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Comparative study of the production of rhamnolipid biosurfactants by *B. thailandensis* E264 and *P. aeruginosa* ATCC 9027 using foam fractionation

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ABSTRACT

Biosurfactants are surface-active agents that are produced by a variety of microorganisms including yeasts, filamentous fungi and bacteria. In this work, we report on the ability of *Pseudomonas aeruginosa* ATCC 9027 and *Burkholderia thailandensis* E264 to produce rhamnolipids via a 10-L bioreactor and their recovery through foam fractionation studies in a continuous stripping mode. The recovery of Rha-C_{10}-C_{10} (mono-rhamnolipids) produced by *P. aeruginosa* ATCC 9027 increased (from 6% to 96%), whilst the enrichment decreased (from 2.9 to 1.2) with the increasing airflow rate. These results are consistent with foam fractionation of a single surfactant system with stable foam. The recovery and enrichment of Rha-Rha-C_{14}-C_{14} (di-rhamnolipids) produced by *B. thailandensis* E264 (and an unknown molecule) in a single-component system were found to display different characteristics. Both recovery and enrichment were found to decrease with the airflow rate. It is postulated that a competitive adsorption process could occur between the smaller molecule identified by electrospray ionisation–mass spectrometry (ESI–MS) and Rha-Rha-C_{14}-C_{14}.

1. Introduction

Biosurfactants are surface-active molecules produced by different microorganisms such as bacteria, yeasts and fungi. Biosurfactants are advantageous over conventional surfactants with regard to their lower toxicity, biodegradability, specific activity at extreme temperatures, pH and salinity [1]. However, the use of biosurfactants has been restricted due to the high cost of production, downstream purification and separation [2]. There is still no economical and reliable downstream technology for the recovery and purification of rhamnolipids at the industrial scale. In the case of biosurfactant production, the downstream processing accounts for 70–80% of the entire production costs [3,4]. Several extraction and purification steps are involved for obtaining reasonably pure biosurfactants from fermentation. The appropriate approach for downstream processing depends on the type and nature of the substrates, the fermentation technique and the type and physicochemical properties of the excreted biosurfactants [5,6]. The most common isolation techniques for biosurfactants includes precipitation, solvent extraction and chromatographic purification. The extraction of low molecular weight biosurfactant normally involves an optional precipitation step and the use of different organic solvents according to hydrophobicity and hydrophilic–lipophilic balance (HLB) value of compounds [7]. However, these traditional recovery methods require volatile organic solvents and chemical substances such as chloroform and methanol–ethanol mixtures, which are expensive and toxic to health and also cause air pollution [8,9].

Over the years, foam fractionation has drawn attention due to its low cost, low space requirements, effectiveness and possibility of continuous product removal and in situ recovery. In addition, foam fractionation is more environmentally friendly compared to the other available methods as it does not require solvents and other chemicals [5,10–14].

Foam fractionation belongs to the group of adsorptive bubble separation techniques and is based on the preferential adsorption of surface-active molecules onto the gas–liquid interfaces. In a foam fractionation process, gas is sparged through a liquid pool at the bottom of a vertical column. Surfactants adsorb onto the rising air bubbles in the liquid pool generating stable foam that rises up the column. As the foam rises up, the column drainage occurs due to

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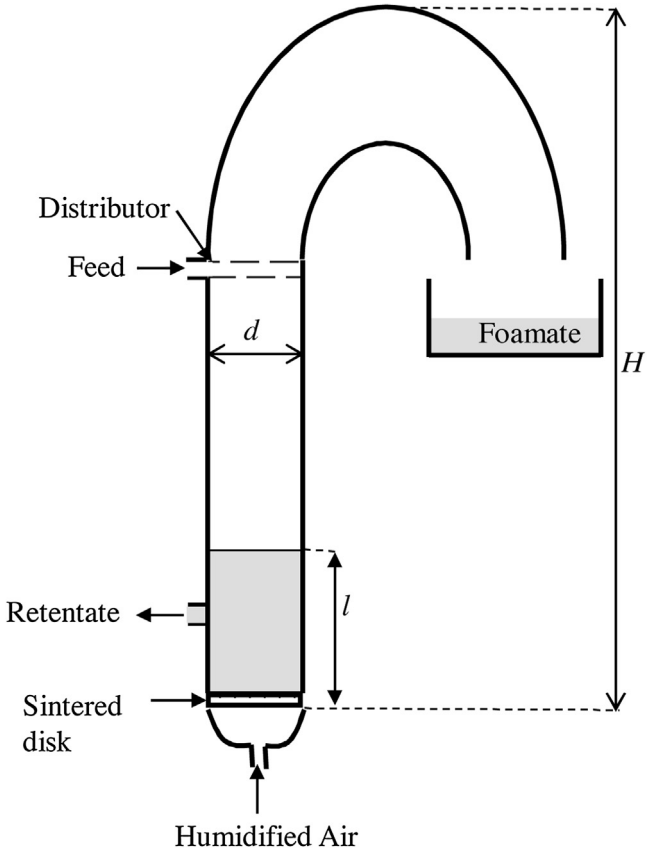

Fig. 1. Schematic diagram of foam fractionation experimental set up.

gravity and capillary suction effects. Thus, the foam collected at the top of the column is drier and contains higher average surfactant concentration than the feed or the bottom liquid pool. This foam product, called the foamate, was collapsed to form an enriched surfactant solution [10].

Studies have demonstrated that foam fractionation can be utilised to purify and recover biosurfactants such as surfactin, rhamnolipids and hydrophobin [5,13–15]. The rhamnolipid used in most existing foam fractionation studies is produced from the microorganism strain *Pseudomonas aeruginosa*; however, no study of the foam fractionation of rhamnolipids produced from *Burkholderia thailandensis* has been reported to our knowledge.

In this present study, foam fractionation in a continuous stripping mode was used for the recovery of mono-rhamnolipids and di-rhamnolipids produced by *P. aeruginosa* ATCC 9027 and *B. thailandensis* E264; the biosurfactant production was done using proteose–peptone ammonium salts (PPGAS) and nutrient broth medium supplemented with glucose and glycerol as a carbon source for each microorganism, respectively. The effects of airflow rate on the foam properties and foam fractionation separation efficiency of rhamnolipid from both microorganisms were investigated.

2. Materials and methods

2.1. Bacteria strains and culture conditions

P. aeruginosa ATCC 9027 and *B. thailandensis* E264 were maintained on nutrient agar slants at 4 °C and were subcultured every month. Each slant was used to obtain a bacterial suspension, with the optical density (570 nm) adjusted to yield 10^7 CFU/mL for each of the strains used.

The standard medium for the production of rhamnolipids by *P. aeruginosa* ATCC 9027 was PPGAS (proteose peptone glucose ammonium salt) medium (1 g/L NH_4Cl, 1.5 g/L KCl, 19 g/L Tris-HCl, 10 g/L peptone and 0.1 g/L $MgSO4{\cdot}7H_2O$) at pH 7.4. The fermentation medium contained the same growth medium, with glucose (5 g/L), as a carbon source. For the production of rhamnolipids by *B. thailandensis* E264, the media used was nutrient broth (8 g/L) with glycerol (20 g/L).

2.2. Production of rhamnolipids

An Electrolab FerMac 360 fermentation unit was used to perform batch cultivation of *P. aeruginosa* ATCC 9027 and *B. thailandensis* E264. The microorganisms used in this study were aerobically (0.5 vvm) incubated in PPGAS medium and nutrient broth, at 37 °C and 30 °C, respectively, at 400 rpm for 72 h for *P. aeruginosa* ATCC 9027 and 120 h for *B. thailandensis* E264.

2.3. Foam fractionation experiments

A continuous foam fractionation system in a stripping mode was used in this study. In this mode, the feed is injected near the top of column into the rising foam. Below the feed point, a relatively wet rising foam is created; above the feed point, the liquid in the foam drains and the foam becomes drier [10]. Adsorption of surface-active molecules to the foam air–water interface occurs as the feed drains downwards through the wet rising foam, resulting in the foamate collected from the top of the column being enriched and the retentate collected from the bottom of the column being depleted. About 4 L of rhamnolipid fermentation broth was fed into the top of the straight section of a "J"-shaped glass column of diameter, d, 50 mm and exposed height, H, 350 mm via a peristaltic pump and a metal tube distributor at a feed flow rate of 15 mL/min, as previously described by Winterburn et al. [15]. Fig. 1 shows the schematic diagram of the foam fractionation column. Humidified air was sparged through a sintered glass disk into a liquid pool creating overflowing foam. The initial composition of the liquid pool at the bottom of the column was the same as the feed, and this liquid exited the column through an exit port such that a constant liquid level, l, of 100 ± 10 mm was maintained throughout the experiment. The enriched overflowing foam was collected at the open end of the "J"-shaped section. The liquid pool and foam that exited the foam fractionation column is referred to as the retentate and foamate, respectively, throughout this study.

Foam fractionation experiments were performed for four different airflow rates, while all other process parameters such as feed flow rate were kept constant. The airflow rates used for *P. aeruginosa* ATCC 9027 were 0.1 and 1.2 L/min and for *B. thailandensis* E264 were 1.2 and 3 L/min. The range of airflow rates was selected to enable the production of stable foam and to allow rhamnolipid separation through foam fractionation [5]. Each airflow rate was determined in duplicate with fresh fermentation broths for each run. The standard deviation values of recovery and enrichment for each airflow rate were within 0.2% and 0.1, respectively.

Foam fractionation was performed for 4 h to ensure steady-state conditions, and the feed, retentate and foamate samples were collected every half an hour. The foamate samples were made airtight and kept at 4 °C overnight. The feed, retentate and foamate samples were analysed for rhamnolipid concentration.

Mass balances were conducted for mono-rhamnolipid and di-rhamnolipid for all the runs and were closed to within ±20%. The separation performance of the foam fractionation process was characterised by the enrichment and recovery (Eqs. (1) and (2)).

$$\text{Enrichment} = \frac{C_F}{C_i} \quad (1)$$

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