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A further study of the recovery and purification of surfactin from fermentation broth by membrane filtration

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ABSTRACT

Surfactin is a bacterial lipopeptide produced by *Bacillus subtilis* and is a powerful surfactant, having also antiviral, antibacterial and antitumor properties. The recovery and purification of surfactin from complex fermentation broths is a major obstacle to its commercialization; therefore, a two-step membrane filtration process was developed using a lab scale tangential flow filtration (TFF) unit with 10 kDa MWCO regenerated cellulose (RC) and polyethersulfone (PES) membranes at three different transmembrane pressure (TMP) of 1.5 bar, 2.0 bar and 2.5 bar. Two modes of filtrations were studied, with and without cleaning of membranes prior to UF-2. In a first step of ultrafiltration (UF-1), surfactin was retained effectively by membranes at above its critical micelle concentration (CMC); subsequently in UF-2, the retentate micelles were disrupted by addition of 50% (v/v) methanol solution to allow recovery of surfactin in the permeate. Main protein contaminants were effectively retained by the membrane in UF-2. Flux of permeates, rejection coefficient (*R*) of surfactin and protein were measured during the filtrations. Overall the three different TMPs applied have no significant effect in the filtrations and PES is the more suitable membrane to selectively separate surfactin from fermentation broth, achieving high recovery and level of purity. In addition this two-step UF process is scalable for larger volume of samples without affecting the original functionality of surfactin, although membranes permeability can be affected due to exposure to methanolic solution used in UF-2.

1. Introduction

Surfactin is a cyclic lipopeptide biosurfactant, 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_{13-15} β-hydroxy fatty acid [1]. Natural surfactin is a mixture of isoforms which slightly differ in their physicochemical properties due to variations in the chain length and branching of its hydroxy fatty acid component; as well as substitutions of the amino acids components of the peptide ring [2]. A variety of important applications and physiological activities have been proposed for surfactin, including reports that it has hemolytic, antiviral, antibacterial and antitumor properties [3]. On account of its biodegradability and its broad range of functional characteristics, it possesses a huge potential for applications in industrial processes, especially those involving surfactant activities.

The total quantity of chemical surfactants and biosurfactants produced in the US and worldwide was estimated at more than 10 billion pounds sterling and 25 billion pounds sterling, respectively [4]. To date, biosurfactants have not been able to compete economically with its chemically synthesized counterparts due to its high production costs, which range between 2 US$ per kg and 3 US$ per kg and are 20–30% more expensive [5]. The major obstacle for the commercialization of surfactin is its recovery and purification from complex fermentation broths. Downstream processing in many biotechnological processes is responsible for up to 60% of the total production cost [6]. Sigma Chemical Co. currently markets surfactin for research purposes at around US $20 per mg. Extensive efforts should be made to improve the production efficiency and recovery bioprocess in order to optimize surfactin recovery and purification. Hence research and development activities that could lead to productivity compatible with economic needs of surfactin are very important.

One of the unique characteristics of surfactin is that above the critical micelle concentration (CMC) associates to form micelles, with molecular diameter of around 8–9 nm [7]. Surfactin molecules in this form can be retained in the retentate of ultrafiltration (UF) membranes of certain molecular weight cut-offs (MWCOs). Mulligan and Gibbs [8] successfully employed this principle for the recovery and purification of surfactin and rhamnolipids from complex fermentation broths with one step of UF. Recovery and

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purification of surfactin from broth samples with one-step of UF was further studied by Sen and Swaminathan [9] by using a stirred cell device and evaluating some filtration characteristics and their effects on the recovery and purity of the final products. In this latter study, they managed to recover surfactin with a purity value based on CMC of 70%. Another reported method by Lin and Jiang [10] for the recovery and purification of surfactin from fermentation broths consists of two-step UF. In that study, they reported recovery of 95% of surfactin from their fermentation broth, although there was no report on the purity of the final product. Recently, Chen et al. [11] proposed a method that requires extra steps of pretreatment of fermentation broth including, acid precipitation to recover surfactin and redissolution of precipitate in NaOH at pH 11 followed by filtration with two-stage dead-end UF process. This process offers high recovery and high purity of surfactin but the extra steps taken would further add to the complexity of the process and could have an affect to the final cost of surfactin production. Furthermore, no measurement on the functionality of surfactin final product was conducted which is important in order to evaluate the efficiency of the whole process.

In our previous study [7], we have managed to achieve high recovery and high purity of surfactin from fermentation broth by a two-step UF process with a dead-end stirred cell device. In that study, we use two types of UF membranes, regenerated cellulose (RC) and polyethersulfone (PES) of 10 kDa MWCO in both steps of filtration at constant transmembrane pressure (TMP) of 2.0 bar. Furthermore the size and surface charge measurements carried out demonstrated that disruption of surfactin micelles, aggregation of protein contaminants and electrostatic interactions between surfactin molecules and the membrane surface have a major influence on its selective separation [7]. As most UF modules in industrial applications are operated in the cross-flow mode, in this study it is proposed to investigate the scalability of this two-step UF process using a lab scale cross-flow filtration unit. In cross-flow mode, the feed is pumped across or tangentially to the membrane surface. Cross-flow is advantageous in that it limits the build up of solids or particles on membrane surface, resulting in less cake build up and less cake resistance on the membrane, hence a higher average flux can be achieved during operation [12]. Furthermore, in this study we also analysed the different isoforms of surfactin produced in the fermentation as well as characterizing its surface activity in order to evaluate the effects of the two-step UF process on surfactin functionality and purity. For a procedure or a method to be successful and potentially to be commercialized, it must be able to achieve high recovery, high purity and fully functional final product.

2. Materials and methods

2.1. Culture conditions, media and fermentation

The strain used in the fermentation was *B. subtilis* ATCC 21332, which was obtained from the American Type Culture Collection (Rockville, MD, USA). Fermentation to produce surfactin was conducted with a 5-l bioreactor (BioFlo 110 New Brunswick Scientific, UK) by using Cooper's medium formulation [13]. Fermentation conditions were set to be operated under depleted oxygen conditions [7]. Culture broth samples of approximately 40 ml were taken during the course of the fermentation at regular intervals in order to determine biomass and surfactin content. Similarly, temperature, dissolved oxygen and pH values were recorded prior to each sampling procedure. The final fermentation broth obtained was harvested, clarified by centrifugation for 10 min at 8000 rpm at room temperature in order to remove biomass, divided in fractions and finally frozen for further studies.

2.2. Recovery and purification of surfactin by a two-step UF process

Two-step UF process to recover and purify surfactin from fermentation broth was operated in a cross-flow mode with a lab scale tangential flow filtration (TFF) unit (Millipore, USA). Two-step of UF was conducted with two sets of membrane materials of Ultracel regenerated cellulose and Biomax polyethersulfone of 10 kDa MWCO with an effective area of 50 cm^2 (Pellicon XL 50, Millipore, USA). The driving force for permeate flow is the pressure gradient that exists through the membrane at each point along the membrane surface [14]. This pressure gradient is referred to as transmembrane pressure, which is calculated as:

$$\mathrm{TMP} = \frac{P_{\mathrm{in}} + P_{\mathrm{out}}}{2} - P_{\mathrm{per}} \approx \frac{P_{\mathrm{in}} + P_{\mathrm{out}}}{2} \quad (1)$$

where P_{in}, P_{out} and P_{per} are the inlet, outlet and permeate side pressure of the membrane module, respectively.

Throughout the UF procedures, the flow rate across the membrane (filtration rate) was estimated by collecting permeates of certain volumes during an exactly controlled period of time. Such permeates were then weighed and translated to volume according to the density of the solution. Flux of permeates was calculated by using the equation as follows:

$$\mathrm{flux\,(LMH\ or\ L/m^2h)} = \frac{\mathrm{flow\ rate\,(ml/min)}}{\mathrm{membrane\ area\,(cm^2)}} \times 600 \quad (2)$$

Surfactin concentration at each fraction of the filtration was analysed by HPLC and the rejection of surfactin by a membrane was defined as rejection coefficient (R) defined as:

$$R = 1 - \frac{C_{\mathrm{p}}}{C_{\mathrm{F}}} \quad (3)$$

where C_{P} and C_{F} are the concentration of surfactin in the permeate and feed, respectively.

In the first step of UF (UF-1), 200 ml of fermentation broth containing surfactin micelles were concentrated in the retentate and flow rate was monitored during the course of UF. The resulting retentate of approximately 20 ml was diluted 1:10 in a solution of methanol 50% (v/v) and ultrafiltered again in the second step of ultrafiltration (UF-2) under the same conditions. This two-step UF process was conducted in two different filtration modes. The first mode is by cleaning the UF membrane after UF-1 before proceeding to UF-2 and both steps were conducted at three different TMP: 1.5 bar, 2.0 bar and 2.5 bar. The second mode is by proceeding to UF-2 without cleaning the membrane after UF-1 and both steps were conducted at TMP of 2.0 bar. Each filtration step and mode were conducted at room temperature. After each step of UF, membranes were cleaned by rinsing with distilled water for approximately 30 min, followed with (0.1 M NaOH solution—1.5 h) for RC membrane and mixed cleaning solution (0.5 M NaOH + 0.10% SDS—2–3 h) for PES. Both membranes were then rinsed with distilled water for approximately 30 min for the final step of the cleaning procedures and then stored in ethanol 10% (v/v) solution at 4 °C.

2.3. Membrane permeability measurements

2.3.1. Membrane flux measurements

At each step of UF, permeability of RC and PES membranes used in the lab scale tangential flow filtration unit was evaluated by measuring the flux of distilled water before and after the cleaning procedures, by using Eq. (2). Membranes flux measurements were carried out at room temperature and at constant TMP of 1.5 bar and 2.0 bar for RC and PES membranes, respectively.