

Removal of Pb(II) from water using keratin colloidal solution obtained from wool

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Abstract The aim of this study is to investigate the use of keratin colloidal solution, which was obtained from wool, for the removal of Pb(II) from water. The addition of keratin colloidal solution (15 g L⁻¹; 0.30 mL) to a Pb(II) solution (1.0 mM; 0.90 mL; pH 5.0) resulted in the formation and precipitation of a Pb-keratin aggregate. Measurement of the Pb(II) and protein concentrations in the supernatant solution revealed that 88 and 99% of the Pb(II) and keratin protein were removed from the solution, respectively. The maximum Pb(II) uptake capacity of keratin in the colloidal solution was 43.3 mg g⁻¹. In addition, the Pb-keratin aggregate was easily decomposed via the addition of nitric acid, which enabled the recovery of Pb(II). However, aggregation did not occur in solutions with Pb(II) concentrations below 0.10 mM. Therefore, we used a keratin colloidal solution encapsulated in a dialysis cellulose tube to remove Pb(II) from 0.10 mM solutions, which enabled the removal of 95% of the Pb(II). From these results, we conclude that keratin colloidal solution is useful for the treatment of water polluted with Pb(II).

Keywords wool; keratin colloidal solution; Pb(II); removal

1 Introduction

Lead is extensively used in modern industry to manufacture a variety of products such as lead-acid batteries, radiation shields, and paint. It is well known that lead is toxic to humans, aquatic organisms, and other life forms (Goyer 1993; Demayo et al. 1982). To preserve aquatic environments, improvements to existing methods and further development of treatments to remove lead from wastewater are needed. Conventional methods of removing Pb(II) from waste water include adsorption, ion exchange (Ahmed et al. 1998), electrochemical processes (Ottosen et al. 2001), and chemical precipitation (Matlock et al. 2002).

Adsorption techniques currently play an important role in the removal of Pb(II) from waste water; accordingly, various materials have been developed as adsorbents (Kwon et al. 2010; Moriwaki et al. 2011; Wang et al. 2002). In particular, activated carbon is frequently used as an adsorbent for heavy metals including Pb(II) (Goel et al. 2005); however, activated carbon is relatively expensive. Furthermore, the interaction between activated carbon and Pb(II) is very strong, thus making it difficult to elute Pb(II) and recycle the activated carbon. For this reason, the development of low-cost adsorbent materials for the removal of Pb(II) from wastewater is targeted.

Wool is used in a large quantity for fabric. However, most discarded wool is not reused or recycled (Kimura 2009). Therefore, an abundance of wool can be obtained at low cost. Wool is rich in keratin, which contains a high proportion of amino acids that have a high affinity for ionic species such as metal ions (Balköse and Baltacığlu 1992). Furthermore, keratin is biodegradable, and several safe methods of keratin biotransformation into valuable products have been reported (Korniłowicz-Kowalska and Bohacz 2011). Accordingly, it is expected that keratin obtained from wool can be used as a low-cost and safe compound for the removal of metal ions.

Several examples of the utility of keratin powder obtained from biological resources, such as

sheep hooves, chicken feathers, and human hair, as a heavy metal adsorbent have been reported (Souag et al. 2009; A-Villarreal et al. 2011; Tan et al. 1985). Kar et al. reported that the adsorption of Pb(II) using keratin fiber obtained from feathers increased after the size of the keratin fiber was decreased from 5000 to 15 μm , thereby enlarging the surface area available for adsorption (Kar and Misra 2004).

Our group has established a method for the extraction of keratin from knitting wool (Fujii and Kato 2007). Using this technique, keratin colloidal solution, which comprises dispersed keratin particles, can be obtained.

In this study, keratin colloidal solution derived from wool was used to remove Pb(II). It is predicted that keratin colloidal solution will be more efficient than keratin powder for the removal of Pb(II) because of the relatively large surface area of keratin colloid particles. The objective of this study is to elucidate the use of keratin colloidal solution obtained from wool as a novel, environmentally friendly material for the removal of Pb(II).

2 Material and methods

2.1 Materials

Lead nitrate, cadmium nitrate, zinc chloride, mercury(II) chloride, tris-hydrochloride, thiourea, urea, keratin powder, and 2-mercaptoethanol were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Distilled water was produced using an automatic water distillation apparatus (Barnstead NANOpure II, Thermoscientific, Boston, USA). White knitting wool was purchased from Motohiro & Co., Ltd. (Kyoto, Japan).

The keratin colloidal solution was prepared according to a slightly modified procedure reported by

Fujii and Kato (2007). For 3 days, knitting wool (8.0 g) was mixed with a solution (200 mL; pH 8.5) containing 25mM tris–hydrochloride, 2.6 M thiourea, 5.0 M urea, and 150 mM 2-mercaptoethanol. The temperature during the extraction treatment was maintained at 50 °C using an incubator (Soft Incubator SLI-450D, EYELA, Tokyo, Japan). The mixture was then filtered and centrifuged at 11000 g for 10 min at room temperature. The obtained supernatant was dialyzed using a cellulose dialysis tube (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The obtained keratin colloidal solution was evaluated by measuring the distribution of particle size, zeta voltage, and protein quantity. The distribution of particle size and zeta voltage was measured using a Zetasizer Nano Series instrument (Sysmex, Hyogo, Japan). The average size of the keratin colloid particles was 80 nm and the zeta voltage showed a peak at -41.5 mV, which indicates that the keratin colloid particle was dispersed with a negative charge in water. The quantity of protein in the solution was assessed via Bradford colorimeter analysis using a plate reader (Moder 550, Bio-Rad Japan, Tokyo, Japan). The absorbance of the solution was measured at 495 nm. The quantities of proteins in the obtained keratin colloidal solutions ranged from 20 to 25 g L⁻¹. The solution was diluted with distilled water to a protein quantity of 15 g L⁻¹ for the Pb(II) removal tests. The solution was kept in the refrigerator at 5 °C, and used within a month of its production.

2.2 Aggregation properties of keratin colloidal solution

To investigate the interaction between keratin colloid and a metal ion, the keratin colloidal solution was mixed with the aqueous solution containing a kind of metal ion. For the test, keratin colloidal solution (1.0 mL; 15 mg of keratin protein), an aqueous solution containing a metal ion (9.0 mL; 0.50 or 1.0 mM; pH 5), and distilled water (2.0 mL) were mixed in test tubes. Cu(II), Ca(II), Hg(II), Fe(II), Cd(II), or Pb(II) was applied to the test. The aggregation of keratin colloid was checked with

eyes.

2.3 Removal of Pb(II) using keratin colloidal solution

The general procedure for evaluating the removal of Pb(II) using keratin colloidal solution was as follows: Keratin colloidal solution (0.10 or 0.30 mL; 15 g L⁻¹) and distilled water (0.20 or 0 mL) were added to a Pb(II) solution (0.90 mL; pH 5) in a sampling tube. The Pb concentration of the solution before adding keratin colloidal solution and distilled water was set at 1.0 mM. The total volume of the final solution was 1.2 mL. To avoid adsorption of Pb on the glass vessel during the adsorption tests, the pH of the Pb solutions was adjusted to 5 using HCl and/or NaOH (Fan et al. 2008). Subsequently, the solution was mixed using an Intelli Mixer SLRM-2M (Seoulin Co., Seoul, Korea) at 10 rpm for 10 min and then centrifuged at 11000 g (Centrifuge 5415R, Eppendorf, Tokyo, Japan). A 0.80 mL aliquot of the supernatant was then extracted. Next, the Pb(II) concentration of a portion of the supernatant (0.50 mL) was quantitatively assessed using inductively-coupled plasma atomic emission spectrometry (ICP-AES).

The experimental operating parameters of the ICP-AES instrument (SPS 3100; SSI Nanotechnology, Tokyo, Japan) were as follows: RF power of 1.2 kW, plasma-gas flow rate (Ar) of 16 L min⁻¹, carrier-gas flow rate of 1.0 L min⁻¹, and auxiliary-gas flow rate of 1.0 L min⁻¹. The working wave-length to identify Pb was 220.353 nm. The detection limit for Pb was 10 µg L⁻¹, and the limit of determination was 30 µg L⁻¹. The remainder of the supernatant (0.30 mL) was used to determine the quantity of proteins via Bradford colorimeter analysis. The surface morphology of the Pb-keratin aggregate was visualized using a scanning electron microscope (JSM-6010LA; JEOL,Ltd., Tokyo, Japan). The sample was sputtered with platinum using a JFC-1600 instrument (JEOL,Ltd., Tokyo, Japan).

2.4 Adsorption isotherm measurement

The adsorption capacities and isotherms for Pb(II) of keratin were determined. The experiments were performed under various conditions including different initial concentrations of Pb(II) and amounts of keratin colloidal solution. All experiments were conducted in the lab at 20 °C. In a sampling tube, keratin colloidal solution (0.10 mL; 15 g L⁻¹) and distilled water (0.20 mL) were added to a Pb(II) solution (0.90 mL; 0.30- 2.0 mM; pH 5). The Pb concentrations of the solution before adding keratin colloidal solution and distilled water were ranged from 0.30 to 2.0 mM. The resultant solutions were stirred at 10 rpm for 10 min using a shaker. The tested suspension was centrifuged and the supernatant was analyzed for Pb(II) concentration via ICP-AES.

The obtained adsorption data were fitted to Langmuir and Freundlich adsorption isotherm equations. The Langmuir adsorption isotherm assumes monolayer adsorption onto a surface with a finite number of identical sites (Langmuir, 1918). In contrast, the Freundlich equation is empirically based on sorption onto heterogeneous surfaces.

2.5 Removal of Pb(II) using keratin colloidal solution encapsulated in a dialysis cellulose tube

Keratin colloidal solution (15 g L⁻¹; 2.0 mL) encapsulated in a dialysis cellulose tube (dialysis membrane size 8; Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used for the removal of lead from a 0.10 mM Pb(II) solution. The average pore size of the tube was 15- 50 Å and the molecular weight cut-off is 14000. The tube was immersed for 24 h in the Pb(II) aqueous solution (20 mL; 0.10 mM). After immersion, the concentration of the Pb(II) solution was determined using ICP-AES, as described above.

2.6 Removal of Pb(II) from a solution containing multiple metal ions using keratin colloid

To elucidate the influence of the presence of other metal ions such as Zn(II), Cd(II), and Hg(II) on Pb(II) removal using keratin colloidal solution, the adsorption of Pb(II) onto keratin colloid particles in binary and ternary metal solutions was observed using 0.90 mL of solution containing metal ions (pH 5) and 4.5 mg of keratin protein. The concentrations of metal ions in the initial solution were set at 1.0 mM.

3 Results and Discussion

3.1 Aggregation of keratin colloid with various metal ions

To investigate the interaction between keratin colloid and various metal ions, the keratin colloidal solution was mixed with various aqueous solutions of metal ions. When the ion concentration of the solution (9.0 mL, pH 5) was 1.0 mM and the quantity of added keratin was 15 mg, aggregates of keratin with Cu(II), Hg(II), Pb(II), and Cd(II) formed. Aggregation of keratin with Ca(II) and Fe(II) was not observed. The aggregate of keratin with Cu(II) was pale blue, while the other aggregates were colorless. When the metal ion concentration was 0.50 mM and the quantity of added keratin was 15 mg, both Cu(II) and Pb(II) formed aggregates with keratin colloid particles; however aggregation was not observed for Hg(II), Cd(II), Ca(II), or Fe(II). In the case of Zn(II), the aggregation with keratin was not observed under the condition. The degree of aggregation of the keratin colloid particles was determined by measuring the amount of protein removed from the solution. The removal levels of the proteins were 71 and 92% for Cu(II) and Pb(II), respectively.

Furthermore, when 3.0 mL of keratin colloidal solution (45 mg of keratin protein) was added to 9.0 mL of an aqueous solution containing Cu(II), Ca(II), Hg(II), Fe(II), Cd(II), or Pb(II) (1.0 mM, pH 5), only Pb(II) formed aggregates with keratin. Therefore, keratin colloidal solutions effectively remove Pb(II) from solution, because Pb(II)-keratin aggregates were most reliably formed compared to the other metal ions.

Various conditions, such as interactions of a flocculant with suspended matters and abundance ratios of components in a suspension, affect the coagulation sedimentation. The adsorption properties for metal ions of chitin modified cysteine were reported (Shao et al. 2002). The increasing rate of the adsorption capacity of Pb(II) by the modification of cysteine to chitin was higher than those of other metal ions, such as Zn(II), Cd(II), Cu(II), and Ni(II). Keratin contains a high proportion of cysteine. The high adsorption capacity of cysteine to Pb(II) would be related to the high ability of Pb(II) to aggregate keratin colloidal particles.

The aggregate of keratin with Pb(II) was observed by scanning electron microscopy (SEM; Pb(II): 1 mM; keratin: 1.5 mg; volume of mixed solution: 1.2 mL; **Fig. 1**). The SEM photograph shows that the aggregate comprises of spherical particles around 100 nm in diameter. The spherical particles are thought to be keratin colloid particles, since the average diameter of keratin colloid particles is 80 nm. It was also confirmed by energy dispersive spectrometric (EDS) analysis that the aggregate contains lead.

3.2 Pb(II) removal by keratin colloidal solution

The percent removal of Pb(II) was determined by measuring the concentration of Pb(II) in the supernatant of the Pb(II) aqueous solution with keratin colloidal solution. When 0.10 mL of keratin colloidal solution was added to 0.90 mL of the Pb(II) solution (1.0 mM; pH 5) in a sampling tube,

31% of the Pb(II) was removed (Table 1, entry 1). From this result, it is evident that the keratin colloidal solution can be used to remove Pb(II) from water. The percent removals of Pb(II) under various conditions were also measured and are summarized in Table 1.

The quantities of protein in the supernatant obtained under the conditions listed in Table 1 decreased more than 99% after the addition of the Pb(II) aqueous solution to the keratin colloidal solution with the expectation of entry 9 (Table 1). First, various initial concentrations of Pb(II) were studied (Table 1, entries 1-8). The percentage of Pb(II) removed increased as the initial concentrations of Pb(II) decreased. In addition, the absorption capacities of keratin colloid particles tended to increase as the initial concentration of Pb(II) increased. This is due to the interruption of the equilibrium of Pb-keratin adsorption that occurs when the Pb(II) concentration increases. In addition, Pb(II) did not aggregate with keratin when the initial concentration of Pb(II) was below 0.20 mM.

Next, the quantity of keratin added was varied (Table 1, entries 1 and 9-13). The results show that greater amounts of keratin lead to higher Pb(II) removal percentages. However, adequate aggregation for the removal of Pb(II) did not occur when the Pb(II) solution (1.0 mM) was mixed with less than 0.75 mg or more than 4.75 mg of keratin. The maximum removal percentage of Pb(II) was 87%, which was achieved by mixing a 0.30 mM Pb(II) solution with 1.5 mg of keratin and by mixing a 1.0 mM Pb(II) solution with 4.5 mg of keratin (Table 1, entries 8 and 18, respectively). Therefore, it is evident that those ratios of Pb(II) to keratin ($\text{Pb(II)} / \text{keratin} = 0.3 \text{ mM} / 1.5 \text{ mg}$, and $1.0 \text{ mM} / 4.5 \text{ mg}$) are the most favorable for the formation of aggregates.

The effect of the solution pH on Pb(II) removal was also assessed (Table 1, entries 13-16). The percentage of Pb(II) removed did not change significantly in the pH range 3-6. However, keratin aggregates were not observed at pH 2. When the pH value of the solution was less than 2, the adsorption of Pb(II) would be prevented by the protonation of the adsorption sites of the keratin

colloid particles.

Next, the effect of changing the agitation time on the adsorption of Pb(II) by keratin colloid particle was studied in order to determine the optimal equilibration time for maximum adsorption (Table 1, entries 13 and 17-20). The percentage of Pb(II) removed was in the range 85-87% and was not affected by varying the agitation period from 10 s to 10 min. This result suggests that this method does not require long equilibration times and can therefore be used for the rapid removal of Pb(II) from water. The rapid aggregation of keratin with Pb(II) may be related to the efficiency of the colloid particle diffusion.

Pb-keratin aggregates were easily digested by the addition of nitric acid in order to recover Pb(II). Accordingly, 1.0 mL of 60% HNO₃ was added to the Pb-keratin aggregate (initial Pb(II) concentration: 1.0 mM; keratin quantity: 4.5 mg) after centrifugation and decantation. The solution was then heated and analyzed for Pb(II) content using ICP-AES. The recovery of adsorbed Pb(II) was 82%. This result indicates that Pb(II) in water can be recovered using simple acid digestion.

Next, Pb(II) removal with keratin colloid using filtration was studied. Accordingly, 3.0 mL of keratin colloidal solution (15 g L⁻¹) was added to 9.0 mL of Pb(II) solution (1.0 mM; pH 5). The solution was then filtered through a paper filter (5A, Advantec, Tokyo, Japan) and the Pb(II) concentration of the filtrate was analyzed using ICP-AES. The percentage of Pb(II) removed was 91%. Therefore, Pb(II) was efficiently removed by the addition of keratin colloidal solution followed by filtration.

The efficiency of the removal of Pb(II) using keratin colloid particles was compared with that of knitting wool, which is the source of the keratin colloidal solution, and commercial keratin powder. The adsorbent (4.5 mg) was added to the Pb(II) solution (1.0 mM; pH 5) and the solution was agitated for ten minutes. The Pb(II) concentration of the supernatant was analyzed and these results were used to determine percentage of Pb(II) removed (Table 2).

The percentage of Pb(II) removed and the adsorption capacity of the keratin colloid particles were higher than those of white knitting wool and keratin powder. The relatively low amount of Pb(II) removed using wool is likely due to the presence of the cuticle cells, which are known to act as barriers to the diffusion of chemicals into wool (Wen et al. 2010). Keratin colloid particles also removed Pb(II) from water more efficiently than keratin powder. This is likely due to the differences in the surface areas (Kar and Misra 2004) and diffusive properties of these materials.

The data of the Pb(II) removal from water using various adsorbents were listed in Table 2. Based on the comparison between the adsorbents in Table 2, the feature of the Pb removal using keratin colloidal solution is that the method can be applied to the Pb(II) removal from the solution contains high level of Pb(II) in a short agitation time.

3.3 Adsorption mechanism of keratin colloid particles for Pb(II)

The adsorption mechanism of keratin colloid particles for Pb(II) were determined in batch adsorption experiments.

The Langmuir isotherm model ($R^2 = 0.99$, **Fig. 2**) describes the isotherm better than the Freundlich isotherm model ($R^2 = 0.86$), which suggests that the adsorption of Pb(II) on the keratin colloid occurs via monolayer adsorption onto the surface of the particles. It is well known that keratin contains a large amount of cysteine and cystine. Therefore, there are many thiol and amino groups at the surface of the keratin particles. The result of adsorption isotherm of keratin colloid that the Langmuir isotherm model fits the adsorption better than Freundlich model indicates that these functional groups act as Pb(II) adsorption sites and adsorb Pb(II) on one on one. It is thought that the functional groups at the surface of keratin colloidal particles interact with Pb(II). It was reported that cystine-modified baker's yeast was applied to the adsorbent for Pb(II) and Cd(II), and FTIR

spectroscopy revealed that carboxyl, amide, and hydroxyl groups on the biomass surface interacted with these metal ions (Yu et al. 2007).

The parameters obtained from the Langmuir and Freundlich isotherms of the keratin colloid particles are summarized in Table 3 along with the reported parameters for the Langmuir isotherms of several adsorbents. Keratin colloid particles had a maximum uptake capacity of 43.3 mg g⁻¹ and an equilibrium constant of 0.10. The maximum uptake capacity of keratin colloid particles for Pb(II) was higher than that of activated carbon (Mishra and Patel 2009) and comparable to those of other biomaterial adsorbents (35-70 mg g⁻¹) (Low et al. 2000; Saeed et al. 2005; Bulut and Baysal 2006).

3.4 Pb(II) removal by keratin colloidal solution encapsulated in a dialysis cellulose tube

The percentage of Pb(II) removed from a 0.10 mM solution (equally 21 ppm) by the immersion of a keratin colloidal solution encapsulated in a dialysis cellulose tube for 24 h was 95%. The Pb(II) level of the treated aqueous solution was 1.1 ppm. The keratin colloidal solution in the dialysis tube removed Pb(II) from the solution and induced the adsorption of Pb(II) onto the tube although the aggregation of keratin colloid particles was not observed. Accordingly, it is found that keratin colloidal solutions can be used for the removal of Pb(II) from <0.2mM Pb(II) aqueous solutions using a dialysis cellulose tube. However, the removal of Pb(II) from water by the presented method was not enough to apply to the clean-up technology for drinking water.

3.5 Addition of keratin colloidal solution to aqueous solutions containing multiple metal ions

When 0.90 mL of Pb(II) solution (1.0 mM) was mixed with keratin colloidal solution (4.5 mg of keratin), aggregation of keratin with Pb(II) was observed (Table 1, entry 13). However, Cd(II),

Zn(II), or Hg(II) solutions (0.90 mL, 1.0 mM) mixed with keratin colloidal solutions containing 4.5 mg of keratin did not result in the formation of aggregates. The adsorption of Pb(II) on keratin colloid particles in binary and ternary metal solutions was observed in order to elucidate the influence of the presence of other metal ions such as Zn(II), Cd(II), and Hg(II) on Pb(II) removal by keratin colloidal solution (Table 4).

The removal of Pb(II) from binary solutions of Zn(II), Cd(II), and Hg(II) ions clearly declined. Interestingly, Zn(II), Cd(II), and Hg(II) were also removed by adsorption onto the keratin aggregate with Pb(II). In particular, the percentage of Hg(II) removed was high. In the case of binary solutions with Hg(II), the removal of Pb(II) drastically decreased compared to those of Zn(II) or Cd(II) binary solutions. Therefore, it is likely that the adsorption sites of the keratin colloid particles were competitively filled by these ions. Zn(II), Cd(II), and Hg(II) would be adsorbed on the keratin colloid particles, but the aggregation of keratin with these ions would not be occurred in the absence of Pb(II).

There are several examples that the concentration of Zn(II) in waste water was higher than that of Pb(II) (Karvelas et al. 2003). Therefore, there is a possibility that the presence of Zn(II) in a waste water avoids the Pb(II) removal from the waste water by the presented method.

The percentage of Hg(II) removed was higher than the percentage of Pb(II) removed in the Pb(II) and Hg(II) binary solution. This suggests that the adsorption of Hg(II) on keratin colloid particles is higher than that of Pb(II) although Pb(II) aggregates with keratin more efficiently than Hg(II).

In the case of ternary metal solutions, the percentages of Pb(II) removed decreased in the presence of other metal ions. The percentages of Cd(II) and Zn(II) removed also decreased in the presence of Hg(II). From these results, it is thought that the adsorptive interaction of Hg(II) with keratin is stronger than those of Cd(II) and Zn(II). Accordingly, it was determined that the present method

could be applied for the removal of several ions from water. However, the sedimentation behavior of keratin colloidal solution must be elucidated in order to optimize various adsorption parameters for the application of this method to waste water containing several metal ions.

4 Conclusions

A novel application of keratin colloidal solution, which is obtained from bioresources, to the removal of Pb(II) from wastewater was presented in this study. We discovered that Pb(II) can be removed from water simply through the addition of keratin colloidal solution followed by separation of the formed aggregate. Discarded wool fabrics were used as the source material of the keratin colloidal solution. Therefore, this method contributes to the reuse of waste materials. In summary, the advantages of using keratin colloidal solution for the removal of Pb(II) are as follows:

1. The source material for the keratin colloidal solution is cost-effective.
2. Keratin is biodegradable and environmental-friendly under controlled conditions for its decomposition.
3. Pb(II) is rapidly removed without a lengthy agitation time.
4. Pb(II) adsorbed on the material was recovered by simple acid digestion.

In particular, this method is ideal for the primary treatment of wastewater that contains high level of Pb(II). Moreover, it is expected that keratin colloidal solution is useful not only for the removal of Pb(II) but also for the extraction of valuable resources such as rare metals from environmental water or wastewater.

The economic potential of biomaterial is an important issue in its application to environmental purification. This can be estimated by the production cost of the material. The source material and reagents for the preparation of the keratin colloidal solution are inexpensive. However, a week is

required to prepare the keratin colloidal solution. The development of a time efficient method for the preparation of the keratin colloidal solution would improve the economic feasibility of this material. In addition, there are several issues to apply the presented method to the waste water clean-up for Pb(II). For example, the aggregation of keratin colloid with Pb(II) could be influenced and interrupted by the presence of diverse anions or organic components in a waste water. Further study about the influence of environmental matrices on the aggregation of keratin with Pb(II) are required in order to use this technique at field sites.

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Figure captions

Fig. 1 Scanning electron micrograph of a keratin aggregate with Pb(II) ($\times 15000$).

Fig. 2 Langmuir plot of Pb(II) adsorption on keratin colloid particles. C_{eq} is the equilibrium concentration of Pb(II) (mg L^{-1}) and q_{eq} is the observed uptake capacity ($\text{mg g}_{\text{adsorbent}}^{-1}$).

Table 1 Removal (in percent) of Pb(II) from water using keratin colloidal solution under various conditions

Entry	Pb(II) initial solution (mM) ^a	pH value of Pb(II) solution	The quantity of added keratin (mg)	Agitation (min)	Removal of Pb(II) (%)	Adsorption capacity mg(Pb)/g(keratin)
1	1.0	5	1.5	10	31	39
2	2.0	5	1.5	10	17	43
3	1.2	5	1.5	10	26	39
4	0.90	5	1.5	10	33	37
5	0.70	5	1.5	10	41	36
6	0.50	5	1.5	10	58	36
7	0.40	5	1.5	10	71	32
8	0.30	5	1.5	10	87	35
9	1.0	5	0.75	10	6.7	17
10	1.0	5	2.25	10	45	37
11	1.0	5	3.0	10	62	39
12	1.0	5	3.75	10	67	33
13	1.0	5	4.5	10	85	34
14	1.0	3	4.5	10	71	29
15	1.0	4	4.5	10	77	32
16	1.0	6	4.5	10	81	34
17	1.0	5	4.5	0.17	85	35
18	1.0	5	4.5	1	87	35
19	1.0	5	4.5	3	86	35
20	1.0	5	4.5	5	86	35

^a The Pb concentration before adding keratin colloidal solution and distilled water was listed.

Table 2 Removal (in percent) of Pb(II) from water using various adsorbent

Adsorbent	Pb solution (mM)	The quantity of the Pb solution (mL)	The quantity of added adsorbent (mg)	Agitation (min)	Removal of Pb(II) (%)	Adsorption capacity mg(Pb)/g(adsorbent)	Reference
keratin colloid particle	1.0	0.90	4.5	10	85	34	This study
keratin powder	1.0	0.90	4.5	10	14	5.6	This study
white knitting wool	1.0	0.90	4.5	10	5.5	2.2	This study
scoria	1.0	50	3000	1440	94	3.3	Kwon et al (2010)
distillery sludge ^a	0.48	100	50	1440	–	37.76 (pH 5)	Nadeem et al (2008)
TiC	0.0048	50	50	120	82	0.82	Moriwaki et al (2011)

^a The data of the untreated distillery sludge were listed.

Table 3 Langmuir and Freundlich parameters of keratin colloid particle and other adsorbents.

Adsorbent	Pb(II) initial concentration (mg/L)	Pb(II) removal (%)	Langmuir parameters ^a			Freundlich parameters ^b			Reference
			q_{\max}	b	r^2	K_F	1/n	r^2	
Keratin colloid particle	207	85	43.3	0.103	0.994	1.57	0.261	0.862	This study
NaOH-treated spent grain	200	62	35.5	c	c	c	c	c	Low et al. (2000)
Black gram husk	10	99.7	49.97	0.590	0.999	7.33	0.452	0.941	Saeed et al. (2005)
Wheat bran	200	96	68.97	0.080	0.9913	9.21	0.3993	0.8695	Bulut and Baysal (2006)
Activated carbon	c	c	6.68	0.050	0.90	0.85	2.27	0.87	Mishra and Patel (2009)

^a Langmuir parameters are q_{\max} : Langmuir constant related to the maximum adsorption capacity, b: Langmuir constant related to the energy of adsorption, and r^2 : the correlation coefficient.

^b Freundlich parameters are K_F : Freundlich constant related to the adsorption capacity, 1/n: Freundlich constant related to the adsorption intensity, and r^2 : the correlation coefficient.

^c The data were not listed in the references.

Table 4 Removal of Pb(II) from single, binary and ternary metal solutions by the treatment of keratin colloidal solution

Metal ion	Metal removal (%) ^{a,b}						
	Single metal solution	Binary metal solution			Ternary metal solution		
		Pb+Zn	Pb+Cd	Pb+Hg	Pb+Zn+Cd	Pb+Zn+Hg	Pb+Cd+Hg
Pb(II)	85±2.1	72±0.9	66±1.3	54±2.1	65±1.3	55±0.6	57±1.6
Cd(II)	c	-	18±0.80	-	11±1.3	-	8.7±0.8
Zn(II)	c	14±1.5	-	-	15±1.0	3.2±1.1	-
Hg(II)	c	-	-	64±2.1	-	69±1.0	73±2.5

^a Experimental conditions: additive keratin colloid particle 4.5 mg, metal concentration 1.0 mM each, pH 5.

^b All values are mean of three independent observations.

^c The aggregation of keratin was not observed under the condition as described above.

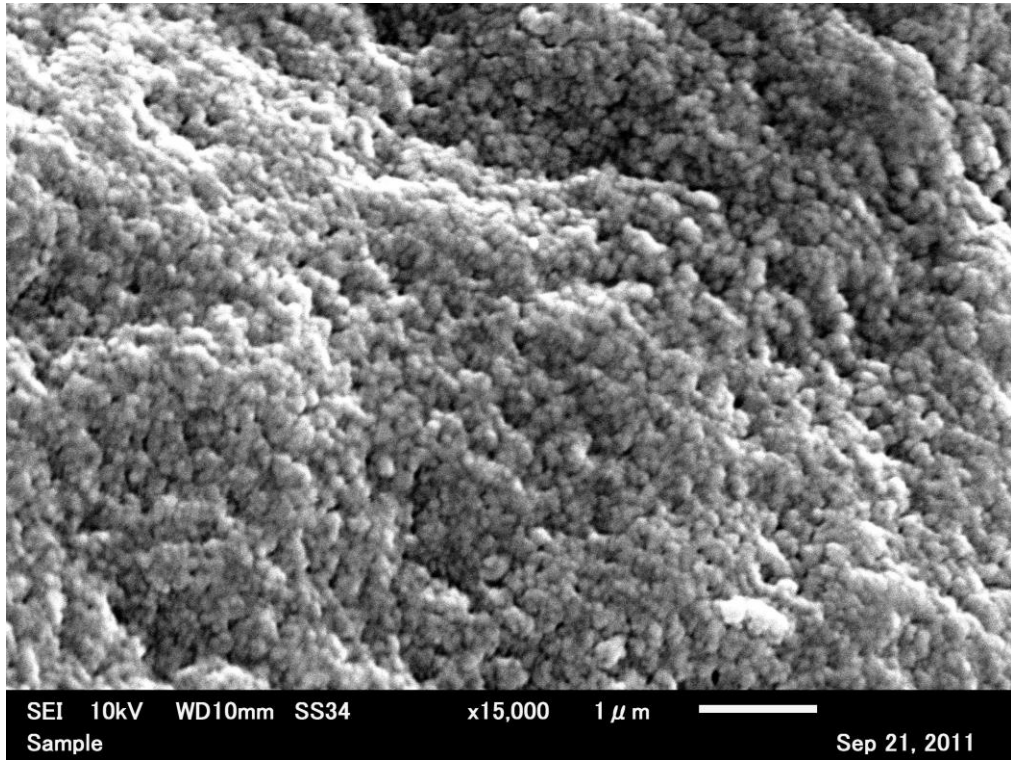


Fig. 1

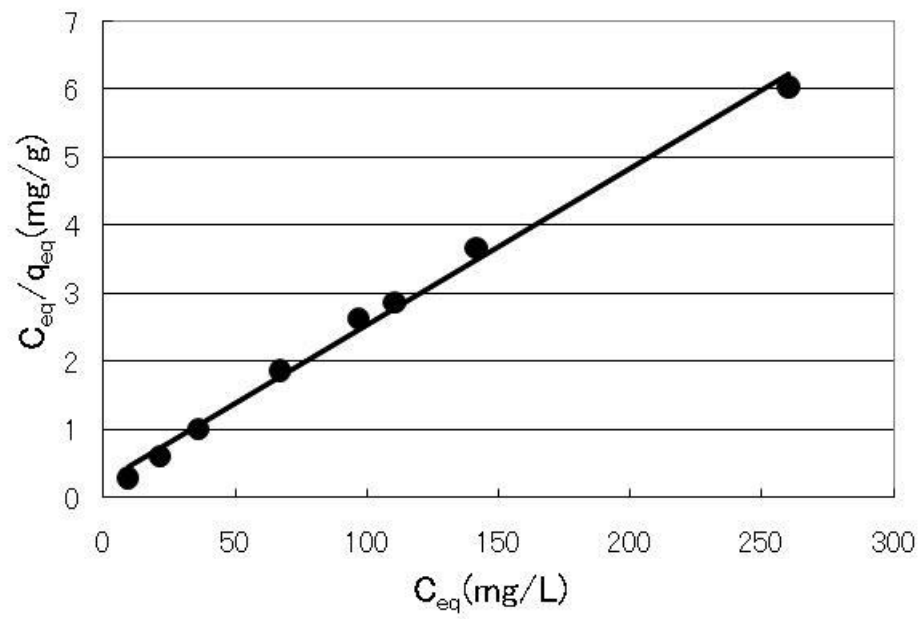


Fig. 2