



## Enhanced rhamnolipids production by *Pseudomonas aeruginosa* based on a pH stage-controlled fed-batch fermentation process

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### HIGHLIGHTS

- ▶ A novel fermentation of rhamnolipids by *Pseudomonas aeruginosa* was established.
- ▶ The rhamnolipids production reached 70.56 g/L.
- ▶ The rhamnolipids production was 2.93 times that of the original value.
- ▶ We have converted more substrate to rhamnolipids rather than the biomass.
- ▶ The highly related fermentation kinetics model in batch fermentation was established.

### ARTICLE INFO

#### Article history:

Received 3 January 2012

Received in revised form 23 March 2012

Accepted 23 April 2012

Available online 2 May 2012

#### Keywords:

Rhamnolipids

Fed-batch fermentation

Optimization

*Pseudomonas aeruginosa*

Kinetics

### ABSTRACT

Rhamnolipids find broad applications as natural surfactants, emulsifiers, and antibiotics because of their low toxicity, high biodegradability and environmental soundness. In this study, a pH stage-controlled process of fermentation of rhamnolipids by *Pseudomonas aeruginosa* O-2-2 was established. A yield of 24.06 g/L in batch fermentation was achieved in a 5-L fermentor via the optimization of stirring speed. By controlling pH, rhamnolipid production was increased to 28.8 g/L, an improvement of 19.7%, and more substrate was converted to rhamnolipids rather than to biomass. Fermentation kinetics models for cell growth, product synthesis and substrate consumption based on the pH stage-controlled fermentation indicated that rhamnolipid production could be further improved by fed-batch fermentation. Rhamnolipid production reached 70.56 g/L, an improvement of 193%, in the pH stage-controlled fed-batch fermentation when the stirring speeds was controlled at 500 rpm and the fermentation temperature was maintained at 30 °C.

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### 1. Introduction

Surfactants have been broadly used as emulsifying agents in the cosmetic and food industries, components in domestic and industrial cleaning products, and additives in the construction and petroleum industries (Banat et al., 2000). Most surfactants are produced by chemical methods, but biosurfactants, which can be produced by microbes, have various advantages including low toxicity, high biodegradability, high surface activity and an environmentally-friendly nature. Biosurfactants have been used in a number of fields including environmental protection, oil recycling, food processing and the biomedical industry (Bertrand et al., 1994; Cameotra and Makkar, 2004; Rodrigues et al., 2006). Rhamnolipids are one of the most important categories of biosurfactant because they have valuable applications in environmental protection and

biomedicine (Clifford et al., 2007; Maier and Soberon-Chavez, 2000; Rahman et al., 2002). For example, rhamnolipids can remove polyaromatic hydrocarbons and chemical oxygen demand components from petrochemical wastewater (Sponza and Gok, 2010). Many microorganisms, including *Pseudomonas aeruginosa*, can produce rhamnolipids (Hörmann et al., 2010; Rooney et al., 2009; Soberon-Chavez et al., 2005).

A number of studies have attempted to improve rhamnolipid production in submerged and solid state fermentation (Hori et al., 2011; Oliveira et al., 2009; Vasileva-Tonkova et al., 2011). However, the yields of rhamnolipids in most of the studies were below 30 g/L (Clarke et al., 2010; Oliveira et al., 2009; Wu et al., 2008). In one study, a yield of 70 g/L with 167 h of fermentation was achieved, but, the fermentation process was complicated and very long (Giani et al., 2000). Therefore, a simple liquid fermentation processes with high rhamnolipid productivity is needed.

Fermentation kinetics are the prerequisite to achieve industrial-scale production and some parameters of the fermentation kinetics

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