Acute toxicity evaluation of several compounds involved in fossil fuels biodesulphurisation studies

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The increasing use of fossil fuels has led to increased emissions of sulphur oxides into the air, which is a major cause of acid rain. Legislation already adopted in 2009 stipulates that the maximum level of sulphur allowed in fuels is only 10 ppm. The process of hydrodesulphurization (HDS) used in refineries is based on very expensive physico-chemical techniques, and has limitations in the removal of organic sulphur. As for stricter legislation on the maximum levels of sulphur in fossil fuels, the most HDS recalcitrant compounds needs to be removed. This implies an increase in the intensity of the physical-chemical treatment and inherently its associated costs. As a result, the recalcitrant compounds to HDS represent a significant barrier to the achievement of very low levels of sulphur in some petroleum fractions.

The alternative to the physical-chemical treatment could be the use of biological processes (biodesulphurisation) which is more effective for the desulphurization of fossil fuels, especially as the removal of sulphur covalently bound to organic matrices. The biodesulphurisation (BDS) occurs in more mild conditions of operation under conditions of atmospheric pressure and temperature, giving greater specificity of reaction due to the nature of the biocatalysts, not requiring molecular hydrogen. Thus, in the last 15 years there has been an increase of studies involving the use of microorganisms with the ability to specifically remove the HDS recalcitrant sulphur compounds.

Several model compounds such as dibenzothiophene (DBT), DBT sulphone or benzothiophene (BT) are used in BDS studies to characterise organic sulphur in coal, coal tars and crude oils. The desulphurising microorganisms are able to remove the sulphur atom from these compounds and use it in their metabolism. However, such compounds are very toxic to the cells. The aim of this work was to evaluate the toxicity of several compounds used in BDS studies, such as DBT and its derivatives and organic solvents used to dissolve these hydrocarbons, to two typical desulphurising strains, namely: *Gordonia alkanivorans* strain 1B and *Rhodococcus eritropolis* strain D1.

The toxicity bioassays evaluated the inhibitory effect of the studied compounds to the described bacteria by measuring the respiration rate (mg O_2/l) under defined conditions in the presence of different concentrations of those compounds. The inhibitory or toxic effect of each chemical at a specific concentration is expressed as a percent of the baseline respiration rate. From these results the several IC_{50}s were estimated and are described in Table 1. These toxicity values showed that strain 1B was less sensitive for almost all of the hydrocarbons, which is an important advantage considering the desulphurisation of fossil fuels process. On the other hand, strain 1B was more sensitive to dimethylformamide (DMF), a typical solvent used in BDS studies. However, a good correlation can be observed between IC_{50}-1B *versus* IC_{50}-D1 (IC_{50}-D1 = 0.504 x IC_{50}-1B + 2.84; $r^2 = 0.908$, $p < 0.05$).

Table 1. Acute toxicity of several compounds involved in the biodesulfurisation studies. Comparison of the sensitivity of both bioassays for toxicity evaluation (Sensitivity Factor).

Chemical	1B Toxicity Bioassay		D1 Toxicity Bioassay		R[b]
	IC_{50}[a]	r^2	IC_{50}	r^2	
BT	1.61 mM	0.904	1.15 mM	0.963	1.4
Ethanol	10.56%	0.995	10.52%	0.992	1.0
DMF	6.56%	0.963	9.91%	0.940	0.7
2-HBP	0.62 mM	0.962	0.52 mM	0.950	1.2
DBT	44.35 mM	0.906	24.21 mM	0.913	1.8

[a] IC_{50} = inhibitory concentration that causes an inhibition of 50% of the bacterial baseline respiration rate

[b] Sensitivity Factor (R): 1B-EC_{50} / D1-EC_{50}

This study also shows the high toxicity of 2-Hydroxybiphenil (2-HBP), the final microbial product from DBT desulphurisation, to both desulphurising microorganisms tested. The physiological response of 2-HBP concentration was also studied in a bioreactor system using both strains.

Keywords: biodesulphurisation; fossil fuels; *Gordonia alkanivorans*; *Rhodococcus eritropolis*; acute toxicity