**TITLE:** RAMNOLIPIDS PRODUCTION BY STATIC-SUBMERGED FERMENTATION USING MEMBRANES OF BACTERIAL CELLULOSE AS SOLID SUPPORT

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**ABSTRACT:**

Biosurfactants have attracted attention because of their low toxicity, high biodegradability, and good ecological acceptability. However, their application still limited because of their high cost of production in comparison to the chemically synthetized congeners. Rhamnolipid production by static-submerged fermentation using membranes of bacterial cellulose as solid support (SSbF-BCM) is an alternative promising method. SSbF-BCM avoid foaming formation, a severe problem in submerged fermentation, in a low-cost process, with no need for additional expend with aeration and agitation. One other advantage of this process is the possible reuse of the cellulose-bacterial membranes for several times. Given that, the present work aimed to compared the rhamnolipid production by SSbF-BCM to other two different processes named, submerged fermentation (SbF) and submerged-static fermentation (SbSF). In addition, we determined the surface-active properties [emulsification index (EI) and micellar critical concentration (MCC)] and the congeners composition of those rhamnolipids produced by SSbF-BCM. The production of rhamnolipid reached by SSbF-BCM (3.7 g/L) were two to four times higher than those reached by the other evaluated processes. The surface-properties of the rhamnolipids produced by SSbF-BCM were very similar to those reported to rhamnolipids produced by submerged liquid fermentation, with a critical micelle concentration of 42.3 mg/L and an emulsification index at 24 h of over 80% against gasoline, kerosene and hexane. The congener composition determined by mass spectroscopy showed a higher percentage of the di-rhamnolipid congeners (over 95 %), being the congener Rha-Rha-$C_{10}$-$C_{10}$ the most abundant (over 66 %), which

might be a very interesting advantage in the obtainment of single structure in high abundance for refined industrial application, such as in the pharmaceutical and cosmetic industries. Our results suggest that the production of rhamnolipid by static-submerged fermentation using membranes of bacterial cellulose as solid support presents great potential to make biotechnological production of biosurfactants feasible.

**Keywords:** Bacterial cellulose membranes, biosurfactants, *Pseudomonas aeruginosa*, rhamnolipids, static submerse fermentation

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