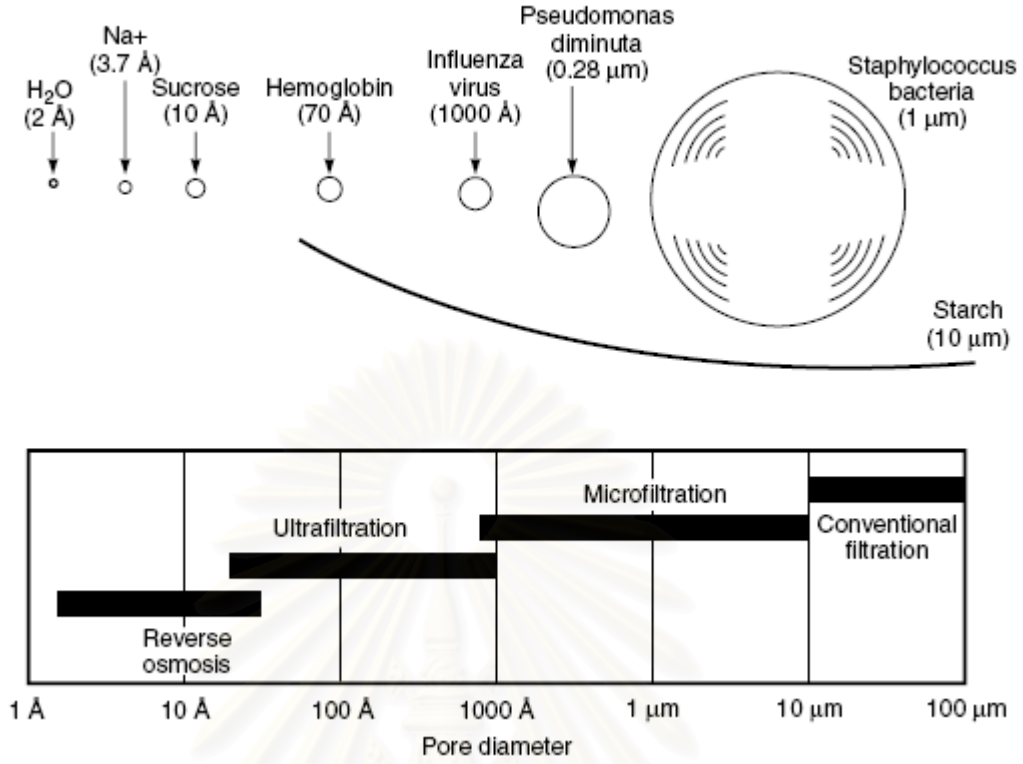


Figure 2.15 Membrane processes primarily based on species size (Baker, 2000).

CHAPTER III

LITERATURE REVIEW

Cellulose, an unbranched polymer of β-(1,4)-linked glucopyranose residues synthesized by plants or bacteria, is the most plentiful biopolymer existed on earth. The development of cellulose membrane from plant for membrane separation has been done for a long time, while bacterial cellulose almost aimed at medical purpose. Recently, bacterial cellulose membrane for membrane separation has become more attractive due to its outstanding characteristics.

This chapter reviews the history of bacterial cellulose and the development of cellulose membrane from plant.

3.1 Bacterial cellulose historical reviews

Over a century ago, Brown (1886) described a bacterium aceti, the major source of acetic acid, which can produce a tough gelatinous film of nearly pure cellulose. This solid membrane was soluable in amoniacal copper hydroxide and gave reducing sugars when hydrolysed with sulphuric acid. Since cotton also gave these reactions, this was the first report about the production of cellulose from microorganism. In the beginning, this bacterium was referred to several names for example *Acetobacterium xylinum* (Ludwig 1898) and *Bacterium xylinodes* (Henneberg 1906). It was later referred to as *Acetobacter xylinum* (Bergey *et al.* 1925), and this has become the official name according to the International Code of Nomenclature of Bacteria (1990 revision). Since that time, several groups have tried to elucidate cellulose formation in living systems by studying this organism.

Initial intensive studies on bacterial cellulose synthesis, using *Acetobacter xylinum* as a model bacterium, were started by Hestrin *et al*. They published several papers on the synthesis of bacterial cellulose. Their first publication was a short note (Aschner and Hestrin, 1946) describing microscopic examination of cellulose from bacteria. They were able to discern a web of thin, discrete fibrils by using a dark-field condenser. In a series of papers published in the 1950's, this group reported on the factors that affected the formation of bacterial cellulose (Schramm *et al.*, 1954)), the inhibitors and substrates of bacterial cellulose production (Schramm *et al.*, 1957), and the enzyme systems and intermediate products involved in bacterial cellulose production (Gromet, 1957, 1962).

Interest in *A. xylinum* was heightened when it was discovered as being the microorganism responsible for producing Nata by Philippines investigator (derived from latin word *natare* which means "to float") from fermenting coconut water (Africa, 1949 and Mendoza, 1953). Nata de Coco was a popular food or dessert produced in the Philippines. It was not until 1967 however, that the identity of the organism responsible for the formation of Nata de coco was established as *Acetobacter xylinum* and that Nata was indeed nearly pure cellulose (Dimaguila *et al*, 1967).

The cellulose pellicle produced by bacterium has been postulated to have many objectives. Since the cellulose matrix was less dense than water, it was first postulated that it provided a floating support with easy access to oxygen for the obligate aerobe nature of the organism (Hestrin and Schramm, 1954). Other possible objectives included protection for *A. xylinum* cells from the killing effects of UV light (Williams *et al.*, 1989), enemy and heavy metal ion. There were two steps of bacterial cellulose formation. Firstly, the polymerization of several β-1,4 glucans occurred at

an extrusion site. Parallel glucan chains then formed aggregates, minimizing entropy and facilitating the formation of cellulose I. The adjacent glucan aggregated, then crystallized into microfibrils, and finally these microfibrils aggregated into discontinuous bundles. The pellicle normally covered the entire surface of the medium that was exposed to air. Bacterial cellulose microfibril occurred in a dimensions ranging from 2 to 4 nm diameters (Yamanaka *et al.*, 1989) and the fibril branched out in three-way branching points as shown in figure 3.1.

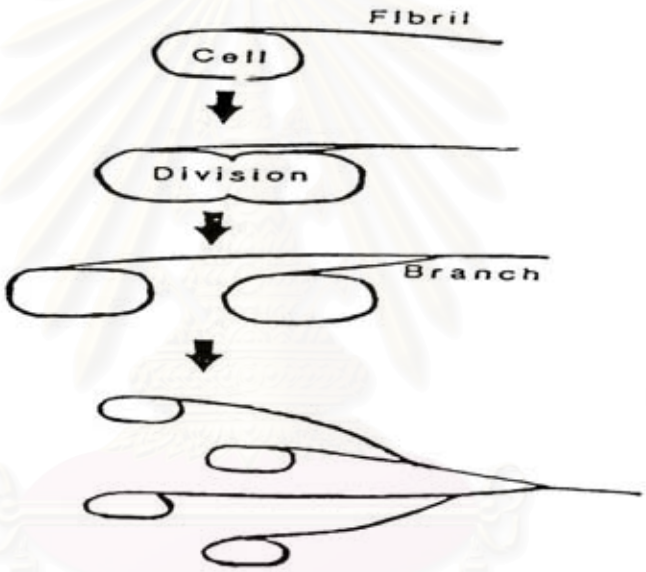


Figure 3.1 Three-way branching points mechanism of bacterial cellulose.

Using transmission electron microscopy (TEM), biosynthetic pathway involved in the production of cellulose from simple sugars had been investigated (Colvin *et al.*, 1977). This group observed both *A. xylinum* and *A. acetigenus* cells encased in a smooth or gently undulating envelope. They also observed a sheath up to 100 nm wide of an amorphous gel surrounding the fibrils, and postulated that chains of highly hydrated, intermediate polyglucans were released from the cell, and these

chains came together to form a cellulose fibril external to the cell but within the cell envelope. The authors also derived a diagram of the lipid-phosphate-carbohydrate intermediate pathway from substrate to cellulose that occurred in an *A xylinum* cell. Their pathway is shown in figure 3.2.

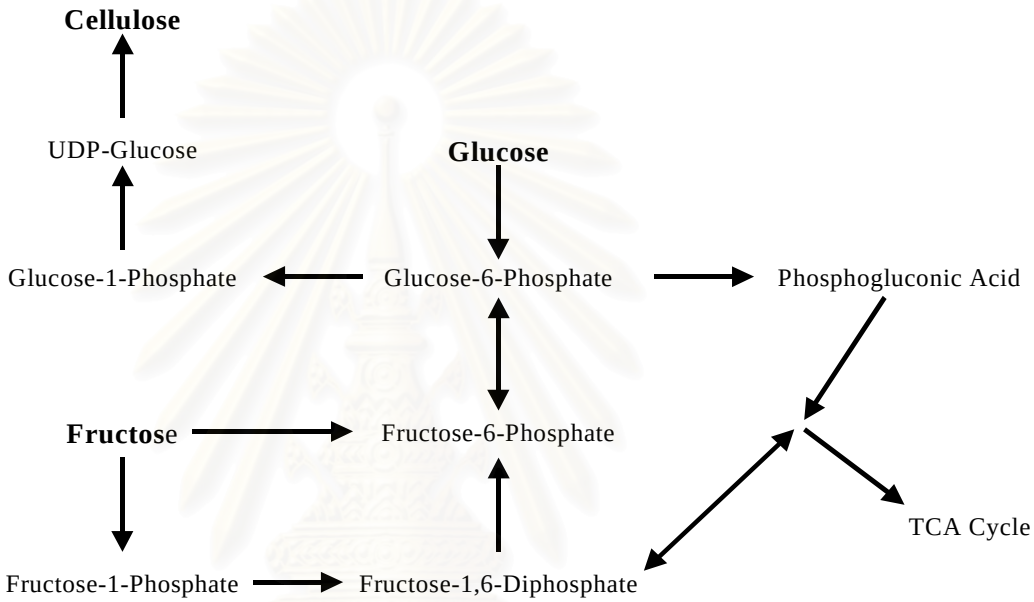


Figure 3.2 A simplified version of the pathway of glucose and fructose to cellulose in *Acetobacter xylinum* (Colvin *et al.,* 1977).

Tayama *et al.* (1985) studied the polysaccharide structure produced by *Acetobacter aceti subsp. Xylinum*. They postulated that polysaccharide (AM-2) was a branched polymer consisting of D-glucose, L-rhamnose, D-mannose, D-glucuronic acid and O-acetyl in the mole ratio 4:1:1:1:1 respectively. The main chain was ß-1,4-linked glycose and L L-rhamnosyl-(1→6)-β-D-glucosyl-(1→6)-D-glucosyl-(1→4)-D-glucuronosyl-(1→2)-D-mannose were the side chains as shown in figure 3.3.

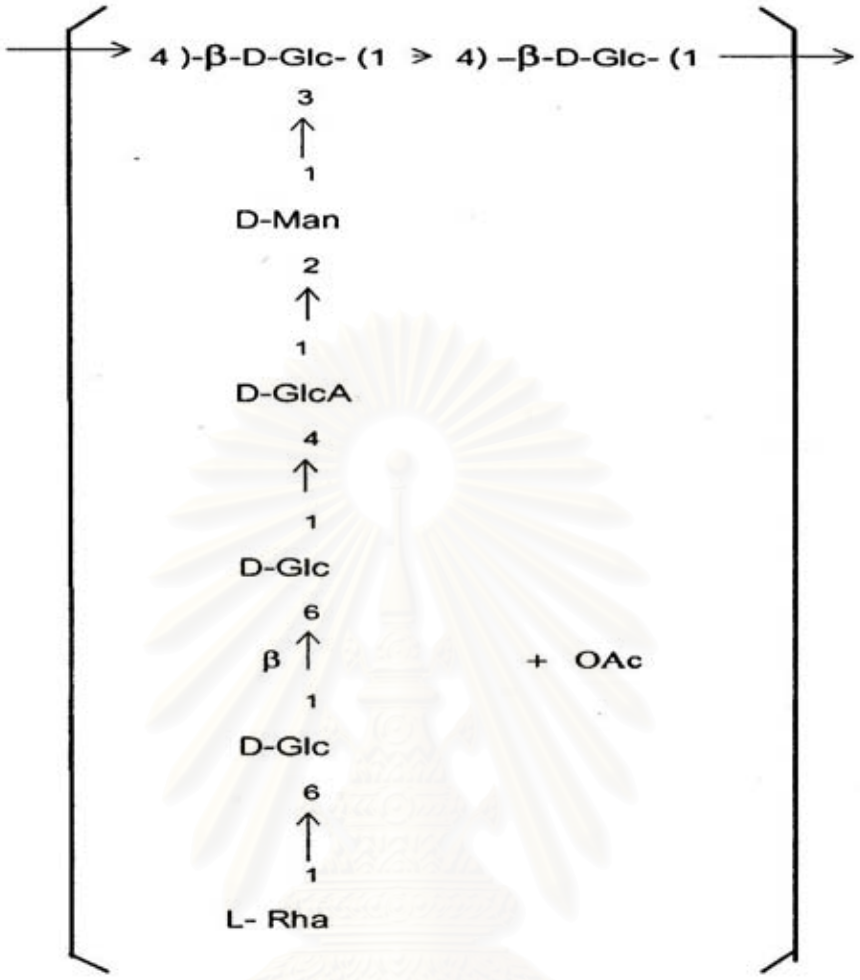


Figure 3.3 The structure of polysaccharide AM-2 (Tayama *et al*., 1985).

It has been investigated that the excellent mechanical properties of bacterial cellulose resulting from its extensive intermolecular hydrogen bonding network. Though identical in chemical composition, the mechanical properties and structure of bacterial cellulose differ from those of plant cellulose. Bacterial cellulose has high mechanical properties like tensile strength and modulus.

Yamanaka *et al.* (1989) reported that bacterial cellulose had mechanical properties that were superior to many synthetic fibers. Bacterial cellulose displayed a

Young's modulus value (a measure of shape retention) of 30 GPa, approximately 4 times greater than any organic fiber. Also, the measured tensile strength is about five times greater than polyvinyl chloride or polyethylene films (Japan Industrial Journal May 15, 1987). They compared the values of mechanical properties of bacterial cellulose with other non-oriented organic sheets as shown in Table 3.1

Type of material	Young's modulus(GPa)	Tensile strength(MPa)
Bacterial cellulose	15-35	200-300
Polypropylene	1-1.5	30-40
PE-teraphthalate	3-4	50-70
Cellophane	2-3	20-100

Table 3.1 Mechanical properties of bacterial cellulose and other organic materials (Yamanaka *et al.*, 1989).

Recently, a direct measurement by Guhados and co-workers (2005) revealed a Young's modulus of 78 ± 17 GPa for bacterial cellulose fibers with diameters ranging from 35 to 90 nm. With the use of atomic force microscope, elastic moduli of suspended fibers could be measured by performing a nanoscale three-point bending test, in which the center of the fiber was deflected by a known force. This technique was applied by modeling the deflection measured at several points along a suspended fiber to obtain more accurate data.

3.2 Development of regenerated cellulose membrane process

Regenerated cellulose membranes from plants have been widely utilized in membrane separation technique such as dialysis, ultrafiltration, reverse osmosis and purification of mixtures. Many extensive regenerated cellulose membrane productions have been studied, but they were mostly developed from plant cellulose. Cellulose could be extracted from its primitive resource and then was processed into its derivative *via* chemical methods.

In 1857 the Swiss chemist Matthias Eduard Schweizer discovered that cotton could be dissolved in cuprammonium solution (ammonial copper oxide solution) and then regenerated in a coagulating bath at room temperature (Schweizer, 1857). He previously observed that ammonia reacted with metal oxide to form a basic compound. The colour of the solution was due to the complex cupric tetramide hydroxide ($Cu(NH_3)_4(OH)_2$) . He was generally credited as the inventor of the solution although he did not apply for a patent for his invention. The fibre process was however invented by a French Chemist, Louis-Henri Despeissis who in 1890 worked on spinning fibres from Schweizer's solution. He extruded the cuprammonium solution of cellulose into water, with dilute sulphuric acid being used to neutralise the ammonia and precipitate the cellulose fibres. This process was still used, most notably by Asahi in Japan where sales of artificial silk and medical disposable still provided a worthwhile income (Wooding, 2001). However, the relative high costs associated with the need to use cotton cellulose and copper salts prevented cuprammonium process from reaching the large scale of many manufactures.

The English chemist Charles Frederick Cross and his collaborators Edward John Bevan and Clayton Beadle discovered the viscous process in 1981. They found that cotton or wood cellulose could be dissolved by treating alkaline cellulose with carbon disulfide (Cross *et al.*, 1983). This process was patented in 1982. This solution formed cellulose xanthate, which was soluable in a dilute sodium hydroxide solution. The resulting solution was very viscous, which was commonly referred to as viscous process. Now regenerated cellulose membrane prepared from the viscose process is still imperative although the process has been met with the increasing environmental problem.

Filho and de Assuncão (1993) prepared regenerated cellulose membranes from sugar-cane bagasse in cuprammonium process for application of hemodialysis. Purified sugar-cane bagasse was dissolved in ammonium copper (II) hydroxide (cuprammonium) and regenerated in the form of a membrane by successive reactions with 3 M NaOH and 2 M HCl. Purification was determined by iodometric analysis of the Cu(II) content in the produced membranes and by UV/Vis spectroscopy of cellulose and lignin solutions. Unfortunately, this process was not successful since it led to dangerously high residual levels of Cu(II).

Okajima *et al*.(1996) investigated regenerated cellulose membranes from its cuprammonium hydroxide solution by selecting the species of aqueous coagulants (H^+, Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+} with various counter ions), and prepared the cellulose membranes from two types of cellulose. The controlling of the morphology of the membranes from cellulose/cuprammonium hydroxide solution could be possible by simply selecting the cationic species of coagulants**.** The porous structure of the regenerated cellulose membranes had basically porous structure more or less, constituted by collision of secondary particles during coagulation.

However, the production of regenerated cellulose membranes including cellophane usually required a strong alkali condition and was associated with problem of discarding pollution. Moreover, an alternative method to prepare cellulose membranes from hydrolysis of asymmetric cellulose acetate membranes was also in strong alkali solution. Therefore, an attempt to develop a non-polluting process based on organic and aqueous solvents of cellulose was carried on. The practical solvents and methodology should be simpler than the conventional method, had a strong power to dissolve cellulose and environmentally hazardous materials and side products in general had to be avoided.

Isagai *et al.* (1998) studied on the dissolution of a number of cellulose from plant using NaOH as a solvent. It was shown that the optimum conditions involved swelling cellulose in 8-9 % by weight NaOH and then freezing it into a solid mass by holding it at -20 °C. This was followed by thawing the frozen mass. All plant-derived cellulose samples were successfully dissolved in the NaOH solution by this procedure.

Zhou *et al*. (2000) studied on the dissolution of cellulose from cotton using a novel solvent, namely, NaOH/urea aqueous solution. The dissolution using NaOH/urea aqueous solution as solvent of cellulose including cotton linter, bagasse, alkali-soluble cellulose and Bemliese was studied by solubility analysis, viscometry and light scattering. It was found out that the addition of 2-4 wt% urea significantly improved the solubility of cellulose in 6-8 wt% NaOH aqueous solutions. These solvents had a strong power to dissolve cotton linters and other natural cellulose with many significant advantages including non-pollution, simple and safe dissolution procedure.

Moreover, the regenerated cellulose membranes blended with other natural polymers for the application in the separation field have been also prepared from the solvents, indicating a good miscibility with alginate, chitin and konjac glucomannan.

Yang *et al.* (2000) prepared blend membranes from cellulose (cotton linter) and alginate for development a new blend membrane for the pervaporation application. The procedure was by coagulating the mixture of 8 wt.% cellulose cuoxam and 8 wt.% aqueous sodium alginate solution and then cross-linked by using Ca^{2+} bridge in 5 wt.% $CaCl_2$ aqueous solution. It was found out that the cellulose/alginate (8:2, by weight) blend membrane cross-linked with Ca^{2+}, showed the higher pervaporation separation factor for 90% alcohol feed mixture at 60°C and the higher permeation flux at lower temperature than that of uncross-linked membrane. Its tensile strength was 12 times higher than that of alginate membrane.

Zhou *et al*. (2001) prepared blend membranes from cellulose (cotton linter) and alginate in NaOH/Urea aqueous solution. Regenerated cellulose blend membranes were satisfactorily prepared in 6 wt% NaOH/4 wt% urea aqueous solution by coagulating with 5 wt% $CaCl_2$ aqueous solution, and then treated with 3 wt% HCl. The crystalline state of the blend membrane prepared from this solution was broken completely, and the crystallinity of the blend membranes decreased with an increase of alginate ratio.

Yang *et al*. (2002) prepared blend membranes from cellulose (cotton linter) and konjac glucomannan (KGM) in NaOH/thiourea aqueous solution by coagulating with 5 wt% $CaCl_2$ aqueous solution. It was found out that the new crystalline plane formed due to the resemble structure and interaction between cellulose and KGM. With an increase of the KGM content, pore size and water permeability of the blend

membranes rapidly increased, then reached a plateau of about 50 nm at KGM weight ≥30%. The tensile strength and breaking elongation in dry state for the blend membranes slightly decreased, and then significantly decreased at KGM weight ≥50%. The content of through pores of the cellulose membrane blended with KGM increased, and the pore size distribution became wider, compared with pure cellulose membrane. KGM played an important role in the formation of through pores for the microporous membrane leading to higher water permeability.

CHAPTER IV

EXPERIMENTAL

The experimental systems and procedures used in this research are divided into three parts:

1. Materials and equipments.
2. Preparation of membranes.
3. Characterization of membranes.

4.1. Materials and equipments

4.1.1 Bacterial cellulose

The bacterial cellulose (BC) was kindly provided by the laboratory of Pramote Thamarat, the Institute of Research and Development of Food Product, Kasetsart University as sheets of Nata de Coco. Nata de Coco is the gel-like cellulose pellicle formed on the surface of the media of *Acetobacter xylinus* cultures by the fermentation of coconut water. The sheets of bacterial cellulose were shredded and were then dipped for 36 hours in 10 % wt solution of NaOH for deproteination and were then rinsed several times with distilled water, until a neutral pH was attained in the drained liquid. The specimens were dried for a week under vacuum at 25 °C prior to use. Unless otherwise noted, all of the chemical reagents used in this work were of analytical grade, and were purchased from commercial sources in Thailand.

4.1.2 Chemicals

The details of chemicals used in this experiment are shown in Table 4.1

Table 4.1 The chemicals used in this experiment

Chemical	Grade	Supplier
Calcium chloride ($CaCl_2$)	Analytical	Italmar Co., Ltd. France
Hydrochloric acid (HCl)	Analytical	Merck, Germany
Sodium alginate (Na-alginate)	Analytical	Carlo Erba, Italy
Sodium hydroxide (NaOH)	Analytical	Carlo Erba, Italy
Urea (NH_2CONH_2)	Analytical	Carlo Erba, Italy

4.1.3 Equipments

1. Blender (Philips Cucina).
2. Centrifuge (Kubota 5100, Japan).
3. Vaccuum oven.
4. CHN-Elemental analyzer (LECO CHN-2000 Analyzer, USA).
5. X-ray Fluorescence (XRF) Spectrometers (OXFORD ED 2000, UK).
6. Scanning electron microscopy, SEM (JOEL JSM-5410LV, Japan).
7. Chemisorption analyzer (Micrometrics ASAP2020, USA).
8. Fourier Transform Infrared (FT-IR) spectrometer (Nicolet SX-170, USA)
9. Universal testing machine (LLOYD 2000R, UK).
10. Water vapor permeation tester (Lyssy L80-4000, Switzerland).

4.2 Preparation of membranes

4.2.1 Dissolution of bacterial cellulose in NaOH

For solubility experiments, an amount of 3 g of bacterial cellulose was scissored into small pieces, then suspended in 97 g NaOH aqueous solution at various concentrations namely 1,2,3,4,5,6,7,8,9 and 10 % (w/v) concentration. These suspensions were stirred for 10 mins to obtain slurry at room temperature, and then cooled to -5 °C in a refrigerator and held at -5 °C until it became a solid frozen mass overnight. The frozen solid was then allowed to thaw and stirred extensively at room temperature to obtain the bacterial cellulose solutions. To isolate the insoluble parts, the whole solutions were isolated by centrifuging at 6000 rpm at 10 °C for 30 minute. The insoluble parts were neautralized with 1% NaOH, 0.5% NaOH, dilute acetic acid and distilled water, respectively, then dried in vacuum oven at 35 °C for 24 hours and weighed. Solubility (S_a) of bacterial cellulose was calculated as follow:

$$S_a = \frac{w_0 - w_1}{w_0} x100\%$$

Where w_1 and w_0 denoted the weight of insoluble and initial weight of bacterial cellulose, respectively.

The solubility experiments were repeated three times, and the average value was considered as S_a.

4.2.2 Dissolution of bacterial cellulose in NaOH/urea

To search for the optimum combination of urea in NaOH solutions, the solubility of bacterial cellulose in the presence of various urea concentrations of 1,2,3,4,5 and 6 wt% in the prepared NaOH solution were examined. The procedure for the dissolution of bacterial cellulose in NaOH/urea was performed following the previous description (4.2.1). The solvents were prepared immediately prior to use to minimize moisture uptake.

4.2.3 Preparation of BC–Alginate blend membranes

The optimum composition of NaOH and urea solution obtained from the previous study (4.2.1 - 4.2.2) was used for the membrane preparation. An amount of 3 g of bacterial cellulose was dissolved in the NaOH/urea aqueous solution, followed the previous description. Sodium alginate (SA) at 3% (w/v) was dissolved in distilled water at room temperature to obtained gel-like solution. The bacterial cellulose solution was mixed with SA solution to produce mixtures having weight ratio of 100/0, 80/20, 60/40, 40/60, 20/80, 0/100, respectively. The mixtures were stirred energetically at room temperature for 24 hours to form clear solutions. The casting solutions were spread over a teflon plate. The thickness of spread casting solution was controlled by manually adjusting the height of the casting blade. Thickness of the cast membranes was measured by micrometer (Mitutoyo, Tokyo, Japan) at various parts of a particular membrane. The water content in the solutions was allowed to evaporate at room temperature for 1 day before coagulating in a 5 wt% $CaCl_2$ aqueous solution for

30 min, followed by neutralization by 1 M HCl aqeous solution for 10 min. After that, the fabricated membrane was washed with distilled water until a neutral pH was attained in the drained liquid and air dried at room temperature. The membranes coded weight ratios of bacterial cellulose and alginate are listed in Table 4.2.

Membrane number	BC:SA(w/w)	Coagulant
RBC	100:0	$CaCl_2$/HCl
RBC-A1	80:20	$CaCl_2$/HCl
RBC-A2	60:40	$CaCl_2$/HCl
RBC-A3	40:60	$CaCl_2$/HCl
RBC-A4	20:80	$CaCl_2$/HCl
SA	0:100	$CaCl_2$/HCl

Table 4.2 The blend ratios and coagulants of membranes.

4.2.4 Preparation of porous membrane

In this study, supercritical drying method was applied for the preparation of porous membranes. Firstly, membranes of each composition were dipped in distilled water for 24 hours. After that, to replace water with ethanol, the swollen membranes were immersed in 10, 30, 50, 70 % (w/v) ethanol for 30 mins in each step and in 100 % (w/v) ethanol for 1 h, respectively. Lastly, the swollen membranes were dried by using supercritical drying method

In supercritical drying method, the membranes were placed in a vessel inside the high-pressure cell with inner diameter 10 cm. The cell was immediately filled with supercritical CO_2 and controlled at temperature = 40 °C and pressure = 1200 Psi (the critical point of carbon dioxide; P_c = 1072 Psi and T_c = 31 °C). Temperature and pressure were selected such that the CO_2 and ethanol inside the membrane were fully miscible. Subsequently, the cell was flushed by adding fresh CO_2 at the same conditions of pressure and temperature in order to replace the residual ethanol inside. The addition was performed for 2 hours and then the system was slowly depressurized at a constant rate of 150 psi/min to remove CO_2.

For comparison purpose, freeze-drying method was also applied with the samples of the fabricated BC membrane and the selected BC/alginate blend membrane. In the freeze-drying process, the swollen membranes were dried by a sublimation of the water component in an iced membrane. The membranes were freeze-dried in a ZIRBUS sublimator 400 (ZIRBUS Technology, Bad Grund, Germany). The shelf temperature was lowered to −45 °C, and freezing was completed within 2 hours. After that, the shelf temperature was increased to -10 °C to complete drying.

4.3 Characterization of membranes

4.3.1 Elemental analysis of membrane

The membranes were cut into particle-like size and vaccum-dried for 24 h before the measurement of elemental contents analysis. The content of nitrogen in the

membranes was determined by elemental analyzer (LECO CHN-2000 Analyzer). The contents of calcium and sodium in the dried membranes were determined with an X-ray Fluorescence (XRF) Spectrometer (OXFORD ED 2000).

4.3.2 Scanning electron microscope (SEM)

Scanning electron micrographs were taken with JOEL JSM-5410LV microscope at Scientific and technological research equipment centre, Chulalongkorn University. The free surface were coated with gold, subsequently their surfaces were observed and photographed. The coated specimens were kept in dry place before experiment. SEM was obtained at 15 kV which is considered to be a suitable condition since too high energy can be burn the samples.

4.3.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is used primarily to identify the chemical structure of the membrane. FTIR spectra of the membranes were recorded with a Nicolet FT-IR Spectrometer (SX-170) at Scientific and technological research equipment centre, Chulalongkorn University.

4.3.4 Equilibrium water content (EWC)

Equilibrium water content (EWC) or percent water absorption was determined by immersing the preweighted of dried membrane in distilled water at room

temperature until equilibration. The membrane was then removed from the water. After excess water at the surface of the membrane was blotted out with Kimwipes paper, the weight of the swollen membrane was measured and the procedure was repeated until there was no further weight change. Water content was determined by gravimetric method (Kim *et al*., 1996) and calculated using the following formula:

$$EWC(\%) = \frac{W_h - W_d}{W_d} x100$$

Where W_h and W_d denoted the weight of hydrate and dry membrane, respectively.

4.3.5 Tensile properties testing

Tensile strength of the membranes were measured by a LLOYD 2000R" universal testing machine (at Polymer Engineering Laboratory, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University). The test conditions follow ASTM D882. The determination of tensile property was done under the following conditions: sample width, 10 mm; length, 10 cm (50 mm between the grips), stretch rate, 2 mm/min. At least five specimens were used for each blend composition.

4.3.6 BET Surface analysis

Pore size and surface area of the membranes were measured by a Brunauer-Emmett-Teller (BET) surface area analyzer (Model ASAP 2020) at Department of Chemical Technology, Faculty of Science, Chulalongkorn University. The samples

were placed in the sample cell, which was then heated up to 100 °C and held at this temperature for 2 hours. The sample was cooled down to room temperature and ready to measure the surface area. There were three steps to measure the surface area: adsorption step, desorption step and calibration step.

4.3.7 The water vapor permeability measurement

Water vapor transmission rate (WVTR) of the bacterial cellulose membrane and the optimum bacterial cellulose/alginate blend membrane with area of 50.00 cm^2, were measured on water vapor permeation tester; Lyssy L80-4000 (at Thailand institute of scientific and technological research). The test conditions followed ISO 15106-1. The determination of WVTR was done under the following conditions: temperature, 38 °C; % Relative Humidity, 90%. The principle of this electronic tester was similar to that of conventional method. One side of the membrane was exposed to the water vapor. As water solubilized into the membrane and permeates through the sample material, nitrogen swept the opposite side of the film and transported the transmitted water vapor molecules to the calibrated infrared sensor. The response was reported as a transmission rate.

CHAPTER V

RESULTS AND DISSUSIONS

5.1 Bacterial cellulose dissolution

It has been widely known that cellulose does not melt, but it will undergo thermal degradation at high temperature (Nishino *et al*., 2004). Cellulose must therefore be converted into a liquid form by either direct solution or by derivitization and subsequence dissolution of the derivative. The strong inter-chain and intra-chain hydrogen bonding involved in the crystalline regions make reactions and dissolution of cellulose difficult. There are a number of liquefaction methods available, however, these process lead to problematic environmental loads (Klemm *et al*., 2005).

5.1.1 Freeze thaw process

When bacterial cellulose was placed in a solution of NaOH/urea solution and allowed to stir at room temperature for an extended period, it was transformed into a highly swollen gel. However, there was no identification of complete solution. After freezing the cellulose solution completely and then allowing it to thaw, clear solution was obtained as shown in figure 5.1.

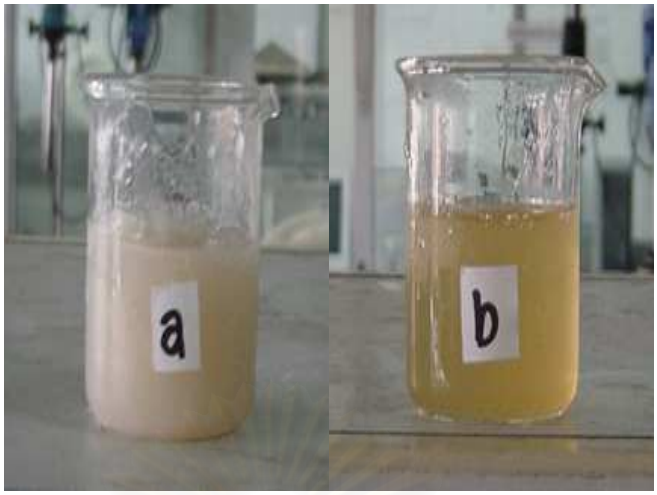


Figure 5.1 Bacterial cellulose in solvent: (a) before and (b) after freeze thaw process.

Isogai and Atalla (1998) investigated the condition for dissolving microcrystalline cellulose in NaOH solution. They concluded that the key points in dissolving microcrystalline cellulose procedure were the complete freezing of the mixture into a tight solid mass and then thawing. This was because the freeze thaw process disrupted hydrogen bonding between and within cellulose chains (Cuculo *et al.*, 1994). Using NH_3 –structure solvent, successive cycling between -40 °C and 30 °C transformed cellulose from the crystal I formation to II then III then amorphous. Once the cellulose was amorphous, dissolution in the solvent system occurred. Therefore, the freeze thaw process increased interaction between solvent and cellulose (Frey *et al*., 2005).

5.1.2 Influence of NaOH concentration

The cellulose solvents could solvate cellulose by disrupting hydrogen bond and thus dissolving the adduct form (Klemm *et al.*, 2005). In the present work, bacterial cellulose solutions were prepared by using solvent solutions with at various NaOH concentrations from 1-10 % (w/v)

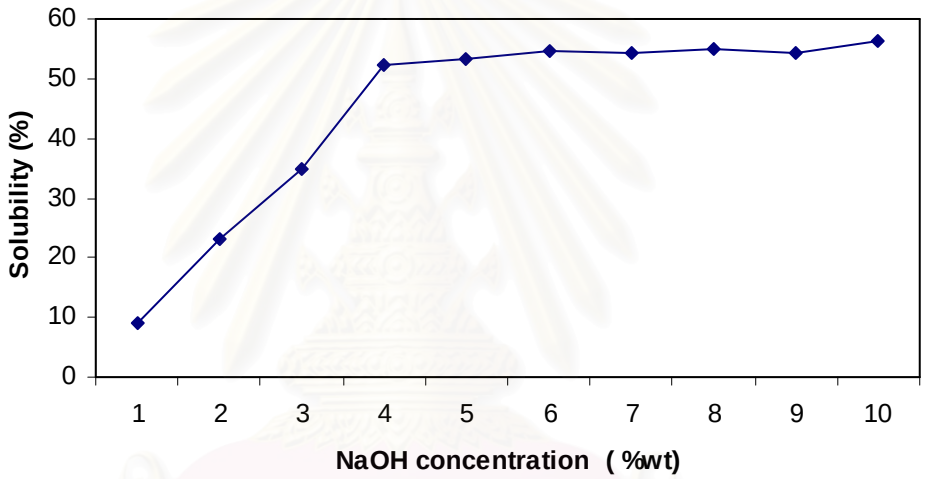


Figure 5.2 Effects of NaOH concentration on the solubility of 3% wt bacterial cellulose.

Figure 5.2 shows the results of the solubility of bacterial cellulose in the presence of different NaOH aqueous solution concentrations. The amount of bacterial cellulose was fixed at 3% by weight. As NaOH concentration increased, the solubility of bacterial cellulose increased. The solubility linearly increased from 9.12% to 52.13 % when the NaOH concentration increased from 1% to 4% (w/v). However, the solubility slowly increased from 52.13% to 56.43%, when the NaOH concentration was increased from 4% to 10% (w/v). Therefore, increasing NaOH concentration

more than 4% w/v had slightly effect on the solubility. The results revealed that the optimum NaOH aqueous solution concentration was 4 % wt.

Zhou *et al.* (2001) studied the dissolution of cellulose from plant and found that 6% w/v of NaOH concentration was the optimal concentration for dissolving plant cellulose. The optimum conditions involved swelling cellulose in NaOH and then followed by freeze thaw process. The freeze thaw process resulted in partial cleavage of intra-molecular hydrogen bonds between the hydroxyl groups on C3 of pyranose rings and the ring oxygen atoms of adjacents anhydroglucose units. This change might be the key factor in increasing the solubility of cellulose in the aqueous NaOH.

5.1.2 Influence of urea concentration

Addition of urea could improve BC solubility in NaOH solution. Figure 5.3 shows the results of the solubility of bacterial cellulose in the presence of various urea concentrations in 4% by weight NaOH solution. The amount of bacterial cellulose was fixed at 3% by weight. The solubility of BC dramatically increased from 77.67 to 92.33 % when the urea aqueous solution concentration increased from 1% to 3% (w/v). Nevertheless, the solubility only gradually increased from 92.33% to 93.21% at the urea concentration varies from 3% wt to 8% (w/v). Addition of urea 3% w/v in 4% wt NaOH solution significantly enhanced the solubility of 3% wt BC from 52.13% to 92.33%. The results revealed that the optimum concentrations of NaOH and urea in the aqueous solution for dissolution of 3% wt BC were 4 and 3% (w/v), respectively.

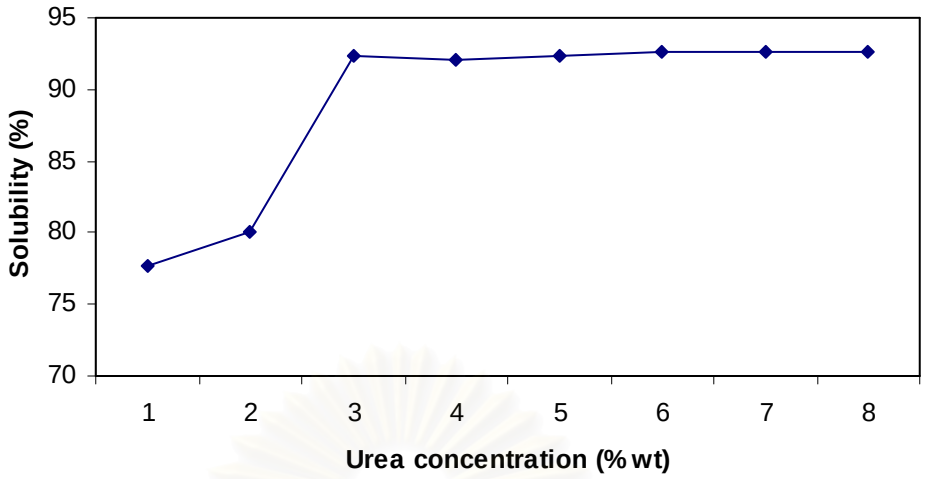


Figure 5.3 Effects of urea concentration on the solubility of 3% wt bacterial cellulose in 4% wt NaOH.

The presence of urea significantly enhanced the solubility of plant cellulose in NaOH aqueous solution (Chen *et al.*, 2006). Zhou *et al.* (2000) studied on the dissolution of plant cellulose and found that the addition of 2-4 wt% urea significantly improved the solubility of cellulose in 6-8 wt% NaOH aqueous solutions. Plant celluloses were completely dissolved in 6 wt% NaOH/4 wt% urea aqueous solution. In addition, it had been investigated that NaOH/urea aqueous solutions as non-derivatizing solvents broke the intra- and inter-molecular hydrogen bonding of cellulose and prevented the approach toward each other of the cellulose molecules, leading to the good dispersion of cellulose to form an actual solution. (Cai and Zhang, 2005)

5.1.3 Influence of bacterial cellulose concentration

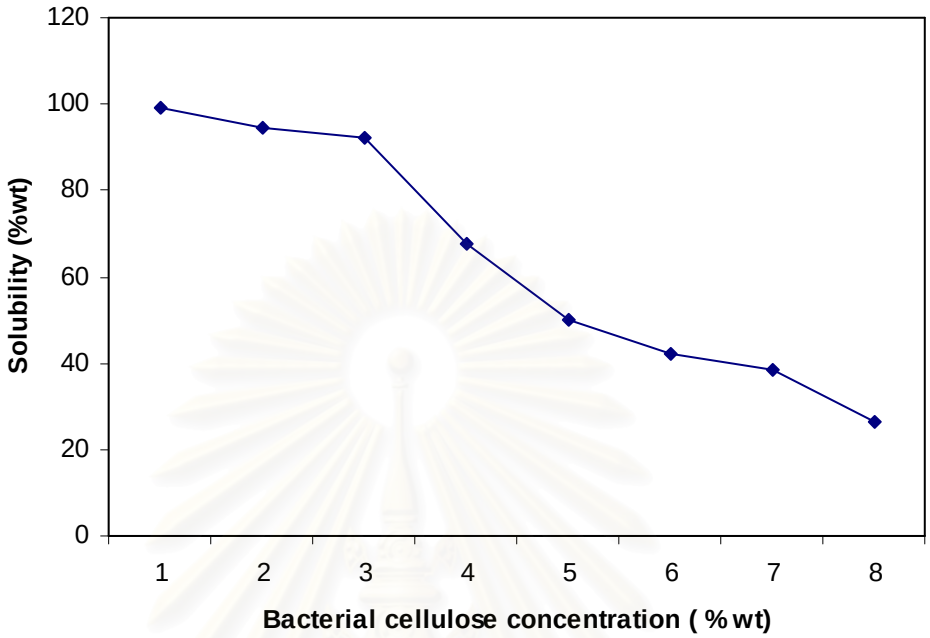


Figure 5.4 Effects of cellulose concentration on the solubility of bacterial cellulose in 4 wt% NaOH / 3 wt% urea aqueous solution.

Figure 5.4 showed the solubility of BC in 4% wt NaOH/ 3% urea aqueous solution. The solubility of BC gradually decreased relatively from 99.12% to 92.33%, when the BC concentration was increased from 1 % to 3 % (w/v). The solubility of BC dramatically decreased from 92.33% to 26.34% when the BC concentration was increased from 3 % wt to 8 % (w/v). Due to the poor solubility at high concentration of BC, to obtain solubility more than 90%, the BC concentration in the solution should not be greater than 3% w/v. The poor solubility of hydrogel cellulose in NaOH was previously investigated (Gavillon and Budtova, 2005). Generally, the more concentration of polymer in gel form increased, the smaller the free volume where solvent could move was available. According to the hydrogel formation of BC, the solubility of high BC concentration was limited. In the membrane fabrication,

cellulose concentration should be high enough to form membranes with integral structures (Kuo and Hong, 2005). Therefore, 3 % by weight of bacterial cellulose concentration was used as the casting solution in this study.

5.2 Membrane formation

The standard immersion precipitation (Zhou *et al.*, 2002), in which the cellulose solution was cast on a glass plate and immersed into an HCl bath with a dilute acid concentration, was exploited to develop membranes. In the present work, it was found that with the standard procedure no membrane but granular cellulose particles were obtained. During the cellulose regeneration, the flakes and granular particles instead of intact thin membrane were obtained. Therefore, this method was incapable of fabricating membrane in this study. The similar formation was previously reported in the casting of the solution with low cellulose concentration (Kuo and Hong, 2005).

To solve this problem, the cast process was developed by increasing the cellulose concentration. The water content of the casting cellulose solution was spontaneously evaporated for 24 hours until a thin dense layer was obtained. The casting solution was spread over a teflon plate, coagulating in a 5 wt% $CaCl_2$ solution for 30 min and regeneration by 1 M HCl solution for 10 min, respectively. After that, the fabricated gel membrane was washed with distilled water until a neutral pH was attained. Supercritical CO_2 drying method was then applied for porous preparation of the membrane.

5.3 Membrane compositions

Elemental analysis revealed the absence of nitrogen in the membrane. Therefore, urea was completely removed from the membrane during the coagulation and washing. The X-Ray Fluorescence Spectroscopy (XRF) results indicated that calcium (Ca) in the all membranes was not exceeds 0.01% and no sodium (Na) peak. Therefore, NaCl and NaOH were also totally removed from the developed membrane. The membrane compositions analyzed by XRF are summarized in Table 5.1.

The result was similar to that of regenerated cellulose membrane from cotton in NaOH/urea aqueous solution prepared by Zhou *et al.* (2002). This co-worker found that N element content in the membranes from cotton did not exceed 0.1% and the calcium and sodium in the membrane were nearly zero. Nakamura *et al.* (1991) suggested that a small amount of alginate-calcium complex as calcium bridges existed in the alginate membrane as shown in figure 5.5. The calcium could not be substituted by sodium ion.

Membrane	Elemental content (%) by XRF	
	Na	Ca
RCA	-	623 ppm
RCA1	-	0.003
RCA2	-	0.005
RCA3	-	0.017
RCA4	-	0.011
SA	-	0.019

Table 5.1 The membrane elemental content analyzed by X-Ray Fluorescence Spectroscopy (XRF).

The NaOH/urea aqueous solution was simpler than viscous process and there was no hazardous material side product. Industrial tests had shown that cellulose dissolved in NaOH/urea aqueous solution could be processed without any problems on viscous spinning machines (Klemm *et al*., 2005).

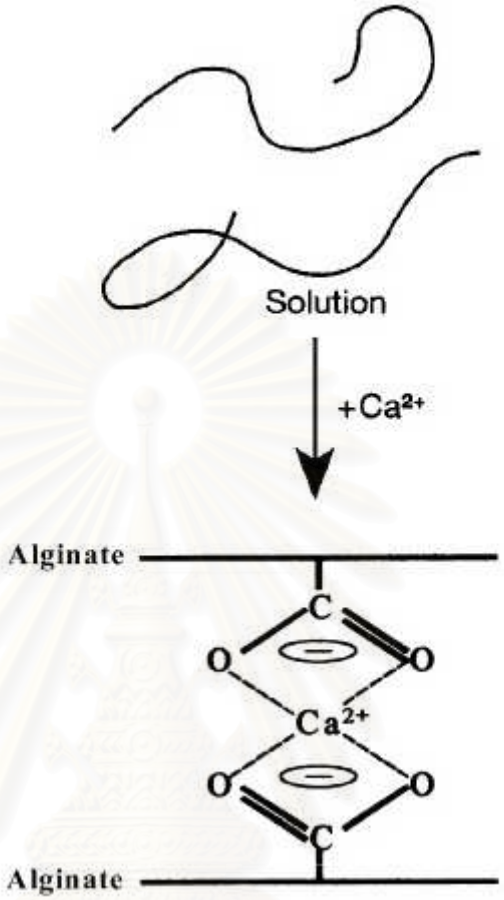


Figure 5.5 Alginate gel network formation by crosslinking with Ca^{2+} ion.

5.4 FTIR analysis of the blend membrane

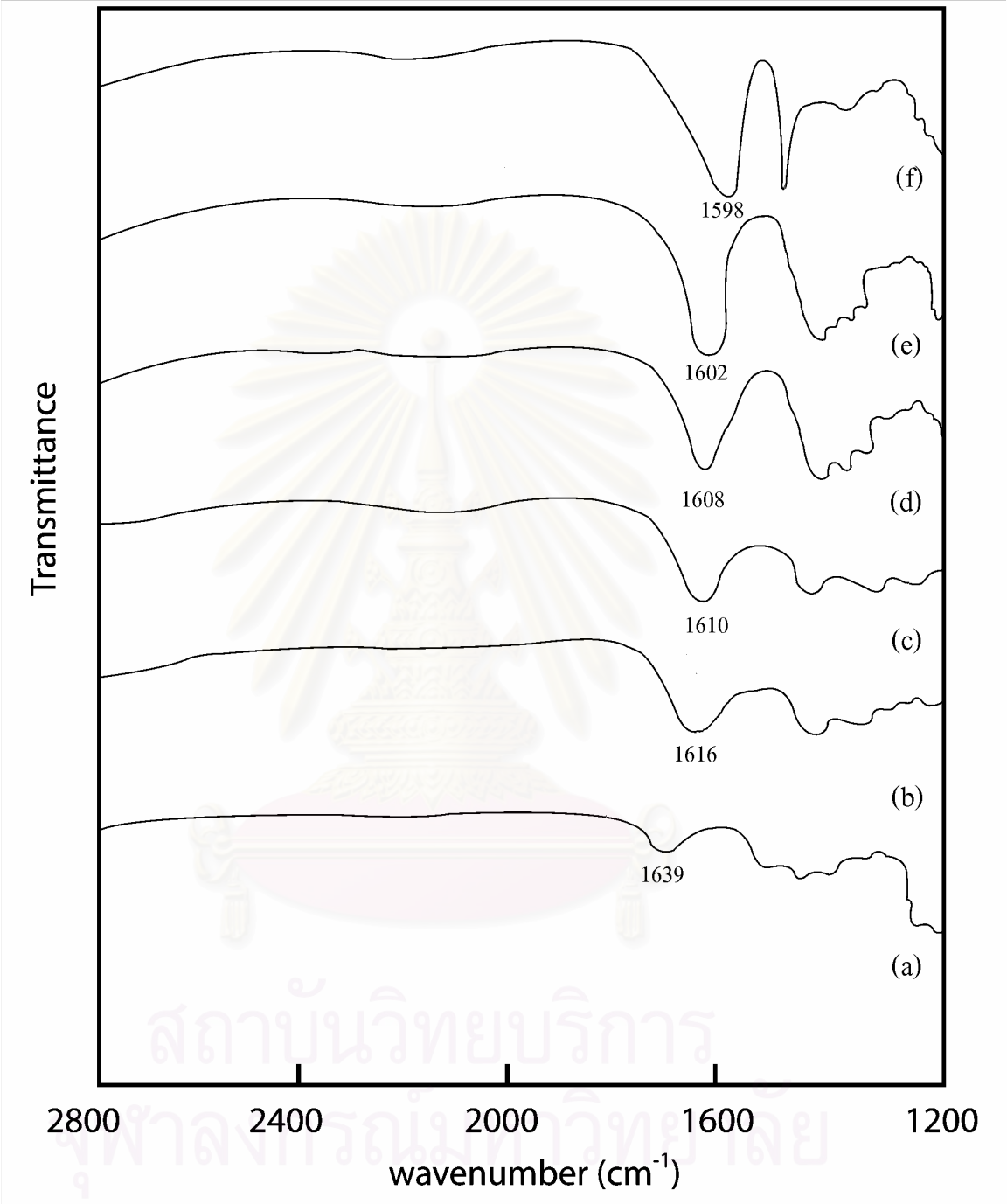


Figure 5.6 The FTIR spectra of the bacterial cellulose blend membranes: (a) BC; (b) BC/SA 80/20;(c) BC/SA 60/40;(d) BC/SA 40/60; (e) BC/SA 20/80; (f) Alginate (SA).

The nature of mixing between the two polymers was of interest to the study. FTIR spectroscopy was one of the most powerful techniques for the investigation of multi-component systems. This analysis provided information on the blend composition as well as on the polymer-polymer interaction (Lee *et al*., 1994) and has often been used as a useful tool in determining specific functional groups or chemical bonds that existed in a material. The presence of a peak at a specific wave number would indicate the presence of a specific chemical bond. Figure 5.6 showed the FTIR spectra of the bacterial cellulose, alginate and the blend membranes measured at wave number ranging from 2800-1200 cm^{-1}. The bacterial cellulose membrane showed a band at 1639 cm^{-1} as in figure 5.6(a), which was attributed to glucose carbonyl of cellulose. The strong band of the alginate membrane at 1598 cm^{-1} was assigned to the carboxyl group as shown in figure 5.6(f). Interestingly, the carboxyl group bands for blend membranes [figure 5.6 (b-e)] were shifted from 1598 cm^{-1} to 1602, 1608, 1610 and 1616 cm^{-1} , respectively, when compared to that of alginate membrane. The result might imply that there were the specified intermolecular hydrogen bonds between hydroxyl group of cellulose and carboxyl group of alginate forms. The similar observations previously described for preparing cotton cellulose/alginate blend membranes for the pervaporation application by Yang *et al*. (2000). The FTIR spectra of bacterial cellulose/alginate blend membranes [figure 5.6 (b-e)] were characterized by the presence of absorption bands of the pure components, whose intensities were roughly related to the blending ratio.

5.5 Equilibrium Water Content

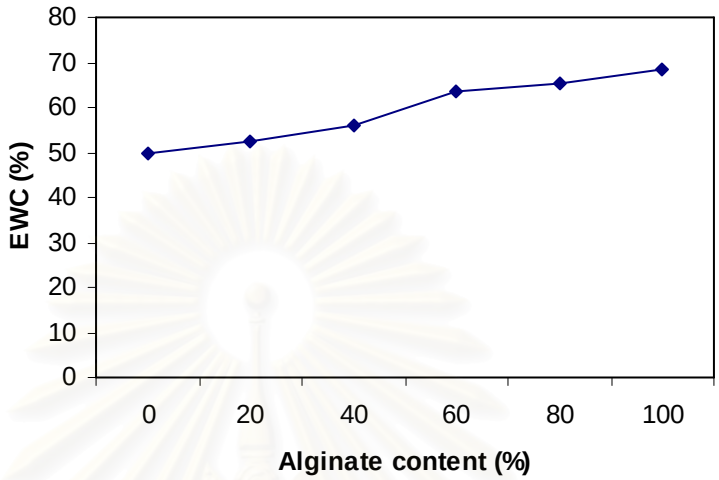


Figure 5.7 The Equilibrium Water Content (EWC) of the bacterial cellulose/alginate blend membranes as a function of alginate content.

Figure 5.7 illustrated the equilibrium water content (EWC) of the membrane as a function of alginate content. The EWC of bacterial cellulose membrane was approximately 50 % whereas the EWC of alginate membrane was around 70%. It was found that the EWC of the blend membranes increased as alginate content. The EWC of the blend membranes were in the range of 50 % to 70%. Khor *et al.* (1997) suggested that the ability to adsorb water of polysaccharide films depended on the method to prepare the films. The hydrogen bonds in cellulose could form very tightly packed crystallites. These crystals were sometimes so tight that water could not penetrate them. Therefore, the reducing of hydrogen bonding of cellulose chains in the membrane could result in increasing of water absorption ability of the membrane. In this study, the increasing of alginate content in the membrane resulted in increasing

of water absorption ability of the blend membranes. Lee *et al*. (1997) suggested that the water molecules were easily adsorbed into the alginate membrane crosslinked with glutaradehyde due to its high hydrophilic property.

5.6 Mechanical properties of the membranes

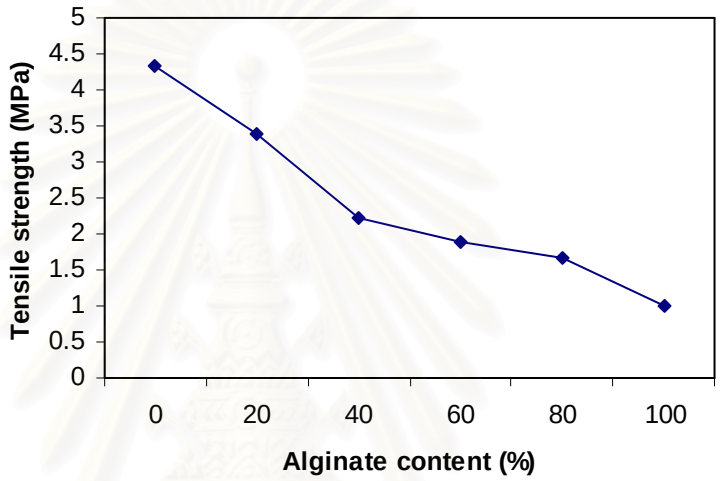


Figure 5.8 The tensile strength of the bacterial cellulose/alginate blend membranes as a function of alginate content.

Generally, for applications, membranes could be subjected to various kinds of stress during usage. The determination of the mechanical properties of the developed membranes, therefore, was useful for practical use. Figure 5.8 showed the tensile strength of bacterial cellulose/alginate blend membranes as a function of alginate content. The tensile strength of bacterial cellulose membrane with the thickness of 0.083-0.091 mm was 4.32 MPa whereas the tensile strength of alginate membrane was 1.01 MPa. The decrease of the tensile strength of the blend membranes depended upon the amount of alginate content. The tensile strength of the blend membrane

significantly decreased from 4.32 to 2.23 MPa when alginate content was increased from 0% to 40% but slightly decreased from 2.23 MPa to 1.01 MPa when alginate content was increased from 40% to 100%.

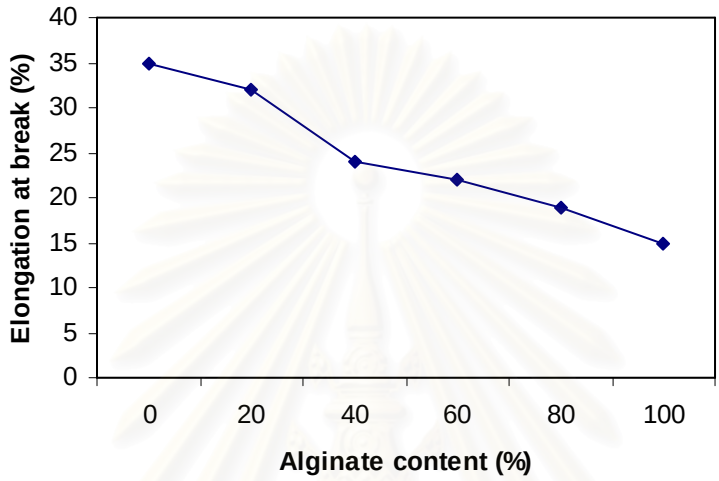


Figure 5.9 The elongation at break of the bacterial cellulose/alginate blend membrane as a function of alginate content.

Figure 5.9 showed the elongation at break of the blend membranes with the thickness of 0.083-0.091 mm as a function of alginate content. The elongation at break of bacterial cellulose membrane was 35% whereas the elongation at break of alginate membrane was 16%. It was found that the elongation at break of the blend membranes also decreased upon the amount of alginate content. The blend membranes had the elongation at break higher than the pure alginate membrane but lower than the bacterial cellulose membrane.

It was found that the effect of alginate content on mechanical properties of the bacterial cellulose/alginate membranes was similar to that of cotton cellulose/alginate blend membranes. Zhang *et al.* (1997) prepared the ion exchange blend membranes of cotton cellulose and alginate from the solution of cuoxam solvent system. They found that the mechanical properties of blend membranes decreased with increasing alginate content. The similar result was reported in the study of cotton cellulose/alginate blend membrane from the solution of NaOH/urea solvent (Zhou *et al.*, 2001). The sodium alginate membrane was usually water soluble and mechanically weak but its mechanical properties were obviously improved by introducing cellulose and cross-linked with Ca^{2+}.

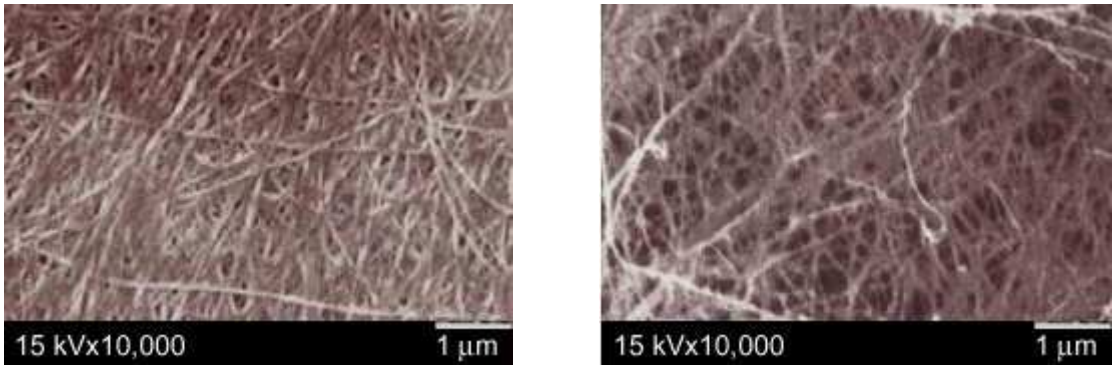
The reason for the difference in the mechanical properties of cellulose/alginate blend membranes might be due to the occurrence of some specific interactions between cellulose and alginate molecules based on their structure similarity. These interactions were supposed to be hydrogen bonding formations between the hydroxyl groups of cellulose and the carboxyl groups of alginate. Yang *et al.* (2000) suggested that the presence of alginate chains enhanced the molecular motion of cellulose in the blend, and perturbed the strong hydrogen bond of pure cellulose due to the formation of new intermolecular interaction between the two biopolymers.

5.7 Surface morphology

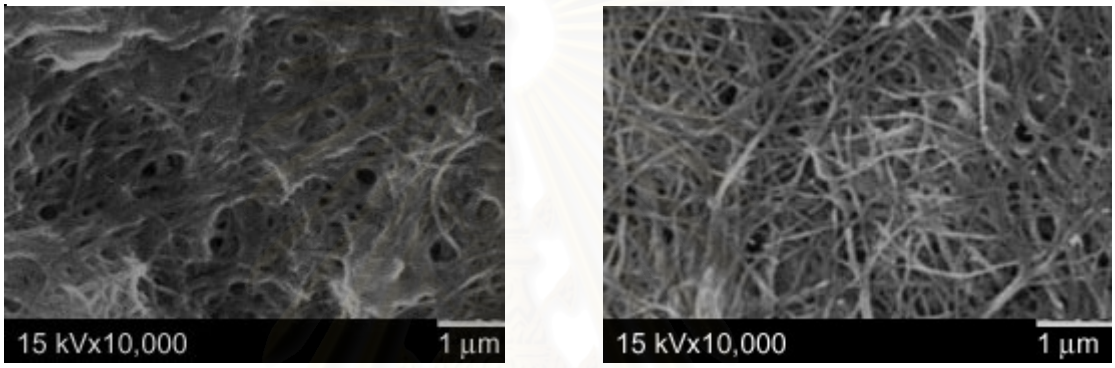
Figure 5.10 showed the micrograph of surfaces for membrane before and after using supercritical drying method. The SEM investigation of the blend membranes showed the difference between regenerated pure bacterial cellulose (RBC-A) and the bacterial cellulose/alginate blends. RBC-A1 – RBC-A4 referred to the 80/20, 60/40, 40/60 and 20/80 bacterial cellulose/alginate blend, respectively. Besides alginate membrane (figure 5.11), the pore sizes were obtained after using supercritical drying method.

In the case of blend membrane, RBC-A1 was the only composition that displays homogenous structure, which exhibited a certain level of miscibility of the blend. This composition showed obviously homogeneous pore structure than other compositions namely RBC-A2, RBC-A3, and RBC-A4. It was shown that fibers were orderly nonwoven when it contained 20 % by weight of alginate. Moreover, the apparent pore size of blend membrane decreased with increase in the percent of alginate. With alginate content more than 20 % by weight, the miscibility of the blend membrane was less orderly.

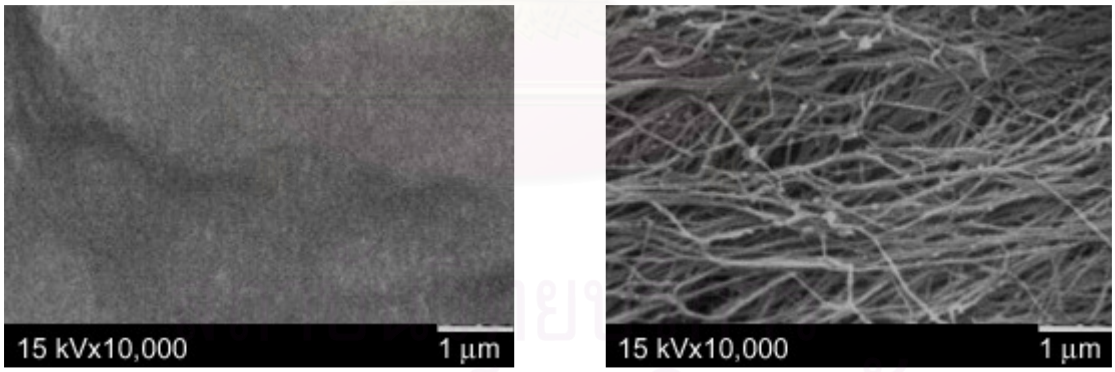
From the tensile test data of this study, it was shown that the tensile strength of the membranes decreased with increasing alginate content. Besides, the micrograph data showed that RBC-A1 exhibited the most orderly alignment of the fibers on its surface. The results revealed that the optimum alginate ratio of blend membrane was 20% by weight alginate content.



BC from biosynthesis (Sangruangroj *et al.*, 2006)

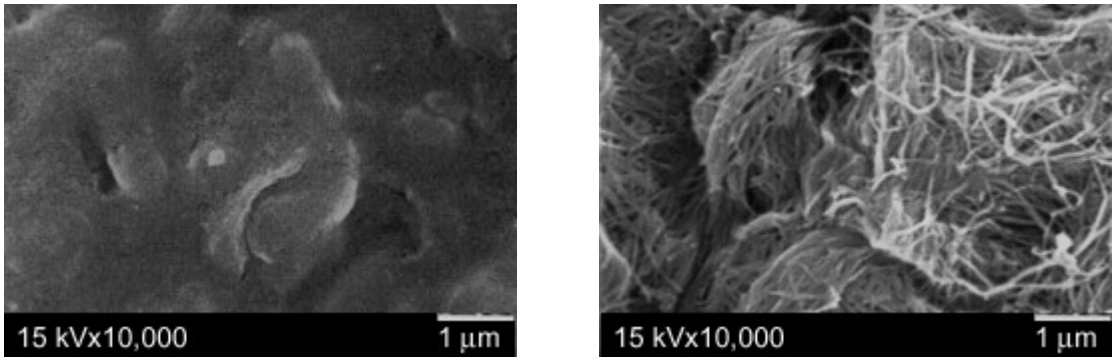


RBC

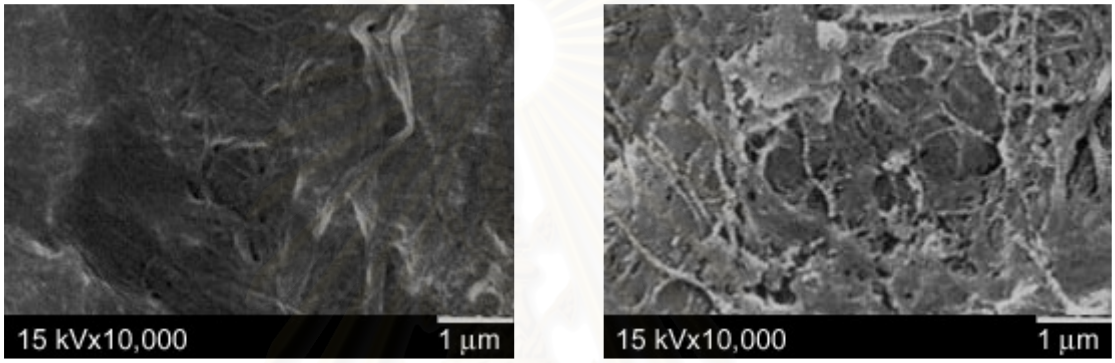


RBC-A1

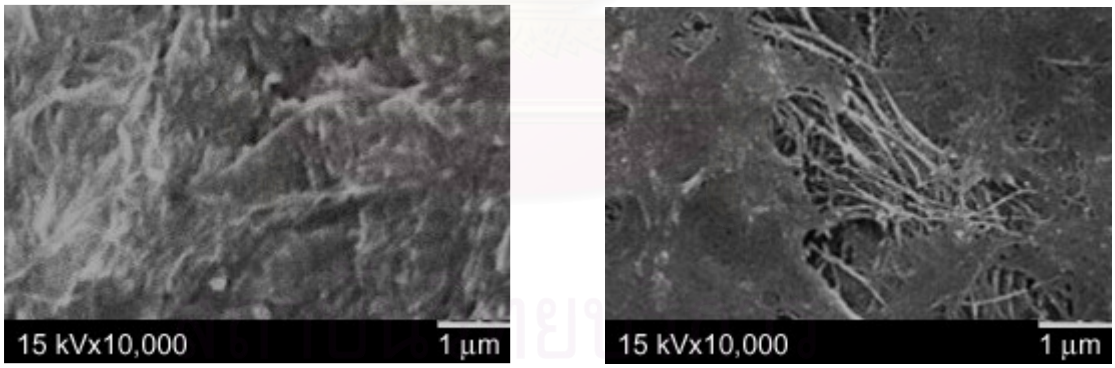
Figure 5.10 SEM of the surface of bacterial cellulose membrane before (left) and after (right) using supercritical drying method.



RBC-A2



RBC-A3



RBC-A4

Figure 5.10 (cont) SEM of the surface of bacterial cellulose membrane before (left) and after (right) using supercritical drying method.

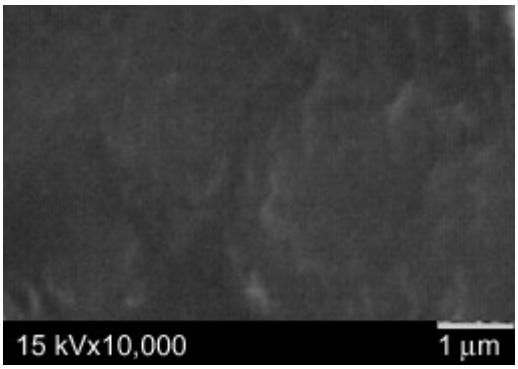
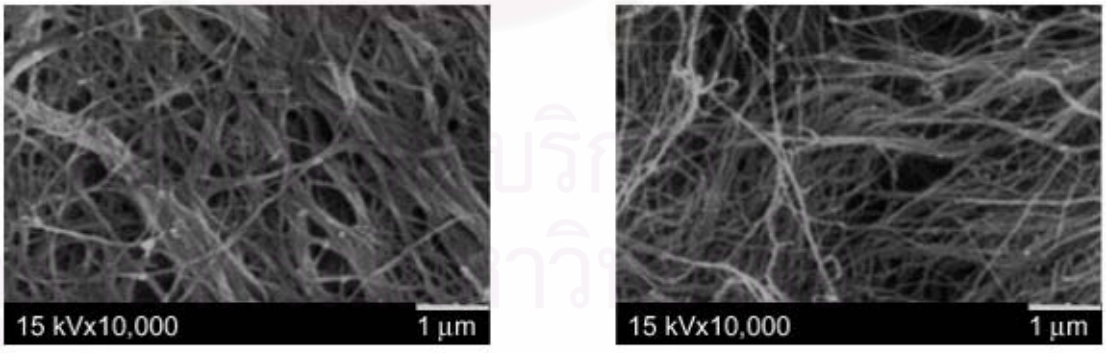


Figure 5.11 SEM of the surface of alginate membrane (SA) after using supercritical drying method.

Freeze drying method was used to prepare porous membrane compared to supercritical point drying method. Figure 5.12 showed the micrograph of surfaces of RBC and RBC-A1 membrane after using freeze drying method. From the observation, the fibers of both RBC and RBC-A1 were less orderly nonwoven than the membrane obtained from supercritical drying method.



RBC RBC-A1

Figure 5.12 SEM of the surface of bacterial cellulose membrane after using freeze drying method.

The micrograph of the surface of regenerated cellulose membrane from plant was shown in figure 5.13. Since bacterial cellulose was synthesized extracellularly into nanosized fibrils by the bacteria *Acetobactor Xylinum*, the diameter of bacterial cellulose was significantly less than that of plant cellulose. The bacterial cellulose membrane was nanofiber network structure while plant-derived cellulose was microfiber network structure.

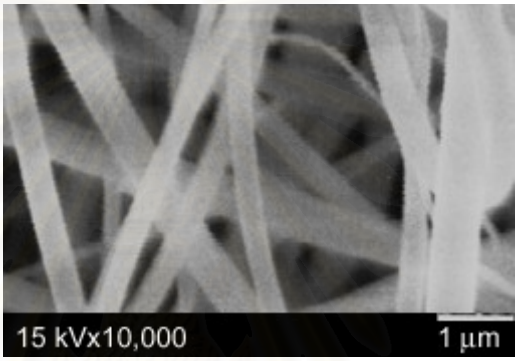


Figure 5.13 SEM of the surface of commercial grade-regenerated cellulose membrane from plant (Whatman membrane filter).

5.8 Water vapor permeability test

Figure 5.13 showed the water vapor transmission rate (WVTR) of the bacterial cellulose/alginate blend membranes. The water vapor transmission rate of bacterial cellulose membrane was 2,504 g/m^2 day and that of the alginate membrane was 5,609 g/m^2 day. The blend membranes had remarkable increasing in WVTR as compared to bacterial cellulose membrane. The water vapor transmission rate for the blend membrane with 20% alginate content was 5,119 g/m^2 day or 2.1 times compared to the pure bacterial cellulose membrane.

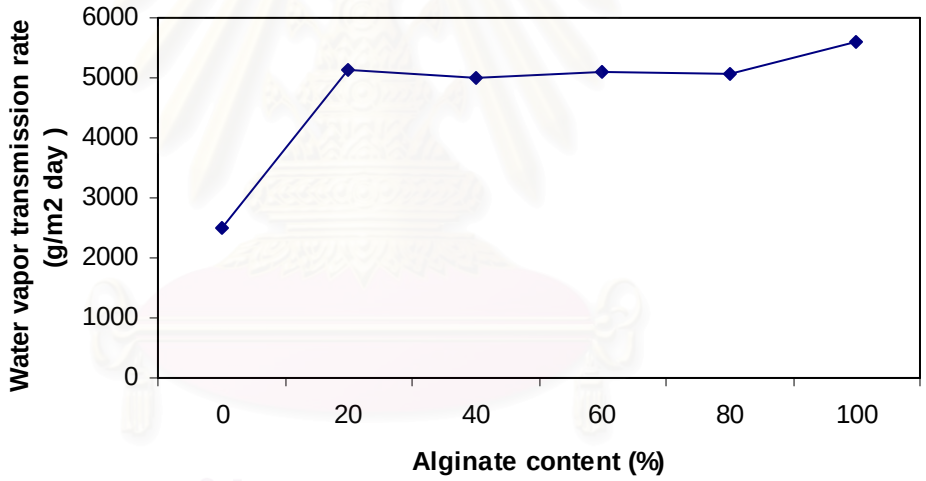


Figure 5.14 The water vapor transmission rate of the bacterial cellulose/alginate blend membranes as a function of alginate content.

The water vapor transmission rate of blend membranes were higher than that of pure bacterial cellulose membrane which might be attributed to the strong interaction between water molecules and $–COO^-$ and –OH functional groups of alginate in the blend membrane (Yang *et al.*, 2000). The dramatically increasing of water vapor transmission rate of the blend membrane as compared to pure bacterial cellulose membrane was possibly due to the high hydrophilic property of alginate. The quantities of water molecules which were bind to the surface of the blend membranes were higher than that of pure bacterial cellulose membrane. As a result, it led to the difference of concentration of water molecules on both top and bottom of the membrane surface. This concentration difference was a driving force for water transport through the membrane.

5.9 Porosity

Surface area and porosity were important characteristics, capable of affecting the quality and utility of many materials. BET method was one of the most powerful techniques for estimating the surface area of material by physical adsorption of gas molecules (Brunauer, Emmett and Teller, 1938).

In this research, pure regenerated bacterial cellulose membrane (RBC) and the optimal ratio of bacterial cellulose/alginate blend membrane (RBC-A1) were characterized by BET analyzer. The result data were summarized in Table 5.2.

Membrane	Pore diameter (A°)	Surface area (m^2/g)
BC (Sangruangroj *et al*., 2006)	224	12.62
RBC	12.63	17.57
RBC-A1	10.60	19.50

Table 5.2 Surface area and pore diameter of the membrane analyzed by BET analyzer.

The result data was shown that regenerated BC membrane and blend membrane had the pore sizes much lessen than that of BC membrane from biosynthesis (Sangruangroj *et al.*, 2006) while the surface area was not slightly increased from the latter. The pore size of new nonwoven RBC and RBC-A1 membranes in this research was 12.63 A° and 10.59 A°, respectively, which were in the range of nanoporous membrane.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The nanoporous membranes were satisfactory prepared by bacterial cellulose and bacterial cellulose/alginate in NaOH/urea aqueous solution. Bacterial cellulose concentrations lower than 3% by weight were completely dissolved in 4% wt NaOH/3% wt urea solution. The optimum conditions involved with swelling cellulose in NaOH/urea and then followed by freeze thaw process.

The mechanical properties of the blend membranes were slightly reduced compared to the regenerated pure bacterial cellulose (RBC) membrane while the water vapor permeability and the percent water absorption were higher than that of the latter. The tensile strength, the elongation at break and the percent water absorption of the regenerated pure bacterial cellulose membrane were 4.32 MPa, 35.20% and 49.67%, respectively. The water vapor transmission rate was 2,504 g/m^2 day and the pore size was 1.26 nm. The tensile strength, the elongation at break and the percent water absorption of the blend membrane with 20% by weight alginate content were 3.38 MPa, 31.6% and 52.25%, respectively. The water vapor transmission rate was 5,609 g/m^2 day and the pore size was 1.06 nm. As information on SEM, the RBC blend membrane with 20% by weight alginate exhibited the most orderly alignment of fibers on its surface.

6.2 Recommendations for future studies

Based on this study, further studies for the improvement of regenerated bacterial cellulose membrane are recommended.

1. The in-depth research of porogent used or method for alteration of pore size of regenerated bacterial cellulose membrane.

2. The study of membrane restructuring development by blending BC with other natural polymer.

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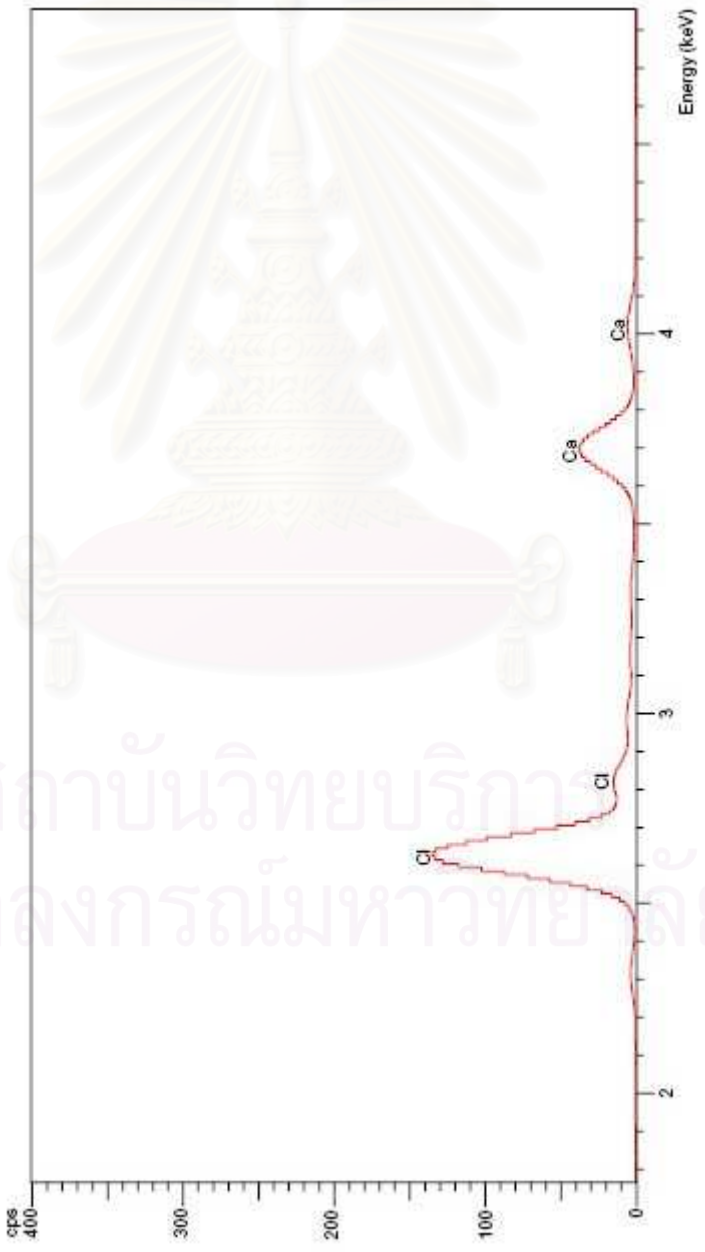
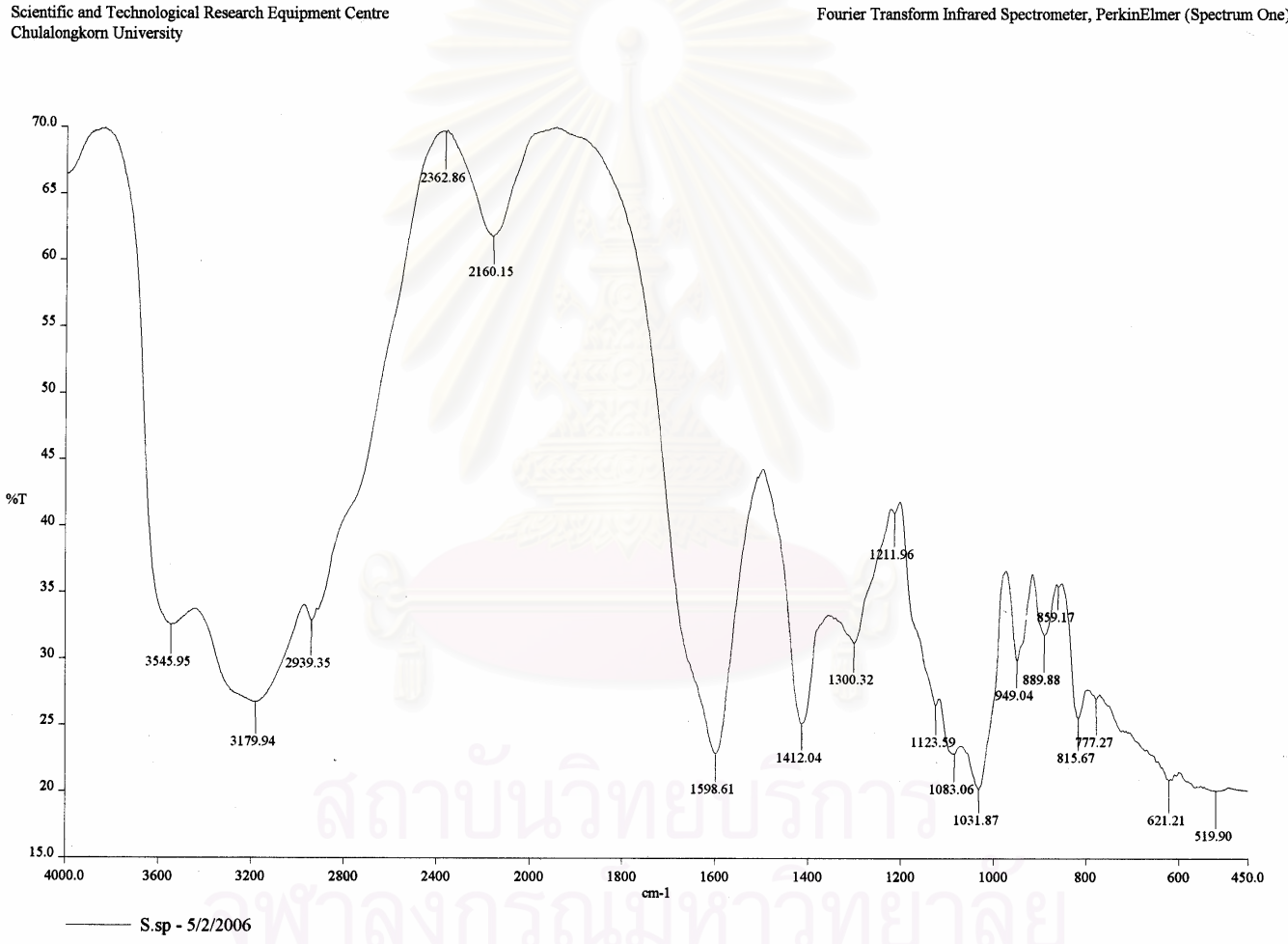
APPENDICES

APPENDIX A

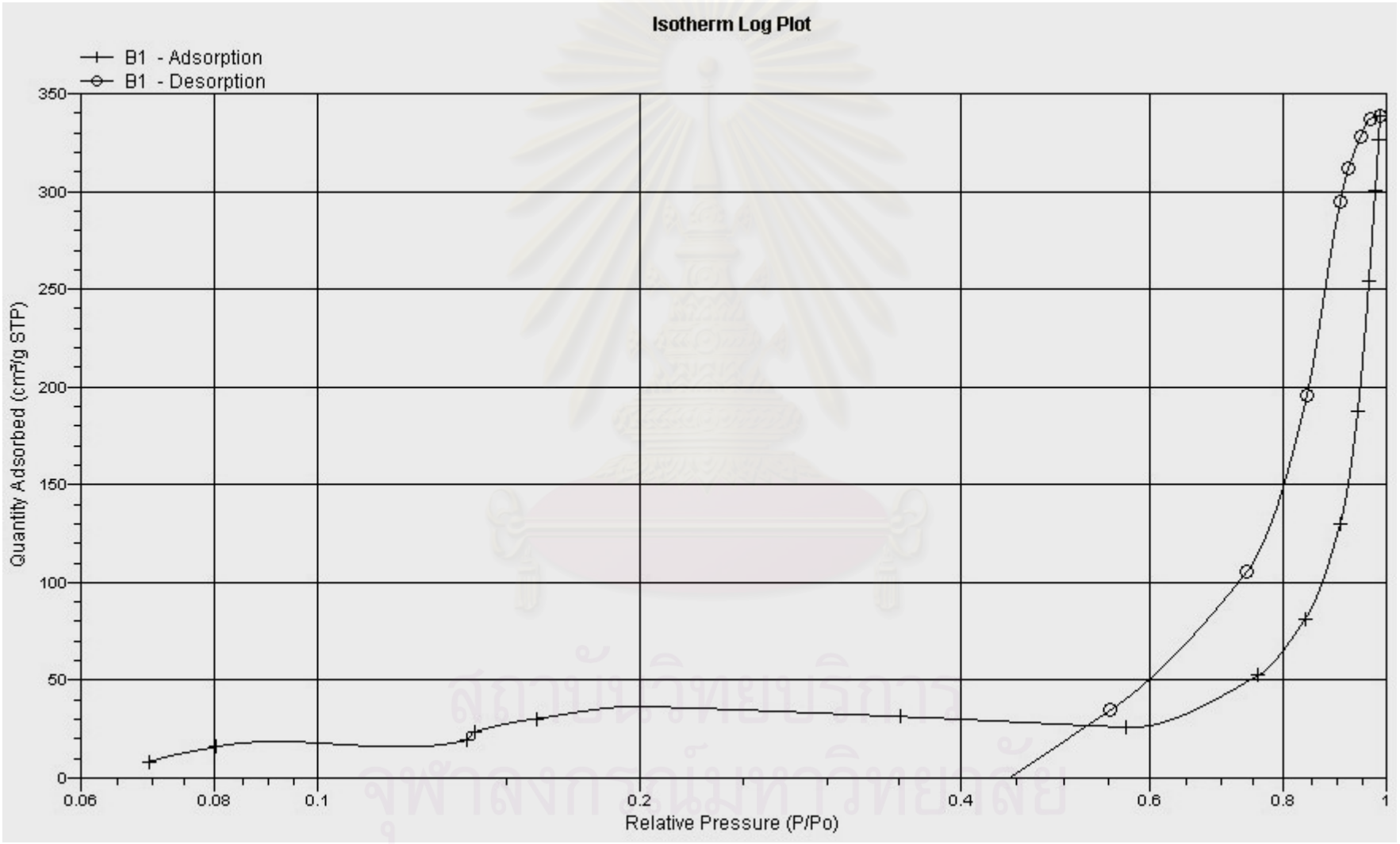
GRAPHICAL DATA OF MEMEMBRANE CHARACTERIZATION

Example of XRF spectra data





Example of data from BET analysis



APPENDIX B

DATA OF EXPERIMENTS

Table B1 Data of Figure 5.2

NaOH concentrations (%wt)	Solubility (%wt)				
	1	2	3	Average	SD
1	9.62	8.29	9.45	9.12	0.59
2	25.20	22.71	21.54	23.15	1.53
3	35.05	34.65	35.09	34.93	0.20
4	55.05	52.01	49.33	52.13	2.34
5	52.13	51.37	55.92	53.14	1.99
6	56.23	52.81	55.30	54.78	1.44
7	54.00	51.00	57.96	54.32	2.85
8	57.09	57.85	50.45	55.13	3.32
9	59.01	56.82	46.82	54.23	5.31
10	56.00	55.03	58.26	56.43	1.35

Table B2 Data of Figure 5.3

Urea concentrations (%wt)	Solubility (%)				
	1	2	3	Average	SD
1	77.49	75.09	80.43	77.67	2.18
2	78.77	84.00	77.29	80.02	2.88
3	90.21	94.36	92.42	92.33	1.70
4	91.95	93.91	90.20	92.02	1.52
5	91.96	91.98	93.20	92.38	0.58
6	94.05	92.65	91.22	92.64	1.16
7	92.30	94.23	91.36	92.63	1.19
8	90.64	95.78	91.50	92.64	2.47

Table B3 Data of Figure 5.4

BC concentrations (%wt)	Solubility (%)				
	1	2	3	Average	SD
1	100	98.92	98.44	99.35	0.47
2	91.76	96.09	96.13	94.66	2.05
3	90.21	94.36	92.42	92.33	1.70
4	69.11	68.22	65.20	67.51	1.68
5	54.46	50.41	45.49	50.12	3.67
6	45.00	38.09	43.30	42.13	2.94
7	39.02	37.77	38.92	38.57	0.57
8	27.98	28.25	22.79	26.34	2.51

Table B4 Data of Figure 5.7.

Alginate concentrations (%wt)	Equilibrium Water Content (%)				
	1	2	3	Average	SD
0	50.89	44.05	54.07	49.67	4.18
20	53.24	54.60	48.91	52.25	2.43
40	55.53	57.79	54.92	56.08	1.24
60	64.23	64.41	61.65	63.43	1.13
80	65.01	67.33	64.10	65.48	1.36
100	67.96	68.87	68.40	68.40	0.37

Table B5 Data of Figure 5.8

Alginate concent (%wt)	Tensile strength (MPa)						
	1	2	3	4	5	Average	SD
0	4.49	3.96	5.68	3.74	4.73	4.32	0.73
20	4.00	3.08	2.83	3.31	3.68	3.38	0.42
40	1.95	2.09	2.36	2.64	2.11	2.23	0.24
60	1.74	1.90	1.66	2.02	2.13	1.89	0.17
80	1.47	1.77	1.63	1.82	1.66	1.67	0.12
100	0.98	1.11	0.89	1.09	1.08	1.03	0.08

Table B6 Data of Figure 5.9

Alginate concent (%wt)	Elongation at break (%)						
	1	2	3	4	5	Average	SD
0	39	34	40	32	31	35.20	3.66
20	36	30	28	30	34	31.60	2.94
40	20	21	26	29	24	24.00	3.29
60	20	21	19	23	26	21.80	2.48
80	17	22	17	22	18	19.20	2.32
100	14	18	14	16	15	15.40	1.50

Table B7 Data of Figure 5.14

Alginate content (%wt)	Temperature (°C)	% RH	WVTR (g/m^2 day)
0	38	90	2,504
20	38	90	5,119
40	38	90	5,012
60	38	90	5,106
80	38	90	5,056
100	38	90	5,609

CURRICULUM VITAE

Mr. Thapanar Suwanmajo was born on July 31st ,1979 in Bangkok, Thailand. He received the Bachelor Degree of Chemical Engineering from Faculty of Engineering, Chulalongkorn University in 2002. He continued his Master's Study at Chulalongkorn University in June, 2003.