Design of Environmental Processes Laboratory, School of Chemical and
Environmental Engineering, Technical University of Crete

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

Design of Environmental Processes Lab
Technical University of Crete
School of Chemical and
Environmental Engineering
biosolids 2energy
LIFE B2E4 sustainable - WWTP
www.biosolids2energy.eu
Life
GREEN FUND

Optimization of a biphasic biodesulfurization system

T.P. Silva[1], S.M Paixão[1], J.C. Roseiro[1] and L. Alves[1]

[1]LNEG – Laboratório Nacional de Energia e Geologia, IP, Unidade de Bioenergia e Biorrefinarias;Estrada do Paço do Lumiar, Lisboa, Portugal
Corresponding author email: luis.alves@lneg.pt

keywords: *biodesulfurization; model fuel; biorefinery.*

Introduction

Many of the new generation fuels, although more sustainable, share some of the problems inherent to fossil fuels. Depending on the biomass/material that originated them, they can present different contaminants that can lead to environmental problems. Sulfur is one of the most common and problematic contaminants in fuels. It is released into the atmosphere in the form of SO_x, leading to the formation of acid rains, which cause drastic environmental and infrastructural problems, as well as several types of health issues. High sulfur concentrations in fuels also result in a loss of efficiency of motors and energy generation systems, mostly due to corrosion and catalyst poisoning.

The current thermochemical desulfurization process, hydrodesulfurization (HDS), is energy demanding, pollutant and has low efficiency against more complex organosulfur molecules. This led researchers to look for new alternatives. Biodesulfurization (BDS), is, as the name implies, the biological removal of sulfur from fuels using microorganisms as living biocatalysts. If correctly employed this process could be more efficient and less pollutant, since microorganisms directly target the sulfur atoms, even those present in complex molecular structures, such as dibenzothiophene (DBT). Moreover, microbial activity occurs at much lower temperatures and pressures, without the need for metal catalysts, resulting in a lower energy demand.

While BDS is a promising technology, it is still at a low development stage, mostly due to some bottlenecks, which have been hindering its large-scale application. Similarly, to other biotechnological processes, it presents lower reaction rates, when compared to HDS, since it depends on the use of living organisms as catalysts. Furthermore, it must be performed under conditions that allow the microorganisms to maintain biological activity, limiting the range of applications. These conditions vary greatly depending on the microorganism selected, and their optimization can significantly increase the biodesulfurization activity of a biocatalyst.

Traditionally, most optimization work has been performed under conditions ideal for bacterial growth, without taking into consideration the conditions that the biocatalysts will be subjected to when in contact with actual fuels in a refinery environment. To better understand microbial behavior and increase BDS activity, it becomes fundamental to study how these microorganisms respond in biphasic systems, if high hydrocarbon ratios and lower water availability are tolerable, and how to compensate for any metabolic activity loss.

Gordonia alkanivorans strain 1B is a bacterium that has demonstrated great potential to be used in a BDS biorefinery. Not only can it remove sulfur from complex compounds, but it seems to increase its efficiency with more complex molecules, such as DBT and its derivatives (Silva et al., 2020). Furthermore, it can simultaneously produce high added value products (carotenoids and surfactants) and can be cultivated with agro-industrial residues as nutrient sources. To this date, most works published with this strain were performed in aqueous medium. This work focusses on the optimization of biodesulfurization conditions in a biphasic system with n-heptane as a model fuel. Using the Doehlert distribution for two factors (X_1: n-heptane:water ratio and X_2: initial cell concentration) an experimental design was performed and evaluated in terms of biodesulfurization activity.

Materials and methods

Microorganism and culture medium: This work was performed using the bacterium *Gordonia alkanivorans* strain 1B, cultivated in a SFMM medium with fructose (Alves et al., 2005; Pacheco et al., 2019).

Experimental design methodology: Following a Doehlert uniform shell design, an experimental distribution for two factors was used to produce response surfaces (Doehlert, 1970). Seven conditions were tested in duplicate, varying the ratio of n-heptane: water (X_1) between 0.1 and 0.9 and initial cell concentration (X_2) between 5 and 20 g/L (dry cell weight). Response was evaluated in terms of 2-HBP produced (the result of DBT biodesulfurization) in μM and results were collected at 3 and 6 h. The model used to express this response was a second-order polynomial model: $Y_i = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_{12} + \beta_{11}X_{12} + \beta_{22}X_{22}$ where Y_i, is the response from experiment i, β are parameters of the polynomial model and X is the experimental factor level (coded units).

Biodesulfurization assays: Cells were collected and centrifuged to obtain a concentrated cell suspension, which was then diluted in the supernatant to obtain different concentrations. n-Heptane was used as a model fuel with a concentration of 1.13 mM of DBT. Cells and n-heptane were placed in 30 mL screw cap tubes, at different ratios and the tubes were placed in an incubator at 30°C and 150 rpm.

Analytical methods: Cell concentration was evaluated through dry cell weight and 2-HBP concentration was determined through HPLC.

Results and discussion

G. alkanivorans strain 1B was able to desulfurize regardless of the ratio of n-heptane it was subjected to, indicating a significant resistance to solvents, which is fundamental for this bioprocess.

In terms of biodesulfurization activity, results demonstrate that both factors tested had a significant influence, but only for n-heptane: water ratios below 50% (0.5), with higher ratios the influence of these factors was greatly reduced. For ratios between 0.1 and 0.5, n-heptane: water ratio was the most influential factor, regardless of cellular concentration. This factor showed a clear negative influence, since any increase resulted in a loss of desulfurization activity. Conversely, within the same interval, any increase in cell concentration had a positive influence on the response, resulting in a biodesulfurization increase. Time was also shown to have a positive influence on most results, since desulfurization increased from 3 h to 6 h, with the exception of the 10% (0.1) ratio where the 1.13 mM of DBT were completely desulfurized in first 3 h. This indicates that, with an aqueous phase of at least 50%, it is possible to compensate for an increment in the fuel ratio by increasing cell concentration, and that this effect is greater for lower fuel ratios. According to this experimental design the best conditions for biodesulfurization are 10 % fuel (0.1 ratio) with 20 g/L biocatalyst and 3 h of biodesulfurization.

Conclusions

This work demonstrates that by increasing cell concentration it is possible to use greater fuel:water ratios maintaining high desulfurization activities, thus reducing reaction times and increasing process efficiency. Further optimization work is still needed, however, this gives important clues for the development of a large scale biodesulfurization process.

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, C., 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.

Doehlert, D.H. (1970). Uniform Shell Designs. J. R. Stat., 19(3), 231-239.

Pacheco, M., Paixão, S.M., Silva, T.P. and Alves, L., 2019. On the road to cost-effective fossil fuel desulfurization by: Gordonia alkanivorans strain 1B. RSC Adv.,9, 25405-25413.

Silva, T.P., Alves, L. and Paixão, S.M., 2020. Effect of dibenzothiophene and its alkylated derivatives on coupled desulfurization and carotenoid production by *Gordonia alkanivorans* strain 1B. J. Environ. Manage., 270, 110825.