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# Improving Biosurfactant Recovery from *Pseudomonas aeruginosa* Fermentation

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## 1. Introduction

Surface active agents or surfactants or are amphiphilic compounds that are chemically synthesized which can greatly reduce the surface tension of a liquid. They are widely used industrially for various purposes such as detergents, wetting agents, foaming agents, emulsifiers, dispersants, lubricants and penetrants (Mulligan & Gibbs, 1993). To date, a large majority of surfactants used are chemically synthesized including alkylbenzene sulfonates (detergents) and lauryl sulfate (foaming agent) (Mukherjee *et al*., 2006). These synthetic surfactants have been used in the oil industries to aid the clean up of oil spills, rapidly removing large amounts of oil from the ocean/soil surface (Banat, 1995). However, much like the oil they remove, these compounds exhibit poor biodegradability, are toxic to the environment and consequently, have limited applications.
In recent years, environmental compatibility and biodegradability have become increasingly important factors in the selection of industrial chemicals (Banat, 1995). For this reason, natural biosurfactants appear as a promising candidate to replace or reduce the usage of chemically synthesized surfactants. Biosurfactants are surface active biomolecules produced by a variety of microorganisms such as bacteria, yeast and fungi. As with surfactants, they too are amphipathic molecules, comprising hydrophilic and hydrophobic domains. The simultaneous existence of these domains provides biosurfactants the ability to partition themselves at the interphases between different fluid phases (Banat *et al*., 2000). They show similar capability of reducing the surface and interfacial tensions using the same mechanisms as the synthetic surfactants. Owing to their unique characteristics which include lower toxicity, higher biodegradability, environmental compatibility and stable activity at extreme pH, salinity and temperature, biosurfactants have gained attention and importance in various fields (Maier, 2003; Mullican *et al*., 2005).

### 1.1 Downstream processes

In general, biosurfactants are still unable to compete with the synthetic surfactants for commercial purposes due to their high production and recovery costs. As reported by Mukherjee *et al*., (2006), three main factors that hinder the commercialization of biosurfactant are: i) the high cost of raw materials; ii) the high recovery and purification costs; and iii) the low yields in the production processes. Thus, in order to reduce the

production cost of biosurfactants and to increase the efficiency of biosurfactant production, several techniques and approaches have been adopted worldwide. Inexpensive alternative substrates, optimized culture conditions in bioreactor operations, cost-effective recovery processes and strain improvements have been investigated to enhance biosurfactant yields (Chen *et al*., 2006; Deleu and Paquot, 2004; Joshi *et al*., 2008; Yeh *et al*., 2006).

Efficient downstream processing techniques are required to minimize the overall production costs of any biotechnological products, including biosurfactants. Moreover, approximately 60% of the total biosurfactant production expenditure is from the downstream processes. Thus, it is prudent to recover and purify the biosurfactants in a cost-effective manner as this will contribute significantly in minimizing the total cost of production. In most reported studies, biosurfactants are recovered from the culture media using a combination of several techniques such as precipitation, centrifugation and solvent extraction (Desai and Desai, 1993). However, the solvents that are generally used for biosurfactant recovery, such as methanol, chloroform, acetone and dichloromethane are air-polluting, costly and toxic to environment.

### 1.2 Foam fractionation

Foam fractionation has drawn the most attention for the recovery of surface active molecules as this technique offers high effectiveness and requires a low cost of operation. It was originally proposed by Leonard and Lemlich in 1965 and has recently been practiced by a number of researchers (Davis *et al*., 2001; Chen *et al*., 2006a; Chen *et al*., 2006b; Sarachat *et al*., 2010). In this technique, foam is allowed to overflow from the bioreactor through a fractionation column, resulting in a highly concentrated product. To date, due to the outstanding features of this technique, such as high effectiveness, high purity of product, low space requirements and environmentally friendly, there are a number of reports that presented foam fractionation as one of the most proficient methods in biosurfactant recovery (Chen *et al*., 2006a; Davis *et al*., 2001; Noah *et al*., 2002; Sarachat *et al*., 2010).

The present study focused on the production and recovery of a biosurfactant produced by *Pseudomonas aeruginosa* isolated from local crude oil sample. This bacterium was found to produce rhamnolipid, a glycolipid-type of biosurfactant. The study was initiated with the cultivation of *Pseudomonas aeruginosa* USMAR-2 in a bioreactor, followed by the simultaneous recovery of rhamnolipid. This resulted in a process with a combined rhamnolipid production and recovery. An integrated foam recycler was employed to fractionate the foam produced and recycle the froth containing biosurfactant into the reactor. Several parameters were manipulated including aeration and agitation rate to improve the rhamnolipid recovery efficiency and productivity. The main objective in this study is to use the foaming problem as a key to purify and concentrate the rhamnolipid by recovering the overflowing foam from the modified bioreactor.

## 2. Methodology

### 2.1 Foam fractionation

Batch cultivation was carried out in 3.0 L bioreactor (Bioflo 115, New Brunswick, USA), integrated with a foam fractionation system for the primary rhamnolipid recovery. The foam fractionation system consisted of two main parts: i) a 3.0 L bioreactor and ii) a foam recycler system (Figure 1). The foam recycler system was equipped with a foam collector vessel (500 mL flask) and a foam recycler pump. The rapid stirring and aeration supplied

during the fermentation ensured excessive foam and the overflowed foam was allowed to flow out of the bioreactor through an integrated foam tube. The resulting foamate was directed into the foam collector vessel and was continuously recycled into the bioreactor using the foam recycler pump until the rhamnolipid concentration in the foam remained constant. The foamate containing the concentrated product can be directly used for a suitable application or can be further purified should a higher concentration of rhamnolipid be required.

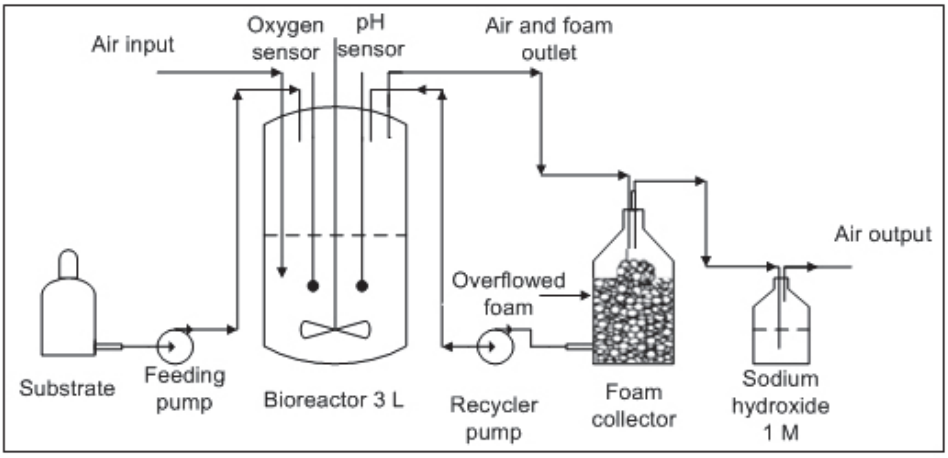


Fig. 1. Schematic figure of modified bioreactor with integrated foam recycler system

### 2.2 Aeration and agitation rate

The agitation speed and the aeration rate were manipulated throughout the study by setting the desired parameter values in the bioreactor controller, Biocommand OPC Version 1.30 (New Brunswick Scientific, USA).

### 2.3 Rhamnolipid recovery (%) and rhamnolipid enrichment

The formulas used to calculate the rhamnolipid recovery and enrichment in foam fractionation are as follows:

a. Rhamnolipid recovery, (%) =

$$\frac{\text{rhamnolipid concentration in foamate}}{\left(\begin{array}{c}\text{rhamnolipid concentration in foamate}\\ +\\ \text{rhamnolipid concentration in culture}\\ \text{when foaming ceases}\end{array}\right)} \text{x}100$$

b. Rhamnolipid enrichment, $E_R$ =

$$\frac{\text{rhamnolipid concentration in foamate}}{\text{rhamnolipid concentration remaining in the bioreactor}}$$

## 3. Results and discussion

In a normal bioprocess practice, foam formation is avoided at all costs as it will cause several problems, such as stripping of product, nutrients and cells into the foam. In most fermentation systems, the excessive foam can be controlled chemically or mechanically. The addition of chemical antifoams can often suppress a highly foaming culture. However, this technique will raise the cost of cultivation and lower its productivity as the antifoam (usually a surface tension lowering substance) can be costly and its presence may reduce the oxygen transfer rate and the nutrient uptake (Davis *et al*., 2001). Moreover, antifoam often works upon addition, but the foam build-up will soon ensue as the fermentation progresses. On the other hand, mechanical foam breakers or any mechanical devices are only applicable for a moderately foaming culture as this technique can cause high energy consumption (Heinzle *et al*., 2006).

The simultaneous rhamnolipid production and primary recovery using the foam fractionation system developed in this work was able to address the foaming problem associated with rhamnolipid production without the addition of an antifoam agent. As reported by Yeh *et al*., (2006), the biosurfactant product preferentially distributed into the foam fraction, resulting in a higher concentration of biosurfactants in the foam. Consequently, this approach gave a concentrated biosurfactant product while at the same time, alleviated the foaming problem.

### 3.1 Effect of different aeration rate

Besides establishing a bioreactor design that can control foaming and recover the product, the other interest in this work is to study the effect of aeration and agitation speed towards rhamnolipid production and foam formation. These two parameters are highly correlated with the oxygen transfer efficiency in the bioreactor (David *et al*., 2001, Yeh *et al*., 2006). Thus, the effect of the aeration rate towards rhamnolipid production was investigated by fixing the stirrer speed at 400 rpm. As indicated in Figure 2, the cell dry weight of *P. aeruginosa* was 6.96 g/L when the aeration rate was set at 1.0 vvm. A similar fermentation run, aerated at 0.5 vvm gave a cell dry weight of 5.88 g/L. Interestingly, as depicted in Figure 3, the rhamnolipid concentrations obtained with both aeration rates (0.5 vvm and 1.0 vvm) were not significantly different, 1.15 g/L and 1.41 g/L, respectively. Thus, 0.5 vvm was used in subsequent experiments (Table 1). However, fixing the agitation rate at 400 rpm with either 0.5 vvm or 1.0 vvm resulted in no overflowing foam. It is tempting to speculate that a higher agitation speed is needed to enhance the foam formation. Thus, it is essential to find a suitable agitation rate, leading to a condition that is favorable for foam formation while maintaining a high rhamnolipid concentration.

### 3.2 Effect of different agitation rates with foam recycler system

Yeh and co-workers (2006) reported that efficient mass transfer and sufficient oxygen supply played major roles in rhamnolipid production. The influence of agitation rates in enhancing rhamnolipid yield and productivity was further investigated. The highest rhamnolipid concentration in the foamate (2.93 g/L) was achieved using the foam recycler system, with the bioreactor aerated at 0.5 vvm and agitated at 500 rpm. As shown in Figure

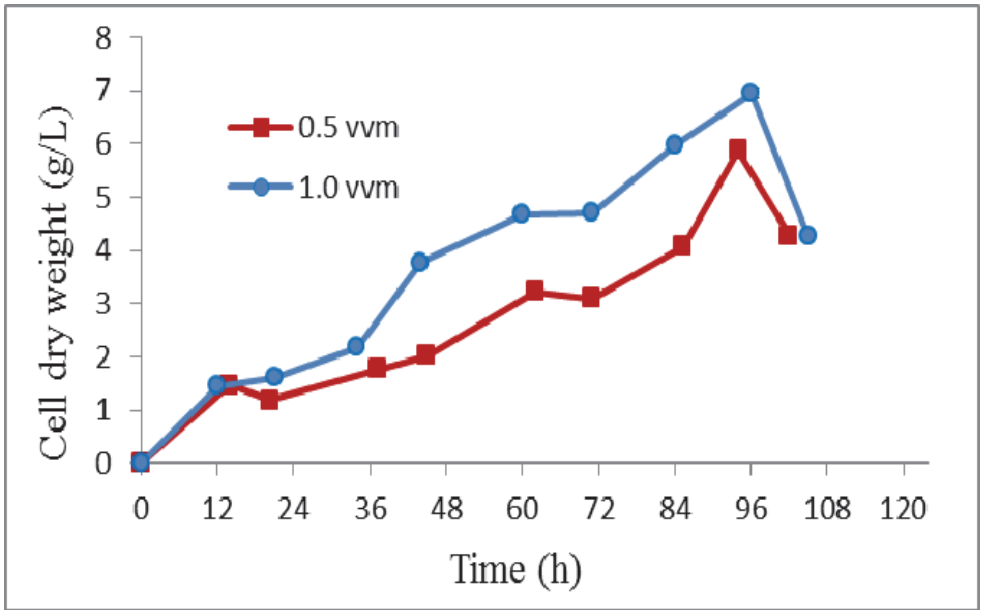


Fig. 2. Time profile of cell dry weight of *P. aeruginosa* USM AR-2 at different aeration rates

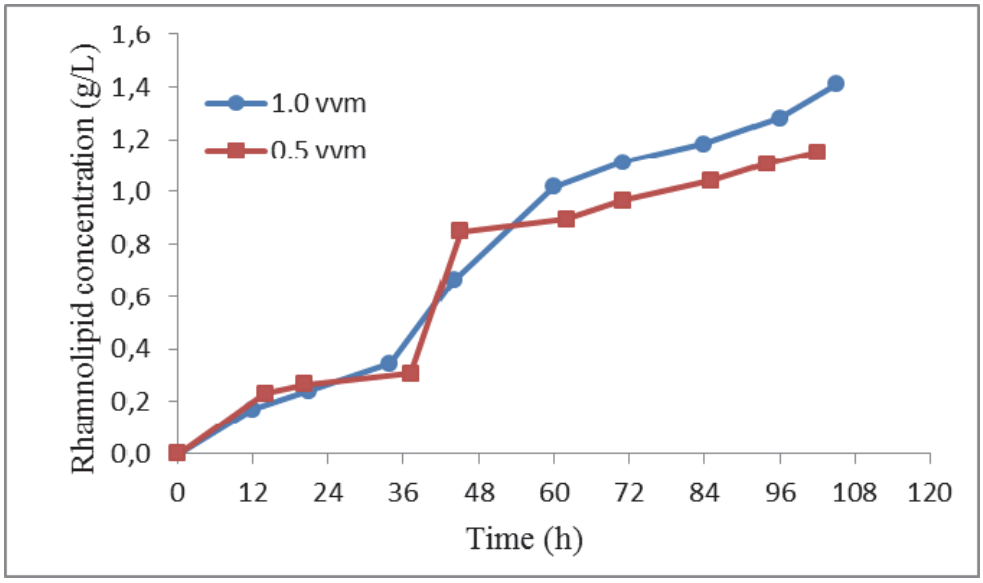


Fig. 3. Time profile of rhamnolipid concentration produced by *P. aeruginosa* USM AR-2 at different aeration rates

5, the highest concentration of rhamnolipid was detected from the recycled foam, not from the culture broth. This finding provides valuable information regarding the rhamnolipid enrichment and recovery process as summarized in Table 1, confirming that rhamnolipid was being concentrated in the foam.

Thus, this approach would be beneficial for subsequent downstream processes, providing an alternative source for rhamnolipid recovery as it was recovered from the froth rather than the spent broth. In addition, it presents a significant process cost reduction since the foamate is much smaller in volume relative to the spent broth. Rhamnolipid productivity in the foam recycler system was double the value of that in the conventional cultivation. In particular, the foam recycler system improved rhamnolipid production but did not contribute much to the growth of *P. aeruginosa* USM AR-2 in the cultivation system. The highest biomass densities for both conventional and foam recycler system remained similar (Figure 4). However, when the conventional cultivation was employed, approximately 50% of the culture broth was stripped from the vessel as a result of severe foaming during cultivation. It was assumed that this contributed to the lower concentration of rhamnolipid (1.15 g/L) as indicated in Figure 5. As summarized in Table 2, rhamnolipid production in the modified bioreactor from this work is highly competitive when compared with the findings from relevant studies done by several researchers (Table 2).

| Cultivation condition | | | Yield [a] | Productivity [b] | Rhamnolipid enrichment | Rhamnolipid recovery (%) | Remarks |
|---|---|---|---|---|---|---|---|
| Aeration rate (vvm) | Agitation rate (rpm) | Foam recycler system | | | | | |
| 1.0 | 400 | √ | 0.203 | 0.015 | - | - | No overflow foam |
| 0.5 | 400 | √ | 0.196 | 0.012 | - | - | No overflow foam |
| 0.5 | 500 | - | 0.144 | 0.014 | - | - | 50 % of culture broth was loss |
| 0.5 | 500 | √ | 0.233 | 0.028 | 1.89 | 65.40 | Overflowed foam was recycled |

[a] the yield of rhamnolipid on biomass (g $g^{-1}$)
[b] volumetric production rate (g $L^{-1}$ $h^{-1}$)

Table 3. Summary of results obtained in an integrated bioreactor for the production of rhamnolipid produced by *P. aeruginosa* USM AR-2

Direct utilization of the foam produced can be applied in many fields, such as in bioremediation where the foam can be used to flush contaminated soils. For example, in a study conducted by Mulligan & Wang, 2006, the feasibility of rhamnolipid foam to enhance the remediation of heavy metals in contaminated soils was evaluated.

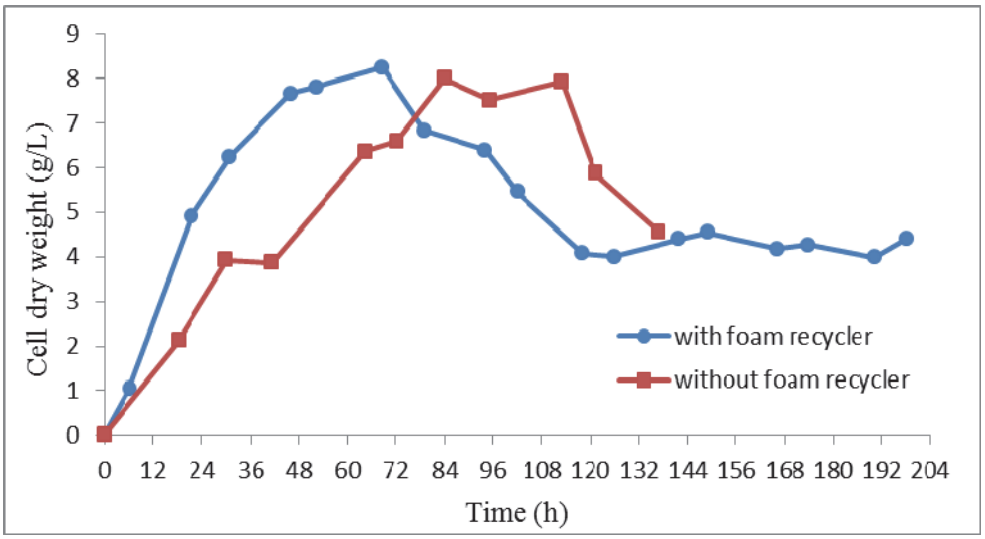


Fig. 4. Time profile of cell growth during batch fermentation agitated at 500 rpm under an aeration rate of 0.5 vvm

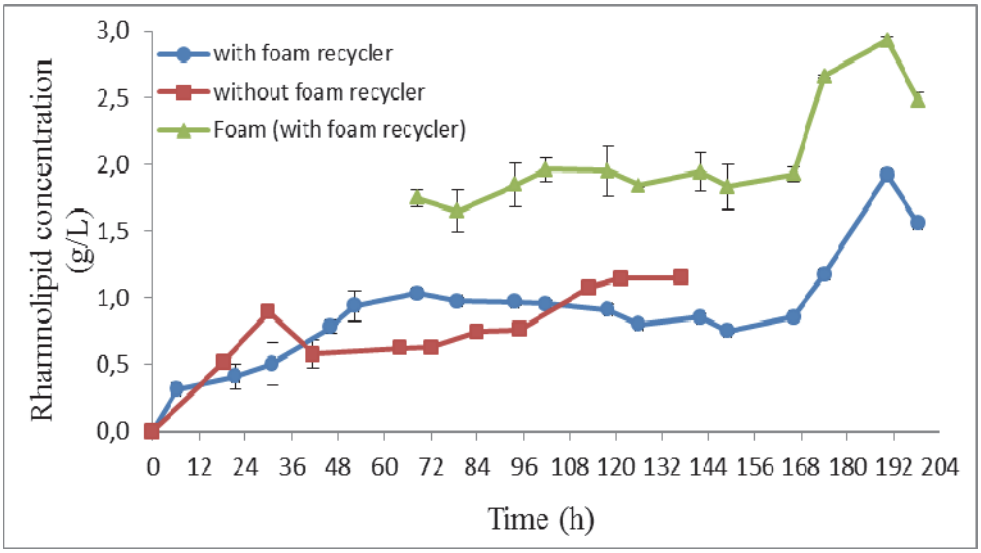


Fig. 5. Time profile of rhamnolipid concentration during batch fermentation agitated at 500 rpm under an aeration rate of 0.5 vvm

| Biosurfactant | Microorganism | Strategy | Highest biosurfactant (g/L) | Biosurfactant recovery (%) | Carbon source | Remarks | References |
|---|---|---|---|---|---|---|---|
| Rhamnolipid | *Pseudomonas aeruginosa* USM-AR2 | An integrated bioreactor with foam recycler system. The foam was continuously recycled back into the bioreactor. | 2.93 | 65.40 | Diesel oil | Batch | This work |
| | *Pseudomonas aeruginosa* | A foam fractionation column was integrated to bioreactor and sintered glasses were used to generate air bubbles with different pore size. | - | 97.00 | Palm oil | Batch | Sarachat *et al.*, (2010) |
| Surfactin | *Bacillus subtilis* | A foam collector and a cell recycler were integrated into the bioreactor. A solid carrier was added into the fermentation broth. | 6.45 | - | Glucose | Batch | Yeh *et al.*, (2006) |
| | | A foam fractionation column was integrated to the bioreactor with its lower end penetrated the headspace of the fermenter. The overflowed foam was collected. | 2.25 | 92.00 | Glucose | Batch | Chen *et al.*, (2006a) |
| | | Continuous cultivation with an integrated foam column. A mechanical rotor was used to break the foam and the resulting foamate was collected. | 0.929 | 28.70 | Glucose | Continuous cultivation | Chen *et al.*, (2006b) |
| | | The foam was collected continuously whereby the foam collection was achieved using an inverted funnel. | 3.74 | - | Potato process effluent | Continuous cultivation using airlift bioreactor, | Noah *et al.*, (2002) |
| | | Foam was allowed to flow out of the bioreactor and the foamate was collected in a separate vessel. | 1.67 | 60 | Glucose | Batch | Davis *et al.*, (2001) |

Table 4. Comparison of foam fractionation for biosurfactant recovery from this work with other studies

## 4. Conclusion

At the end of this experiment, 2.93 g/L rhamnolipid was successfully obtained from the foam collected. The suitable agitation rate is very important to maintain the homogeneity of the medium and bacterial cells in the bioreactor. Using this combination (500 rpm and 0.5 vvm), a uniform distribution of the gas phase and sufficient gas-liquid mass transfer was achieved. With the foam recycler system, the foam was successfully fractionated and yielded a much higher rhamnolipid concentration. Severe foam formation was kept under control throughout the cultivation without the addition of an antifoam agent. Further research is needed to improve the system, namely to enhance the production where the other modes of cultivation (fed-batch or continuous) can be explored.

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