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Application of DNA condensation for removal of mercury ions from aqueous solutions

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ABSTRACT

DNA has a unique character that allows it to combine with various chemical substrates at the molecular level, and the DNA binding with chemical pollutants can cause serious damage to the organism. The purpose of this research was to apply the strong bonding character of DNA for the removal of mercury ions. In this research, we used DNA condensation promoted by the action of DNA condensing agents, such as cetyltrimethylammonium bromide and a commercially available combination flocculant made of zeolite, to precipitate out the DNA bound with mercury ion in an aqueous solution. When solutions of mercury at 0.02–100 ppm (parts per million) concentrations at a pH range of 2–11 were treated with double-stranded DNA followed by the condensing agent, more than 95% of the mercury ions could be removed after simple filtration or sedimentation.

1. Introduction

Mercury and its derivatives have been used for thousands of years and have afforded many benefits in human culture, but expanded use of mercury compounds and cumulated mercury wastes have caused serious damages to the environment as well as to human health. Therefore, environmental remediation for mercury pollution, that is, the removal of metallic, ionic, and organic mercury substances from environmental media, has been considered to be one of the most important aims for environmental scientists and engineers. Accordingly, various kinds of adsorbents for mercury in aqueous solutions, like synthetic polymers, surface modified materials and so on, have been investigated in the last several decades [1–24]. Some of these sorbents are suitable to use in a large-scale apparatus for the treatment of a large amount of waste solutions, but the disadvantage of such practical-scale mercury decontamination process would be to operate for a small amount of waste solution. For small laboratories of high schools and colleges, where a few amounts of mercury salts are used, small-scale treatment of waste solutions by industrial processes might be a problem due to bad cost and scale performances. It is worth searching for a high performance adsorbent which works for the treatment of a small amount of waste solutions with hazardous materials.

We focused our attention on DNA as we considered the issue of mercury adsorption. DNA is a commonly existing macromolecule in life, and it has never been considered that DNA and nucleic acids, except pathogenic viruses, are hazardous to humans or cause significant environmental damages. At the molecular level, DNA can interact with many kinds of chemical substances by means of widely known intermolecular bonding mechanisms, such as +/− charge (or dipole) attraction, hydrophobic (or hydrophilic) interaction, hydrogen bonding, and intercalation. Among such DNA-combining chemicals, there are many chemical pollutants such as heavy metal compounds and persistent organic pollutants (POPs). For example, mercury and mercury ions strongly interact with DNA, and the bond formation of DNA with mercury causes various harmful effects in humans [25–28].

Viewed from another perspective, however, such characteristic binding interactions of DNA with mercury could be useful to reduce (uptake) mercury and its compounds from various media. Although this idea has not been considered practical, there have been reported a few laboratory-scale studies in which researchers used designed materials consisting of DNA for removing mercury ions or POPs from aqueous solutions [29–32]. These reported successes have supported our work using untreated DNA, but until recently there were two major problems to be solved before DNA could be applied for environmental treatment. One was the high cost of DNA, and the other was the difficulty of separating DNA complexes with pollutants from aqueous solutions. Recently, the development of a method for accomplishing large-scale preparation of DNA from major wastes disposed of by the sea food industry resolved the first problem. Because DNA is a hydrophilic polymer with many negative charges on the surface, it is not easy to separate the DNA complex with pollutants from an aqueous colloidal

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solution. The desired DNA separation was accomplished using DNA condensation which gave tightly compacted and easily precipitated DNA particles [33–37], and in this way the second problem was solved. In this paper, a report of our preliminary studies on the application of the complex formation of DNA for environmental remediation, we describe the removal of mercury ions from an aqueous solution by the combination of the complex formation of DNA with mercury ions and DNA condensation protocols.

2. Experimental

2.1. General procedures, materials, and instruments

Double-stranded high-molecular-weight DNA (L-DNA; >10,000 base pairs) and double-stranded low-molecular-weight DNA (S-DNA; 500–2000 base pairs) were obtained from Nippon Chemical Feed Co., Ltd., and Wako Pure Chemical Industry Co., Ltd., respectively, and were used as received. The concentration of DNA (C_{DNA}) as used in this paper represents the molar concentration (M) of the nucleotide pair per volume. Since an average molecular weight of nucleotides is generally considered to be 333, a DNA solution of C_{DNA} = 100 μM corresponds to 67 mg/L, that is equal to 67 ppm. Artificial zeolite powder, Circulash®, prepared from fly ash of coal incineration [38,39] and the combination flocculant, named Zeflock®, made of zeolite, both of which are available in industrial scales, were obtained from Chubu Electric Power Co., Inc., Japan. Cetyltrimethylammonium bromide (CTAB), a 1.00 g/L standard solution of mercury, dithizone (diphenylthiocarbazone), analytical grade carbon tetrachloride, and humic acid were purchased from Wako Pure Chemical Industry Co., Ltd. Water using in this research was purified using a Millipore Corp. Milli-Q Labo®. UV spectra were taken on a JASCO® V-550 spectrometer.

2.2. Determination of mercury ions (C_{Hg}) by dithizone method

To a mixed solution of 1.0 M sulfuric acid (4.0 mL) and 6.0 M acetic acid (0.40 mL) were added a mixture of a sample solution (1.0 mL) and a 0.002% solution of dithizone in carbon tetrachloride (5.0 mL) at 20 °C. The mixture was vigorously shaken for 1 min, and the bottom layer (1.0 mL) was subjected to UV analysis. Absorbance at 485 nm was measured, and concentrations of mercury (C_{Hg}) were calculated based on a calibration curve. If necessary, the sample solutions were diluted or concentrated to appropriate concentrations before subjecting them to the UV analysis.

2.3. General procedure for the removal of mercury ions, DNA condensation using CTAB

To water (0.50 mL) were added 500 μM solution of mercury (0.022 mL) and 5.0 mM solution of L-DNA (0.11 mL), and the volume of the solution was adjusted to 0.90 mL by the addition of water, and the mixed solution was stirred gently at 20 °C for 15 min. Next, a 5.0 mM solution of CTAB (0.11 mL) was added, and the total volume was adjusted to 1.1 mL by the addition of water. These operations gave the sample solution, the final concentrations of which are as follows: C_{Hg} = 2.0 ppm (parts per million) = 10 μM, C_{DNA} = 500 μM, and C_{CTAB} = 500 mM. After 30 min, this solution was subjected to centrifugal separation (15,000 rpm, 30 min), and the top layer (1.0 mL) was subjected to an analysis of mercury ions.

2.4. General procedure for the removal of mercury ions, DNA condensation using the combination flocculant

A sample solution (1.1 mL) of C_{Hg} = 2.0 ppm and L-DNA (C_{DNA} = 500 μM) was stirred gently at 20 °C for 15 min. To this was added the combination flocculant (Zeflock®, 10 mg), and, after 15 min, the mixture was separated by filtration through a filter paper. The resulting clear solution filtrate (1.0 mL) was subjected to an analysis of mercury ions.

3. Results and discussion

3.1. Mercury removal by the action of the DNA condensing agent: control experiments without DNA

A solution of mercury (C_{Hg} = 20 ppm) was stirred with 500 μM concentration of CTAB at room temperature (20 °C) for 30 min. After high-speed centrifugal separation (15,000 rpm, 30 min), a further decrease of mercury ion concentration was not detected based on the UV analysis. On the contrary, it is known that zeolite has ion exchange ability and can absorb mercury ions in an aqueous solution [6,9,10,38,39]. When the same solution was treated with 10 mg of the combination flocculant (Zeflock®) followed by centrifugal separation (3000 rpm, 3 min), the concentration (C_{Hg}) decreased from 20 to 14 ppm. The efficiency increased with increasing the amount of the combination flocculant, and 92% mercury removal efficiency was accomplished in the presence of 50 mg of Zeflock®.

3.2. Mercury removal using DNA condensation generated by CTAB

An aqueous solution of high-molecular-weight DNA (L-DNA; >10,000 base pairs) was treated with CTAB at room temperature, and the DNA concentration was monitored using UV absorption at 285 nm after the high-speed centrifugal separation. An equimolar amount of CTAB to the amount of nucleosides was necessary to complete DNA separation from the colloidal solution, and the following mercury removal experiments were carried out under the condition $C_{CTAB} = C_{DNA}$. The results of mercury removal using L-DNA or S-DNA (500–2,000 base pairs) and CTAB ($C_{CTAB} = C_{Hg}$) under similar conditions are summarized in Table 1. When solutions of mercury (C_{Hg} = 2.0 ppm) were treated with 100 μM and 500 μM of L-DNA at room temperature for 15 min followed by 100 μM and 500 μM concentrations, respectively, of CTAB, 68% and 97% amounts of mercury were removed from the resulting clear solutions (entries 3 and 6 in Table 1), respectively. The 15-min reaction period was long enough to complete the complex formation of DNA with mercury, and the removal efficiency using L-DNA was 73% in the case of 24-h reaction under the conditions C_{Hg} = 2.0 ppm and C_{DNA} = 100 μM (entry 4). In the mercury solution (C_{Hg} = 2.0 ppm), the removal efficiencies promoted by various concentrations of L-DNA (10–500 μM) are given in entries 1–3, 5 and 6, and comparative results employing S-DNA at the same mercury concentration (C_{Hg} = 2.0 ppm) are given in entries 7–11. It is obvious by these

Table 1
Removal efficiency of Hg^{2+} using S- or L-DNA and CTAB.[a].

Entry	DNA		Hg^{2+} removal efficiency/%
	Type	C_{DNA}/μM	
1	L-DNA	10	11
2	L-DNA	40	18
3	L-DNA	100	68
4	L-DNA	100	73[b]
5	L-DNA	200	90
6	L-DNA	500	97
7	S-DNA	10	15
8	S-DNA	40	18
9	S-DNA	100	71
10	S-DNA	200	89
11	S-DNA	500	94

[a] Unless otherwise stated, these entries were carried out under the following conditions: C_{Hg} = 2.0 ppm for 15-min and C_{CTAB} = 500 μM.
[b] The reaction period of Hg^{2+} and DNA was 24 h.

Table 2
Removal efficiency of Hg^{2+} using L-DNA and CTAB.[a]

Entry	Hg^{2+} (C_{Hg}/ppm)	DNA (C_{DNA}/μM)	Hg^{2+} removal efficiency/%
1	0.20	10	9
2	0.20	20	13
3	2.0	20	12
4	0.20	40	19
5	2.0	40	18
6	0.020	60	29
7	0.2	60	35
8	2.0	60	37
9	20	60	38
10	0.20	80	47
11	2.0	80	49
12	0.020	100	66
13	0.20	100	66
14	2.0	100	68
15	100	100	35
16	0.20	200	88
17	2.0	200	90
18	0.020	500	94
19	0.20	500	98
20	2.0	500	97
21	20	500	93
22	100	500	90

[a] For the DNA precipitation, an equimolar amount of CTAB ($C_{CTAB} = C_{DNA}$) was used.

results that the Hg^{2+} removal efficiency increased with increased DNA concentration, and the difference in DNA molecular weight (L- or S-DNA) did not afford significant effects. At least 200 μM concentration of DNA was necessary to perform the satisfactory Hg^{2+} removal (efficiency over 90%).

Mercury solutions of a wide concentration range (C_{Hg} = 0.020–100 ppm) were treated with various concentrations of L-DNA, and the results are shown in Table 2. It is obvious that the removal efficiency of mercury was affected not by the concentration of mercury (C_{Hg}) but by the concentration of DNA (C_{DNA}). The DNA concentration of 500 μM was necessary to perform satisfactory removal for the wide concentration range of mercury solutions as shown in entries 18–22 in Table 2. Here, when 0.020 ppm solution of mercury was treated with 500 μM L-DNA, the final concentration of 0.04 ppb (parts per billion) was under the lower detection limit of the analysis method (entry 18). Therefore, the resulting solution was concentrated to the 1/10 volume before subjecting it to the mercury ion analysis. Based on these results, if the treatment using 500 μM DNA is repeated 3 times for a mercury solution with an initial concentration of 100 ppm, the final concentration would go down below 1/10 value of the Japanese Effluent Standard 0.5 ppb.

It is worth noting that L-DNA (C_{DNA} = 500 μM) could adsorb almost the same amount of mercury (C_{Hg} = 100 ppm = 500 μM): see entry 22 in Table 2. When an excess amount of mercury (C_{Hg} = 100 ppm = 500 μM) was treated with 100 μM DNA, the removal efficiency was not the stoichiometric value (20%) but 35% (entry 15). Since CTAB itself could not uptake mercury from solutions, the result meant that 1.75 times more mercury than that of DNA was absorbed during the formation of precipitates. The following experiments using the dissociated single-strand DNA chain, which was prepared from the double-strand DNA by denaturation (thermal chain-dissociation), showed the importance of the DNA double helix structure for binding with mercury ions. A 60 μM solution of single-strand L-DNA was prepared by heating the solution at 80 °C for 10 min followed by quenching at 0 °C for 1 min, and this solution (C_{Hg} = 2.0 ppm) was used for the mercury removal. The mercury removal efficiency significantly decreased from 37% (untreated, entry 8 in Table 2) to 25%. The lower efficiency caused by the single-stranded DNA chain reverted within 5 min due to quick renaturation (recombination to the double strand). The result indicated that one of the major mercury removal mechanisms was metal binding on the site between the nucleotide base pairs performing the duplex. This mechanistic speculation was rationalized by studies on DNA complex formation with transition metals. It is known that the DNA heterocyclic base pair coordinated to the metal to give DNA–metal complexes in which the metal atom cross-linked between the two DNA strands such as thymine-Hg(II)-thymine [40–47].

According to the cross-link bonding of the metal ion with the DNA duplex, the maximum adsorption amount of mercury is estimated as the same amount of DNA base pairs (=C_{DNA}). However, it is obvious that DNA adsorbed more than the stoichiometric amount of mercury during complexation and DNA compaction induced by CTAB. Indeed, in the case of C_{Hg} = 100 ppm and C_{DNA} = 500 mM (entry 22 in Table 2), at least 1.5 equivalent amount of mercury was considered to be additionally absorbed by binding mechanisms other than cross-linking. In fact, several different binding mechanisms of DNA with metals have been discovered [48–52]. Consequently, DNA condensation and precipitation can uptake a few times more amounts of mercury by external as well as internal bindings. The speculated mechanism is illustrated in Fig. 1.

3.3. *Mercury removal using DNA condensation performed by the combination flocculant, Zeflock®*

As described at the preceding section, the mercury removal method using DNA condensation carried out by the action of CTAB is effective, but this method is not satisfactory for practical usage, due to the necessity of high-speed centrifugal separation for precipitation of the DNA–mercury complexes. Another problem is that the discharged water of this treatment might be contaminated with a significant amount of surfactant CTAB. To avoid these problems, we looked for other DNA condensing agents that

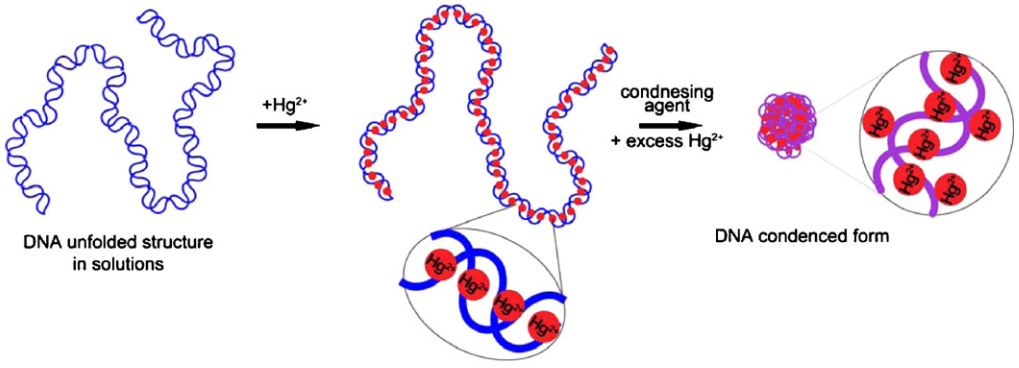

Fig. 1. Possible mechanism for Hg^{2+} removal by DNA.

Table 3
Removal efficiency of Hg^{2+} using L-DNA and the combination flocculant, Zeflock®.[a].

Entry	Hg^{2+} (C_{Hg}/ppm)	DNA (C_{DNA}/μM)	Hg^{2+} removal efficiency/%
1	2	10	54
2	0.02	40	63
3	0.2	40	64
4	2	40	85
5	20	40	64
6	2	100	89
7	20	100	90
8	100	100	83
9	0.02	500	97
10	0.21	500	98
11	2	500	99
12	20	500	96
13	100	500	94

[a] For the DNA precipitation, 10 mg of Zeflock® was used.

would be able to afford more bulky and easily separable DNA precipitates and create less of an environmental load. In previous studies, such DNA precipitation and condensation was generally carried out by the action of polycationic agents such as polyamine and metal ions with multiple positive charges [33–37]. In the present study, the DNA condensing agents as well as CTAB produced small particles of compacted DNA with at most 0.1 μm diameter. On the other hand, the combination flocculant made of zeolite powder, the average diameter of which was 20 μm, could afford more bulky precipitates that seemed to be easily separable from a colloidal solution even by simple sedimentation. Indeed, L-DNA (C_{DNA} = 100 μM) was completely separated by the action of the combination flocculant (Zeflock®, 10 mg per 1.10 mL solution) followed by practical centrifugal separation (3000 rpm, 3 min) or filtration through a filter paper. Similarly, S-DNA (500 μM) was separated in the presence of the flocculant (10 mg). When solutions of mercury (C_{Hg} = 0.020–100 ppm) were treated with L-DNA and the combination flocculant (10 mg) under the same conditions, up to 99% removal efficiency was accomplished. The results using Zeflock® are given in Table 3. Due to the cooperation with the mercury removal capacity of the combination flocculant itself, the method using Zeflock® (given in Table 3) performed better than did the methods using CTAB (given in Table 2).

3.4. Effects of pH and contaminates to the mercury removal efficiency

Sample solutions (C_{Hg} = 2.0 ppm) in strong to weak acidic conditions (initial pH values of 1, 2, and 4) were treated with L-DNA (C_{DNA} = 100 μM) and CTAB (C_{CTAB} = 500 μM), and the results are given in Table 4 (entries 1–3). The removal efficiency at pH 1 (30%) was rather low compared with the removal under neutral pH condition (68% in entry 4). Although it is well known that degradation of DNA occurs in a strong acidic solution, the results of sample solutions at pH 2 and 4 were not significantly different from one another (entries 2 and 3). Because of buffer effects caused by coexisting nucleic bases and phosphate groups in DNA, this method has widely applicability to acidic solutions. The similar tendency was observed in the cases using Zeflock®, and the original high mercury removal efficiency (89% in entry 8) was maintained under conditions of pH 2 and 4 (entries 6 and 7). Therefore, it is not necessary to neutralize acidic waste solutions which are generally discarded by chemical laboratories before the treatment with DNA.

Effects of coexisting organic as well as inorganic substances are given in Table 4, entries 9–14. Metals ion and organic contaminants which could interact with DNA seemed to disturb the mercury adsorption, but the presence of copper or magnesium ion even at the same molar concentration of mercury (C = 10 μM; that is C_{Hg} = 2.0 and equals to 0.64 and 0.24 ppm of Cu^{2+} and Mg^{2+}, respectively) in the sample solution did not decrease the mercury removal efficiency of DNA (C_{DNA} = 40 μM) and Zeflock® (entries 10–13, cf. entry 9). Although a naturally occurring acidic polyphenol derivative, humic acid, seems to interact with both mercury and DNA, the method using Zeflock® reduced the mercury concentration in a 100 ppm suspension of humic acid (entry 14).

Table 4
Removal efficiency of Hg^{2+} using L-DNA and CTAB or the combination flocculant, Zeflock®, under various conditions.[a].

Entry	C_{DNA}/μM	Condition		Condensing agent	Hg^{2+} removal efficiency (%)
		pH	Contaminant (C/ppm)		
1	100	1	Non	CTAB	30
2	100	2	Non	CTAB	71
3	100	4	Non	CTAB	58
4	100	[b]	Non	CTAB	68
5	100	1	Non	Zeflock®	10
6	100	2	Non	Zeflock®	91
7	100	4	Non	Zeflock®	90
8	100	[b]	Non	Zeflock®	89
9	40	[b]	Non	Zeflock®	85
10	40	[b]	Cu^{2+} (0.064)	Zeflock®	86
11	40	[b]	Cu^{2+} (0.64)	Zeflock®	84
12	40	[b]	Mg^{2+} (0.024)	Zeflock®	83
13	40	[b]	Mg^{2+} (0.24)	Zeflock®	83
14	40	[b]	Humic acid (100)	Zeflock®	83

[a] These entries were carried out at C_{Hg} = 2.0 ppm.
[b] These treatments were carried out under ambient pH condition.

To examine contamination with organic chemicals, we measured chemical oxygen demand (COD) values of the sample solutions before and after the precipitation treatment with CTAB or Zeflock® by permanganate titration. COD values of 500 μM solutions of L-DNA and CTAB were 230 and 205 ppm, respectively, and high-speed centrifugal separation of the condensed DNA precipitates decreased the COD value to 88 ppm. The DNA separation treated with Zeflock® followed by filtration gave the solution a COD value of 82 ppm. Because Zeflock®, which consists of inorganic materials such as zeolite, does not affect COD, the organic contaminates in the solutions treated with CTAB as well as Zeflock® were regarded as soluble peptides originating from major impurities of L-DNA.

3.5. General remarks, scope and limitations

Various polymer-based systems for mercury removal have been developed, which are mainly based on new synthetic polymers [8,12,19], modified natural polymers [1,2,5], colloidal systems fabricated from or modified by polymers [13,18,22,23]. Therefore, all hitherto reported systems require chemical synthesis or chemical modification to prepare desired mercury adsorbents. In contrast, the DNA utilized in our study, being the waste product of fishery industry, requires no chemical modification and, as a result, the proposed method is simple. On the other hand, interaction of Hg^{2+} with DNA (pK_b = 4–6) is estimated by reported stability constants (pK = 5–6) [53] and the value of pK = 4–5 calculated from our data on DNA binding with Hg^{2+}. The value is somewhat lower in comparison to the binding constant of sulfur-containing ligands, the pK_b value of which is on the order of several tens, and strong chelating agents such as EDTA (pK_b = 21.5). However, in terms of performance per purification cycle (95% and higher under conditions of sufficient amount of DNA) and the lowest values of residual Hg^{2+} after purification (below 0.5 ppb), our method represents a good and simple alternative approach to earlier reported systems for mercury removal.

It is important to notice that mercury exists as pollution not only in form of Hg^{2+} ion, but also in a form of organic mercury (e.g. methyl mercury), which is even more toxic. According to available data

on binding constants between DNA and mercury and organic mercury [53], DNA interaction with organic mercury is characterized by higher values of binding constants compared to DNA binding with ionic Hg^{2+}, therefore, we suppose higher mercury removal efficiencies of organic mercury than Hg^{2+} by the method proposed in the present study.

The scope of possible for Hg^{2+} removal solutions according to the proposed method has the following limitations. Due to relatively low DNA binding constants with Hg^{2+}, the strong Hg^{2+} complexation agents in solutions is expected to interfere with the binding of mercury with DNA and lower the efficiency of mercury removal according to difference in Hg^{2+} binding constants. These limitations are not specific to the proposed DNA method, but rather common for most of hitherto reported polymeric systems except those with very high binding constants. On the other hand, the presence of inorganic cations has a little influence on the mercury binding and, subsequently, on removal efficiency. In particular, cations of group 1 and 2 elements interact with DNA through weak interaction with phosphate groups [54,55], thus do not influence DNA interaction with mercury by intercalation mechanism. Some transition metal ions such as Cu^{+}, Ag^{+}, and so on interact with DNA by a similar intercalation mechanism as Hg^{2+} and can be taken up along with mercury. However, since the binding constant of mercury with DNA is high among the transition metals [54], for example, pK of DNA binding with Cu^{+} is on the order of 4 [56], the uptake efficiency of mercury has no significant changes as was confirmed by our experiments on Cu^{+} inhibition of Hg^{2+} removal.

4. Conclusion

Since mercury removal using CTAB is conceptually simple, the method will be useful for the study of DNA binding with various chemicals not only for the removal of other pollutants, but also for the concentration or extraction of rare metal elements. The mercury removal method using DNA and the combination flocculant made of zeolite (Zeflock®) has the following advantages: (i) it is applicable for a wide mercury concentration range (C_{Hg} = 0.02–100 ppm), (ii) it is applicable for acidic solutions (pH > 2) and solutions including other metal ions and humic substances, (iii) it has high removal efficiency, (iv) it involves simple operations and it is easy to repeat the removal cycle for perfect treatment, (v) it requires the use of neither hazardous chemicals nor expensive artificial materials, and (vi) it will be useful for the treatment of industrial waste materials. In comparison to costs for treatment a mercury solution (C_{Hg} = 6 ppm), this method, which can be estimated to require ca. USD 5.5/L as the sum of prices of chemicals and a cost for treatment of hazardous solids, is cheaper than the draining cost (USD 12/L) of the mercury swage in our department. We conclude that this method is suitable for the practical treatment of laboratory scale that means small volume, low concentration, and low contamination of waste solutions.

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