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Cleaning Potential of Surfactin on Fouled Ultrafiltration (UF) Membranes

(Potensi Pembersihan Surfaktin untuk Membersihkan Jerapan pada Membran Ultrapenurasan(UF))

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ABSTRACT

Biosurfactants are microbially produced surface active agents that offer better biodegradability and lower toxicity than chemically synthesized surfactants because of their biogenetic origin. One of the most surface-active biosurfactants known is surfactin, a cyclic lipopeptide produced by various strains of Bacillus subtilis. *In this study, the cleaning potential of surfactin on ultrafiltration (UF) membranes fouled with BSA was studied using centrifugal UF devices of 50 kDa and 100 kDa MWCO polyethersulfone (PES) membranes. Mechanisms of bovine serum albumin (BSA) displacement by surfactin on fouled UF membranes were studied using dynamic light scattering (DLS) technique and surface tension measurements. Hydrodynamic diameter and surface tension measurements of BSA-surfactin mixtures showed that the surfactin was efficient in displacing BSA fouled on UF membranes due to strong electrostatic repulsive interactions involved at pH8.5. This study demonstrated that surfactin can be used to effectively clean fouled UF membranes.*

Keywords: Critical Micelle Concentration (CMC); hydrodynamic diameter; surface tension measurements; surfactin; ultrafiltration (UF)

ABSTRAK

Biosurfaktan merupakan agen aktif permukaan yang mempunyai keupayaan biodegradasi yang lebih baik dan sifat toksik yang lebih rendah berbanding surfaktan sintesis kimia disebabkan oleh ciri biogenetik asalannya. Salah satu biosurfaktan yang paling aktif permukaan diketahui ialah surfaktin, sejenis lipopeptida berbentuk siklik yang dihasilkan oleh pelbagai strain Bacillus subtilis. *Di dalam kajian ini, keupayaan pembersihan surfaktin terhadap membran ultrapenurasan (UF) yang dijerap dengan albumin serum lembu (BSA) telah dikaji dengan menggunakan alat pengempar UF bermembran polietersulfon (PES) 50 kDa dan 100 kDa MWCO. Mekanisme pembersihan membran yang dijerap dengan albumin serum lembu (BSA) oleh surfaktin telah dikaji dengan menggunakan teknik selerakan cahaya dinamik (DLS) dan pengukuran tegangan permukaan. Pengukuran diameter hidrodinamik dan tegangan permukaan ke atas sebatian kompleks BSA-surfaktin menunjukkan surfaktin berkesan di dalam pembersihan membran UF yang dicemari BSA berdasarkan interaksi tangkisan elektrostatik yang kuat pada pH8.5. Kajian ini menunjukkan bahawa surfaktin boleh digunakan secara efektif untuk membersihkan jerapan pada membran UF.*

Kata kunci: Diameter hidrodinamik; kepekatan kritikal misel (CMC); pengukuran tegangan permukaan; surfaktin; ultrapenurasan (UF)

INTRODUCTION

Biosurfactants have attracted greater interests in the last few years compared with their chemical counterparts due to their lower toxicity and better biodegradability (Lang & Wagner 1993). Unlike chemical surfactants, biosurfactants are easily degraded and particularly suited for environmental applications. Increasing environmental concern among consumers and regulators are forcing industries to search for alternative products which are more environmentally-friendly (Muthusamy et al. 2008). Biosurfactans are generally considered as low or non-toxic products and therefore, are suitable for pharmaceuticals, cosmetic and food uses or additive. Biosurfactants have many potential applications in the food processing industry due to their antiadhesive, antifungal, antimicrobial and antiviral properties, as well as being highly surface-active. Several biosurfactants have shown antimicrobial activity against bacteria, fungi, algae and viruses (inactivation of enveloped virus such as herpes and retrovirus was observed with 80 mM of surfactin) (Muthusamy et al. 2008). Surface activity, antiadhesive and antimicrobial properties of biosurfactants in particular have attracted attention as a new tool to inhibit and disrupt biofilms and films of food related contaminants formed on food contact surfaces in processing equipments (Nitschke & Costa 2007). Bacterial biofilms present in food industry surfaces are potential sources of contamination that can lead to food spoilage and disease transmission (Muthusamy et al. 2008).

Biosurfactants have been demonstrated to have many interesting biological activities, but relatively little has been done to study their basic application as detergents. One potential field of application for biosurfactants is for removing fouling from membranes filtration. Membrane application in food and dairy industry particularly

faces important issue of membrane fouling by organic molecule adsorption such as proteins, which leads to the blockage of the membrane and thereby reduces the throughput or flux (Chen et al. 2006; Nigam et al. 2008). To overcome membrane fouling, cleaning of membrane can be carried out using cleaning agents such as acids, alkalis, surfactants, disinfectants and combined cleaning materials (Kazemimoghadam & Mohammadi 2007). It is essentially important to understand the interactions of biosurfactants and proteins at interfaces and in bulk solution to evaluate its cleaning potency. In general the effects of surfactants and proteins depend on the nature of both molecules. The structures of proteins and surfactants differ greatly and as a result of this so does their behaviour at interfaces and in bulk solution. Proteins are complex macromolecules, with different levels of structure. Amino acids with hydrophobic side chains tend to be hidden in the core of the molecule allowing it to be soluble in an aqueous environment. However, some proteins have hydrophobic and aromatic groups on the surface of the molecule which allow them to adsorb more readily to hydrophobic structures (Mackie & Wilde 2005). Surfactants and proteins stabilize an interface via different and incompatible mechanisms. Surfactants rely on a high degree of surface mobility to stabilize an interface following the Gibbs-Marangoni mechanism, while proteins stabilize an interface by forming an immobile viscoelastic network which sometimes is referred to as a two-dimensional gel (Gunning et al. 2004). In this study, bovine serum albumin (BSA), a well characterized globular protein has been chosen as a protein model. It is relatively large globular protein (66.3 kDa) and consists of 607 amino acids with 17 disulfide bonds and one free cysteine group (Heerklotz & Seelig 2001). It has relatively high water solubility because it contains a large number of ionisable amino acids and it also binds many types of amphiphilic biological molecules (Gunning et al. 2004).

One of the most surface-active biosurfactants known is surfactin, a cyclic lipopeptide produced by various strains of *Bacillus subtilis*. Surfactin consists of a heptapeptide headgroup with the sequence Glu-Leu-D-Leu-Val-Asp-D-Leu-Leu closed to a lactone ring by a $C_{13\text{-}15}$ β-hydroxy fatty acid (Heerklotz & Seelig 2001). It was reported by Ishigami et al. (1995) that the excellent surface-active behaviour of surfactin was attributed to the ease of pilling of molecules organized in β-sheet formation after reaching its critical micelle concentration (CMC). Surfactin have also been found to inhibit the adhesion of pathogenic organisms to solid surfaces or the infection sites; and effective in inactivation of viruses by incorporating into the lipid bilayer which induce a complete disintegration of the envelope which contains the viral proteins involved in virus adsorption and penetration (Seydlová & Svobodová 2008).

Interactions of surfactin-protein can be studied in terms of hydrodynamic diameter and surface tension measurements. Hydrodynamic techniques may help to recognize when a protein has lost its non-covalent structure as the increase in hydrodynamic diameter can be associated to the unfolding of a protein molecule (Adel et al. 2008). It is well known that globular proteins may exist in at least four different conformations: native, molten globule, pre-molten globule and unfolded; which can be discriminated by the degree of compactness of the polypeptide chain. Dynamic light scattering (DLS) can provide a direct hydrodynamic diameter measurement of a protein molecule which may indicate the molecule's compactness and the degree of unfolding (Adel et al. 2008). In addition, DLS can also provide information on a protein molecule overall shape and distribution of configuration of an unfolded chain (Adel et al. 2008). Another simple and useful technique which can be used to study surfactin-protein interactions is surface tension measurements (Wei et al. 2003). Surface tension is a useful method to study the formation of aggregates and the effects of changes in the micellization process reflect on the surface properties of surfactant-polymer complexes (Santos et al. 2003). Furthermore, the low surface activity property of the proteins can become relatively relevant, considering the reflected effects on the surface of solutions containing both components (Santos et al. 2003). The objective of this study was to investigate the cleaning potential of surfactin on fouled UF membrane with BSA. In addition, the mechanism of BSA displacement by surfactin was investigated using several techniques such as DLS and surface tension measurements.

Materials and Methods

BSA of 96% purity was obtained from Sigma (Dorset, U.K.) and no further purification was done before use. Surfactin was obtained after recovery and purification from fermentation broth by a two step ultrafiltration (UF) process described in detail in Isa et al. (2007). It was freeze-dried prior to use. Sample solutions at pH 8.5 and pH 3.6 used in this study were prepared using 20 mM phosphate and 20 mMTris-HCl buffer solutions, respectively using Milli-Q water.

Ultrafiltration (UF) Centrifugal Device

Membrane cleaning studies were conducted using a centrifugal UF device of 50 kDa (Vivaspin 6) and 100 kDa (Vivaspin 20) MWCO polyethersulfone (PES) membrane (Sartorius, Germany). Vivaspin 6 and Vivaspin 20 have an effective membrane area of 2.5 cm^2 and 6.0 cm^2, respectively. Fouling of membranes was conducted by filtering BSA solution (0.5 wt%, pH 3.6) at 3500 rpm for 10 min. Below its isoelectric point (IEP) of pH 4.71 – pH 4.84, BSA will be positively charged; and at pH 4 it will have a surface charge of 21.4 mV (Fuda et al. 2004). According to Salgin et al. (2006), PES membranes at pH 3.78 have a surface charge of -36.84 mV and -22.79 mV at ionic strength of 0.01 M KCl and 0.1 M KCl, respectively.

The highest degree of adsorption of BSA on PES membrane was by filtration of BSA solutions at pH 3.78 as strong electrostatic attractions were involved (Salgin et al. 2006). In this study, filtration of BSA solution (0.5 wt%) at pH 3.6 with PES membranes was chosen as the optimal conditions for membrane fouling as strong electrostatic attraction interactions between the oppositely charged BSA and PES membranes were involved. BSA concentration was measured using bicinchoninic acid method (BCA) (Smith et al. 1985), in order to determine the amount of BSA adsorbed onto membranes. The difference between the mass of BSA in the feed sample and the sum of BSA (mg) in the retentate and permeate sample (after centrifugation) was accounted as the amount of BSA adsorbed onto a membrane. The amount of BSA adsorbed (at pH 3.6) onto a membrane is then defined using the following equation:

$$\text{Adsorbed BSA per surface area (mg/cm}^2\text{)} = \frac{M_{BSA(feed)} - M_{BSA(after\ centrifugation)}}{\text{Membrane area (cm}^2\text{)}} \quad (1)$$

where $M_{BSA(feed)}$ is the mass of BSA (mg) in the feed and $M_{BSA(after\ centrifugation)}$ is the sum of BSA (mg) in the retentate and permeate (after centrifugation).

The amount of BSA (mg) adsorbed onto a membrane was then used to determine the amount of surfactin needed to prepare solutions of varying molar ratios of BSA-surfactin of 2 and 4. Rejection coefficient (R) of BSA (at pH 3.6) on 50 kDa and 100 kDa PES membranes were measured using the following equation:

$$R = 1 - \frac{C_P}{C_F}, \quad (2)$$

where C_P(mg/L) and C_F(mg/L) are concentration of BSA in the permeate and feed, respectively.

Cleaning of membranes with surfactin solutions and comparison of membranes flux at various conditions were conducted at 3500 rpm for 5 min of centrifugation using deionized water. All centrifugations were conducted at room temperature and in triplicates.

DYNAMIC LIGHT SCATTERING (DLS)

Measurement of hydrodynamic diameter of BSA at fixed concentration of 0.5 wt% with increasing concentration of surfactin for preparation of BSA-surfactin mixtures of various molar ratios and CMC value of surfactin were determined using ZetaSizer Nano ZS system (Malvern, UK). Each sample was prepared in 20 mM phosphate buffer solutions at pH 8.5. This instrument, which contains a 4 mW He-Ne laser operating at a wavelength of 633 nm determines the light scattered at a certain angle which relates to Brownian motion of the particles, their diffusion coefficient and their size. The size of a particle is calculated from the translational diffusion coefficient by using the Stokes-Einstein equation. The diameter that is measured in DLS is a value that refers to how a particle diffuses within a fluid and it is referred to as a hydrodynamic diameter. Each measurement was conducted at room temperature and in triplicates.

SURFACE TENSION MEASUREMENTS

Surface tension measurements were obtained using a digital tensiometer (K11, KRÜSS, Germany) by using a Wilhelmy plate method. A platinum plate of known perimeter was vertically suspended from a precision balance. The platinum plate was submerged into each solution and then slowly pulled through the air-water interface, to measure the surface tension (mN/m). The platinum plate was cleaned by rinsing with ethanol and Milli-Q water and was flamed with a Bunsen burner to remove any residual deposits. The platinum plate was then cooled before each measurement. Measurements of surface tension of surfactin in the absence and presence of BSA at fixed concentration of 0.5 wt% were done at pH 8.5. All samples for surface tension measurements were prepared in 20 mM phosphate buffer solutions. Each measurement was conducted at room temperature and in triplicates.

RESULTS AND DISCUSSION

MEMBRANE CLEANING STUDIES

Cleaning of fouled UF membranes with BSA were conducted by filtrations with surfactin solutions and evaluation of cleaning were based on the relative flux of membranes at various conditions. According to Al-Amoudi et al. (2008), flux measurement is a direct assessment of fouling and cleaning process on membranes and it is usually accepted that flux decline of membranes is caused by adsorption or crystallization due to excessive pore blocking and/or cake formation. Figures 1 and 2 show the relative flux of new membranes, fouled and cleaned membranes with surfactin solutions of molar ratios to BSA adsorbed onto membranes of 2 and 4 at pH 8.5 using 50 kDa and 100 kDa MWCO PES membranes, respectively. In addition, Table 1 shows rejection coefficient (R), total recovery and amount of BSA adsorbed per surface area on 50 kDa and 100 kDa membranes at pH 3.6.

Referring to Figures 1 and 2, flux of fouled membranes decreased 42% (± 1%) and 22% (± 3%), respectively, compared with the flux of new membranes, indicating some degree of concentration polarization and fouling of the membranes by BSA. Table 1 shows a higher rejection coefficient (R) and total recovery of BSA with 50 kDa membranes compared with 100 kDa, which were to be expected with the molecular weight of BSA of 66.3 kDa. Although BSA should be able to permeate 100 kDa membranes, high rejection coefficient (R) was caused by severe concentration polarization that formed secondary membrane layer and decreasing the apparent MWCO and permeability, thus impeding further permeation of BSA molecules. Table 1 also shows the amount of adsorbed

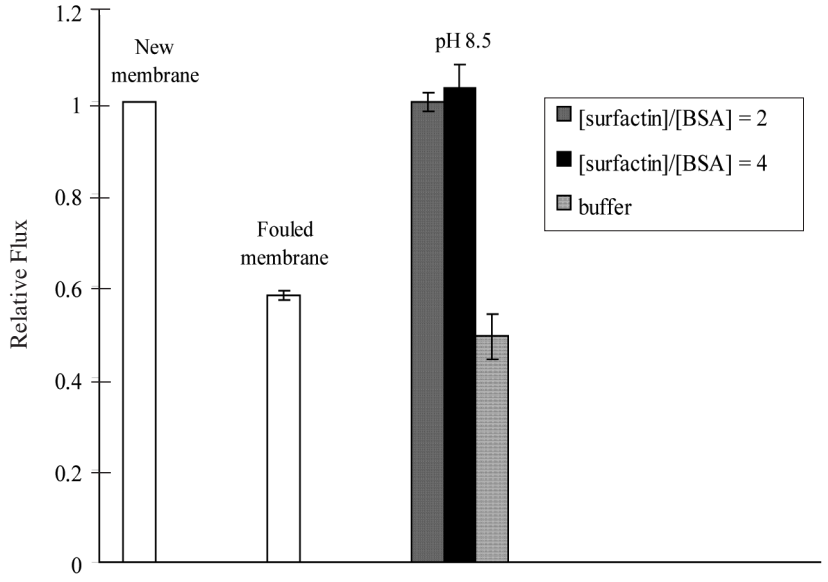

FIGURE 1. Relative flux of new 50 kDa MWCO membranes, fouled and cleaned 50 kDa MWCO membranes with surfactin solutions at pH 8.5

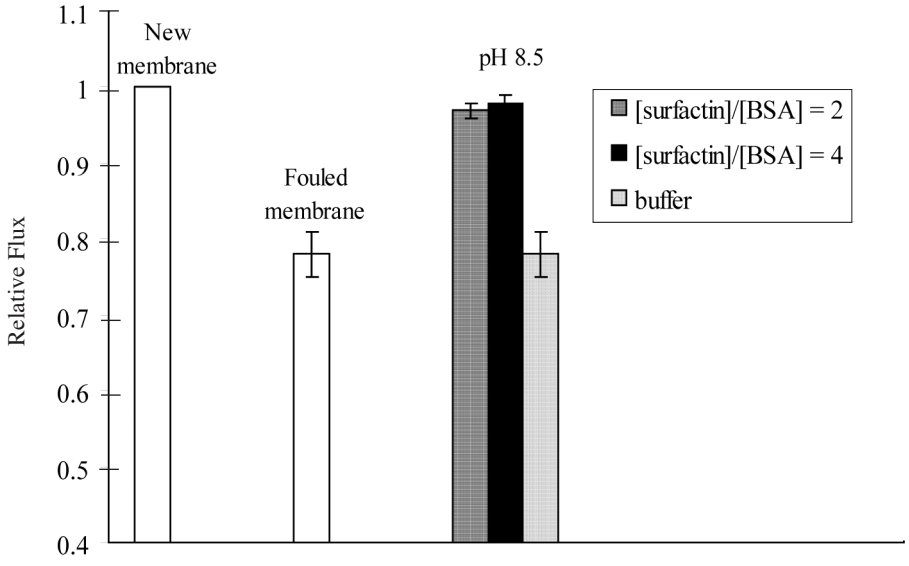

FIGURE 2. Relative flux of new 100 kDa MWCO membranes, fouled and cleaned 100 kDa MWCO membranes with surfactin solutions at pH 8.5

TABLE 1. Rejection coefficient (R), total recovery and adsorbed BSA ratio onto UF membranes of 50 kDa and 100 kDa at pH 3.6

Membranes (MWCO)	Rejection coefficient (R)	Total recovery (%)	Adsorbed BSA per surface area (mg/cm^2)
50 kDa	0.99 ± 0	96.9 ± 0.1	0.30 ± 0.02
100 kDa	0.87 ± 0.04	89.5 ± 2.6	0.90 ± 0.23

BSA per surface area was higher with 100 kDa membranes compared with 50 kDa.

Cleaning of fouled membranes with surfactin solutions at pH 8.5 in Figures 1 and 2 was efficient and showed some degree of BSA removal. Cleaning of fouled 50 kDa and 100 kDa membranes achieved at least 97% of flux recovery from the initial flux of new membranes, and showed no significant difference between molar ratios of surfactin-BSA of 2 and 4. Cleaning of fouled membranes with buffered solutions at pH 8.5 showed no significant effect on flux

improvement of fouled UF membranes. In order to get an improved insight into the mechanism of cleaning of fouled UF membranes with BSA, further experiments were carried out with surfactin-BSA solutions mixtures of various molar ratios using DLS and surface tension measurements.

SURFACTIN-BSA INTERACTIONS IN BULK SOLUTION

Hydrodynamic Diameter of BSA. DLS technique was used to study the interactions of BSA and surfactin at various molar ratios. Figure 3 shows the changes of mean hydrodynamic diameters of BSA at different molar ratios to surfactin at pH 8.5.

As shown in Figure 3, there was no significant change in the conformation of BSA with increasing concentration of surfactin at pH 8.5. At pH 8.5, both BSA and surfactin are negatively charged and at this pH hydrophobic interaction between the two are expected to be minimal and repulsive electrostatic interactions will be predominant. At pH 8.5 conformation of BSA remained the same as there was no significant change of its mean hydrodynamic diameters, which means there were neither occurrence of BSA unfolding or formation of surfactin-BSA complexes.

Surface Tension Measurements. Interactions of surfactin-BSA in bulk solution can be studied as well with surface tension measurements. According to Kwaambwa and Maikokera (2007), when strong interactions between surfactant and protein exist, the surface tension curve of protein-surfactant mixtures would deviate from that of surfactant. The binding of surfactant to protein is expected to affect protein surface activity, either due to formation of surfactant-protein complexes which have a different surface activity, or due to a decrease in the concentration of free surfactant molecules (Magdassi et al. 1995). Surface tension of surfactin in the absence and presence of BSA conducted at pH 8.5 is shown in Figure 4.

Surface tension profile of surfactin in the presence of BSA showed no significant deviation in comparison to the profile with the presence of BSA, indicating neither occurrence of binding of surfactin to BSA nor formation of surfactin-BSA complexes. At pH 8.5, interactions between surfactin and BSA were predominately hydrophobic and less electrostatic attraction interactions between the two. The difference between surface tension profiles of surfactin in the absence and presence of BSA were conditions that favour the formation of surfactin micelles. Higher surface tension profile of surfactin in the presence of BSA was as a result of stronger electrostatic repulsive interactions in the solution mixture increasing the value of surfactin CMC. According to Heerklotz and Seelig (2001), stronger electrostatic repulsions between the peptide head group of surfactin molecules can lead to a higher CMC for surfactin. In the case of surfactin with the presence of BSA, it is likely that surfactin will have a higher CMC as both surfactin and BSA were negatively charged, thus repelling each other. Surface tension profile of surfactin in the absence of BSA was lower compared with that of surfactin in the presence of BSA. Without BSA in the solution mixtures, conditions were more favourable for the formation of surfactin micelles as less electrostatic repulsive interactions between surfactin and BSA were involved. Results obtained in Figures 3 and 4 show interaction between surfactin and BSA at pH 8.5 is minimal and repulsive electrostatic attraction is the predominant interactions as both surfactin and BSA at this pH is negatively charged.

Surfactin CMC Determination. One of the most interesting characters of surfactin is its excellent surface-active behaviour which is attributed to the ease of pilling of surfactin molecules organized by β-sheet formation after reaching CMC (Ishigami et al. 1995). Surfactin CMC in this study was determined with DLS technique, as shown in Figure 5.

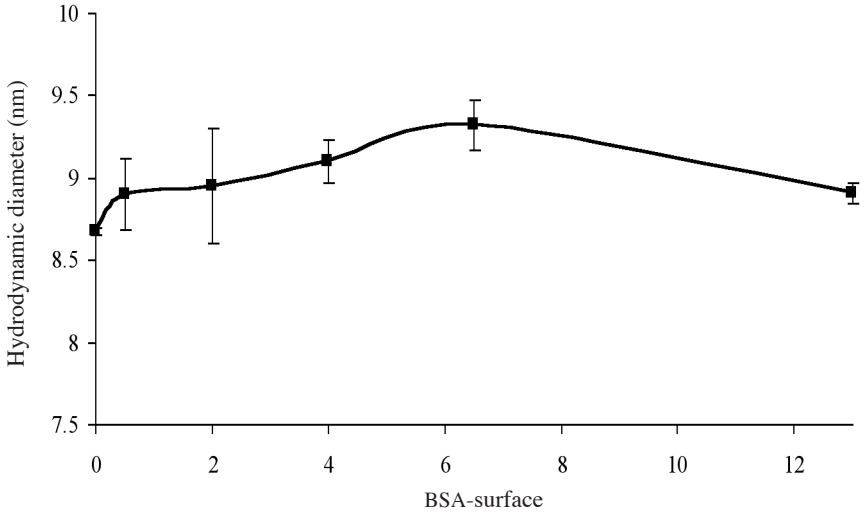

FIGURE 3. Mean hydrodynamic diameters of BSA at different molar ratios to surfactin at pH 8.5

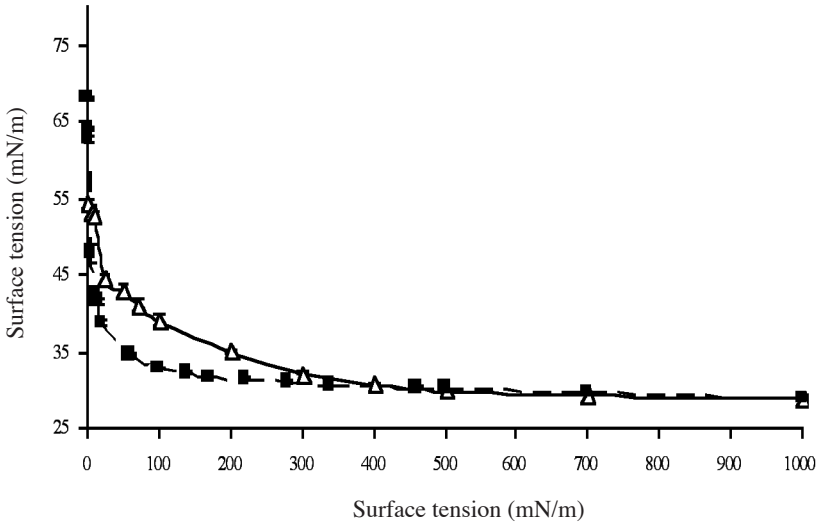

FIGURE 4. Surface tension of surfactin in the absence (<) and presence of BSA (Δ) at pH 8.5

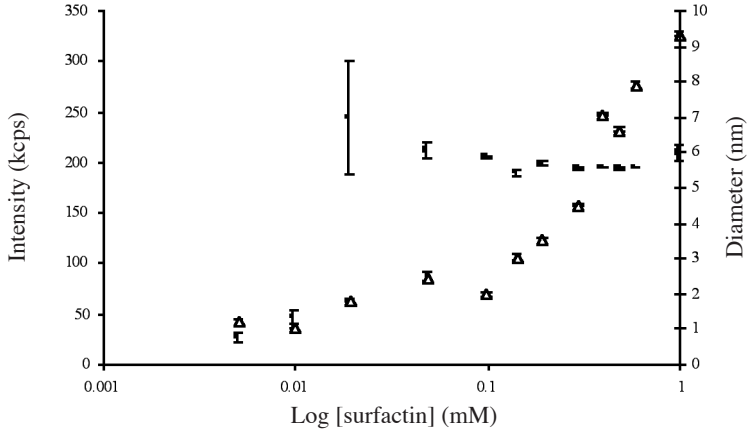

FIGURE 5. Surfactin CMC determination with DLS; (<) diameter, (Δ) intensity

Figure 5 shows surfactin molecules starting to form micelles at a concentration as low as 20 μM. The formation of surfactin micelles can be seen by the increased intensity of light scattered and presence of particles with diameter in the range of 6-7 nm with increasing concentration of surfactin solutions. Surfactin CMC can vary depending on the ionic strength of the solution as electrostatic repulsion between peptide molecules is stronger at lower ionic strength (Maget-Dana & Ptak 1992). Surfactin CMC determination by Ishigami et al. (1995) using surface tension measurements showed surfactin reaching CMC at a concentration of 9.4 μM in 200 mM $NaHCO_3$ at pH 8.7; while according to Heerklotz and Seelig (2001), surfactin CMC to be 7.5 μM in 100 mMNaCl, 10 mMTris, 1 mM EDTA at pH 8.5 which was determined using isothermal titration calorimetry (ITC). Figure 5 also shows surfactin has an excellent detergency property due to its very low CMC value which is much lower in comparison to anionic sodium dodecyl sulphate (SDS) which has CMC value of 8.1 mM (Malhotra & Coupland 2003).

Mechanism of BSA Displacement. Figures 1 and 2 show surfactin solution at pH 8.5 was efficient to clean both fouled 50 kDa and 100 kDa MWCO UF membranes with BSA and no significant effect in terms of different molar ratios of surfactin-BSA used. At pH 8.5, both surfactin and BSA were negatively charged. According to Isa et al. (2007) and Fuda et al. (2004), surfactin (pH ~7.7) and BSA (pH 8) had a surface charge of approximately -27 mV and -36.6 mV, respectively. DLS and surface tension measurements of surfactin-BSA solutions mixtures at pH 8.5 showed minimal interactions between surfactin and BSA. Effective displacement of BSA fouled on UF membranes by surfactin at pH 8.5 was as a result of strong electrostatic repulsion between both molecules permitting effective displacement of BSA with the more surface-active surfactin. As shown in Figure 5, surfactin is a highly surface-active molecules which can form micelles structure with a concentration as low as 20 μM. Electrostatic repulsive interactions of surfactin-BSA on UF membranes are the most predominant interactions for effective displacement or removal of

BSA with the more surface-active surfactin. Overall there were no significant effects of increasing molar ratios of surfactin-BSA from 2 to 4 in the membrane cleaning studies. Overall, results obtained in this studies show surfactin can be applied effectively to clean fouled UF membranes by BSA and only requires minimum quantity due to its very low CMC value.

Conclusion

Surfactin was effective in cleaning fouled UF membrane with BSA as strong electrostatic repulsive interactions between both components were involved at pH 8.5. Surfactin is very surface-active and this unique character is attributed to the ease of pilling of surfactin molecule in β-sheet formation after reaching its CMC, which is ~20 μM in 20 mM phosphate buffer pH 8.5 at room temperature. Although surfactin is an anionic biosurfactant, its very low CMC value and hydrophilic-lipophilic balance (HLB) have close resemblances to non-ionic surfactants. Understanding surfactin functionality and interactions with protein is very important to evaluate its potential in certain area of application particularly as a cleaning agent for food processing equipments. Surfactin application as a cleaning agent for food processing equipments is advantageous as it has antiadhesive, antibacterial, antifungal and antiviral properties which can minimize the possibility of cross contamination; moreover it has a very low CMC which makes it a very powerful surfactant. In addition, surfactin has a lower toxicity and it is more biodegradable compared with chemical surfactants. Further studies of surfactin cleaning potential could be applied with different types of proteins and biofilms as these two are major source of problems for the cleaning of food processing equipments. To complement further advancement of surfactin applications in many areas, emphasis on surfactin production, recovery and purification methods that are cost-effective and environmentally-friendly should also be taken in order for surfactin or other biosurfactants to compete with their chemical counterparts, which have more competitive market price and more established areas of industrial applications.

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