CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Ethanol

Ethanol (C_2H_5OH) (ethyl alcohol, grain alcohol, or drinking alcohol) is a volatile, flammable, and colorless liquid. Although ethanol has been used in many industries, it can be commonly classified based on its end use into three main industries (Berg, 2004; Rosillo-Calle and Walter, 2006):

a) Portable alcohol: the ethanol that used as a principle component in beverage products, such as beer, wine, and liquors. This type of alcohol also includes that used in drug and cosmetics industries.

b) Industrial alcohol: the ethanol which cannot be consumed directly but used in the production, such as acetic acid production, medical industry, and aseptic production. Ethanol is generally used as a solvent in various industries.

c) Alcoholic fuel: because of the petroleum shortage and environmentally toxic concern, ethanol has received worldwide attention to be used as an alternative fuel for several decades. It can be used directly, blended with the petroleum fuel, or used as an octane booster.

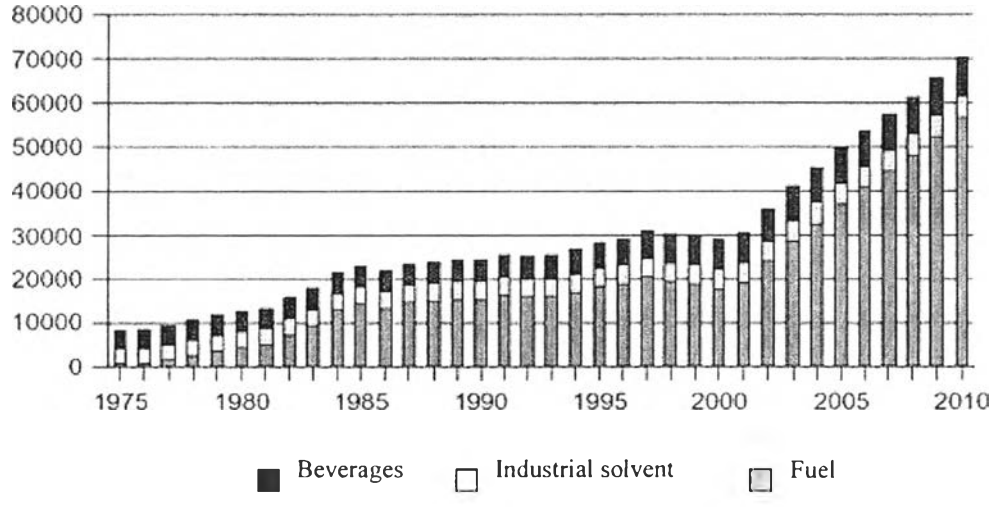

Figure 2.1 Growth rate of ethanol (million liters) between 1975 and 2010, according to an industry (Berg, 2004).

2.1.1 Production of Ethanol

Ethanol can be divided based on its production routes into two categories; a synthetic ethanol and a bioethanol.

a) Synthetic ethanol: the ethanol derived from the chemical processes. Ethanol is produced via the hydration of the petroleum product — ethylene (Clark, 2002).

$$CH_2{=}CH_{2\,(g)} + H_2O_{\,(g)} \rightleftharpoons CH_3CH_2OH_{\,(g)}$$

b) Bioethanol: the ethanol produced by biological processes, fermentation, or agriculture crop utilization. Crops (sugarcane, wheat, and corn) are the most essential types of bioresources that are utilized for bioethanol production (Singh Nigam *et al.*, 2011). Feedstocks containing sugar, or materials that can be converted into sugars, such as starch or cellulose, also can be used (Singh *et al.*, 1995; Verma *et al.*, 2000; Aggarwal *et al.*, 2001).

Although both synthetic and fermented ethanol are chemically identical, more than 95 % of overall output of ethanol is derived from fermentation process due to a lower overall cost and able to be adapt to use numerous other agriculture sources (Berg, 2004; Suksaroj, 2009).

2.2 Fermentation

An enormous potential of microorganisms for industrial usages has been discovered about a decade. Fermentation is a process in which energy is generated by the oxidation of organic compounds, such as carbohydrates like sugar. These organic compounds are converted into a variety of products depended on the microorganisms used in the process. In general, fermentation is a process that takes advantages from microorganisms, yeast, mold, and bacteria, together with an appropriate controlled environment to stimulate cell growth or enzyme production. The fermentation products can be classified into four types (Shuler and Kargi, 2002):

a) Microbial cell, such as Baker's yeast production for bakery use and single cell protein (SCP) production as an animal feed.

b) Microbial enzyme, which mainly used in food industry and goods production, like amylase, lipase, and protease.

c) Metabolite substance, a product of metabolism process, such as ethanol, butanol lysine, and vitamins.

d) Transformation process, a transformation of the product into another similar form which has a higher price, such as the productions of vinegar and antibiotics.

2.2.1 Ethanol Fermentation

Alcoholic fermentation is a degradation of substances by microbial cells in the absence of oxygen or anaerobic respiration. It is a biological process which uses the enzyme produced from yeast to convert a substrate — such as glucose, fructose, and sucrose — into energy, ethanol, and carbon dioxide. Prior to the fermentation process, glucose molecules are broken into two molecules of pyruvic acid through a cycle called

"glycolysis", as shown in the below chemical equation (Shuler and Kargi, 2002). After that, yeast cells transform pyruvate to acetaldehyde, which acts as the last electron accepter, and yield the ethanol product.

$$C_6H_{12}O_6 + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H_2O + 2H^+$$

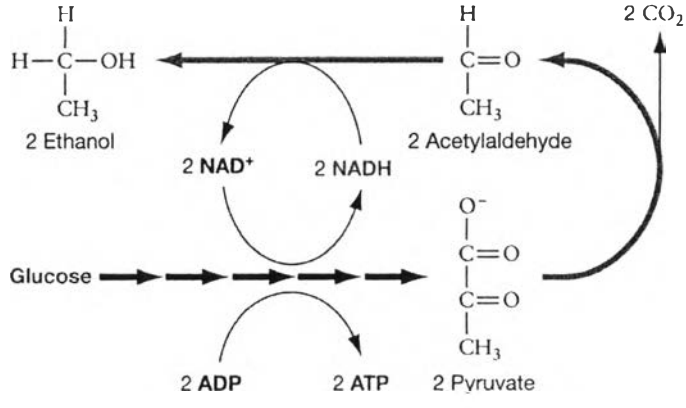

Figure 2.2 Ethanol fermentation pathway (Farabee, 2007).

The usage of bioethanol as biofuel is considered to be both renewable and environment-friendly; therefore, several developments of fermentation production of bioethanol have been made. The technologies or methods used to enhance the fermentation production include the utilization of the genetically-modified microorganisms. Nonetheless, the long-term effects of genetic modification microorganism are still doubted. Another potential method is to incorporate the engineering process to improve the fermentation step, such as the use of continuous fermentation instead of a batch type in order to prevent product inhibition (Shuler and Kargi, 2002). The microorganism activities can be stop due to the product inhibition, which always occurs when the system contains a high concentration of ethanol product. Although the continuous process can solve this problem, it requires high investment and

Although the continuous process can solve this problem, it requires high investment and better process control. As a result, cell immobilization fermentation strategy, which possesses economical advantage and also be able to eliminate the product inhibition, has been developed (Rakin *et al.*, 2009).

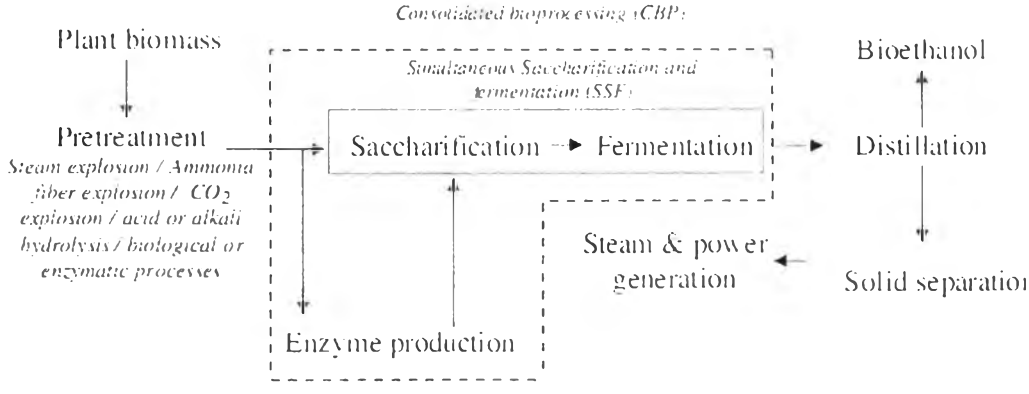

Figure 2.3 Various processes for the production of bioethanol from lignocellulosic biomass (Singh Nigam *et al.*, 2011).

2.3 Cell Immobilization

Cell immobilization is defined as "the physical confinement of localization of intact cells to a certain defined region of space with preservation of some desired catalytic activity" (Karel *et al.*, 1985). Cell immobilization can be done by physical attachment, covalent bonding, and entrapment of microbial cells on the substrates. Nevertheless, among these three techniques, the most widely practical method is the immobilization by cell entrapment (Kumakura *et al.*, 1991). The immobilization of microbial cells shows advantages over free cells system in both economical and technical points of view. This fermentation strategy can eliminate the product inhibition and also enhance the production yield of ethanol (Pilkinton *et al.*, 1997; Rakin *et al.*,

2008). Moreover, the immobilized cell can create the cellular stability and ease of cell recovery, reutilization (Prasad and Mishra, 1995), as well as downstream processing.

Naturally, many microorganisms have a capability to adhere to the carrier closed to nutrient and food supply. In other words, this biological system occurs in a natural state (Nedovic *et al.*, 2001). Cell immobilization can be divided into four main categories:

2.3.1 Adsorption to a Pre-Formed Carrier

The earliest type of cell immobilization is based on the adsorption of microbial cells on external solid surface. Microbial cell can be attached by Van der Waals forces, electrostatic interaction, covalent bonding, and physical entrapment in the pores of substrates (Nedovic *et al.*, 2001). For the adsorption of yeast cells, the cultivation substrates can be divided in two general types: (a) materials with the yeast restricted to its external surface and (b) porous materials that allow the cell adsorption inside the material.

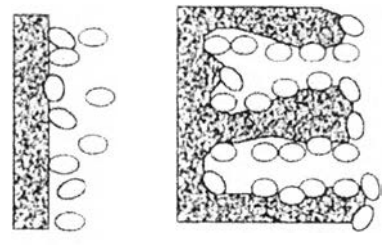

Figure 2.4 Adsorption to a performed carrier (Nedovic *et al.*, 2001).

2.3.2 Cell Entrapment

The entrapment methods are based on the inclusion of cells within a rigid network to prevent the cells from diffusing into surrounding medium while still allowing

penetration of substrate. The porous beads — calcium alginate, kappa, carrageenan, and pectate gel — are normally used as the matrices (White and Perto, 1978; Onaka *et al.*, 1985; Nedovic *et al.*, 1993; Domeny *et al.*, 1996; Pilkinton *et al.*,1997).

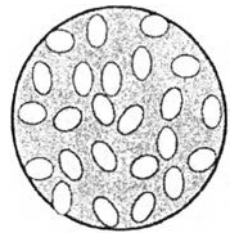

Figure 2.5 Cell entrapment within the carrier (Nedovic *et al.*, 2001).

This method provides extremely high cell loading and high fermentation rates but a low mass transfer of the yeast cells and substrate together with gel degradation are the limitations of cell entrapment strategy (Pilkinton *et al.*, 1997).

2.3.3 Self-Aggregation

Self-aggregation of cells can either occur naturally (Stratton *et al.*, 1994) or artificially induced by crosslinking agent. This technique is based on the use of highly concentrated of flocculent yeast strain in order to eliminate the product inhibition during fermentation process. Although this is the simplest and cheapest immobilization technique, it is the most sensitive to a change in the cultivation environment (Nedovic *et al.*, 2001).

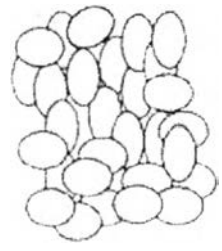

Figure 2.6 Self aggregation of microbial cells (Nedovic *et al.*, 2001).

2.3.4 Containment of Cells Behind Carrier

The cells are confined to a space bounded by a semipermeable barrier or immobilized within a membrane. However, this procedure for the preparation of cell carriers is complex.

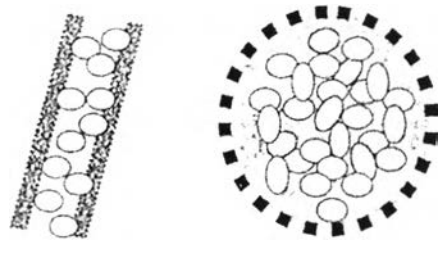

Figure 2.7 Containment of cells behind carrier (Nedovic *et al.*, 2001)

The polymeric materials generally used as the cell immobilization matrix possess a porous structure, in which cells are able to grow up inside their pores (Kumakura *et al.*, 1991). Various kinds of support materials for cell immobilization have been reported, including calcium alginate, κ-carragenan gel, polyacrylamide, g-alumina (Nursevin *et al.*, 2003), and orange peel (Plessas *et al.*, 2007). The cultivation substrates should also provide the ease of nutrient accessibility for cell activities and have enough strength to withstand the fermentation condition.

Kumakura *et al.* (1991) investigated the effect of the matrix's pore size on the ethanol production of yeast cells. The ethanol productivity of the immobilized yeast cells increased with an increase in the size of porous support. Moreover, the results also showed that a hydrophilic polymer matrix was the most suitable for the growth of yeast.

Winkel *et al.* (1993, 1995) integrated a novel immobilization with a continuous beer production. The biorector was designed to allow the immobilization of the yeast cells inside them. In the bioreactor, multichannel porous silicon carbide immobilization rods were arranged. The results indicated that the open pores of silicon carbide with the size ranging from 40 μm to 60 μm promoted yeast colonization and maximized mass transfer between the yeast cells and the liquid flowing through the channels.

Plessas *et al.* (2007) prepared biocatalyst by immobilizing *Saccharaomyces cerevisiae*, baker's yeast strain, on orange peel in order to use in alcoholic fermentation. Yeast cell densely attached on the surface of the peels due to natural entrapment, physical absorption via electrostatic forces, or covalently binding between the cells and the peels. The fermentation time decreased twice, compared to free cells system.

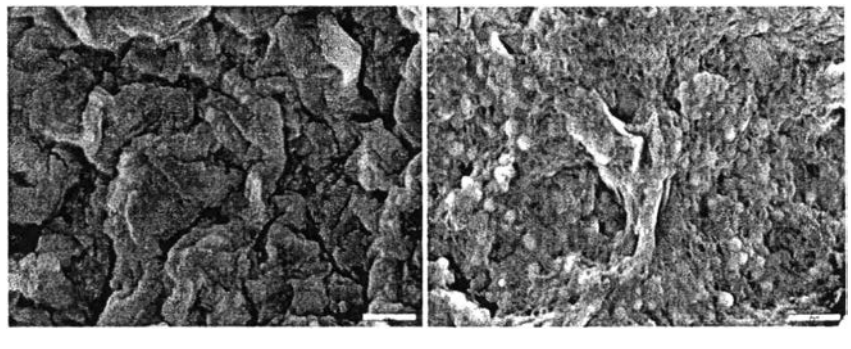

Figure 2.8 Electron micrographs of the surface of orange peel, before and after yeast immobilization at a magnification of 1200 (Plessas *et al.*, 2007).

As shown in Figure 2.9, the immobilized cells show a faster decrease in sugar brix density, implying a faster conversion of sugar to ethanol. Moreover, it was also reported that the immobilized cell system showed higher cell viability during the repeated batch fermentation, indicating that the immobilized cells tolerated the product inhibition.

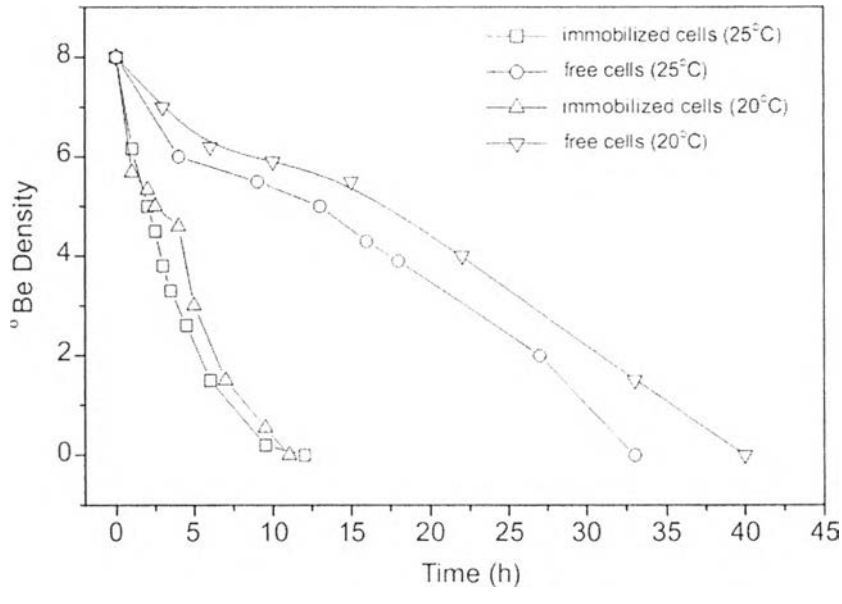

Figure 2.9 Kinetics of glucose fermentation by free cell and immobilized cell (Plessas *et al.*, 2007).

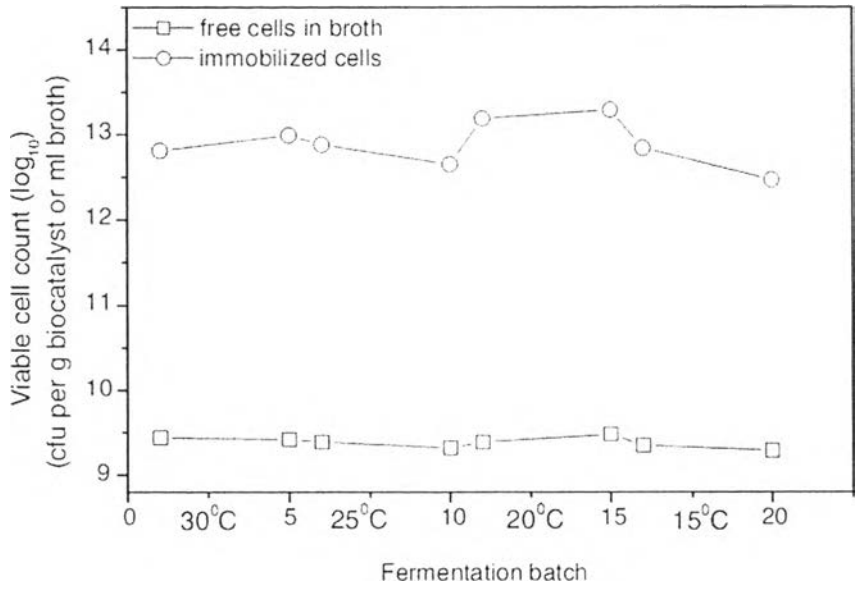

Figure 2.10 Viable cell counts of free and immobilized cells during repeated batch fermentations of glucose (as log10 colony forming unit per gram (cfu/g) of orange peel or log10 cfu/ml of fermentation broth) (Plessas *et al.*, 2007).

However, to use immobilization technology in the production of sustainable fuel — ethanol, the polymer matrix must be cheap and should be achievable with minimum addition cost. The matrix is preferentially renewable and biodegradable. For example, Chen *et al.* (2007) used a natural plant sponge, which have high porosity, stable physical properties, and low cost, for the bioethanol fermentation via the cell immobilization technique.

2.4 Silk Fibroin (SF)

Silk, "the queen of fibers", is a continuous protein fiber produced by silkworm, *Bombyx mori*, during its cocoon formation. Silk is generally known in the textile industry for its luster and mechanical properties. The mature silkworms build their

cocoons by extruding a viscous fluid from special glands in their bodies before passing through a spinneret, and yielding a single fiber (Sonthisombat and Speakman, 2004).

Silk cocoons mainly comprise of two proteins named as fibroin and sericin of about 70 % and 30 %, respectively. Two strands of fibroin are bonded together to form a core structure located in the center of the silk fiber to provide the strength. These two fibroin strands are cover by a sticky layer of sericin coating, which acts as a natural glue for maintaining the shape of cocoon (Wu *et al.*, 2006; Oh *et al.*, 2007) but concealing the unique luster of silk. Silk sericin is known as an amorphous material and can be removed by boiling the cocoons in a hot water. The sericin removal process is called "silk degumming". After the sericin removal, the remaining silk fibroin shows the biological responses comparable to those of other biomaterials used in biomedical fields because sericin is found to be a major cause of adverse problems with biocompatibility and hypersensitivity to silk (Altman *et al.*, 2002).

Table 2.1 Composition of silk produced by *Bombyx mori* silkworm (Gulrajani, 1988)

Component	Percent (%)
Fibroin	70 – 80
Sericin	20 – 30
Wax matter	0.4 – 0.8
Carbohydrates	1.2 – 1.6
Inorganic matter	0.7
Pigment	0.2
Total	100

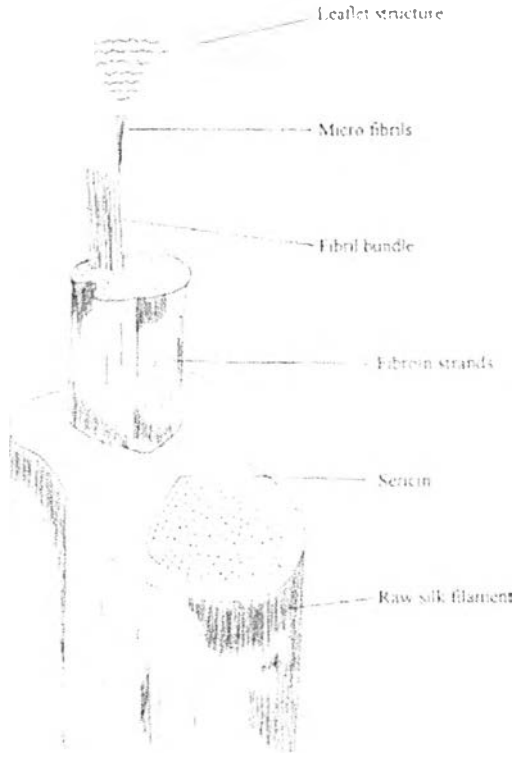

Figure 2.11 Physical structure of raw silk fiber (Sandoz Colour Chronicale, 1990).

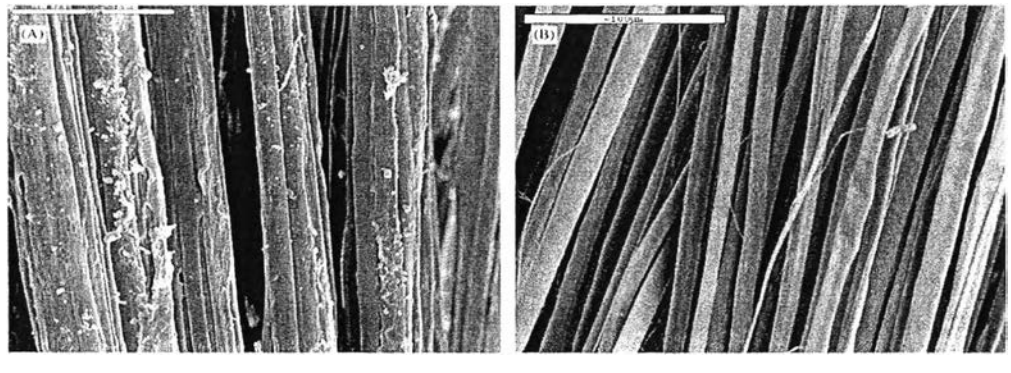

Figure 2.12 Scanning electron micrograph (SEM) of virgin silk produced by *B. mori* (a) before and (b) after extraction of sericin protein coating (Altman *et al.*, 2006).

2.4.1 SF as a Biomaterial

A biomaterial is a non living material used in a medical device that intended to interact with a biological system (Teddai *et al.*, 2001). The ability of biomaterial to response in a specific situation is known as biocompatibility. Several researchers have investigated SF as one of the promising resources of biotechnology and biomedical materials due to its unique properties, including good biocompatibility and biodegradability.

Minoura *et al.* (1995) compared the abilities of SF and silk sericin films to that of collagen for the potential use as a support of L-929 fibroblast attachment and growth. The result indicated that the ability of SF and collagen were equivalent. Therefore, the degummed silk was found to be a suitable matrix for cell and tissue culture.

Inuoya *et al.* (1998) investigated the use of SF film in the cultivation of human cells (human colon adenocarcinoma, SE116; human mouth epidermoid, KB; human lung carcinoma, QG56) in a comparison with the use of a collagen matrix. After 5 days of the cell culture, both of the film supported equivalent amount of cell growth.

SF provides processability in an aqueous-based system for the fabrication of a versatile of materials in difference forms. Hence, an aqueous SF solution represents a good starting material to prepare SF-based film, powder, gel, membrane, and sponge (Zhang *et al.*, 1998; Altman *et al.*, 2003).

Wongpanit *et al.* (2007) reported the culture of L-929 fibroblast cell line on the chitin whiskers reinforced SF sponge. The nanocomposite sponges exhibited the absence of the cytotoxicity as well as enhanced the cell spreading.

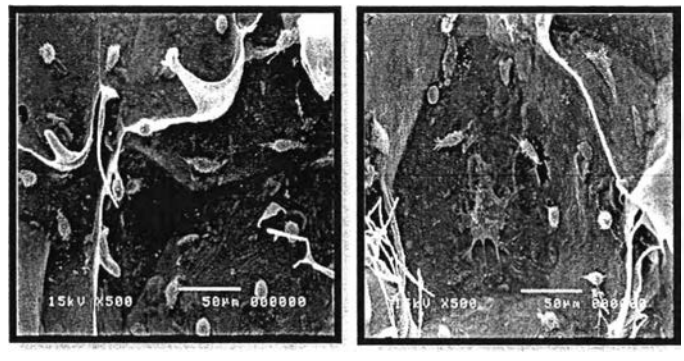

Figure 2.13 SEM micrographs of mouse connective tissue cells cultures attached on methanol-treated SF sponges for (a) 6 and (b) 24 h of cultivation (Wongpanit *et al.*, 2007).

Li *et al.* (2002) demonstrated the degradation behavior of porous SF sheets by *in vitro* enzymatic method with α-chymotrypsin, Collagenase IA, and protease XIV. The pore size of fibroin sheet increased with increasing the degradation time before the sheet finally collapsed and became shapeless. After the degradation with protease XIV, more than 50 % of the degraded products were found to be free amino acids.

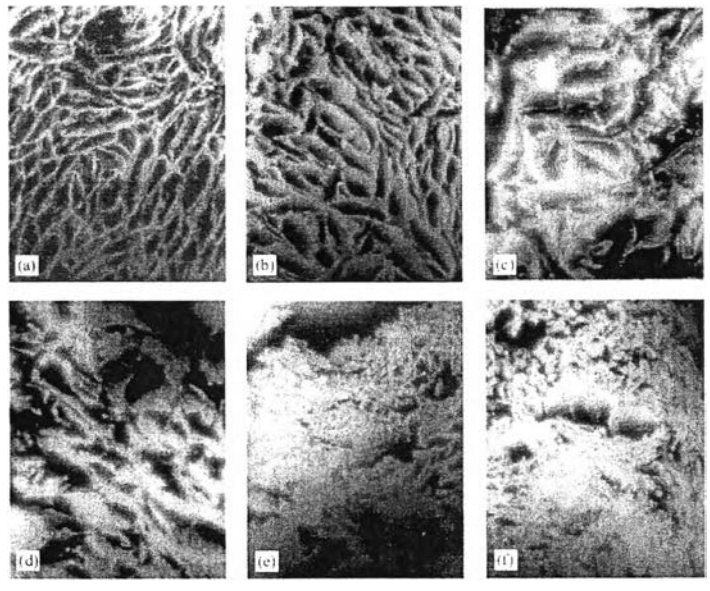

Fig 2.14 SEM micrographs of porous SF sheet (a) before degradation and after degradation by protease XIV for (b) 1 day, (c) 6 days, (d) 9 days, (e) 12 days, and (f) 15 days (Li *et al.*, 2002).

2.4.2 Structure of SF

2.4.2.1 Primary Structure

SF is an insoluble protein containing up to 90 % of the amino acids, like glycine (Gly), alanine (Ala), and serine (Ser). The sequence of amino acids of SF can be expressed as a hexaamino acid $[gly\text{-}ala\text{-}gly\text{-}ala\text{-}gla\text{-}ser]_n$.

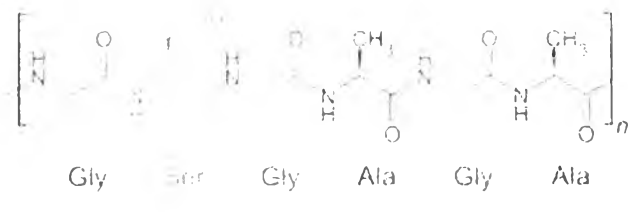

Figure 2.15 Primary structure of SF.

2.4.2.2 Secondary Structure

SF can exist in a number of different conformations known as random coil conformation, Silk I, and β-sheet structure (Silk II). The latter conformation (Silk II) is the solid fibroin that can be found in the spun silk thread while Silk I is a meta-stable form stored in the silk gland of the silkworms (SJ *et al.*, 1999). The amount of β-sheet structure, Silk II, of *B. mori* SF is around 40 % to 50 % (Gosline *et al.*, 1999).

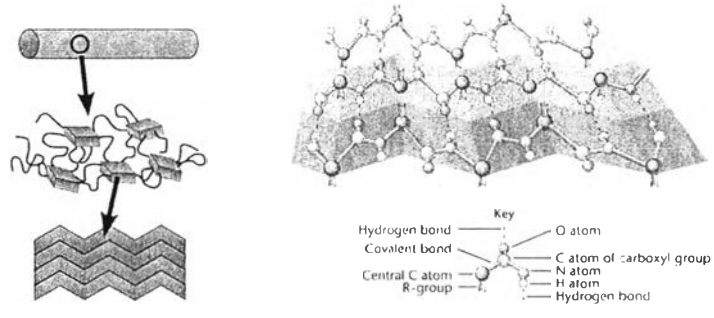

Figure 2.16 (a) Structure of single SF composed of amorphous and crystalline region (Kraig Biocraft Laboratories, Inc., 2008) and (b) β-sheet conformation of the crystalline region of SF.

The β-sheet structure can be induced by several treatments, such as heating, immersion in methanol, shearing, blending with others natural polymers (sodium alginate (Liang *et al.*, 1992), chitosan (Chen *et al.*, 1997) and cellulose (Freddi *et al.*, 1995)) or synthetic polymers (poly (vinyl alcohol) (Li *et al.*, 2001) and poly (acrylamide) (Freddi *et al.*, 1999)) and using chemical crosslinking agent (Rujiravanit *et al.*, 2003).

Noshiki *et al.* (2002) investigated the conformation transition of SF by blending with microcrystalline cellulose (MCC). The shifts of amide I and amide II of silk I to silk II were observed in the Fourier transform infrared (FTIR) spectra,

indicating the formation of β-sheet structure at the MCC content in the range of 70 % to -90 %. It was proposed that the contact between highly ordered surface of MCC and SF promoted the transformation of silk I to silk II. A higher crystalline content of silk fibroin also enhanced the mechanical properties of the composite film.

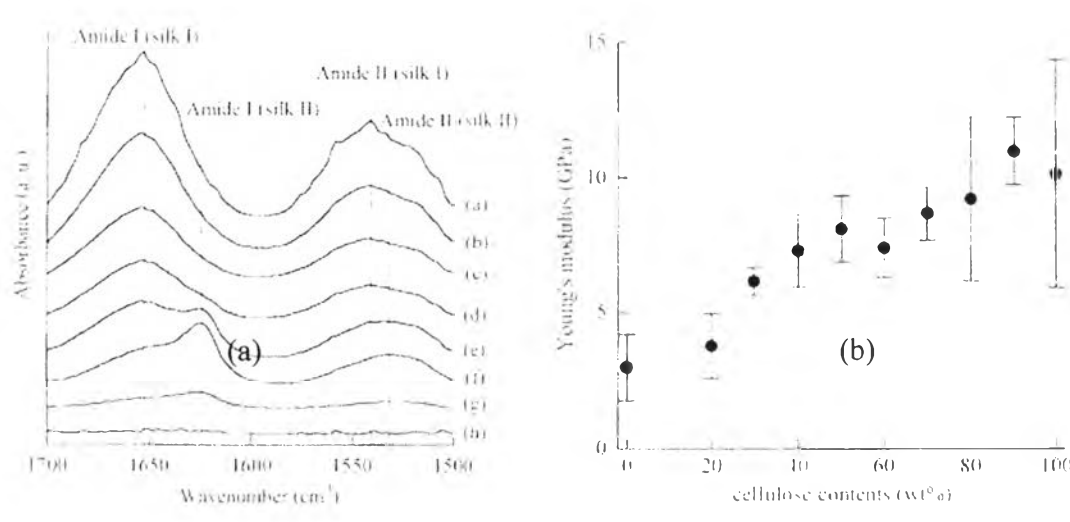

Figure 2.17 (a) FTIR spectra of SF–MCC composite films at SF/MCC ratio of (a)100/0, (b) 80/20, (c) 60/40, (d) 40/60, (e) 30/70, (f) 20/80, (g) 10/90, and (h)0/100. (b) Young's modulus of SF/MCC composite films (Noshiki *et al., 2002).*

Wongpanit *et al.* (2007) studied the effect of methanol treatment on the β-sheet formation of SF component in the chitin whiskers reinforced SF sponge after immersion in a 90% (v/v) methanol solution for 10 min. From the obtained FTIR spectra, a new absorption shoulder, which is the characteristic absorption band of β-sheet structure, was observed. The intensity of this peak was also found to increase with increasing chitin whiskers content.

2.5 Cellulose (CL)

Cellulose, the most abundant natural polysaccharide on earth, is the main constituent of the cell wall in all plants for maintaining their structure. CL is also present in bacteria, fungi, algae, and even in some animals (O'Sullivan, 1996). CL is a fibrous, tough, and water-insoluble substance. The structure of CL contains unbranched homopolysaccharide composed of β-D-glucopyranose unit linked by β-(1,4)-glycosidic bonds (Purves, 1954; Marchessault and Sundararajan, 1983) with a syndiotactic configuration (Dufresneetal, 1997) bonded covalently together into long chains.

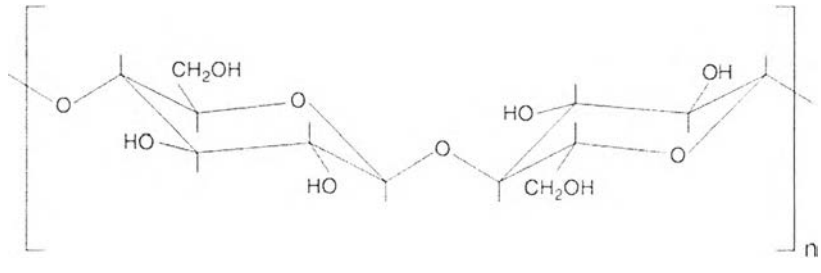

Figure 2.18 Chemical repeating units of CL (Guenet., 2008).

CL chains are biosynthesized and aggregate to form microfibrils by hydrogen bondings between hydroxyl groups and oxygen of adjacent molecule (Samir *et al.*, 2004). The microfibrils consist of monocrystalline CL domains linked together by amorphous domains (Oaksman *et al.*, 2006). The diameter of microfibril, depending on their source, ranges from about 2 nm to 20 nm while their length can reach several tens of microns. The hydrogen bonds continue in the same plane with other chains as well as in planes above and below this plane to form strong fibrous bundles.

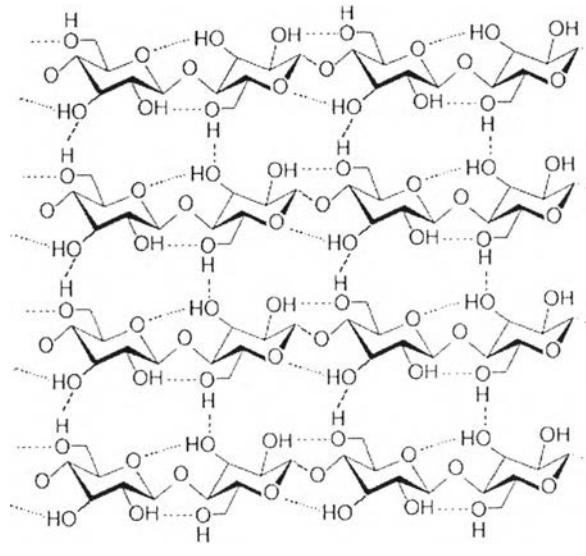

Figure 2.19 Inter- and intra- hydrogen bonding in CL (Sameer *et al.*, 2008)

CL chains are biosynthesized and aggregate to form microfibrils by hydrogen bondings between hydroxyl groups and oxygen of adjacent molecule (Samir *et al.*, 2004). The microfibrils consist of monocrystalline CL domains linked together by amorphous domains (Oaksman *et al.*, 2006). The diameter of microfibril, depending on their source, ranges from about 2 nm to 20 nm while their length can reach several tens of microns. The hydrogen bonds continue in the same plane with other chains as well as in planes above and below this plane to form strong fibrous bundles.

2.5.1 Cellulose Whiskers (CLWs)

Whiskers are stiff rod-like particles which have been grown under certain conditions that lead to the formation of high-purity single crystals (Milewski, 1994) with a variety of aspect ratios *(L/d)* depended on their sources. The whiskers with high aspect ratio give the best reinforcing effect (Samir *et al.*, 2005). In order to obtain the whiskers, a specific acid hydrolysis step is generally used for the digestion of amorphous cellulosic domains (Siqueira *et al.*, 2008). Under controlled conditions, the disruption of

amorphous regions, which acted as structural defects surrounding and embedded within CL microfibrils, occurred and the remaining substance was microcrystalline segment.

The use of sulfuric acid for CLWs preparation leads to a more stable whisker colloid suspension, compared to that prepared by using hydrochloric acid (Araki *et al.*, 1998). The sulfuric acid hydrolysis yields a negatively charged surface of the resultant CLWs, whereas the hydrochloric whiskers are not charged. The surface charge is one of the main parameter controlling the CLWs interaction and the rheological behavior of their suspensions (Samir *et al.*, 2004).

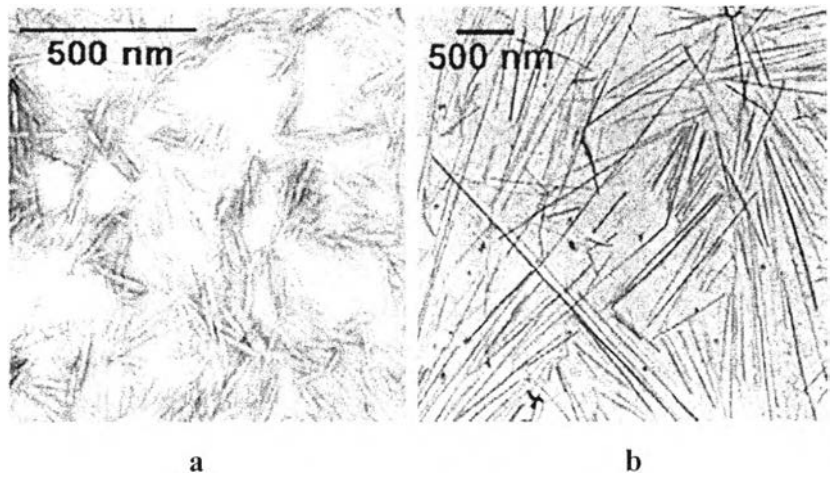

Figure 2.20 Transmission electron micrographs (TEM) of a dilute suspension of hydrolyzed (a) cotton (b) tunicin CLWs (Samir *et al.*, 2004).

The whiskers obtained from acid hydrolysis have highly ordered structure, resulting in high strengths and significant improvement in electrical, optical, magnetic, and conductive properties. The whiskers have received attention to be used as natural nanofillers for reinforcing polymer matrix in biocomposites due to not only their high tensile strength but also others advantages, such as renewable nature, low energy consumption, low density, and low cost (Samir *et al.*, 2004). Another reason why the whiskers have been widely used is an increase in the production of green materials based

on natural resources. Therefore, many researches try to utilize the CLWs as reinforcing nanofillers in environmental friendly matrices.

Noshiki *et al.* (2005) studied the composite material of SF and tunicin whiskers. The SF was prepared by dissolving silk cocoons in lithiumthiocyanate, LiSCN, and the composites were fabricated by using the solution casting technique. The FTIR result indicated that there was a transition of SF conformation from a random coil to an ordered structure, which was related to the highly ordered structure of tunicin CLWs.

Siquera *et al.* (2008) prepared cellulosic nanoparticles, both CLWs and microfibrillated cellulose (MFC), from sisal for being used as reinforcement in polycarpolactone (PCL), a biodegradable matrix. The presence of the whisker fillers increased the glass transition, crystallization, and melting temperatures as well as the degree of crystallinity of the PCL matrix so the CLWs probably acted as a nucleation site. For the MFC-reinforced composite, the modulus was higher than that of the whisker-reinforced composite. Moreover, the introduction of MFC did not increase the degree of crystallinity of the matrix and its melting point tended to decrease. This should be caused by the entanglement of MFC that tended to confine the polymer matrix and restricted its crystallization.

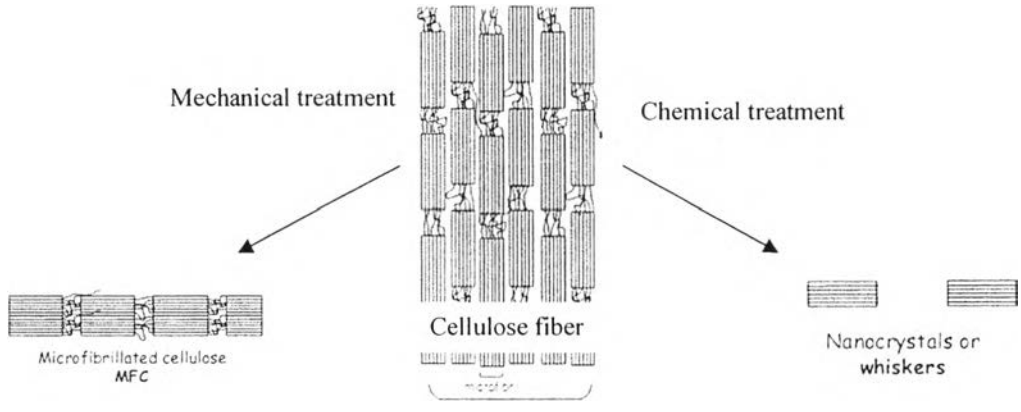

Figure 2.21 The distinguish between MFC and CLWs (adapted from Senechal *et al.*, 2010).

Bras *et al.* (2010) incorporated the whiskers obtained from sugarcane bagasse in NR latex in order to prepare nanocomposites by using the casting/evaporation technique. The length of the incorporated whiskers was in the range 82nm to104 nm while their width ranged from 4 nm to12 nm. The aspect ratio of the obtained bagasse whiskers (about 13) was close to that of whiskers isolated from cotton (about 11 to 12). The prepared composite films were homogeneous. The stress-strain curve revealed that the Young's modulus and tensile strength significantly increased after the addition of the whiskers to the NR matrix, while the elongation at break of the obtained nanocomposite decreased. Adding bagasse whiskers also resulted in an increase in the moisture sorption of the nanocomposite films due to the hydrophilic nature of the whiskers.

The use of microparticles and nanoparticles from natural resources as reinforcement in composite has gained a lot of attention. However, there are a few of researches prepared the whisker from banana tree. Banana plant fibers are fibrous remaining parts of pseudo-stems which left over after banana cultivation. The banana, fruit, constitutes only 12 % by weight of the plant. Thus, the remaining parts become agriculture cellulosic-based waste (Elanthikkal *et al.*, 2010). According to a high content

of cellulose in the residue parts, the rachis, pseudo-stem, and leaf sheath, banana fiber has attracted interest for potential uses as a reinforcing material.

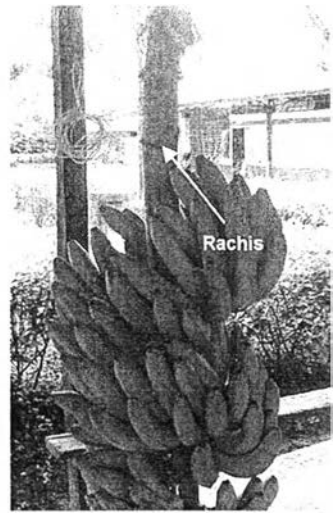

Figure 2.22 Banana rachis (Zuluaga *et al.*, 2007).

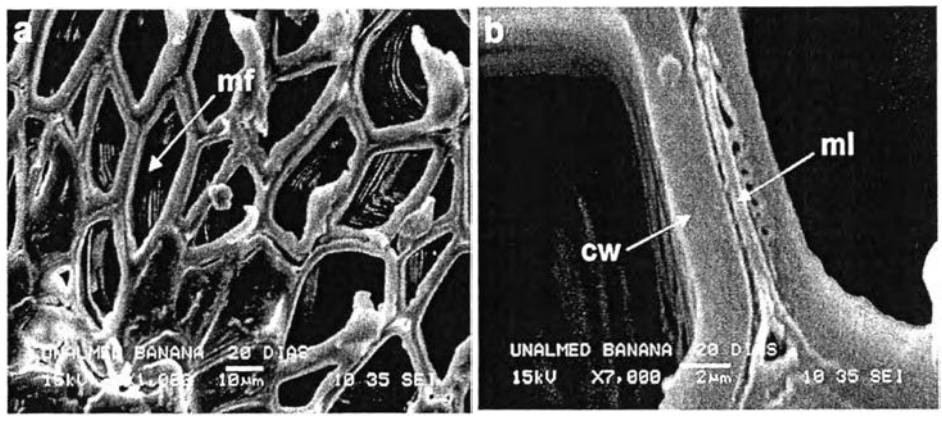

Figure 2.23 SEM micrographs of the vascular bundles in banana rachis showing (a) microfibril (mf) and (b) cell wall (cw) as well as middle lamella (ml) (Zuluaga et al., 2009).

The isolation of cellulose fibers requires the removal of other components — such as lignin, hemicelluloses, and pectin — from the banana fibres. For pectins and hemicelluloses, are solubilized and removed by alkaline solution treatment of the banana fibers. To breakdown phenolic compounds and molecule having chromophoric groups presented in lignin, the bleaching treatment was performed (Elanthikkal *et al.*, 2010).

Zuluaga *et al.* (2007) isolated cellulose microfrils from banana farming residues. The banana rachis was passed through mechanical and chemical treatments. Two different chemical treatments — peroxide/organosolv (PO) and peroxide/homogenization (PH) — were investigated. The width of individual cellulose microfibrils obtained from both treatments was close to 5 nm. However, the PO-microfibrils had a higher cystallinity due to more efficient removal of non-cellulosic, hemicelluloses, and lignin substances.

Elanthikkal *et al.* (2010) examined the possible use of sulfuric acid in acid hydrolysis of banana fibres and the effect of hydrolysis condition on the resultining whiskers. The results showed that not only the acid concentration but also the reaction time used in hydrolysis affected the dimensions of the obtained CLWs.