SOLUBILIZATION AND IMMOBILIZATION OF TOXIC METALS BY BACTERIA (SLOVAK REPUBLIC AND ITALY)

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Abstract
Microorganisms play important roles in the biogeochemical cycling of metals. Some microbial processes release metals thereby increasing their mobility, which may increase bioavailability and potential toxicity, whereas other processes result in immobilization and reduce bioavailability. The relative balance between mobilization and immobilization varies depending on the microorganisms, their environment and changing physico-chemical conditions. As well as being a key component of natural biogeochemical cycles for metals, these processes may be exploited for the treatment of contaminated solid and liquid wastes. This paper describes two selected aspects, which illustrate the key importance in effecting changes in metal solubility, namely bioleaching by aerobic chemoautotrophic sulphur and ferrous oxidisers (bacteria genera *Thiobacillus*) and metal sulphide precipitation by anaerobic dissimilatory sulphate reducers (bacteria genera *Desulfovibrio* and *Desulfotomaculum*).

Introduction
Environmental contamination by toxic metals is very significant problem of contemporary period. The application of biological processes is appropriate concept for the solution of the environment metal contamination. Biological processes for the bioremediation are dependent on the nature of the site and chemical environment. In soils or sediments the potential ecological or public health hazard posed by toxic metals depends upon the form in which metals occur. Mineral components may contain considerable quantities of metal, which are unavailable to organisms, but soluble metal species have greater mobility and bioavailability. Biological processes can either solubilize metals, thereby increasing their bioavailability and potential toxicity, or immobilize them and reduce their bioavailability (Gadd, 2000). The relative balance between mobilization and immobilization varies depending on the microorganisms involved, their environment and associated physico-chemical conditions. As well as being a key component of natural biogeochemical cycles for metals these processes may be exploited for the treatment of contaminated soil and liquid wastes. Metal mobilization can be carried out under the thumb of various microorganisms and processes include autotrophic and heterotrophic leaching, chelation by microbial metabolites and siderophores, and methylation, which can result in volatilisation. Similarly many organisms can contribute to immobilization by sorption to cell components or exopolymers, transport and intracellular sequestration or precipitation as organic and inorganic compounds, e.g. oxalates in fungi, sulphides in sulphate-reducing bacteria (Gadd and White, 1993). In the context of bioremediation the solubilization of metal contaminants provides a route for removal of metals from soils, sediments and industrial wastes by e.g. bacterial leaching. Alternatively processes such as precipitation may enable metals to be transformed in situ into insoluble forms. Immobilization processes are particularly applicable to removing metals from mobile phases such as ground waters and leachates. This paper describes two biological processes, which are of significance in determining metal mobility in environment of mines, namely bioleaching by aerobic chemoautotrophic sulphur and ferrous oxidisers (bacteria genera *Thiobacillus*) and metal sulphide precipitation by anaerobic dissimilatory sulphate reducers (bacteria genera *Desulfovibrio* and *Desulfotomaculum*).

A pivotal ecological role of sulphate-reducing bacteria and sulphur oxidizing bacteria result from their participation in the sulphur cycle, which is a major biogeological cycle in the nature. Sulphur is widely distributed on the earth and occurs predominantly bound as sulphides, in the form of sulphates or even as elemental sulphur. In the nature sulphur circulates permanently because it is continuously oxidized or reduced by chemical or biological processes. In a biogeochemical sulphur cycle (Fig. 1) the biological transformations may have either assimilatory or dissimilatory metabolic functions. With the exception of animals and humans, most plants, fungi and bacteria are capable of performing an assimilatory reduction of sulphate to sulphide, which is necessary for the biosynthesis of sulphur containing cell compounds. On the other hand the energy producing dissimilatory sulphur metabolism is restricted to a few groups of bacteria. These groups include MO such as: anaerobic dissimilatory sulphate reducers (bacteria *Desulfovibrio, Desulfotomaculum, Desulfomonas,….*), anaerobic dissimilatory sulphur reducers (bacteria such as *Desulfuromonas,…*), anaerobic phototrophic sulphur oxidisers (some cyanobacteria and most anoxygenic phototrophic bacteria), aerobic chemotrophic sulphur oxidisers (bacteria such as *Thiobacillus, Sulfolobus, Thiospira, Thiobacterium, ….*), anaerobic chemotrophic sulphur oxidisers (bacteria such as *Thiobacillus denitrificans, Thiomicrospira denitrificans,..*) (Rehm and Reed, 1981).

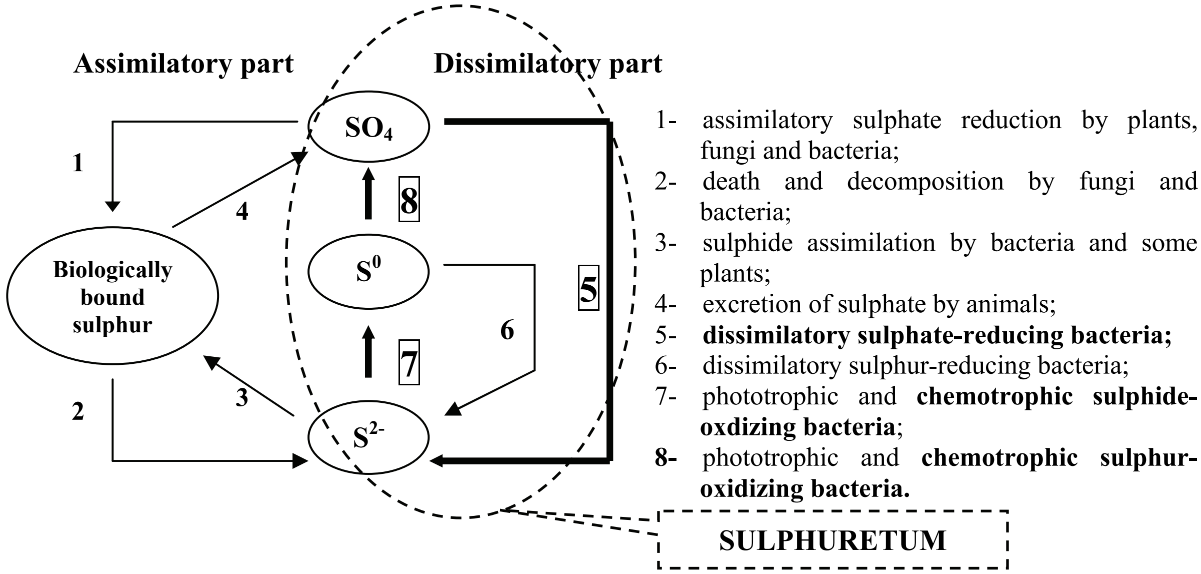

Figure 1. The biological sulphur cycle (Rehm and Reed, 1981; Luptakova et al., 2002).

The microbial population of this dissimilatory part is called "sulphuretum". Its activities are the fundamentals of many negative and positive processes in the nature and industry. Negative processes of sulphuretum include e.g. the aerobic or anaerobic biocorrosion of steel and concrete, the production of acid mine drainage, the decomposition of historical monuments etc.. Positive processes of sulphuretum include e.g. the bioleaching by aerobic chemoautotrophic sulphur and ferrous oxidisers (bacteria genera *Thiobacillus*) and the metal sulphide precipitation by anaerobic dissimilatory sulphate reducers (bacteria genera *Desulfovibrio* and *Desulfotomaculum*) (Luptakova et al., 2002).
The typical species of SRB is *Desulfovibrio desulfuricans* (*D. desulfuricans*), which may reduce sulphates autotrophically with hydrogen gas, as shown in reaction (1), as well as heterotrophically with an organic electron donor like lactate, as shown by reaction (2) (Rehm and Reed, 1981):

$$4\,H_2 + SO_4^{2-} \xrightarrow{SRB} H_2S + 2\,H_2O + 2\,OH^- \qquad (1)$$

$$2\,C_3H_5O_3Na + MgSO_4 \xrightarrow{SRB} 2\,CH_3COONa + CO_2 + MgCO_3 + H_2S + H_2O \qquad (2)$$

SRB can produce a considerable amount of hydrogen sulphide, which reacts easily in aqueous solution with the cations of heavy metals, forming metal sulphides that have low solubility. Reaction (3) summarises this type of reaction where Me^{2+} is the cation of a particular metal:

$$Me^{2+} + H_2S \longrightarrow MeS + 2\,H^+ \qquad (3)$$

On the basis of the complex evaluation of theoretical and practical knowledge obtained up published in the previous studies, it is possible to propose the following integrated biological approach utilizing chemoautotrophic leaching by sulphur-oxidizing bacteria followed by sulphate reduction and metal sulphide precipitation for the removal of toxic metals from soils (Fig. 2). In the integrated process sulphur-oxidizing bacteria which produce sulphuric acid are employed to leach metals from soils both by the breakdown of sulphide minerals and by liberation of acid-labile forms from e.g. hydroxides, carbonates or sorbed metals. The metals are released as an acidic sulphate solution, which enables both a large proportion of the acidity and almost the entirety of the metals to be removed in a subsequent process using bacterial sulphate reduction.

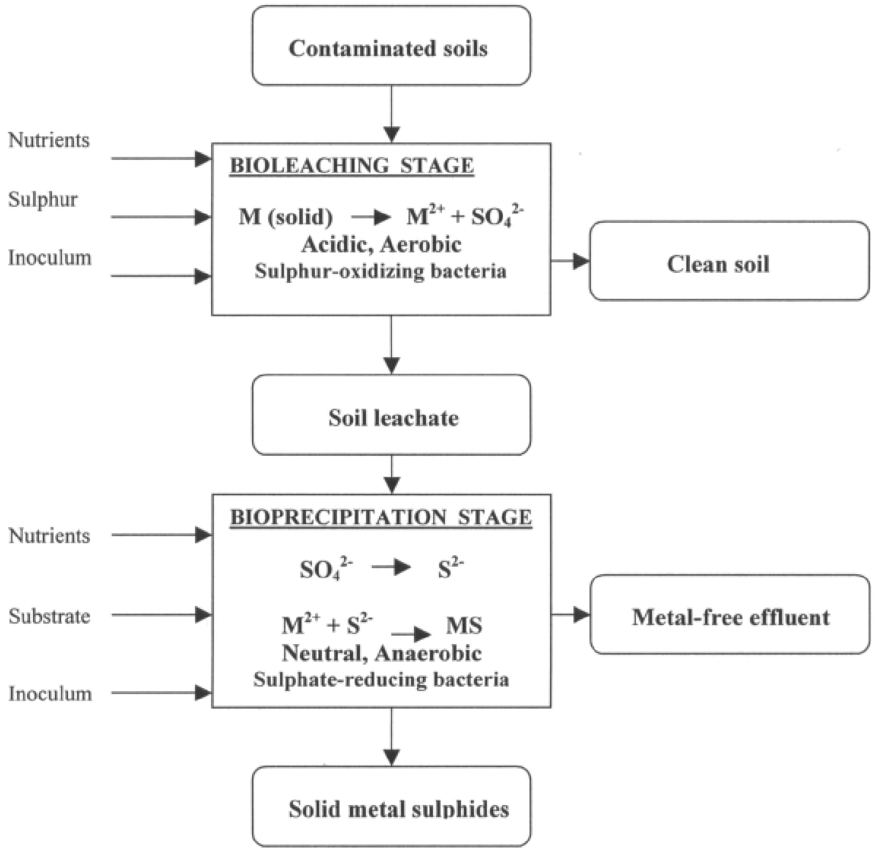

Figure 2. Diagram showing the integrated process for bioremediation of metal-contaminated soils (Eccles and Holroyd, 1996).

Conclusions

The bacterial processes described in this paper are significant components of metal biogeochemistry although details such as the global scale and environments affected vary. The bioleaching by sulphur-oxidizing bacteria and the bioprecipitation by sulphate-reducing bacteria have received the most extensive practical applications to date of the processes discussed. However since the chemistry and biology of individual polluted sites largely dictates the bioremediation method to be applied, it can be expected that a wider range of processes will be applied in the future.

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