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BIOSURFACTANT PRODUCTION ON AIR LIFT BENCH REACTOR

Brenda Novais Santos[1], Ítalo Waldimiro Lima de França[1], Maria Cristiane Rabelo[2], Vania Maria M. Melo[3], Sueli Rodrigues[2] and Luciana Rocha Barros Gonçalves[1*]

[1] Universidade Federal do Ceará, Departamento de Engenharia Química

[2] Universidade Federal do Ceará, Departamento de Engenharia de Alimentos

[3] Universidade Federal do Ceará, Departamento de Biologia

*E-mail: lrg@ufc.br

ABSTRACT

This work aimed to investigate the biosurfactant production by Bacillus subtiulis ICA56 (isolated from a Brazilian mangrove) on air lift bench reactor, and evaluate its properties as a tensoative. Even though all biosurfactants advantages over chemical surfactants, the large scale production of biosurfactant is usually limited to the over production of foam, causing a possible product loss. Air lift bench reactor is an option for this issue. So, this reactor apparatus has been applied for biosurfactant production by Bacillus subtilis ICA56. Aeration and pH control have showed a straight-forward relation with biosurfactant production. It has been produced around 1,92 g.L^{-1} of lipopeptide biosurfactant, and a completely O_2 consumption, after 33 hours of assay, using aeration about 4L.min^{-1}. It has also been observed a reduction on surface tension about 42% during the cultivation, reaching about 31 mN.m^{-1}, showing its efficiency as a tensoative.

1. INTRODUCTION

Biosurfactants are amphiphilic surfactant molecules produced by microorganisms including bacteria and fungi. Biosurfactants have been attracted much attention from industry in recent years, due to their advantages over chemical synthetic surfactants: lower toxicity, higher biodegradability, better environmental compatibility, specific activity under extreme conditions (temperature, pH, and salinity) and the ability to be synthesized from renewable feedstocks (Reis *et al.,* 2013).

The industrial interest on biosurfactants is based on their functional properties: emulsification, separation, solubilisation, corrosion inhibition, chelating agent, reduction of liquid viscosity and reduction of surface tension. So, biosurfactants might be useful for environmental applications and for different industry fields such as petroleum, food production, chemistry, cosmetics and pharmaceutics (Pacwa-Płociniczak *et al.,* 2011). Some factors are reported to limit the large scale for biosurfactant production: low productivity on conventional stirred batch reactors (quite foam production), high cost for culture medium and purification process. In the last years, some authors have report strategies to overcome these problems: unusual reactor apparatus, such as air lift bench reactor and the use of renewable feedstocks, such as glycerol (Sousa *et al*., 2012; Franca *et al*., 2015).

Among different biosurfactants and producing microorganisms, the production of lipopeptide biosurfactants, mostly produced by *Bacillus* sp., is highlighted due to its considerable efficiency and commercial interest. Surfactin is one of the most powerful biosurfactants and it has gained attention for industrial application, since it's supoosed to reduce surface tension of water from 72 to 27 mN m^{-1} even at low concentrations (CMC ≅ 20 $mg.L^{-1}$) (Reis *et al.,* 2013).

In this context, this study aimed to evaluate the effect of aeration on the production of biosurfactant by *Bacillus subtilis* ICA56, using an air lift bench reactor with glycerol as carbon source. The tensoative capacity of the biosurfactant produced was also evaluated, as an indirect measure of biosurfactant's efficiency.

2. MATERIAL AND METHODS

2.1. Microorganism

The strain of *Bacillus subtilis* ICA56 was previously isolated from a Brazilian mangrove soil. The 16S rRNA gene sequence can be accessed in the *GenBank* (KM235112).

2.2. Inoculum and cultivation on air lift bench reactor

The stains was initially cultivated (inoculum) in mineral medium (Franca *et al.,* 2015) using glucose as carbon source. After 24 hours, some amount of this cultivate (250mL) was transferred to the air lift bench reactor up to a final volume of 2500 mL, with a mineral medium (Sousa et al., 2012) using glycerol as carbon source (20 $g.L^{-1}$). The effect of aeration on biosurfactant production was evaluated (4 and 5 $L.min^{-1}$ O_2). The assays were performed at 30 °C during 72 hours, with controlling and measurement for pH and O_2 saturation, and periodic sampling. Cell growth was determined by measuring the optical density (OD) on spectrophotometer at 600 nm. Then, cells were removed by centrifugation at 10,000 × g for 20 min and the cell-free supernatant was assayed to measure pH, surface tension and biosurfactant concentration.

2.3 Surface tension

Surface tension was determined according to the *De Nöuy ring* method at 30 ◦C by using a tensiometer (Kruss K6).

2.4 Crude biosurfactant concentration

An aliquot of 20 mL of the cell-free fermented broth was collected and the pH was adjusted to 2.0 with 3 M HCl. This solution was maintained for 12 h (overnight) at 4 ◦C (allowing the biosurfactant precipitation), then centrifuged at 10,000 × g and 4 ◦C for 15 min, dried (50 °C for 24 hours) and finally weighted (Perreira *et al.* 2013).

3. RESULTS AND DISCUSSION

Initially, the capacity of *Bacillus subtilis* ICA56 to grown and produce biosurfactant on air lift bench reactor, using glycerol as carbon source and continuous O_2 pumping (4 $L.min^{-1}$), was evaluated. Figure 1 presents the biomass production and O_2 consumption and Figure 2 shows the reduction on surface tension during the assay.

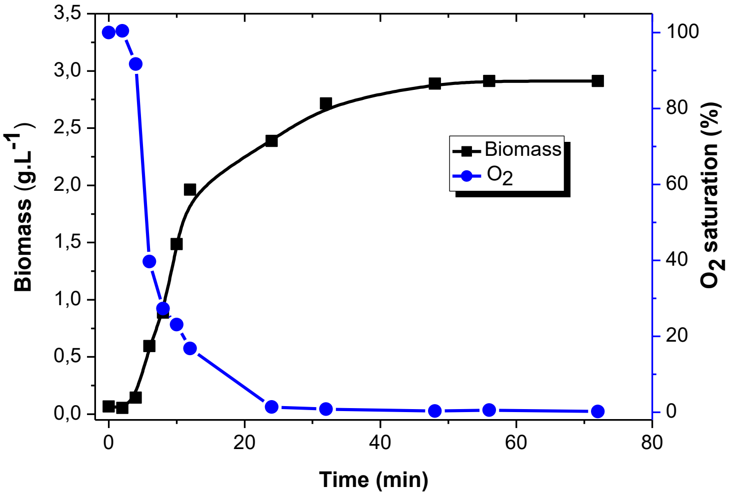

Figure 1 – Biosurfactant production on air lift bench reactor at 30 °C, 4 L.min^{-1}(O_2) and pH 7.

The Figure 1 shows the straight forward relationship between O_2 consumption and cell growth. The O_2 consumption follows directly the beginning of log phase (around 5 hours). Fahim *et al.* (2012) reported the O_2 mass transfer is an important factor for lipopeptide biosurfactant production by *Bacillus subtilis* strains, and aeration and stirring play an important role on it. High productivity on biosurfactants depends on O_2 mass transfer from gas phase to the bulk phase, but high aeration and stirring might reach low yields, since there is a considerable production of foam, and probably occurs a product loss (Reis *et al.,* 2013). So, unusual apparatus for biosurfactant production are promising, since it might solve the issue for excess foam. Air lift bench reactor is reported to be a good alternative for conventional stirred batch reactor, once it's supposed to achieve high aerating and stirring (then enhancing O_2 mass transfer), regarding the low foam production (Fahim *et al.,* 2012; Reis *et al.,* 2013). It was observed a reduction for lag time (from 9 to 5 hour), when compared to stirred reactor (Franca et al., 2015), indicating that air lift reactor promoted a faster strain growth.

Table 1 presents the biomass, surface tension and crude biosurfactant production on air lift bench reactor at different aeration, and also a comparative study with a stirred reactor for biosurfactant production by *Bacilllus subtilis*. A higher yield for biosurfactant production was reported on air lift bench reactor compared to a stirred reactor (on the same aeration). It was reported an improvement about 35% on biosurfactant production on air lift bench reactor, using the same condition for a stirred reactor (4L.min^{-1}). An efficient mixing, provided for this reactor apparatus, probably caused this high yield (Reis *et al.,* 2013). Even though, a lower biomass production has been observed. Comparing results for 4L.min^{-1} e and 5L.min^{-1}, it can be observed a higher lipopeptide biosurfactant production on the lower aeration. It seems that enhancing aeration, it's supposed to provide an ideal condition to the strain to grow, and maintain its metabolism, but not necessarily produce biosurfactant. A higher biosurfactant production was reported on the lower aeration (4L.min^{-1}). This is a typical behavior of secondary metabolites, but further studies are required to certify it. The biosurfactant produced was able to reduce surface tension to around 30 mN.m^{-1}, showing its efficiency as a tensoative. According to Mulligan (2005) an effective surfactant is supposed to reduce surface tension of water from 72 to less than 35 mN m^{-1}.

Table 1 – Comparative for biomass, reduction on surface tension, crude biosurfactant production for air lift bench reactor (A. L.) and stirred reactor (S. R.)

Reactor apparatus	Biomass ($g.L^{-1}$)	Surface tension ($mN.m^{-1}$)	Biosurfactant production ($g.L^{-1}$)	Reference
S. R.: 4L.min^{-1} - 200 rpm	3,10	30,15	1,29	Franca *et al.,* 2015
A. L.: 4L.min^{-1}	1,71	31,7	1,92	Author
A. L.: 5L.min^{-1}	3,16	32,7	1,15	Author

4. CONCLUSIONS

It is possible to produce biosurfactant by *Bacillus subtilis* ICA56 on air lift bench reactor, since this reactor apparatus promotes efficient mixing on the system, regarding low foam production. The cell growth seemed to be related to the aeration, but not necessarily the biosurfactant production. For 4 L.min^{-1} of O_2, it was reported a biomass production about 1,71 g.L^{-1}, and biosurfactant production around 1,92 g.L^{-1}, presenting the capacity of the strain to produce biosurfactant at high yields using renewable feedstocks (glycerol). It was observed a higher biosurfactant production compared to a stirred reactor using the same aeration condition (literature). The biosurfactant produced was able to achieve about 30 mN.m^{-1} on surface tension, indicating it as an efficient tensoative.

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