Process Biochemistry 51 (2016) 820–827

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

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

M.A. Díaz De Rienzo*, I.D. Kamalanathan, P.J. Martin

School of Chemical Engineering and Analytical Science, The University of Manchester, Manchester, M13 9PL, UK

ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form 7 April 2016
Accepted 8 April 2016
Available online 11 April 2016

Keywords:
Biosurfactants
Rhamnolipids
Foam fractionation
Pseudomonas
Burkholderia

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

* Corresponding author.
E-mail addresses: mayri.diazderienzo@manchester.ac.uk, mayriales@gmail.com (M.A. Díaz De Rienzo).

http://dx.doi.org/10.1016/j.procbio.2016.04.007

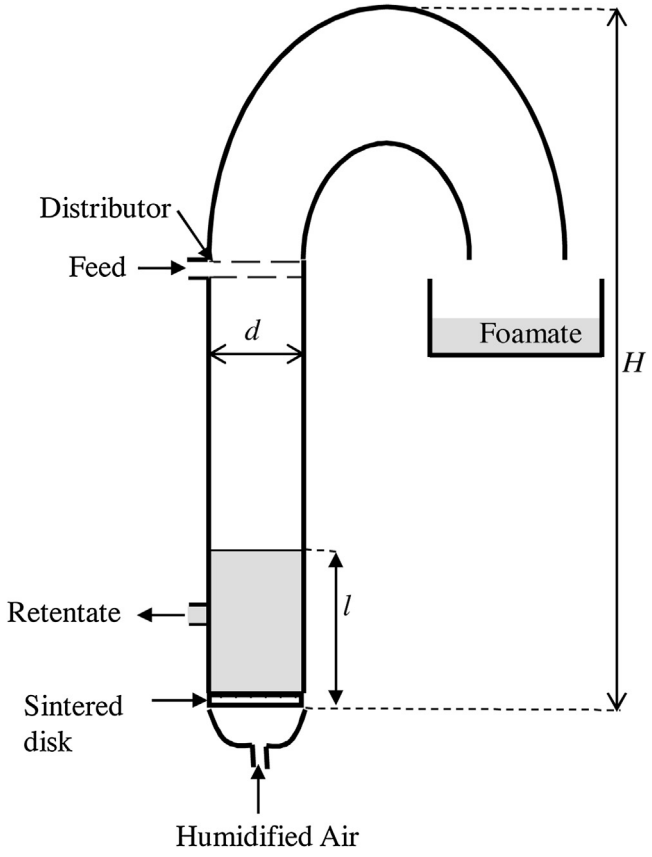

Fig. 1. Schematic diagram of foam fractionation experimental set up.

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)).

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

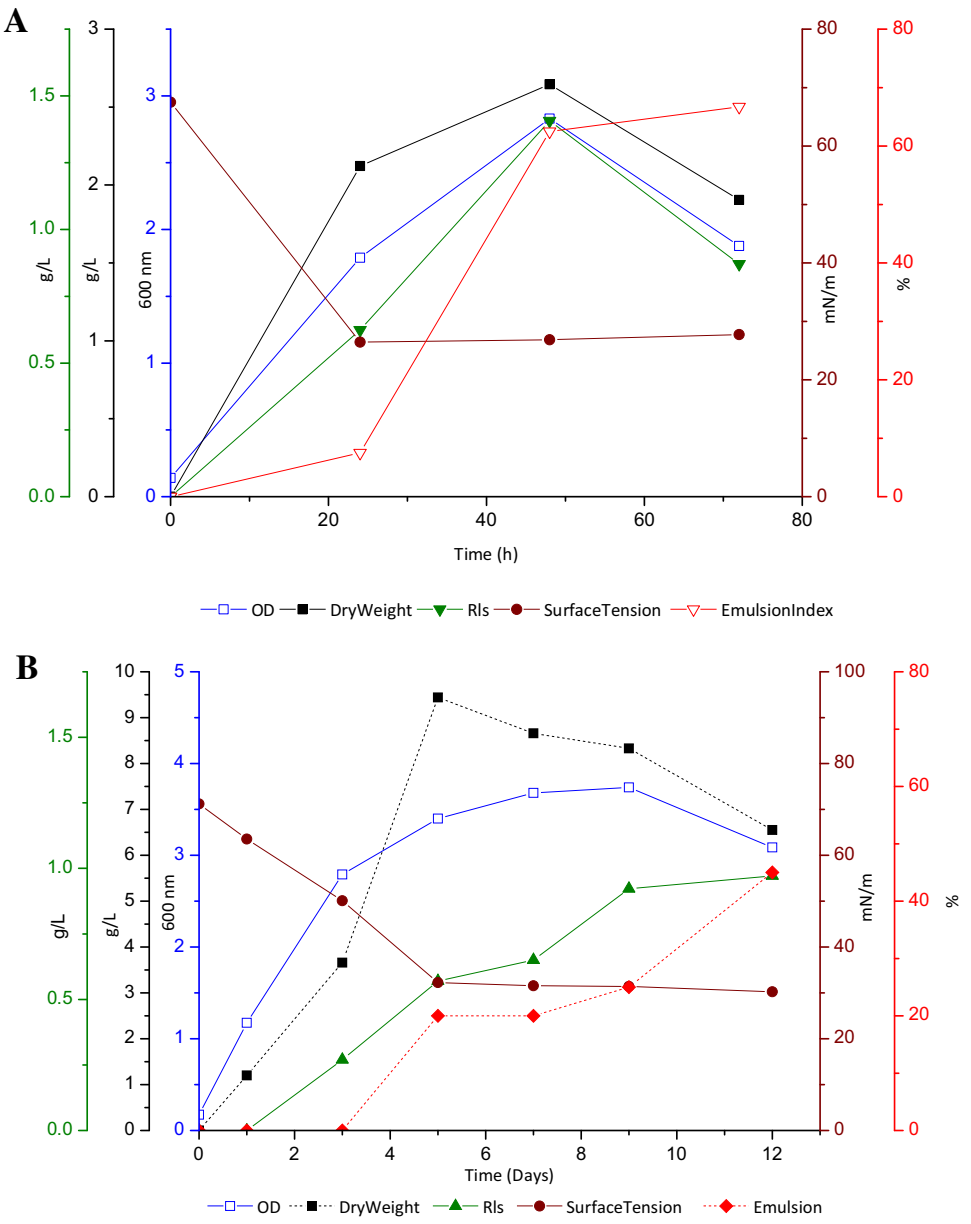

Fig. 2. Rhamnolipid production profile by *P. aeruginosa* ATCC 9027 (A) and *B. thailandensis* E264 (B). Rhamnolipids were extracted from cell free supernatant by acidification to pH 2–3 and solvent extraction with ethyl acetate. RL crude yields (g/L) were obtained by removing traces of aqueous phase with $MgSO_4$ and rotary evaporating to dryness. Yields were gravimetrically determined. Biomass (g/L), emulsion index (%) and surface tension (mN/m) and OD (600 nm) was obtained over time.

$$\text{Recovery} = \frac{C_F V_F}{C_i V_i} \times 100 \quad (2)$$

Where C_F is the concentration of rhamnolipid (g/L) in the foamate, C_i is the concentration of rhamnolipid (g/L) in the feed, V_F is the volumetric flow rate of foamate (L/s) and V_i is the volumetric flow rate (L/s) of the feed.

2.4. Rhamnolipid extraction

Rhamnolipid mixtures were extracted from feed, retentate and foamate samples from the foam fractionation process. The pH of each fraction was adjusted to 2.0 using hydrochloric acid (HCl) and an equal volume of ethyl acetate was added. The mixture was vigorously shaken for 5 min and allowed to set until phase separation. The organic phase was removed and the extraction process was further repeated twice. The obtained rhamnolipid product was concentrated from the pooled organic phases using a rotary evaporator. The viscous yellowish product obtained was dissolved in chloroform/methanol at 2:1 ratio and concentrated again by evaporation of the solvent at 45 °C.

2.5. Analytical methods and measurements

2.5.1. Cell biomass analysis

Microorganism cultures were centrifuged at 11,000*g* for 10 min. The supernatant was stored at −20 °C, until further use for the additional experiments. Cell pellets were washed thrice with distilled water under the same conditions of centrifugation and dried at 60 °C to a constant weight. The cell growth was monitored by measuring the weight (g) of the dry pellets and the OD_{600nm} of each culture before centrifugation.

2.5.2. Surface tension measurements

Surface tension was evaluated in 10 mL aliquots of fermented cultures in the absence of biomass, using a Krüss Tensiometer

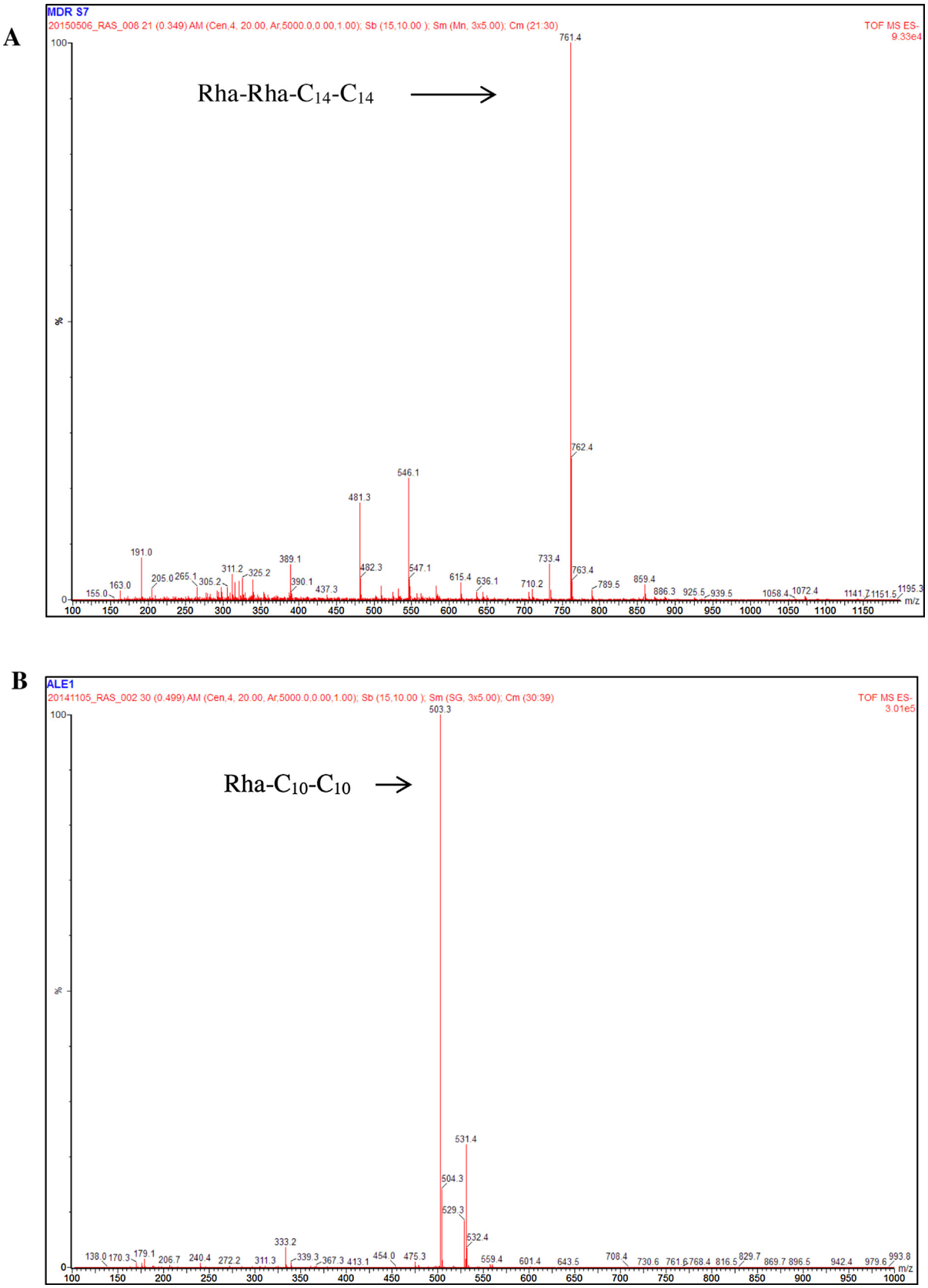

Fig. 3. ESI-MS analysis. Spectrum of partially purified extracts from fermented cells of (A) *B. thailandensis E264* and (B) *P. aeruginosa ATCC 9027* (Rha: rhamnose molecules) in the feed fraction.

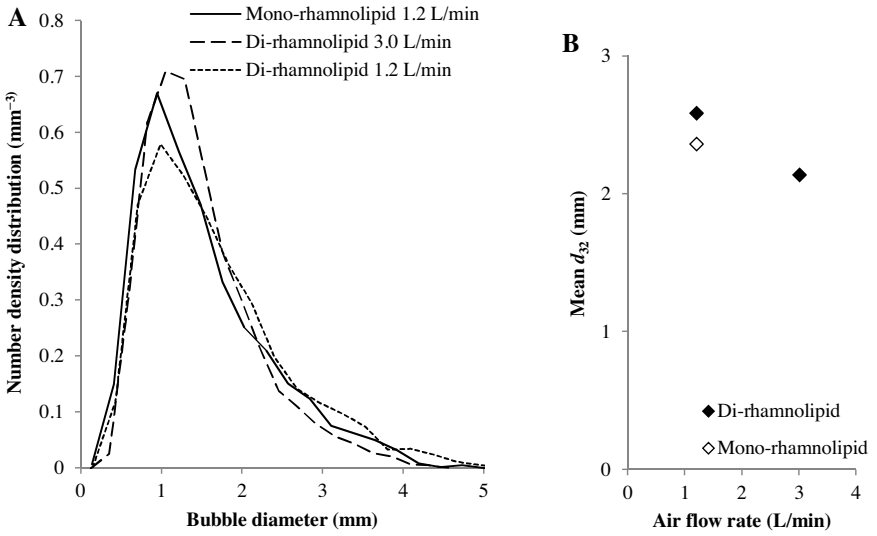

Fig. 4. Bubble size diagrams. (A) Bubble size distribution (Air flow rates are given in the legend). (B) Sauter mean diameter, d_{32}.

K11 Mk4. Distilled water was used to calibrate the instrument and measurements were performed in triplicate, using each culture media as a control. The critical micelle concentration (CMC) was determined from a plot of surface tension against rhamnolipid concentration by identifying the concentration at which surface tension plateaued.

2.5.3. Emulsifying capacity determination

The emulsifying capacity was measured by adding 5 mL of kerosene to 5 mL of the aqueous sample. The mixture was vortexed at a high speed for 2 min, and after 24 h, the height of the stable emulsion layer was measured. The emulsion index E is calculated as the ratio of the height of the emulsion layer and the total height of liquid (Eq. (3)):

$$E = \frac{h_{emulsion}}{h_{total}} \times 100\% \quad (3)$$

2.5.4. Measurement of foam bubble sizes in the foam fractionation column

The bubble size was measured by performing batch foam fractionation experiments and then collecting the foam produced in a glass container of square cross section with a glass prism attached to one face of the container.

A Canon EOS 7D camera with a macro lens was used to capture images which were then analysed using ImageJ, an open-source image processing and analysis package. A circular sticker of diameter 19 mm was placed on the container on the same plane as the foam. The images were calibrated by calculating the number of pixels per unit area for the known area of the circular sticker. The bubbles were assumed to be spherical; hence, the bubble diameter could be calculated [16].

2.5.5. Electrospray ionisation–mass spectrometry analysis

For mass analysis, partially purified rhamnolipid preparations (the feed, foamate, or retentate) were dissolved in water and characterised by electrospray ionisation–mass spectrometry (ESI–MS) using a Waters LCT mass spectrometer in negative-ion mode

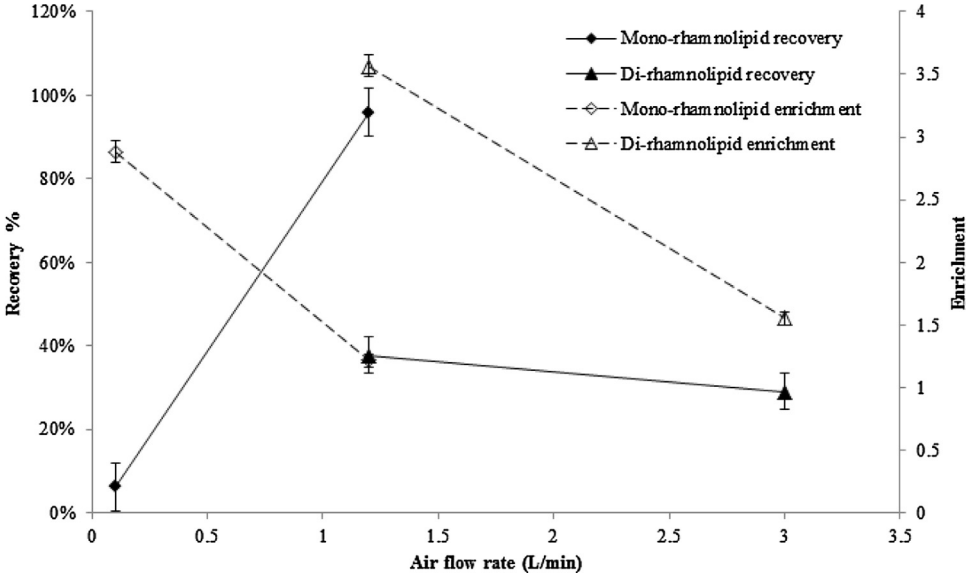

Fig. 5. Recovery and enrichment of Rha-C_{10}-C_{10} (mono) and Rha-Rha-C_{14}-C_{14} (di) against air flow rate. Error bars represents standard error.

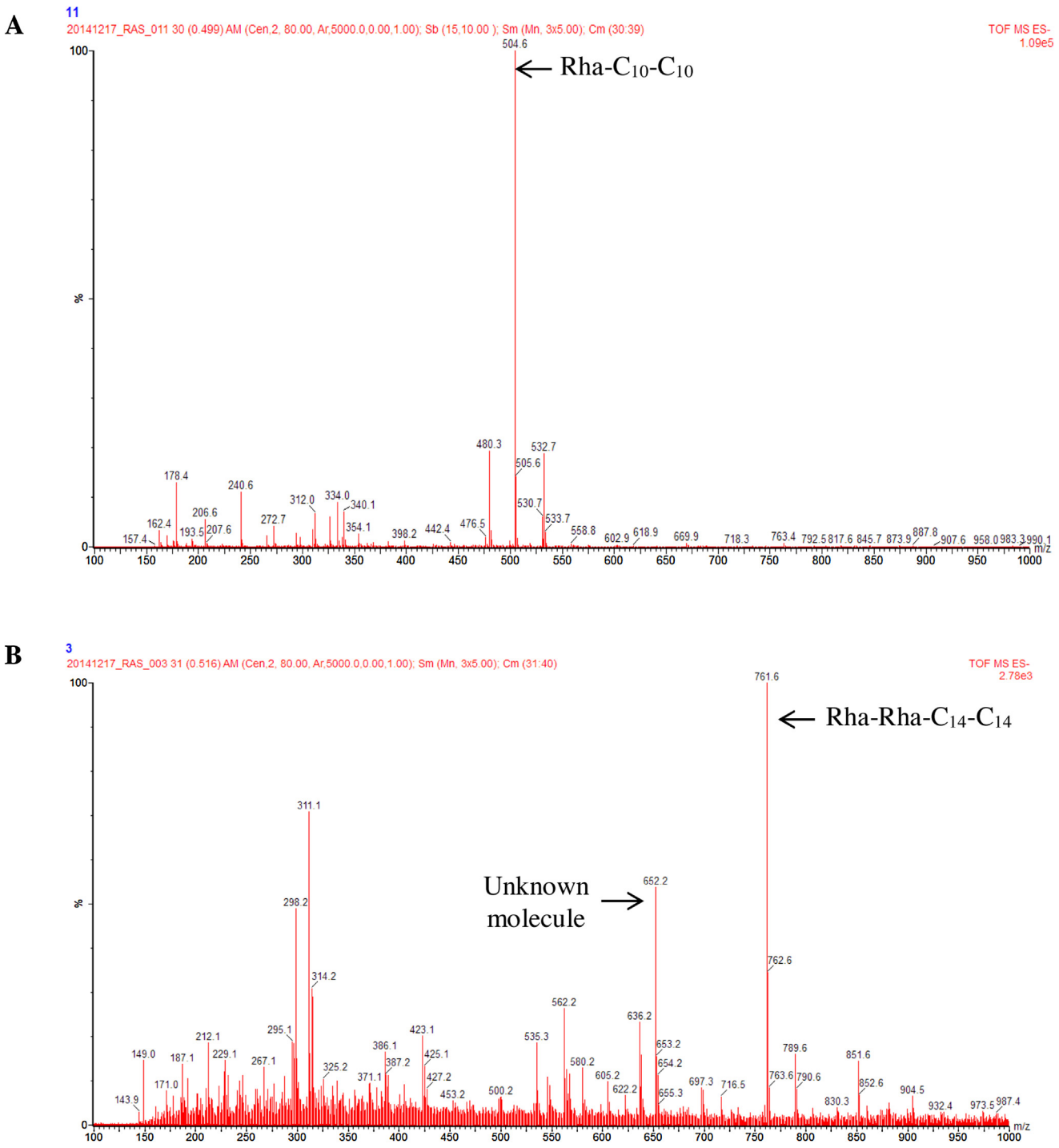

Fig. 6. ESI–MS analysis. Spectrum of partially purified extracts from (A) *P. aeruginosa* ATCC 9027 and (B) *B. thailandensis* E264 (Rha: rhamnose molecules) in the foamate fraction recovered at 1.2 L/min air flow rate.

previously tuned and calibrated with sodium fluoride (NaF). A 20-μL sample was injected into a mobile phase consisting of 50% acetonitrile and 0.1% formic acid using a Waters Alliance 1170 HPLC (high-performance liquid chromatography) system.

2.5.6. Protein assay

A Quick Start™ Bradford protein assay was used for protein quantification. Approximately 20 μL of the test sample was taken in an Eppendorf tube. Then 1 mL of the 1X dye reagent was slowly added with agitation. After thorough mixing, the tube was stoppered and left at 25 °C for 5 min. A bluish solution was obtained, and its absorbance was measured at 595 nm using a UV/Vis spectrophotometer (Shimadzu Uvmini-1240). The amount of protein was calculated in terms of μg/L of BSA (bovine serum albumin) in the test sample using a calibration curve of the coloured solution obtained from the reaction between the 1X dye reagent and the BSA in the concentration range of 0–1200 μg/L.

3. Results and discussion

3.1. Fermentation process

P. aeruginosa ATCC 9027 and *B. thailandensis* E264 were able to produce rhamnolipids under aerobic conditions. After 72 h, *P. aeruginosa* ATCC 9027 was able to produce rhamnolipids on PPGAS medium at 37 °C, using glucose (5 g/L) as the carbon source. On the contrary, *B. thailandensis* E264 was able to produce rhamnolipids on nutrient broth using glycerol (20 g/L) as the carbon source.

The rhamnolipids produced by *B. thailandensis* E264 reduced the surface tension of water to 32 mN/m while displaying a critical micelle concentration value of 225 mg/L, whereas those produced by *P. aeruginosa* ATCC reduced the surface tension of water to 24 mN/m (with a CMC of 20 mg/L). This indicated that both molecules were structurally different. These values are similar to those previously reported for *P. aeruginosa* sp. with values between 25 and 30 mN/m [17]. Dubeau et al. [18] reported for first time the ability of *B. thailandensis* to produce rhamnolipids, with a reduction of the surface tension of 42 mN/m, while in the present study, the reduction displayed 10 units lower than expected, more likely due to an improvement in the purification of the product analysed. The different microorganisms were assessed for their ability to form stable emulsion from their cell-free broth. The results showed that the highest percentage of emulsion (65%) was for rhamnolipids produced by *P. aeruginosa* ATCC 9027 (Fig. 2A) in contrast to the emulsion obtained (42%) for *B. thailandensis* E264 (Fig. 2B); the percentage of emulsion is another parameter that could indicate the presence of different molecules.

For both *B. thailandensis* E264 and *P. aeruginosa* ATCC 9027, the results from ESI–MS analysis revealed the presence of different types of rhamnolipids. In the case of *B. thailandensis*, E264, a dominant peak in the ESI–MS, was shown as a pseudomolecular ion of *m*/*z* 761 in the negative-ion mode (Fig. 3A), a value that is compatible with a compound consisting of two L-rhamnose molecules as well as two β-hydroxytetradecanoic acids. A corresponding di-rhamnolipid, 2-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxytetradecanoyl-β-hydroxytetradecanoate (Rha-Rha-C_{14}-C_{14}), with a molecular weight of 762 Da, has been previously reported from *Burkholderia pseudomallei* and *Burkholderia plantarii and B. thailandensis* itself [18]. The same ESI–MS method was used to confirm the rhamnolipid production by *P. aeruginosa* ATCC 9027. The presence of the mono-rhamnolipid rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate Rha-C_{10}-C_{10} was revealed as a predominant peak of *m*/*z* 503 (Fig. 3B). The analysis of *B. thailandensis* E264 cultures revealed long-chain rhamnolipids, with an HLB value of 9.2, under the conditions tested in this work. However, the rhamnolipids produced by *P. aeruginosa* ATCC 9027 had an HLB value of 6.5, under the conditions tested in this work. These values were calculated based on Griffin's method [19]. This confirms the hypothesis presented where both microorganisms produce different molecules that could have different application from a biotechnology point of view, suggesting that Rha-C_{10}-C_{10} and Rha-Rha-C_{14}-C_{14} could be used as an emulsifier and a wetting agent, respectively.

3.1. Foam fractionation studies

In this study, the effect of airflow rate on the foam bubble size was determined and related to the separation performance. The foam bubble size has two major impacts on the foam fractionation process: first on the surface area available for rhamnolipid adsorption and the other on the foam stability, liquid fraction of foam and foam structure. Foams with larger bubble sizes tend to be drier due to an enhanced foam drainage resulting in higher enrichment, while smaller bubbles are wetter yielding high recovery. The most commonly reported bubble diameter in the literature is the Sauter mean diameter d_{32} as it represents the surface area of bubbles, which is essential for the performance of the foam fractionation process in terms of surfactant adsorption [20]. The foam bubble size in the column was measured for *B. thailandensis* E264 at airflow rates of 1.2 and 3 L/min and for *P. aeruginosa* ATCC 9027 at an airflow rate of 1.2 L/min. Fig. 4A and B shows the bubble size distributions and Sauter mean bubble diameter, respectively, for *P. aeruginosa* ATCC 9027 and *B. thailandensis* E264 at different airflow rates.

The bubble size distributions for *B. thailandensis* E264 at 1.2 and 3 L/min overlap each other suggesting that a change in the airflow rate does not affect the bubble size. Similarly, the bubble size distribution for *P. aeruginosa* ATCC 9027 at an airflow rate of 1.2 L/min overlaps that of *B. thailandensis* E264, thus suggesting that the type of microorganism does not affect the bubble size distribution of rhamnolipids.

However, the mean d_{32} diameter calculated for *B. thailandensis* E264 at 1.2 L/min is slightly greater than that calculated at 3 L/min. Likewise, the mean d_{32} diameter calculated for *P. aeruginosa* ATCC 9027 at 1.2 L/min is less than the bubble diameter for *B. thailandensis* E264 at the same airflow rate. Therefore, the airflow rate and type of organisms have a small effect on the foam bubble size in the foam fractionation column; however, this effect on the foam fractionation process was not investigated.

The foam fractionation separation performance was evaluated in terms of recovery and enrichment. Fig. 5 shows the recovery and enrichment variation with an increasing airflow rate for Rha-C_{10}-C_{10} and Rha-Rha-C_{14}-C_{14}, based on dry weight analysis of the foamate fraction.

The results show that the recovery and enrichment of Rha-C_{10}-C_{10} increased and decreased, respectively, with an increasing airflow rate, as is expected for a single-component system. However, both recovery and enrichment of Rha-Rha-C_{14}-C_{14} decreased with the increasing airflow rate. The recovery decreased from 38% to 29%, while the enrichment decreased from 3.57 to 1.55. In order to investigate this behaviour, a protein assay was performed on the feed, retentate and foamate samples for both Rha-C_{10}-C_{10} and Rha-Rha-C_{14}-C_{14}. The feed, retentate and foamate samples from *P. aeruginosa* were analysed, without any detection of proteins in any fraction. The samples from *B. thailandensis* reacted in a different way; although no proteins were present in the feed and retentate samples, approximately 20 μg/mL protein was detected in the foamate samples at both airflow rates as shown in Table 1. It is believed that proteins were present in the fermentation samples of *B. thailandensis*; however, they were too dilute for detection by a colorimetric method.

Table 1
Protein concentration in the free-cell culture medium, the foamate or retentate of *B. thailandensis E264.*

Microorganisms	Air flow rate (L/min)	Protein Concentration (μg/mL)		
		Feed	Retentate	Foamate
B. thailandensis E264	1.2	–	–	19.33
B. thailandensis E264	3	–	–	20.60

– Not detected.

The presence of proteins in the foamate samples of *B. thailandensis* E264 and their absence in *P. aeruginosa* ATCC 9027 suggest that the different recovery and enrichment trends with the increasing airflow rate observed for Rha-Rha-C_{14}-C_{14} could be due to competitive adsorption between the rhamnolipids and proteins on the foam surface.

These results are further supported by an ESI–MS analysis on the foamate samples of *P. aeruginosa* ATCC 9027 and *B. thailandensis* E264 obtained at an airflow rate of 1.2 L/min shown in Fig. 6. In

addition to the desired Rha-Rha-C_{14}-C_{14} component, the ESI–MS data revealed an unknown molecule for *B. thailandensis* E264, while for *P. aeruginosa* ATCC 9027, only the expected Rha-C_{10}-C_{10} component was present. These results suggest that the foam fractionation method could be used for efficient recovery and enrichment of Rha-C_{10}-C_{10} and Rha-Rha-C_{14}-C_{14}; however, the optimal process conditions are specific for each congener.

4. Conclusions

P. aeruginosa ATCC 9027 produces Rha-C_{10}-C_{10} and *B. thailandensis* E264 produces Rha-Rha-C_{14}-C_{14} under the conditions tested in this study. It was demonstrated that foam fractionation in a continuous stripping mode can be used to purify and recover rhamnolipids produced by both microorganisms. *P. aeruginosa* ATCC 9027 was shown to behave as a single-component system, while the recovery and enrichment trends of *B. thailandensis* E264 with the increasing airflow rate indicated the presence of other surface-active components in the fermentation broth, thus resulting in competitive adsorption in the foam fractionation column. These results demonstrate that foam fractionation is promising as a primary purification and recovery step for rhamnolipids produced by *P. aeruginosa* ATCC 9027 and *B. thailandensis* E264. However, to fully understand the potential of foam fractionation for biosurfactant recovery, the integration of fermentation and foam fractionation is vital.

Acknowledgement

The authors are grateful for financial support from the UK Engineering and Physical Sciences Research Council (EP/I024905) which enabled this work to be conducted.

References

[1] H. Amani, M.R. Mehrnia, M.H. Sarrafzadeh, M. Haghighi, M.R. Soudi, Scale up and application of biosurfactant from *Bacillus subtilis* in enhanced oil recovery, Appl. Biochem. Biotechnol. 162 (2010) 510–523.
[2] H.E. Connolly, P.K.S.M. Rahman, I.M. Banat, R.A. Lord, Resource recovery and reduction of oily hazardous wastes via biosurfactant washing and bioremediation, in: P. Grazyna (Ed.), Trends in Bioremediation and Phytoremediation, Research Signpost, Trivandrum, India, 2010, pp. 157–172.
[3] R. Marchant, I.M. Banat, Microbial biosurfactants: challenges and opportunities for future exploitation, Trends Biotechnol. 30 (2012) 558–565.
[4] K.K.S. Randhawa, P.K.S.M. Rahman, Rhamnolipid biosurfactants—past, present, and future scenario of global market, Front. Microbiol. 5 (2014) 454.
[5] T. Sarachat, O. Pornsunthorntawee, S. Chavadej, R. Rujiravanit, Purification and concentration of a rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* SP4 using foam fractionation, Bioresour. Technol. 101 (2010) 324–330.
[6] R. Sen, T. Swaminathan, Characterization of concentration and purification parameters and operating conditions for the small-scale recovery of surfactin, Process Biochem. 40 (2005) 2953–2958.
[7] I.M. Banat, M.A. Díaz De Rienzo, G.A. Quinn, Microbial biofilms: biosurfactants as antibiofilm agents, Appl. Microbiol. Biotechnol. 98 (24) (2014) 9915–9929.
[8] Y. Zhang, R.M. Miller, Enhancement of octadecane dispersion and biodegradation by a *Pseudomonas* rhamnolipid surfactant (biosurfactant), Appl. Environ. Microbiol. 58 (1992) 3276–3282.
[9] M. Rudden, K. Tsauosi, R. Marchant, I.M. Banat, T.J. Smyth, Development and validation of an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for the quantitative determination of rhamnolipid congeners, Appl. Microbiol. Biotechnol. 99 (21) (2015) 9177–9187.
[10] L. Xueliang, P. Stevenson, Foam Engineering: Fundamentals and Applications, John Wiley & Sons, Ltd., West Sussex, United Kingdom, 2012, pp. 307–324.
[11] S. Mukherjee, P. Das, R. Sen, Towards commercial production of microbial surfactants, Trends Biotechnol. 24 (2006) 509–515.
[12] P.J. Martin, M. Swain, R.C. Darton, Riser design in foam fractionation, in: E. Sorensen (Ed.), Distillation and Absorption, IChemE, Rugby, UK, 2006, pp. 657––666.
[13] C.Y. Chen, S.C. Baker, R.C. Darton, Batch production of biosurfactant with foam fractionation, J. Chem. Technol. Biotechnol. 81 (2006) 1923–1931.
[14] C.Y. Chen, S.C. Baker, R.C. Darton, Continuous production of biosurfactant with foam fractionation, J. Chem. Technol. Biotechnol. 81 (2006) 1915–1922.
[15] J.B. Winterburn, A.B. Russell, P.J. Martin, Integrated recirculating foam fractionation for the continuous recovery of biosurfactant from fermenters, Biochem. Eng. J. 54 (2011) 132–139.
[16] P.J. Martin, H.M. Dutton, J.B. Winterburn, S. Baker, A.B. Russell, Foam fractionation with reflux, Chem. Eng. Sci. 65 (2010) 3825–3835.
[17] N. Samadi, N. Abadian, R. Ahmadkhaniha, F. Amini, D. Dalili, N. Rastkari, E. Safaripour, F.A. Mohseni, Structural characterization and surface activities of biogenic rhamnolipid surfactants from *Pseudomonas aeruginosa* isolate MN1 and synergistic effects against methicillin-resistant *Staphylococcus aureus*, Folia Microbiol. 57 (6) (2012) 501–508.
[18] D. Dubeau, E. Deziel, D.E. Woods, F. Lepine, Burkholderia thailandensis harbors two identical rhl gene clusters responsible for the biosynthesis of rhamnolipids, BMC Microbiol. 9 (2009) 263.
[19] W.C. Griffin, HLB classification of surface-active agents, J. Soc. Cosmet. Chem. 1 (5) (1949) 11–26.
[20] A. Hofmann, G. Schembecker, J. Merz, Role of bubble size for the performance of continuous foam fractionation in stripping mode, Colloids Surf. A 473 (2015) 85–94.