- Mining wastewaters -

Removal of aluminium from mining water using sulfate-reducing bacteria

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Abstract

The mechanism of biosorption of aluminium by 5 strains of sulfate-reducing bacteria isolated from uranium mine tailings was examined. One strain, designated UFZ B 406 not of mining site origin was also used. The mechanism of aluminium biosorption was found to be a passive one. Freezing and thawing of the cells resulted in higher biosorption of aluminium, whereas heat treatment or the uncoupler carbonyl-cyanide-m-chlorophenylhydrazone (CCCP) showed no effect. The pH-value had significant influence on aluminium ion adsorption, the highest was found at pH 3 and 5, the lowest at pH 7. Aluminium biosorption decreased after the addition of magnesium or the presence of iron sulfide precipitates. The suitability of the isolates for the removal of aluminium from waste water is discussed.

1. Introduction

Acid mine drainage from abandoned uranium mines and dumps poses a significant threat to the environment. As result of bacterial leaching these drainage waters are very acidic with pH values between 1 and 2 and contain high concentrations of metals, particularly iron, uranium, aluminium and magnesium [1]. A microbial decontamination process to increase the pH and to immobilize metals as insoluble sulfides utilizes the ability of sulfate-reducing bacteria to reduce sulfate ions to sulfide ions [2, 3].

Aluminium, known to have toxic effects [4] cannot be removed from the water by precipitation since the sulfides are soluble in water; they have to be immobilized by other methods. As the pH increases through the reduction of sulfate by sulfate-reducing bacteria a large amount of aluminium precipitates as the hydroxide. Depending on the concentration of inorganic (e.g. F^-) or organic (humic acids) complexing agents present high concentrations of aluminium ions (as Al^{3+}) remain in solution. Reports on aluminium accumulation have so far been restricted to *Bacillus* species [5, 6].

In this work we investigated the ability of sulfate-reducing bacteria to remove aluminium from waste water and the mechanism behind it.

2. Materials and methods

2.1. Organism isolation and cultivation

Water samples were taken from sampling wells on a waste dump of a uranium processing plant in Dresden-Gittersee (Germany). For the isolation of sulfate-reducing bacteria a modification of Postgate's medium was used [3]. For biosorption experiments on plates 10 mM aluminium sulfate was added to the medium. Iron sulfate was omitted to exclude any interaction or competition for binding sites between aluminium and iron. The plates were inoculated with 1 ml of cell suspension and incubated for 7 to 10 days at 30°C in anaerobic jars. UFZ B 406 was isolated from the mud of a waste water pond of a disused sugar factory as described previously [2].

2.2. Protein measurements

Protein was determined according to the modified method of Bradford [3].

2.3. Qualitative aluminium biosorption experiments

For a fast qualitative assessment of the ability of the isolates to accumulate aluminium, a modification of the method of Pümpel et al. [7] was used. After the colonies had grown to a size of approximately 1 to 2 mm in diameter the plates were overlaid with a layer of molten agar. After 12 to 24 hours the plates were covered with 3 ml of a 0.1 % (w/v) alizarin red S solution (sodium alizarin sulfonate; Sigma, Germany). 1 % acetic acid (v/v) was added until the original orange red color had disappeared. After a few minutes a bright red aluminium-alizarin complex formed and isolates which have the ability to adsorb aluminium were at this stage seen as purple colonies. After 24 hours light circles were recognizable around the colonies. More alzarin s solution was added to check for possible degradation of the dye by bacteria. As the light circles around the colonies remained unchanged the aluminium had to have been accumulated by the bacterial cells.

2.4. Quantitative aluminium biosorption assay

The aluminium sulfate solution used in all experiments had a concentration of 10 mM and a pH value of 7 unless stated otherwise.

For the direct and indirect assays 1 ml cell suspension samples were incubated for 2.5 hours with 30 ml or 10 ml of 10 mM $Al_2(SO_4)_3$. After incubation cells were harvested by centrifugation at 10 000 *g* for 10 minutes and washed with distilled H_2O. The supernatant and the control were used for indirect biosorption measurements by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

For direct biosorption assays the pellet was resuspended in 10 M nitric acid (1 ml) and the volume made up to 10 ml. The samples were centrifuged at 10 000 *g* for 10 minutes. Aluminium concentrations in the supernatants were measured on the same day using ICP-AES. All determinations were carried out in triplicate.

2.5. Staining procedures and microscopy

The Gram-stain was carried out using a commercial Gram-staining kit by Merck (Germany). For the volutin staining procedures Neisser-dyes were applied: Solution A: 0.1 g methylene blue, 5 ml glacial acetic acid (100 %), 5 ml ethanol (96 %, v/v), 100 ml dH_2O. Solution B: 10 ml crystal violet (3.3 %, v/v in 96 % ethanol, v/v). Solution C: 0.33 g chrysoidine Y, 100 ml distilled H_2O. The cells were dried on a fatfree microscope slide, heat fixed and covered with a freshly prepared mixture of solution A and B (2:1). After 15 minutes the dye was removed by tilting the slide for the excess dye to run off. Solution C was applied for counterstaining for 45 seconds and then washed off with water. The preparation was air dried and examined under a light microscope (Axioskop, Zeiss, Germany).

3. Results and discussion

3.1. Isolation of strains for biosorption of aluminium

Agar plates were inoculated with sample material and after 10 days of incubation a number of colonies had formed on all plates. Some of the colonies were black indicating the reduction of sulfate to sulfide. From these plates single black colonies were taken, transferred to new plates and incubated with 10 mM aluminium sulfate.

Five isolates were selected for further investigation: Iso 2 was chosen because of its fast growth compared to the other isolates and its ability to eliminate aluminium from the medium. Al-Tol 4 was isolated directly from plates with aluminium and tested positive, however it was found to grow comparatively slowly. Iso 16 as well as Iso 21 were tolerant to aluminium, yet both isolates were unable to accumulate aluminium. Iso 21 formed colonies which were visible after only 3 days. Even though Iso D was not tolerant to aluminium, it showed the highest level of aluminium sorption. It grew very slowly on the original isolation medium described above.

3.2. Measurement of aluminium biosorption

The amount of aluminium which is taken out of the medium by bacterial cells can be determined using two different approaches. The direct method whereby the aluminium which has been fixed by the cells is brought back into liquid phase using a suitable eluent. This however will in most cases destroy the cells. The indirect method measures the concentration of aluminium before and after incubation of the cells and determines fixation through the difference. This method is the one that has been most commonly used in the past for experiments carried out with other metals [8]. There have been no reports so far on a suitable eluent for detaching aluminium from bacterial cell walls or washing it out of cells. EDTA cannot be used as an eluent as it binds aluminium which could influence the determination of aluminium with ICP-AES.

In Figure 1 fixation of aluminium ions by the different isolates is shown. Nitric acid at a concentration of 10 M was found to be useful for the purpose of getting the aluminium off the cells and data of the aluminium measurements were compared to data from the indirect method. The values obtained using the indirect method are slightly higher. This could be due to aluminium adsorption to the walls of the glass, dilution or other experimental errors. For these reasons all further measurements in this study were carried out using the direct method.

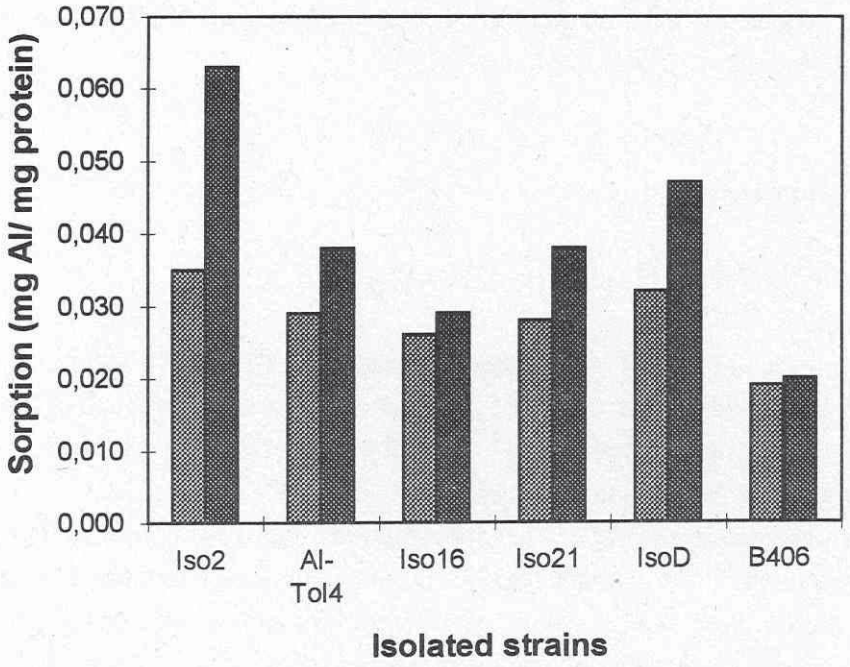

Fig. 1. Comparison of the direct and indirect method for quantifying aluminium biosorption. Data represents mean of 5 batch cultures of each isolate in this and all subsequent experiments unless stated otherwise. ▒ direct method, ▓ indirect method

3.3. Influence of iron sulfide on biosorption of aluminium

The enrichment medium for the selection of sulfate-reducing bacteria contained iron sulfate. During growth of the bacteria it was reduced to iron sulfide which precipitated as black flakes. Figure 2 shows a comparison of elimination of aluminium from the medium by cells grown in the presence and absence of iron sulfide. While there is little difference in the fixation of aluminium by strain Iso 2, more aluminium is fixed by cells of strain UFZ 406 in the absence of iron sulfide. Therefore all subsequent experiments were carried out with cells grown without iron.

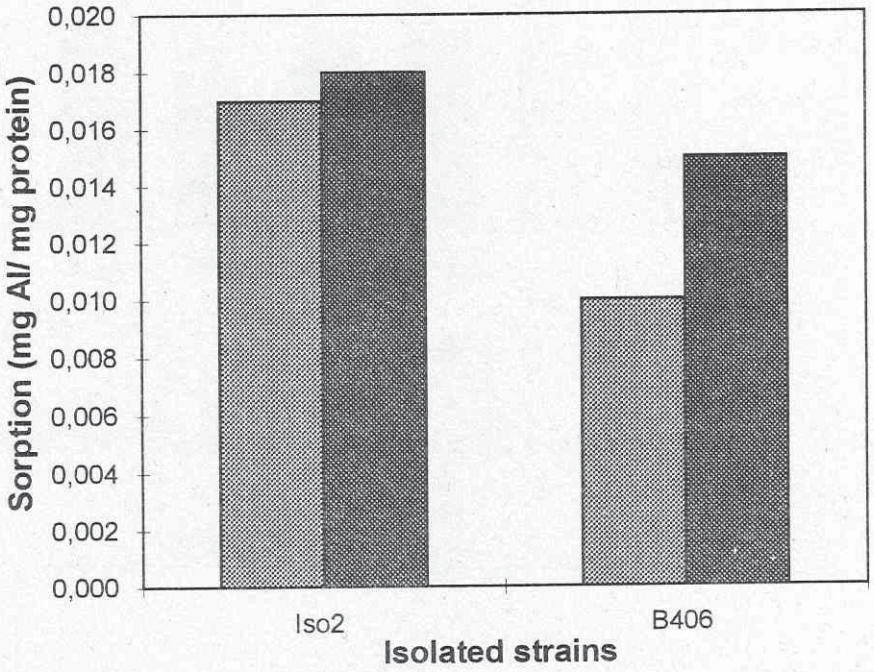

Fig. 2. Influence of iron sulfide on the accumulation of aluminium by strains Iso 2 and UFZ B 406. ▒ medium with iron sulfide, ▓ medium without iron sulfide

3.4. Mechanism of elimination of aluminium

In order to investigate whether the process of elimination is uptake and subsequent accumulation inside the cell or adsorption and accumulation outside the cell, 20 mM lactate as energy source was added to the samples (Figure 3). The values for samples with added lactate were slightly higher than controls. Only Iso 16 showed a greater difference, 0.007 mg aluminium/mg protein.

Incubation of the cells of the strains Iso 2 and UFZ B 406 with the uncoupler carbonyl-cyanide-m-chlorophenylhydrazone, CCCP (50 µM) or heat treatment (98°C, 30 minutes) showed little or no effect compared to the controls (Figure 4). There was no difference in aluminium biosorption between the treated and untreated samples of Iso 2 and little difference (0.002 mg aluminium/mg protein) between control samples of UFZ B 406 and treated ones.

The relationship between biomass concentration and total aluminium fixed was linear, higher protein concentrations resulted in higher biosorption values (data not shown).

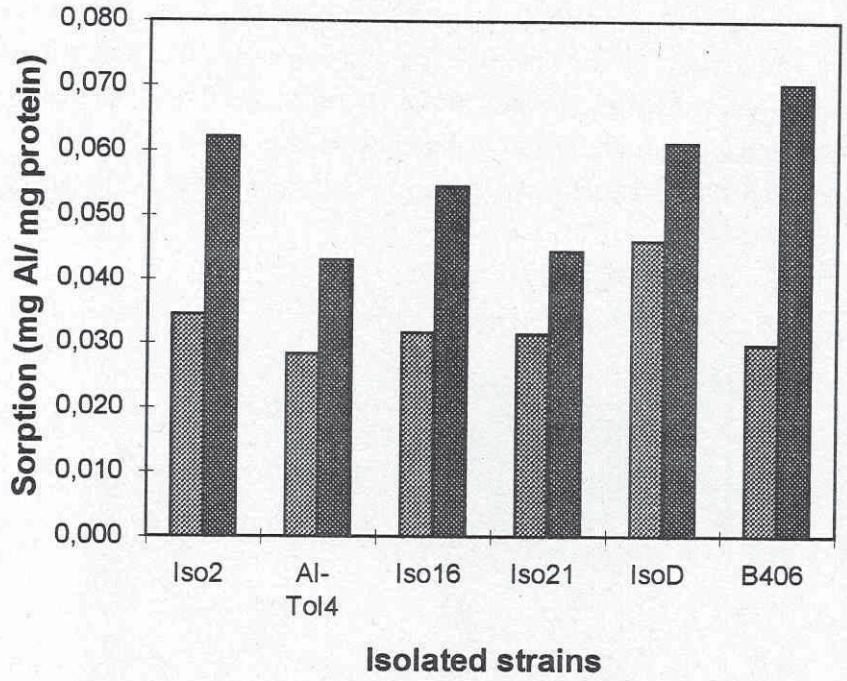

Fig. 3. Aluminium ion fixation with and without additional lactate. ▒ with 20 mM lactate, ■ control

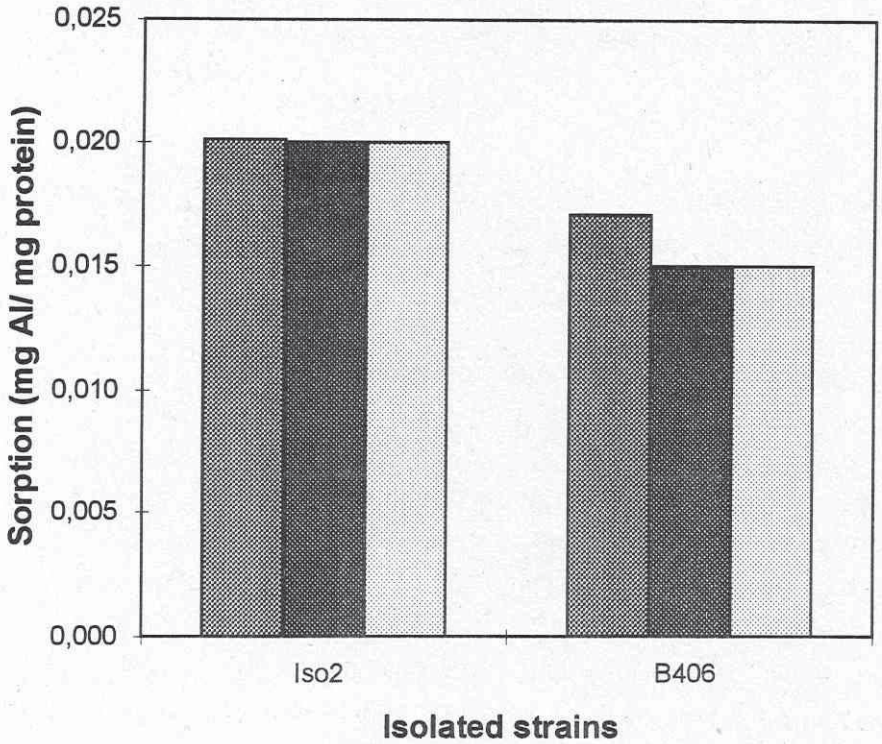

Fig. 4. Influences of CCCP or heat treatment on aluminium ion sorption by strains Iso 2 and UFZ B 406. ▒ 50 mM CCCP, ▒ heat treatment, ■ control

3.5. Influence of freezing and thawing of cells on the removal of aluminium from the medium

Cells of 5 isolates and strain UFZ B 406 were frozen and the effect of freezing and thawing on fixation of aluminium was investigated (Figure 5). More aluminium was bound to treated cells than to untreated cells. There was no correlation between the accumulation of aluminium to living cells or to frozen cells. Strain UFZ B 406 which removes the least in the control experiments showed the highest values with frozen and thawed cells.

Frozen and thawed cells of both strains used accumulated more aluminium than untreated ones. This is possibly due to a breaking of the cells and subsequent availability of additional binding sites. It indicates that process of fixation of aluminium by sulfate-reducing bacteria is a passive one.

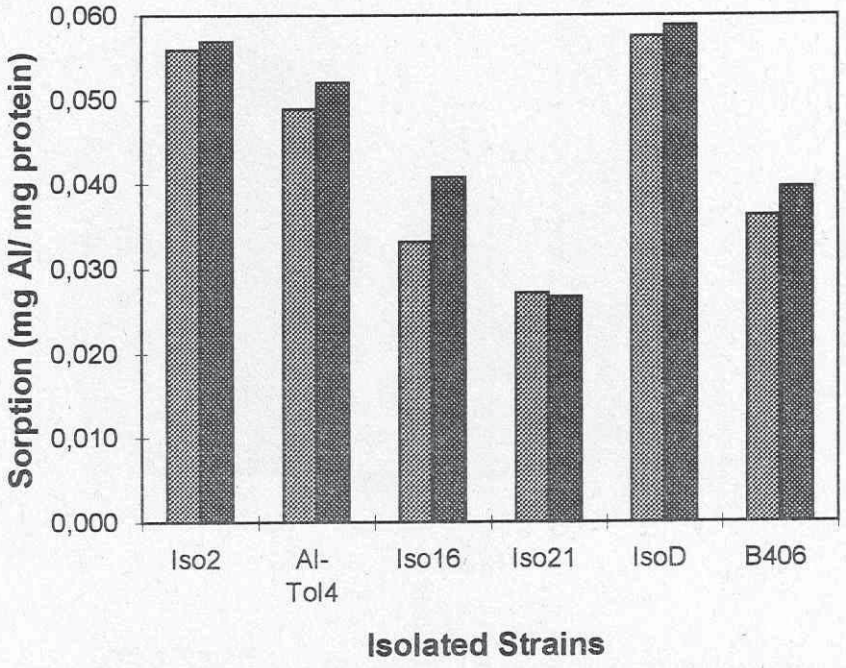

Fig. 5. Effect of freezing and thawing on aluminium accumulation by different isolates ▒ cells frozen and thawn, ▓ control

3.6. Influence of pH on biosorption

The influence of the pH on biosorption of aluminium is shown in Figure 6. Cells of isolates Iso 2 and UFZ B 406 were incubated in 10 mM aluminium sulfate solution at pH 3, pH 5, pH 7 and pH 9. The highest accumulation of aluminium by the cells were found at pH values of 3 and 5, the least at pH 7.

This quite significant influence of the pH on the metal-cell interaction is in agreement with studies by Hughes and Poole [9]. At first sight it seems contradictory that biosorption is lowest

at neutral pH where the cellular complex formers as well as the metal ions are dissociated and highest at lower pH values where the complex forming (acid) groups are increasingly protonated. Lower biosorption at pH 7 could be due to the presence of tartaric acid as soluble complexing agent in addition to the cellular ligands. At neutral pH much of the aluminium is in a stable aluminium-tartrate complex and not free for adsorption to cellular structures. Lowering the pH results in more free Al^{3+}-ions which are now available for binding, and tartaric acid in protonated form does no longer act as complex former. At alkaline pH aluminium forms very stable $Al(OH)_4^-$ complexes which, being anions do not react with anionic cellular ligands.

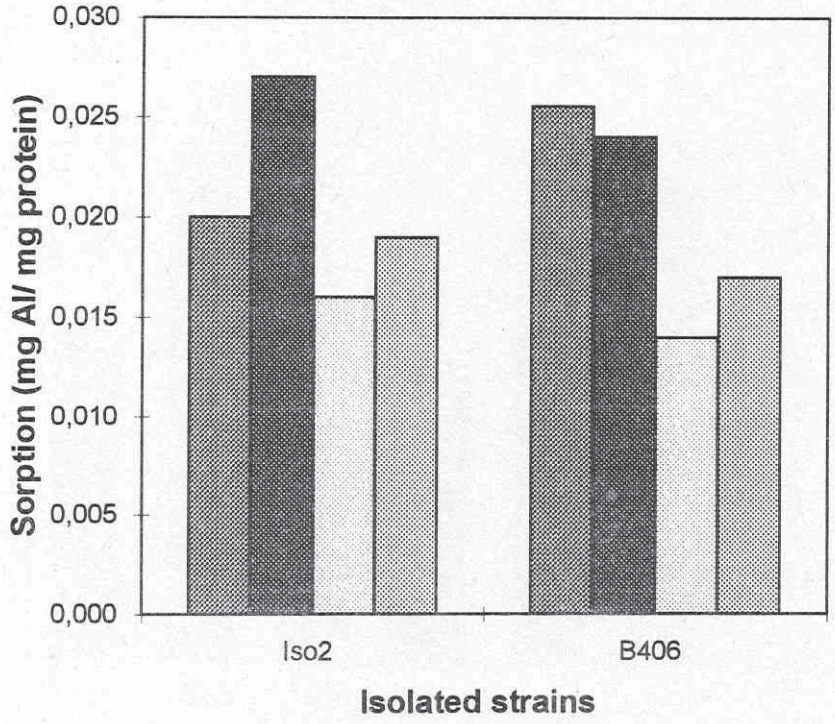

Fig. 6. Effect of pH on aluminium fixation by strains Iso 2 and UFZ B 406.
pH 3, pH 5, □ pH 7, pH 9

3.7. Influence of Mg^{2+}-ions on biosorption

Most waste waters are contaminated with more than one metal species. Competition for binding sites between metals is therefore an important issue in biosorption processes. Acid mine water from uranium mines in eastern Germany contains high concentrations of aluminium (around 90 mM) as well as high concentrations of magnesium (around 130 mM). The influence of different concentrations of magnesium ions on the biosorption of aluminium by strain Iso 2 was investigated. Figure 7 shows the highest accumulation at 10 mM and 50 mM magnesium ions, higher magnesium concentrations in the medium result in lower biosorption of aluminium. The presence of magnesium ions had negative influence on the accumulation of aluminium

even though magnesium and aluminium ions differ in charge and size. However, because both have similar chemical properties, they are hard ions and not readily polarized, there seems to be competition for binding sites at the cell surface. With the aim of removing aluminium from waster water it means that the presence of high concentrations of magnesium has strong influence on the magnitude of biosorption and has to be taken into consideration.

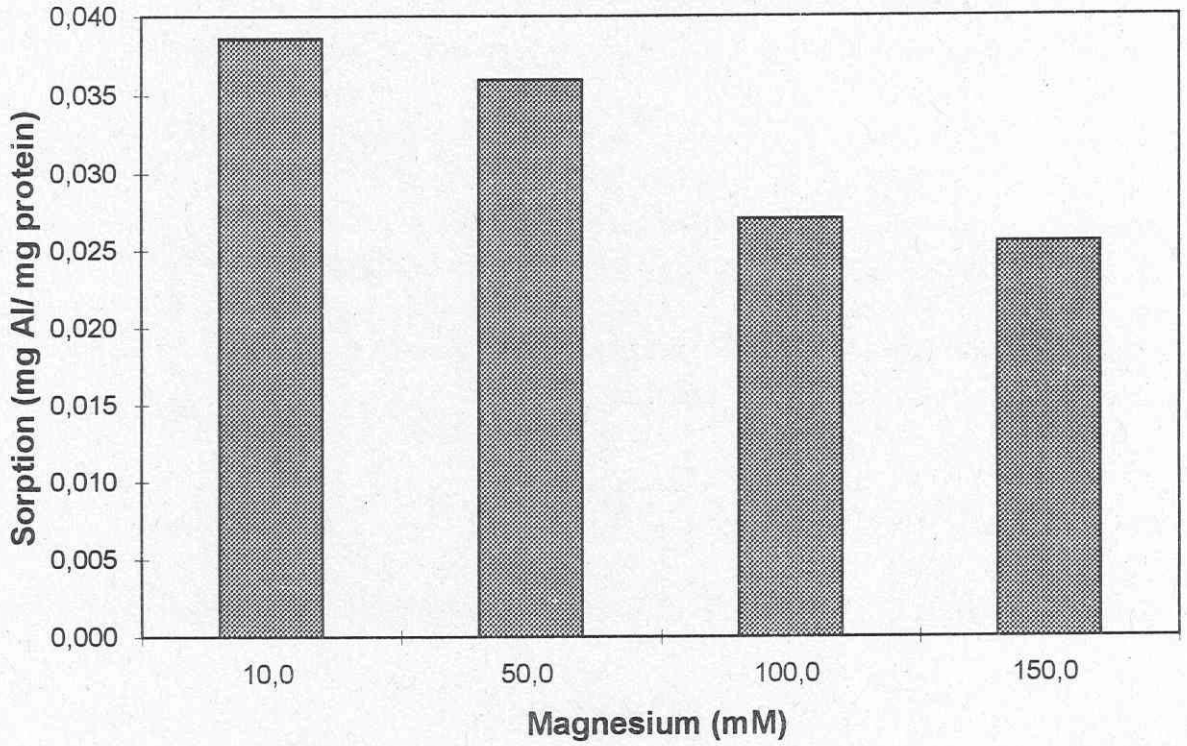

Fig. 7. Influence of different magnesium concentrations in the medium on aluminium ion biosorption by strain Iso 2.

3.8. Staining

Isolate Iso 2 stained Gram-positive. A number of cells with spores in the center were observed. UFZ B 406 stained Gram-negative and spores were not found.

Peptidoglycan is the major site of metal complexation, in particular the carboxylgroups of the glutamate rests [10]. As Iso 2 is a Gram-positive bacterium it is highly probable that the peptidoglycan layer is the main site of biosorption. Peptidoglycan is structurally quite stable and not susceptible to moderate heat treatment. Because of the murein layer which makes up to 50 % of the cell wall dry mass Gram-positive bacteria should passively bind more metal ions than Gram-negative bacteria. This confirms the findings in this study where Iso 2, a Gram-positive bacterium adsorbs more aluminium ions than UFZ B 406 which is Gram-negative.

Volutin could have been another possible site for aluminium accumulation. However, the results of the volutin staining procedures were negative, no polyphosphate granules were detected.

4. Conclusions

Bioremediation of acidic waste water with high concentrations of metals using sulfate-reducing bacteria is a viable option [2, 3, 11]. In the past biosorption of aluminium has not yet been examined.

The results of this study suggest that the process of aluminium biosorption by sulfate-reducing bacteria is a passive one and occurs on the surface of the cells. It is independent of the physiological status of the cells, they can be alive or dead, intact or broken.

Whether elimination of aluminium from waste water by biosorption is a realistic approach remains to be seen. However, using sulfate-reducing bacteria would solve three problems simultaneously: the acidic waters are neutralized, metals are precipitated as sulfides or hydroxides and aluminium is fixed and collected.

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