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A new impetus for biodesulfurization: bypassing sulfate inhibition in biocatalyst production†

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Biodesulfurization is a biotechnological process that employs microorganisms as biocatalysts to remove sulfur from fuels usually at mesophilic conditions, targeting recalcitrant organosulfur compounds without affecting their hydrocarbon structure. One of the bottlenecks hindering its large-scale application is the inhibition of biodesulfurization activity by easily metabolized sulfur compounds, such as sulfates, even at residual concentrations. This increases production costs by requiring high-purity sulfur-free nutrients or complex induction steps to prevent/revert inhibition. The objective of this work was to bypass this limitation and demonstrate that it is possible to produce biocatalysts with biodesulfurization activity using sulfate as the only sulfur source, without employing inducers or genetic manipulation, simply by adjusting the sulfur : carbon ratio in continuous culture. With this goal, the bacterium *Gordonia alkanivorans* strain 1B was cultivated in a chemostat with a medium containing 10 g L^{-1} of fructose as the carbon source and different sulfate concentrations (12–50 mg per L SO_4^{2-}) using Na_2SO_4. Then the bacteria were employed as biocatalysts in biodesulfurization assays with a recalcitrant organosulfur compound (dibenzothiophene). Under these conditions it was observed that 2.2 $mg_{sulfate}$ $g_{fructose}^{-1}$ ensured a biodesulfurization activity of 6.1 μmol g_{DCW}^{-1} h^{-1}, 15% greater than previously reported for this strain with an inducer, without limiting biocatalyst production. This novel procedure was further applied to another biocatalyst, *Rhodococcus erythropolis* strain D1, validating its wide applicability to other desulfurizing microorganisms. Overall, these results indicate a previously unknown regulation mechanism dependent on relative sulfur concentration, which influences cellular responses and regulates biodesulfurization activity, allowing the use of easily metabolized sulfur sources to produce cost-effective biocatalysts for biodesulfurization.

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1. Introduction

To prevent the negative effects of sulfur emissions, most fuels have strict limitations on sulfur content and, therefore, must be subjected to desulfurization before commercialization. Fuels, such as diesel and gasoline, are typically subjected to a catalytic desulfurization process known as hydrodesulfurization (HDS). Despite its wide application, this is an energy-intensive process that presents low efficiency when dealing with sulfur in complex organosulfur compounds, such as dibenzothiophene (DBT), which are abundant in these fuels. To effectively treat them, operation conditions must be so severe that it can result in the loss of fuel calorific value.[1]

Biodesulfurization (BDS) is a biotechnological process that proposes the use of living microorganisms as biocatalysts to actively remove sulfur from fuels. Through their enzymatic pathways, microorganisms access the sulfur in fuels and either incorporate it into their biomass or use it for their metabolic activity. BDS has been studied for several decades, and many breakthroughs have been achieved in this field. The first BDS biocatalysts showed it was possible to remove sulfur from fuels at mesophilic conditions (usually between 20–37 °C and 1 atm) without hydrogen or metal catalysts, and since then many other microorganisms have been isolated with different BDS abilities.[2–4] However, what cemented BDS as a potential alternative/complementary process to HDS was the discovery of the 4S pathway. This metabolic pathway allows microorganisms to remove sulfur from recalcitrant sulfur compounds, without damaging their hydrocarbon structure nor generating toxic/hazardous pollutants, maintaining the calorific value of the fuel through a potentially more sustainable process.[5] Furthermore, the discovery and sequencing of the *dsz* operon, encoding the main BDS enzymes, has allowed the exploration of different genetic engineering methods for optimization, while the discovery of other genes connected to this pathway, with a direct and indirect effect on gene expression or enzyme

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