for Nickel(II) concentrations in the range of 5-25 mg/L. EC_{50} values of the isolates were determined by probit analysis. *Synechocystis* sp. BASO403 (EC_{50} of 17.41 mg/L) and *Synechocystis* sp. BASO404 (EC_{50} of 2.56 mg/L) were selected from among the isolates based on their capacity to produce extracellular polysaccharides (EPS) which were 143 mg/L and 44 mg/L, respectively, for bioaccumulation at 15 mg/L Ni(II) concentration. The BASO404 isolate displayed a higher Ni(II) accumulation (95%), especially on the cell surface than BASO403 (51%) after a 7-day incubation period. A reverse relationship between EPS capacity and Nickel bioaccumulation ($p < 0.01$) was indicated, but a positive correlation was observed between EPS capacity and Nickel toxicity ($p < 0.01$). It is suggested that EPS from two isolates of *Synechocystis* sp. plays a protective role for Ni(II) toxicity.

doi:10.1016/j.jbiotec.2010.09.083

[P-E.62]

Bioleaching of hexavalent chromium from soils using *Acidithiobacillus thiooxidans*

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Keywords: Bioleaching; Hexavalent chromium; *Acidithiobacillus thiooxidans*; Soils

The continuous and growing degradation of the environment, due to several anthropogenic activities, is a main concern of the scientific community. Consequently, the development of low cost techniques to clean air, water and soils are under intense investigation. In this study, the focused problem is the soil contamination by hexavalent chromium, which is known for its several industrial applications - production of stainless steel, textile dyes, wood preservation and leather tanning - its high toxicity and mobility.

Bioleaching has been presented as a low cost effective technique to decontaminate soils polluted with heavy metals. Sulphur oxidizing bacteria, like *Acidithiobacillus thiooxidans*, were already applied with this technique as they produce sulphuric acid, lowering the pH and promoting the dissolution of heavy metals. On the other hand, it also known that polythionates, generated during the oxidation process, have high reducing power. Considering this information and since few studies have been made concerning the bioleaching of hexavalent chromium from soils, this work pretended to investigate this matter.

Specifically, eighteen Erlenmeyers flasks (250 mL) with a working volume of 150 mL, containing 10% ($^{V}/_{V}$) of inoculum (*Acidithiobacillus thiooxidans* DSM 504), 90% ($^{V}/_{V}$) of growing medium (DSM 35) and 3% ($^{W}/_{V}$) of contaminated soil were agitated in a rotary shaker, at 150 rpm, for 70 days. Also three controls were undertaken by sterilizing the soil and/or suppressing the inoculum. Two operation temperatures - 26 °C and room temperature - and different levels of soil contamination were evaluated within this work. Finally, the composition, richness and structure of soil microbial communities, before and after the contamination/decontamination processes, were assessed through denaturating gradient gel electrophoresis (DGGE), of the amplified 16S rRNA gene fragments of the DNA extracts of the soil samples.

This study presented bioleaching as a competitive technique in soil cleaning, as it is efficient and inexpensive.

doi:10.1016/j.jbiotec.2010.09.084

[P-E.63]

Identification of the third glutathione S-transferase gene involved in the stereospecific cleavage of β-aryl ether in *Sphingobium* sp. strain SYK-6

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Keywords: glutathione S-transferase; lignin; beta-aryl ether; *Sphingobium*

The degradation of arylglycerol-β-aryl ether is the most important process in microbial lignin catabolism. *Sphingobium* sp. SYK-6 degrades guaiacylglycerol-β-guaiacyl ether (GGE) to α-(2-methoxyphenoxy)-β-hydroxypropiovanillone (MPHPV), and then the ether linkage of MPHPV is cleaved to generate α-glutathionyl-β-hydroxypropiovanillone (GS-HPV) and guaiacol. Three alcohol dehydrogenase genes are involved in the degradation of four stereoisomers of GGE. The *ligD* gene is essential for conversion of (α*R*,β*S*)-GGE and (α*R*,β*R*)-GGE into (β*S*)-MPHPV and (β*R*)-MPHPV, respectively, while LigL and LigN transform (α*S*,β*R*)-GGE and (α*S*,β*S*)-GGE to (β*R*)-MPHPV and (β*S*)-MPHPV, respectively. LigF and LigE, which belong to glutathione S-transferases (GST), catalyze the nucleophilic attack of glutathione on Cβ of (β*S*)-MPHPV and (β*R*)-MPHPV, respectively. In these processes, *ligF* is crucial, however, a participation of an alternative GST gene was suggested in the degradation of (β*R*)-MPHPV. In order to isolate the gene for the (β*R*)-MPHPV degradation, a cosmid library was screened using a fluorescent assay substrate for the cleavage of β-aryl ether. A positive clone, pAD12, which did not contain either *ligF* or *ligE*, was isolated. The 4.6-kb BamHI fragment of pAD12 conferred the ability to convert only (β*R*)-MPHPV on *E. coli*, indicating that the gene included in this fragment encodes (β*R*)-MPHPV-specific GST. The nucleotide sequence of the fragment revealed an open reading frame of 843 bp (*ligE2*). The deduced amino acid sequence of *ligE2* showed 54 and 22% identity with those of *ligE* and *ligF*, respectively. Disruption of *ligE2* in SYK-6 resulted in a reduced conversion rate for MPHPV, suggesting the involvement of *ligE2* in (β*R*)-MPHPV degradation. The *ligE ligE2* double mutant no longer degraded one half of racemic MPHPV. All these results indicate that both *ligE* and *ligE2* alone contribute to the degradation of (β*R*)-MPHPV. This work was supported in part by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (Rural Biomass Research Project BM-D1310).

doi:10.1016/j.jbiotec.2010.09.085