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**Biodesulfurization biorefinery using *Gordonia alkanivorans* strain 1B:
Life cycle inventory of the integrated process**

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Introduction

High sulfur concentrations are a problem common to fossil fuels and derivatives (such as oil and coal), as well as many new generation fuels and biofuels (such as pyrolysis oils, syngas, biogas or even biodiesel). If the sulfur present in these fuels is released into the atmosphere it can result in SO₂/SO_x emissions, leading to environmental damage, and health issues. Transportation fuels have sulfur limits that go below 5000 ppm in ships, 3000 ppm in airplanes and 10 ppm in cars, and without treatment fuels can have several thousand ppm of sulfur. As such, they must be submitted to desulfurization, typically through a thermochemical process known as hydrodesulfurization, in which H₂ is combined with the fuel at high temperatures and pressures, in the presence of metal catalysts. However, this process has significant environmental impacts. Usually, it depends on hydrogen and heat/steam produced from natural gas, totalizing 4.17 kg natural gas per 2.89 kg sulfur removed. It also involves high electricity and water consumption (approximately 2.9 kWh and 86.9 kg, respectively, per 2.89 kg sulfur removed). Furthermore, these impacts are greater for lower sulfur demands (Burgess & Brennan, 2001). Thus, there has been a search for alternative/complementary processes, one of which is biodesulfurization (BDS). It consists of the use of microorganism that consume the sulfur present in the fuels, at ambient temperature and pressure, without the need for metal catalysts. BDS still presents several bottlenecks, common to many microbial processes, such as low conversion rates and high production costs for the microbial biocatalyst. To surpass these limitations researchers have pursued different strategies: minimization/optimization of culture medium and culture conditions; employment of cheaper alternative nutrient sources; exploitation of added value products.

Gordonia alkanivorans strain 1B is a bacterium known for its biodesulfurization properties. It has demonstrated several characteristics which make it interesting: it can perform BDS of different compounds, several of which extremely recalcitrant for the thermochemical process; it has very low nutritional needs; it can be cultivated on several alternative carbon sources; it has been shown to produce two different types of added value products: carotenoids and biosurfactants (Alves et al., 2015; Silva et al., 2020, 2022). Therefore, *G. alkanivorans* strain 1B is the ideal candidate for a biodesulfurization biorefinery, that simultaneously removes sulfur from fuels and produces carotenoids and biosurfactants.

To correctly assess the overall environmental impact of the BDS biorefinery, it becomes fundamental to perform a life cycle assessment (LCA). According to ISO 14040, LCA is a technique for the assessment of environmental impacts of a product or process. It depends on the correct compilation of a process inventory, accounting for all the relevant inputs and outputs of the production system; the evaluation of the environmental impacts of each individual step of the process; the interpretation of the results within the goal and scope of the study. Through LCA it becomes possible to assess a product's impact throughout its life, from the production process to its disposal, or from any two points in between.

The present work is an initial life cycle assessment study on a biodesulfurization biorefinery, in which the process system will be defined, an inventory will be elaborated, and inputs and outputs will be accounted for. The goal of this study is to evaluate the CO_{2eq} emissions resulting from a biodesulfurization biorefinery using the bacterium *G. alkanivorans* strain 1B, to better understand its potential as a more sustainable alternative to the current thermochemical processes.



Materials and methods

Life cycle methodology: The present work follows the methodology defined by the ISO 14040 (ISO 14040–14044), composed of four major steps: (1) definition of goal, scope and boundaries; (2) elaboration of inventory (Life Cycle Inventory, LCI); (3) assessment of impacts (Life Cycle Impact Assessment, LCIA); (4) analysis. In the present study only the 1st and 2nd steps will be addressed.

Data source: The data was collected from bench scale assays, performed under the scope of the project GreenFuel (PTDC/EAM-AMB/30975/2017).

Microorganism: The microorganism used in this work was the bacterium *Gordonia alkanivorans* strain 1B, isolated in our lab from samples of hydrocarbon contaminated soils (Alves et al., 2005).

Results and discussion

The LCI of the BDS biorefinery process was performed, from the moment the sulfur rich fuel enters the biorefinery, to its conversion to a low-sulfur fuel. This process encompasses three major steps: Production of bacteria with biodesulfurization; Separation of the oil from the biocatalyst; Valorization of the spent biocatalyst and spent culture medium, through carotenoid and biosurfactant extraction.

In the first step the major inputs considered were the sulfur rich fuel, the components of the medium needed to cultivate the bacteria and the energy needed for sterilization and maintenance of the ideal growth and biodesulfurization conditions (ex: temperature 30°C and constant agitation). In the second step, inputs were mostly the energy needed for the separation process, namely through centrifugation. In the final step, the inputs considered were the organic solvents needed to extract the added value products and the energy spent in the extraction process and separation of the products of interest from the solvents.

In terms of outputs, 5 major outputs were identified: low-sulfur fuel, carotenoids, biosurfactants, biogenic CO₂-rich air and spent bacterial biomass. The CO₂ rich gas stream can be used as a carbon source to cultivate autotrophic microalgae, which can then be combined with the spent bacterial biomass and be converted through hydrothermal liquefaction into a biofuel.

Conclusions

The results obtained in this work will guide the development of future strategies to improve the sustainability of the biodesulfurization biorefinery, helping to identify which steps should be eliminated/optimized. Furthermore, the LCA resulting from this data, will help to determine if the integrated biodesulfurization approach is superior/less pollutant, than the benchmark hydrodesulfurization contributing towards future decision making.

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