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PRELIMINARY STUDY ON THE EFFECT OF LOW ELECTRIC CURRENT ON PRODUCTION OF BIOSURFACTANT BY *Pseudomonas aeruginosa*

Djaber Tazdaït[1,2*], Rym Salah[1], Fadila Guiddir[1], Nawal Temouche[1], Nadia Abdi[2], Hocine Grib[2], Nabil Mameri[2]

1 Department of Biochemistry and Microbiology, Faculty of Biological and Agronomical Sciences, Mouloud Mammeri University of Tizi-Ouzou, P.O. Box 17 RP 15000 Hasnaoua, Tizi-Ouzou, Algeria.
2 Laboratory of Bioengineering and Process Engineering, National Polytechnic School, Avenue Hacen Badi, El-Harrach, Algiers, Algeria.
**Corresponding author. Emails:*
djaber.tazdait@ummto.dz;djaber.tazdait@g.enp.edu.dz;djabertazdait@yahoo.fr

Introduction and study objectives

Environmental pollution caused by petroleum hydrocarbons is nowadays of great concern as it poses not only serious concerns to human health, but also affects the ecosystems, especially the aquatic ecosystems, in negative ways. It is therefore urgent to think of solutions that would be effective and ecofriendly in solving this issue. It is well established that microorganisms use different strategies to enhance hydrocarbons solubility in water prior to their effective degradation. One of these strategies consists in producing biosurfactants, which are amphiphilic molecules secreted as primary and/or secondary metabolites during microbial growth.

Several studies have been attempted to investigate the effect of different parameters on the performance of biosurfactant production by microorganisms, these parameters include temperature, pH, agitation, and carbon and nitrogen sources. In literature, the studies devoted to the use of electric current are mostly focused on the treatment of wastewaters by electrocoagulation. However, to the best of our knowledge, no studies have been reported on the direct effect of electric current on microbial production of biosurfactants.

The first purpose of this study was to investigate the effect of direct electric current on the performance of a biosurfactant-producing strain *Pseudomonas aeruginosa*, isolated from hydrocarbon-contaminated soil, to produce biosurfactant under aerobic conditions. Besides, the produced biosurfactant was tested as an antibacterial agent against some pathogenic microbial strains.

Methodology

The bacterial strain used in this study was previously isolated from a fuel-contaminated soil collected from a gas station located in Boumerdès, Algeria. The strain was characterized and identified as a member of *Pseudomonas aeruginosa* species. The culture medium (CM) used for biosurfactant production in all experiments was composed of the the following constituents: 5 g of glucose, 1.6 g of K_2HPO_4, 0.4 g of KH_2PO_4, 0.09 g of $MgSO_4$, 15 g of NaCl, 0.1 g of NH_4NO_3, 0.02 g of $CaCl_2$, 0.01 g of $ZnSO_4$, 0.05 g of $FeSO_4 \cdot 7\ H_2O$, 0.008 g of $MnSO_4 \cdot H_2O$, 0.004 g of $CuSO_4 \cdot 5\ H_2O$, 0.01 g of urea, 1 L of distilled water. The bacterial strain (5 mL at 1.5 10^7 CFU/ml) was first subcultured in 1000 mL Erlenmayer flask containing 1000 ml of CM and incubated at 50 rpm, at room temperature (21.7°C ± 3.07°C) for 48h. The effect of direct electric current on the biosurfactant production was evaluated by using an experimental setup, which consisted of 1000 mL glass beaker containing 500 mL of CM pre-inoculated with 500 ml subculture, in which were vertically immersed two electrodes in shape of plates made of stainless steel with dimensions of 10 × 2 × 0.1 cm (L (Length) × W (Width) × T (Thickness)). The two electrodes were connected to a DC power supply with amperage and voltage outputs of 0 to 5 A and 0 to 18 V DC using copper electrical wires. In total six runs were carried out by varying the inter-electrode distance (d) (2, 4 and 6cm) with current density values (D) of 3µA/cm2 for 3 days at room temperature (21.7°C ± 3.07°C), at 150 rpm and pH 7. Besides, control experiment was carried-out under the same conditions for which no electrical treatment was performed. During this incubation period, samples were taken three times a day for Optical Density (OD) measurement at 600 nm, then centrifuged at 5000 rpm for 10 min to discard cells and the supernatant was used for E_{24} determination. On the other hand, the biosurfactant concentration was determined at the end of each experiment using a solvent extraction method.

The biosurfactant produced by the strain was tested for its possible antibacterial effect against three standard pathogen strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* MU50, and *Bacillus aureus* ATCC 14579) and against four pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Candida albicans*) obtained from the Laboratory of Microbiology of Nedir Mohamed Hospital (Tizi-Ouzou, Algeria). The antibacterial activity assessment was performed by the disc diffusion method. Briefly, 6 mm diameter filter papers (Whatman, no. 1) were separately soaked with 100 µL of 0.5%, 1%, 3% and 6% (w/v) crude biosurfactant solutions, and with sterilized distilled water used as a negative control. The discs were air-dried and placed on the center surface of Mueller-Hinton agar plates previously inoculated with 0.1 mL of each bacterial strain containing 10^7 CFU/mL, and then incubated at 37°C for 24 h. At the end of this incubation period, the dishes were visually inspected for a clear zone around discs indicative of growth inhibition. The diameter of clear zone was measured with calliper and averaged.

Results and conclusions

The results clearly showed that direct current application with distance between electrodes of 2 cm yielded the highest biosurfactant production (5.36 g/L), and emulsification index (E_{24}). Besides, the production cost decreases of about 20% (from 0.56 USD/g to 0.45 USD/g) with electric current application (d=2cm, D=3µA/cm^2), which contributes very slightly (4.8×10^{-4} %) in the total energy consumption cost. It was found that the produced biosurfactant is 48 times cheaper than commercial one (Rhamnolipid) marketed by Sigma-Aldrich, USA in 2017. This method might, in the future, lead the way towards profitable application in enhancing biosurfactant production. On the other hand, the produced biosurfactant exhibited a clear antimicrobial effect against the following strains: *Escherichia coli* ATCC 25922, *Escherichia coli*, *Bacillus aureus* ATCC 14579 and *Klebsiella pneumoniae*.