



## Characterization of metal removal by os sepiae of *Sepiella maindroni* Rochebrune from aqueous solutions

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### ABSTRACT

To develop low cost metal adsorbents with less secondary pollution, metal adsorption from the aqueous solutions by the raw os sepiae (ROS) and alkali (NaOH)-pretreated OS (APOS) of the cuttlefish (*Sepiella maindroni* Rochebrune) was characterized. The capacities of adsorption of ROS and APOS were estimated to be 299.26 mg Cu g<sup>-1</sup> and 299.58 mg Cu g<sup>-1</sup>, respectively. Metal adsorption by OS was significantly improved by appropriately increasing initial pH in the solution but hardly affected by temperature change within a wide range of 15–45 °C. Cu adsorption of both ROS and APOS was well described neither by Langmuir model nor by Freundlich model. Metal adsorption by OS fell in the order of Fe > Cu ≈ Cd > Zn in the solution with mixed metals, but followed the sequence of Cd > Cu > Fe ≈ Zn in the solutions respectively, with a single metal of Fe, Cu, Cd and Zn. The changes in Ca amounts in OS and solutions in adsorption strongly correlated with removal efficiencies of the metals. Obvious shifts of stretching bands of numbers of groups in OS after and before adsorption and the pretreatment occurred. It were concluded: (1) that metal adsorption by OS involves ion exchange, which occurred mainly between Ca rather than K and Na that OS itself contains and metals that were added in the solution, (2) that metal adsorption-promoting effects by NaOH pretreatment likely involve deprotonation of surface groups in OS, exposure of more functional groups, and increase in specific surface areas and (3) that related mechanisms for adsorption also likely include surface complexation, electrostatic adsorption and even micro-deposition. The results also indicated that OS is a very promising adsorbent for metal removal from electroplating wastewater.

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### 1. Introduction

Today, ones have to confront a fact that metal contamination becomes more and more serious due to indiscriminate and/or uncontrolled discharge of metal contaminated industrial effluents into the environment during highly industrialized development. Therefore, treatment of metal contamination has become an issue of major concern [1]. Treatment technologies based on physical and/or chemical reaction have been applied for many years. However, the problems in use of the technologies are not economical,

**Abbreviations:** APOS, Alkali (NaOH)-pretreated OS; AAS, Atomic adsorption spectroscopy; Ce, The metal equilibrium concentration; CRE, Cu removal efficiency; OS, Os sepiae; q, The maximum metal adsorption; ROS, Raw os sepiae; rpm, Revolutions per min; SEM, Scanning electron microscopy; XRDs, X-ray diffraction spectrum.

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lead to secondary pollution and/or produce a large amount of toxic chemical sludge which is difficult to treat [2–4]. For treatment of aqueous solutions contaminated with metals, an emerging method is called adsorption [4], which is based on adsorbents made of biomaterials [3,5] or non-biomaterials [2,6,7]. In choice and application of the adsorbents, the following factors must be considered: low cost, high efficiency, minimization of chemical or biological sludge, adsorbent regeneration and metal recovery [6]. For example, microbial biomass-based adsorption is very economical [1,3], but this technology will not allow separation of the metals from solution if the metal adsorption is continuously operated in dynamic continuous-flow systems. Even though the problems of metal separation associated with microbial adsorption systems can be solved by some measures such as immobilization, operating costs and adsorbent regeneration will be problematic [8]. Therefore, it is necessary to further develop some low-cost and more efficient adsorbents with less secondary pollution [2,6,7].

Cuttlefish (*Sepiella maindroni* Rochebrune) is the common seafood. The os sepiae (OS), also called cuttlefish bone, is the waste by-product after processing cuttlefish, with the annual

production of 40,000–50,000 tons in China. The OS is the internal shell of the cuttlefish and has long been used as the Chinese traditional medicine [9]. The chief constituent of the OS is 80–85% of calcium carbonate, a little sodium chloride, trace calcium phosphate, and 10–15% of organic matter. It occurs in commerce in white, oval-oblong, flattened masses, 10–25 cm long and 4–7.5 cm broad, which consist of a hard, outer, concave chitinous coat (<http://www.henriettesherbal.com/eclectic/bpc1911/sepia.html>). In addition to use as the medicine, little is known about other uses of the OS. In the previous tentative, we found that the OS has a strong ability to remove the heavy metals from the aqueous solution. The aim of this study is to characterize the removal of heavy metals by OS from the aqueous solution to supply some essential knowledge of future use of the OS in removal of heavy metals.

## 2. Materials and methods

### 2.1. Preparation of OS and metal-containing solutions

The OS of *S. maindroni* in this study was purchased from the local Chinese medicine market in Nanning. The OS was brushed to remove any adhered materials, after which they were pulverized and then sieved through 200 meshes.

The aqueous solutions tested were ones containing  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  or mixed heavy metals ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{Fe}_2(\text{SO}_4)_3$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ ). The concentrations used would be indicated in the related experiments. All of the inorganic chemicals used were of analytical grade. All solutions used were prepared using distilled deionized water and then adjusted to the required pH values by adding HCl or NaOH.

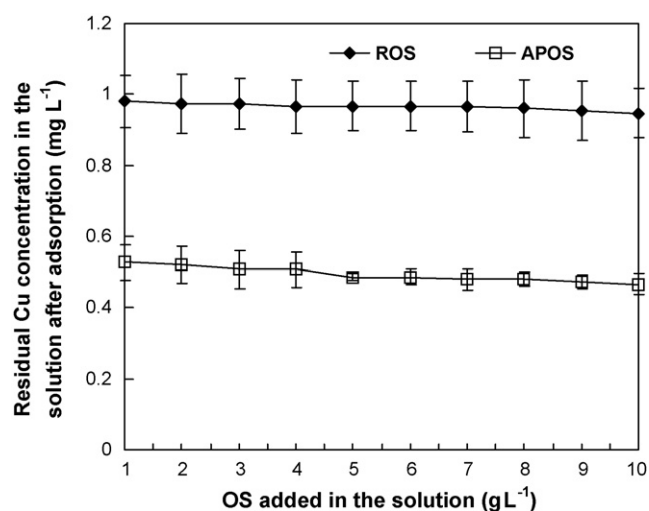
Electroplating wastewater was provided by a local electroplating factory and characterized by light brown owing to a higher concentration of Fe. The atomic absorption spectroscopy (AAS) analysis indicated that the wastewater had a pH of 3 and contained  $\text{Cu}^{2+}$  ( $9.4 \pm 0.1 \text{ mg L}^{-1}$ ),  $\text{Fe}^{3+}$  ( $232.7 \pm 1.2 \text{ mg L}^{-1}$ ) and  $\text{Zn}^{2+}$  ( $75.5 \pm 0.8 \text{ mg L}^{-1}$ ). According to data provided by sampling plant, chemical oxygen demand as well as biological oxygen demand of the electroplating wastewater were  $1400 \text{ mg L}^{-1}$  and  $550 \text{ mg L}^{-1}$ , respectively.

### 2.2. Pretreatment of the OS

Prior to use as an adsorbent, the powered OS was treated for 60 min by shaken culture at 100 rpm in the solution with NaOH ( $1 \text{ mol L}^{-1}$ ), after which the OS were collected by 5 min centrifugation at  $1600 \times g$ . The resulting alkali-pretreated OS was named APOS. The OS parallel-treated in distilled deionized water was used as a control and named raw OS (ROS).

### 2.3. Adsorption experimental procedures

Adsorption experiments were conducted in batches in 250 ml Erlenmeyer flasks containing 100 ml solution with or without complementation with the metal salts. The powered OS was then added to each flask, which was subsequently sealed with a cap and underwent shaken culture at 200 rpm at the indicated time and temperatures. The solution treated in the flasks was centrifuged at  $4200 \times g$  for 5 min to collect the supernatant and/or the OS precipitate. The removal efficiency of specific metals in the solution was calculated as:  $Q = (C_0 - C_1)/C_0$ , where  $Q$  is the removal efficiency of the specific metal (%),  $C_0$  is the initial concentration of the specific metal in solution ( $\text{mg L}^{-1}$ ), and  $C_1$  is the residual concentration of the specific metal in solution after adsorption ( $\text{mg L}^{-1}$ ). The specific metals were analysed by AAS.



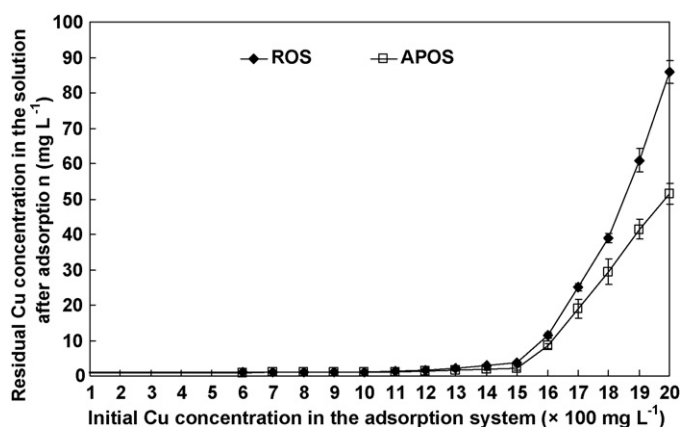
**Fig. 1.** CRE changes with OS dosage in the Cu-added solution. Adsorption was conducted for 60 min at  $32^\circ\text{C}$  in 100 ml solution containing  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  ( $100 \text{ mg L}^{-1}$ ) and different dosages of OS. The initial pH of the solution was adjusted to 5. The error bars represent standard deviation of the mean from three batches of experiments. APOS: alkali (NaOH)-pretreated OS, CRE: Cu removal efficiency, OS: os sepiae and ROS: raw os sepiae.

### 2.4. Ion exchange analysis in metal adsorption

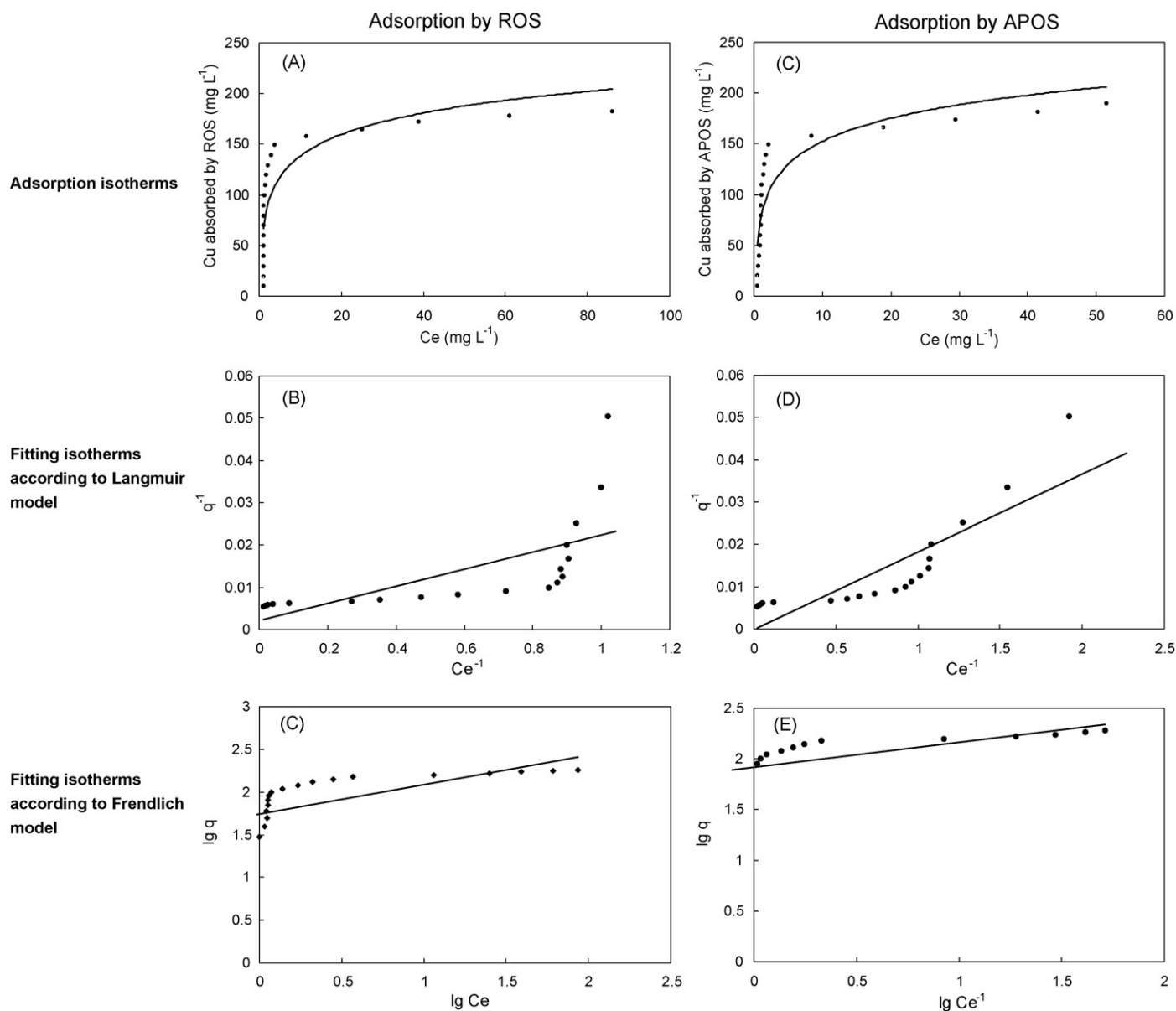
Ion exchange experiment was performed in the 100 ml solution containing OS (1 g), and with or without addition of metal(s). The adsorption experiment was conducted for 1 h at  $37^\circ\text{C}$  by shaken culture at 100 rpm. After adsorption, the resulting adsorption solution was immediately centrifuged for 5 min at  $1600 \times g$ , and the supernatant and the precipitated OS were then analysed for metal content by ASS.

### 2.5. Metal adsorption–desorption experiment

Metal adsorption–desorption experiment was performed in the electroplating wastewater as described by Liu et al. [10]. Metal adsorption was carried out with ROS. Desorption of metal adsorbed on the ROS was conducted in ethylenediamine tetraacetate solution ( $0.5 \text{ mmol L}^{-1}$ ).



**Fig. 2.** CRE change with Cu-added concentrations. Adsorption was conducted for 60 min at  $32^\circ\text{C}$  in 100 ml solution containing OS ( $5 \text{ g L}^{-1}$ ) and different concentrations of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . The initial pH of the solution was adjusted to 5. The error bars represent standard deviation of the mean from three batches of experiments. APOS: alkali (NaOH)-pretreated OS, CRE: Cu removal efficiency, OS: os sepiae and ROS: raw os sepiae.



**Fig. 3.** Adsorption isotherms of OS. The construction of adsorption isotherms was based on the data shown in Fig. 2. APOS: alkali (NaOH)-pretreated OS, Ce: the metal equilibrium concentration ( $\text{mg L}^{-1}$ ), OS: os sepiae,  $q$ : The maximum metal Cu adsorption ( $\text{mg g}^{-1}$ ) and ROS: raw os sepiae.

## 2.6. AAS assay

The AAS assay was conducted on a Hitachi Z-8000 atomic absorption spectrophotometer (Hitachi, Tokyo, Japan) equipped with a graphite tube atomizer following standard procedures. All assays were conducted in triplicate and reported in the mean values  $\pm$  the standard deviation.

## 2.7. Construction of adsorption isotherms

Construction of adsorption isotherms was performed strictly as indicated by the literature [11].

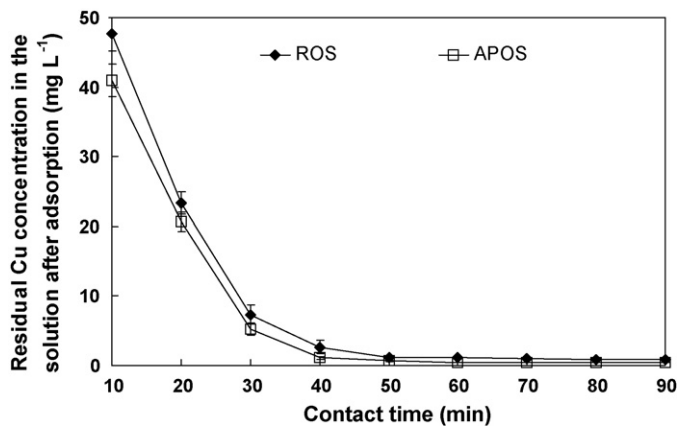
## 2.8. Analysis of infrared absorbance spectra

The OS materials (ROS and APOS) prepared in ion exchange experiment were dried at  $70^\circ\text{C}$  and analysed for infrared

absorbance spectra to identify the molecular functional groups. Infrared absorbance spectra were determined by the Fourier transform infrared spectrometer (Nicolet, USA) as the method of potassium bromide pellet. All parameters for analysis were default settings.

## 2.9. Analyses of X-ray diffraction spectrum (XRDS) as well as scanning electron microscopy (SEM)

The OS materials (ROS and APOS) prepared in ion exchange experiment were analysed for their structures by SEM and for quantification of metal elements by XRDS. The pretreatments of the materials for SEM and XRDS analyses were conducted as the conventional methods. According to the standard procedures, the SEM observation was conducted on a HITACHI S-3400N (Japan); XRDS analysis was performed on an EDAX Genesis (USA).



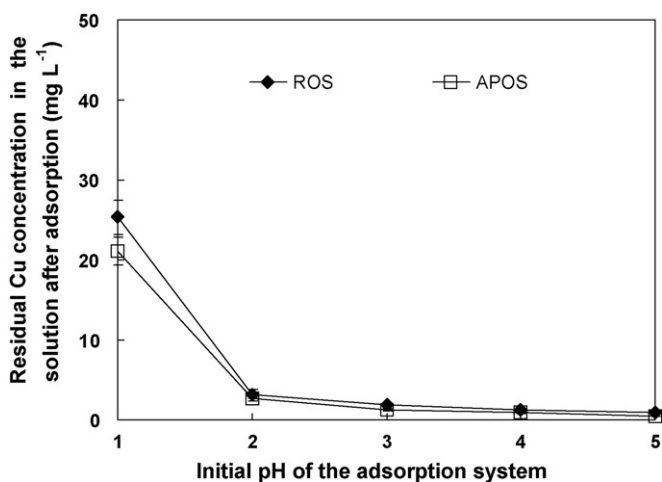
**Fig. 4.** Effects of contact time on CRES. Adsorption was conducted for indicated time at 32 °C in 100 ml solution containing OS (5 g L<sup>-1</sup>) and CuSO<sub>4</sub>·5H<sub>2</sub>O (100 mg L<sup>-1</sup>). The initial pH of the solution was adjusted to 5. The error bars represent standard deviation of the mean from three batches of experiments. APOS: alkali (NaOH)-pretreated OS, CRE: Cu removal efficiency, OS: os sepiae and ROS: raw os sepiae.

### 3. Results and discussion

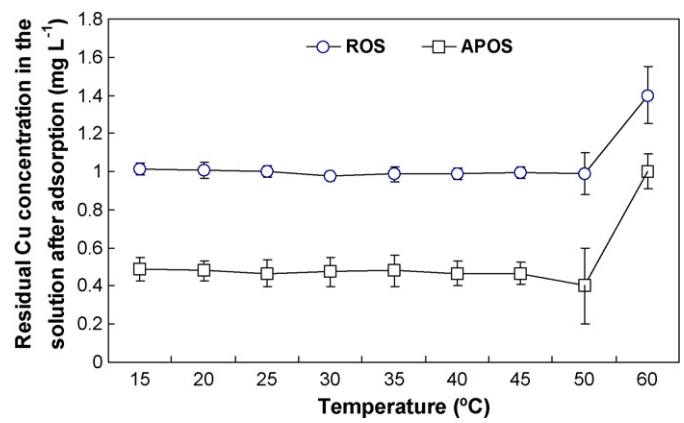
#### 3.1. Copper (Cu) removal efficiencies (CREs) from the CuSO<sub>4</sub>·5H<sub>2</sub>O added solution

To date, many industrial, agricultural and seafood by-products have received considerable interest for metal removal due to their excellent metal-binding capacities and low cost as compared to activated carbon and non-biomass adsorbents such as chitosan, zeolites, clay, fly ash, coal and oxides [2,6]. In comparison with these adsorbents reported, the OS is very easy to be processed into particles or powder upon request.

Nowadays, the Cu-containing wastewater is very common. Therefore, we first evaluated the Cu removal by OS from Cu-containing solution (Fig. 1). As a result, the CREs of ROS and APOS were 99% and 99.6%, respectively, even if at a lowest dose used (1 g L<sup>-1</sup>), suggesting that OS has stronger ability to remove the Cu from the solution and that its CRE can be significantly ( $p < 0.05$ ) improved by pretreatment with NaOH compared to unpretreatment.



**Fig. 5.** Effects of initial pH of the solution on CRES. Adsorption was conducted for 60 min at 32 °C in 100 ml solution with different initial pHs and containing OS (5 g L<sup>-1</sup>) and CuSO<sub>4</sub>·5H<sub>2</sub>O (100 mg L<sup>-1</sup>). The error bars represent standard deviation of the mean from three batches of experiments. APOS: alkali (NaOH)-pretreated OS, CRE: Cu removal efficiency, OS: os sepiae and ROS: raw os sepiae.



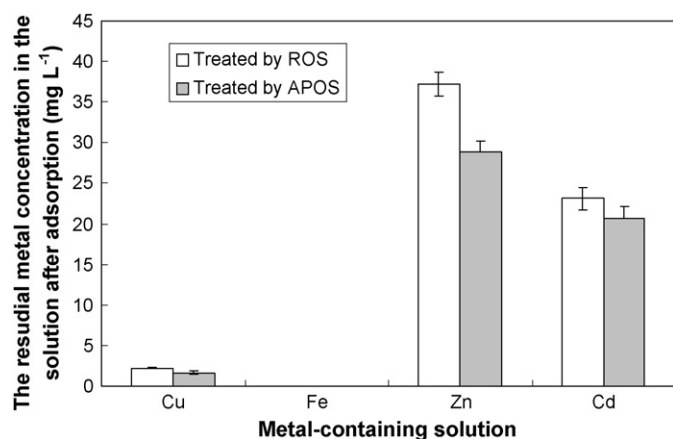
**Fig. 6.** Effects of temperature on CRES. Adsorption was conducted for 60 min at different temperatures in 100 ml solution containing OS (5 g L<sup>-1</sup>) and CuSO<sub>4</sub>·5H<sub>2</sub>O (100 mg L<sup>-1</sup>). The initial pH of the solution was adjusted to 5. The error bars represent standard deviation of the mean from three batches of experiments. APOS: alkali (NaOH)-pretreated OS, CRE: Cu removal efficiency, OS: os sepiae and ROS: raw os sepiae.

To enhance the metal adsorption capacity of the adsorbents, the adsorbents usually suffer treatment before use with some chemicals such as alkali and acid [2,3,12,13]. However, pretreatment effects depend to a great extent on the property of the adsorbents [13,14]. Usually, surface molecular groups of the adsorbents have more or less protons with a positive electrical charge, and/or can be protonated. Protonation of surface groups can weak metal absorption efficiency of the adsorbents likely because of repulsive force resulting from the same electrical charges between the protons and metal cations [15]. NaOH is generalized to be a typical industrial catalyst [16] and has deprotonation effects [17]. Additionally, the OS contains neutral mucus, plenty of neutral mucopolysaccharide and alkaline phosphatase [18]. The structures of mucopolysaccharides are easily changed in alkali solution [19]. It is thus inferred that one of reasons for the increased CRE of the OS following the NaOH-based pretreatment is likely associated with deprotonation of surface groups of the OS and in part with exposure of more functional groups that can bind metal ions to the OS surface.

#### 3.2. Estimation of capacity of maximum Cu adsorption of the OS

To estimate the ability of maximum Cu adsorption of the OS, we conducted an adsorption experiment in 100 ml adsorption system that contained different initial concentrations of CuSO<sub>4</sub>·5H<sub>2</sub>O (Fig. 2). Both ROS and APOS still showed higher CREs (over 99%) when CuSO<sub>4</sub>·5H<sub>2</sub>O added in the solution were at 1,500 mg L<sup>-1</sup>. However, the CREs of both ROS and APOS began to sharply decline when CuSO<sub>4</sub>·5H<sub>2</sub>O added was over 1,500 mg L<sup>-1</sup> and decreased to 95.7% and 97.4% at 2,000 mg L<sup>-1</sup>, respectively. Therefore, based on the CREs at 1,500 mg L<sup>-1</sup>, the capacities of adsorption of ROS and APOS were estimated to be 299.26 mg Cu g<sup>-1</sup> and 299.58 mg Cu g<sup>-1</sup>, respectively.

Because the above Cu adsorption experiments were conducted under the constant conditions of 32 °C, initial pH 5 and OS dose (5 g L<sup>-1</sup>), the resulting data were used to construct adsorption isotherms (Fig. 3). However, adsorption of both ROS and APOS were well described neither by Langmuir model nor by Freundlich model because there were no strong correlation between the maximum adsorption capacities ( $q$  values) of OS and the metal equilibrium concentrations ( $C_e$  values) in the solutions, with very low correlation coefficients. These results suggest that the single Cu<sup>2+</sup> adsorption by OS is different from that by many other biomass, whose adsorption could be fit for Langmuir model and/or Fre-



**Fig. 7.** Metal removal by OS from the mixed heavy metal solution. Adsorption was conducted for 60 min at 32 °C in 100 ml solution with OS (5 g L<sup>-1</sup>) and mixed heavy metal salts of CuSO<sub>4</sub>·5H<sub>2</sub>O, Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, ZnSO<sub>4</sub>·7H<sub>2</sub>O and CdCl<sub>2</sub>·2.5H<sub>2</sub>O (100 mmol L<sup>-1</sup> each). The initial pH of the solution was adjusted to 5. The error bars represent standard deviation of the mean from three batches of experiments. APOS: alkali (NaOH)-pretreated OS, OS: os sepiae and ROS: raw os sepiae.

undlich model, but similar to that by non-biomass [3]. These results also strongly imply that Cu adsorption by OS is not a simple electrostatic adsorption equilibrium process, likely involved in other events such as complexation reaction as well as ion exchange indicated by the follow-up experiments in this study. The reason is

likely associated with OS composition not only containing large amount of calcium as non-biomass but also having a small amount of organic matter as biomass.

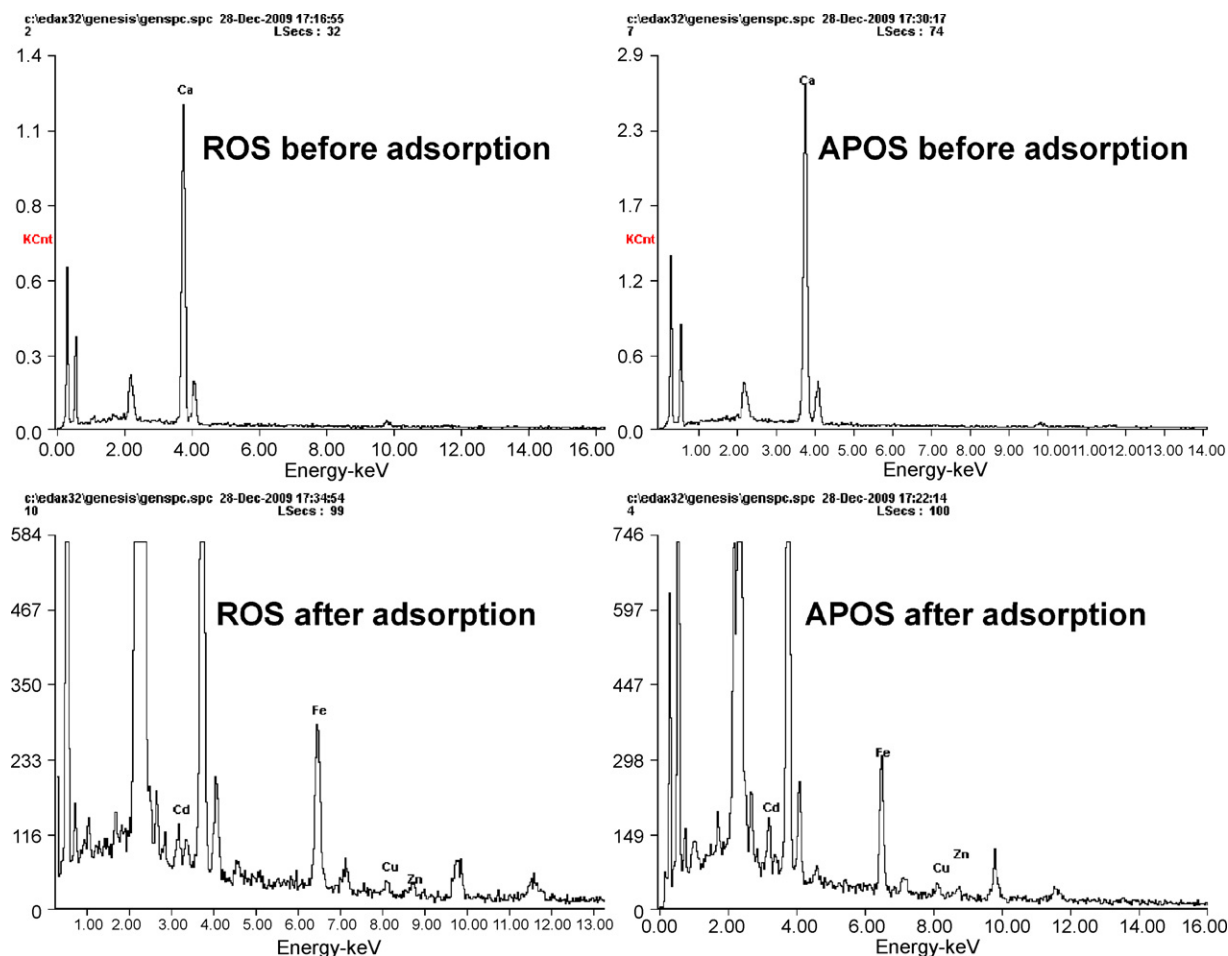
The highest CRE reported before was obtained by using citric-acid modified soybean hulls (CMSH), reaching 154.9 mg g<sup>-1</sup> [13]. The estimated CREs of ROS and APOS are about two fold higher than that of the CMSH. Taken together, the highest metal adsorption capacity for specific adsorbents seems to change with conditions of the adsorption.

### 3.3. CRE changes with contact time

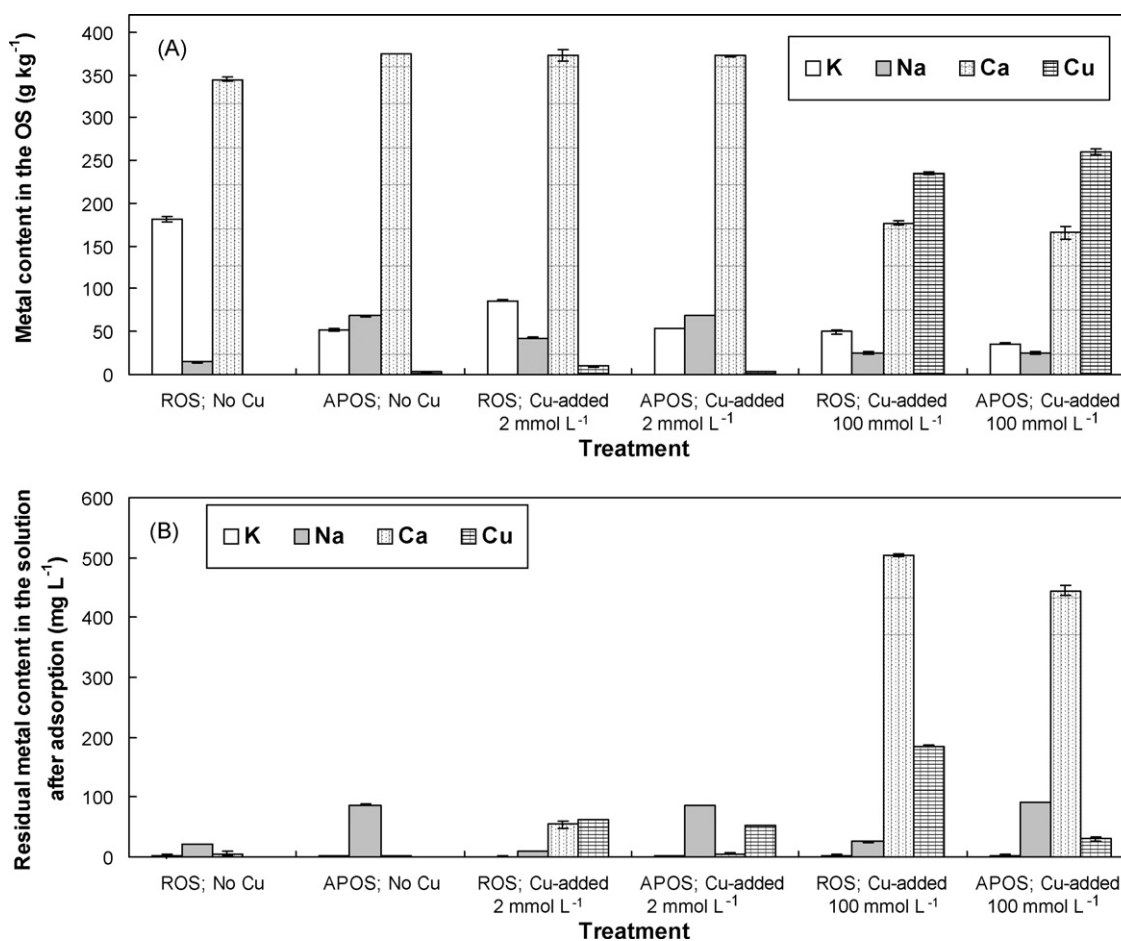
The contact time for metal removal affects to some extent the running cost in practice. We therefore assayed change in CRE over contact time (Fig. 4). As a result, the CREs of ROS and APOS were all increased with increasing contact time within 40 min. Differences between ROS and APOS in adsorption capacity at each of the time points were significant ( $p < 0.05$ ). The longer contact (over 40 min) did not significantly improve CREs, which were maintained at a level of about 99%. These results indicate that Cu adsorption by OS is a rapid process, similar to the metal biosorption by fungal biomass [3].

### 3.4. The effect of initial solution pH on the CRE

Actual industrial waste water is complicated by variable pH. Therefore, pH is an important factor affecting the metal adsorption



**Fig. 8.** Identification of metals in OS by XRDs. Adsorption was conducted for 60 min at 32 °C in 100 ml solution with OS (5 g L<sup>-1</sup>) and mixed heavy metal salts of CuSO<sub>4</sub>·5H<sub>2</sub>O, Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, ZnSO<sub>4</sub>·7H<sub>2</sub>O and CdCl<sub>2</sub>·2.5H<sub>2</sub>O (100 mmol L<sup>-1</sup> each). The initial pH of the solution was adjusted to 5. APOS: alkali (NaOH)-pretreated OS, OS: os sepiae, ROS: raw os sepiae and XRDs: X-ray diffraction spectrum.



**Fig. 9.** Ion exchange effects in the solution with a single Cu. Adsorption was conducted for 60 min at 32 °C in 100 ml solution with OS (5 g L<sup>-1</sup>) and CuSO<sub>4</sub>·5H<sub>2</sub>O (100 mmol L<sup>-1</sup>). The initial pH of the solution was adjusted to 5. (A) Metal content in the ROS. (B) Residual metal content in the solution after adsorption. The error bars represent standard deviation of the mean from three batches of experiments. APOS: alkali (NaOH)-pretreated OS; OS: *Os sepiae* and ROS: raw *os sepiae*.

by the adsorbents [2,3]. In this study, the CREs of ROS and APOS were lowest when the initial pH was 1, but the former CRE was only 74.7% and significantly ( $p < 0.05$ ) lower than 79% of the latter at this initial pH level. When initial pH changed from 2 to 5, the CREs were maintained at the level of at least 97% (Fig. 5) and showed no significance between ROS and APOS (Fig. 5).

For this experiment, the kinetic behavior of adsorption by OS could be divided clearly into first order reaction at low initial pH of less than 2, which confers OS on lower adsorption capacities and the second order reaction. In the second order reaction, OS adsorption capacity becomes stable and is highest.

Decreased adsorption efficiency at low pH often occurs in metal adsorption by adsorbents, but the mechanisms related remain unclear [3]. As for decreased OS adsorption under lower pH of less than 2, the likely reasons were associated with protonation and/or with increase in hydronium ions [H<sub>3</sub>O<sup>+</sup>] of surface groups of adsorbents [3], all of which can result in electrostatic repulsive force that strongly restrict the approach of metal cations to the adsorption sites. Additionally, pH not only alters site dissociation on the surface of the adsorbents but also strongly affects the solution chemistry of the metals [3].

The optimal pH for the adsorption of metal ions varies with adsorption systems [13,20]. For example, the optimal pH value for the Cu removal by *S. cerevisiae* is from 5–9 [20], whereas a broader pH range from 1.5 to 9.0 was found to be optimum for metal removal by industrial by-products such as iron/steel slag [13]. The pH values with less than 2 is extreme conditions, and hardly occur in the industrial effluents, therefore, initial pH in the adsorption

system seems not to be a major factor limiting metal removal by OS from the aqueous solution.

### 3.5. The effect of temperature on the CRE

Generally speaking, the temperature from most of the industrial effluent does not change greatly, but it is a factor that has to be considered in metal removal because of its effect on active binding sites in the adsorbents [21], and most importantly, because metal adsorption reactions are normally exothermic [22]. Effects of temperatures on Cu adsorption were assayed. Within a temperature range from 15 to 45 °C, the CREs of both ROS and APOS were maintained approximately at the level of 99% or more (Fig. 6). Over 45 °C, the CREs of both ROS and APOS were obviously decreased. Anyway, the former CRE was significantly ( $p < 0.05$ ) lower than the latter's at all the corresponding time points in the adsorption system. All these results support an opinion that temperature has an effect on the biosorption of metal ions, but only a limited extent within a certain range [3].

Similarly to adsorption with pH change (Fig. 5), OS adsorption also showed an obvious reaction order at the indicated temperatures: higher adsorption capacity at lower temperatures ( $\leq 45$  °C) as well as lower adsorption capacity at higher temperatures ( $> 45$  °C). The probable reasons are that higher temperatures can cause damage to the active binding sites in the biomass [21], on the other hand, increase Cu<sup>2+</sup> entropy, consequently promoting desorption of absorbed Cu<sup>2+</sup> from the adsorption sites of OS. The superiority of the OS lies in its having a stable and higher ability to

remove Cu from the aqueous solution in a wider temperature range.

### 3.6. Metal removal from the mixed heavy metal solution by OS

For the metal removal, the challenge is from the industrial effluent that contains various ionic components. Therefore, we examined the ability of the OS to remove metals from the solution containing mixed heavy metals of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{Fe}_2(\text{SO}_4)_3$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$  (Fig. 7). The results showed that ROS and APOS showed better adsorption. However, the removal efficiencies of individual metals by ROS and APOS followed the order of  $\text{Fe}$  (100%) >  $\text{Cu}$  (97–98%) >  $\text{Cd}$  (76–79%) >  $\text{Zn}$  (62–71%).

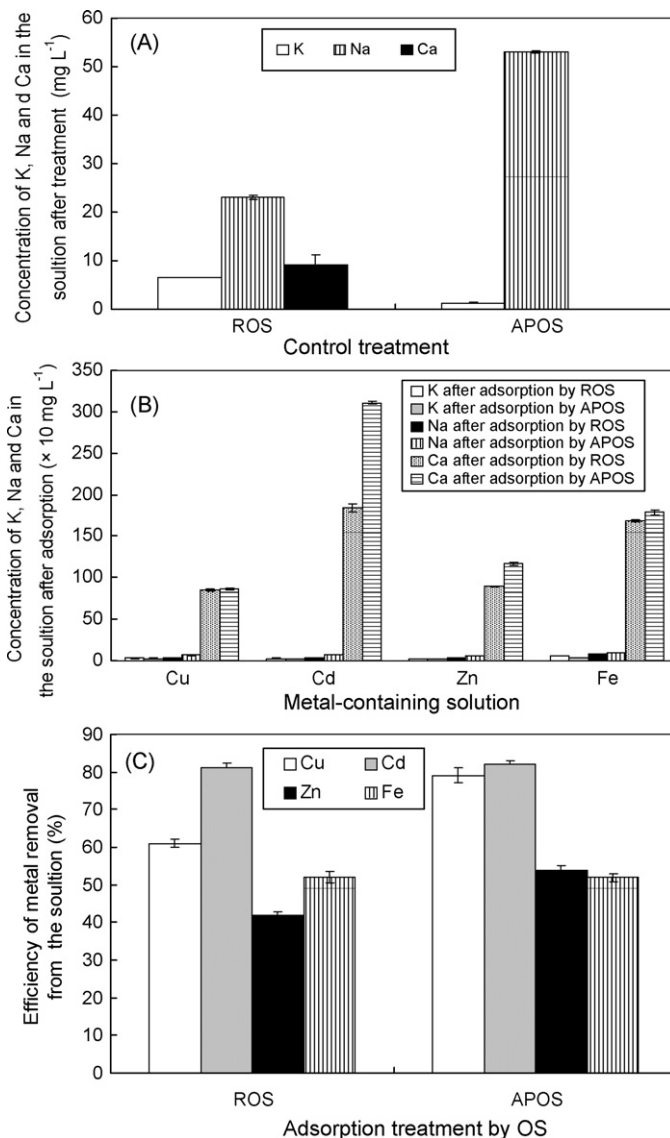
After adsorption in above solution containing the mixed metal salts (Fig. 7), metals in OS were identified by XRD (Fig. 8). Very obviously, Ca content in OS was much higher before adsorption than after adsorption. Ca content in APOS was higher than that in ROS before adsorption, suggesting that pretreatment by NaOH can increase exposure of more  $\text{Ca}^{2+}$  in OS and thereby promote exchange of  $\text{Ca}^{2+}$  in OS with metal ions in the solutions. As shown in Fig. 8, after adsorption, sequence of contents (% w/w) of metals detected was  $\text{Fe}$  (54.7%) >  $\text{Cd}$  (14%) >  $\text{Cu}$  (11.4%) >  $\text{Zn}$  (10.7%) in ROS and  $\text{Fe}$  (63.8%) >  $\text{Cd} \approx \text{Cu}$  (15%) >  $\text{Zn}$  (14%) in APOS, roughly correlating with the sequence of residual concentrations of the corresponding metals in the solution after adsorption (Fig. 7).

Differences of individual metals in the removal efficiencies is a phenomenon common to metal removal by using adsorbents from the wastewater containing multiple metals [2,3]. Fe preferential adsorption by OS from the solution with mixed metal salts is likely because Fe adsorption sites differ from those of the other three metals and is therefore not affected by competition for adsorption sites. On the other hand, the adsorption sites for Fe in OS are maybe more than those of Cu, Cd and Zn. Certainly, it cannot be ignored that  $\text{Fe}^{3+}$  has stronger electrostatic force than those of  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  in electrostatic adsorption because of its trivalent characteristics. Sequence of adsorption for divalent ions of Cu, Cd and Zn was likely associated with potential competition for adsorption sites because they all belong to divalent cations [23]. Additionally, the stability of metals in the adsorbents after adsorption is also one of factors affecting adsorption effects, which seems to depend on various factors including adsorbents and adsorption conditions. It was reported by Sakai [24] that once in the state of metal–protein complexes formed by adsorption by sea urchin egg the stability of Cu, Cd and Zn fell in the order of  $\text{Cu} > \text{Cd} > \text{Zn}$ .

### 3.7. Ion exchange in the metal removal as well as identification of metal elements in OS

Some studies have indicated that ion exchange occurs in the metal solution [10], therefore constituting an important mechanism for metal removal by adsorbents. Therefore, the ion exchange experiment was conducted. In the experiment, it was also found that OS itself is a material rich in K, Na and Ca, of which the content fell in the order of  $\text{Ca} > \text{Na} > \text{K}$  (Fig. 9A). After adsorption, the more Cu was absorbed in the OS, the lesser the native ions in the OS lost. Content of Na and Ca in the OS was significantly increased in the solution while content of the residual Cu in the solution significantly reduced (Fig. 9B).

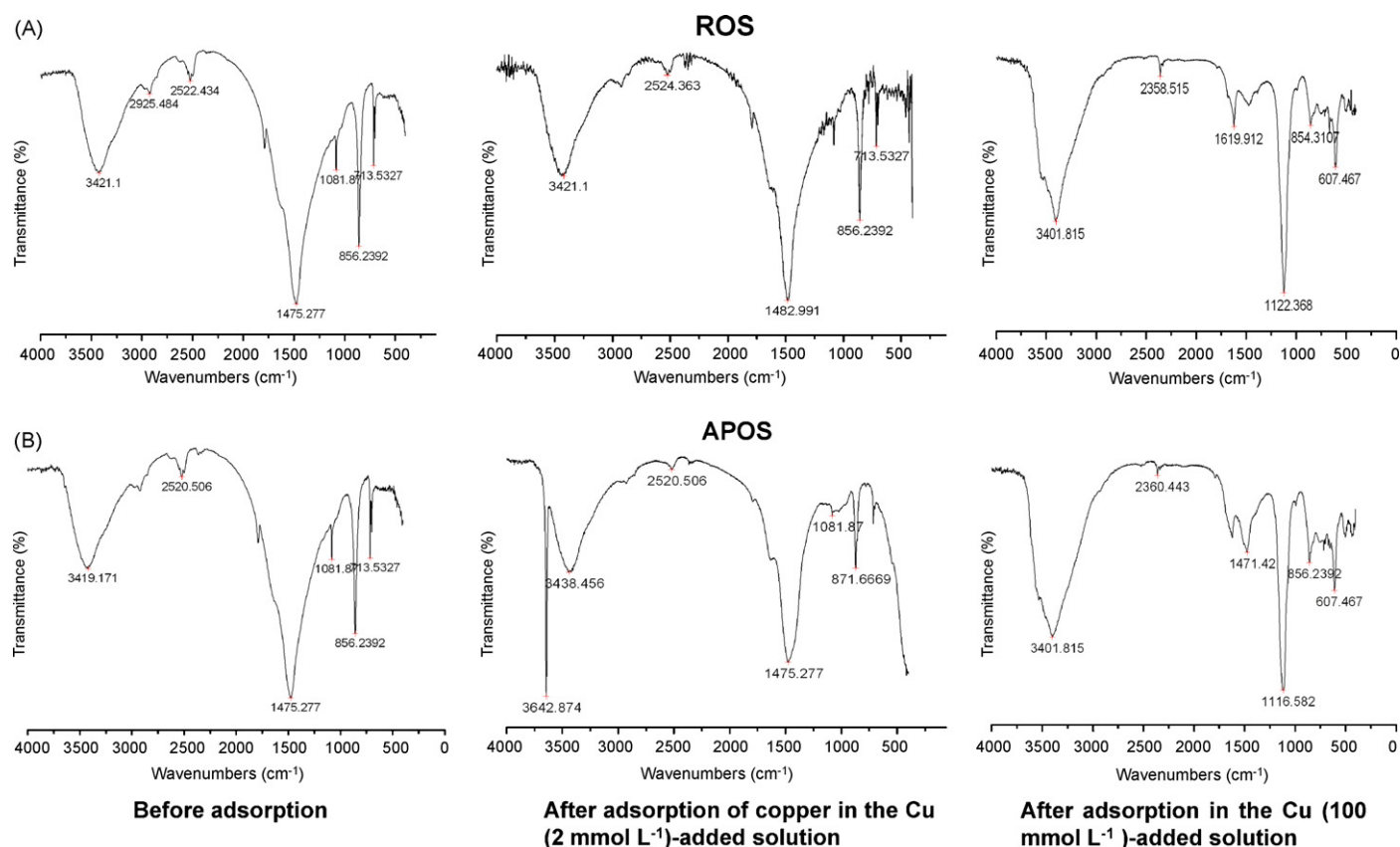
Another batches of comparative ion exchange experiments (Fig. 10) were performed in the solution with higher concentration ( $100 \text{ mmol L}^{-1}$ ) of a single metal salt:  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{Fe}_2(\text{SO}_4)_3$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ , respectively. Similar to changes shown in Fig. 9B was that only Ca concentrations were greatly increased in the solutions after adsorption while K and Na concentrations were not significantly altered at the statistic level ( $p < 0.05$ ) (Fig. 10A and B). Ca concentrations in the solutions after adsorption



**Fig. 10.** Ion exchange effects in the solution with mixed metal salts. Adsorption was conducted for 60 min at 32 °C in 100 ml solution with OS ( $5 \text{ g L}^{-1}$ ) and mixed metal salts ( $100 \text{ mmol L}^{-1}$  each  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{Fe}_2(\text{SO}_4)_3$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ ). The initial pH of the solution was adjusted to 5. The error bars represent standard deviation of the mean from three batches of experiments. APOS: alkali (NaOH)-pretreated OS; OS: *Os sepiae* and ROS: raw *os sepiae*.

by APOS were much higher than those in the solutions after adsorption by ROS (Fig. 10B). Unlike adsorption in the solution with mixed metals (Fig. 7), Ca exchange capacity occurred in Cd-containing solutions (Fig. 10B) as well as efficiencies of Cd removal from the solutions (Fig. 10C) were highest.

Taken together, metal adsorption by OS was involved in ion exchange, which occurred mainly between Ca rather than K and Na that OS itself contains and metals that were added in the solution. This is likely associated with valence matching between the ions. On the other hand, ions of equivalent valence probable have the same adsorption sites in the adsorbents. The changes in Ca amounts strongly correlated with removal efficiencies of metals but changes in both were out of proportion. This reflects likely existence of a dynamic and reversible process of exchange-adsorption during adsorption by OS.



**Fig. 11.** Infrared absorbance spectra of OS. (A) ROS, (B) APOS. Adsorption was performed in the 100 ml adsorption system containing OS (5 g L<sup>-1</sup>) and CuSO<sub>4</sub>·5H<sub>2</sub>O (2 mmol L<sup>-1</sup> and 100 mmol L<sup>-1</sup>, respectively). The adsorption experiment was conducted for 1 h at 37 °C. APOS: alkali (NaOH)-pretreated OS, OS: os sepiae and ROS: raw os sepiae.

### 3.8. Functional groups in the OS

It has been found that metal removal by biomass materials from aqueous solutions depends largely on the functional groups on the surface of the biomass [4,10]. We therefore analysed the changes of functional groups of ROS and APOS before and after adsorption of Cu by using the infrared absorbance spectra. The result indicated that before adsorption OS itself is rich in a variety of functional groups (Fig. 11A), at least including  $\text{-CH=CH-}$  at 713.5327 cm<sup>-1</sup>,  $\text{CH}_2=\text{C} < \text{RR}'$  at 856.2392 cm<sup>-1</sup>,  $\text{-C-NH}_2$  at 1081.87 cm<sup>-1</sup>,  $\text{-NH}_3^+\text{X}^-$  at 2522.434 cm<sup>-1</sup>,  $\text{-C-CH}_3$  and  $\text{-CH}_2-$  at 2925.484 cm<sup>-1</sup> and  $\text{-OH}$  at 3421.1 cm<sup>-1</sup>. Most of the functional groups in ROS also presented in APOS, however, no groups of  $\text{-C-CH}_3$  and  $\text{-CH}_2-$  were found in APOS (Fig. 11B).

As for ROS after adsorption in the solution with 2 mmol L<sup>-1</sup> CuSO<sub>4</sub>·5H<sub>2</sub>O (Fig. 11A), the wave absorption peaks (WAPs) of  $\text{CH}_2=\text{C} < \text{RR}'$ ,  $\text{-C=O-O-}$ ,  $\text{-C-CH}_3$  and  $\text{-CH}_2-$  disappeared, the WAP of  $>\text{N-CH}_3$  occurred at 1482.991 cm<sup>-1</sup>, and the WAP of  $\text{-C-NH}_2$  shifted from 1081.87 to 856.2392 cm<sup>-1</sup>. Interestingly, different from the group changes after adsorption in the solution with 2 mmol L<sup>-1</sup> CuSO<sub>4</sub>·5H<sub>2</sub>O was that, after adsorption in the solution with 100 mmol L<sup>-1</sup> CuSO<sub>4</sub>·5H<sub>2</sub>O,  $\text{-NH}_3^+\text{X}^-$  appeared at 2358.515 cm<sup>-1</sup>,  $\text{-R-NH}_2$  at 1619.912 cm<sup>-1</sup>, two groups of  $\text{-C-NH}_2$  respectively at 854.3107 cm<sup>-1</sup> and 1122.368 cm<sup>-1</sup>, and  $\text{-C}\equiv\text{CH}$  at 307.467 cm<sup>-1</sup>.

As for APOS after adsorption in the solution with 2 mmol L<sup>-1</sup> CuSO<sub>4</sub>·5H<sub>2</sub>O (Fig. 11B),  $\text{-CH=CH-}$  disappeared;  $\text{-OH}$  shifted from 3419.171 to 3642.874 cm<sup>-1</sup>; a new group of  $\text{-NH}$  occurred at 23438.456 cm<sup>-1</sup>. After adsorption in the solution with 100 mmol L<sup>-1</sup> CuSO<sub>4</sub>·5H<sub>2</sub>O,  $\text{CH}_2=\text{C} < \text{RR}'$  appeared

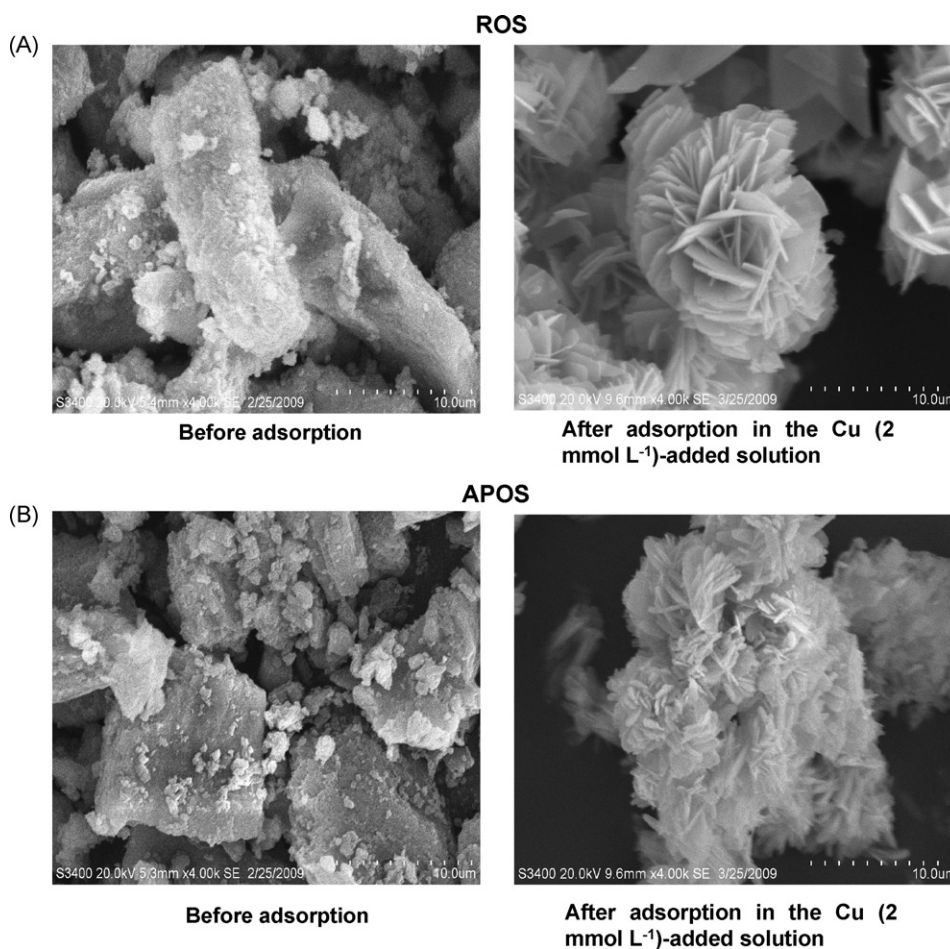
at 871.6669 cm<sup>-1</sup>;  $\text{-C-O-C-}$  at 1471.42 cm<sup>-1</sup>;  $\text{-NH}_3^+\text{X}^-$  at 2360.443 cm<sup>-1</sup>;  $\text{-C}\equiv\text{CH}$  at 607.467 cm<sup>-1</sup>.

The results strongly indicate that pretreatment with NaOH released and/or modified binding sites in OS.

Metal adsorption onto biomass-based adsorbents is closely related to surface groups or organic substances such as chitin, acetamido, alcoholic, carbonyl, phenolic, amido, amino, sulphhydryl groups and many atoms such as N, O and S [4,25]. These groups greatly determine adsorption capacity, and are associated with adsorption mechanisms such as electrostatic adsorption, adsorption-complexation on biosurface, ion exchange, microprecipitation and metal hydroxide condensation onto the biosurface [6]. Occurrence of so many groups is reasonable in OS partly because it contains a hard, outer, concave chitinous coat. Shifts of stretching bands of negatively charged groups such as  $\text{-OH}$  and  $\text{-NH}_3^+\text{X}^-$  strongly suggest that mechanisms of OS adsorption likely involve electrostatic adsorption and metal hydroxide condensation, while shifts of stretching bands of those N-containing groups such as  $\text{-NH}_3^+\text{X}^-$ ,  $\text{N-CH}_3$ ,  $\text{-C-NH}_2$  and  $\text{-NH}$  also imply that there exist adsorption-complexation followed by microprecipitation in the OS adsorption [25].

It cannot fully be determined that the all the groups with shifts of stretching bands are necessarily associated with Cu adsorption. However, such shifts may mean the complexing of the groups by dative coordination to metals [26]. That shifts of stretching bands of groups in OS varied with increasing CuSO<sub>4</sub>·5H<sub>2</sub>O concentrations implies that metal-adsorbing groups in the adsorbents are not invariable, but changeable with increasing metals in the solution due either to saturation of adsorption sites or to requirement for dative coordination. Changes in many groups together with high



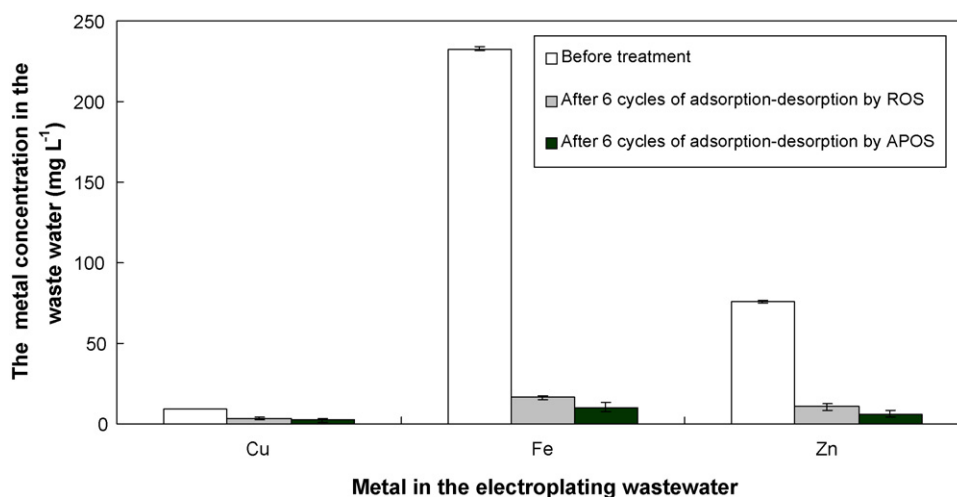


**Fig. 12.** Ultrastructure of OS before and after adsorption of Cu. (A) ROS, (B) APOS. Adsorption was conducted for 60 min at 32 °C in 100 ml solution with OS (5 g L<sup>-1</sup>) and CuSO<sub>4</sub>·5H<sub>2</sub>O (100 mmol L<sup>-1</sup>). The initial pH of the solution was adjusted to 5. The ultrastructure was observed by SEM. APOS: alkali (NaOH)-pretreated OS, OS: os sepiae, ROS: raw os sepiae and SEM: Scanning electron microscopy.

metal adsorption capacity of OS suggest that plentiful groups with strong dative coordination are one of prerequisites for high metal adsorption capacity of adsorbents, and can partly explain why Cu adsorption of both ROS and APOS could not be well described by both Langmuir and Freundlich models.

### 3.9. Ultrastructure of the OS

Ultrastructures of ROS and APOS were observed by SEM. As a result, ROS (Fig. 12A) showed no difference from APOS (Fig. 12B) in the ultrastructure before and after adsorption of Cu in the solution



**Fig. 13.** Metal removal by OS from electroplating wastewater after 6 cycles of adsorption–desorption. Adsorption was conducted for 60 min at 32 °C in 100 ml electroplating wastewater with OS (5 g L<sup>-1</sup>). Desorption of metal adsorbed on the OS was conducted in ethylenediamine tetraacetate solution (0.5 mmol L<sup>-1</sup>). The initial pH of the solution was adjusted to 5. The error bars represent standard deviation of the mean from three batches of experiments. APOS: alkali (NaOH)-pretreated OS, OS: os sepiae and ROS: raw os sepiae.

containing 2 mmol L<sup>-1</sup> CuSO<sub>4</sub>·5H<sub>2</sub>O. The most significant change was that after adsorption both ROS and APOS presented a rose-like shape. Seemingly, rose-like crystals of APOS were denser than those of ROS. This is likely that APOS obtained larger specific surface areas than ROS owing to NaOH treatment. Although it was unknown how such changes of OS in ultrastructures after adsorption formed, this change was certainly related with the adsorption of Cu.

### 3.10. Metal removal from the actual electroplating wastewater by the ROS and APOS

Cycle use of the absorbents is directly associated with the cost of wastewater treatment in metal removal. We evaluated metal removal effects by ROS and APOS after adsorption–desorption from actual electroplating wastewater (Fig. 13). After 6 cycles of adsorption–desorption, ROS exhibited removal efficiency of 93%, 86.1% and 61.2%, respectively, for Fe, Zn and Cu while APOS showed higher removal efficiency, with 95.6% for Fe, 91.8% for Zn and 77.4% for Cu. The sequence of Cu and Zn adsorption by OS in this industrial wastewater was opposite to the result of adsorption in the solution with mixed metals (Fig. 7) likely because of great difference between solutions. Usually, a part of metals in electroplating wastewater is in complexation. After NaOH pretreatment, an alkaline microenvironment likely formed on the OS surface. Such alkaline microenvironments can effectively cause disintegration of the complex, thereby enable dissolution of complex metals from the complex [27], and consequently increase chance of metal adsorption by OS. Certainly, alkaline microenvironments can also facilitate metal microprecipitation. As shown in Fig. 12, NaOH pretreatment did increase the specific surface areas of OS. All these factors gave APOS a metal adsorption capacity higher than that of ROS in actual electroplating wastewater.

## 4. Conclusions

OS of *S. esculenta* is rich in K, Na and Ca. Cu adsorption by ROS can be significantly improved by pretreatment with NaOH and by appropriately increasing initial pH in the solution. However, the Cu adsorption is hardly affected by temperature change within a wide range of 15–45 °C. Metal adsorption-promoting effects by NaOH pretreatment likely involve deprotonation of surface groups in OS, exposure of more functional groups, and increase in specific surface areas. Cu adsorption by OS is not a simple electrostatic adsorption equilibrium process. High metal adsorption of OS is likely associate with plentiful groups with strong dative coordination. Metal adsorption by OS falls in the order of Fe > Cu ≈ Cd > Zn in the solution with mixed metals of Fe, Cu, Cd and Zn, but follows the sequence of Cd > Cu > Fe ≈ Zn in the solutions respectively, with a single metal of Fe, Cu, Cd and Zn. The mechanisms of metal adsorption by OS depends partly on ion exchange, which occurred mainly between Ca rather than K and Na that OS itself contains and metals that were added in the solution. Ion exchange efficiencies are determined greatly by valence matching between ions. It can be also further reasoned out that related mechanisms also likely include electrostatic adsorption, surface complexation and even micro-deposition. OS is a very promising absorbent for metal removal from aqueous solutions, with the high capacities of multi-metal adsorption in the solutions.

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