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Frothability and surface behavior of a rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* MA01

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ABSTRACT

In this work, surface activity and frothability of rhamnolipid biosurfactant produced by a *Pseudomonas aeruginosa* MA01 were studied and compared with conventional flotation frothers, i.e. methyl isobutyl carbinol (MIBC), pine oil, Dowfroth-250 (DF-250), and Aerofroth-65 (A-65). FTIR and ES-MS analysis indicated that the product contained two types of commonly found rhamnolipids: L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (RL-1) and L-rhamnosyl L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (RL-2). Surface tension measurements showed that the rhamnolipid product reduces surface tension more effectively than frothers due to higher molecular weight and the presence of multiple oxygenated groups in its structure, producing more viscous liquid film, which was confirmed with film elasticity calculations. Both surface tension and elasticity values followed the order: rhamnolipid > A-65 > DF-250 > pine oil > MIBC. Frothability of the tested surfactants gave the order: rhamnolipid > A-65 > DF-250 > MIBC > pine oil.

1. Introduction

The presence of a frothing agent is vital to every flotation process. In addition to the formation of froth, a frother has a significant effect on the increase in air dispersion in the flotation machine, reduction in coalescence of individual bubbles in the pulp and decrease in the rate at which the bubbles rise to the surface [1]. Frothers increase the strength of the bubbles and the stability of the froth formed as a result of the rise of mineralized air bubbles to the surface of the pulp. These factors play a significant role in the kinetic viability of the process, and with the overall recovery and grade that can be achieved from a flotation cell or circuit [2].

Today, however, synthetic chemicals from petroleum origins are now coming at a turning point for their hazardous problems to living organisms. Concerns about environmental protection and health safety have introduced an interest in developing biomaterials such as naturally occurring surfactants (biosurfactants) [3]. Surfactants (surface active agents) are amphiphilic molecules with both hydrophilic and lipophilic moieties and biosurfactant is microbial originated surfactant produced as co-metabolite by particular microorganisms. Because of its advantages over the synthetic surfactants, i.e. high effectiveness, low toxicity to microorganisms and environmental compatibility, biosurfactants have more and more environmental applications in recent years [4]. Given these features as well as structural similarities, rhamnolipid seems to be well suited to apply in the mineral processing industry as a substitute case for conventional chemical frothers.

Among the various species of biosurfactants much work has been done on rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa* strains. Two types of rhamnolipids (RLs) are mainly produced by *P. aeruginosa* under usual growth condition: they are mono-(RL-1) and di-rhamnolipids (RL-2) and have two hydrophobic chains. As shown in Fig. 1, the hydrophilic moieties of RL molecules are the rhamnosyl and carboxylic acid groups. RL-1 has a single rhamnosyl group whereas RL-2 has two rhamnosyl groups joined to each other with an ester bridge [5].

Numerous articles consider the surface behavior and micellization kinetics of rhamnolipid biosurfactants either in the presence or in the absence of an electrolyte [e.g. 5–11]. Generally, micelle formation and micellar growth are enhanced by adding an electrolyte to a surfactant solution, which causes a decrease in the repulsive forces between similar charges. Large aggregates also rearrange their micellar shapes to spherical or ellipsoidal form with the addition of salt. Özdemir and Malayoglu [12] investigated wetting characteristics of rhamnolipids by measuring contact angles of the aqueous solutions of the RL mixture as a function of biosurfactant concentration. Detailed studies on surface forces and properties of foam films from RL biosurfactants have been performed by Cohen and Exerowa [13]. The curves of the RL foam films and the directly measured disjoining pressure isotherms

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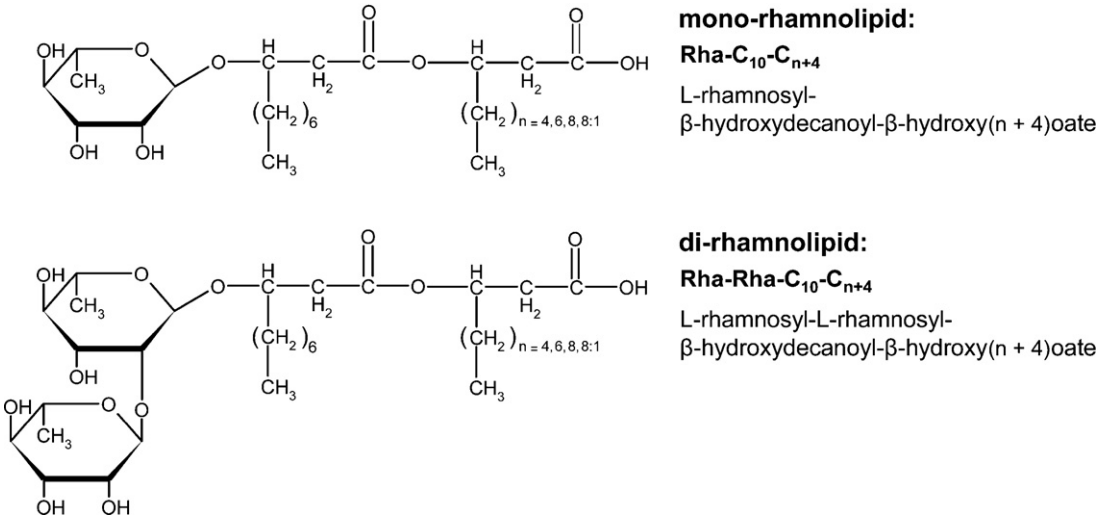

Fig. 1. General structure of rhamnolipid biosurfactants produced by *Pseudomonas aeruginosa* strains.

indicated the ranges of action of the DLVO and non-DLVO surface forces. Recently, Fazaelipoor et al. [14] used rhamnolipid in coal flotation. Froth characterization in their study showed that the biosurfactant was superior to methyl isobutyl carbinol (MIBC) in terms of froth height and stability. However, low purity (<50%) of the rhamnolipid they used challenges the reported results.

In this investigation, a high potential biosurfactant producing strain was isolated from spoiled apples according to Kitamoto et al. [15]. During screening and isolation processes, oil displacement test [16] and extraction of biosurfactant [17] were used as criteria for biosurfactant production. Then, the surface behavior and frothing characteristics of the rhamnolipid product were studied by measuring surface tension and frothability and calculating film elasticity. Then, results were compared to those from some frothers conventionally applied in mineral flotation.

2. Materials and methods

2.1. Bacterial strain and culture medium

Selected isolate was identified as *P. aeruginosa* MA01 using morphological, biochemical and molecular methods. Biochemical identification of selected isolate was done using standard biochemical test (API 20E kit, bioMérieux, Mercy, France). The results were analyzed using APIWEB® Software. Sequence analysis of conserved "housekeeping" genes such as the bacterial 16S rRNA gene are increasingly being used to identify bacterial species in clinical practice and scientific investigations. Molecular identification was carried out according to Lotfabad et al. [18]. Sequence homologies were examined using BLAST version 2.2.12 of the National Center for Biotechnology Information [19]. Multiple sequence alignments were carried out using ClastalW and a consensus neighbor-joining tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) software (version 4.0) [20].

The composition of medium for biosurfactant production was as follows (g/l): soybean oil 40.0, $NaNO_3$ 3.0, $MgSO_4{\cdot}7H_2O$ 0.25, KH_2PO_4 0.25 and yeast extract 1.0. Seed cultures were prepared using a nutrient broth medium by overnight incubation in a rotary shaker at 200 rpm and 30 °C.

2.2. Biosurfactant production

Seed culture was inoculated (2%, v/v) in 2 l flasks containing 700 ml of production medium using soybean oil as sole carbon source. Then it was incubated at 200 rpm and 30 °C for 10 days in a shaker incubator. At the end of the incubation period the extraction of biosurfactant was carried out using acid precipitation–solvent extraction method [17]. Briefly, culture medium was centrifuged (Sigma model 6-16K, Germany) 16,000 × *g* for 15 min at 4 °C to remove bacterial cells. The cell free supernatant was acidified with 6 N HCl to pH 2 and stored overnight at 4 °C for better precipitating of biosurfactant. The resulted precipitate was harvested by centrifugation (18,000 × *g*, 50 min, 4 °C) and extracted several times with ethyl acetate at room temperature. Then it was centrifuged at 18,000 × *g* for 15 min at 4 °C and the organic phase was transferred to a new flask. Finally the solvent was completely evaporated using rotary evaporator (Buchi model R-200, Germany) at 35 °C. About 9 g of a viscous honey-colored biosurfactant product was extracted per liter of the culture medium.

Fractionation of crude rhamnolipid to its mono and di-rhamnolipid components was carried out using column chromatography according to the methods of Sánchez et al. [7].

2.3. Structural characterization of biosurfactant

The crude biosurfactant had some impurities. Therefore the purification of crude biosurfactant was carried out using column chromatography according to the method of Sánchez et al. [7] with minor modifications. Briefly, a slurry of 20 g silica gel 60 in chloroform was poured into a glass column chromatography (2 cm × 40 cm). 2 g of crude biosurfactant was dissolved in 4 ml chloroform and loaded onto the column. The purification was carried out by washing the column at flow rate of ca. 1 ml/min with chloroform (to elute neutral lipids), followed by chloroform:methanol 50:5 (to elute monorhamnolipid fraction) and finally chloroform:methanol 50:50 (to elute dirhamnolipid fraction). Then purified fractions were mixed and the solvents were removed under vacuum and finally, freeze-dried.

Each fraction was analyzed using Fourier-transform infrared (FTIR) spectroscopy (Nicolet 6700 FTIR, Madison, WI) and ES-MS in an Agilent model LC-MSD-Trap-VL (Classic series, United States) equipment. For FTIR analysis, purified biosurfactant sample was placed in between two CaF_2 windows, without spacers, and the set was mounted in a thermostated cell holder. The sample holder was thermostated at 25 °C using a Peltier device (Proteus system from Nicolet). Each spectrum was obtained by collecting 256 interferograms with a nominal resolution of 2 cm^{-1}. The equipment was continuously purged with dry air in order to minimize the