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Enhanced rhamnolipids production via efficient foam-control using stop valve as a foam breaker

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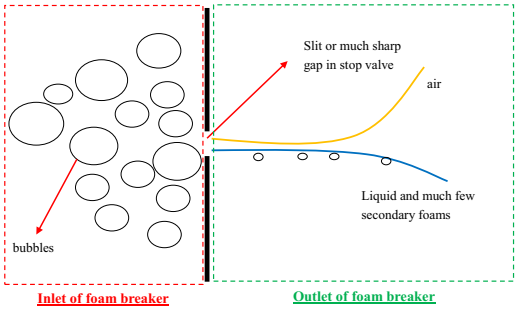
HIGHLIGHTS

- Stop valve at its tiny opening can readily break foams in rhamnolipid fermentation.
- Efficient foam-control by stop valve improved rhamnolipid fermentation efficiency.
- High shear force and fast air separation could explain foam-breaking by stop valve.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a stop valve was used as a foam breaker for dealing with the massive overflowing foam in rhamnolipid fermentation. As found, a stop valve at its tiny opening could break over 90% of the extremely stable rhamnolipid foam into enriched liquid when foam flows through the sharp gap in valve. The efficient foam-control by the stop valve considerably improved the rhamnolipid fermentation and significantly enhanced the rhamnolipid productivity by 83% compared to the regular fermentation. This efficient foam breaking was mainly achieved by a high shear rate in combination with fast separation of air from the collapsed foam. Altogether, the stop valve possessed a great activity in breaking rhamnolipid foam, and the involving mechanism holds the potential for developing efficient foam breakers for industrial rhamnolipid fermentation.

1. Introduction

Biosurfactants have attracted great interest as potential alternatives for synthetic surfactants in the last years due to being superior environmentally friendly and not harmful for human health. As reported, the market for these "green" surfactants is expected to increase to $2.8 billion by 2023 (Sekhon and Rahman, 2014). Consequently, enhancing the productivity as well as the production scale to achieve industrial production of biosurfactants is urgently desired (Sekhon and Rahman, 2014). Among the biosurfactants, rhamnolipid is well-accepted as the most promising one.

Rhamnolipids are glycolipid biosurfactants largely produced by various strains of *Pseudomonas aeruginosa* and related species, comprising one or two rhamnose molecules and up to three molecules of hydroxyl fatty acids containing 8–14 carbons (Long et al., 2013a). Owing to their excellent surface/interfacial activities and their multifunctional as well as eco-friendly properties, rhamnolipids have a variety of potential applications in agricultural, envi-

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ronmental, petrochemical, pharmaceutical, cosmetics, or food industries (Liu et al., 2016; Long et al., 2013a,b; Sekhon and Rahman, 2014), holding a great potential as eco-friendly alternatives to synthetic surfactants. However, their commercialization is largely limited by the high production cost (Gudiña et al., 2016; Moya Ramírez et al., 2016; Sekhon and Rahman, 2014).

Extensive efforts have been put into reducing the production cost of rhamnolipids by constructing high-yield recombinant mutant strains (Zhao et al., 2015a) *via* using inexpensive agro-industrial by-products or wastes as the sole carbon source (Gudiña et al., 2015, 2016; Moya Ramírez et al., 2016), and developing economically viable downstream processes (Heyd et al., 2008; Long et al., 2012). However, the rhamnolipid productivity in bioreactors as well as their production scale-up were both limited by the severe foam problem in fermentation (Chayabutra et al., 2001; Sha et al., 2012b). As the dominant foaming components in culture broth, the biosurfactant rhamnolipids can generate massive foams under aeration and intensive agitation during its fermentation (Long et al., 2016). Such severe foaming can largely limit the working efficiency of a bioreactor (normally no more than 50%). Moreover, the massive foam overflowing from the bioreactor can suspend the fermentation by bringing detrimental impacts, such as loss of product, nutrients, and cells, and caused sterilization and contamination problems (Pornsunthorntawee et al., 2009). Hence, the severe foaming in rhamnolipid fermentation should be either avoided or efficiently conrolled.

Although anaerobic (Zhao et al., 2015b) or solid fermentation processes (Camilios-Neto et al., 2011) have been developed to avoid foam formation, aerobic fermentation still seems to be the sole approach for the high-yield production of rhamnolipids (Müller et al., 2010; Zhu et al., 2012). In this respect, efficient foam-control is the most urgently required technology in rhamnolipid fermentation. Typical foam-control is carried out *via* adding chemical anti-foam or defoamer (Sha et al., 2012b; Zhu et al., 2012) to prevent foam formation or escape. This approach is very costively and can even cause undesirable impacts upon the fermentation as well as downstream separation owing to the massive addition of antifoam caused by the extremely stable foam in rhamnolipid fermentation (Long et al., 2016; Sha et al., 2012a). Hence, the restriction of foam are very important for the aerobic rhamnolipid fermentation (especially in high-yield production of rhamnolipid), and ex-situ foam breaking by means of mechanical breakers could be an alternative to overcome these difficulties (Gong et al., 2015). So far, the extensively used mechanical rotary devices (Hoeks et al., 2003) cannot effectively break rhamnolipid foam, and even aggravate the foam problem by producing more stable secondary foam (Long et al., 2016).

Interestingly, rhamnolipid foam was previously observed to be readily disrupted at crossing a ball valve at a tiny opening (unpublished data), implying the valve could be a promising new foam breaker for challenging the serious foaming problem in rhamnolipid fermentation. Hence, in this study, the applicability of a valve as a foam breaker will be investigated for ex-situ dealing with the massive overflowing foam in rhamnolipid fermentation. To this purpose, the foam-breaking efficiency and capacity of this valve will be evaluated.

2. Materials and methods

2.1. Materials

Rhamnolipids extract (over 90% purity) used as foaming agent was obtained from Huzhou Gemking Biotechnology Co., Ltd. (Huzhou, China). The chemical surfactants sodium dodecyl sulfate (SDS) and polysorbate 20 (Tween 20) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The remaining chemicals were purchased from a local supplier and were of reagent grade.

2.2. Strain and culture medium

The strain *Pseudomonas aeruginosa* ZJU211 (CCTCC M209237), isolated from heavily oil-contaminated soil, was used to produce rhamnolipids. The culture medium comprised: colza oil, 6% (v/v), 2.0 g/L $NaNO_3$, 1.0 g/L NaCl, 1.0 g/L KCl, 0.1 g/L $CaCl_2{\cdot}2H_2O$, 6.5 g/L KH_2PO_4, 11.0 g/L $Na_2HPO_4{\cdot}12H_2O$, 0.25 g/L $MgSO_4$, and 2 mL/L of a trace element solution containing: 0.08 g/L $FeCl_3{\cdot}6H_2O$, 0.75 g/L $ZnSO_4{\cdot}7H_2O$, 0.08 g/L $CoCl_2{\cdot}6H_2O$, 0.075 g/L $CuSO_4{\cdot}5H_2O$, 0.75 g/L $MnSO_4{\cdot}H_2O$, 0.15 g/L H_3BO_3, and 0.05 g/L $Na_2MoO_4{\cdot}2H_2O$. The pH of the medium was adjusted to 6.8 using 1 M NaOH solution prior to autoclaving.

2.3. Rhamnolipid fermentation

A single *P. aeruginosa* colony on an agar plate was inoculated into 30 mL of Luria-Bertani (LB) medium in a 150 mL flask and cultured in a shaking incubator (ZWY-2102C, Zhicheng, Shanghai, China) at 37 °C and 220 rpm for 48 h. Then, the culture was inoculated at 3% (v/v) into 100 mL of culture medium in a 500 mL flask and grown for 48 h as the seed for batch fermentation of rhamnolipids in a bioreactor.

For rhamnolipid fermentation, a 10-L stirred bioreactor (GUJS–10C, Eastbiotech, Zhenjiang, China) equipped with an integrated process control system for temperature, pH, p_{O2}, and airflow was used. The fermentation was performed under agitation with 300 rpm at 37 °C for 96 h, and 2% (v/v) of colza oil was fed at 48 and 60 h, respectively. After fermentation, the rhamnolipid yield and broth volume were recorded.

During fermentation, samples were routinely collected for the offline analysis of cell and rhamnolipid concentration. The culture broth was centrifuged (H-1650 R, Xiangyi, Hunan, China) at 15,000 rpm (13,800*g*) at 4 °C for 5 min. The supernatant was used for measuring the rhamnolipid content applying the anthrone-sulfuric acid assay, while the sediment was suspended in deionized water for detecting the cell content (Long et al., 2016). All assays were carried out in triplicate.

2.4. Foam-breaking and its efficiency

Rhamnolipid or chemical surfactant solution at 5 L was sparged in the 10-L bioreactor under agitation at 300 rpm for generating foam. The overflowing foam was disrupted by guiding it through the valves with a very confined opening. After breaking, the enriched liquid (collapsed film) and foams were pumped back to the bioreactor *via* a silicon tube using a peristaltic pump (BT300M, Langer, Baoding, China).

Like the ex-situ foam-control, the broth containing large amounts of cells should be pumped back to the bioreactor avoiding oxygen limitation and contamination. The pump efficiency of the collapsed foam is reflected by the volume and liquid fraction of the foam. The foam volume reduction ratio (*FVE*) as well as the liquid fraction of the foam (η) were used as more practical alternatives to evaluate the foam-control efficiency. The *FVE* was calculated according to the equation

$$\mathrm{FVE}[\%] = \frac{F_0 - F_c}{F_0} \times 100 \quad (1)$$

where F_0 and F_c are the foam flow rates (L/min) when the valve is fully or tiny opened, respectively.

The liquid fraction of the foam η was calculated based on the equation

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