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Characteristics and mechanisms of Cu(II) biosorption by disintegrated aerobic granules

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ABSTRACT

Disintegrated aerobic granules (DAG) as an effective biosorbent had great potential to remove Cu(II) from aqueous solution. The effects of solution pH value, contact time, initial Cu(II) concentration on the biosorption were investigated. Kinetic studies indicate that pseudo-second-order model with correlation coefficients of 0.9999 best fits the Cu(II) biosorption process. Investigation of the biosorption mechanisms shows that Cu(II) biosorption is associated with a significant release of Ca(II). The adsorption capacity of extracted extracellular polymeric substances (EPS) was 2.34 times as much as that of pristine DAG, indicating the significant role of EPS in adsorption. In order to determine the role of different functional groups, DAG was chemically modified to block specific functional groups and was then used in the adsorption of Cu(II). The anionic carboxyl group, was identified as the key binding site for the cationic Cu(II). Results reveal that ion exchange is the most important biosorption mechanism but other mechanisms to some extent like electrostatic interaction, involving in functional groups, also play a part.

1. Introduction

To date, aerobic granules process have been a promising technology for wastewater treatment owing to its advantages such as strong structure, excellent settleability, and high biomass retention [1,2]. The aerobic granular sludge has been used for the treatment of intermediate- and high-strength wastewaters containing organics, nitrogen, phosphorus, and heavy metals [3–6]. However, larger aerobic granules often disintegrate into detached fragments due to diffusion limitation [3,7–9] which would further result in failure in wastewater treatment and leave disintegrated aerobic granule (DAG) to be treated. So, a suitable reuse of the DAG is in accordance with sustainable development and the needs for the development of sludge disposal methods.

Extracellular polymeric substances (EPS) play an important part in the formation of aerobic granules. EPS mainly consist of polysaccharides (PS) and proteins (PN). In our previous work, EPS played an important role in the binding of Zn(II) and Co(II) to aerobic granules [10]. Since the amount of EPS increased during the storage of aerobic granules [11,12], it will be a promising technique to reuse DAG as metals ion adsorbent directly.

This study aimed to investigate the performance and mechanisms of the adsorption of heavy metals by DAG as a novel biosorbent. Cu(II) was selected as a typical candidate of heavy metals pollution. In order to achieve this purpose, the study was conducted as follows: Adsorption performance of DAG in the removal of Cu(II) from aqueous solution was evaluated under different pH conditions. Kinetics models were explored to describe the experimental data under two different Cu(II) concentrations. The percentages of ion exchange and electrostatic interaction played in the biosorption were also evaluated. It is expected that the findings of our research could provide useful information for the selection of cost-effective biosorbents and enrich the current knowledge on aerobic granules self-disintegration.

2. Materials and methods

2.1. Cultivation and storage of aerobic granules

Aerobic granules were cultivated in an internal-circulate sequencing batch reactor (SBR) fed with glucose as the sole carbon source as described by [11]. Briefly, the reactor was fed with synthetic wastewater and operated sequentially in 6 h cycles, with 2 min of substrate filling, 355 min of aeration, 2 min of settling and 1 min of effluent withdraw. After about 450 days operation, the aerobic granular sludge in SBR gradually began to disintegrate. The collocted aerobic granules were washed with tap water thrice and centrifuged at 3500 × *g* for 10 min to remove supernatant and then were transferred into a 1 L glass bottle with a total liquor volume of 0.8 L, resulting in 7000 mg/L dry weight granules and 3.3 mg/L

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dissolved oxygen (DO) finally. The bottle was sealed with a rubber stopper, and then stored at 4 °C for 24 months.

2.2. Chemicals

Stock solution of Cu(II) (approximately 2000 mg/L) was prepared by dissolving $CuCl_2{\cdot}2H_2O$ (analytical grade, obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) in deionized water and further diluted to the concentrations required for the experiments. The pH was adjusted by adding 0.01 or 0.1 mol/L HCl and 0.01 or 0.1 mol/L NaOH. Concentration of the free Cu(II) ion was calculated after calibrating with potential response of the copper ion-selected electrode (Ruosull Technology Ltd., Shanghai, China) [13].

2.3. Batch biosorption experiments

Biosorption experiments were conducted using 50 mL shaking flasks, to which 25 mL of Cu(II) working solution (40 and 80 mg/L) and 0.4 g wet weight (proportion of water 96.68%) of biosorbent were added. These flasks were reciprocated end-over-end in a water-bath shaker (temperature 35 ± 1 °C) with a shaking rate of 200 rpm. All the experiments were carried out in duplicates, and abiotic controls with the same Cu(II) solution were run in parallels. After 3 h of adsorption, the Cu(II) solutions were allowed to settle for 30 s and the supernatant was then taken out to determine the residual Cu(II) concentration. In the effect of pH study, the Cu(II) solution (fixed as 80 mg/L) was adjusted to the desired pH (2–5) with HCl or NaOH.

In the kinetic sorption experiments, series of 50 mL glass beaker containing 0.4 g of DAG and 25 mL of Cu(II) solution were shaken at 200 rpm at natural pH. Samples of each glass beaker were withdrawn at required time intervals and then analyzed for residual Cu(II) concentrations in the solution.

2.4. Ion exchange procedure

In order to investigate the mechanisms of heavy metal biosorption by DAG, the amounts (meq/g) of biosorbed heavy metals on DAG as well as light metal ions (Ca(II), Mg(II) and K(I)) released into the solution during biosorption experiment were analyzed by Inductively Coupled Plasma Emission Spectrometry (IRIS Intrepid II XSP, American) [14]. The initial pH was kept at 5.0 ± 0.1 [15,16] and all the experiments were conducted at 35 ± 1 °C.

2.5. Chemical blocking of functional groups

The DAG were chemically treated in different methods to block the functional groups as carboxyl, amino, phosphate and hydroxyl groups to understand the role of these groups in the present adsorption process. These functional groups were blocked according to the following reactions [17–19]:

$$\text{R-COOH} + \text{CH}_3\text{OH} \xrightarrow{\text{H}^+} \text{R-COOCH}_3 + \text{H}_2\text{O} \quad (1)$$

$$\text{R-NH}_2 + 2\text{HCHO} + 2\text{HCOOH} \rightarrow \text{R-N(CH}_3)_2 + 2\text{CO}_2 + 2\text{H}_2\text{O} \quad (2)$$

$$\text{R-PO}_4\text{H}_2 + (\text{Et})_3\text{PO}_3 + \text{CH}_3\text{NO}_3 \rightarrow \text{R-PO}_4(\text{Et})_2 \quad (3)$$

$$\text{R-CH}_2\text{OH} + (\text{CH}_3\text{CO})_2\text{O} \rightarrow \text{R-CH}_2\text{OCOCH}_3 + \text{CH}_3\text{COOH} \quad (4)$$

The modified DAG was thoroughly washed with deionized water till reaching a neutral pH, and used for Cu(II) adsorption at pH 5 with Cu(II) concentration at 80 mg/L.

2.6. SEM, EDX and FT-IR analysis

The surface morphologies and elemental compositions of DAG before and after Cu(II) biosorption were examined by SEM (S-570, Japan) coupled with EDX (Oxford INCA X, Japan). FT-IR spectra of the pristine and chemically modified DAG before and after adsorption were recorded on a FT-IR spectrometer (Avatar 370, USA) to identify and evaluate the functional groups that might be involved in the sorption process. The functional groups were also characterized with the binding energies of C, N and O atoms by EDX to further understand the role of functional groups in biosorption of Cu(II) in detail. Before analysis, the biomass was freeze-dried and then grounded to powder.

2.7. EPS extraction and chemical analysis

In this study, two kinds of biosorbents were used, i.e., DAG and EPS extracted from DAG. DAG taken from the parent glass bottle were gently washed three times using tap water, and were designated as DAG. To extract the EPS, part of DAG were centrifuged at 3000 rpm for 15 min and then the pellets were resuspended in a buffer solution consisting of 2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9 mM NaCl and 1 mM KCl (pH 7) to their original volume (30 mL). Later, the solution was transferred to an extraction beaker, followed by the cation exchange resin (CER) addition with a dosage of 75 g/g volatile suspended solids (VSS) [14,20]. These suspensions were stirred for 60 min at 200 rpm. After removing CER by settlement and removing remaining sludge components by high speed centrifugation at 8000 rpm for 60 min, the supernatants were then filtered through 0.22 μm cellulose acetate membranes and used as the EPS fraction for chemical analysis and adsorption experiments. The content of PS and PN was determined by the phenol/sulphuric acid method and modified Lowry method, respectively [21,22], The (suspended solids) SS and VSS of the sludge were determined according to the Standard Methods [23].

3. Results and discussion

This mature granule was nearly spherical in shape and buff in color which had a compacted and integrated structure (Fig. 1a) with several small peaks on the surface. Most of granules were disintegrated during long term storage. DAG was in an irregular structure, and had a large hollow (Fig. 1b) or even split into detached fragments completely. Prior to biosorption tests, DAG were gently washed with deionized water three times.

3.1. Biosorption kinetics

Fig. 2 shows the relationship between contact time and Cu(II) adsorption by DAG at different metal concentrations of 40 and 80 mg/L. Results vividly indicate that the uptake of Cu(II) by DAG rapidly reaches equilibrium within 35 and 20 min for metal concentrations of 40 and 80 mg/L, respectively. Moreover, it is noticeable that the rate of Cu(II) uptake was very rapid in the first 10 min. This rapid initial biosorption was consistent with the results reported by Gai et al. [24] in which the time required for equilibrium was 30 min at an initial Cu(II) concentration of 125 mg/L. It also can be seen from Fig. 2 that the initial metal concentrations of Cu(II) has significant impact on equilibrium. This could be attributed to the different amount of metal ions on the surface of the granules and the higher driving force and the active sites [25]. Whereafter the Cu(II) concentration in solution decreases, the lower concentration leaded to a lower driving force, and the remaining active sites with lower affinities are slowly occupied.

Lagergren pseudo-first-order and second-order kinetic models [26,27] were used to evaluate the kinetics of the Cu(II) biosorption

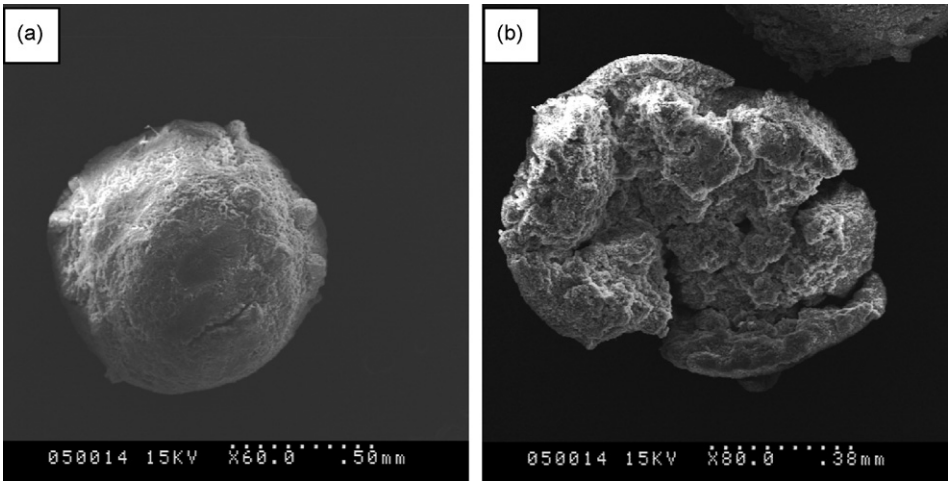

Fig. 1. SEM images of integrated (a) and disintegrated (b) aerobic granules.

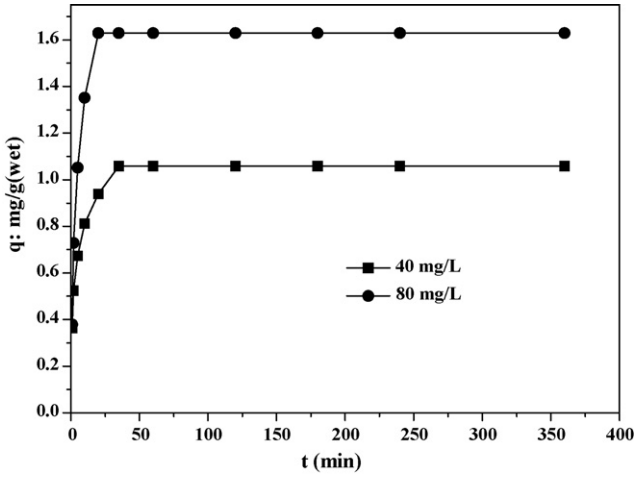

Fig. 2. Effect of contact time and initial Cu(II) concentration on the Cu(II) biosorption with DAG: 35 ± 1 °C; adsorbent dose = 16 g/L.

on DAG. The first order rate expression of Lagergren is given as:

$$\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} \quad (5)$$

where q_e and q_t are amounts of Cu(II) adsorbed (mg/g) at equilibrium and time t (min), respectively, and k_1 is the rate constant of pseudo-first-order (1/min).

The sorption kinetics may be described by a pseudo-second-order model. The second order kinetic model is expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (6)$$

where k_2 is the equilibrium rate constant of pseudo-second-order sorption (g/mg min).

Adsorption kinetic constants and correlation coefficients at different initial Cu(II) concentrations are given in Table 1. Compared with the pseudo-first-order equation, it can be noticed that the correlation coefficients of the pseudo-second-order kinetic model completely reach 0.9999 at different initial Cu(II) concentrations and the calculated adsorption capacities (q_e) matched well with the experimental ones ($q'_{e,exp}$). Therefore, the pseudo-second-order model was more suitable to describe the biosorption kinetic process accurately. The pseudo-second-order rate expression is in accordance with the reaction mechanism involving valency forces and ion exchange. The biosorption of Cu(II) onto DAG may be controlled by the chemical processes involving valency forces and ion exchange [25,28], which is used to discuss on the subsequent biosorption mechanism.

For the initial concentration of 40 mg/L Cu(II), the maximum adsorption amount was 49.39 mg/g SS for DAG in this study. While Gai et al. [24] reported that, the maximum adsorption capacity was 39.84 mg/g SS at 250 mg/L of Cu(II) for integration aerobic granules (IAG). The compared results demonstrated that DAG could be used as an effective adsorbent to remove Cu(II) probably due to its larger specific surface area.

3.2. Effect of initial pH on Cu(II) biosorption

The effect of pH on removing Cu(II) from aqueous solution by DAG was studied at various pH (ranking from 2 to 5) [17,29]. Fig. 3 shows the effect of pH on Cu(II) adsorption onto DAG and the shifts in pH after adsorption. The pH of the equilibrated solution increased at initial pH (2–4), while it decreased when the initial pH was 5. Considering the PZC value of lying between pH 3 and 4 for many microbial species [24], this may be due to the continuous protonation and deprotonation of DAG at initial pH in the range of 2–5. At lower pH (2–3), the H_3O^+ and Cu(II) protons compete for binding sites on the cell wall, and result in lower Cu(II) uptake. As the pH increased from 3 to 5, the Cu(II) uptake also increased due to the attractive electrostatic interactions [30] and reached 2.08 mg/g at pH 5. In this study, experiments were not conducted beyond pH 5.5 in order to avoid Cu(II) precipitation in the form of hydroxide [31].

Table 1
Comparison of pseudo-first-order and pseudo-second-order kinetic model parameters obtained at different initial Cu(II) concentrations.

C_0 (mg/L)	Pseudo-first-order			Pseudo-second-order			Measured
	K_1 (1/min)	q_e (mg/g)	R^2	K_2 (g/(mg min))	q_e (mg/g)	R^2	$q'_{e,exp}$ (mg/g)
40	0.0888	0.6559	0.9779	0.8833	1.0622	0.9999	1.0578
80	0.1602	1.3403	0.9869	0.3812	1.6399	0.9999	1.6290

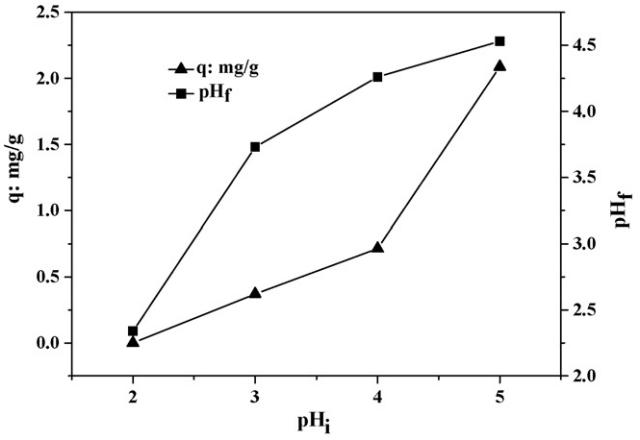

Fig. 3. Effect of initial pH on the Cu(II) biosorption with DAG: 35 ± 1 °C; 3 h; C_0 = 80 mg/L, adsorbent dose = 16 g/L.

3.3. SEM and EDX analysis

In the present study, SEM and EDX were conducted to investigate the metal interaction with the biomass before and after Cu(II) biosorption. The results are presented in Fig. 4 and Table 2. A lot of cavities and coccoid bacteria (Fig. 4a and c) were present on the coarse surface of the granule before Cu(II) biosorption respectively. After contacting with metal ions, the surface of biomass became more compact and less sharply defined with the presence of some particles (Fig. 4b) and the coccoid bacteria appeared somewhat crinkled (Fig. 4d). These changes could be the result of chelation between Cu(II) and macromolecular weight organics such as polysaccharide or protein. Similar findings were previously reported by [32] in the investigation of Cd(II) and Cu(II) removal from aqueous solution using fungi *Botrytis cinerea*.

Table 2
Elemental compositions of DAG before and after biosorption.

Element	Before biosorption		After biosorption	
	wt%	at%	wt%	at%
C	45.39	52.59	32.26	39.03
N	10.41	10.35	9.33	9.68
O	41.09	35.74	35.74	49.64
Mg	0.39	0.22	0.33	0.20
P	0.92	0.41	1.93	0.91
S	0.83	0.36	0.72	0.33
K	0.05	0.02	0.02	0.01
Ca	0.92	0.32	0.21	0.08
Cu	–	–	0.55	0.13

Table 2 shows that C, O, P and Ca constitute the four major elements of DAG. As for heavy metals, element Cu was not detected in DAG, while very small amount of K and Mg at the respective (dry) weight percentage of 0.05% and 0.39% were found in pristine DAG. After Cu(II) biosorption, a remarkable amount of Cu (0.55%) was adsorbed by DAG, while the Ca, K and Mg peaks decreased from 0.92%, 0.05%, 0.39% to 0.21%, 0.02%, 0.33%. These

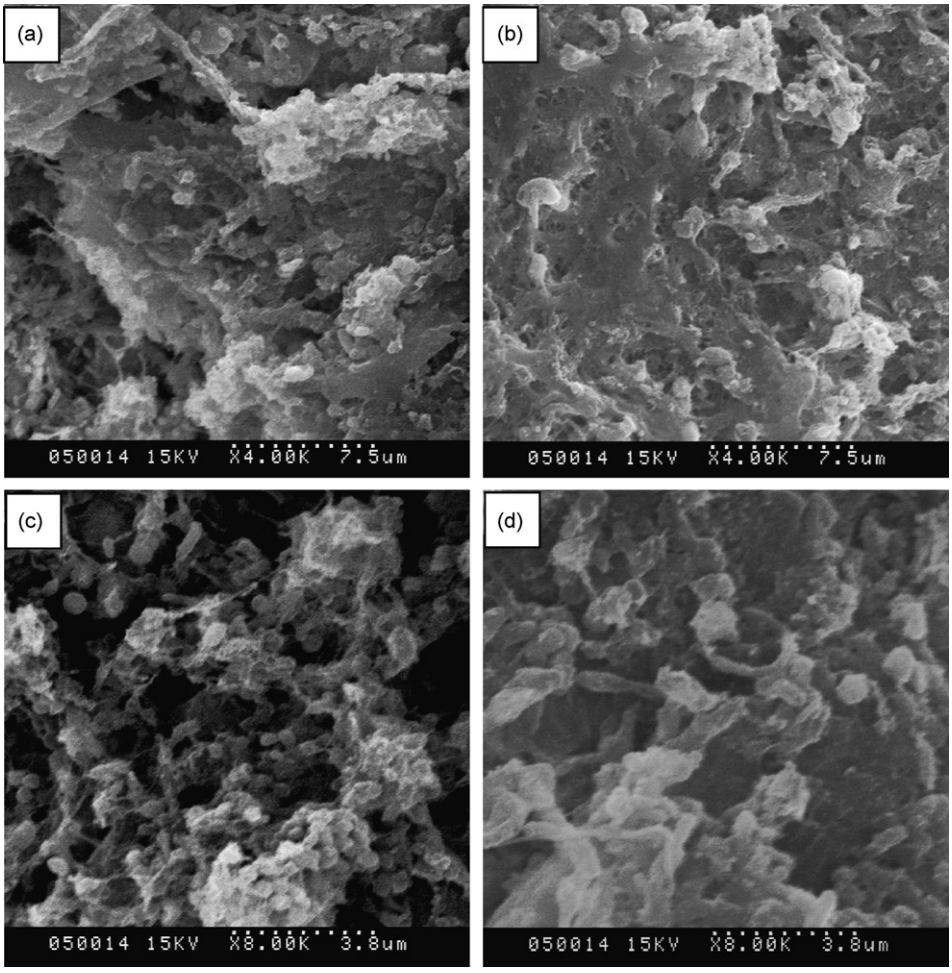

Fig. 4. SEM images of aerobic granular biomass before and after biosorption. (a) and (c) Before Cu(II) biosorption, (b) and (d) after Cu(II) biosorption.

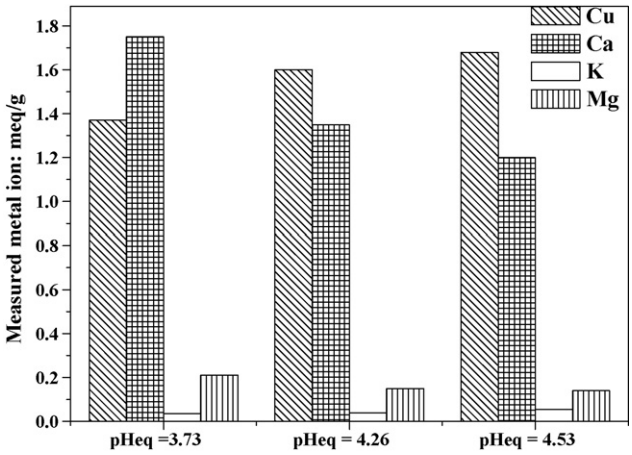

Fig. 5. K(I), Ca(II) and Mg(II) released from DAG and Cu(II) uptake capacity during Cu(II) biosorption at an initial Cu(II) concentration of 80 mg/L (temperature $35 \pm 1\,^{\circ}C$).

results implied that the ion exchange was involved in the Cu(II) biosorption.

3.4. Ion exchange-associated biosorption

The biosorption mechanisms, including ion exchange, physical adsorption, chelation, complexation, possibly are involved in the heavy metals adsorption processes.

As shown in Fig. 5, some light metals, e.g. K(I), Mg(II) and Ca(II) were released from the aerobic granules during the Cu(II) biosorption by DAG. The respective amount of Ca(II) and Mg(II) ions released from DAG tended to decrease with the increase of pH from 3 to 5. However, the K(I) released from aerobic granules varied little and was the least at pH 3. The total amount of K(I), Ca(II) and Mg(II) released accounted for 96.13 and 83.04% of the Cu(II) biosorption capacity at pH 4 and 5 respectively. Thus, the ion exchange mechanism would be involved in the Cu(II) biosorption by DAG. Moreover, it can be seen in Fig. 5 that the amount of metal ions released from aerobic granules is in the order of K(I) < Mg(II) < Ca(II) at the given pH. For example, at pH 5, the percentage of K(I), Mg(II) and Ca(II) released over adsorbed Cu(II) is 3.27%, 8.33% and 71.42%, respectively. The elemental composition analysis of DAG through EDX revealed that the Ca, Mg and K contents accounted for 32.00%, 22.00% and 2.00% by dry weight respectively in all element composition. The content of K(I) was lowest, while the amount of Ca(II) was the dominant metal element among them, indicating that ion exchange may be involved in the adsorption of Cu(II).

Results indicate that the release of light metals from DAG during the biosorption process can be mainly explained by the ion exchange mechanism. But the amount of Cu(II) adsorbed from solution was not in accordance with the amount of Ca(II) + Mg(II) + K(I) released into aqueous solution. This difference can be explained by the unmeasured light metals released or by other mechanisms, e.g. chemisorption involving valency forces, during the biosorption process [33]. The amount of light metal ions released from the aerobic granules exceeded the amount of adsorbed Cu(II) at pH 3. The large amount of the released Ca(II) at pH 3 may also be attributed to the effect of H-ion through ion exchange. Therefore the exchangeable light metal ions were in excess. It could be concluded that lower pH had a positive effect on the release of Ca(II).

3.5. EPS-associated Cu(II) biosorption by DAG

DAG and EPS extracted from the same amount of DAG were both used as biosorbents to remove Cu(II) from aqueous solution. In this study, the content of overall EPS was 164.79 mg/vss with 139.30 mg/vss of PS and 25.49 mg/vss of PN. The biosorption capacity of EPS towards Cu(II) was about 3.82 mg/g, while it was 1.63 mg/g for DAG. The contribution of extracted EPS as a biosorbent of Cu(II) was 2.34 times as much as the adsorption capacity of pristine DAG, indicating the significant role of EPS in the adsorption process. Previous research also shows the involvement of EPS in the heavy metal biosorption [15,17].

However, EPS on DAG could not act efficiently mainly due to the obstruction of the spatial structure of granules. For this markedly improved adorption of Cu(II), besides the contribution of EPS, it could be also attributed to involve in the use of a buffer consisting phosphate during EPS extraction, which can precipitate with Cu(II).

3.6. FT-IR spectra and Cu(II) biosorption by chemically modified DAG

FT-IR is a useful tool to provide direct information about the presence of functional groups in a molecular in the range of 400–4000 cm^{-1}. Fig. 6 shows the FT-IR spectroscopy of pristine, Cu-loaded and chemical modified DAG. Wave numbers for main bands are summarized in Table 3.

As seen in the spectrum of DAG, a broad and intense peak at 3424.62 cm^{-1} is due to the stretching vibration of –OH and $-NH_2$. The bands at 2923.66 and 2849.26 cm^{-1} would be due to an asymmetric vibration and a symmetric vibration of CH_2 respectively. A distinct band at 1650.56 cm^{-1} was the result of C=O and C–N (amide I) stretching vibration of protein, while the amide II bands at 1543.06 and 1556.55 cm^{-1} showed stretching vibration of C–N and deformation vibration of N–H (amide II) of protein. The bands at 1454.33 and 1401.16 cm^{-1} corresponding to C–H bending from proteins may also have a contribution from an amine III. The absorption band at 1046.75 cm^{-1} may be susceptible to overlap C–OH stretching of polysaccharide groups and the P–O vibration of the phosphate groups [10,13,17]. Thereby, functional groups such as carboxyl, amino, phosphate and hydroxyl groups were proved to present on DAG.

After adsorption of Cu(II), peaks of –OH, $-NH_2$ stretching and C=O, C–N (amide I) moved to higher wave numbers while C–N stretching and N–H (amide II) deformation shifted to a lower wave number (Table 3), indicating different functional groups would be involved in the biosorption process of Cu(II). The broad stretching absorption band at 3424.62 cm^{-1} shifted to 3435.11 cm^{-1}, but it is difficult to distinguish which group causes the shift. Band at 2849.26 cm^{-1} which could also be assigned to N–H stretching of amine I or II and –OH stretching of polymeric compounds disappeared after Cu(II) adsorption. These changes of wave numbers provided evidence for the involvement of amine and hydroxyl in the carboxyl groups in the reaction. The band at 1046.75 cm^{-1} was dominated by a sequence of peaks due to C–OH and C–O–P stretching vibrations of polysaccharides [10,13,14,19], showing the presence of phosphate and hydroxyl groups in the biomass. After the exposure to Cu(II), –OH and $-NH_2$ stretching moved to a higher wave number (1046.75 cm^{-1}). It indicates the important roles of amine and hydroxyl groups played in the adsorption process. The EDX analysis also shows the presence of C, N, O and P on the surface of the biomass before biosorption (Table 2). The atomic mass percentages of C and N decreased after adsorption which confirm the important roles of amine and carboxyl groups in the adsorption process.

Functional groups which contained carboxyl, amino, phosphate and hydroxyl groups were investigated on their role in biosorpion of metal ion. The blocking of functional groups resulted from chemical treatments was examined with FT-IR. Fig. 6M_1 shows the FT-IR spectrum of biomass treated with methanol and hydrochloric acid. The peak area at 3432.93 cm^{-1} notablely became smaller than that

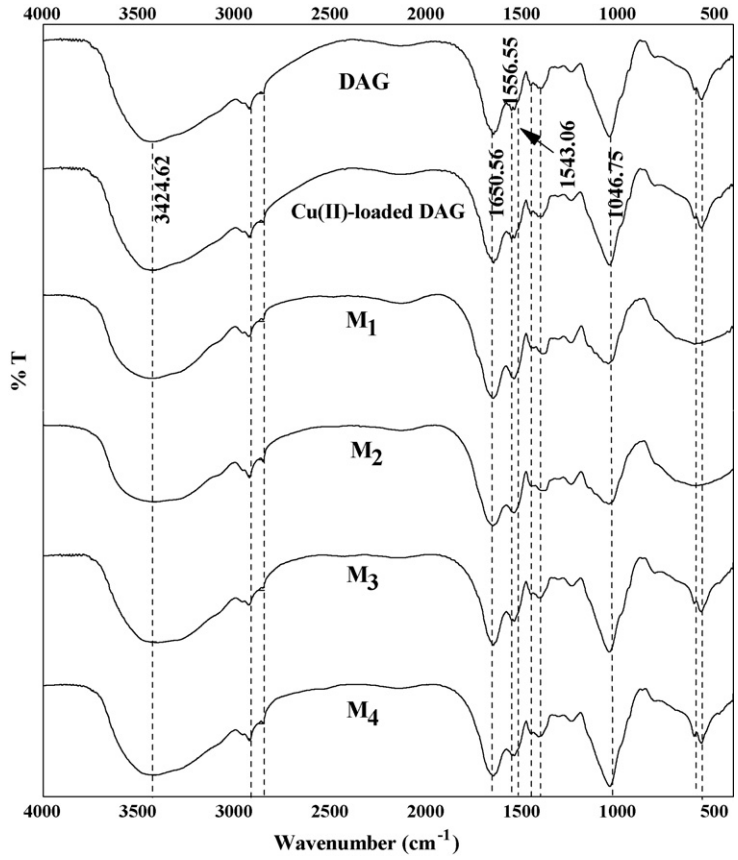

Fig. 6. Wave numbers (cm^{-1}) of dominant peaks obtained from FT-IR spectra of pristine, Cu(II)-loaded and modified DAG (M_1 – carboxyl blocking, M_2 – carboxyl blocking, M_3 – carboxyl blocking, M_4 – carboxyl blocking).

of the pristine DAG, which suggests the successful blocking of carboxyl group as a result of the esterification reaction. The treatment also resulted in a trough at 1309.68 cm^{-1} which is indicative of the presence of methanol, showing that traces of methanol remained on the biomass even after thorough washing. Fig. 6M_2 shows the FT-IR spectrum which was obtained for biomass treated with formic acid and formaldehyde. The peak area at 3432.93 cm^{-1} became smaller like Fig. 6M_2 showed. The peaks of C–N, N–H (amine II) at 1556.55 cm^{-1} disappeared, indicating the effective methylation of amine group during the chemical modification process. Fig. 6M_3 shows the FT-IR spectrum of biomass which received nitromethane and triethyl phosphite treatment. This spectrum shows an increase of the trough at 1046.75 cm^{-1}, which could be due to the esterification of phosphate groups. Fig. 6M_4 shows the FT-IR spectrum of biomass which treated with 0.1% acetic anhydride dissolved in ultra dry petroleum ether. The change of peak area at 3422.28 cm^{-1} for the OH stretching was very minimal and this region was susceptible to water adsorption involving in the blocking of hydroxyl groups. This spectrum also shows a reduction of the trough from 1046.75 cm^{-1} to 1045.39 cm^{-1}, which could be due to the acetylation of hydroxyl groups.

FT-IR of pristine DAG (Fig. 6) and EDX (Table 2) displayed that different functional groups such as carboxyl, amino and phosphate groups present on DAG were chemically modified. The effects of chemical modification on Cu(II) adsorption are shown in Table 4. It is clear that blocking of amino and hydroxyl groups leads to equivalent decrease by 22.2% on Cu(II) adsorption, indicating the important role of amino and hydroxyl groups. Phosphate group

Table 3
Wave numbers for main bands in FT-IR.

Functional group	Wave number (cm^{-1})					
	Pristine DAG	Cu-loaded DAG	Carboxyl blocking	Amine blocking	Phoshpate blocking	Hydroxy blocking
–OH and $–NH_2$ stretching	3424.62	3435.11	3432.93	3422.20	3408.15	3422.28
C–H asymmetric stretching of $–CH_2$	2923.66	2925.57	2923.24	2923.51	2926.35	2922.94
C–H symmetric stretching of $–CH_2$	2849.26	–	–	2852.86	–	2843.78
C=O and C–N (amide I) stretching	1650.56	1650.73	1651.83	1652.55	1651.73	1650.68
C–N stretching and N–H (amide II) deformation	1556.55	1555.60	–	–	–	–
	1543.06	1542.77	1543.72	1543.77	1543.77	1542.91
C–H bending	1454.33	1454.18	1446.65	1452.13	1463.09	–
	1401.16	1399.83	1393.97	1379.82	1416.60	1416.36
C–O deformation			1309.68			
	1244.41	1241.69	1246.97	1245.81	1241.76	1242.98
C–O–P, C–OH stretching	1046.75	1045.81	1052.09	1049.87	1047.29	1045.39

Table 4
Effects of specific functional group blocking on Cu(II) biosorption.

Type of modified	q_e (mg/g)	Decrease (%)
Cell wall	40.71	–
–COOH block	21.93	46.1
$–NH_2$ block	31.66	22.2
$–PO_4$ block	40.71	–
–OH block	31.66	22.2

modification by actylation had negligible effect on the adsorption capacity. However, the adsorption capacity of DAG significantly decreased by 46.1% due to carboxyl groups blocking. This implies that carboxyl groups of the biomass play a major role in the adsorption of Cu(II). The cationic Cu(II) may be adsorbed to the biomass through the electrostatic attraction with the negatively charged carboxyl groups.

In order to elucidate the performance and mechanisms clearly and completely, the extended studies will be considered to be done, such as continuous flow column tests, the modeling and simulation in the future. Moreover, searching for effective means to increase the content of carboxyl groups is greatly valuable to improve the adsorption capability of DAG. We believe DAG will be probable to widely apply for wastewater treatment containg heavy metals through further study.

4. Conclusions

DAG as a novel biosorbent holds greater potential for the removal of Cu(II) than IAG, and Cu(II) biosorption capacity increased with the increasing of pH (3–5). Pseudo-second-order kinetic model fit the biosorption process accurately. Ion exchange and electrostatic interaction were the main mechanisms involved in Cu(II) removal. Cu(II) biosorption was associated with a significant release of Ca^{2+} from DAG. EPS played an important role in biosorption, but its efficiency was restricted by the spatial structure of granules. Functional groups of DAG contributed to Cu(II) adsorption in the order of $–COOH > –NH_2 \approx –OH > –PO_4$.

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