CHAPTER II

LITERATURE REVIEW

2.1. Surfactant

Surfactant is defined as surface active agents were produced either chemically or biologically and have wide ranging of properties used in various applications (Al-Araji *et al.*, 2007). Chemical surfactants were derived from nonrenewable feedstock while biosurfactants synthesis by aerobic microorganisms in aqueous media (Muthusamy *et al.*, 2008). Generally, chemical surfactant is toxic to environments, have low biodegradability and their manufacturing processes and byproducts can be environmentally hazardous (Maier and Soberon-Chavez, 2000). These characteristics are undesirable because of the increasing environmental concerns among the public and policy of most governments regarding pollutant control, thus biosurfactants became potential candidates to replace chemical surfactants for many applications in various fields (Banat *et al.*, 2000).

2.1.1. Chemical surfactants

Chemical surfactants are widely used in many industrial applications industrial purposes because of its competitive price compared to its counterpart. The usage of chemical surfactants included in enhancing the bioavailability of hydrophobic compounds, to desorb them from solid surface or to increase the apparent water solubility (Najafi *et al.*, 2011). Characterization of chemical surfactants depending on the precise chemical nature exist and the properties exerted such as emulsification, detergency and foaming. The number and arrangement of the hydrocarbon groups with the position of the hydrophilic groups determine the surface-active properties of the molecule as an example, C12 to C20 regarded as the range that covering optimum detergency, whilst wetting and foaming are best achieved with shorter chain lengths. Therefore structure-performance relationships and chemical compatibility are the key elements in chemical surfactant formulations.

2.1.2.Biosurfactant

Pattanathu *et al* (2008) describes biosurfactant as amphiphilic biological compounds synthesized extracellularly or as part of the cell membrane and were produced by a

variety of yeast, bacteria and filamentous fungi in aqueous fermentation media (Chen *et al.*, 2007) excreted as secondary metabolites (Georgiou *et al.*, 1992). Biosurfactants can be classified into several groups in terms of their chemical composition and their microbial origin as shown in Table 1. Biosurfactants structure consisting of hydrophilic moiety and hydrophobic moiety (Desai and Banat, 1997).

Hydrophilic moiety made up by amino acids, peptides, anions or cations, monosaccharides, disaccharides, or polysaccharides. The hydrophilic moiety can be carbohydrate, amino acid, cyclic, peptide, phosphate, carboxylic acid or alcohol (Pattanathu *et al.*, 2008). While, hydrophobic moiety consisting of unsaturated, saturated, or fatty acids (Desai and Banat, 1997). Hydrophobic moiety is a hydrocarbon part which is less soluble in water and this part of the molecule contains a long chain of fatty acids, hydroxyl fatty acids, hydroxyl fatty acids or α-alkyl-β-hydroxy fatty acids. The hydrophobic moiety is usually a C8 to C22 alkyl chain or alkyl aryl that may be linear or branched (Van Ginkel, 1989). Besides, biosurfactants demonstrate a large variety of isoforms which differ by variation of the length and branching of their hydrophobic components as well as by amino acid components in their peptide ring (Bonmatin *et al.*, 2003).

Biosurfactants is better chemical surfactant such as less toxic, more biodegradable, environmentally friendly, and do not lose physicochemical properties at different temperatures and pH levels (Mulligan, 2005). In addition biosurfactants have been found to possess several unique therapeutic and biomedical properties (Singh and Cameotra, 2004) and were able to exert antiadhesive action against several pathogenic microorganisms (Heinemann *et al.*, 2000). Due to their attractive properties, biosurfactants are gaining significant interests for its applications in various fields such as in food, medical, pharmaceutical, cosmetics and agriculture (Banat *et al.*, 2000). The interest in biosurfactants has increased considerably because of these favorable features and it can be potential alternatives to replace chemically synthesized surfactants.

Table 1: Major types of biosurfactants produce by microbes.

Biosurfactants class	Microbes
Glycolipids	
Rhamnolipids	*Pseudomonas aeruginosa*
Trehalose lipids	*Rhodococcus eritropolis, Arthobacter sp.*
Sophorolipids	*Candida bombicola, Candida apicola*
Mannosylerythritol lipids	*Candida antartica*
Lipopeptides	
Surfactin/iturin/fengycin	*Bacillus subtilis*
Viscosin	*Pseudomonas fluorescens*
Lichenysin	*Bacillus licheniformis*
Serrawettin	*Serratia marcescens*
Phospholipids	*Acinetobacter sp., Corynebacterium lepus*
Fatty acids/neutral lipids	
Corynomicolic acids	*Corynebacterium insidibasseosum*
Polymeric surfactants	
Emulsan	*Acinetobacter calcoaceticus*
Alasan	*Acinetobacter radioresistens*
Liposan	*Candida lipolytica*
Lipomanan	*Candida tropicalis*
Particulate biosurfactants	
Vesicles	*Acinetobacter calcoaceticus*
Whole microbial cells	*Cyanobacteria*

Source: (Desai and Banat, 1997; Rosenberg and Ron, 1999)

2.1.3. Surfactin

Among many classes of biosurfactants, lipopeptides group becoming great interest among researcher because of their high surface activities and therapeutic potential (Nitschke and Pastore, 2004; Nitschke and Pastore, 2006). Surfactin was classified under lipopetides excreted by *Bacillus sp.* (Cooper *et al.*, 1981). *B. subtilis* is a sporulating rod bacteria which thrive in the soil and non-pathogenic to human being (Zweers *et al.*, 2008) permits its application in various fields and it is one of the most studied Gram-positive bacteria (Driks, 2002). *Bacillus subtilis* is a well-known producer of lipopeptides (Katz and Demain, 1977). Different strains of *Bacillus* produce a different lipopeptide family and a single strain can also produce lipopeptides with different peptide cores such as surfactin, fengycin and iturin (Liu *et al.*, 2009). The ability of *B. subtilis* to produce lipopeptide has been documented for over 50 years (Xiao *et al.*, 2008; Liu *et al.*, 2009) and created a great potential application for pharmaceutical and biotechnological fields (Kowall *et al.*, 1998; Mulligan, 2005).

Surfactin exhibit diverse biological activities such as antimicrobial properties (Fernandes *et al.*, 2007), hemolytic properties, antifungal, antiviral and antimycoplasma (Singh *et al.*, 2004). In addition, they have antitumor activity against Ehrlich's ascites carcinoma cells, and can inhibit the cyclic adenosine 3,5-monophosphate phosphodiesterase (Davies *et al.*, 2001; Fernandes *et al.*, 2007). The diversity of surfactin can be enhanced by the combinatorial biosynthesis and precursor-directed biosynthesis. This leads to the exploration of surfactin derivatives with a linear structure or a small ring size (Chiocchini *et al.*, 2006). Therefore, novel surfactin derivatized lipopeptides with modified properties from *Bacillus sp.* can be continuously explored (Yakimov *et al.*, 1997).

Surfactin as shown in Figure 1 is a heptapeptide linked to a β-hydroxy fatty acid chain of 13-16 carbon chains. Surfactins demonstrate a variety of isoforms which differ by variation of the length and branching of their fatty acid components as well as by amino acid component in their peptide ring (Bonmatin *et al.*, 2003; Kowall *et al.*, 1998). Surfactin constitutes of a mixture of isoforms and traditional methods have not

been able to establish direct quantitative analyses for individual isoforms concentration and chemical structure.

Figure 1: Primary structure of surfactin (Maget-Dana & Ptak, 1995).

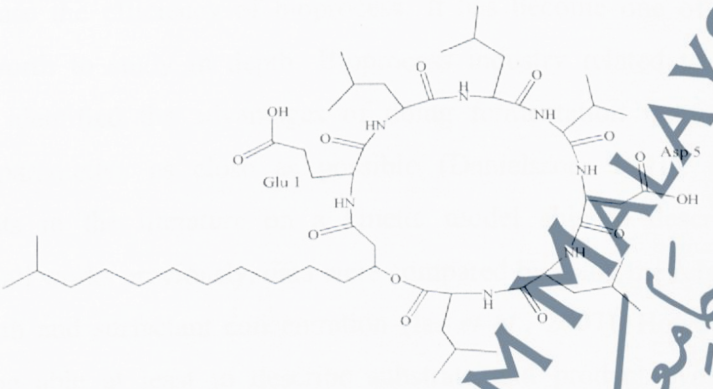

Figure 2: Three-dimensional structure of surfactin peptide moiety. Backbone atoms are displayed in grey. The heavy atoms of amino acid residues (1 to 7) are illustrated. Pale grey represents hydrophobic residues 2, 3, 4, 6, 7 and the attachment of the lipidic chain. Acidic residues 1 and 5 are in black and dark grey, respectively (Peypoux *et al.*, 1999).

Figure 2 shows the three-dimensional structure of surfactin determind by high resolution Nuclear Magnetic Resonances (NMR) integrated with molecular imaging techniques (Peypoux *et al.*, 1999). On one side of the molecule residues 2 and 6 are

facing each other in the acidic Glu-1 and Asp-5 side chains, which define a minor polar domain (Bonmatin *et al.*, 1995).

2.2. Kinetic Studies

Knowing how the fermentation behavior of *B. subtilis* provides vital knowledge in order to increase the efficiency of bioprocess. It has become one of the essential aspects and worth to study in depth. Bioprocess industry related to fermentation activities has identified the advantages of doing fermentation monitoring of the fermentation parameters as close as possible (Danielsson, 1991). Very limited reference exists in the literature on a kinetic model able to describe surfactin production. In all works previously, data were compared by using the relation between bacterial growth and surfactant concentration (Isa *et al.*, 2007). However, a kinetic model must be able at least to describe substrate and product evolutions under operational fermentation conditions (Rodrigues *et al.*, 2006).

Fermentation monitoring covers a vast area and involved with numerous analytical fields (Danielsson, 1991) because of the effective control of fermentation processes demands the measurement as many significant parameters as frequently as possible (Bradley *et al.*, 1991). Kinetic equations that describe the growth of a microorganism on a substrate are important factors in understanding the phenomena of bioprocess. A variety of mathematical models have been proposed to describe the dynamics of metabolism of a microbial population towards bioproduct (Okpokwasili *et al.*, 2008). In all previous studies, analytical data were compared using the relationship between bacterial growth and surfactin concentration (Isa *et al.*, 2007). The Monod equation widely used to describe growth-linked substrate utilization. However, a kinetic model should be at least able to explain substrate and product evolutions under operational fermentation conditions (Rodrigues *et al.*, 2006).

On-line and off-line measurements are frequently used as the main source of information about the condition state of the fermentation behavior with combination of model-based calculations to have the estimations of fermentation condition for monitoring purposes (Dondo, 2001). However, the qualitative and quantitative composition of a fermentation broth is very often difficult to determine accurately

because the raw fermentation broth itself is a complex component contain of nutrient, cell debris, cells, waste products, and interested products. Besides the chemical and physical properties of the broth are continuously changing with time. Therefore, it is important to have an accurate and consistent set of approaches for measurement purpose of each parameter considered in the fermentation process.

The determination of reliable kinetic constants of a fermentation process is a difficult task due to limitations in the usual laboratory procedures to measure biomass, substrate concentrations and also due to the dynamic response of the cells under different environmental conditions. The measurement of fermentation process parameters in fermentation technology has been well investigated by previous study (Danielsson, 1991) however, the technique can be improvised with the advancement of analytical tools present nowadays. Monitoring of the fermentation process includes a wide range of analytical methods to efficiently measure the fermentation parameters (Bradley *et al.,* 1991). Therefore, the development of a fermentation process model for scale-up and bioreactor design is necessary. Development of bioprocesses is another important aspect of biosurfactant production using various media. Several different issues need to be looked into before a standard procedure is laid out for setting up the process at industrial levels.

2.2.1. Fermentation Broth

The fermentation broth is very complex solution where the microorganisms grow and reproduce makes the characterization difficult to achieve (Oka *et al.*, 1993; Pursell *et al.,* 2004). The fermentation broth supplies the microorganisms with all the nutrients the microorganisms need to grow and produce the various fermentation products (Cooper *et al.*, 1981). Fermentation broth may have wide range of compounds contains all three main phases which are solid, liquid, gases and their possible interactions such as raw substrates (Chen *et al.*, 2007), surfactin (Isa *et al.*, 2008, Isa *et al,* 2007), microorganisms and its derivative components (Pursell *et al.*, 2004), chemical additives added to the fermentor (Cooper *et al.*, 1981; Isa *et al.*, 2007).

The qualitative and quantitative composition of a fermentation broth is very often difficult to determine accurately because the medium itself contains a complex

component, besides the chemical and physical properties of the broth are continuously changing with time (Buttler *et al.*, 1996). The composition of raw fermentation broth was well characterized by Mulligan and Gibbs (1990) as shown in Table 2. Hence the development method of detection of the major component in fermentation broth is important works in order to characterize the final media composition.

Table 2: Components classified in the raw fermentation broth [a].

Macromolecules	Mid-molecules	Small molecules
Surfactin micelle (30,000–100,000)	Surfactin monomer (1036)	Alcohols (46)
Polysaccharides		MS medium (80–400)
Peptides		Glycine (75)
Proteins		Alanine (89)
		Phosphate (100)
		Serine (105)
		Threonine (119)
		Phthalic acid (150)
		Amino acid (200)

[a] The numeral in the parentheses indicates the molecular weight in g/mol.

Source : Mulligan and Gibbs, 1990

2.2.2. Detection of Surfactin in Fermentation Broth

Research on formulation, characterization and pharmacokinetics studies of surfactin, render and accurate, effective, quick and reproducible analytical method for identification and quantification of surfactin. Identification and quantification of surfactin produced by *B. subtilis* strain is complex since it produces a series of isoforms which slightly differ in their physiochemical properties due to (a) variations in the chain length and branching of its hydroxy fatty acid component (Hosono and Suzuki, 1983) as well as (b) substitutions of the amino acid components of the peptide ring (Peypoux *et al.*, 1991) besides the complexity of fermentation broth (Oka *et al.*, 1993).

Earlier studies for surfactin identification and quantification involved measurement of the surface of interfacial tension of fermentation culture broth or by thin-layer chromatography (Cooper *et al.*, 1981). However, neither of these methods is satisfactory for quantitative analysis of surfactin because the presence of impurities that might influence the quantitative analysis. In contrast, High Performance Liquid Chromatography (HPLC) assay is specific for surfactin identification and the quantification is highly sensitive and reproducible. HPLC is one of the best methods used to identify and quantify surfactin and has been reported in various research publication however, most of previous methods used to employ gradient elution causes base line shifting and it is time consuming, which involved total sampling time of more than 30 minutes (Fonseca *et al.*, 2007; Isa *et al.*, 2007; Lin *et al.*, 1998; Wei and Chi, 2002). Delay in total sampling time will result in an increase amount of mobile phase used which is not favorable for the cost effectiveness. The current method should improve to obtain faster analysis, therefore there is a demand for a quicker and reliable improved HPLC method for qualitative and quantitave measurement of surfactin. Reducing the total elution time of surfactin analysis is important for reducing the amount of mobile phase used thus make the method more cost effective and environmentally friendly which can increase competitiveness of the commercial aspect.

2.2.3. Detection of Glucose in Fermentation Broth

One compound present in fermentation broth solutions is carbon feedstock (glucose, fructose, lactose, sucrose, etc.) which are vital sources for cell growth and surfactin synthesis. Glucose was assimilated during bacterial growth and biosurfactant production (Casas *et al.*, 1997; Cooper and Paddock, 1984) until the source depleted. The carbon sources present in fermentation broth could directly impact the yield and quality of the surfactin. Hence, it is desirable to characterize the fermentation broth to optimize media formulation development, manufacturing process performance with nutrient supplementation, and endpoint definition (Hanko and Rohrer, 2000).

Therefore, the measurement of carbon sources present in fermentation broth is particular interest. Biosensor were commonly used to analyze glucose, however, this method cannot simultaneously determine multiple compounds in raw fermentation

broth (Schugerl *et al.,* 1993). Hence, for any method that need to be developed for detection of glucose must satisfy these conditions; (a) allow measurement over the range of interest, (b) respond rapidly to changes in analyte concentration; and (c) their signals must be easily interpretable (Bradley and Schmid, 1991).

One of the techniques suggested for glucose detection is HPLC equipped with detector such as refractive index (RI) (Rodrigues *et al.,* 2006) and light scattering detector (LSD) (Garcı´a-Ochoa and Casas, 1999). HPLC is a very powerful and commonly used technique in off-line analysis for determination of glucose concentration on biosurfactant works (Rodrigues *et al.,* 2006; Garcı´a-Ochoa and Casas, 1999; de Oliveira *et al.,* 2013). The obvious advantage of HPLC is its capability for multicomponent analysis in a complex media. This HPLC protocol bringing us closer to understanding the fermentation process and fermentation kinetics itself. HPLC has been always couple with RI (de Oliveira *et al.,* 2013) as a detector for detection of sugar in biosurfactant works, but is limited by poor sensitivity and specificity (Buttler *et al.,* 1993). Besides, the RI detection only reliably be used when the concentration of the analytes is high and the influence of co-eluting compounds are small (Buttler *et al.,* 1996). A reliable technique for glucose analysis would undoubtedly improve greatly the efficiency of fermentation monitoring and allow the information for development of new feedback control strategies.

2.3 Recovery and Purification of Surfactin

Nowadays, more and more high-value bioproducts that offer advantageous characteristic for mankind being produced by using fermentation techniques. However, it brings new challenges for recovery and purification steps due to the complexity component of fermentation broth (Oka *et al.,* 1993). Till now, surfactin have not been able to compete economically with chemical surfactants because of the uncompetitive price due to downstream process contributed to the major part of the production cost (Keller *et al.,* 2001). A lot of efforts have been done to lower the cost of downstream processing include membrane-based techniques, foam fractionation, extraction, adsorption and liquid membrane extraction.

The conventional method for recovery and purification of surfactin involves

precipitation at low pH followed by extraction with organic solvent such as dichloromethane and chloroform resulted in a low purity. The impurities will co-precipitate and further steps of chromatographic techniques need to be used to improve the purity of the final product. Besides, the chemical extraction method is disadvantageous due to the usage of toxic organic solvents which environmentally hazardous and can result in the loss of surfactin activity besides more than two stages of process involved make this conventional techniques impractical and less attractive to be practiced in industry.

Another technique to recover and purify surfactin involve continuous in situ removal of surfactin from fermentation broths by foam fractionation, which will then continue with acid precipitation (Cooper *et al.,* 1981) and extraction with organic solvents or by blowing foam out of fermentor to be collected, centrifuged, and extracted by acetone precipitation. This method involved with the usage of high amount of organic solvent and it makes the final production cost increase as the functionality of the final product will be doubted

Chen *et al.,* (2007) proposed a method however it requires extra steps of pretreatment of fermentation broth involves acid precipitation to recover surfactin and redissolution of precipitate using sodium hydroxide at pH 11 before applying filtration with two-stage dead end UF technique. This process offers high recovery and high purity of surfactin however the extra steps taken would further add to the complexity of the process and could have an effect to the final cost of surfactin production.

For all of these methods mentioned, no measurement on the functionality of the surfactin final fraction was conducted which is important to evaluate the efficiency of the whole process. Continued efforts must therefore be redirected to improve the downstream processing in order to optimize the yields of biosurfactants.

2.3.1 Membrane Filtration

Membrane filtration is one of the separation process and widely used in various industrial fields around the world where the development of membrane filtration technology begins for the bacteriological analysis of drinking water. In several years

back, membrane filtration has been used for recovering bioprocess products (Hwang and Wang, 2012). Membrane filtration played a major role in purification of many byproducts in which the process has been adopted from technology developed originally for blood fractionation, food, dairy and water industries. The surfactin separation efficiency is the essential issue in developing commercial scale processes. From previous scholar, membrane process has been found to be more environmentally friendly and economical (Isa *et al.,* 2007). By times, new membranes have been created to meet the requirement of industries (Reis and Zydney, 2007) and can be classified into four major groups which are microfiltration, ultrafiltraton, nanofiltration and hyperfiltration which differ in their pore size.

2.3.2. Principle of Membrane Filtration

The first practical application of membrane filtration technology is in the analysis of water for coliform bacteria. The principle of membrane filtration is to separate different molecules based on size (Nielsen, 2000), it acts like selective sieving mechanism, where certain molecules will pass through membrane (Cuperus and Nijhuis, 1993) known as permeate while the feed retained by the membrane known as retentate. Permeation of certain molecules from a solution results in an increase in the concentration of the remaining particles in the retained solution. The membrane filtration process can be divided into different categories based on the driving force which are concentration, electrode potential difference and pressure.

Under pressure driven membrane filtration processes, there are four types of filtration classified which are microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). The classification depends on the basis of the size of the molecules or components passing through the membrane filtration process (Mistry, 2002). Our interest in this is to use the ultrafiltration (UF) membrane which the pore size ranges from 1.0 nm to 0.05 μm to segregate surfactin from raw fermentation broth.

The UF system was selected in this study because of the ability of the UF membranes to separate surfactin components from raw fermentation broth, where small molecules

such as water, minerals, sugars, amino acid pass through the membrane while large molecules such as surfactin are fully or partially retained (Wagner, 2001).

2.4. Ultrafiltration

Ultrafiltration (UF) is a pressure-driven membrane separation technique for dissolved and suspended species based on the size and molecular scale (Keller *et al.,* 2001) widely used in various chemical and biochemical processes. The UF process essentially involves no phase change or chemical reagents (Chen *et al.,* 2007), the technique became the alternatives for downstream process because it is easy to scale-up and became economically attractive in recent years (Zondervan and Roffel, 2008). UF is a promising technology especially for bioactive compound using pressure ranging between 1 bar to10 bar as the driven force (Nielsen, 2000). The favorable characteristics of UF like minimize physical damage of biomolecules from shear effects, minimal denaturation, high recovery yield, and the avoidance of resolubilization make it excellent for downstream applications. UF membranes are characterized in terms of their ability to retain proteins of a particular molecular weight (MW) and thus the terms of molecular weight cut-off (MWCO) are used to define the size of protein or any other molecules that would be retained by a UF membrane.

UF system has been used as an effective process to segregate surfactin molecules from the fermentation broth as reported by a previous study. The effectiveness of applying UF in recovery and purify surfactin because of the surfactin is able to form micelles that big enough to be rejected by membrane within the critical micelle concentration size. At concentrations above the critical micelle concentration (CMC), surfactin molecules readily associate to form supramolecular structures such as micelles or vesicles, with nominal molecular diameters up to two to three orders of the magnitude larger than that of single unassociated molecules (Isa *et al.,* 2007). Theoretically, surfactin micelles can be retained by any membranes with the pore within the CMC size.

In this study, cross-flow ultrafiltration equipped with polyethersulfone membrane (PES) and regenerated cellulose membrane (RC) each with MWCO 10 kDa and 30 kDa were used for filtration of raw fermentation broth of *B. subtilis*. The aim of this

work is to evaluate which type of membrane better in terms of recovery and purity of final surfactin fractions. The objective was to investigate the effects of the type's membrane with different MWCO operated under different TMP on the recovery and purification of final surfactin fractions.

Several researchers had applied membrane system for extraction of biosurfactants were Isa *et al.*, (2007, 2008), Lin and Jiang (1997), Mulligan and Gibbs (1990), Sen and Swaminathan (2005) and Chen *et al.*, (2008a) and many more. Among previous study, Mulligan and Gibbs (1990) successfully recover rhamnolipids (one type of biosurfactants) from complex fermentation broth with one step UF.

Recovery and purification of surfactin from broth sample with one step of UF was further studied by Sen and Swaminathan (2005) by using a stirred cell device and evaluating some filtration characteristics and their effects on the recovery and purity of the final products This works managed to recover surfactin with a purity value based on critical micelle concentration (CMC) of 70%. Recovery of surfactin achieved up to 95% by Lin and Jiang (1997) however no report on the purity of the surfactin by using all of these methods.

Isa *et al.* (2008) used a two-step tangential membrane filtration process to recover and purify surfactin from the complex fermentation broth of *B. subtilis* ATCC 21332 and found that PES with a MWCO of 10 kDa was suitable for surfactin purification due to the high recovery rate besides the TMP had no significant effect on the filtration rate and surfactin rejection.

Juang *et al.* (2008) used dead-end ultrafiltration of fermentation broths. The broth was pre-treated by centrifugation under a centrifugal effect of 10,000 x g. He reported that the filtration resistance due to solute concentration polarization dominated the flux decline, while the sum of resistances due to cake formation and solute adsorption contributed below 3% of the overall resistance.

Chen *et al.* (2008a) used cross-flow ultrafiltration equipped with PES membrane with a MWCO of 100 kDa to purify acid-based pre-treated fermentation broths. The filtration rate increased with increasing cross-flow velocity, but decreased with

increasing initial surfactin concentration and TMP. There is demand to develop environmentally and economically method to increase purity and recovery yield of surfactin.

2.5. Principle of Membrane Ultrafiltration

UF system efficiency was evaluated by its ability to remove suspended/colloidal particles via a sieving mechanism based on the size of the membrane pores relative to that of the particulate matter for the application of solid-liquid separation and low–molecular weight compound separation. According to Cheryan (1998), the UF mostly used for aqueous fluid treatment and its application system in consideration of this parameter which are (i) rejection of impurities, (ii) retention and concentration variable and (iii) permeation and purification of valuables.

The term MWCO always expressed in Daltons (Da, a unit of mass) to describe the lowest molecular weight solute that is generally retained by the membrane. The MWCO levels for UF membrane range from 10 kDa up to 500 kDa. Theoretically, UF membrane with a specific MWCO permeates molecules with a molecular weight exceeding the MWCO. However, the MWCO values are not absolute because the degree of a particular solute is retained by a UF system is not entirely dependent on its molecular weight. Another factor such as the shape of solute, its association with water and its charge must considered in the selection in UF system. These characteristic knowledge are important to understand the UF and the flux of whole process. The basic removal mechanism in UF need to consider is the hydrophobic interactions between hydrophobic organics and hydrophobic tail groups of surfactants and electrostatic interactions between inorganic pollutants and hydrophilic head groups of surfactants (Cheryan, 1998).

2.6. Factors Affecting of UF

According to Cheryan (1998), they are six common factors affecting the retentivity of UF membranes which are (i) size of molecules, (ii) shape of molecules, (iii) membrane configuration, (iv) type of membrane materials, (v) presence of other solutes, (vi) absorption of solutes by the membrane and (vii) type of feed flow.

2.6.1. Size of Molecules

UF membranes have very broad pore size distributions which covering more than one order of magnitude. The water and low molecular weight solutes will pass through the membrane while the suspended solids and solutes of high molecular weight will be retained on the membrane (Cheryan, 1998).

2.6.2. Shape of Molecules

In UF, the size and shape of molecules are the most important parameters affecting the separation (Jauregi *et al.,*2013). The shape and conformation of macromolecules are also affected by ionic strength, temperature, and interaction with other components. The differences in shape could be the reason the selection of UF system. A linear molecule has a greater probability of passing through the pore than a globular molecule of the same molecular weight.

2.6.3. Membrane Configuration

The different types of membrane configuration are affected the rejection coefficient and permeate flux. Therefore, rejections coefficient and flux for the same membrane could be different in different module designs, such the rejection coefficient of flat-sheet membrane configuration rejections are different with hollow fiber membrane configurations.

2.6.4. Type of Membrane Materials

Different membrane materials with the same nominal MWCO will appear to give different solute rejection (Isa *et al.*, 2008). As an example, regenerated cellulose (RC) membrane has broader pore size distribution compared to PES membrane, thus make RC membrane have higher rejections and show less deviation between observed and actual rejections and less effect of TMP on rejection (Isa *et al.,* 2007; Isa *et al.,* 2008). These phenomena are probably related to fouling effects, hydrophobicity, charge and surface roughness.

2.6.5. Presence of Other Solutes

Low molecular weight solute presents in fermentation broth whose molecular size is smaller than the membrane pore will be freely permeated. No rejection observed to these small molecules. The permeability of individual components in a mixture

depends on the relative sized of these components and the pores. Their presence in the feed solution will not usually affect the permeability of large molecules, such as protein, unless they interact with the large molecules and cause molecular changes. However, if there are two or more high molecular weight solutes in the feed solution, their retention will be different than if they were present individually for the smallest of the macromolecules. This is due to concentration polarization and the formation of a secondary dynamic membrane by the macromolecule on the synthetic membrane filter that inhibits passage of the smaller molecules (Cheryan, 1998).

2.6.6. Absorption of Solutes by The Membrane

Solute-membrane interactions that results in the physical adsorption of the solute by the membrane, whether on the surface or in the pores will decrease the yield of the solute. The adsorption of solutes depends on the nature of solutes and type of membrane. This adsorption phenomenon affect apparent rejection coefficient values, since the solute will not appear in permeate in full concentration until the adsorption sites are completely saturated (Cheryan, 1998).

2.6.7. Type of Feed Flow

Currently, there are two types of feed flow, which are dead-end filtration mode and crossflow filtration mode (Isa *et al.*, 2007). The feed plays a very important role as it affects the permeate flux which determines the performance and efficiency of the whole process.

In dead-end filtration, the feed flow enters perpendicular to the membrane surface, essentially all of the flow which can permeate passes through the membrane. By time more and more liquid will pass through the membrane, make more particles build up on top of membrane surface forms a layer until the flow resistance becomes too large make the flux cannot be maintained. At that point, the operation must be shut down, the solids need to be removed, and the flow restarted (Humphrey and Keller II, 1997). Hence, the smaller particle concentration in permeate will decrease while the cost of separation due to the higher operational costs such as pumping cost will increase (Baker, 2005).

While the feed flow parallel to the membrane surface known as the cross flow. Theoretically, cross flow mode avoids deposition of large particles on the membrane surface by the sweeping effects. The non-permeating species tend to be swept along the velocity of the retentate stream (Humphrey *et al.,* 1997). Cross flow filtration results in less fouling and maintain flux longer in comparison to the dead-end flow (Isa *et al.,* 2008). The cross flow filtration process were demonstrated the concentration polarization will taken an effect at much longer time. In membrane separation, the concentration polarization is unavoidable phenomena however we could minimize the effects.

2.7. Factors Affecting Membrane Separation

Without requiring phase change make membrane separations as a potential alternative to replace any other downstream techniques, membrane separations already replaced several conventional downstream processing. The membrane separations becoming an increasingly important tool for separation and concentration of a variety of materials ranging from oil/water emulsion to waste sludge. Although there is considerable interest in the use of membrane technology, its efficient operation is hindered by two major problems, namely concentration polarization and fouling.

2.7.1. Concentration Polarization

Concentration polarization (CP) defined as the accumulation of solute species at the upstream surface of the membrane (Delaney *et al.,* 1977), it is becoming a huge problem resulted in reduction of the permeate flux. Hence, the result from this phenomenon can be seen from the decreasing driving force for filtration or an increasing resistance against transport of the permeating species during the filtration (Fernandes *et al.,* 2007). CP is considered to be a hydrodynamic/diffusion phenomenon (Glover *et al.,* 1974; Glover, 1980) but this problem can be alleviated by operating the system at a higher feed velocity if the system can tolerate (Merin *et al.,* 1980). Sablani *et al.* (2001) had considered CP to be reversible and can be controlled in a membrane module such as velocity adjustment or pulsation.

CP appears to be one of the problems occurs in membrane processing because of the fundamental limitations of mass transfer and the existence of the boundary layer (Zhao *et al.,* 2000). By the appearance the polarizing layer, the concentration of the

solution on the membrane will become higher make the permeate flow decreased. It often leads to major losses in membrane hydraulic permeability and alters the solute rejection characteristics of the membrane (Iritani *et al.,* 2002). There are interplay between the adsorption and concentration of protein molecule in UF that caused flux decline in constant pressure mode (Gekas *et al.,*1993). In addition, recent works try to reduce the permeate flux decline by applying of model (Ghosh *et al.,* 2007). These models apply a different mechanism to explain the solute accumulation on the membrane. Generally, it can be subdivided into resistance models, gel polarization models and osmotic pressure models or a combination of any of these models.

2.7.2. Membrane Fouling

Fouling involves the adsorption of particles (foulants) present in the feed stream transported across the membrane, by times it would create a barrier makes the permeating species unable to pass through. Some typical foulants are protein, lipids, bacteria and any other molecules present in raw fermentation broth. The basic mechanisms of fouling have been studied by previous researcher (Howell *et al.,* 1980; Matthiasson, 1983; Aimar *et al.,* 1988); foulants could enter the module in the feed as particulates, gel or soluble, high molecular-weight species, or they may precipitate from solution as part of the feed permeates. There is a flux towards the surface of the membrane, caused by the flow of material through the membrane, these foulants tends to migrate to the membrane surface. Sablani *et al.,*(2001) suggest by adjusting the operation parameters will reduce the fouling effect.

The causes appear in membrane fouling may be due to these following mechanisms which are:

(1) Formation of a dynamic membrane (suface layer or cake layer) on the front face of the membrane. Fouling occurs predominantly on the membrane surface where the dynamic membrane controls membrane behavior because the interaction between membranes surfaces with feed solution.

(2) Fouling within the membrane structure. Previous studies were found that they were proteins deposits within the membrane pores as well as on the surface (Labbe *et*

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al., 1990; Attia *et al.*, 1991). However, the amount of protein deposited within the pores is small compared with that on membrane surface (Zhao *et al.,* 2000).

(3) Fouling at the entrance. Previous work (Le and Howell, 1984; Devereux and Hoare, 1986; Weldring and van't Riet, 1988) had considered the deposition of solute species on the surface of the membrane that some way obstructed the pore entrances. The loss of effective membrane surface porosity is dependent upon the size of the deposition molecule and the pore size, the net effect of this blockage is the permeate flux would reduce.

This fouling problem can be reduced by ameliorates the effects of fouling which are:

(1) Maintaining the velocities on the feed side of the membrane as high as possible. The higher the velocity, the more shearing action is exerted near the membrane surface and the greater will be the tendency to resuspend surface coating in the feed stream.

(2) The used of cleaning cycle. This means of cleaning involve stopping the feed and taking all modules out of service at the same time. Cleaning solutions either dissolved the solids or reduce their physical bond with the membrane surface, so that they can sweep away.

(3) The using of blowback, or reverse flow from the permeate side of the membrane to the feed side. Blowback is affected by increasing the pressure of the permeate stream to a value greater than the feed pressure.

2.8. Factors Affecting Membrane Fouling

Fouling is a result of specific interactions between the membrane and feed solution, and between the adsorbed solute and other solutes in the feed solution. Each component in the feed solution will react differently with the membrane such as conformation, charge, zeta potential, hydrophobic, interaction and other factors will have a significant bearing on these membrane-solute interactions. According to Cheryan (1990), membrane material properties, solute properties, and operating

parameters affecting fouling phenomenon. Besides, physical process factors, such as cross-flow velocity, pressure and temperature could give an effect on fouling.

2.8.1. The effect of Feed Solution

2.8.1.1. Concentration

From previous studies (Olson *et al.*, 1977; Balmann and Nobrega, 1989), by increasing the feed concentration will result in a decreasing in permeate flux, but has little effect on the membrane retention characteristic, except if the component size present in the feed solution changes with concentration. By increasing the feed concentration will give a little effect on irreversible membrane fouling but causes an increase in reversible gel formation. When internal membrane fouling dominates, increasing concentration increases the rate of membrane fouling. At higher concentrations, cake or surface fouling is likely to dominate.

2.8.1.2. pH and Ionic Strength

pH and ionic strength are the issued need to be considered (Muller and Hayes , 1973; Hayes *et al.*, 1974) as the raw fermentation broth of *B. subtilis sp.* contains protein (Mulligan, 2005). Proteins are complex molecules and their aggregation or interaction with the membrane surface, which influenced by the pH and ionic strength currently not clearly understood. Generally, flux becomes lower at the isoelectric point of the protein and is higher as the pH is moved away from the isoelectric point because it will affect the solubility and conformation of feed components. The solubility of a protein is generally lower at the isoelectric point. However, if there are presents of salts that decrease in solubility and precipitate on the membrane then flux could be decreased at higher pH (Cheryan, 1998)

The explanations could be made with the observed membrane behavior with pH and ionic strength that are; (1) changes in protein conformation and stability would affect the tendency of the protein to deposit on the membrane; (2) changes in the proteins effective size alter the porosity of the dynamic membrane; (3) changes in the charge difference between the protein and the membrane surface affect protein adsorption or deposition (Zhao *et al.,* 2000).

These three explanations have some validity based on previous study because the contribution of each factor is dependent upon the protein type, conformation and pretreatment, and the type of membrane material used.

2.8.1.3. Component Interaction

In a complex feed solution contain various sizes of molecules, there have various interactions between the membrane and different kind of solutes in the feed stream. The presence of larger molecules in the feed solution will cause a steric hindrance to the passage of smaller molecules through the membrane. This occurs because the larger component forms dynamic membranes thus decrease the porosity than the original membrane, or because the larger component interacts within the membrane pores. In some case, specific component interaction in the feed solution may also affect the retention of different components (Blatt *et al.*, 1970).

As for example, proteins is a large size components molecules present in feed solution which contributes into fouling problems in membrane systems. The decrease in flux has been correlated with increasing protein deposition on the membrane. On the other hand, proteins also will be affected by the pH, ionic strength, shear, heat treatment and other environmental factors (Marshall *et al.*, 1993). Besides, proteins have the multiplicity of functional groups, the charge density within protein molecules, the varying degrees of hydrophobicity, and the complex secondary and tertiary structure that allows a protein to interact with other feed components, as well as the membrane itself.

2.8.1.4. Prefiltration and The Removal of Aggregates

A series of studies showed the removal of large molecular weight compounds by prefiltration or any other treatment techniques to remove unwanted component resulted in an improvement in the permeate flux in the membrane system (Tanny *et al.*, 1982; Merin *et al.*, 1983). Large molecules that potentially block the membrane pores such as protein aggregates will result in disproportionate loss of flux, and may act as seeds or catalyst for the formation of a protein fouling layer on the membrane surface.

2.8.2. The Effect of Membrane Material and Physico-Chemical Properties

Two characteristic of membrane appear to influence fouling, viz: (a) the pore size, porosity and the morphology of the membrane surface, and (b) the physico-chemical properties of the membrane. The proper form of the membrane must be considered for the UF process to minimize the membrane fouling because different types of membrane configuration have different susceptibilities to fouling. For example, hollow fibers membrane are the most susceptible and plate-and-frame configurations are the least influence by fouling.

2.8.2.1. Pore Size

Numerous examples showed membrane fouling is more severe with increasing pore size (Gatenholm *et al.,* 1988; Nobrega *et al.,* 1989). Pores size and solutes in a feed solution are becoming important factors need to be considered in the membrane filtration process. Larger pore membranes have higher flux than tighter membrane. If the size of the particle to be separated is the same order of magnitude as the range of pore sizes being used, some of the smaller particles in that feed sample could lodge in the pores without necessarily going through them. This physical blockage of the pores will cause a rapid drop in flux in the first few minutes of operation. In contrast, if the pores are much smaller than the particles to be separated, the particles will not get caught within the pores but will roll off the surface under the shear forces generated by the flow.

2.8.2.2. Porosity and Pore Size Distribution

Most UF membranes have a wider pore size distribution. The flow through the pores dominates the total permeate flow and as a consequence, the permeate flux profiles are very sensitive to fouling or plugging of the pores. Membrane fouling will change the pore size distribution and pore density of the membrane. Thus the permeate flow, component retentions and membrane selectivity change as the membrane fouls with time.

2.8.2.3. Physico-chemical Properties

Many situations arise where physico-chemical interactions occur between solution species and membrane materials. For example macrosolute such as proteins can bind

to the polymer surface by a variety of mechanisms (Hofstee, 1982) including electrostatic interaction, hydrophobic effects, charge transfer (hydrogen bonding and π- π bonding) or a thorough combination of these.

Physico-chemical properties includes charge effects and hydrophobility.

(1) Charge effects: The charges on a membrane are strongly dependent upon the membrane materials, the pH and ionic strength of the feed solution (Heinemann *et al.,* 1988; Brink and Romijn, 1990). A study carried out by Nakao *et al.,* (1988) shows that operation with a membrane of similar charge of the protein can enhance the permeate flux if concentration polarization minimized. With membrane of large pore size, greater protein selectivity may be possible.

(2) Hydrophobility: a series of investigation prove that protein are generally less easily adsorbed to hydrophilic membrane than hydrophobic membrane, besides, there is the potential for permeate flux improvement for hydrophilic membrane (Fane and Fell, 1987; Stengard, 1988). However, when concentration and total protein deposition are high, the effect of hyrophobility is masked by the effects of concentration polarization may be due to increased protein denaturation at the surface of the membrane (Sheldon *et al.,*1991).

Membrane material properties closely related to the passage of molecule through the membrane. In order to measure the relative hydrophilicity of a membrane, we always consider wettability of the membrane by measuring the contact angle of water with the membrane surface. Otherwise, a hydrophobic material would repel the water, causing it to have a high value of the contact angle. For hydrophilic material, a drop of water would spread out on the surface resulting in a zero or low contact angle. The appropriate membrane should hydrophilic (water-attracting) for the aqueous feed stream. For hydrophobic (water-repelling) material, it will adsorb hydrophobic components resulting in fouling.

Besides, any factors such as the roughness of the membrane surface need to be in consideration. As examples, cellulosic membranes like RC membrane foul less

compared to the other polymeric membranes like PES membrane. This is because the surface of cellulosic membrane appears to be smooth and uniform. In contrast, polyamide thin-film composite membrane appear to have protuberance on the surface which could act as hooks for suspended matter in the feed, thus leading to greater fouling. In addition, the largest protein deposit occurred with the most heterogenous membrane (Suki *et al.,* 1988).

It is a vital part to understand the physiochemical characteristics of individual feed components in relation to membrane process. This is must to be considered because solutes are the main causes that will deposit onto a membrane surface or into membrane pores thus degrades membrane performance.

2.8.3. The Effect of The Process Variables

Process parameters such as temperature, feed flow and pressure could have great influence on membrane fouling.

2.8.3.1. Transmembrane Pressure

Transmembrane pressure (TMP) could be one of factor affecting fouling phenomena. By increasing the TMP in the low pressure range (< 4 bar) initially resulted in an increase in permeate flux, but also an increase in the fouling rate (Forman *et al.,*1990; Jonsson, 1986). Membrane retention increases with the increase in membrane fouling and appears to remain constant only at very low pressure and low concentrations. The optimum pressure must be obtained to maximize the permeate flux, while the optimum pressure decreases with the increasing of membrane pore.

When TMP in the pre-gel region, flux increases as pressure increases, though usually not linear for macromolecules feeds. As pressure increases further, the CP layer reaches a limiting concentration, and flux becomes independent of pressure. Increasing pressure above a critical point may result in a lower flux (Cheryan, 1998). Fouling mechanism in UF of colloidal suspensions has been studied. In the UF of macromolecular solutions, they found that the higher concentration and TMP, the greater the forces of interaction between the macromolecules and with the surface of the membrane (Bacchin *et al.,* 2002).

2.8.3.2. Temperature

Increasing the temperature generally results in an increase in the permeate flux due to the dual effect of lowering the permeate viscosity, which assist flow rate an increasing diffusivity, which assist the dispersion of the polarized layer both in UF and MF (Attia *et al.,* 1991; Scott, 1988). Fouling on membrane surface may be reduced due to the increase in diffusivity and a lessening of concentration polarization. On the other hand, the removal of the surface layer may lead to greater internal fouling (Attia *et al.,* 1991)

According to Hagen-Poiseuille model stated, by increasing temperature should result in higher flux. At temperatures below 30°C, flux decrease with increasing temperature because of a decrease in solubility of the feed stream. Moreover, if the temperature is increased further it will give the beneficial effects (lower viscosity, higher diffusivity), this will outweigh the detrimental effects and may result in a net increase in flux (Cheryan, 1998).

2.8.3.3. Crossflow Velocity and Turbulence Promoters

Increasing the cross-flow velocity generally results in an improvement in permeate flux in both UF (Blatt *et al.,* 1970; Nakanishi and Kessler, 1985) and MF (Attia *et al.,* 1991). By increasing the crossflow velocity could decrease the membrane fouling thus the effective pore size increases. However, internal fouling rather than surface fouling occurs the cross-flow velocity has limited the effect (Bowen and Gan, 1991). Various means of creating greater turbulence at the membrane surface generally result in an improvement in mass transfer and a higher membrane flux.

High shear rates generated at the membrane surface tend to shear off deposited material and thus reduce the hydraulic resistance of the fouling layer. At low pressure (low permeation velocity), shear forces are high enough to minimize deposition of all particles on the membrane surface. At high pressure or high flux, particles move to the membrane surface at a much faster rate than their removal by shear, leading to greater fouling (Cheryan, 1998).