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(54) Title: A PROCESS FOR THE REMOVAL OF HEAVY METALS BY ACTINOMYCETE

(57) Abstract: The present invention provides a process for the uptake of heavy metals from an aqueous solution an alkalophilic or alkalotolerant actinomycete PTA 3422 by contacting with the aqueous solution.

## A PROCESS FOR THE REMOVAL OF HEAVY METALS BY ACTINOMYCETE

### Field of the invention

The present invention relates to a process for the removal of heavy metal ions from aqueous solutions using metal tolerant alkalophilic actinomycete.

### Background of the invention

Rapid and large scale industrialization has led to generation of large quantities of waste water. Waste water from many industrial processes such as metal finishing, electroplating, paint and the like contain several heavy metal ions which are toxic in nature and harmful to the environment while a few of such metal ions such as gold or silver or platinum and the like are valuable. It is therefore extremely important to recover metal ions from wastes in order to control pollution and recover valuable metals. The following are major sources of heavy metal pollution: (a) mining and processing of minerals; (b) nuclear and other industrial effluents; (c) brewing and distillery wastes; (d) biocides and preservatives including organo metallic compounds; (e) leather industry; (f) metal refining and metal cleaning; (g) electroplating, metal processing and dyeing industry, and the like.

Heavy metals which commonly occur as pollutants in effluents are Cu, Zn, Cd, Fe, Hg, Pb, Ni, Ag, Au and radionuclides such as U, Th and the like. According to the US Environmental Protection Agency, the acceptable value of heavy metal ion is usually less than 1 mg/L (1ppm). Although the total amount of metals released by such activities is large, concentrations are typically low rendering most prior art recovery techniques impractical. The existing processes for removal of heavy metals include solvent extraction, hydroxide precipitation (lime addition), evaporative concentration, electrowinning, membrane concentration, ion exchange processes, adsorption, reverse osmosis and biological methods. However, all the above methods known in the art suffer from several disadvantages. The prior art methods mentioned above require high energy, have limited applicability, low absorption capacity, etc. As an example, chemical precipitation produces wet bulky sludge and requires a filter for the polishing stage. Ion exchange adsorption, reverse osmosis and solvent extraction are not only relatively expensive and involve elaborate and costly equipment but also the operation costs are very energy intensive.

Several attempts have been made to use microbial biomass for metal removal (Volesky and Holan, 1995; Rani Gupta et al., 2000). Living and non-living cells have been used to concentrate metal ions from aqueous solutions which is a rapid and reversible phenomenon. US Patent 4320093 (1982) discloses a technique for the removal of U and Th from solutions using a fungal microorganism *Rhizopus arrhizus*. Koglev et al report the use

of bioabsorbents prepared from *Aspergillus*, *Penicillium*, *Trichoderma* or *Micrococcus* biomass (US Patent 5789204, 1982).

Dick and Macasakie report the use of *Acinetobacter* for removal of metals by the precipitation of the metal as insoluble metal phosphates due to the phosphate produced by the organism by enzymatic reaction (US Patent 5520811, 1996). Brierley et al describe the preparation of biomass product by alkaline treatment to increase the metal uptake by several folds. Among the various bacteria and fungal cultures tested *B. subtilis* was found to be best (US Patent 4690894, 1987). Volesky describes the immobilized biomass of a marine alga for the removal of gold (US Patent 4769223, 1988).

Amoroso et al report the isolation of 25 new strains of *Streptomyces* from polluted sites which are disclosed as being capable of growth in the presence of 5.9 to 59 ppm of Ni [Amoroso, M. J., Schubert, D., Mitscherlich, P., Schumann, P., Kothe, E, (2000) Evidence for high affinity nickel transporter genes in heavy metal resistant *Streptomyces*, *J. Basic Microbiol*, **40**, 295 – 301]. However, this reference is silent on the use of these organisms for the removal of heavy metals including Ni from aqueous solutions. Similarly, Bose and Paul report the screening of 32 isolates of actinomycetes for tolerance to Cr+6 in concentrations ranging from 200 – 1000 ppm in agar medium [Bose, M., and Paul, A. K., (1999), Chromium resistant actinomycetes: their tolerance to other metals and antibiotics, *Acta. Microbiol. Immunol. Hung.*, **46**, 25 – 32]. However, this reference is silent on the use of any of the disclosed organisms for the removal of metals from aqueous solutions.

Biosorption of metals is due to the binding of metal ions to various cell constituents, primarily the cell wall. As organisms vary widely in cell wall composition, their capacity to bind metals also varies. Thus different organisms bind metals to different extents.

While bacteria, fungi, yeast and algae have been studied for heavy metal removal, there are very few reports on the use of mycelial actinomycetes (Mattuschka and Straube, 1993; Kefala et al, 1999) and fewer where live actinomycetes cultures have been employed. No reports have been located in prior art literature relating to the use of alkalophilic or alkalotolerant actinomycete for heavy metal biosorption, bioaccumulation or removal.

### **Objects of the invention**

The main object of the invention is to provide a process for the removal of heavy metal ions from media/aqueous solutions using alkalophilic or alkalotolerant actinomycetes.

It is another object of the invention to provide a process for the removal of toxic heavy metal ions from media/aqueous solutions using alkalophilic or alkalotolerant actinomycetes thereby reducing the level of pollution in waste water and effluent streams.

It is another object of the invention to provide a process for the recovery of valuable heavy metal ions such as gold, silver and platinum from media/aqueous solutions using alkalophilic or alkalotolerant actinomycetes.

### **Summary of the invention**

The present invention relies on the ability of alkalophilic actinomycete to tolerate and adsorb heavy metals during growth. Significant quantities of heavy metals are also taken up from aqueous solutions by pre-grown biomass. The term 'biomass' as used herein refers to the cellular mass of the microorganism produced as a result of growth.

Accordingly the present invention provides a process for the uptake of heavy metals from aqueous solution comprising using an alkalophilic or alkalotolerant actinomycete to uptake the heavy metals.

In one embodiment of the invention, the heavy metal uptake from the aqueous solution is achieved by contacting the said aqueous solution with the alkalophilic or alkalotolerant actinomycete biomass.

In another embodiment of the invention the heavy metal uptake from aqueous solution is achieved by growing the alkalophilic or alkalotolerant actinomycete in aqueous solution.

In another embodiment of the invention, the alkalophilic actinomycete comprises PTA 3422.

In another embodiment of the invention, the heavy metal ion is selected from the group comprising of Co, Cu, Cr, Ni, Pb, Zn, Ag, Cd, Pt, Au and any mixture thereof.

In another embodiment of the invention, the process comprises growing PTA 3422 in a medium containing carbon and nitrogen sources and metal ions selected from the group consisting of cobalt chloride, cupric chloride, potassium dichromate, nickel nitrate, lead nitrate, zinc sulphate and any mixture thereof, in a temperature ranging between 20 - 45°C and a pH in the range of 7.5 to 11 for a period of 1 - 4 days with constant stirring.

In another embodiment of the present invention, the process comprises incubating the said metal solutions singly or in combination with a pre-grown biomass of PTA 3422 at a temperature in the range of 15 - 50°C at a pH in the range of 3 - 11 for 10 minutes to 24 hours with shaking/stirring.

In another embodiment of the invention, the heavy metal is recovered from the biomass by the use of dilute acids or salts or chelating agents.

In a further embodiment of the invention, the dilute acid is selected from the group consisting of hydrochloric acid, lactic acid, nitric acid and sulphuric acid.

In a further embodiment of the invention, the salts are selected from the group consisting of sodium chloride,  $MgSO_4$  and  $Na_2SO_4$ .

In a further embodiment of the invention, the chelating agent comprises EDTA.

### **Brief description of the accompanying drawings**

Figure 1 is a graphical representation of the effect of nickel concentration in the biomass in accordance with example 3.

Figure 2 is a graphical representation of the effect of pH on biosorption of nickel in accordance with example 4.

Figure 3 is a graphical representation of the effect of co-ions on the adsorption on Ni in the biomass in accordance with example 5.

Figure 4 is a graphical representation of the effect of Co-ions on the uptake of Zn in accordance with example 7.

### **Detailed description of the invention**

The invention relates to a process for the removal of heavy metal ions using an alkalophilic actinomycete isolate. The isolate PTA 3422 grows at alkaline pH and can tolerate and grow in the presence of high levels of heavy metals such as chromium ions and produce alkaline protease (Indian Patent Applications 373/Del/94 and 431/Del/97). This organism can also tolerate other heavy metal ions such as Co, Cu, Ni, Pb and Zn. The published reports on heavy metal removal pertain predominantly to studies on bacteria growing at neutral or slightly alkaline pH. There are no reports on tolerance to heavy metals or accumulation by mycelial actinomycetes. Actinomycete designated PTA 3422 is isolated from its natural habitat of Sambhar Lake, Rajasthan, India.

The culture grows at alkaline pH in the presence of high concentration of Cr (up to 5000 ppm) supplemented either as  $Cr^{3+}$  or  $Cr^{6+}$  to the medium. PTA 3422 formed whitish to pale cream coloured aerial mycelium on alkaline mikami agar (g/L: beef extract 5; peptone 5, yeast extract 1.5 and glucose 1.5). Cell wall analysis indicated the presence of only m-DAP. The culture grows well between pH 7 – 11 and 25 to 45°C and utilises various inorganic sources such as  $NaNO_3$ ,  $KNO_3$ ,  $Ca(NO_3)_2$ ,  $NH_4NO_3$ ,  $(NH_4)_2SO_4$ , as well as organic nitrogen sources such as peptone, yeast extract, casein, soyabean meal, sugars such as glucose, galactose, mannose, and sugar alcohols such as glycerol, mannitol, sorbitol and starch as carbon sources.

The morphological characteristics of the organism when grown on various agar media are provided in Table 1 below:

**Table 1: morphological and cultural characteristics of organism grown on different agar cultures**

| Medium                 | Morphological and cultural characteristics   |
|------------------------|--|
| Inorganic salts medium | Good growth with white aerial mycelium ++  |
| Sucrose nitrate        | Very good growth with luxurious white aerial mycelium raised colonies, no diffusible pigment formation +++   |
| Glucose nitrate        | Very good growth with luxurious white aerial mycelium raised colonies, no diffusible pigment formation   |
| Nutrient               | Good growth and good sporulation with cream coloured colony, no diffusible pigment formation   |
| Glucose asparagine     | Good growth with white aerial mycelium, no diffusible pigment formation  |
| Glucose asparagine     | Good growth with white aerial mycelium, no diffusible pigment formation  |
| Tyrosine agar          | Good growth in the form of vegetative mycelium. No aerial mycelium formation. Only orange coloured substrate mycelium with orange coloured-diffusible pigmentation |
| Mikami + 100 ppm Ni    | Good growth in the form of vegetative mycelium. Inhibits aerial mycelium formation   |
| Mikami + 100 ppm Pb    | The reverse of the colony deep brown with greyish aerial mycelium. No inhibition of growth   |
| Mikami + 100 ppm Zn    | Good growth and aerial mycelium and good sporulation. No diffusible pigmentation   |

\* pH of the medium made alkaline by addition of sterile sodium carbonate

The heavy metal uptake from the aqueous solution is achieved by contacting the said aqueous solution with the alkalophilic or alkalotolerant actinomycete biomass or by growing the alkalophilic or alkalotolerant actinomycete in said aqueous solution. The heavy metal ion can be any of Co, Cu, Cr, Ni, Pb, Zn, Ag, Cd, Pt, Au and any mixture thereof.

In one feature of the invention PTA 3422 is grown in a medium containing carbon and nitrogen sources and metal ions selected from cobalt chloride, cupric chloride, potassium dichromate, nickel nitrate, lead nitrate, zinc sulphate and any mixture thereof, in a temperature ranging between 20 – 45°C and a pH in the range of 7.5 to 11 for a period of 1 – 4 days with constant stirring. The metal solutions singly or in combination can also be incubated with a pre-grown biomass of PTA 3422 at a temperature in the range of 15 – 50°C at a pH in the range of 3 – 11 for 10 minutes to 24 hours with shaking/stirring. The heavy metal is recovered from the biomass by the use of dilute acids such as hydrochloric acid, lactic acid, nitric acid and sulphuric acid or salts such as sodium chloride, MgSO<sub>4</sub> and Na<sub>2</sub>SO<sub>4</sub> or a chelating agent such as EDTA etc.

The removal of nickel above 80 – 90 % (loading capacity between 26 – 32 mg/g) is many fold higher when compared to 5mg/g reported for *Streptomyces noursei* reported by

Mattuschka and Straube (1993). Similarly, the zinc uptake of 30 – 35 mg/g of native biomass is comparable to values reported in prior art (Puranick and Pakniker, 1995; Mameri et al, 1999). These values can be further increased by appropriate pre-treatment of the biomass. The ability of the biomass to remove Ni and Zn from the mixture of other heavy metals is advantageous as the effluents often contain more than one heavy metal.

#### Example 1 (comparative)

Spores from 4 – 7 day old alkaline Mikami slant were transferred to liquid medium of the same composition (Indian Patent Application Nos. 343/Del/94; 431/Del/97). After 24 hours of growth, 10% (v/v) vegetative growth was transferred to alkaline liquid medium containing 20 –100 ppm of Co, Cu, Cd, Cr, Ni, Pb, Zn singly or in combination and incubated at 28°C, with shaking at 180 – 220 rpm. The organism grew well without significant inhibition and the biomass dry weight ranged between 1.5 – 2.5 g/L. the uptake of the metal depended on the metal ion as is evident from Table 2.

**Table 2: Uptake of metals during growth of PTA 3422**

| Metal    | Uptake (mg/g)          |
|----------|------------------------|
| Chromium | 3.06                   |
| Cobalt   | 4.63                   |
| Copper   | 5.62                   |
| Lead     | 4.67                   |
| Nickel   | 6.58                   |
| Zinc     | 2.41                   |
| Ni + Pb  | Ni = 6.59<br>Pb = 0.26 |
| Ni + Zn  | Ni = 1.20<br>Zn = 3.17 |

#### Example 2

PTA 3422 was grown in liquid alkaline Mikami for 24 hours. The biomass was filtered, washed thoroughly and suspended in deionized water and adjusted to pH of 6 – 7. It was then mixed with one of the following metal solutions (100 ppm effective concentration)

viz. silver, cobalt, copper, nickel, zinc (adjusted to pH 5 – 7) and incubated with shaking at 28°C. After one hour, the biomass was filtered, washed thoroughly and metal content in the biomass was estimated by atomic absorption spectrometer after acid digestion of the biomass. The removal of silver, copper cobalt from the solution ranged from 60 – 82% (see Table 3).

**Table 3:**

| Metal    | Adsorption (%) | Uptake (mg/g) |
|----------|----------------|---------------|
| Chromium | 40.23          | 23.60         |
| Cobalt   | 60.76          | 22.86         |
| Copper   | 81.96          | 35.05         |
| Nickel   | 74.50          | 26.60         |
| Silver   | 75.00          | 28.57         |
| Zinc     | 69.96          | 33.21         |

### Example 3

PTA 3422 was grown in liquid alkaline Mikami for 24 hours. The biomass was filtered, washed thoroughly and suspended in deionised water and adjusted to neutral pH. It was then mixed with nickel solution (to give varying effective concentrations of 10 – 100 ppm) and incubated with shaking at 28°C. After one hour, the biomass was filtered, washed thoroughly and nickel content in the biomass was estimated by atomic absorption spectrometer after acid digestion of the biomass. The metal content in the biomass increases with increasing concentration and 70 – 95% nickel was removed from solution. (figure 1).

### Example 4

PTA 3422 was grown in liquid alkaline Mikami for 24 hours. The biomass was filtered, washed thoroughly and suspended in deionized water and adjusted to different pH in the range of 3 – 8. It was then mixed with nickel solution of corresponding pH (adjusted previously in the range of 3 – 8) and incubated with shaking at 28°C. After one hour, the biomass was filtered, washed thoroughly and nickel content in the biomass was estimated using atomic absorption spectrometer after acid digestion of the biomass. The metal content in the biomass increases with increase in pH up to 7 and decreased with further increase. About 90% of the Ni was removed from the solution within 1 hour. (Figure 2).

### Example 5

PTA 3422 was grown in liquid alkaline Mikami for 24 hours. The biomass was filtered, washed thoroughly and suspended in deionized water and adjusted to pH 7. It was then mixed with nickel solution containing one or more heavy metals adjusted to pH 7 and incubated with shaking at 28°C. After one hour, the biomass was filtered, washed thoroughly and nickel content in the biomass was estimated using atomic absorption spectrometer after acid digestion of the biomass. The nickel content in the biomass is unaffected by the presence of other metals such as Co, Cr, Pb, and Zn in the solution. (Figure 3).

### Example 6

PTA 3422 was grown in liquid alkaline Mikami for 24 hours. The biomass was filtered, washed thoroughly and suspended in deionized water and adjusted to pH 6. It was then mixed with zinc solution adjusted to pH 6 and incubated with shaking at 28°C. After one hour, the biomass was filtered, washed thoroughly and zinc content in the biomass was estimated using atomic absorption spectrometer after acid digestion of the biomass. The zinc content in the biomass and percentage zinc removal from the aqueous solution ranged between 10 – 35 mg/g and 70 to > 90% respectively depending on the initial zinc concentration (25 – 100 ppm).

### Example 7

PTA 3422 was grown in liquid alkaline Mikami for 24 hours. The biomass was filtered, washed thoroughly and suspended in deionized water and adjusted to pH 7. It was then mixed with a mixture of metal containing zinc (adjusted to pH 7) and incubated with shaking at 28°C. After one hour, the biomass was filtered, washed thoroughly and zinc content in the biomass was estimated using atomic absorption spectrometer after acid digestion of the biomass. The zinc content in the biomass was around 14 mg/g and zinc removal was not affected by the presence of other metals such as Ni, Cd, Cr, Pb and > 90 % zinc was removed from the solution. (Figure 4).

### Example 8

PTA 3422 was grown in liquid alkaline Mikami for 24 hours. The biomass was filtered, washed thoroughly and suspended in deionized water and adjusted to pH 7. It was then mixed with 100 ppm of nickel and incubated with shaking at 28°C. After one hour, the

biomass was filtered, washed thoroughly and nickel content in the biomass was estimated using atomic absorption spectrometer after acid digestion of the biomass. The nickel in the biomass was recovered by incubating with an eluent for 2 hours with shaking. The recovery of nickel was between 50 – 98% depending on the choice of eluent as given in Table 4.

**Table 4: Recovery of Ni from biomass**

| Eluent                                | Total nickel recovered (mg) | % Recovery |
|---------------------------------------|-----------------------------|------------|
| Water                                 | 0.05                        | 3.42       |
| 0.1 M NaCl                            | 0.675                       | 46.23      |
| 0.1 M Na <sub>2</sub> CO <sub>3</sub> | 0.082                       | 5.62       |
| 0.1 M Na <sub>2</sub> SO <sub>4</sub> | 0.746                       | 51.09      |
| 0.1 M HCl                             | 1.196                       | 81.92      |
| 0.1 M MgSO <sub>4</sub>               | 0.743                       | 50.89      |
| 0.1 M Lactic acid                     | 1.42                        | 97.26      |
| 0.1 M EDTA                            | 1.362                       | 93.29      |

#### **Advantages of the invention**

1. The process of the invention results in reduction of pollution due to the absence of toxic metals such as Ni in the effluent stream
2. The process also provides an economical route to recover precious metals such as gold, silver or platinum in good yield.

**We claim:**

1. A process for the uptake of heavy metals from an aqueous solution comprising using an alkalophilic or alkalotolerant actinomycete PTA 3422 to uptake the heavy metals.
2. A process as claimed in claim 1 wherein the heavy metal uptake from the aqueous solution is achieved by contacting the said aqueous solution with the alkalophilic or alkalotolerant actinomycete biomass.
3. A process as claimed in claim 1 wherein the heavy metal uptake from the aqueous solution is achieved by growing the alkalophilic or alkalotolerant actinomycete in said aqueous solution.
4. A process as claimed in claim 1 wherein the heavy metal ion is selected from the group comprising of Co, Cu, Cr, Ni, Pb, Zn, Ag, Cd, Pt, Au and any mixture thereof.
5. A process as claimed in claim 1 wherein the process comprises growing PTA 3422 in a medium containing carbon and nitrogen sources and metal ions selected from the group consisting of cobalt chloride, cupric chloride, potassium dichromate, nickel nitrate, lead nitrate, zinc sulphate and any mixture thereof, in a temperature ranging between 20 - 45°C and a pH in the range of 7.5 to 11 for a period of 1 – 4 days with constant stirring.
6. A process as claimed in claim 5 wherein the said carbon sources are selected from the group consisting of sugars comprising at least one of glucose, galactose and mannose, sugar alcohols comprising at least one of glycerol, mannitol and sorbitol, and starch.
7. A process as claimed in claim 5 wherein said nitrogen source is selected from the group consisting of NaNO<sub>3</sub>, KNO<sub>3</sub>, Ca(NO<sub>3</sub>)<sub>2</sub>, NH<sub>4</sub>NO<sub>3</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, peptone, yeast extract, casein and soyabean meal.
8. A process as claimed in claim 1 wherein the process comprises incubating the said metal solutions singly or in combination with a pre-grown biomass of PTA 3422 at a temperature in the range of 15 - 50°C at a pH in the range of 3 – 11 for 10 minutes to 24 hours with shaking/stirring.
9. A process as claimed in claim 1 wherein the heavy metal is recovered from the biomass by the use of dilute acids or salts or chelating agents.
10. A process as claimed in claim 9 wherein the dilute acid is selected from the group consisting of hydrochloric acid, lactic acid, nitric acid and sulphuric acid.
11. A process as claimed in claim 9 wherein the salts are selected from the group consisting of sodium chloride, MgSO<sub>4</sub> and Na<sub>2</sub>SO<sub>4</sub>.
12. A process as claimed in claim 9 wherein the chelating agent comprises EDTA.

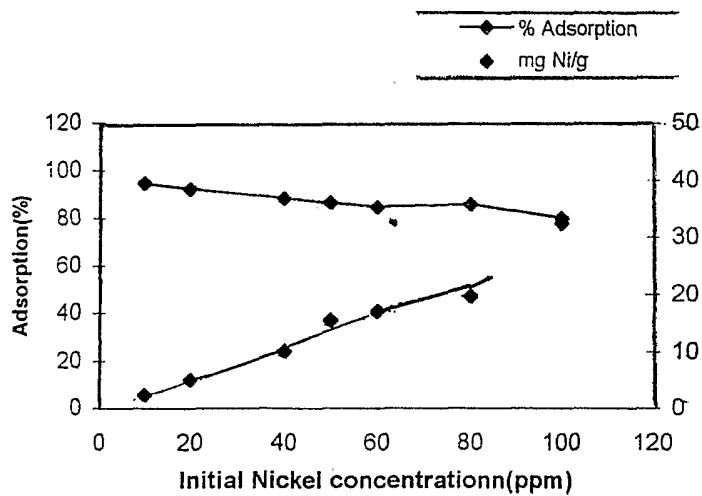


Fig. 1: Effect of Ni concentration

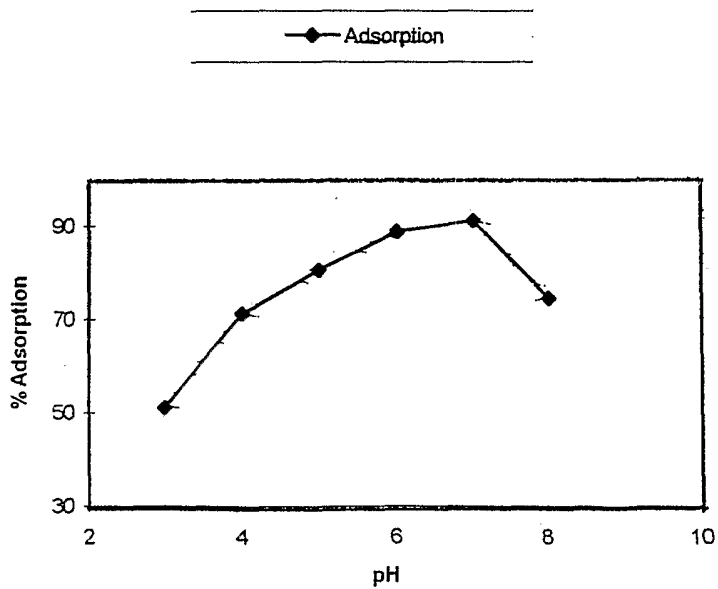


Fig. 2: Effect of pH on Biosorption of Nickel

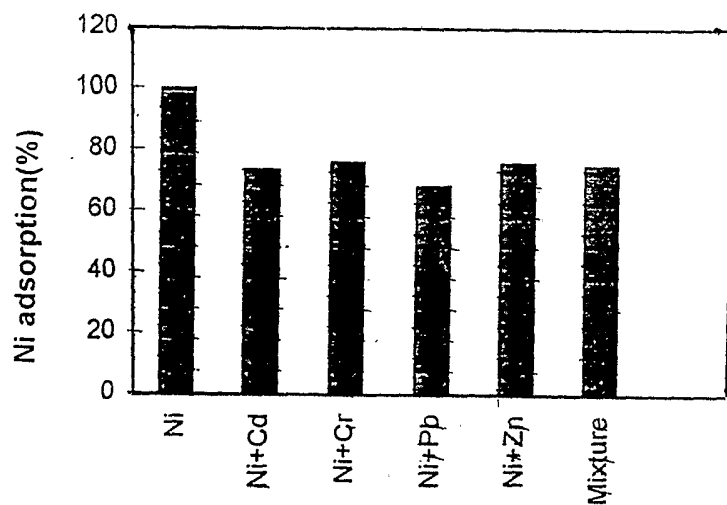


Fig. 3 : Effect of co-ions on adsorption of Ni

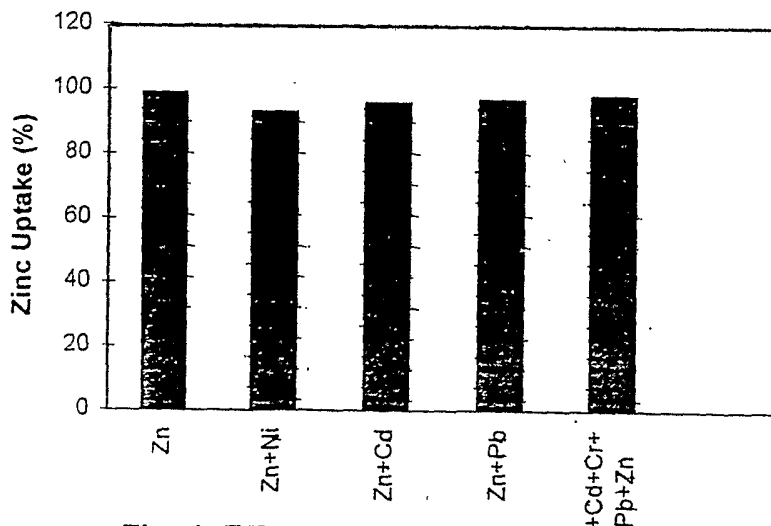


Fig. 4: Effect of Co-ions on uptake of Zinc

INTERNATIONAL SEARCH REPORT

Int Application No  
PCT/IN 01/00143

| <p><b>A. CLASSIFICATION OF SUBJECT MATTER</b><br/>IPC 7 C02F3/34</p> <p>According to International Patent Classification (IPC) or to both national classification and IPC</p>   |  |   |  |  |                       |   |   |             |
|---|--|---|--|--|-----------------------|---|---|-------------|
| <p><b>B. FIELDS SEARCHED</b></p> <p>Minimum documentation searched (classification system followed by classification symbols)<br/>IPC 7 C02F C22B C12R</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched</p> <p>Electronic data base consulted during the international search (name of data base and, where practical, search terms used)<br/>EPO-Internal, WPI Data, PAJ, COMPENDEX, BIOSIS</p>  |  |   |  |  |                       |   |   |             |
| <p><b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b></p> <table border="1"> <thead> <tr> <th>Category *</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>X</td> <td> <p>KEFALA M. I. ET AL.: "Biosorption of cadmium ions by Actinomycetes and separation by flotation" ENVIRONMENTAL POLLUTION, vol. 104, 1999, pages 283-293, XP002191058 cited in the application page 285, left-hand column, line 15 - line 44; figure 2 page 292, left-hand column -right-hand column</p> <p style="text-align: center;">---<br/>-/--</p> </td> <td>1,2,4,9, 11</td> </tr> </tbody> </table>  |  |   | Category *   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. | X | <p>KEFALA M. I. ET AL.: "Biosorption of cadmium ions by Actinomycetes and separation by flotation" ENVIRONMENTAL POLLUTION, vol. 104, 1999, pages 283-293, XP002191058 cited in the application page 285, left-hand column, line 15 - line 44; figure 2 page 292, left-hand column -right-hand column</p> <p style="text-align: center;">---<br/>-/--</p> | 1,2,4,9, 11 |
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| X   | <p>KEFALA M. I. ET AL.: "Biosorption of cadmium ions by Actinomycetes and separation by flotation" ENVIRONMENTAL POLLUTION, vol. 104, 1999, pages 283-293, XP002191058 cited in the application page 285, left-hand column, line 15 - line 44; figure 2 page 292, left-hand column -right-hand column</p> <p style="text-align: center;">---<br/>-/--</p>  | 1,2,4,9, 11   |  |  |                       |   |   |             |
| <p><input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.      <input type="checkbox"/> Patent family members are listed in annex.</p>  |  |   |  |  |                       |   |   |             |
| <p>* Special categories of cited documents :</p> <table border="0"> <tr> <td style="vertical-align: top;"> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="vertical-align: top;"> <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>* &amp; * document member of the same patent family</p> </td> </tr> </table> |  |   | <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> | <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>* &amp; * document member of the same patent family</p> |                       |   |   |             |
| <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p>  | <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>* &amp; * document member of the same patent family</p> |   |  |  |                       |   |   |             |
| <p>Date of the actual completion of the international search</p> <p style="text-align: center;">11 March 2002</p>   |  | <p>Date of mailing of the international search report</p> <p style="text-align: center;">03/04/2002</p> |  |  |                       |   |   |             |
| <p>Name and mailing address of the ISA</p> <p>European Patent Office, P.B. 5818 Patentlaan 2<br/>NL - 2280 HV Rijswijk<br/>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br/>Fax: (+31-70) 340-3016</p>  |  | <p>Authorized officer</p> <p style="text-align: center;">Liebig, T</p>                                  |  |  |                       |   |   |             |

## INTERNATIONAL SEARCH REPORT

Int'l Application No  
PCT/IN 01/00143

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |   |                       |
|--|---|-----------------------|
| Category *   | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
| A  | <p>MATIS K A ET AL: "FLOTATION OF CADMIUM-LOADED BIOMASS" BIOTECHNOLOGY AND BIOENGINEERING. INCLUDING: SYMPOSIUM BIOTECHNOLOGY IN ENERGY PRODUCTION AND CONSERVATION, JOHN WILEY &amp; SONS. NEW YORK, US, vol. 44, no. 3, 1 July 1994 (1994-07-01), pages 354-360, XP000443857<br/>ISSN: 0006-3592<br/>the whole document</p> <p style="text-align: center;">---</p> | 1-12                  |
| A  | <p>MATIS K A ET AL: "Biosorptive Flotation in Metal Ions Recovery" SEPARATION SCIENCE AND TECHNOLOGY, vol. 29, 1994, pages 1055-1071, XP008000877<br/>the whole document</p> <p style="text-align: center;">---</p>   | 1-12                  |
| A  | <p>AMOROSO M J ET AL: "SCREENING OF HEAVY METAL-TOLERANT ACTINOMYCETES ISOLATED FROM THE SALI RIVER" JOURNAL OF GENERAL AND APPLIED MICROBIOLOGY, vol. 44, no. 2, April 1998 (1998-04), pages 129-132, XP008000895<br/>the whole document</p> <p style="text-align: center;">-----</p>  | 1-12                  |