

Proceedings of

1st International Conference on Sustainable Chemical & Environmental Engineering



SUST
ENG
2022

31st Aug - 4th Sep 2022

Rethymno, Crete, Greece

hybrid event

www.susteng.eu

ISBN: 978-618-86417-0-9



www.susteng.eu





A novel microbial biosurfactant/bioemulsifier: Production and characterization

T.P. Silva¹, S.M. Paixão¹, J. Tavares¹ and L. Alves¹

¹LNEG-Laboratório Nacional de Energia e Geologia, IP, Unidade de Bioenergia e Biorrefinarias, Lisboa, Portugal
Corresponding author email: susana.alves@lneg.pt

keywords: biosurfactants; bioemulsifiers; *Gordonia alkanivorans* strain 1B.

Introduction

Currently there is an immediate need for new and more sustainable production methods in most industries. The detergent industry, frequently associated with negative environmental impacts, is also in need of new alternatives, such as biosurfactants/bioemulsifiers (BS/BE). These are naturally synthesized compounds, classified as amphiphilic, for having both hydrophobic and hydrophilic properties. Their application results in a reduction of the surface tension between two immiscible phases, facilitating the mixture of different substances such as water and oil, or water and air (Tavares et al., 2021).

BS/BE present several advantages over conventional detergents, they have lower toxicity and greater biodegradability, resulting in lower negative impacts to both consumers and ecosystems. Furthermore, these compounds present antibiotic, antiviral and antioxidant properties. They can be used in a broad range of pH, temperature and salinity and are effective at small concentrations. This makes them interesting for many other industries, such as food, cosmetics, pharmaceutical and chemical.

BS/BE are commonly produced by microorganisms found in particular environments such as oil wells, hydrocarbon contaminated soils and solid waste lixiviates. These compounds facilitate the access to hydrophobic nutrient sources abundant in these environments, while also increasing the resistance of the microorganisms to such toxic environments.

Gordonia alkanivorans strain 1B, is a bacterium with significant biotechnological potential, which was isolated from oil contaminated soils (Alves et al., 2005). It is mostly known for its biodesulfurizing properties, carotenoid production and broad catabolic range (Silva et al., 2016). The present work focuses on the potential of this strain to produce BS/BE compounds, initial purification and characterization.

Materials and methods

Microorganism and culture media: All assays were performed using the bacterium *Gordonia alkanivorans* strain 1B, isolated in our laboratory, cultivated in a sulfur-free mineral (SFM) medium (Alves et al., 2005).

Preliminary assays: *G. alkanivorans* strain 1B was cultivated with three different individual carbon sources, fructose and glucose, simple sugars commonly used and easily consumed by the bacteria, and olive oil a known hydrophobic inducer. Two different sulfur sources were also tested for their influence on BS/BE production, sodium sulfate (Na_2SO_4) and dibenzothiophene (DBT). Fructose and glucose solutions and olive oil were filter sterilized and added to the medium to obtain 10 g/L of carbon source. Na_2SO_4 or DBT were added as sulfur source to an initial concentration of 300 mg/L and 300 μM respectively. After the growth ended cells were separated through centrifugation and cell-free supernatants were used in emulsification assays (E24) to determine BS/BE production.

BS/BE cellular activity: Bacteria were cultivated with fructose and SO_4 , separated through centrifugation and resuspended in ultra-pure water, to obtain different concentrations of cell suspension. These were used in emulsification tests (E24) to determine cellular BS/BE activity.

BS/BE production and concentration: Bacteria were cultivated with fructose and SO_4 and cell free supernatants were obtained through centrifugation. The cell-free liquids were then mixed with n-heptane in a 4:1 ratio, shaken vigorously and left to rest overnight. After observing the separation of three phases, the middle layer containing the BS/BE was collected, and freeze-dried to obtain a raw BS/BE powder, used in the characterization assays.

Emulsification assays (E24): Cell-free supernatants were mixed with olive oil in a 1:1 ratio in 20 ml tubes, shaken for 60 s and left to rest for 24 h. Emulsification activity was detected by the presence of a layer containing a mixture of water and oil, an emulsion. Relative quantification was obtained by measuring the height of the emulsion and comparing it to the total height of the liquids.



Critical micelle concentration (CMC): Several solutions of crude BS/BE powder were prepared with ultra-pure water, to obtain concentrations between 1.37 mg/L and 841.2 mg/L. These were then taken to a Kruss K12 Mk6 tensiometer and used to determine the influence of the BS/BE concentration on the surface tension (ST) of water. Results obtained in mN/m were plotted and used to calculate CMC for the crude BS/BE. The process was repeated with SDS (0.024–4.723 g/L) and Tween 80 (0.18 and 106.24 mg/L), as benchmark to correctly evaluate the results obtained.

Chemical characterization: Using the raw powder, chemical characterization by CHNS elemental analysis was performed following the ISO 16967:2015.

Results and discussion

The initial screening revealed that strain 1B can produce extracellular substances with emulsifying properties with different degrees of activity depending on culture conditions. The best results were observed using hydrophobic inducers such as olive oil or DBT, while the worst results were obtained combining glucose and sulfate. Nonetheless, activity was detected for every condition tested, with the highest being obtained for fructose and DBT at an E24 of 55.6% with complete emulsification of the hydrophobic layer.

In the assays with bacterial biomass, the highest concentration tested was able to generate an E24 of 95%, with complete emulsification of the aqueous phase. Even when diluted 16 times, a strong emulsion with an E24 of 77% was still observed. This clearly attests to the emulsifying properties of the cells, demonstrating the presence of BS/BE substances not just released to the culture medium, but also adhered to the cells.

Based on the preliminary results, the strain 1B was cultivated in a larger volume, and a method was developed to extract/concentrate the BS/BE compounds, resulting in raw BS/BE powder. This powder was resuspended at different concentrations and taken to a tensiometer, where it was able to lower the surface tension of water from 71.71 to 45.92 mN/m, with only 33.28 mg/L, reaching a CMC of 16.94 mg/L. Even in an unpurified form, these results are similar to what was obtained for Tween 80 (CMC= 6.4 mg/L) and superior to SDS (CMC= 1836.9 mg/L), indicating the potential of this extract.

Finally, the raw powder was taken to an elemental analyzer to access its CHNS composition, revealing that it was mostly constituted by carbon and hydrogen (41.5% and 8.3% respectively) followed by nitrogen (3.4%) and only residual sulfur (<0.3%). These results were within what would be expected and point to a protein content of 21.25%, indicating they are not the main component.

Conclusions

Results show that the strain 1B can produce extracellular and cell-bound compounds with BS/BE properties, even without the presence of hydrophobic inducers. Further work still needs to be performed towards purification and characterization of these substances, however an unpurified extract clearly demonstrated properties equal/superior to some conventional surfactants, proving its potential as a future sustainable alternative to these substances.

Acknowledgements: This work was financed by national funds through FCT (Fundação para a Ciência e a Tecnologia) in the scope of the project GreenFuel (PTDC/EAM-AMB/30975/2017). Tiago P. Silva also acknowledges FCT for his PhD financial support (SFRH/BD/104977/2014).

References

- Alves, L., Salgueiro, R., Rodrigues, ., Mesquita, E., Matos, J. and Gírio, F.M., 2005. Desulfurization of dibenzothiophene, benzothiophene, and other thiophene analogs by a newly isolated bacterium, *Gordonia alkanivorans* strain 1B. *Appl.Biochem. Biotechnol.* 120(3), 199–208.
- Silva, T.P., Paixão, S.M. and Alves, L., 2016. Ability of *Gordonia alkanivorans* strain 1B for high added value carotenoids production. *RSC Adv.*, 6(63), 58055–58063.
- Tavares, J., Alves, L., Silva, T.P. and Paixão, S.M., 2021. Design and validation of an expeditious analytical method to quantify the emulsifying activity during biosurfactants/bioemulsifiers production. *Colloids Surf. B*, 208, 112111.