

Heavy metal extraction from electroplating sludge using *Bacillus subtilis* and *Saccharomyces cerevisiae*

Schwermetallgewinnung aus Galvanikschlamm durch *Bacillus subtilis* und *Saccharomyces cerevisiae*

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Abstract

Every year, a large amount of heavy metal-bearing sludge is produced by the electroplating industry and has to be categorized as hazardous waste. However, besides its environmental toxicity, electroplating sludge can also be considered as a resource, due to its high concentration of valuable metals like copper, chromium, nickel, iron, and zinc. Microorganisms capable of adsorbing and accumulating these metals may be used in cost-effective and non-polluting methods for the treatment of such sludges.

In the present study, the extraction of copper, chromium, nickel, iron, and zinc from electroplating sludge via *Bacillus subtilis* and *Saccharomyces cerevisiae* was investigated. Both organisms are characterized by their environmental harmlessness and simple cultivation conditions. Pronounced differences exist in the leaching capabilities of both organisms: *B. subtilis* was able to mobilize all heavy metals contained in the sludge, while *S. cerevisiae* could only remove copper, nickel, and zinc. Furthermore, leaching with *B. subtilis* was significantly more effective than the *S. cerevisiae*-leaching. The former extracted 76% of chromium, 42% of iron, 74% of copper, 59% of zinc, and 51% of nickel from the solids, compared with only 2% of nickel and zinc, and 16% of copper leached by *S. cerevisiae*.

Kurzfassung

In der Galvanikindustrie fallen jährlich große Mengen an Galvanikschlamm an, die als gefährliche Abfälle deklariert und entsorgt werden müssen. Neben ihrer Toxizität für die Umwelt sind diese Industrieabfälle in Zeiten immer knapper werdender Rohstoffe aufgrund ihrer Metallgehalte jedoch auch eine nutzbare Ressource. Die Extraktion von Metallen mit Hilfe von Mikroorganismen ist eine kostengünstige und umweltfreundliche Recyclingmethode für derartige Industrieabfälle, die auch bei geringen Metallkonzentrationen effektiv eingesetzt werden kann.

In dieser Arbeit wurde die Extraktion von Kupfer, Zink, Chrom, Nickel und Eisen aus Galvanikschlamm durch Laugung mit den Mikroorganismen *Bacillus subtilis* und *Saccharomyces cerevisiae* durchgeführt. Beide Organismen zeichnen sich durch ihre Unbedenklichkeit für Mensch und Umwelt sowie eine einfache Kultivierung aus. Die Versuche zeigen deutliche Unterschiede im Laugungsvermögen beider Organismen. Während *B. subtilis* alle aufgeführten Metalle aus den Schlamm extrahierte, war *S. cerevisiae* dagegen nur in der Lage Kupfer, Nickel und Zink in Lösung zu bringen. Auch erzielte die Laugung mit *B. subtilis* eine höhere Extraktionsrate als die Laugung mit *S. cerevisiae*. Durch Letztere wurden je 2% des ursprünglich im Schlamm enthaltenen Nickels und Zinks sowie 16% des Kupfers in Lösung gebracht, während die Laugungsraten für *B. subtilis* 76% Chrom, 74% Kupfer, 59% Zink, 51% Nickel und 42% Eisen betrugen.

1. Introduction

People's need of inorganic raw materials strongly increased since the beginning of industrialization. The development of new technologies and the globalization of markets in recent years led to an increasing mineral resources demand across the world. Apart from special materials for key technologies, such as tantalum (Ta) and niobium (Nb), classical raw materials like iron (Fe), copper (Cu), chromium (Cr) and zinc (Zn) are still widely important. For instance, since 1995, the world Cu-production increased by 65% (Angerer et al. 2009). In spite of rising recycling rates, extensive mining operations are necessary to accommodate this increasing demand of raw materials, which is often accompanied by serious environmental problems.

In addition to mining, subsequent processing of metal surfaces (e.g., electroplating) may also cause environmental problems. Many basic commodities, such as cars, food containers, and electrical appliances, are embellished or protected from abrasion and/or corrosion through targeted surface treatment. Heavy metals like Cu, Cr, Ni, or Zn are often used as coating materials and accumulate in the waste water of pre-treatment, coating, finishing, and purging baths from the respective process stages. Then, after a detoxification of cyanide, nitrite or Cr (VI) containing waters, the metals are precipitated as more or less insoluble hydroxides or alkaline salts (Hartinger 1991) and they sediment as electroplating sludge. Due to the contained heavy metals, the material must be categorized as hazardous waste, according to the European Waste Catalogue (Commission Decision 2000/532/EC). In Germany, every year about 80,000 t of electroplating sludge accumulate, which amount to some 1% of the overall hazardous waste (Fresner et al. 2003), while the USA generate about 1.3 million t of wet sludge annually (US EPA 1996). The disposal is expensive (about 300 €/t), because special precautions have to be taken.

Beside its environmental toxicity, hazardous sludge can also be considered as a resource and not as waste, due to its high concentration of valuable metals, especially in times of growing shortages of raw materials. However, only a small fraction of all the sludge is currently recycled, the rest is still dumped.

Today, the most common recycling methods for electroplating sludge are pyro- or hydrometallurgical treatments. The former process involves a thermal treatment of the sludge in a furnace (Fresner et al. 2003), where it is often just used as an additive to ores. The success of this method is limited by its high energy consumption, emissions, and the resulting slag. Hydrometallurgical treatment involves selective leaching of the sludge (often with acids like sulphuric acid; Sethu et al. 2008, Silva et al. 2005a) and the precipitation from solution by chemicals or by electrolysis (Silva et al. 2005b, Fresner et al. 2003). Li et al. (2010) enhanced a two-stage acid leaching of electroplating sludge via ultrasound. A substantial disadvantage of wet chemical methods is the use of various

chemicals, which cause more waste and may harm the environment. Approaches to biological treatment of hazardous waste are often limited to the investigation of *Acidithiobacillus* (e.g., Bayat & Sari 2010, Yan et al. 2008). Thus, further development of non-polluting techniques to segregate heavy metals from electroplating sludge is necessary, as well as waste reduction methods.

Bioleaching is generally regarded as an environmentally friendly method for removing metals and should also be used for metal recovery from electroplating sludge. By employing biotechnology in raw materials production, only small amounts of chemical additives and energy are needed, because microorganisms can be easily cultivated, which is advantageous in relation to traditional extraction techniques.

The aim of the present study was the remobilization of heavy metals from electroplating sludge containing Cu, Cr, Ni, Zn, and Fe in laboratory scale via baker's yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) and the hay bacillus *Bacillus subtilis* (*B. subtilis*). Additionally, the metal uptake capacity of both microbes should be compared. *S. cerevisiae* is ecologically harmless, a cheap by-product of the fermentation industry, and can be easily cultivated at large scale (Wang & Chen 2006). It is also known to adsorb and/or to accumulate metals such as Cu, cobalt (Co), cadmium (Cd; e.g., Brady & Duncan 1994, Cojocaru et al. 2009), Cr, Zn, tin (Sn), and Ni (e.g., Volesky 1990, Padmavathy 2008). The aerobic Gram-positive bacteria *B. subtilis* is also an effective metal adsorbing bacterium (e.g., Fein et al. 2001, Beveridge & Murry 1976). This study focuses on the metal extraction from the sludge and not on the reutilisation.

2. Materials and methods

2.1. Origin and characterization of the sample material

The sample material used in this study is a classical electroplating sludge from a local electroplating company, which uses Cu-, Ni-, Zn-, Sn-, and Cr-electrolytes.

At a macroscopic scale, the sludge is fine-grained, consist of moist lumps of different size, and it appears brown or greyish-green, according to the degree of dryness and subsequent oxidation (Fig. 1). Due to the small grain size, individual components are difficult to distinguish even under the microscope. However, very small metallic particles (probably the results of instrument abrasion) were detected by suspending the sludge in water.

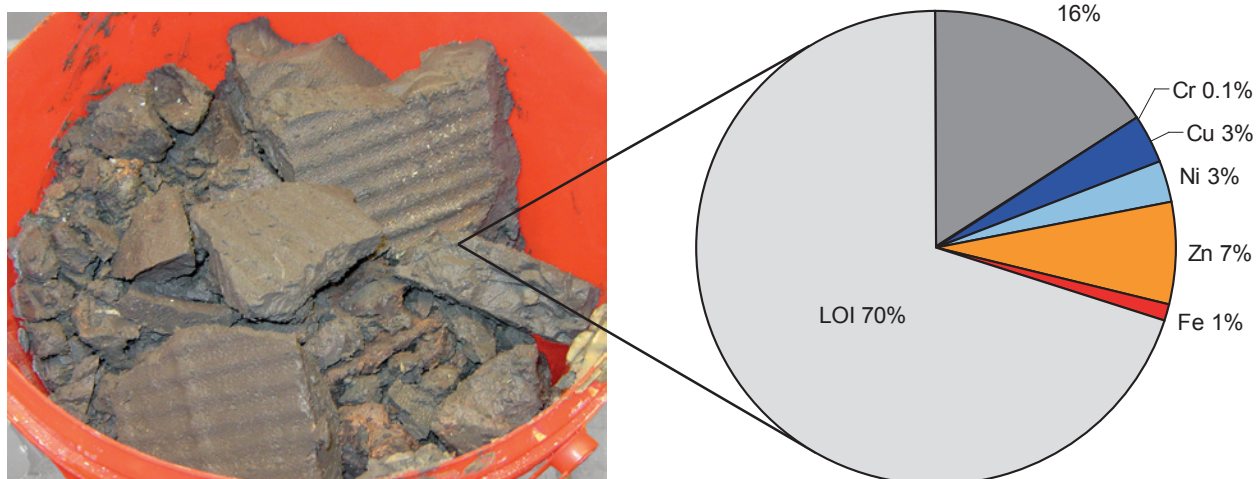


Fig. 1. Sample of moist electroplating sludge, accumulated through the treatment of wastewaters and residues from electroplating processes (left), and its chemical composition, quantified by X-ray fluorescence spectroscopy (right); LOI = loss on ignition.

Abb. 1. Probe des durch die Behandlung von Abwässern und Rückständen aus galvanisierenden Prozessen anfallenden Galvanikschlammes (links) sowie seine chemische Zusammensetzung (rechts); LOI = loss on ignition (Glühverlust).

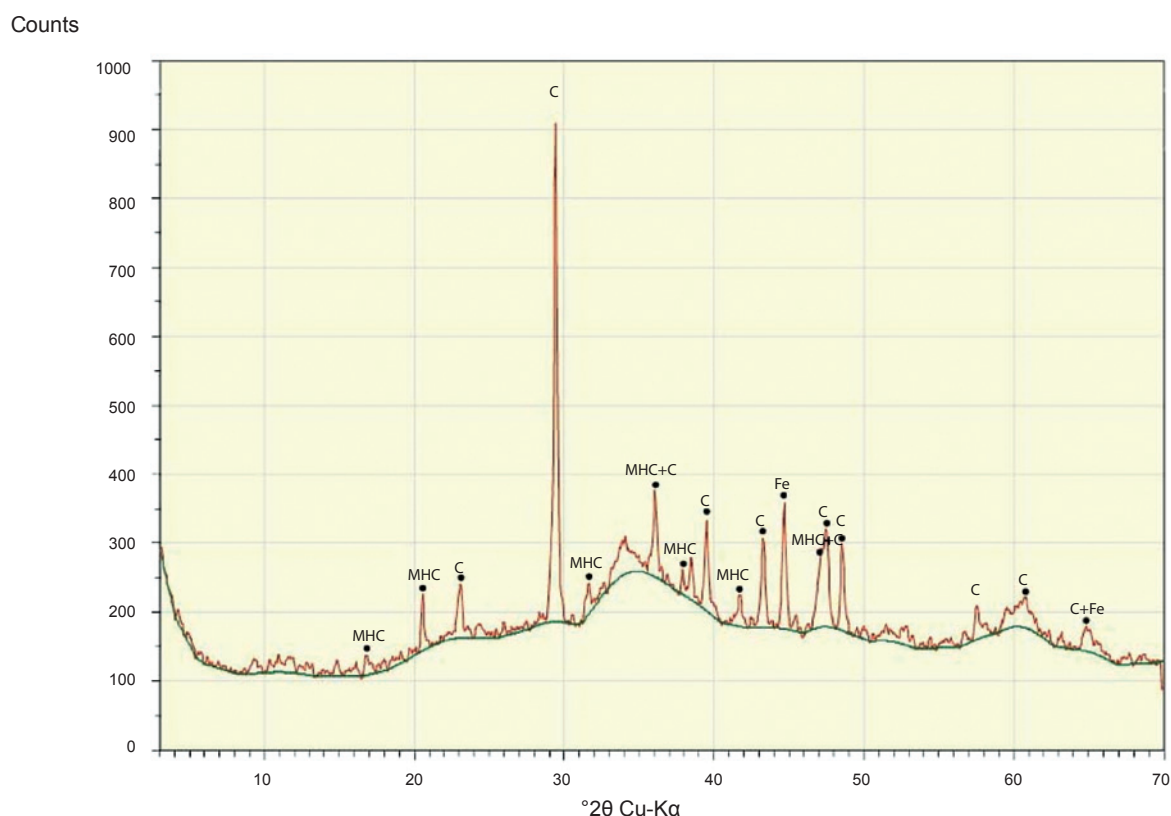


Fig. 2. X-ray diffraction diagram of the electroplating sludge; the main peaks are identified as calcite (C), monohydrocalcite (MHC), and iron (Fe).

Abb. 2. Röntgendiffraktionsdiagramm des Galvanikschlammes mit den identifizierten Komponenten Calcit (C), Monohydrocalcit (MHC) und Eisen (Fe).

The mineralogical composition was investigated by X-ray diffraction analysis (PW 1729/40, Philips). The baseline shows several broader peaks, typical for more or less X-ray amorphous material. Some well-identifiable

peaks can be attributed to calcite and monohydrocalcite (Fig. 2). Furthermore, the observed Fe-peaks are probably related to the abrasion dust of the machines. Despite an intensive data base research no other peaks could be

identified. Since all metal-hydroxides and metal-salts contained in electroplating sludge have lattice-anomalies (or just lattice-fragments; Hartinger 1991), it is difficult to obtain a meaningful X-ray diffraction analysis.

For further characterization, major and minor elements were quantified by X-ray fluorescence spectroscopy (NITON XL 3t 900S GOLDD He Mining, Thermo Scientific). The dried sludge contains 0.11 wt% Cr, 3.23 wt% Cu, 2.64 wt% Ni, 6.97 wt% Zn, and 1.19 wt% Fe. Loss on ignition (> 950°C) is 70 wt%. The complete major element analysis is shown in Fig. 1.

2.2. Growth conditions of microorganisms

The *B. subtilis* cells were cultured at 30°C in a liquid nutrient medium (Merck KGaA, Darmstadt), containing peptone from meat (5 g/l) and meat extract (3 g/l). *S. cerevisiae* was cultivated at 30°C in a growth medium composed of yeast extract (5 g/l) and glucose (20 g/l; both Merck KGaA, Darmstadt). Both organisms were cultured under microbiological standard conditions and were supplied by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig). The cell density of both main cultures was about 10^8 – 10^9 cells per millilitre.

2.3. Experiments

For pre-treatment, the electroplating sludge was ground and dried for 72 h at 70°C in an oven.

Since water is the main component of the microorganisms' culture medium, a baseline leaching with 200 ml of distilled water and 100 g of dried sludge was conducted to establish its metal extraction rate. Both media were mixed on a shaker (Schüttelmaschine LS20, Gerhardt) at room temperature. The leaching time was 9 days. Each day a sample was taken from the leaching fluid and analyzed for Cu, Cr, Ni, Zn, and Fe. The pH-value was also monitored daily.

For the experiments with the microorganisms, 200 ml of the main culture and 200 ml of the respective culture medium were added to every 20 g of dried sludge. In the following, the samples were placed on the shaker to improve the oxygen supply for the microorganisms and to allow a better mixing of both components. The leachates were sampled every 5–7 days and analyzed for Cu, Cr, Ni, Zn, and Fe. Furthermore, the respective pH-values were measured. All analyses were conducted by Analytisches Zentrum Berlin Adlershof GmbH (AZBA, Berlin) with ICP/OES according to DIN EN ISO 11885. After 40 (*S. cerevisiae*) and 48 (*B. subtilis*) days, respectively, the experiments were terminated.

3. Results

3.1. Leaching with distilled water (baseline)

Leaching with distilled water only resulted in a minor metal removal from the sludge (Fig. 3a). Concentrations of up to 0.025 g/l of Zn and 0.008 g/l of Cr were observed. The Zn concentration of the leachate varied significantly, while the amount of Cr was constant over the entire leaching period. At the beginning of the experiment, Cu was hardly mobilized from the sludge. However, after 6 days, Cu concentrations increased and the values reached those of Cr (maximum 0.008 g/l); Ni (0.002 g/l) and Fe remained mostly unaffected.

3.2. Leaching with both microbial species

Immediately after the addition of microorganisms to the sludge, an interaction was noticed causing a colour change. The *B. subtilis* culture first became dark green and changed into a deep blue after 1–2 hours. Leaching with *S. cerevisiae* brought a dark blue-green colour.

In these experiments, *S. cerevisiae* hardly mobilized any Cr and Fe (Fig. 3b), while the amount of dissolved Cu increased to 0.26 g/l within 5 days. Afterwards, the Cu-values varied between 0.13 g/l and 0.26 g/l until the end of experimentation. The maximum removal of Ni (0.024 g/l) from the sludge is reached at around 5 days, then the Ni-concentration decreased to 0.005 g/l. The Zn-concentrations increased to 0.075 g/l within 12 days and subsequently decreased to 0.005–0.04 g/l until the end of the experimentation.

The *B. subtilis* culture showed a continuous uptake of all main metals until day 19 (Fig. 3c). In the following, the concentrations of all metals decreased significantly. For Cr, the maximum enrichment in solution was about 0.042 g/l; successive re-mineralization reduced this value to less than 0.002 g/l. Similar observations were made for Cu (maximum 1.2 g/l, minimum 0.16 g/l), Ni (maximum 0.67 g/l, minimum 0.006 g/l), Zn (maximum 2.07 g/l, minimum 0.005 g/l), and Fe (maximum 0.25 g/l, minimum < 0.002 g/l).

The efficiency of the respective leaching agent (percentage of overall remobilized material) is shown in Fig. 4. As illustrated, *B. subtilis* is much more able to leach the investigated elements than *S. cerevisiae*. The yeast basically fails to remove any of the metals to any significant amount (with the exception of Cu), while *B. subtilis* extracted large quantities of Ni (51%), Cu and Cr (~ 75%), Fe (~ 42%), and Zn (~ 59%).

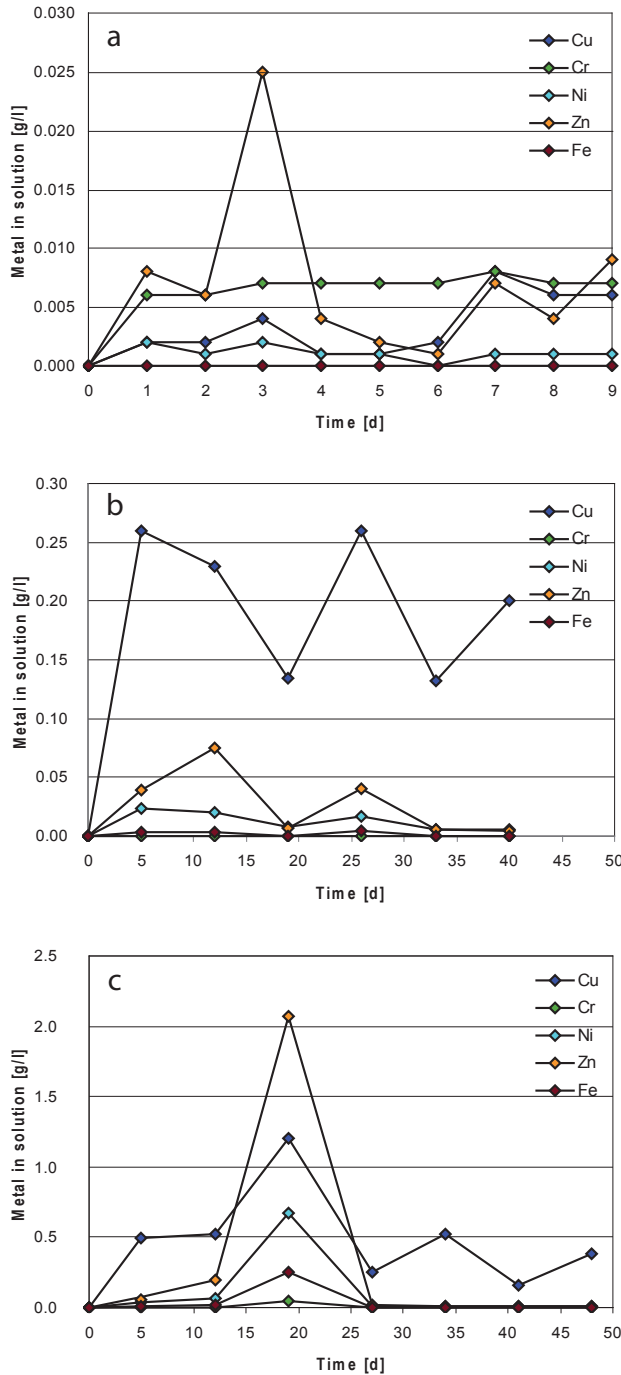


Fig. 3. Leaching rates of Cu, Cr, Ni, Zn, and Fe depending on leaching time with **a**, distilled water, **b**, *S. cerevisiae*, and **c**, *B. subtilis* as leaching agents.

Abb. 3. Laugungszeitreihe der Wertkomponenten Cu, Cr, Ni, Zn und Fe mit **a**, destilliertem Wasser, **b**, *B. subtilis* und **c**, *S. cerevisiae* als Laugungsmedien.

3.3. pH-value

With the exception of an initial rise, the leaching fluid of the *B. subtilis* culture showed nearly constant pH-values over the whole period of 48 days (Fig. 5). The contact

of this leaching fluid with the sludge caused an increase of the pH-value from 7 to final values between 8.17 and 8.54. The trend of the pH-curve shows a similar development using *S. cerevisiae* as leaching agent, although the pH-values are generally slightly lower (7.78–8.23; the starting pH was 6.6) than using *B. subtilis*. A minor increase in pH-values was observed towards the end of the leaching period.

Afterwards, the extraction rates of each metal were compared with the corresponding pH-values. *S. cerevisiae* shows no significant dependency of metal uptake and pH-values for Cu, Ni, and Zn (Fig. 6). As Fe and Cr were hardly mobilized, no plots are shown for neither of them. *B. subtilis* shows increasing Cu-, Ni-, and Zn-contents in solution with increasing pH-values. Fe in solution increases up to nearly constant pH-values of ~ 8.5 (Fig. 6).

4. Discussion

4.1. Leaching time

The colour change at the beginning of both microbiological leaching experiments indicated a rapid dissolution of metals within a few hours. The increase of metal uptake within the first 5 days may be due to metal adsorption on functional groups, which are situated on the microorganisms' surfaces such as phosphate-, carboxyl-, hydroxyl-, and amino-groups (Vecchio et al. 1998, Urrutia & Beveridge 1993) and interact with the metal cations. This passive biosorption proceeds fast (Wang & Chen 2006). The relatively constant progression of the yeast-leaching gives evidence for firm metal binding over 40 days, no remarkable desorption was observed. The metal uptake via *B. subtilis* also increased rapidly, but this increase continued up to day 19. A slow sorption of metals could be an indicator for an active biosorption. Thereby, metal ions penetrating the cell membrane enter into the cells (Wang & Chen 2006) and larger quantities of metal ions can accumulate (Brady & Duncan 1994). An argument against active biosorption would be the decrease of metals in the leaching fluid after 19 days. This suggests metals' desorption and re-accumulation in the sludge, what occurs at passive biosorption, when metal ions bound on the cells' surfaces are eluted by other ions or acids (Wang & Chen 2006).

4.2. pH-value

In contact with the sludge, the pH-values of both species, the yeast and the bacteria, increased due to carbonates

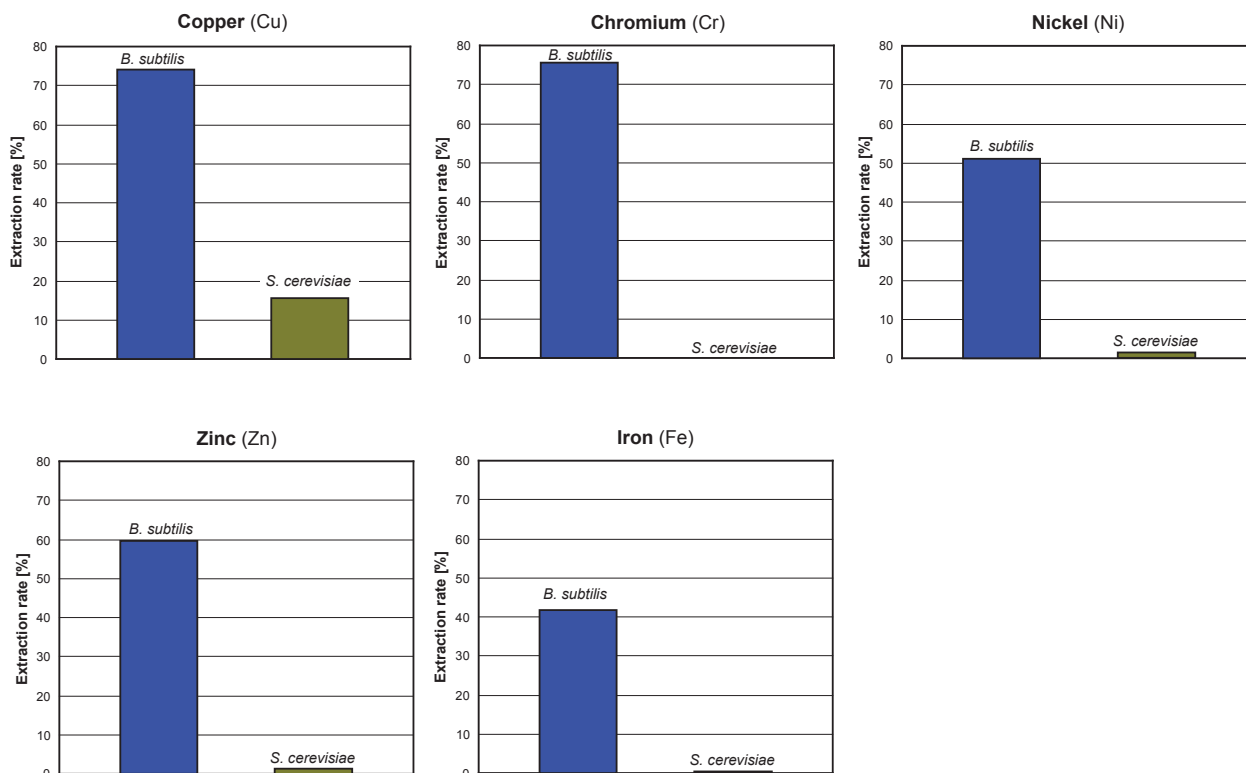


Fig. 4. Comparison of the maximum metal-extraction rates achieved with *B. subtilis* and *S. cerevisiae* as leaching agents for the metals Cu, Cr, Ni, Zn, and Fe, regardless of leaching-time.

Abb. 4. Vergleich der maximalen Metallextraktionsraten, die durch *B. subtilis* und *S. cerevisiae* unabhängig von der Laugungsdauer erreicht wurden.

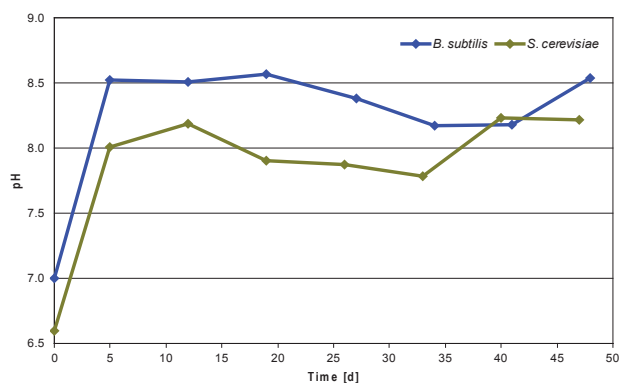


Fig. 5. Development of pH-values depending on the leaching-time for leaching experiments with *B. subtilis* and *S. cerevisiae*.

Abb. 5. Verlauf des pH-Wertes während der Laugung mit *B. subtilis* und *S. cerevisiae* in Abhängigkeit von der Zeit.

contained in the sludge. In the existing pH-intervals, a dependency of leaching rate and pH-value was noticed only for leaching of Cu, Zn, and Ni via *B. subtilis*. Larger leaching rates were reached at increasing pH-values. In case of *S. cerevisiae*, the leaching rates vary independently of pH-value. The pH-values of both experiments range in small intervals (between 8.17 and 8.57, 7.78 and 8.23, respectively), so it is generally difficult to recognize a dependency.

The pH-value is one of the most important factors for biosorption of heavy metals. The availability of functional groups for heavy metal cations is, amongst others, influenced by the pH-value. With increasing pH-value, the respective groups deprotonated, become negatively charged and may bind metal cations (Naja et al., 2010). For Cu-, Cr-, and Zn-adsorption via *S. cerevisiae*, Mapolelo & Torto (2004) reported optimal pH-values of > 5. All pH-values of the leaching fluids within this study are > 7.7 and thus should have influenced the metal uptake capacity positively.

5. Conclusion

B. subtilis is capable of extracting Cu, Cr, Zn, Ni, and Fe from hazardous electroplating sludge. *S. cerevisiae* was only able to extract Cu, Ni, Zn, and some Fe; Cr was not soluble.

Compared to *S. cerevisiae*, *B. subtilis* is a significantly better extraction medium for all investigated metals. Up to 76.36% of primary contained Cr was dissolved from the sludge via *B. subtilis*, as well as 74.30% of Cu, 50.76% of Ni, 59.40% of Zn, and 42.02% of Fe. The

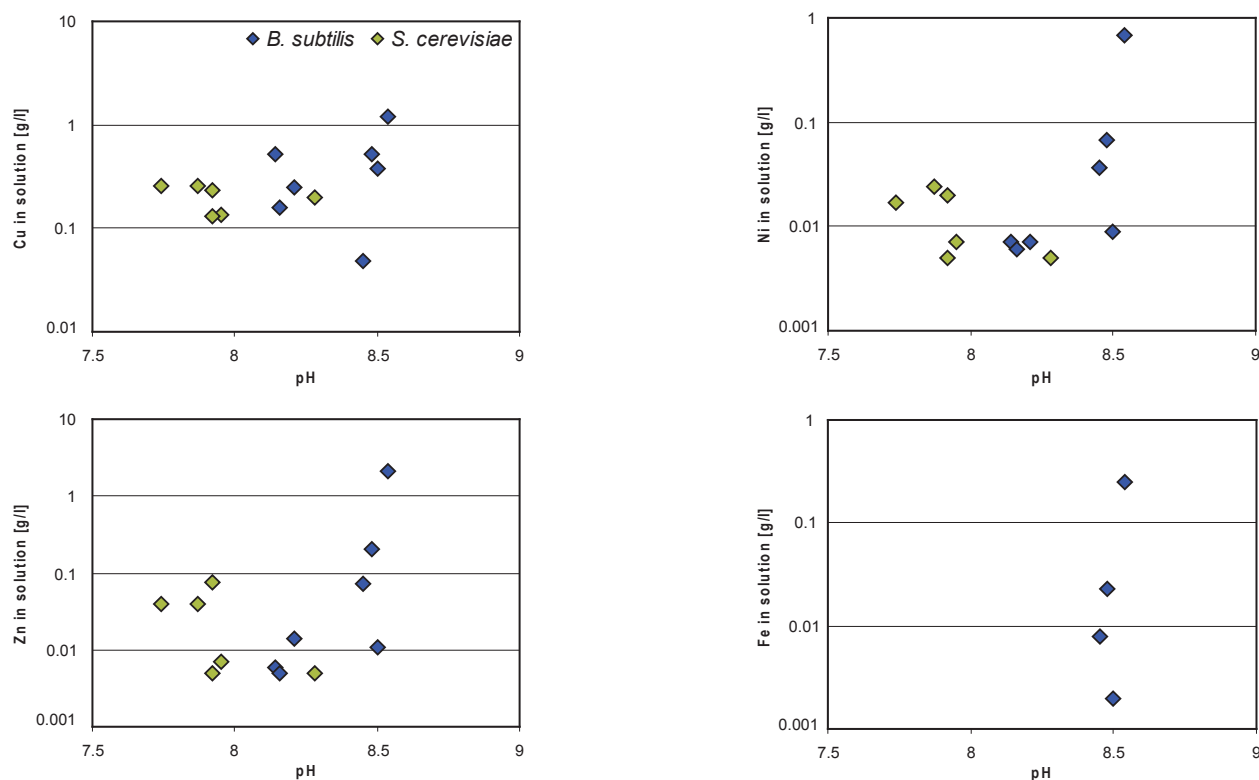


Fig. 6. Comparison of metal-extraction rates with the corresponding pH-value for leaching experiments with *B. subtilis* and *S. cerevisiae* for Cu, Ni, Zn, and Fe.

Abb. 6. Vergleich der Metallextraktionsraten mit den entsprechenden pH-Werten der Laugungsexperimente mit *B. subtilis* und *S. cerevisiae* für die Metalle Cu, Ni, Zn und Fe.

S. cerevisiae culture extracted only 1.8% of Ni, 2.15% of Zn, 16.10% of Cu, < 1% Fe, and no Cr.

Leaching electroplating sludge via *B. subtilis* is an environmentally friendly technique for metal extraction and much more effective than leaching via *S. cerevisiae*. Compared with other extraction methods, like wet-chemical treatments, lesser extraction rates were achieved in this study. For example, Li et al. (2010) achieved extraction rates of 97.42% for Cu, 98.46% for Ni, 98.63% for Zn, 98.32% for Cr, and 100.00% for Fe from electroplating sludge via ultrasonically enhanced two-stage acid leaching. Sethu et al. (2008) obtained maximum 95% of Cu from electroplating sludge using sulphuric acid as dissolution reagent; Silva et al. (2005a) extracted maximum 88.6% of Cu, 98.0% of Ni and 99.2% of Zn, also via sulphuric acid leaching.

Even though these techniques provide higher extraction rates, microbiological methods, as presented in this study, ensure a much simpler and more ecological treatment of hazardous waste, e.g., due to absence of concentrated sulphuric acid and high reaction temperatures.

Changing a few of the experiments' parameters may even heighten the extraction rates of bioleaching electroplating sludge. As microorganisms are only able to adsorb a certain amount of metals, an increased number of leaching runs could boost the extraction rates. Leaching the sludge within a "fresh" state, before it solidifies with advancing age and metal extraction becomes diffi-

cult due to a smaller contact surface, would also raise the effectivity of bioleaching. In times of increasing raw material prices, microbiological metal production from hazardous waste must be regarded as a profitable alternative to common extraction techniques.

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