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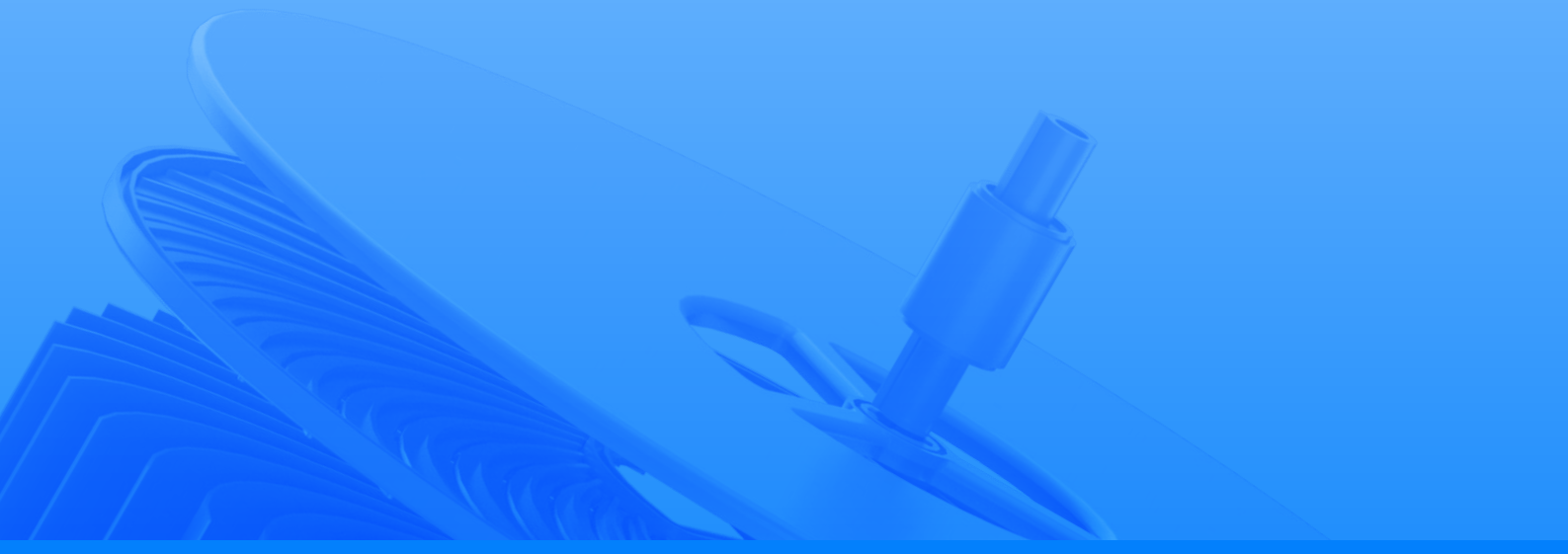
A Benchmark Comparison

Novel membrane stirrer prevents excessive foaming in rhamnolipid production using *Pseudomonas putida*



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Introduction

Biosurfactants as Rhamnolipids (RLs) are naturally occurring amphipathic surface-active agents produced by microorganisms as yeast and bacteria. RLs are eco-friendly alternatives to petrochemical-derived synthetic surfactants with wide-ranging applications across various industries including environment, petroleum, agriculture, food and pharmaceuticals.

Apart from the typical microbial cultivation challenges, producing biosurfactants is especially prone to extensive foaming. When gas bubbles are introduced into the fermentation broth, the biosurfactants adsorb at the gas-liquid interfaces, forming bubbles with micelles stabilizing the foam (see Fig. 1) [1]. Foaming in bioprocesses can reduce product quality and quantity (e.g., by protein denaturation) as well as productivity and result in a loss of the whole-cell biocatalyst [2-4]. In this application note, fermentation experiments using *Pseudomonas putida* KT2440 SK4 strains were conducted to establish a benchmark run comparing RL titers in the BioThrust Mem*Stir* with a conventional stirred-tank reactor (STR) at a 2 L scale. The Mem*Stir* demonstrated a space-time yield (STY) of up to 118 mg_{RL} L^{-1} h^{-1}, ranking it high in industrial RL production with both conventional and complex fermentation methods.

Objective

- Investigate foaming, oxygen supply solutions & transmembrane pressure (TMP) in RL production and its impact on product quantity.
- Performance analysis of BioThrust's Mem*Stir* with a conventional STR in RL batch processes using *Pseudomonas putida*.
- Getting an insight into a complex RL production using a fed-batch process.

Key Results

1. Foam-free batch and fed-batch state-of-the-art fermentation.
2. More than 3-fold increase of rhamnolipid production.
3. Increased productivity due to 65 % increased batch volume.
4. The Mem*Stir* enabled a superior STY of up to 118 mg_{RL} L^{-1} h^{-1}.

Materials & Methods

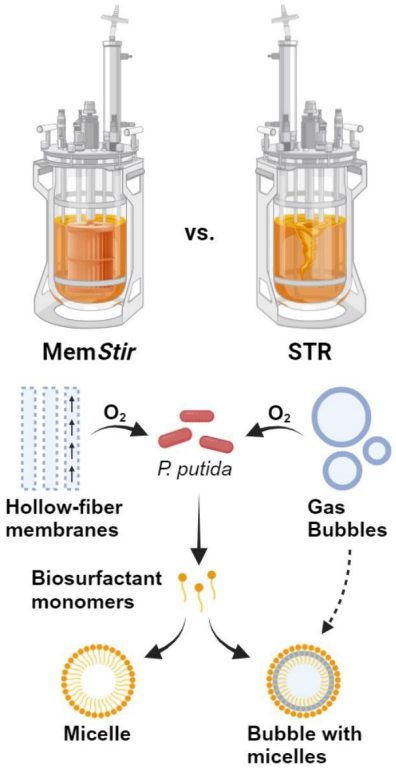

Figure 1: Overview of RL production with *P. putida* by either conventional sparged bubble-aeration in an STR (A) or membrane aeration with BioThrust's Mem*Stir* (B). Created with BioRender.com.

Specifications

- **Bacterial strains**:
  P. putida KT2440 SK4 and *P. putida* $KT2440_{\Delta flag}$ SK4
- **Culture medium:**
  - Preculture 1: Lysogeny Broth (LB).
  - Preculture 2: 3x buffered Minimal Salt Medium (MSM) with 10 g L^{-1} glucose.
- **Bioreactor:**
  Working volume of 1.2 L (STR) and 2 L (Mem*Stir*).
- **Process modalities:**
  Batch and Fed-Batch
  (DO controlled feed: 200 g L^{-1} glucose).
- **Process time:**
  Batch: 10 h, Fed-Batch: 18 h.

Abbreviations

RL = Rhamnolipid
STR = Stirred-tank reactor
STY = Space-time yield
SK4 = Wild type containing RL synthesis promoters
LB = Lysogeny broth
CDW = Cell Dry Weight
XO_2 = O_2 content in the feed gas
MSM = Minimal salt medium
DO = Dissolved oxygen
GLC = Glucose
rpm = Revolutions per minute
TMP = Transmembrane pressure

Parameter	Mem*Stir*	STR
Stirrer	100 – 350 rpm	300 – 1200 rpm (Rushton turbines)
DO	>30 %	>30 %
pH	7.0 (Base adjusted)	7.0 (Base adjusted)
Temperature	30 °C	30 °C
Aeration	1 L/min	0.12 L/min
TMP	0 – 0.3 bar	–
Antifoam	-	23 g/L

Results

For the first time a novel membrane impeller, the Mem*Stir* was developed and patented (W0 2021/152128) and applied for a benchmark run for a foam-free RL production (see Fig. 1). Please refer to the Technical Application Note for further details. The benchmark fermentation was conducted in a conventional STR using submerged bubble-aeration and a high titer RL-producing recombinant *P. putida* KT2440$_{\Delta flag}$ strain [5]. The Mem*Stir* batch fermentation was conducted in biological duplicates with *P. putida* KT2440 SK4 for 10 h. The Mem*Stir* replaced the stirrer and ring sparger as the mixing and aeration unit for bubble-free aeration of the bioreactor. Figure 3A and 3C show the cell dry weight (CDW), glucose (Glc), ammonium sulfate ($(NH_4)_2SO_4$), and RL concentrations within the conventional bioreactor process (A) and within the Mem*Stir* process (C). Figures 3B and D show the DO and O_2 content in the feed gas XO_2 as well as the stirring rate within the conventional bioreactor (B) and the Mem*Stir* (D).

Batch Benchmark

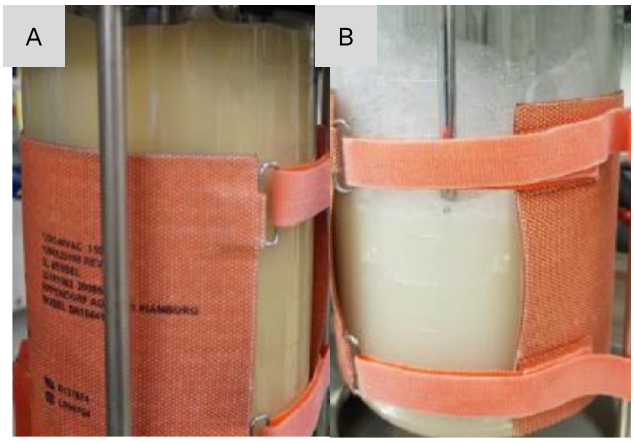

Figure 2: Images of batch fermentations of *P. putida* in BioThrust's Mem*Stir* (A) and in an STR with conventional stirring and aeration (B). Working volumes of the conventional STR experiments were reduced due to occurring foam formation.

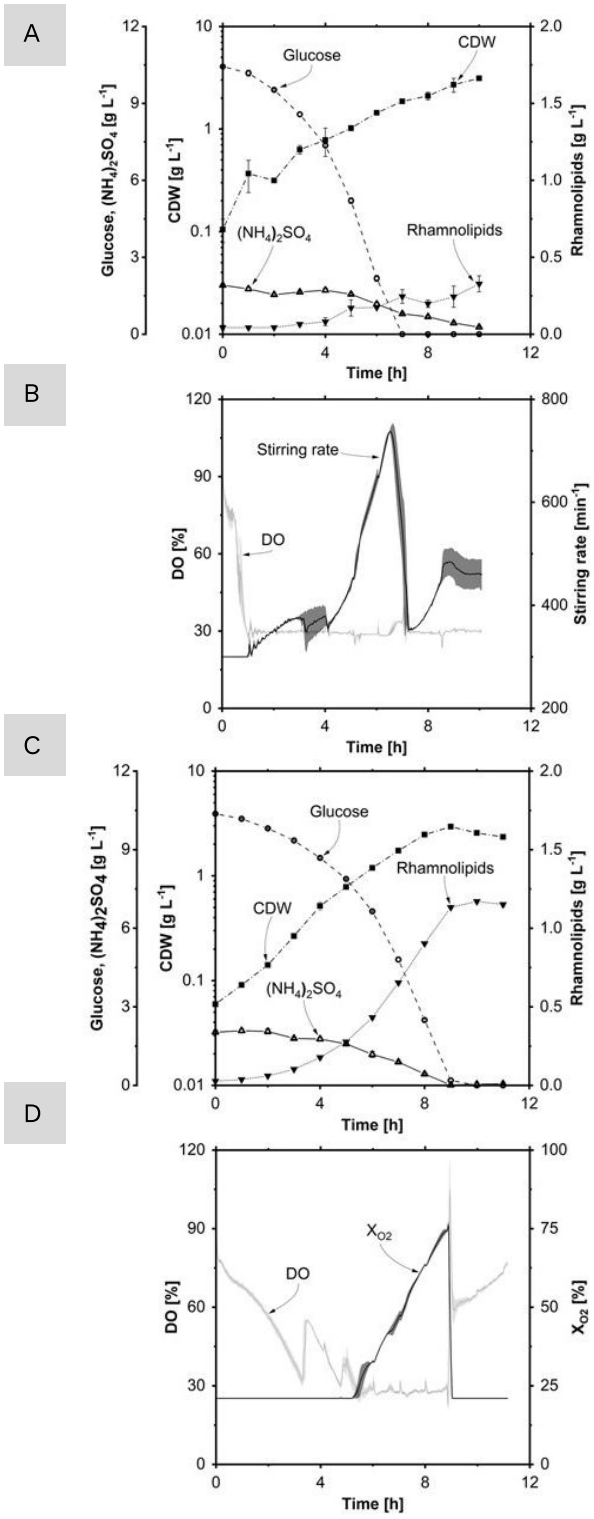

Figure 3: Results of the batch cultivations with *P. putida* KT2440$_{\Delta flag}$ SK4 in the conventional STR (A, C) and with *P. putida* KT2440 SK4 in the Mem*Stir* STR (B, D). (A) and (C) are displaying the cell dry weight (CDW), glucose (Glc), ammonium sulfate ($(NH_4)_2SO_4$), and rhamnolipid (RL) titers over time. (B) and (D) indicate the DO, stirring rate and O_2 content in the feed gas XO_2 over time. Experiments were conducted as biological duplicates, whereas off-line samples were analyzed as technical triplicates.

In the Mem*Stir* a maximum biomass concentration of 2.57 g L^{-1} with a μ_{max} of 0.59 h^{-1} was achieved. The DO was maintained at or above 30 % throughout the cultivation. At the end of the fermentation, a RL titer of 1.17 g L^{-1} was achieved, which is a measured 3.5-fold higher than in the benchmark STR fermentation using bubble-aeration. The culture volume was 1.7-fold higher in contrast to the benchmark fermentation. Thus, higher product amounts per batch can be achieved using the Mem*Stir*. Neither foam formation nor oxygen limitation were observed over the fermentation time. More RL accumulated in the Mem*Stir* process, increasing the titer approximately 3.3-fold with an STY of 118 mg_{RL} L^{-1} h^{-1}.

Fed-Batch investigation

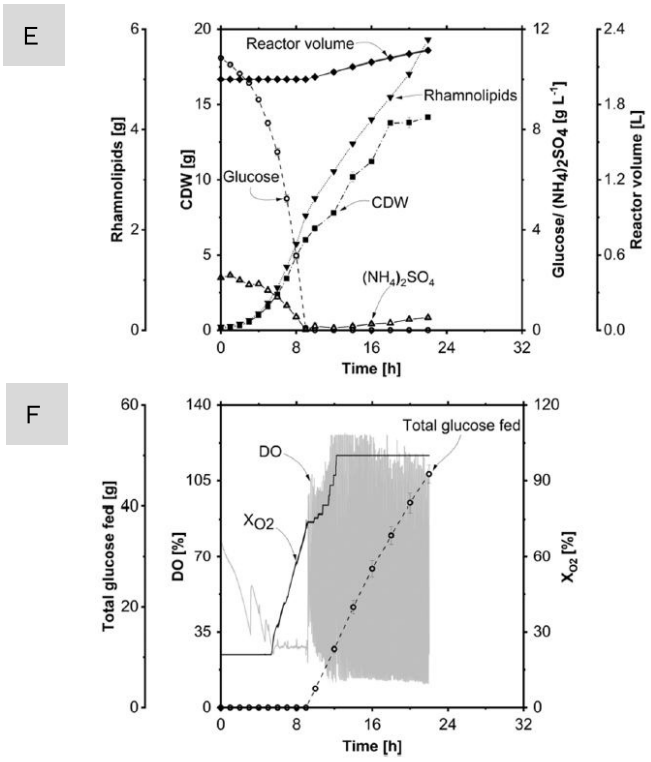

Figure 4: Fed-Batch cultivation of *P. putida* KT2440 SK4 in the Mem*Stir* setup (n = 2) displaying CDW, Glc, $(NH_4)_2SO_4$ and RL titers (E) as well as DO, stirring rate and XO_2 (F). Off-line samples were analyzed as technical triplicates.

The DO-based fed-batch fermentation was conducted in biological duplicates with *P. putida* KT2440 SK4 (see Fig. 4). A concentrated MSM containing 200 g L^{-1} glucose was used for feeding. Fermentation began with a batch phase that concluded after glucose depletion at 9 hours. Following depletion, DO levels rose to 70 %, prompting the start of the feed phase (Fig. 4F). At the end of the fermentation, 5.8 g rhamnolipids (2.6 g L^{-1}) and 14.1 g CDW (6.3 g L^{-1}) were reached (Fig. 4E). Compared with the batch fermentation with the SK4 strain, the RL titer (2.6 g L^{-1}) was increased by 2.2-fold. Furthermore, a STY of 118 mg_{RL} L^{-1} h^{-1} was achieved, equaling to the batch fermentation.

Discussion

The benchmark experiment comparing BioThrusts Mem*Stir*-driven bioreactor with a conventional STR demonstrated several advantages:

1. Due to no foam formation, the working volume could be increased to the maximum value recommended by the reactor manufacturer, which in turn allows for a more accurate assessment of the reactor's productivity.

2. RL bioprocesses will no longer require a continuous supply of antifoam, reducing downstream processing costs, purification expenses, and potential product loss [6].

3. BioThrust's Mem*Stir* achieved a more than 3-fold higher rhamnolipid titer compared to the conventional STR. In the Mem*Stir* aerated batch as well as fed-batch fermentations, a STY up to 118 mg_{RL} L^{-1} h^{-1} was reached.

The Mem*Stir* bioreactor demonstrates the feasibility of prolonged operation with minimal risk of batch failure, thereby increasing the potential yield. Both batch and fed-batch processes exhibited product titers and STY that are competitive with those reported in recent literature.

Conclusion

In this study, also published by Bongartz et al., (2023), high-titer RLs were produced by bubble-free aeration, preventing any foam formation and oxygen limitation during the fermentation process. Compared to previous reports about recombinant rhamnolipid batch production with *P. putida* KT2440, obtained product titers were greatly increased to conventional processes. Overall, cell concentrations and STY can be ranked in a higher performance category. While there are other process strategies that increase the system effectivity even further, results of the fed-batch shows a promising potential to further exceed current industrial standards when optimizing operation and feeding strategies.

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