

Description

Heavy-Metal-Adsorbent Composition

Technical Field

The present invention relates to a heavy-metal-adsorbent composition containing dead bacterial cells, and to a method for removing heavy metals from a heavy-metal-containing medium such as environmental water by use of the heavy-metal-adsorbent composition.

Background Art

Environmental pollution caused by heavy metals such as cadmium, copper, zinc, chromium, and lead has grown problematic all over the globe, because these metals are biologically toxic even if used in trace amounts. The coagulation-sedimentation method is widely known as a means for removing heavy metals. This method is inexpensive, but a large amount of sludge having a high concentration of heavy metals is produced thereby, and thus the sludge must be processed in a final disposal facility. Meanwhile, the ion exchange method is known to produce only a small amount of sludge, but this method does not yet prevail because of its high cost.

Recently, there have been many reports disclosing methods of removing heavy metals by use of biomass such as bacteria, yeast, fungi, or algae. Some methods focusing on

live biomass were reported, but other methods using dead biomass are considered more practical from the standpoint that they do not require cultivation of biomass in the environment. Biomass is thought to specifically adsorb heavy metals such as cadmium, copper, zinc, chromium, and lead, and moreover, the adsorbed heavy metals can be collected through desorption of the metals. Furthermore, use of biomass is advantageous in that biomass is generally biodegradable and is thus environmentally friendly. For use of live biomass, such as live bacteria, the bacteria are first subjected to culturing and then to washing with water, calcium nitrate solution (pH 4.0) (Mullen MD et al. Appl. Environ. Microbiol. 1989: 55, 3143-3149) or a phosphate buffer (Kurek E et al. Appl. Environ. Microbiol. 1982: 43, 1011-1015).

For use of dead biomass, a variety of methods have been reported. As an example, dead biomass is subjected to treatment with an alkaline solution (Brierley et al., USP 4,992,179), followed by washing. Other methods include autoclave treatment (Seki H et al. J Colloid Interface Sci. 1998: 197, 185-190) and treatment with potassium cyanide and ethanol (Kurek E et al. Appl. Environ. Microbiol. 1982: 43, 1011-1015). Specifically, Brierley et al. discloses that alkaline treatment of bacterial biomass such as *Bacillus subtilis* increases the amount of gold, silver, copper, or lead adsorbed per unit weight of the biomass. The present inventors have also investigated amount of heavy metals adsorbed onto various bacteria treated with alkaline

solutions, and have found that the alkaline treatment increases the amount of adsorption per unit weight. However, it also turns out that the alkaline treatment considerably lowers the quantity of bacteria. Therefore, after all, alkaline treatment is not necessarily advantageous.

Disclosure of the Invention

In view of the foregoing, the present inventors have carried out studies focusing on the following two aspects: methodology for bacterial treatment and identification of bacteria exhibiting high heavy-metal adsorption capacity. Through the studies, they have found that acid-treated cells of bacteria selected from among *Bacillus* sp. KRI-02 or analogues thereof, *Bacillus licheniformis*, and *Staphylococcus* sp. KRI-04 or analogues thereof are useful as heavy-metal adsorbents, because, as compared with the case of alkaline treatment, the weight of the cells does not significantly drop after the cells undergo the acid treatment; the amount of heavy metals adsorbed per unit weight of bacterial cells increases; and the bacterial cells can be regenerated through acid treatment, thereby leading to completion of the invention.

Accordingly, the present invention provides a heavy-metal-adsorbent composition containing bacterial cells obtained from acid treatment of bacteria selected from among *Bacillus* sp. KRI-02 or analogues thereof, *Bacillus licheniformis*, and *Staphylococcus* sp. KRI-04 or analogues

thereof.

The present invention also provides a method of removing heavy metals from a heavy-metal-containing medium, characterized by treating a heavy-metal-containing medium with a heavy-metal-adsorbent composition containing bacterial cells obtained from acid treatment of bacteria selected from among *Bacillus* sp. KRI-02 or analogues thereof, *Bacillus licheniformis*, and *Staphylococcus* sp. KRI-04 or analogues thereof.

The present invention also provides a bacterium selected from among *Bacillus* sp. KRI-02 (FERM BP-8165), *Bacillus licheniformis* KRI-03 (FERM BP-8167), and *Staphylococcus* sp. KRI-04 (FERM BP-8166).

The present invention also provides a heavy-metal-adsorbing apparatus comprising (A) at least one heavy-metal-adsorbing reservoir containing the above-mentioned heavy-metal-adsorbent composition, and (B) an acid-containing reservoir.

By use of the heavy-metal-adsorbent composition of the present invention, it has become possible to efficiently remove heavy metals from, for example, river water, lakes and marshes, and industrial effluents.

Brief Description of the Drawings

Fig. 1 shows the results of a test carried out to investigate adsorption/desorption of strain KRI-02 with respect to Cu.

Fig. 2 shows the results of a test carried out to investigate adsorption/desorption of strain KRI-02 with respect to Ni.

Fig. 3 shows the adsorption capacity of Teflon-containing cell beads with respect to Cu (the first cycle and the tenth cycle).

Fig. 4 shows the adsorption capacity of Teflon-containing cell beads with respect to Zn (the first cycle and the tenth cycle).

Fig. 5 shows the adsorption capacity of heat-treated cell beads with respect to Cu.

Fig. 6 shows the adsorption capacity of heat-treated cell beads with respect to Zn.

Fig. 7 shows the adsorption capacity of heat-treated cell beads with respect to Cu (the first cycle and the tenth cycle).

Fig. 8 shows the adsorption capacity of heat-treated cell beads with respect to Zn (the first cycle and the tenth cycle).

Fig. 9 shows relations between the amount of added cell beads and Zn adsorption.

Fig. 10 shows relations between the number of regeneration cycles of cell beads and Zn adsorption.

Fig. 11 shows adsorption capacity of cell beads with respect to Zn and Fe contained in industrial effluents discharged from a plating factory.

Fig. 12 shows adsorption capacity (repeated use) of

cell beads with respect to Zn and Fe contained in industrial effluents discharged from a plating factory.

Fig. 13 shows relations between adsorption capacity of with respect to Zn and Fe contained in industrial effluents discharged from a plating factory and the number of regeneration cycles.

Fig. 14 shows adsorption capacity of cell beads with respect to Zn contained in industrial effluents discharged from a plating factory, when 5 adsorption reservoirs are employed.

Fig. 15 shows the relation between the number of regeneration cycles in a plating waste water reservoir and Zn adsorption.

Figs. 16 and 17 are schematic illustrations showing an exemplary system for removing heavy metals from waste water.

Fig. 18 refers to the system shown in Fig. 17, and shows flows of waste water streams flowing to 4 beads reservoirs.

Best Modes for Carrying Out the Invention

The present invention employs, as the above-mentioned heavy-metal adsorbent, acid-treated cells of bacteria selected from among *Bacillus* sp. KRI-02 or analogues thereof, *Bacillus licheniformis*, and *Staphylococcus* sp. KRI-04 or analogues thereof. Notably, when treated with acid, these specific bacteria are hardly troubled by significant weight reduction, and therefore increase the quantity of heavy

metals adsorbed onto a unit weight of the cells. Among *Bacillus* sp. KRI-02 and analogues thereof, *Bacillus* sp. KRI-02 (FERM BP-8165) and its analogues are particularly preferred. Among *Bacillus licheniformis*, *Bacillus licheniformis* KRI-03 (FERM BP-8167) and its analogues are particularly preferred. Among *Staphylococcus* sp. KRI-04 and analogues thereof, *Staphylococcus* sp. KRI-04 (FERM BP-8166) and its analogues are particularly preferred. Here, "analogues" of a certain bacterial strain are bacterial strains which belong to the same species of the bacterial strain and exhibit heavy-metal adsorption capacity which is similar to that of the bacterial strain.

No particular limitation is imposed on the type of acid which may be used for the acid treatment of the above bacteria, so long as the acid is able to kill the bacteria, and employable acids include inorganic acids such as hydrochloric acid, sulfuric acid, and nitric acid; and organic acid such as acetic acid, formic acid, valeric acid, propionic acid, oxalic acid, and citric acid. Conditions required for acid treatment are that they attain death of bacteria. As an example of preferred acid treatments, bacteria are treated in an aqueous acid solution of pH -0.5 to 2 for 15 to 150 minutes. Acid treatment temperatures are preferably those at which the bacteria are grown. Preferably, bacteria are washed with water before the acid treatment.

Bacterial cells treated with acid are preferably washed with water so as to have a neutral pH. Although the acid-

treated cells may be added to water to thereby form a suspension, they are preferably employed in a dry form through freeze-drying, spray drying, heating, or similar means.

The resultant acid-treated cells exhibit only very small reduction in their weight, as compared with cells treated with an alkaline solution. Moreover, they show increased heavy metal adsorption capacity as compared with untreated cells. Therefore, as heavy metal adsorbents, these acid-treated cells are by far more useful than untreated cells or cells treated with an alkaline solution.

The body of organism, including bacteria, is constituted of proteins, lipids, glycosides, etc. Therefore, alkaline treatment dissolves proteins and acidic substances. Moreover, most of their constituents are solubilized through hydrolysis, and only insoluble substances are left behind. In contrast, when bacteria are treated with acid, proteins continue to stay in denatured form. Thus it is considered that not only metal ions (which are cations), but also basic substances are forced out of their bodies and an enormous number of pores develop inside their bodies. Thus it becomes easier to accelerate penetration of ions including heavy metal ions and thereby maximize the utility of bonding positions of heavy metal atoms within the bacteria.

Examples of the heavy metals which the acid-treated cells of the present invention can adsorb thereto include Ag, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Pd, and Zn.

The heavy-metal-adsorbent composition according to the present invention may be in the form of the aforementioned acid-treated cells *per se* or may include an aqueous carrier or a solid carrier. Examples of the aqueous carrier include water and aqueous solutions, and examples of compositions prepared therefrom include aqueous suspensions. Examples of the solid carrier include various inorganic carriers and resin carriers. The composition may contain, in addition to the acid-treated cells, other heavy metal adsorbents than the heavy metal adsorbents of the present invention, plasticizers, emulsifiers, lubricants, antistatic agents, foaming agents, fire retardants, fillers, and reinforcing agents.

The concentration of the acid-treated cells present in the heavy-metal-adsorbent composition is preferably 5 to 100 wt.%, more preferably 10 to 100 wt.%, particularly preferably 20 to 100 wt.%, as reduced to the dry weight of the cells.

Examples of the inorganic carrier used as a carrier for the acid-treated cells include silica gel, alumina, glass, diatomaceous earth, and Teflon. Examples of the resin carrier include cellulose, acrylamide derivatives, polysulfone, polyvinyl alcohol, polystyrene, calcium alginate, carrageenan, and polyethylene imine. These inorganic carriers and resin carriers may be employed singly or in combination.

The acid-treated cells are preferably employed in the form of "cell beads," each formed of cells and an inorganic or resin carrier therefor. Among them, cell beads formed of

acid-treated cells and a resin carrier therefor are particularly preferred. The ratio by weight of the acid-treated cells to the inorganic carrier or resin carrier of cell beads is preferably 1:10 to 10:1, more preferably 1:5 to 5:1.

Cell beads may be produced by a variety of methods; for example, an admixture of acid-treated cells and any of the aforementioned carriers is added dropwise to a medium such as liquid nitrogen (dropwise addition method); or alternatively, nuclei are formed from, for example, lactose, and onto the nuclei, an admixture of acid-treated cells and any of the aforementioned carriers is sprayed (granulation method). When cell beads produced through the granulation method are treated with heat, improved water resistance of the beads can be obtained. Moreover, pores may be introduced into the cell beads by freeze-thawing treatment, whereby improved heavy-metal adsorption performance can be obtained. The heat treatment is preferably performed for 2 minutes to 30 minutes at 120 to 250°C, more preferably for 5 minutes to 30 minutes at 150 to 200°C.

When cell beads flocculate during the heavy-metal adsorption treatment, the area connected with heavy metal tends to shrink, so flocculation of cell beads should be refrained preferably. In one preferred flocculation preventing technique, in addition to acid-treated cells and resin, an additive such as Teflon powder, dibutyl phthalate, castor oil, or ethyl acetate is added during preparation of

cell beads. Any of these additives is preferably employed in an amount of 0.05 to 5 times by weight, more preferably 0.1 to 2 times by weight, with respect to the weight of the acid-treated cells. Alternatively, flocculation of cell beads can be prevented by heat treatment of the beads. The conditions of heat treatment are the same as those employed for improving the aforementioned water resistance.

When a heavy-metal-containing medium is treated with the heavy-metal-adsorbent composition of the present invention, heavy metals can be removed from the heavy-metal-containing medium. Examples of the heavy-metal-containing medium include environmental water or soil containing heavy metals, and specifically, mention may be given of river water, lake/marsh water, sewage, industrial effluents, soil, cooling water for incinerator ashes, waste water from plating, and other heavy-metal-containing environmental media. When such heavy-metal-containing media are desired to be treated with the composition of the present invention, the following method may be performed among other methods. Specifically, carriers carrying acid-treated cells immobilized thereon are brought into contact with any of the above-mentioned liquid medium, more specifically, the liquid medium is allowed to pass through a column packed with carriers carrying acid-treated cells immobilized thereon.

Through such a treatment, heavy metals present in the medium are adsorbed by the acid-treated cells contained in the composition of the present invention, resulting in the

removal of the heavy metals. The adsorbed heavy metals are easily released from acid-treated cells by the addition of an organic acid or an inorganic acid—which lowers pH—or by the addition of an chelating agent such as EGTA or EDTA, enabling recovery of heavy metals. Notably, acid-treated cells from which heavy metals have been released is reusable as a heavy-metal adsorbent again.

In order to remove heavy metals from a heavy-metal-containing medium, such as industrial effluent or plating water, by use of the heavy-metal-adsorbent composition of the present invention, preferably, a heavy-metal-adsorbing apparatus including the following (A) and (B) are employed:

(A) at least one reservoir containing a heavy-metal-adsorbent composition, and (B) an acid-containing reservoir. The heavy-metal-adsorption reservoir (A) may serve for continuous use; specifically, after heavy metal adsorption treatment takes place therein, heavy metals are recovered and the heavy-metal-adsorbent composition is regenerated for anew treatment.

Electroplating factories produce, in a post-plating washing step, waste water containing heavy metals at high concentrations. Since high levels of cyan compounds are used in zinc plating, they are decomposed through oxidation in a preliminary treatment. In the case of chromium plating where hexavalent chromium is employed, it is converted into trivalent chromium in a preliminary treatment. Thereafter, caustic soda or a similar substance is added to the plating

waste water containing zinc, nickel, chromium, etc. so as to shift the pH value towards a basic pH. Subsequently, a polymer flocculant is added to sediment insoluble matter in the form of sludge, and the sludge-removed water is discharged. Most part of the sludge is handled by waste disposal contractors, and major portions thereof are transported to final disposal sites for landfill purposes, and only a fraction is reused.

Therefore, if waste water before or after undergoing treatment is treated with the heavy-metal-adsorbent composition of the present invention to thereby remove heavy metals contained therein, there will no longer be a need for a polymer flocculant which otherwise will produce a large amount of sludge. Moreover, through use of the heavy-metal-adsorbent composition of the present invention exhibiting highly specific adsorption capacity, when heavy metals are recovered at high concentrations from the heavy-metal-adsorbent composition which adsorb the heavy metals thereon, not only the life of the final disposal site is prolonged but also, recycling of resources can be realized.

Thus, the heavy-metal-adsorbent composition and the heavy-metal-adsorbing apparatus of the present invention are very useful for collecting heavy metals, particularly from plating waste water.

Examples

Next, the present invention will be described in more

detail by way of examples, which should not be construed as limiting the invention thereto.

Example 1 (Selection and identification of bacteria which adsorb heavy metals (hereinafter referred to as "heavy-metal-adsorbed bacteria"))

(1) Selection of heavy-metal-adsorbed bacteria

A soil sample was suspended in saline and then allowed to stand, and the resultant supernatant was seeded into a Brain Heart Infusion Agar medium containing 1mM heavy metal. One day after the seeding, emerging colonies were selected.

(2) Identification of the obtained colonies

a. Methods

In the first bacterium test, the colonies were observed under an optical microscope (Olympus U-LH1000, Japan) in terms of cell form, Gram stain, presence or absence of spore, and presence or absence of movement with flagella. Additionally, colony forms formed on a Brain Heart Infusion Agar (Becton Dickinson, NJ, U.S.A) + agar medium (B. H. I agar) were observed. Moreover, tests were carried out with respect to catalase reaction, oxidase reaction, acid/gas production from Glucose, and oxidation/fermentation (O/F) of Glucose.

In the second bacterium test, tests regarding biochemical properties of the bacteria were performed by use of an API system (bioMérieux, France: http://www.biomerieux.fr/home_en.htm) in accordance with the

measurement method of the system.

Additional tests regarding physiological properties of the bacteria were performed.

b. Results

The results of the first-stage test are shown in Table 1.

Table 1

Specimen No.		KRI-02	KRI-03	KRI-04
Culture temp. °C		37	37	37
Cell morphology		Rod (1.0 × 3.0 to 5.0 μm)	Rod (0.8 × 2.0 to 4.0 μm)	Coccus (φ0.8 μm)
Gram stain		+	+	+
Spore		+	+	-
Movement		-	+	-
Colony form		Medium: B.H.I agar Culture time: 48h Circular Margin: slight undulate Short and flat Glossy Pale yellow	Medium: B.H.I agar Culture time: 48h Irregular Margin: fimbriate Short and flat Not glossy Pale yellow	Medium: B.H.I agar Culture time: 48h Circular Entire smooth Short and flat Glossy Pale yellow
Culture temp. °C	37	+	+	+
	45	+	+	+
Catalase		+	+	+
Oxidase		-	-	-
Production of acid/gas (glucose)		-/-	-/-	+/-
O/F test (glucose)		-/-	-/-	+/+
Additional test		-	-	Sensitivity against furazolidone +
The genus group which exhibits similar property		<i>Bacillus</i>	<i>Bacillus</i>	<i>Staphylococcus</i>

+: Positive, -: Negative, w: Weak response

The results obtained from the second-stage test and additional tests are shown in Tables 2 to 4.

Table 2 (KRI-02)

Presence or absence of fermentation		
Control -	Glycerol +	Erythritol -
D-arabinose -	L-arabinose +	Ribose +
D-xylose -	L-xylose -	Adonitol -
β -Methyl-D-xylose -	Galactose -	Glucose +
Fructose +	Mannose +	Sorbose -
Rhamnose -	Dulcitol -	Inositol -
Mannitol +	Sorbitol +	α -Methyl-D-mannose -
α -Methyl-D-glucose +	N-acetylglucosamine -	Amygdalin -
Arbutin -	Aesculin +	Salicin +
Cellobiose +	Maltose +	Lactose -
Melibiose -	Sucrose +	Trepase -
Inulin -	Melezitose -	Raffinose -
Starch -	Glycogen +	Xylitol -
Gentiobiose -	D-turanose -	D-lyxose -
D-tagatose -	D-fucose -	L-fucose -
D-arabitol-	L-arabitol-	Gluconate -
Biochemical test		
2-Ketogluconic acid -	5-Ketogluconic acid -	
β -Galactosidase -	Arginine dihydrolase -	
Lysine decarboxylase -	Ornithine decarboxylase -	
Utility of citric acid -	H ₂ S production -	
Urease -	Tryptophan deaminase -	
Indole production -	Acetoin production +	
Biratinase +	Nitrate reduction +	
Additional test		
Growth property at 50°C +		
Growth property under anaerobic condition -		
Growth property in 10% NaCl +		
Hippuric acid salt hydrolysis -		
Casein hydrolysis +		

Table 3 (KRI-03)

Presence or absence of fermentation		
Control -	Glycerol +	Erythritol -
D-arabinose -	L-arabinose +	Ribose +
D-xylose +	L-xylose -	Adonitol -
β -Methyl-D-xylose -	Galactose -	Glucose +
Fructose +	Mannose +	Sorbose -
Rhamnose -	Dulcitol -	Inositol +
Mannitol +	Sorbitol +	α -Methyl-D-mannose -
α -Methyl-D-glucose +	N-acetylglucosamine +	Amygdalin -

Arbutin +	Aesculin +	Salicin +
Cellobiose +	Maltose +	Lactose -
Melibiose -	Sucrose +	Trepase +
Inulin -	Melezitose -	Raffinose -
Starch +	Glycogen +	Xylitol -
Gentiobiose -	D-turanose ±	D-lyxose -
D-tagatose +	D-fucose -	L-fucose -
D-arabitol-	L-arabitol-	Gluconate -
Biochemical test		
2-Ketogluconic acid -	5-Ketogluconic acid -	
β-Galactosidase +	Arginine dihydrolase +	
Lysine decarboxylase +	Ornithine decarboxylase -	
Utility of citric acid -	H ₂ S production -	
Urease -	Tryptophan deaminase -	
Indole production -	Acetoin production +	
Biratinase -	Nitrate reduction +	
Additional test		
Growth property at 50°C +		
Growth property under anaerobic condition +		
Growth property in 10% NaCl +		
Hippuric acid salt hydrolysis -		
Casein hydrolysis +		

Table 4 (KRI-04)

Biochemical test and acidification test	
Urease +	Arginine dihydrolase -
Ornithine decarboxylase -	Aesculin hydrolysis -
Glucose +	Fructose -
D-mannose -	D-maltose +
Lactose -	Trepase +
D-mannitol +	Raffinose -
Reduction of nitrate to sulfite +	Acetoin production -
β-Galactosidase -	Arginine allylamidase -
Alkaline phosphatase -	Pyrrolidonyl allylamidase -
Novobiocin sodium resistance -	Sucrose +
N-acetylglucosamine -	D-turanose -
L-arabinose -	β-Glucuronidase +
D-ribose -	D-cellobiose -

KRI-02 was found to belong to the genus *Bacillus*, but the species of the bacteria cannot be identified from these results. Therefore, the bacterium was named *Bacillus* sp.

KRI-02. KRI-03 was determined to belong to *Bacillus licheniformis* and named *Bacillus licheniformis* KRI-03. KRI-04 was found to belong to the genus *Staphylococcus*, but the species of the bacterium cannot be identified. Therefore, the bacterium was named *Staphylococcus* sp. KRI-04. KRI-02, KRI-03, and KRI-04 were deposited as FERM BP-8165, FERM BP-8167, and FERM BP-8166, respectively, to an independent administrative institution, National Institute of Advanced Industrial Science and Technology, Patent Microorganisms Depositary (Chuo 6, 1-1-1 Higashi, Tsukuba-shi, Ibaraki-ken, Japan (zip code: 305-8566): August 21, 2002).

Example 2

Each of KRI-02, KRI-03, and KRI-04 was cultured in a Brain Heart Infusin medium (Difco) and then washed with water, and 0.5N hydrochloric acid (five times the volume equivalent to the wet weight of the bacterium) was added thereto, whereby the bacterium was suspended therein. Subsequently, the hydrochloric acid-added bacterium was shaken for two hours at 37°C. In addition, comparison study was performed through a method of Brierley et al (USP 4,992,179). Specifically, 3% sodium hydroxide was added to the cultured bacterium in a volume equivalent to five times the wet weight of the bacterium, and then the mixture was shaken for ten minutes at 50°C or 100°C. After completion of shaking, each of the bacteria was thoroughly washed with water and then lyophilized. The results are shown in Table 5. As compared

with the case washed with water (no treatment), the weight of the acid-treated bacterium was found to be reduced by approximately 20%, whereas the weight of the sodium hydroxide-treated bacterium was reduced by 50% or more. In particular, when the sodium hydroxide treatment was performed at 100°C, the reduction in weight reaches over 60%.

Table 5

Treatment		Weight of bacteria (after lyophilization)		
		Upper: weight Lower: wt.% vs. water-washing		
		KRI-02	KRI-03	KRI-04
No treatment		0.653 100	0.257 100	1.313 100
Treatment with acid		0.514 78.7	0.246 95.7	0.930 70.8
Sodium hydroxide	50°C	0.266 40.7	0.074 28.8	0.679 51.7
	100°C	0.134 20.5	0.039 15.2	0.483 36.8

Example 3 (Measurement of the amount of metal adsorbed to bacteria)

A bacterium powder which had been prepared through lyophilization of each of the above bacteria was suspended to a buffer (Tris: 100 mM), to thereby prepare a 60-mg/mL suspension. The bacterium suspension (20 μ L) was added to a 2.4mM aqueous solution (1 mL) of a heavy metal (CdCl_2 , CuSO_4 , ZnCl_2 , or NiCl_2) in Tris (10 mM), and the mixture was stirred for two hours. After completion of reaction, the mixture was centrifuged, and the concentration of the heavy metal in the supernatant was measured by means of an atomic absorption spectrophotometer.

The results are shown in Tables 6 to 9. Amounts of cadmium and copper adsorbed to the acid-treated KRI-02, KRI-03, or KRI-04 were found to increase with acid as compared with those of the water-washing. Although amount of cadmium adsorbed to the sodium hydroxide-treated KRI-02, KRI-03, or KRI-04 was also found to increase as compared with the water-washing, acid-treatment provides larger increase in the amount as compared with sodium hydroxide-treatment. Amounts of zinc and nickel adsorbed to the acid-treated KRI-02, KRI-03, or KRI-04 were found to increase as compared with those of the water-washing. However, treatment with sodium hydroxide (100°C) provides a slight larger intake amount. Comparison of the amount of heavy metal adsorbed to KRI-02, KRI-03, or KRI-04 treated with sodium hydroxide at different temperatures; i.e., at 50°C and 100°C, reveals that treatment at 100°C provides larger adsorbed amount as compared with the case of 50°C.

Table 6

Treatment		Amount of cadmium adsorbed ($\mu\text{mol/g}$)		
		KRI-02	KRI-03	KRI-04
No treatment		207.6	25.1	232.3
Treatment with acid		447.5	371.4	409.9
sodium hydroxide	50°C	200.8	266.6	244.4
	100°C	389.9	346.8	300.7

Table 7

Treatment		Amount of copper adsorbed ($\mu\text{mol/g}$)		
		KRI-02	KRI-03	KRI-04
No treatment		611.2	206.6	474.3
Treatment with acid		723.2	552.5	618.2
sodium hydroxide	50°C	464.7	494.6	546.4
	100°C	671.3	537.6	591.3

Table 8

Treatment		Amount of zinc adsorbed ($\mu\text{mol/g}$)		
		KRI-02	KRI-03	KRI-04
No treatment		219.5	66.8	366.9
Treatment with acid		458.6	385.9	418.6
sodium hydroxide	50°C	322.2	326.4	381.5
	100°C	466.8	390.8	430.1

Table 9

Treatment		Amount of nickel adsorbed ($\mu\text{mol/g}$)		
		KRI-02	KRI-03	KRI-04
No treatment		10.2	42.1	37.1
Treatment with acid		392.3	318.5	330.1
sodium hydroxide	50°C	244.2	293.7	252.1
	100°C	407.0	335.7	322.3

Example 4 (Adsorption and release test)

Lyophilized bacteria (KRI-02) were suspended in 100mM Tris buffer (pH 7.5), to thereby prepare a suspension (60 mg/mL). The bacterium suspension (20 μL) was added to a 2.4mM aqueous solution (1 mL) of heavy metals (CuSO_4 and NiCl_2) in Tris (10 mM), and the mixture was stirred for two hours (at pH 6.0 and pH 7.3). After completion of reaction, the resultant mixture was centrifuged, to thereby separate the supernatant (a) from the bacterium layer. Hydrochloric acid (pH 1.54) was added to the bacterium layer and then

stirred for 30 minutes, and the resultant mixture was again centrifuged to thereby separate the supernatant (b) from the bacteria layer. The concentration of the heavy metals contained in each of the supernatants (a) and (b) was measured by use of an atomic absorption spectrophotometer, whereby amount of the heavy metal adsorbed to or desorbed from the bacterium was determined. The hydrochloric acid-treated bacteria layer was washed with 100mM Tris (pH 7.5) to thereby make the pH conditions neutral, and the procedures for adsorption and desorption of heavy metal were repeated (three times). The results are shown in Figs. 1 and 2. For the second adsorption, both metals showed reduced amount of adsorption as compared with the first adsorption. The second and third adsorptions showed almost a similar level of adsorption. Regarding desorption of Cu, excellent results of 90% or more the amount of adsorption was obtained. In the case of Ni, although the desorption amount is insignificant, in the second and third desorption, almost a comparable level as that of adsorption step was obtained, thus proving that the composition of the present invention is reusable for either metal species.

Example 5

Synthesis of cell beads containing additives

To a 10% (w/v) aqueous solution of polyvinyl alcohol (PVA) (degree of polymerization: 1500 to 1800, saponification degree: 98%), KRI-02 (the same weight as PVA) and an additive

were added, and the mixture was stirred. The thus-prepared suspension was added dropwise to liquid nitrogen through use of a syringe, and the product was subjected to freeze-thawing and lyophilization. The additive was formed of Teflon powder, castor oil, ethyl acetate, and/or dibutyl phthalate.

Example 6

Evaluation of flocculation property of additive-containing cell beads

The gel beads (0.35 g) was investigated in terms of their flocculation property (stability) through use of an ash-cooling water sample (20 mL, pH 7.5) obtained from an incinerator, the water containing alkali metals and alkaline earth metals in greatly larger amounts as compared with heavy metals (cadmium, copper, zinc, and nickel). After shaking had been performed for 12 hours, adhesion or flocculation of the gel was observed with naked eyes. Cell beads containing no additives were found to flocculate. The highest effect of additive for preventing flocculation of cell beads was observed in cell beads containing as an additive a mixture of Teflon powder and dibutyl phthalate. Castor oil and ethyl acetate exhibited the effect in a medium degree.

Example 7

Effect of additive mixing ratio on flocculation of beads

In order to study the effect of mixing ratio of additives or KRI-02 (ratio by weight) on flocculation of

beads, some types of Teflon-containing cell beads were produced. In the same manner as employed in Example 6, flocculation property (stability) of beads (0.35 g) was investigated by use of an ash-cooling water sample (20 mL, pH 7.5). The results indicate that beads containing no cell (KRI-02) flocculate, and that flocculation of beads is prevented by increasing the mixing ratio of components other than PVA (Table 10).

Table 10

Effect of mixing ratio on flocculation of beads

Teflon	1	1	1	0.2	0.2	0.5
PVA	1	1	1	1	1	1
Cell	0	1	2	1	2	2
	C	AA	AA	B	A	A

A, AA: No flocculation, C: Flocculation is observed, B: Slight flocculation is observed

Example 8

Adsorption capacity of Teflon-containing cell beads

Teflon-containing cell beads (Teflon : PVA : cells (KPI-02) = 1:1:2 (ratio by weight)) were formed. Adsorption test was carried out by use of an ash-cooling water sample (20 mL, pH 7.5), and then oxalic acid (20 mL, pH 1.2) was added to the resultant mixture for release test. The adsorption and regeneration procedures were repeated, whereby adsorption capacity of the beads was observed. Adsorption capacity observed in the first cycle was compared with that

observed in the tenth cycle. Even after the beads underwent 10 cycles of use, substantially no reduction in heavy metal (copper or zinc) adsorption capacity of the beads was observed. In addition, adsorption and removal of the heavy metals were successfully performed with substantially no affect by salts contained in the sample in high concentrations (Figs. 3 and 4).

Example 9

Effects of heat treatment on flocculation and adsorption of beads

Gel beads containing KRI-02 (PVA:cell = 1:2 (ratio by weight)) were produced in the above-described method, and the beads were lyophilized and subsequently heat-treated for ten minutes (at 180°C). The gel beads (0.35 g) were investigated in terms of flocculation property (stability) by use of an ash-cooling water sample (20 mL, pH 7.5) in a similar manner as above. In addition, in order to study effect of heat treatment on adsorption capacity of beads, change in concentration of heavy metals (zinc, nickel) and alkaline earth metals (calcium, magnesium) in the ash-cooling water sample were determined through use of an atomic absorption spectrophotometer. Comparison of cell beads which had undergone heat treatment and cell beads which had undergone no heat treatment reveals that, in the sample containing the heat-treated cell beads, no adhesion of cell bead particles to one another was observed; i.e., flocculation was prevented.

On the contrary, in a sample containing cell beads which had undergone no heat-treatment, beads were flocculated to one another, as observed in the previous tests. Concentrations of metals in the ash-cooling water samples reveal that substantially no reduction in adsorption capacity was imposed by heat treatment (Figs. 5 and 6).

Example 10

Change in adsorption capacity of heat-treated beads

After the above-prepared heat-treated beads had been undergone the adsorption process, the beads were collected, and oxalic acid (20 mL, pH 1.2) were added thereto, whereby each metal was released. Subsequently, the beads were washed with Tris (100 mM), and then subjected to another absorption process. These sequential processes (one cycle is referred to as usage cycle) were repeated for investigation of change in adsorption capacity of beads caused by regeneration. Adsorption capacity observed in the first cycle was compared with that observed in the tenth cycle. Even after the beads underwent 10 usage cycles, substantially no reduction in adsorption capacity of the beads against heavy metal (copper or zinc) was observed. In addition, adsorption and removal of the heavy metals were accomplished without being affected by salts contained in the sample in high concentrations (Figs. 7 and 8).

Example 11

Production of cell beads through granulation

KRI-02 which had been crushed to 150 μm or smaller and a PVA (degree of polymerization, saponification degree: 98 to 99%) powder were mixed at a ratio of 2:1 by weight, and the resultant powder mixture was granulated by use of a centrifugal fluidized coating apparatus. Specifically, the powder mixture was stirred with nuclear grains (500 μm) formed of spherical granules (lactose), while an aqueous PVA solution (5%) was sprayed thereto. The granulated product was dried under heat (70°C), and particles having a diameter of 1.4 to 1.7 mm were separated by use of a sieve and then treated for 20 minutes at 180°C.

Example 12

Evaluation of cell beads produced through granulation in terms of water resisting

Each (0.35 g) of the cell beads treated for 20 minutes at 180°C and non-heat-treated cell beads was added to water (20 mL) and then shaken for 24 hours. The non-heat-treated cell beads revealed disintegration of particles several hours after starting of shaking, the water becoming suspended, whereas the heat-treated cell beads maintained their shape form 24 hours after starting of shaking. This shows that when granulated cell beads undergo heat treatment, bacterial cells can be stably immobilized without disintegration of granules.

Example 13

Production of porous beads through freezing

The heat-treated cell beads produced in Example 11 were placed in and washed with water, and the resultant beads to which had absorbed sufficient water were subjected to freeze-thawing and then lyophilization. The freeze-treated beads (0.5 g) were added to a plating waste water sample (20 mL) containing zinc and then stirred, whereby concentration of zinc in the sample as time elapsed was measured from starting of reaction. As a control, cell beads which had undergone only heat treatment were employed. The cell beads which had undergone freeze-treatment exhibited large change in Zn concentration as compared with that of the control. Specifically, when the cell beads which had undergone freeze-thawing and the cells which had undergone lyophilization were employed, concentrations of zinc in the waste water 90 minutes after starting of reaction were 354.1 μM and 332.8 μM , respectively, which are lower than that of control; i.e., 381.6 μM . Thus, by subjecting heat-treated cell beads to freeze-treatment, diffusion of heavy metals in beads can be facilitated, resulting in accelerating reduction in concentration of heavy metals in the liquid.

Example 14

Removal of heavy metal by use of beads

The heat-treated cell beads (Example 11) were placed in 1N hydrochloric acid and then washed with MES buffer (pH 6),

and the resultant beads were added in different amounts (8, 17.5, 25, and 35 mg/mL) to plating waste water samples containing zinc. The mixture was stirred and investigated in terms of change in concentration of zinc. The results are shown in Fig. 9. Change in concentration of zinc was found to depend on the amount of cell beads added to sample. That is, a sample to which cell beads were added in a higher amount exhibited a larger initial gradient of change in concentration. Therefore, by adding the cell beads to waste water containing any heavy metal such as zinc, zinc can be removed so that concentration of zinc can be reduced to a standard value of effluent (75.6 μM) or lower. The cell beads can remove, by way of adsorption, not only zinc, but also harmful heavy metal species such as copper, iron, cadmium, and nickel.

Example 15

Regeneration of beads

The heat-treated cell beads (Example 11, 0.35 g) were washed with MES buffer (pH 6) and then added to a plating waste water sample (20 mL) containing zinc and iron, and the mixture was stirred. After completion of adsorption reaction, the cell beads were collected from the waste water and then added to 1N hydrochloric acid (20 mL) for removal of the heavy metals. Subsequently, the resultant beads were washed with MES, and the waste water was refreshed. These sequential processes for adsorption and desorption were

repeated whereby regeneration of beads were repeated. Concentrations of heavy metals were measured through use of an atomic absorption spectrophotometer. The relationship between the amount of zinc adsorbed (pH 7) and the number of regeneration is shown in Fig. 10. Initial concentrations of zinc and iron at the start of adsorption process were 790 μM and 458 μM in average, respectively. The amounts of zinc and iron adsorbed to cell beads were 36.2 μmol and 4.6 μmol in average, respectively, per gram (dry weight) of cell beads. Even after regeneration was performed 100 times, the form of cell beads was maintained, and the beads exhibited substantially no change in the adsorption amounts of the heavy metals. In addition, 90% or more of the heavy metals adsorbed to cell beads were found to be desorbed. These results indicate that the cell beads have resistance against rapid change in pH, and repeated use of the beads is possible.

Example 16

Use of beads in treatment of waste water produced from plating factory (1) - mass balance in agitation reservoir

Heat-treated cell beads (Example 11, 400 g) were added to a reservoir (50 L) for cyanide plating waste water containing heavy metals, and change in concentrations of zinc and iron was investigated through sampling of water under stirring (revolution speed: 200 rpm). Table 11 shows concentrations of zinc and iron in the cyanide plating waste water (pH 7.5) contained in the reservoir. Zinc and iron had

been present in the waste water at concentrations higher than the standard value of effluent. Fig. 11 shows change in concentrations of the heavy metals after addition of the cell beads. The cell beads were calculated to adsorb zinc and iron in amounts of 53.6 μmol and 12.1 μmol , respectively, per gram (dry) of the beads.

Table 11

Unit: μM

Heavy metal	Secondary reaction water	Threshold
Zinc	894	76.5
Iron	422	179

Example 17

Use of beads in treatment of waste water produced from plating factory (2) - increase in concentration of metals adsorbed to and removed from beads

In a similar method described in Example 16, heat-treated cell beads (Example 11, 400 g) were added to a reservoir (50 L) for cyanide plating waste water, and adsorption and removal (90 minutes) of zinc and iron was performed. Thereafter, the beads were added to a 1N hydrochloric acid solution (20 L) for release of metals and regeneration of the beads. After completion of regeneration of the beads, the beads were washed with an aqueous sodium hydroxide solution (pH 7) and again added to the plating waste water reservoir. These adsorption and release

processes were repeated in order to try concentrations of heavy metals in the hydrochloric acid solution. Change in concentrations of zinc and iron in the cyanide plating waste water is shown in Fig. 12. The results indicate that, through repetition of adsorption processes, the concentration of the heavy metals in the waste water is lowered. When the release and regeneration cycle was repeated 6 times, the zinc concentration became lower than the standard value of effluent ($76.5 \mu\text{M}$), while the iron concentration remained higher than the standard value of effluent ($179 \mu\text{M}$). Change in concentrations of zinc and iron in the hydrochloric acid solution is shown in Fig. 13. The results indicate that the zinc concentration of the hydrochloric acid solution is increased in accordance with the number of regeneration of cell beads. However, the zinc concentration of the waste water decreased through repetition of adsorption, so that the concentration change became small gradually. After regeneration of beads was performed seven times, concentration factor of zinc was 2.1 times. Although iron was also released from the beads to the hydrochloric acid solution as in the case of zinc, the iron concentration was lower than that of zinc, and the iron concentration was not increased. The ratios of zinc concentration to iron concentration in the waste water and in the hydrochloric acid solution to which the heavy metals were released were found to be 2.0 and 7.8 (after the release process was performed seven times), respectively, indicating that, through use of

the beads, zinc is collected at a higher concentration than iron.

Example 18

Use of beads in treatment of waste water produced from plating factory - multiple adsorption

Heat-treated cell beads (Example 11, 400 g) was placed in a cyanide plating waste water reservoir, and adsorption and removal of zinc (90 minutes) were performed (the 1st reservoir). The beads were subjected to release and regeneration in a hydrochloric acid (20 L) and then washed with an aqueous sodium hydroxide solution (pH 7), and the mixture was added to another plating waste water reservoir (the 2nd reservoir). The adsorption and release processes were repeated through use of five plating waste water reservoirs (50 L) in total. Amounts of zinc adsorbed to beads contained in the respective waste water reservoirs 90 minutes after addition were shown in Fig. 14. The results reveal that adsorption of zinc was successfully repeated, and the average amount of adsorbed zinc was found to be 31.1 $\mu\text{mol/g}$. In addition, how the zinc concentration of the hydrochloric acid changes by release of zinc from beads was investigated (Fig. 15). The zinc concentration increased linearly in accordance with the number of regeneration cycle. Therefore, by repeating a cycle of placing the cell beads sequentially in a waste water reservoir and in a release reservoir (hydrochloric acid) (multiple adsorption),

concentration of zinc contained in the release reservoir can be increased.

Example 19

Construction of waste water heavy metal removal system by use of cell beads

A waste water heavy metal removal system shown in Fig. 16 can be established.

A waste water containing heavy metals at high concentrations is added to the leftmost adsorption reservoir 1, to thereby react the water with cell beads contained therein for a predetermined period of time. Subsequently, the waste water is sequentially transferred to the adjacent right adsorption reservoir. Finally, the waste water is treated in the rightmost adsorption reservoir and then removed. The cell beads are transferred from the rightmost reservoir to the leftmost reservoir sequentially, and the resultant beads are acid-treated, whereby adsorbed heavy metals are released for regeneration of the beads. The thus-regenerated cell beads are again transferred to the rightmost reservoir and then to the left reservoir sequentially. By use of cell beads in an amount according to concentration of heavy metals contained in the waste water to be treated, heavy metal concentrations of the waste water can be reduced to their environmental regulations or lower through use of at least three treatment reservoirs. Treatment time is at least one hour. When the heavy metal concentration of the

hydrochloric acid employed for regeneration of the beads becomes high, the hydrochloric acid is refreshed.

Example 20

A heavy metal removal system shown in Fig. 17 can be established.

Different from the case shown in Fig. 16, heavy metals contained in waste water are removed by transferring the waste water from a reservoir to another reservoir, while cell beads are retained in the reservoirs. Specifically, as shown in Fig. 18, continuous treatment of waste water can be performed through use of cell beads in an amount that is appropriate for the heavy metal concentration of the waste water, by transferring waste water containing heavy metals at high concentrations through reservoirs 1→2→1, reservoirs 2→1→2, reservoirs 3→4→3, and reservoirs 4→3→4, and by regenerating, through acid-treatment, the cell beads employed in treatment of high concentration waste water. When the heavy metal concentration of the hydrochloric acid employed for regeneration of the beads becomes high, the hydrochloric acid is refreshed.

Claims:

1. A heavy-metal-adsorbent composition containing bacterial cells obtained from acid treatment of bacteria selected from among *Bacillus* sp. KRI-02 or analogues thereof, *Bacillus licheniformis*, and *Staphylococcus* sp. KRI-04 or analogues thereof.

2. The heavy-metal-adsorbent composition as recited in claim 1, wherein the *Bacillus* sp. KRI-02 or analogues thereof are *Bacillus* sp. KRI-02 (FERM BP-8165) or its analogues; the *Bacillus licheniformis* is *Bacillus licheniformis* KRI-03 (FERM BP-8167) or its analogues, and the *Staphylococcus* sp. KRI-04 or analogues thereof are *Staphylococcus* sp. KRI-04 (FERM BP-8166) or its analogues.

3. The heavy-metal-adsorbent composition as recited in claim 1 or 2, wherein the acid-treated cells are carried by an inorganic carrier or a resin carrier.

4. The heavy-metal-adsorbent composition as recited in claim 1 or 2, wherein the acid-treated cells are cell beads carried by an inorganic carrier or a resin carrier.

5. The heavy-metal-adsorbent composition as recited in claim 4, wherein the cell beads contain Teflon powder, dibutyl phthalate, castor oil, or ethyl acetate, in addition to the carrier.

6. The heavy-metal-adsorbent composition as recited in claim 4, wherein the cell beads are produced through granulation.

7. A method of removing heavy metals from a heavy-

metal-containing medium, characterized by treating a heavy-metal-containing medium with a heavy-metal-adsorbent composition containing bacterial cells obtained from acid treatment of bacteria selected from among *Bacillus* sp. KRI-02 or analogues thereof, *Bacillus licheniformis*, and *Staphylococcus* sp. KRI-04 or analogues thereof.

8. The method as recited in claim 7, wherein the *Bacillus* sp. KRI-02 or analogues thereof are *Bacillus* sp. KRI-02 (FERM BP-8165) or its analogues; the *Bacillus licheniformis* is *Bacillus licheniformis* KRI-03 (FERM BP-8167) or its analogues, and the *Staphylococcus* sp. KRI-04 or analogues thereof are *Staphylococcus* sp. KRI-04 (FERM BP-8166) or its analogues.

9. The method as recited in claim 7 or 8, wherein the heavy-metal-containing medium is selected from among environmental water containing heavy metals or soil containing heavy metals.

10. The method as recited in any of claims 7 through 9, wherein the acid-treated cells are carried by an inorganic carrier or a resin carrier.

11. The method as recited in any of claims 7 through 9, wherein the acid-treated cells are cell beads carried by an inorganic carrier or a resin carrier.

12. The method as recited in claim 11, wherein the cell beads contain Teflon powder, dibutyl phthalate, castor oil, or ethyl acetate, in addition to the carrier.

13. The method as recited in claim 11, wherein the cell

beads are produced through granulation.

14. A bacterium selected from among *Bacillus* sp. KRI-02 (FERM BP-8165), *Bacillus licheniformis* KRI-03 (FERM BP-8167), and *Staphylococcus* sp. KRI-04 (FERM BP-8166).

15. A heavy-metal-adsorbing apparatus, characterized by comprising (A) at least one heavy-metal-adsorbing reservoir containing a heavy-metal-adsorbent composition as recited in any one of claims 1 through 6, and (B) an acid-containing reservoir.

16. The heavy-metal-adsorbing apparatus as recited in claim 15, wherein the (A) heavy-metal-adsorption reservoir is reused after heavy metals are recovered through acid treatment and the heavy-metal-adsorbent composition is regenerated, following heavy metal adsorption treatment.