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Minireview

## Leaching of metals with fungi

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### Introduction

It is one of the future challenges of environmental biotechnology to establish metal winning processes which are based on closed cycle concepts carried out with ecologically sustainable techniques. Furthermore, the methods used at the moment for the recycling of metal containing secondary raw materials should resort to methods which are less polluting and less energy consuming (Burgstaller et al., 1992). What have fungi got to do with this task? In biotechnology fungi have mainly been associated with the production of antibiotics, enzymes and organic acids up to now (Rehm, 1979). However, the ability of a number of fungi to solubilize great amounts of metals from solid materials may open up completely new prospects for the application of fungi. Fungi may contribute to the winning of metals from carbonaceous low-grade ores and mining wastes (Wenberg et al., 1971; Kiel and Schwartz, 1980) as well as from metal containing secondary raw materials and industrial intermediate products like filter dusts (Burgstaller et al., 1990) and slags. Also the detoxification of heavy metal contaminated wastes like sewage sludges (Kuppelwieser, 1989) and coal wastes (Scott et al., 1986) may be done with fungi. Further possible applications include the pretreatment of ores in order to increase the recovery rate of conventional pyrometallurgical methods, for example when aluminium is won from bauxite (Groudev et al., 1982), and the elimination of interfering contaminations of ores like phosphates from iron ores (Parks et al., 1990), or iron from quartz sand (Groudev, 1987).

Almost all knowledge of biohydrometallurgy accumulated up to now deals with chemolithoautotrophic microorganisms of the genus *Thiobacillus*. This is illustrated by the fact that – without one exception (Depaoli, 1990) – no outline about fungal leaching exists. Several facts account for this disregard of fungi as a possible means

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for leaching: (a) heterotrophic microorganisms need a lot of organic carbon source for growth and for the production of great amounts of leaching agents; (b) many biohydrometallurgists are not very familiar with the handling of fungi; (c) the turnover of the leaching material is considerably slower with fungi in comparison to the 'classic' substrate of *Thiobacillus ferrooxidans*, pyrite; (d) there seems to be less experience concerning genetic approaches.

However, on closer inspection, the following counter arguments must be emphasized: (a) a lot of the mentioned metal containing materials increase the pH of the medium and are therefore not a suitable environment for the commercially important acidophilic *Thiobacillus* species; (b) the ability of fungal leaching agents (organic acids) to form complexes with the metal ions is of advantage in order to increase the solubility of metal ions in neutral environments and in order to reduce the toxicity of heavy metal ions; (c) a great part of the mentioned leaching materials contains no energy source for the growth of chemolithoautotrophic bacteria which depend on the oxidation of sulfur or reduced iron and sulfur compounds; (d) tightened environmental standards and subsequently increasing costs for environmental precautions will force the industry to apply more and more non-polluting metal winning processes and to revalue conventional recycling methods for secondary raw materials for their ecologic sustainability; (e) the recycling of secondary raw materials will become more and more important because of the exhaustion of natural resources; (f) the genetics of yeasts are well known and there are also a growing number of publications concerning the transformation of filamentous fungi; (g) if we had as much knowledge about the causal connections of the leaching processes with fungi as we have with autotrophic bacteria, optimizing strategies could be applied much more efficiently.

For these reasons, and if cheap organic wastes like whey permeate or molasses are used as growth substrates, leaching processes with heterotrophic microorganisms should be applicable in special niches where autotrophic bacteria are not feasible and conventional pyro- or hydrometallurgical recycling techniques have environmental, economic or operational disadvantages.

Additionally, ecological implications of fungal leaching are of importance. Fungi contribute to weathering and mineralization of metal containing materials not only in specialized niches such as mining areas but in a wide diversity of habitats. Knowledge about these processes is important in order to avoid the contamination of the environment with heavy metals solubilized from deposited wastes.

The present article will give a brief survey of several diverse subjects which are of importance for leaching processes with fungi. Some of the treated topics may at the first impression appear to be far from biotechnology. But only a profound knowledge of the fundamentals gives us the opportunity to optimize and control a biotechnological process. Therefore, our aim was to collect the widely scattered results and information which are of relevance to the treated subject. Because one can find much information also useful for fungal leaching in reviews concerning chemolithoautotrophic bacteria and geomicrobiology, a selection has been included in the cited literature (Zajic, 1969; Karavaiko et al., 1988; Torma, 1988; Ehrlich, 1990; Rossi, 1990).

### Comparison to *Thiobacillus* spp.

Whereas chemolithoautotrophic bacteria of the genus *Thiobacillus* only need carbon dioxide and a reduced iron or sulfur compound for growth, chemoorganoheterotrophic fungi are dependent on an organic carbon- and energy source. Nevertheless, the fixation of carbon dioxide also exists in fungi: the high efficiency observed when citric acid is excreted by *Aspergillus niger* can only be obtained because high amounts of carbon dioxide are fixed by anaerobic pathways. In addition, the ability to gain energy from the oxidation of thiosulfate can also be found with fungi (Wainwright and Grayston, 1989). This oxidation of elemental sulfur or reduced sulfur compounds leads to the solubilization of metals. However, the rates of this process are much less efficient than with thiobacilli.

Chemolithoautotrophic bacteria and fungi show strikingly different ways of solubilizing metals from solid materials. Chemolithoautotrophic bacteria solubilize solid metal compounds by two mechanisms: (a) in order to gain energy the leaching material is solubilized by enzymically catalyzed reactions which imply a physical contact between the bacterium and the leaching material and lead to the destruction of the mineral; (b) the end products of these reactions, namely sulfuric acid, supplying protons, and ferric iron, supplying oxidizing capacity and protons, contribute also to the solubilization process. Redox processes play a major role.

Contrary to that fungi extract metals only by the excretion of metabolites like protons, organic acids, amino acids, peptides and proteins, and redox processes play a minor role. Enzymically catalyzed solubilization of a metal compound has not been confirmed with fungi up to now. However, a cell-particle contact may also enhance indirect leaching processes. This is observed when citric acid excretion is stimulated by the adsorption of filter dust (Franz et al., 1991) or when iron is reduced by oxalic acid produced by a fungus (Ghiorse, 1988).

*T. ferrooxidans* must transfer 2 mol of electrons from ferrous iron to oxygen in order to produce 1 mol of ATP. This results in a cell yield of 0.5 g of dry weight (experimentally determined). Fungi can synthesize 1 mol of ATP and about 2 g of dry weight from the transfer of 0.66 mol of electrons from glucose to oxygen (Brown, 1988). This corresponds to the oxidation of 0.027 mol of glucose. In other words the turnover of the growth substrate ferrous iron by *T. ferrooxidans* is 74 times faster than that of glucose by a fungus. The leaching materials dealt with in this article contain in most cases no energy source for chemolithoautotrophic bacteria. Therefore ferrous iron, for instance, has to be added. In this case, the efficiency of the metabolism of *T. ferrooxidans* and of a fungus to produce leaching equivalents are placed in the same order of magnitude: 1 mol of ferric iron produced by *T. ferrooxidans* is able to produce a maximum of 3 mol of protons (Smith et al., 1988) or to oxidize 1 mol of a metal ion. From this it can be deduced that between 1 and 1.5 mol of a metal ion, which is twofold positively charged after the reaction, can be solubilized. A fungus can produce up to 1.3 mol of citric acid from 1 mol of sucrose (Rehm, 1979); from this amount of citric acid 3.9 mol of protons are available which can solubilize up to 1.95 mol of a twofold positively charged metal ion. However, the time in which the citric acid is produced, is 10 d

for a fungal leaching process compared to less than 1 h for the oxidation of 1 mol of ferrous iron by *T. ferrooxidans* (Rossi, 1990).

The temperature for optimal growth and activity of the commercially important *Thiobacillus* species and of most leaching active fungi is around 30°C.

The pH range in which growth and leaching activities occur lies between 1.0 and 5.5 with an optimum around 2.4 for the commercially important *T. ferrooxidans*. But attempts have also been made to leach with autotrophic bacteria in a near neutral environment (Iske et al., 1991). The pH optimum for the most frequently used genus *Penicillium* is between 2.0 and 8.0 (Brown, 1988).

Rossi (1990) stated that *T. ferrooxidans* is resistant to heavy metal ion concentrations “of the same order of magnitude as those typical of hydrometallurgical processes”. With regard to fungi it can be postulated that it is possible to find a strain which tolerates the metal ion concentrations occurring in any arbitrary leaching process.

In contrast to thiobacilli, the nature of the leaching active metabolite excreted by a fungus is strongly influenced by the medium composition. This property of fungal metabolism may be of advantage if the leaching agent has to have definite complexing properties in order to improve the solubility of a certain metal ion. For example, with increasing pH of the medium, *A. niger* produces different organic acids in the following order: citric acid, gluconic acid and oxalic acid. The same was found with *Penicillium simplicissimum* (Zanella, 1991; unpublished results).

Important for a future application is the property of fungi to grow on a wide variety of substrates including organic carbon containing wastes from agriculture, food industry or biotechnological processes. However, whether a fungus produces great amounts of metabolites from these substrates or not, has to be tested with each specific substrate.

## **Metal resistance**

Information about the resistance of fungi to heavy metal ions can be found in reviews by Sadler and Trudinger (1967), Babich and Stotzky (1980), Duxbury (1985), Ehrlich (1986), Stotzky and Babich (1986), Collins and Stotzky (1989), Gadd and White (1989), Hughes and Poole (1989), Gadd (1990) and Mehra and Winge (1991).

A great deal of the knowledge concerning the toxicity of heavy metal ions to fungi came from investigations about the prevention and control of fungi by means of fungicides (Newby and Gadd, 1986).

A few indications exist that heavy metals are less toxic to fungi than to heterotrophic bacteria: for example, Bewley (1979) found that – in vitro – filamentous fungi were more tolerant to heavy metals than bacteria. Gadd (1990) reported that bacteria were more sensitive to lead, cadmium or zinc than fungi. Jordan and Lechevalier (1975) found that the same applies to zinc. According to the results of Pümpel and Schinner (1986), significantly more fungi in comparison to bacteria showed primary resistance (without adaptation) to 1 mM silver. And

Bosecker (1989) found that 34 isolated strains of heterotrophic bacteria were sensitive to metal concentrations higher than  $100 \text{ mg l}^{-1}$ . In comparison to that, the primary resistance for yeasts was  $300 \text{ mg l}^{-1}$  and the most resistant filamentous fungi tolerated  $1000\text{--}1500 \text{ mg l}^{-1}$ .

Heavy metals exhibit toxic effects mainly due to four factors: the blocking of essential functional groups of enzymes, the displacement of essential metals, the induction of conformational changes of polymers, and the influence on membrane integrity and transport processes (Ross, 1975; Gadd and White, 1989; Gadd, 1990). For fungi the following order concerning the toxicity of heavy metal ions was determined:  $\text{Ag} > \text{Hg} > \text{Cu} > \text{Cd} > \text{Cr} > \text{Ni} > \text{Pb} > \text{Co} > \text{Zn} > \text{Ca}, \text{Fe}$  (Horsefall, 1956, p. 279; Somers, 1961).

If a fungus shows more or less normal growth in the presence of high concentrations of heavy metals, this can be caused by several mechanisms:

- passive exclusion of the metal from the cell (impermeable membrane; alteration of membrane composition towards impermeability; alteration of the cell wall composition; prevention of uptake by proton competition in an acidic environment; Gadd, 1990);
- alterations in the speciation of the metal (Campbell and Stokes, 1985), for instance, if the metal ion is masked by organic chelators contained in the medium (Ramamoorthy and Kushner, 1975) or produced by the fungus (organic acids, amino acids: Avakyan and Rabotnova, 1971; Avakyan, 1971; extracellular polymers; siderophores; altered cell wall composition: Collins and Stotzky, 1989);
- an active transport system which transports metal ions out of the cell;
- the precipitation of the metal in the form of sulfides (Gadd and Griffiths, 1978) or oxalates (Murphy and Levy, 1983);
- the simultaneous presence of and interaction with other metal ions (Sadler and Trudinger, 1967);
- an enzymatic transformation of the metal ion as it is the case with mercury (Jeffries, 1982);
- the intracellular production of heavy metal binding compounds (methalothioneins and phytochelatins: Meisch et al., 1983; Abass and Razak, 1991; Mehra and Winge, 1991; Glaeser et al., 1991);
- the uptake and intracellular compartmentation in vacuoles and binding to polyphosphates (Gadd and White, 1989);
- the increased formation of melanin (Gadd and White, 1989);
- reduced uptake of heavy metals (Gadd and White, 1989); or
- the intracellular production of low molecular weight organic solutes like glycerol, erythritol, arabitol, mannitol, sorbitol and trehalose (Gadd et al., 1984).

One example for an extreme tolerance to heavy metals is *Penicillium ochrochloron*. Spores of *P. ochrochloron* were described to germinate even in a saturated solution of copper sulfate (Okamoto et al., 1977). The tolerance of *P. ochrochloron* to copper was attributed to the ability of the fungus to control the quantities of copper taken up. This ability was said to involve the decrease of cellular potassium content observed with copper concentrations above  $10\,000 \text{ mg}$

$l^{-1}$ . Additionally – because most copper tolerant microorganisms are acidophilic (*P. ochrochloron* grew at pH 1.5) – a role of protons in regulating metal ion uptake was also proposed: protons should compete with copper ions at the uptake system responsible for copper uptake.

Newby and Gadd (1986) tested the copper tolerance of several fungi. The sensitivity of various yeasts and filamentous fungi to cadmium was investigated by Babich and Stotzky (1977) and Trevors et al. (1986). The same authors published data about the toxicity of lead (Babich and Stotzky, 1979) and zinc (Babich and Stotzky, 1978) to fungi. Certain fungi are able to methylate arsenic and thereby detoxicate it (Summers and Silver, 1978). The resistance of fungi to mercury seems to be widespread (Jeffries, 1982). The toxicity of nickel to fungi has been reviewed by Babich and Stotzky (1983) and fungi tolerant to selenium have been described by Ragab et al. (1986).

In general, inhibition of fungal leaching processes by heavy metal toxicity seems to play a minor role, at least with the most frequently used genus *Penicillium*. This is mainly due to the excretion of organic acids which decreases the toxicity of heavy metal ions. However, if metal tolerant strains have to be found, one possibility would be to screen for halophilic species. The strategy of such strains to cope with high salt concentrations (intracellular production of low molecular weight solutes) seems also to include heavy metal resistance (Gadd et al., 1984).

### **Screening methods and submerged leaching**

Two qualities are necessary for a fungus which is to be used in leaching processes: it must excrete considerable amounts of metabolites (in most cases organic acids) and it must be resistant to heavy metals. The question arises if a screening program should take both criteria into consideration simultaneously. If this is done, strains would be excluded which are not resistant to heavy metals but are able to produce large amounts of metabolites. Such strains could for example be employed in two-step processes (Groudev, 1989; Strasser et al., 1992). Resistance to heavy metals and excretion of organic acids are only in few cases causally connected. An example is the production of oxalic acid by *A. niger*, *Penicillium spinulosum* and other fungi (Murphy and Levy, 1983). Contrary to the induction of the excretion of a metabolite by a heavy metal ion, *A. niger*, for instance, produces great amounts of citric acid only if the medium is deficient of a certain metal, namely manganese (Kubicek and Röhr, 1986).

There is – with the exception of weathering – no causal connection between growth of fungi and the solubilization of metal compounds with fungi as it is the case with thiobacilli. Therefore two different methods have to be applied in order to develop highly efficient fungal leaching processes: as a first step screening programs must be carried out applying direct as well as indirect criteria; secondly, the mechanisms of the excretion of metabolites must be elucidated and subsequently optimized.

Indirect criteria of screening programs are based on the assumption that fungi

which are able to solubilize non-metal compounds of low solubility, for example  $\text{CaHPO}_4$ , are also suitable for metal leaching processes (Schwartz and Nèveke, 1980). However, great care must be taken, because acid production by fungi is very strongly dependent on medium composition and conditions of cultivation (for example, *A. niger*, which solubilizes  $\text{CaHPO}_4$ , was not able to produce great amounts of organic acids in the presence of a filter dust).

This type of screening can be carried out on agar plates turbid with a fine powdered material where a halo around a colony indicates an active strain. The excretion of acids can also be observed on agar plates containing a pH indicator. If a fungus identified that way produces conidia, a further selection can be made according to Röhr et al. (1979): these authors developed a method which permits a differentiation between conidia with high and low acid excretion by means of a specific strain (*p*-dimethylaminobenzaldehyde).

Screening criteria are called direct, if the capacity to solubilize the specific insoluble metal compound which is to be leached, is tested. The detection can be of the same type as with indirect criteria (looking for halos around colonies growing on agar plates, e.g. for the solubilization of zinc oxide). When submerged cultivation is used solubilized metals are quantified by atomic absorption spectrophotometry.

Once promising strains are found, tests with a submerged cultivation technique must be carried out. It is a common problem that the leaching material is often adsorbed to, or included by, the fungal mycelium. This is especially adverse if the leached material and not the solubilized metal is of interest as it is the case with quartz sand after the removal of the contaminating iron. In this case the process must either consist of two separated steps (metabolite production and leaching), or a physical separation of the fungus and the leaching material by means of a dialysis membrane must be carried out, or the leached residue must be separated from the biomass after the leaching process by flotation.

For the exact determination of the yield of the solubilized metal in a submerged leaching process, several points must be carefully examined.

Is there an uptake of solubilized metal ions by the fungus? If so, the leached residue or the biomass must be combusted and yields must be calculated according to the results of this combustion. Does precipitation occur during the leaching process? If so, it must be attempted to separate the precipitate from the leached residue and to quantify the precipitation. Does the fungus produce great amounts of biomass? If so, the displacement of liquid by the biomass must be considered in the calculation of yields. The loss of liquid because of evaporation must also be included in the calculations.

The binding of leaching material to the mycelium also renders the determination of the biomass more difficult. A convenient method to avoid such difficulties is ashing of the biomass/leaching material conglomeration at  $500^\circ\text{C}$ . However, care must be taken if the leaching material contains metal compounds which evaporate at the temperature used for ashing.

Most leaching active fungi which have been isolated and used in the past belong to the genera *Penicillium* or *Aspergillus*. Species from other genera were only

rarely used. Within these two genera many species with the ability to excrete great amounts of organic acids can be found. The most important species are *P. simplicissimum* and *A. niger*.

A promising new candidate for leaching processes would be the copper resistant fungus *P. ochrochloron* (Okamoto et al., 1977) which was described to produce oxalic acid (Moss, 1987).

## **Mechanisms**

In order to optimize a leaching process it is necessary to understand the chemical reactions which take place in the abiotic part of the system as well as the regulatory processes which determine the metabolism of the fungus. The main abiotic components of the system are the insoluble leaching material (a part of which consisting of metal compounds), the components of the medium and the excreted metabolites (which are for instance protons as well as anions originating from the excreted organic acids). Additionally, fungal cells are present which influence reaction equilibriums through active metabolic reactions and passive adsorption properties.

### *Environmental regulation of fungal metabolism*

Generally, fungi acidify the nutrient medium during growth. This acidification can largely be put down to four processes: (a) the excretion of protons via the proton translocating plasma membrane ATPase (the excretion of protons can even be used as a parameter for the determination of growth; Huth et al., 1990); (b) the absorption of nutrients in exchange for protons; (c) the excretion of organic acids; and (d) the acidification brought about through the carbon dioxide produced by the respiratory activity of the fungus.

Although these processes occur also within the frame of a 'normal' metabolism, they usually end at a certain point. The production of great amounts of metabolites is only possible if there is an 'imbalance' of the metabolism. Therefore, it is of utmost importance to know the reasons of such a disorganization.

The best known case of metabolite excretion, caused by an imbalance of metabolism, is the citric acid production by *A. niger* (Kubicek and Röhr, 1986). The main effect is a manganese deficiency which induces the synthesis of a protease. Subsequently this leads to an increased protein turnover and to an elevation of the intracellular ammonium concentration. The ammonium then counteracts the citrate inhibition of the phosphofructokinase.

A short selection of other fungi capable of producing organic acids in amounts relevant for biotechnologists are: *Yarrowia lipolytica* (citric acid), *Mucor* spp. (fumaric acid, gluconic acid), *Rhizopus* spp. (lactic acid, fumaric acid, gluconic acid), *A. niger* (citric acid, oxalic acid, gluconic acid), *Aspergillus* spp. (citric acid, malic acid, tartaric acid, ketoglutaric acid, itaconic acid, aconitic acid), *Penicillium*



spp. (citric acid, tartaric acid, ketoglutaric acid, malic acid, gluconic acid), *Schizophyllum commune* (malic acid), *Paecilomyces variotii* (malic acid).

The nature and amount of organic acids excreted by fungi are mainly influenced by the pH of the medium (production of oxalic acid by *A. niger*: Kubicek et al., 1988; *P. simplicissimum*: Zanella and Burgstaller, 1991, unpublished results), the buffering capacity of the medium (Burgstaller et al., 1991), the carbon source (Xu et al., 1989) and the presence or absence of certain heavy metals and trace elements (Mn, Fe, Cu; Kubicek and Röhr, 1986; Mn; Babenko et al., 1989). Additional factors which are important for the excretion of, for instance, citric acid by *A. niger*, are the balances of nitrogen and phosphate (Kubicek and Röhr, 1986). Operational parameters of growth such as stirrer rate and aeration rate are also of importance because they influence the oxygen supply (Kubicek et al., 1980) and the form of growth (pellets or filamentous; Atkinson and Daoud, 1976).

The fungal cell is necessarily exposed to many environmental influences (Bull and Bushell, 1976; Berry, 1975; Kritsky, 1985). In connection to our subject three groups of environmental influences on the excretion of metabolites will be dealt with briefly. The first one is the alteration of the activity of enzymes and of the regulation of metabolic pathways by heavy metals, the second one deals with environmental influences on transport processes through the plasma membrane, and the third one with the influence of reactive (an)organic surfaces.

It is well known that citric acid production by *A. niger* is strongly influenced by metal ions. For example, an excess of copper ions neutralizes the inhibition of the aconitase by iron. Another example is the stimulating influence of manganese ions on enzymes of the glycolysis, the TCA cycle and the pentose phosphate cycle (Babenko et al., 1989) which occurs during the leaching of manganese ores. It is obviously impossible to control the concentrations of every metal ion which is liberated during a leaching process. However, the addition of specific metal ions in order to stimulate the production of metabolites should be possible.

If metabolites are to be excreted, they must pass the plasma membrane. Because the intracellular pH of fungal cells ranges from slightly acidic to neutral, most of the common metabolites occur in an ionized form. For this reason the membrane permeability for these molecules is rather low, and some sort of driving force for the excretion must be assumed.

The plasma membrane of fungi contains a proton translocating ATPase which is a key enzyme for the fungal cell (Serrano, 1985). It manages, or at least participates, in the control of growth, uptake of nutrients by secondary transport processes, control of intracellular and extracellular pH, germination of spores, and control of polarity of growth. Because the main product of its activity is a proton motive force consisting of a proton gradient (outside acidic) and a transmembrane potential gradient (inside negative) it becomes clear that interactions have to be expected between the environment present in leaching cultures and this enzyme or its products. Possible interactions include the dissipation of the proton gradient by leaching materials with high buffering capacity, a hyperpolarization of the transmembrane potential gradient by the neutralization of the extruded protons, a depolarization of the potential gradient by uptake of metal ions, direct influences

on the activity of the H<sup>+</sup>-ATPase, and changes in membrane permeability. These influences may also affect the excretion of organic acids (Roos and Slavik, 1987). It was shown that citric acid excretion in a leaching culture with *P. simplicissimum* is decreased if the H<sup>+</sup>-ATPase is inhibited by specific inhibitors, for example diethylstilbestrol, sodiumorthovanadate, and miconazole (Burgstaller et al., 1991). Therefore, the investigation of interactions between the environment in leaching cultures and the H<sup>+</sup>-ATPase seems to be important.

The presence of inorganic or organic surfaces may also greatly influence the metabolic activity of the cells. For example, Warscheid (1990) found, that the presence of clay minerals stimulated the excretion of organic acids by chemoorganoheterotrophic bacteria. He attributed this effect to the buffer capacity and the ion exchange capacity of the minerals. The observation that buffering of the medium increases acid production by fungi has also been made by Burgstaller et al. (1991). Stotzky (1980) reported that montmorillonite enhanced certain metabolic activities of heterotrophic microorganisms and protected the cells against heavy metal toxicity.

#### *Abiotic reactions*

Four mechanisms of the solubilization of solid metal compounds can be observed in leaching processes with fungi: acidolysis, complexolysis, redoxolysis (Berthelin, 1983) and the mycelium functioning as a 'sink' (Weed et al., 1969). The first three processes occur through metabolites excreted by the fungus. The fourth process can be observed if the fungus accumulates the metal ion from the solution and – by disturbing the equilibrium between solid and dissolved metal – causes the continuous solubilization of the metal.

By far the most important mechanism is the acidolysis. The oxygen atoms covering the surface of a metal compound are protonated very fast. The protons and the oxygen combine with water and the metal is therefore detached from the surface (Sigg and Stumm, 1989, p. 353). The rate limiting steps are the removal of the metal ion from the surface and the production of the metabolite by the fungus. On the other hand, protons are also able to catalyze solubilization reactions without their neutralization.

The term complexolysis means that a metal ion is solubilized due to the complexing capacity of a molecule (Sigg and Stumm, 1989, p. 353). Reactions of this kind are slower than solubilization with protons. Complexes with high stability constants are formed for example by oxalic acid and iron, magnesium and aluminium, by citric acid and calcium and magnesium, as well as by tartaric acid and calcium, magnesium, iron, silicium and aluminium. Also certain amino acids form stable complexes with metal ions. Complexation plays an important role in enhancing the solubility of a metal ion which has been solubilized via acidolysis. Because it is the final aim of leaching processes to obtain metal ions in solution, this is an important characteristic property of fungal leaching processes. Furthermore, the complexation of a heavy metal often reduces its toxicity for the fungus (Avakyan, 1971; Avakyan and Rabotnova, 1971).

An example of redoxolysis can be found in the reduction of ferric iron and manganese which is mediated by oxalic acid in an acidic environment (Ghiorse, 1988).

### **Fungal leaching techniques**

For the excretion of great amounts of organic acids one of the most critical factors is the availability of oxygen (Kubicek et al., 1980). This means that oxygen may be needed as a substrate for synthesizing an organic acid but also that oxygen plays a role in the regulation of the metabolic pathways involved in acid formation (Kubicek et al., 1980). Because the supply with sufficient oxygen and carbon source as well as the maintenance of a defined metabolic imbalance are essential, an efficient leaching process with a fungus is only thinkable in a bioreactor at the moment.

The characteristics of the various fungal growth forms and acid production processes necessitate the use of special constructed bioreactors. Details can be found in Bull and Bushell (1976), Kristiansen and Chamberlain (1983), Smith et al. (1980), and Brown (1988). For leaching processes a construction as simple as possible is desirable. One possibly promising technique would be the use of biphasic media, for instance a mixture of dextrane and polyethyleneglycol (Nardoslawsky et al., 1991).

Before a process is scaled up, it must be carefully considered if a one-step or a two-step process should be employed. Some useful criteria are the tolerance of the fungus to the material and the metal ions, the possibly necessary presence of the material in order to stimulate the formation and excretion of acid, the advantages or disadvantages of binding of the leaching material and the solubilized metal ions to the fungal biomass, and the possible use of higher temperatures in a two step process.

Only very few papers have been published where fungal leaching was carried out on a greater scale than shake flasks.

King and Dudeney (1987) solubilized aluminium from nepheline by cultivating *A. niger* in a sophisticated semi-continuous fluid bed bioreactor (five liter working volume). The dilution rates were increased from  $0.006 \text{ l h}^{-1}$  to  $0.05 \text{ l h}^{-1}$  during a run which lasted between 10 and 16 days. Aeration was 0.1 l of air per liter of medium per minute (vvm). The main characteristic of the used device was that the medium was circulating until the solid nepheline particles were dissolved. The solubilized aluminium was retained in an ion exchange unit. For this reason the fungus was never exposed to high concentrations of metal ions.

Ilgar and Torma (1989) studied the conversion of sucrose to citric and oxalic acid by *A. niger* in a bioreactor (1.5 l working volume). With the culture fluid obtained after seven days of cultivation and with abiotic solutions of citric and oxalic acids, the authors leached lithium from spodumene (7 d leaching period) and investigated several kinetic parameters in abiotic leaching experiments.

Gupta and Ehrlich (1989) leached manganese and silver from hardshell ore with

*Penicillium* sp. by using a one liter bioreactor in a continuous mode. The dilution rate was  $0.021 \text{ l h}^{-1}$  and one run lasted 15 days. The aeration rate was 1.5 vvm and the stirrer rate 100 rpm to guarantee that the ore particles remained settled. The highest concentrations achieved were  $2.8 \text{ g l}^{-1}$  manganese and  $2.8 \text{ mg l}^{-1}$  silver, respectively.

Strasser et al. (1992) applied a two step process for the removal of iron contamination from quartz sand. Oxalic acid was produced at optimal conditions by *A. niger* in a batch cultivation (two liter working volume; 1 vvm; up to 800 rpm). After 10 d the medium was filtered and leaching was carried out for 4 d. 90% of the iron was removed by this process.

Burgstaller et al. (1992) constructed a ten liter stirred tank reactor modifying the proposals of Babij et al. (1980). Aeration rates up to 1.5 vvm and stirrer rates up to 1000 rpm were used. The authors leached zinc from filter dust of a copper melting plant in a fed-batch process. The influence of various parameters on the solubilization of zinc was investigated.  $30 \text{ g l}^{-1}$  zinc were the limit which could be achieved with the device and the operational parameters used. The process lasted 9 d.

## Metals

*Copper.* As stated by Rossi (1990), the winning of copper from copper sulfide by chemolithoautotrophic bacteria is “the most commonly practiced ore leaching process and is responsible for more than 15% of the world’s primary copper production”. Contrary to that, leaching of copper with fungi was only done on a shake-flask scale. However, there are many copper containing leaching materials which cannot be treated with *Thiobacillus* spp.

Several investigations were carried out to extract copper with fungi from ores which contain carbonates, silicates and oxides. The fungi belonged to the genera *Aspergillus* or *Penicillium*. The leaching of copper sulfides with *A. niger* and *Trichoderma harzianum* has been described as possible but not feasible (Wainwright and Grayston, 1989).

Wenberg et al. (1971) leached copper from materials (0.2–95% Cu) which could not be leached with chemolithoautotrophic bacteria because these materials contained no pyrite or other reduced sulfur compounds. Contrary to that, the materials alcalinized the medium. The fungus was cultivated for five days and the spent medium was shaken with the material for 3 d. Sucrose and glucose were suitable carbon sources for the *Penicillium* strain isolated by the authors, whereas galactose and lactose were not. Only three (cysteine, glutamic acid or methionine), of 18 amino acids tested as nitrogen sources, led to amounts of solubilized copper comparable to the ones with sodium nitrate as nitrogen source. A reduction of copper toxicity increased the rate of copper solubilization with the fungus. In connection with this, growing the fungus in the absence of copper bearing material was more effective than the addition of sodium citrate in the presence of the material. The highest concentration of solubilized copper was  $3038 \text{ mg l}^{-1}$ .

A very detailed investigation concerning copper leaching from a low grade copper ore (1% Cu) containing silicates and carbonates was carried out by Kiel and Schwartz (1980). Via a screening program mostly strains of the *A. niger* group were yielded. *A. niger* solubilized more copper when growing in surface culture than in submerged culture. Incubation times lasted from 4 to 14 d. The solubility of the complexes formed between copper and the organic acids excreted by the fungi was a limiting factor. The authors tested various agricultural waste materials as growth substrate for *A. niger*. A detoxicated sulfite liquor, citrus peels and tomato residues enabled the fungus to leach copper from the ore. Copper concentrations in the medium were 1780 mg l<sup>-1</sup> for experiments in a percolator (pulp density 100%; yield 16%), 1000 mg l<sup>-1</sup> for surface cultures (pulp density 10%; yield 87%) and 620 mg l<sup>-1</sup> for the submerged cultures (pulp density 10%; yield 54%).

Groudev (1979; Groudev et al., 1985) found that carbonate rich copper ores (1% Cu) could only be leached effectively with *T. ferrooxidans* if the carbonate was preneutralized. The leaching experiments with thiobacilli lasted 160 d. *Thiobacillus thioparus*, a strain which is able to oxidize sulphur under neutral conditions, was not effective. When the ores were treated with a citric acid containing spent medium of *A. niger*, yields of copper between 4% and 79% – depending on the type of ore – were achieved.

Metabolites produced by *A. niger* and *P. variotii* (citric and oxalic acid) were able to leach copper from an oxidized copper ore (1% Cu; Dave and Natarajan, 1981). Increasing the inoculum had no positive effect on the solubilization of copper.

A considerable amount of copper was solubilized with *Penicillium notatum* at a pH near neutral from elemental copper and pyrite within 30 days (Siegel, 1973; Puerner and Siegel, 1976; Siegel et al., 1983). *P. notatum* was able to grow in saturated salt solutions (boron, calcium). The spent medium contained 132 mg l<sup>-1</sup> copper after 13 d. The intracellular copper content was 8%, on dry weight basis. Because the hyphae etched the metal surface, the authors postulated that complexing agents contained in hyphal cells or cell walls were responsible for copper solubilization (peptides, D-glucosamine, lysine). A close correlation was found between the melting point of a metal and its corrosion by a fungus (Siegel et al., 1983). For the reported solubilization of copper a mechanical contact was not necessary and metabolites other than protons were assumed to be involved.

Hartmann and Kuhr (1974) reported about the leaching of a low-grade copper ore (0.6% Cu). After 6 d, *A. niger* had solubilized 59% of the present copper.

That copper oxide may be solubilized with ammonia formed during the aerobic decomposition of lucerne due to mould growth was shown by Bloomfield and Kelso (1971). This solubilization occurred at a pH around 8. The formation of cupric ammine complexes was postulated.

Ehrlich (1980) proposed that copper could be solubilized from manganese nodules if the manganese were reduced by oxalic acid produced for example by *A. niger*.

When thymol or other naturally occurring lipids like pine resin are added to a soil, mainly acidophilic, metal resistant bacteria and yeasts are able to survive

(*Pseudomonas* and *Bacillus* species and a few yeasts; Berthelin et al., 1974). The addition of these antimicrobial agents resulted in a shift of the spectrum of the excreted acids and in the increased production of organic acids, combined with a reduced glucose consumption (Munier-Lamy and Berthelin, 1987). This stimulation of organic acid excretion led to an increased solubilization of copper (between day 20 and day 40 of the experiment). The authors determined which species of organic acids were formed (aliphatic acids: isocitric acid, oxalic acid, succinic acid, lactic acid; phenolic compounds: especially coumaric acids and hydroxybenzoic acids). Solubilized copper was present in the form of metallo-organic compounds (associations of metallic cations with organic polyanions) with a molecular weight between 2500 and 3000 or greater than 5000, respectively.

In order to find information about fungal species which are able to solubilize copper the literature about corrosion phenomena is also useful (Staffeldt and Calderon, 1967; Iverson, 1987; Ford and Mitchell, 1990). Staffeldt and Calderon (1967) gave a list of organic acids, amino acids, fatty acids and enzymes which react readily with copper. Iverson (1987) mentions the fungal species *Cerostomella* sp., *A. niger*, *Aspergillus amstelodami*, *Penicillium cyclopium*, *Penicillium brevicompactum* and *P. variotii* which are all able to solubilize copper.

Reagents which are used for metal recovery from ores in hydrometallurgical processes have been reviewed by Smith (1988). Some of these compounds may be of interest because they may also be formed by fungi, for instance fatty acids and cresol.

Leaching of synthetic copper oxide with *A. niger* was carried out by Golab and Orłowska (1988). Spent medium of *A. niger* cultivated in a Czapek Dox medium contained organic acids (tartaric acid, citric acid) and amino acids (histidine, glycine, aspartic acid, glutamic acid, threonine, serine). This spent medium solubilized 790 mg l<sup>-1</sup> copper after 24 h of contact (over 90% of the present copper oxide). Abiotic leaching experiments revealed that especially tartaric acid and also the amino acids produced by *A. niger* (with the exception of glutamic acid) were appropriate leaching agents for copper oxide. Spent medium from *A. niger* cultivated in a molasses medium was less suited for leaching of copper oxide. This was attributed to the higher pH and the precipitation of metals because of the high sugar concentration. The solubilization of copper with a spent medium of *A. niger* cultivated in synthetic media was said to be mainly due to complexolysis. This was concluded from the similar pH values of the spent media from cultivations with and without copper oxide. However, a metal oxide may also go into solution by the action of protons which are thereby neutralized (Burgstaller et al., 1992).

**Zinc.** Several fungal species are able to solubilize zinc from solid materials. Some of these results came from research on the solubilization of micronutrients from metal compounds (oxides, carbonates, silicates, hydroxides, chromates and others) in soils (Webley et al., 1963; Bloomfield and Kelso, 1971; Siegel, 1973; Agbim and Duxtader, 1975; Puerner and Siegel, 1976; Kucey, 1988).

Siegel et al. (1983) found that *P. notatum* corroded metallic zinc most easily among a series of tested metals (Zn > Sn > Al > Pb > Cu > Er > Co).

The stimulating influence of unidentified fungi on the leaching of zinc from industrial waste products with *T. ferrooxidans* was stated by Schäfer (1983).

Zinc from an oxidized lead–zinc ore (20% Zn) was extracted with *P. variotii* (Dave and Natarajan, 1981). It was striking that the pH increased from 7.0 to 7.6 after twenty days of leaching. Whereas leaching of copper with *A. niger* was attributed to the production of organic acids, the mechanism of zinc solubilization with *P. variotii* remained unclear.

Groudev et al. (1985) treated residues from hydrometallurgical processes of zinc winning (17.2% Zn) with a mixture of spent medium from *A. niger*, several mineral acids and the thermophilic bacterium *Sulfolobus* sp. The fungus was cultivated for 10 d. The actual leaching process was carried out at 70°C and lasted 5 h.

The winning of metals from industrial wastes is of growing importance (Förstner et al., 1991). Leaching of such a secondary raw material from a copper works (filter dust from a converter; 50% Zn as ZnO) was carried out with *P. simplicissimum* (Schinner and Burgstaller, 1989). These experiments led to more detailed investigations about the mechanisms of zinc oxide solubilization (Franz et al., 1991; Burgstaller et al., 1991). As mentioned above, the role of the H<sup>+</sup>-ATPase seems to be important in this leaching process (manuscript in preparation). The process with *P. simplicissimum* was carried out successfully in a ten liter bioreactor (Burgstaller et al., 1992). The highest zinc concentration in the spent medium was 30 g l<sup>-1</sup>. At the moment an attempt is made to find a suitable and cheap growth substrate for leaching processes with fungi. It was also tried to apply yeasts to the leaching of this filter dust (Wenzl et al., 1990).

**Nickel.** Nickel was leached from lateritic ores (0.9%–3.1% nickel) with several species of the genera *Penicillium* (*P. simplicissimum*, *Penicillium funiculosum* and others), and *Aspergillus* (Bosecker, 1987, 1988; Bosecker, 1989; Alibhai et al., 1992). *P. simplicissimum* was adapted to tolerate up to 30 000 mg l<sup>-1</sup> nickel. Abiotic leaching experiments with fifteen organic acids revealed that citric acid was the most suitable acid for this leaching process (up to 90% of the nickel extracted with 0.5 M citric acid after 15 d; Bosecker, 1987). With *Penicillium* sp. up to 70% of the nickel were extracted. However, the extraction rate was strongly dependent on the type of the ore (silicatic or limonitic). *A. niger* was also tested but was much more sensitive to nickel and additionally accumulated nickel.

Another possibility of winning nickel was pointed out by Ehrlich (1980). The manganese of ferromanganese nodules can be reduced by oxalic acid which is produced by *Penicillium* spp. or *Aspergillus* spp. The solubilization of manganese concomitantly releases nickel.

**Uranium.** Uranium in the form of oxides, phosphate or thorites was leached from different uranium bearing rocks (0.018–4.40% uranium; Munier-Lamy and Berthelin, 1987; Berthelin et al., 1988). The fungi used were *Aspergillus ochraceus* and *P. funiculosum*. The mechanism of uranium solubilization was attributed to the formation of stable organo-uranyl complexes with citric acid and glutamic acid.

The complexes had molecular weights between 2000 and 2900 which indicated the existence of polyelectrolytes. The fungi also accumulated the uranium. The dissipation of the proton-motive force promoted the uranium uptake.

*Silicates.* A lot of results concerning the solubilization of silicates with fungi has been collected from investigations about weathering processes (Henderson and Duff, 1963; Webley et al., 1963; Wagner and Schwartz, 1965; Boyie et al., 1967; Silverman and Munoz, 1970; Berthelin et al., 1974; Eckhardt, 1979; Karavaiko et al., 1980; Avakyan et al., 1981; Berthelin, 1985; Avakyan, 1985; Krumbein, 1988; Ehrlich, 1990). The topic was reviewed by Silverman (1979), Berthelin (1983, 1988) and, most extensively, by Dacey et al. (1981).

The treatment of silicate minerals will become more and more important, because the increasing depletion of high grade ores will make it necessary to win metals from the abundant low grade silicate minerals. Possible applications include: the destruction of the crystal lattice of silicate minerals in order to solubilize the metals contained in the mineral (e.g. aluminium: Groudev and Groudeva, 1986; potassium: Rossi, 1978; lithium: Ilgar and Torma, 1989; iron: Groudev, 1987; uranium and copper: Munier-Lamy and Berthelin, 1987; nickel: Henderson and Duff, 1963; zinc: Agbim and Doxtader, 1975) and the removal of silicon from low-grade bauxite ores in order to enhance the recovery rate of aluminium won via the Bayer process (Groudev et al., 1982) or from magnesite ores (Mishra et al., 1986).

*Aluminium.* The leaching of aluminium is closely connected to the solubilization of silicates. Rossi (1985) and Rossi and Ehrlich (1990) reported about the solubilization of aluminium from various aluminosilicates. The authors concluded that the use of moulds, although possible, has too many disadvantages which make them unsuitable for commercial application.

A two stage process for the microbial winning of aluminium was described by Groudev et al. (1982), Groudev and Groudeva (1986) and Groudev (1987). The lixiviant was a spent medium from *A. niger* containing citric and oxalic acid acidified with inorganic acids to pH 0.5. The leaching process itself was carried out at 90–100°C. Heating the minerals to 600°C increased the yield and inhibited the leaching of iron which is troublesome at the recovery of aluminium from the pregnant solution. After 3–5 h more than 90% of the aluminium was extracted from thermally activated genthite and vermiculite. Other minerals yielded between 60% and 90%.

Citric acid produced by *P. simplicissimum* was used for extracting aluminium from basalt rock (Mehta et al., 1978, 1979). The biodegradation was followed by scanning electron microscopy. The authors also investigated the influence of the composition of the medium, of the initial pH and of the glucose concentration on the extraction of aluminium. The highest yield was 20% after 30 d.

King and Dudeney (1987) carried out a very detailed study (including kinetic aspects) on the leaching of aluminium from nepheline with *A. niger*. They used a



sophisticated semi-continuous, fluid-bed process equipment which simultaneously made it possible to recover the solubilized aluminium.

Singer et al. (1982) reported the extraction of aluminium from fly ash from a coal-burning power plant with *A. niger*.

The aluminium oxide  $\text{Al}_2\text{O}_3$  is also susceptible to solubilization with organic acids produced by *A. niger* (Golab and Orłowska, 1988).

Bosecker (1989) leached aluminium from silicate waste products from the aluminium processing industry. The author carried out abiotic leaching tests using organic acids which are produced by fungi. Up to 60% of the aluminium was solubilized by citric acid after 15 days.

Information on fungi which are able to dissolve aluminium compounds (*A. niger*, *Aspergillus fumigatus*, *Aspergillus amstelodami*, *P. cyclopium*, *P. brevicompactum*, *Cladosporium resinae*, *Cerostomella* sp., and *P. variotii*) is also available from studies on corrosion effects (Iverson, 1987).

**Iron.** The solubilization of iron with fungi is possible in two ways: by reducing ferric iron to ferrous iron (catalysed by an excreted metabolite), or by solubilizing ferric iron with protons or complexing agents. Both reactions can be carried out with several fungi (*A. niger*: Groudev, 1987; Strasser et al., 1992; *Fusarium* spp., *Actinomucor* spp., *Alternaria* spp.: Ghiorse, 1988) and are supposed to be non-enzymatic by means of excreted metabolites such as oxalic acid, malate, oxaloacetate and pyruvate (Ghiorse, 1988). The reduction process is stimulated in acidic and anaerobic environments (Ghiorse, 1988).

One major field of application for the solubilization of iron is the removal of iron oxide contaminations from quartz sand, clays and kaolin. This topic was investigated thoroughly by Groudev and coworkers (Groudev et al., 1982; Groudev, 1987, 1988; Groudev and Groudeva, 1988a). Spent medium from *A. niger*, containing oxalic, citric and gluconic acid, was acidified to pH 0.5 with hydrochloric acid. With this solution which contained 5–10 g l<sup>-1</sup> oxalic acid, leaching was carried out in a stirred tank at 90°C for 1 to 5 h. A further improvement was achieved with an oxalic acid containing waste solution from tetracycline production. More than 90% of the iron oxide contaminations could be removed.

The removal of iron from bauxites was investigated by Karavaiko et al. (1989).

Strasser et al. (1992) also found that oxalic acid was the most suitable acid for solubilizing iron oxide contained in quartz sand. They optimized the conditions of oxalic acid production by *A. niger* (maximum 0.4 M oxalic acid after 10 d). The solubilization occurred through the effect of protons and subsequent complexation of the ferric iron by oxalic acid. Redox processes and ligand catalysed solubilization played almost no role. More than 90% of the haematite was solubilized in a two stage process after 5 d of incubation.

*P. simplicissimum* was used by Silverman and Munoz (1970) for solubilization of several igneous and metamorphic rocks. Iron was the element which was solubilized to the greatest extent (maximum 64%). The 'weathering' of the minerals was connected with an altered infrared spectrum of the minerals.

*Manganese.* The leaching of manganese ores (mainly oxides) may serve to win the manganese or to extract other valuable metals like copper, nickel and cobalt from these ores. Because the manganese in these ores is trivalent or tetravalent, a reduction must take place to get the manganese soluble. Fungi from the genera *Aspergillus*, *Penicillium* and *Pichia* (Ghiorse, 1988) can reduce manganese only non-enzymically via excreted metabolites (Ehrlich, 1980; Groudev, 1987; Ghiorse, 1988).

Gupta and Ehrlich (1989) presented a profound study on the leaching of manganese and silver from a manganese-containing silver ore. The authors found that material-cell contact is not necessary for the solubilization of manganese. From their results they concluded that *Penicillium* sp. had excreted one or more compounds which are strong reductants. By maintaining a chloride concentration in the medium at which the solubility of silver chloride is minimal, manganese could be leached selectively. The toxicity of silver was possibly decreased by the formation of complexes between silver ions and chloride ions. Only about 1% of the manganese was solubilized by acids or by reducing glucose. Natural as well as synthetic seawater was used as growth medium.

*Other elements.* Up to 80% of the titanium contained in a number of rocks was solubilized with *P. simplicissimum* (Silverman and Munoz, 1971).

Mercury in the form of metallic mercury, mercury oxide and mercury sulfide (HgS) is also susceptible to fungal attack. *Aspergillus clavatus* was able to solubilize these compounds (Puerner and Siegel, 1976). From various tested fungal metabolites L-asparagine was the most active one on mercury compounds.

A series of metals not mentioned up to now in this review are also susceptible to fungal attack. Siegel et al. (1983) reported the following order of 'bio-corrodibility': Zn > Sn > Al > Pb > Cu > Cd > Er > Co.

Phosphorus, which is undesirable in ferromanganese alloys, was successfully removed from manganese ores (Agate, 1985).

Potassium is an important element in agriculture (Rossi, 1985). It can be leached with fungi from aluminosilicates (Rossi, 1978, 1985; Rossi and Ehrlich, 1990).

The extraction of lithium from  $\beta$ -spodumene (LiAl(SiO<sub>4</sub>)) with *A. niger* was carried out by Ilgar and Torma (1989). Investigations of the kinetics of the process included the influence of pulp density, temperature, activation energy, and acid concentration.

Up to now gold has only been solubilized very inefficiently with fungi. Two species which were able to extract gold from gold dust (*Penicillium* sp. and *Candida* sp.) were described by Groudev and Groudeva (1988b, 1990).

The extraction of chromium was possible from solid wastes of a tanning factory (10000 mg kg<sup>-1</sup> chromium). *P. simplicissimum* was able to solubilize 30% of the chromium contained in this waste after seven days (Burgstaller, 1991; unpublished results).

Insoluble phosphates (hydroxyapatite) should be removed from iron ores in order to increase the quality of steel. An unidentified fungus (possibly *Penicillium*

sp.) was described to solubilize such phosphates (Parks et al., 1990). Oxalic acid and minor amounts of itaconic acid were produced by the fungus. Tests with acidified (HCl) spent medium greatly increased the solubilization rate.

Certain fungi (e.g. *Trametes versicolor*, Fredrickson et al. 1990; *Coriolus versicolor*, *Penicillium* sp., *Cunninghamiella* sp., *Candida* sp., *Phanerochaete chrysosporium*, Stewart et al., 1990) are able to degrade oxidized bituminous and lignite coals. Suggested mechanisms are base solubilization, action of extracellular enzymes and solubilization by a siderophore-like product. Besides the use of fungi for an initial processing step in solid fuel processing, they could be applied to remove toxic heavy metals from coal cleaning residues as was described for bacteria by Francis et al. (1988).

## Outlook

We want to stress two arguments which should support the application of fungi in leaching processes in the future. An ecologic argument: industrial processes for the winning of metals should be changed little by little in order to minimize environmental pollution, as well as mass and energy fluxes; and the argument of 'closed-cycle concepts': the establishing of closed cycles is desirable for industrial secondary raw materials as well as for organic wastes.

The key question whether a leaching process with a fungus will be at some time or other economically viable or not, is the availability of a cheap carbon source. The application of wastes from agriculture, food industry or biotechnological processes which contain organic compounds as growth substrates is investigated at the moment by our group. For example, starch and cellulose have proven to be as efficient as glucose (Berthelin, 1985), and molasses as well as whey permeate are also suitable.

It can be predicted that the leaching materials which are most likely to be the first ones to be leached with fungi will share two properties. First, these will be materials which increase the pH of the medium. Secondly, they will have to have a high content of a precious metal, as it is the case for secondary raw materials from the metal winning industry. And the third point is that fungal leaching may be – because of the complexing property of fungal leaching agents – the method of choice where the solubility of the metal to be leached is limited.

A lot of questions remain unanswered. However, the basic research which has been done in this field has demonstrated many potential applications. Additionally, diverse knowledge was gained on the physiology of fungi in connection with heavy metal ions. A great effort will nevertheless be necessary to overcome all limitations towards a commercial application. This effort includes the application of new methods for strain improvement (e.g. fusion of protoplasts in order to enhance the excretion of metabolites; Ferenczy, 1984), the application of sophisticated methods in order to investigate metabolic regulations and transport processes, for example the use of specific inhibitors or of the patch clamp technique, the development of new process control methods such as calorimetric techniques

(Schroeter and Sand, 1992) or biosensors, the design of simple bioreactors which meet the demands of the cultivation of fungi, and, last but not least, the search for cheap growth substrates. Additionally, when the mechanisms of organic acid excretion are better understood, it is thinkable to influence a natural fungal population in order to increase their acid excretion. This would open up the field of an in situ leaching of waste disposal sites, for instance, also for fungi if the problem of the supply with oxygen and carbon source is solved simultaneously. The other side of the coin would be to stop fungal leaching processes in natural environments where they are undesirable.

If all this could be achieved, an increased contribution of fungi to metal leaching processes would stand a chance.

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