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Effect of Cr(VI) on the microbial activity of aerobic granular sludge

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ABSTRACT

The effects of different chromium (Cr(VI)) concentrations (0, 5, 10, 25, and 50 mg/L) on pollutant removal, nitrification ability, and microbial activity of aerobic granular sludge in sequencing batch reactors were analyzed. When compared with the control system, Cr(VI) decreased the average chemical oxygen demand (COD) and ammonia nitrogen removal rates. Significant correlations between the inhibitory rate of specific ammonia utilization rate (sAUR) and Cr(VI) concentration suggested that sAUR inhibition rate could be an effective indicator to predict the biological nitrification process in the aerobic granular sludge system. Although appropriate Cr(VI) concentration stimulated electron transport system activity of the granules, more than 25 mg/L Cr(VI) obviously inhibited the activity. The total polysaccharide and protein contents of the extracellular polymeric substances (EPS) linearly increased with increasing Cr(VI) concentrations of up to 25 mg/L. Furthermore, Cr(VI) exhibited greater inhibitory effects on the nitrification process than on the organic substrate removal process. This may be owing to the lower tolerance of nitrifying bacteria to Cr(VI), when compared with that of organics-degrading heterotrophic bacteria; distribution of nitrifying and heterotrophic bacteria, which occurred at the outer and inner layers of the granular sludge, respectively; and improvement in the COD removal rate of the micro-organisms as a result of EPS secretion.

Keywords: Aerobic granular sludge; Cr(VI); Specific ammonia utilization rate; EST; EPS

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1. Introduction

Toxic heavy metal pollution is a global environmental concern owing to its serious effects on human health. Some of the important sources of heavy metals in urban sewage are human and animal feces, and discharge from industry, hospitals, laboratories, and commercial premises [1]. Among all the heavy metals, chromium (Cr) is often encountered in sewage. Cr is usually found in the environment in trivalent (III) and hexavalent (VI) oxidation states [2]. Although Cr plays an important role in many biological processes and metabolisms, in excessive amounts this heavy metal, including its compounds, is toxic and difficult to remove through biotransformation [3]. Previous studies have shown that Cr(VI) is more toxic than Cr(III), which is considered to be mutagenic and carcinogenic [4,5].

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Since the first report on aerobic granular sludge in the 1990s [6,7], research on this subject has expanded. It has been proved that sequencing batch reactor (SBR) is an established model for aerobic granular sludge cultivation [8,9]. This emerging environmental biotechnological process has been increasingly drawing the interest of researchers in the field of biological wastewater treatment [10–12]. According to studies conducted in the past decade, the biological treatment process using aerobic granular sludge systems is a promising technology for wastewater treatment because of its low space requirement, capacity to treat high loading rates, and ability to achieve simultaneous nutrient removal through nitrification, denitrification, and phosphorus removal [13–15]. When compared with the conventional biological treatment process, aerobic granules have strong microbial structure, excellent stability, and high biomass retention, which all contribute their resistance to toxic compounds [16–18]. Furthermore, aerobic granules exhibit a favorable performance in removing refractory compounds, including heavy metals, from wastewater [19].

However, to date, there are only a few reports on the effect of heavy metals on aerobic granular sludge. Hence, it is important to analyze the influence of heavy metals on the basic characteristics of aerobic granules, as well as determine an accurate and effective monitoring method and index system, which could have a significant impact on the use of aerobic granular sludge in the heavy metal wastewater treatment. Thus, the objective of this study was to investigate the effect of different concentrations of Cr(VI) on aerobic granular sludge in SBRs, focusing on the variations in the pollutant removal capability, nitrification ability, and microbial activity of the aerobic granules. The results obtained could provide the underlying scientific knowledge on the application of aerobic granular sludge technology.

2. Materials and methods

2.1. Operation of the activated sludge process

The SBR was made of polymethyl methacrylate (PMMA), with an effective volume of 4 L and a diameter of 80 mm. A schematic diagram of the SBR is presented in Fig. 1. The daily operation of SBR involved four 6 h cycles, each consisting of four stages: fill (10 min), react (342 min with aeration), settle (3 min), and draw (5 min). Each cycle was performed automatically with a time controller for filling, drawing, and aeration. The feeding water inflow was from the top of the cylindrical reactor through a peristaltic pump, and the effluent was discharged from the lower part of the reactor at an exchange ratio of 75% controlled by a solenoid valve. Oxygen was supplied by porous ceramic air diffusers and the aeration was maintained at 150 L/h. A total of five identical SBRs were operated in parallel. Synthetic wastewater (Table 1) was used as feed, and was supplemented with phosphate buffer to maintain a constant pH of 7.0 ± 0.5. The chemical composition of the influent was as follows: chemical oxygen demand (COD) = 600 ± 100 mg/L and ammonia nitrogen (NH_4^+-N) = 30 ± 10 mg/L.

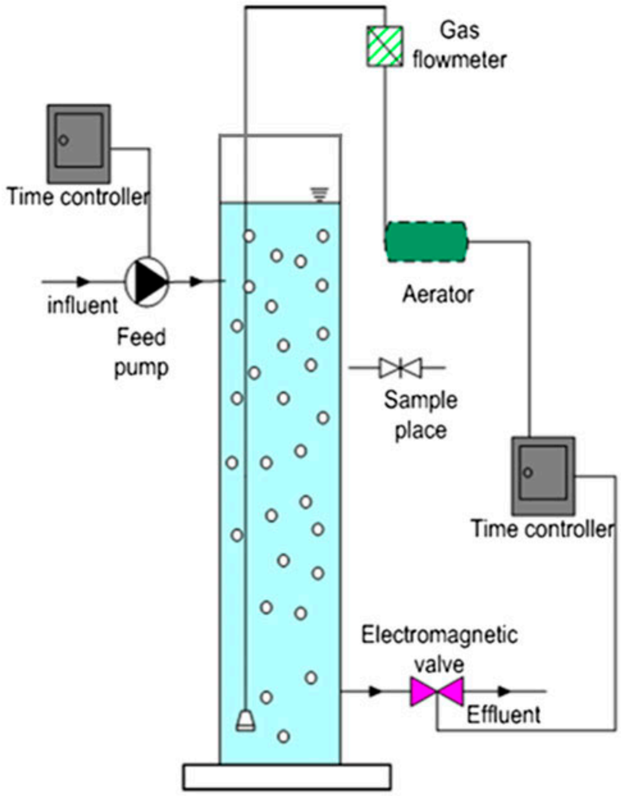

Fig. 1. Schematic diagram of SBR.

The aerobic granular sludge used in this study was cultured from conventional-activated sludge. The seed sludge was obtained from the secondary sedimentation tank (mixed liquor suspended solids (MLSS) = 1830 mg/L) of a municipal wastewater treatment plant in Nanjing, China. The aerobic granular sludge was successfully cultivated in five reactors (SBR-A, B, C, D, and E) for 40 d. The MLSS in each SBR were maintained at a constant concentration of 3.5–4.0 g/L and the particle size was retained between 0.8 and 1.2 mm. As the control system, 0 mg/L Cr(VI) was added into SBR-A. The concentrations of synthetic heavy metal wastewater were according to those in actual urban sewage [20]. A potassium dichromate solution ($K_2Cr_2O_7$) was added into the reactors on the

Table 1
Composition of the synthetic wastewater

Constituents	Concentration (mg L^{-1})	Constituents	Concentration (mg L^{-1})
Glucose	400	$MgSO_4{\cdot}7H_2O$	20
Sodium acetate	400	$FeSO_4{\cdot}7H_2O$	20
Peptone	15	$Co(NO_3)_2{\cdot}6H_2O$	3
KH_2PO_4	60	$CuSO_4{\cdot}5H_2O$	0.05
$CaCl_2{\cdot}2H_2O$	50	$MnCl_2{\cdot}4H_2O$	0.4
		$ZnSO_4{\cdot}7H_2O$	0.5

fourty-first day of the experiment to achieve a constant Cr(VI) concentration of 5, 10, 25, and 50 mg/L in SBR-B, C, D, and E, respectively. The temperatures of the reactors were maintained at 20 ± 2 ℃.

2.2. Analytical methods

(1) The treated effluents were collected everyday in the "draw" stage after the addition of Cr (VI), and COD and NH_4^+-N removal efficiencies were analyzed. The COD and NH_4^+-N concentrations were measured according to the standard methods [21].

(2) Specific ammonia utilization rate (sAUR) [22] was determined using the short-term batch reactors. The initial conditions (including the granular sludge and synthetic wastewater) and operational parameters (including pH, dissolved oxygen, and MLSS) of the batch assays were similar to those of the continuous flow reactors. The sAUR in the short-term batch was calculated by employing the following equation:

$$\mathrm{sAUR} = \frac{dc/dt}{X} = -60\ k\ /\ X \tag{1}$$

where c is the NH_4^+-N concentration in mg/L, t is the reaction time in min, dc/dt is the variation in NH_4^+-N concentration vs. time in mg/(L min), X is the concentration of the MLSS in g/L, and k is the slope rate of the NH_4^+-N concentration decline curve in mg/(L h).

(3) Electron transport system (ETS) activity test [23]. Triphenyltetrazolium chloride (TTC)–ETS was performed to estimate the influence of Cr (VI) on the activity of sludge micro-organisms. The ETS was measured using 2,3,5-TTC. Homogenized samples of mixed liquor were collected from the five batch assay setups for the measurement of the specific substrate removal rates. During the experiment, an aerator and porous ceramic air diffusers were used to provide aerobic conditions. MLSS was also measured to determine the biomass. The ETS activity was calculated as follows:

$$\mathrm{ETS}_{t'} = \frac{D_{485}\ V}{k'_{t'}\ W_{t'}} \tag{2}$$

where ETS_t is the activity of the ETS in μg/(mg h), D_{485} is the absorbance at 485 nm, V is the volume of the extractant in mL, k' is the slope of standard curve in mL/μg, W is the dry weight of the activated sludge in mg, and t' is the incubation time in h.

(4) Extracellular polymeric substances (EPS) include humic-like substances, proteins, polysaccharides, uronic acids, and nucleic acids, with the main compositions being polysaccharides and proteins [24]. EPS were extracted by employing the magnetic stirrer-cation-exchange resin method [25]. The conditions for the extraction were as follows: contact time of 6 h, stirring speed of 1,000 rpm, and resin dose of 60 g/g SS. The EPS protein content was measured by the Coomassie brilliant blue method and the polysaccharide content was measured by the anthrone method [26].

3. Results

3.1. Effects of Cr(VI) on pollutants removal

The index for the evaluation of the effects of Cr(VI) on the pollutants removal ability of aerobic granular sludge included COD and NH_4^+-N removal efficiency. As shown in Fig. 2(a), the control system (SBR-A) exhibited a high and stable organic substrate removal efficiency (indicated by COD) during the entire experimental period, with an average removal rate of 96.32%. When compared with the control system,

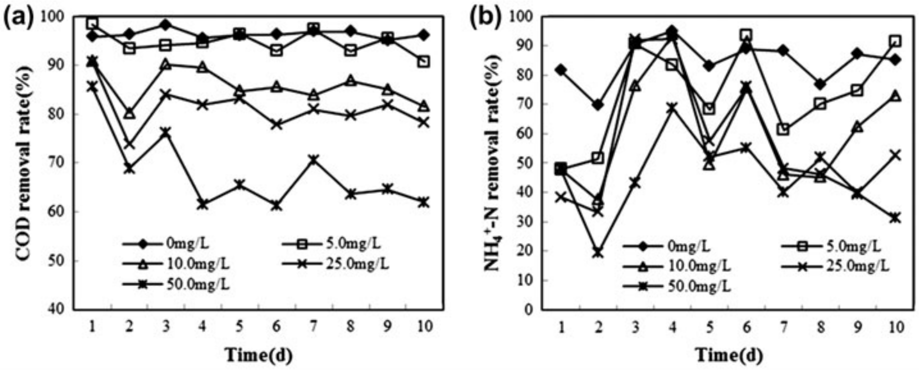

Fig. 2. Effects of Cr(VI) on the pollutants removal performance of the sludge (a) COD removal efficiency (b) NH_4^+-N concentration and removal efficiency.

5 mg/L Cr(VI) (SBR-B) had little impact on the COD removal rate, with the average COD removal rate reaching 94.59%. This suggested that the aerobic granular sludge had strong stability at a Cr(VI) concentration of <5 mg/L. However, 10 and 25 mg/L Cr(VI) (SBR-C and D, respectively) produced an obviously inhibitory effect on the COD removal rate. Owing to the Cr(VI) shock on aerobic granules, the COD removal rates decreased from 95.0 to 80.15 and 73.78% in SBR-C and D, respectively. Soon afterward, the granules gradually adapted to the toxic effects of the metal ions, and the COD removal rates recovered to 84.68 and 83.13% on the fifth day in SBR-C and D, respectively. However, after 10 d of exposure to Cr(VI), the COD removal rates decreased to 81.55 and 78.20% in SBR-C and D, respectively, because Cr(VI) accumulation produced an extended inhibitory effect on the granular sludge. In general, the COD removal rates decreased in the early stage, subsequently increased, and remained steady in the later stage (46–50 d). As 50 mg/L Cr(VI) (SBR-E) was highly toxic, the average COD removal rate dropped to 67.94%.

As presented in Fig. 2(b), the control system (SBR-A) exhibited a high NH_4^+-N removal efficiency, with an average efficiency of 84.57% during the entire experiment period. In contrast, a drastic decrease in NH_4^+-N removal rate was observed after the addition of Cr(VI). The NH_4^+-N removal rate dropped to 47.98, 47.80, 38.26, and 48.02% in SBR-B, C, D, and E, respectively. However, within a few days, the granules gradually adapted to the toxic effects of the metal ions, and on the third or fourth day, the NH_4^+-N removal rates reached a maximum of 90.58, 93.80, 92.24, and 68.65% in SBR-B, C, D, and E, respectively. Nevertheless, as the Cr(VI) accumulation produced an extended inhibitory effect on the granular sludge, the NH_4^+-N removal rates decreased again. Finally, the NH_4^+-N removal rate significantly decreased to <50% in SBR-D and E (25 and 50 mg/L Cr(VI), respectively). In general, the NH_4^+-N removal rates decreased in the early stage, subsequently increased, and decreased again in the later stage, suggesting that Cr(VI) had an obviously inhibitory effect on the NH_4^+-N removal efficiency of aerobic granular sludge.

The average COD and NH_4^+-N removal efficiencies obviously decreased with the increasing Cr(VI) concentration. This reflected the significant inhibitory effects of Cr(VI) on the aerobic granular sludge. The relationship between the Cr(VI) concentrations and average substrate removal efficiencies could be calculated using the following equations:

$$R_{\text{COD}} = -1.348[\text{Cr(VI)}]^2 + 1.072[\text{Cr(VI)}] + 96.783 \quad R^2 = 0.9840 \tag{3}$$

$$R_{\text{NH}_4^+\text{-N}} = -23.398 \ln\,[\text{Cr(VI)}] + 86.61 \qquad R^2 = 0.9559 \tag{4}$$

where R_{COD} and NH_4^+-N are, respectively, the average COD and NH_4^+-N removal efficiencies during the period, when Cr(VI) was continuously fed, and [Cr(VI)] is the Cr(VI) concentration in the influent.

When compared with the control system, addition of 5, 10, 25, and 50 mg/L Cr(VI) decreased the average COD and NH_4^+-N removal efficiency of the aerobic granular sludge from 96.34 to 94.59, 85.85, 81.17, and 67.94% and from 84.57 to 73.24, 60.71, 57.62, and 44.88%, respectively. Furthermore, the average NH_4^+-N removal efficiency decreased more significantly than the average COD removal efficiency with the addition of 5 mg/L Cr(VI) (SBR-B), and similar results were also observed in SBR-C, D, and E, indicating that Cr(VI) could produce greater inhibitory effects on the nitrification process than on the organic substrate removal process.

3.2. Effects of Cr(VI) on the nitrification ability

The sAUR is the most widely used measurement for the determination of microbiological nitrifying ability [27]. Batch test indicates the ammonia removal kinetics and sAUR reveals the impact of Cr(VI) shock on the nitrifying bacteria. In this study, the samples were collected on the tenth day and the nitrification ability was analyzed. Fig. 3 indicates that maximum sAUR was achieved in the control system (SBR-A), whereas the values drastically decreased after the addition of Cr(VI). The sAUR for SBR-A, B, C, D, and E were 1.00, 0.91, 0.68, 0.42, and 0.24 mg N/(g MLSS h), respectively. The sAUR inhibitory rate of Cr(VI) could be defined using Eq. (5) as follows:

$$\text{Inhibitory rate} = -(C' - C_0)/C_0 \tag{5}$$

where C_0 and C' are the sAUR of the control system and other reactors fed with different concentrations of Cr(VI) in mg N/(g MLSS h), respectively.

The inhibitory effects of Cr(VI) on sAUR could be well predicted by a linear equation, which suggests that sAUR inhibition rate is an effective indicator of the activity of nitrifying bacteria, and that this parameter could provide valid predictions of the potential performance of the biological nitrification process in the aerobic granular sludge system.

3.3. Effect of Cr(VI) on the ETS and EPS

3.3.1. ETS

The ETS was used to estimate the influence of Cr(VI) on the activity of sludge micro-organisms, and was measured using TTC. As shown in Fig. 4, appropriate concentration of Cr(VI) could stimulate the activity of the granular sludge, while obvious inhibitory action occurred at concentration >5 mg/L Cr(VI). TTC-ETS increased from 29.29 to 38.36 μg TF/(g MLSS h) at a Cr(VI) concentration of 5 mg/L (SBR-B), when compared with the control system (SBR-A). In contrast, TTC-ETS decreased to 27.16, 19.92, and 16.91 μg TF/(g MLSS h) with the addition of 10, 25, and 50 mg/L Cr(VI) (SBR-C, D, and E), respectively. Thus, the Cr(VI) concentration range that promoted the activity of aerobic granular sludge was 0–5 mg/L, whereas more than 25.0 mg/L Cr(VI) significantly inhibited the activity of the aerobic granular sludge.

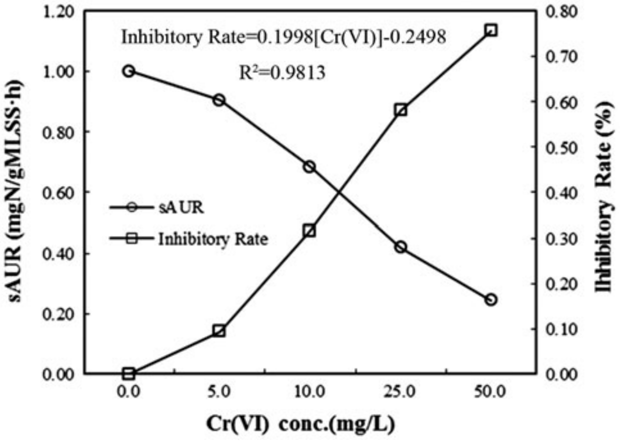

Fig. 3. Inhibitory effects and inhibition rates of Cr(VI) loading on sAUR.

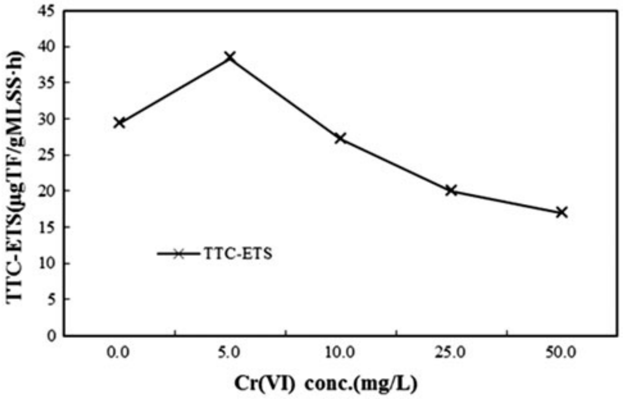

Fig. 4. Effects of Cr(VI) on the ETS activity of the sludge.

3.3.2. Extracellular polymeric substances

The EPS are related to the microbial metabolism and may be more correlated with microbial self-aggregation, affecting various physicochemical characteristics of the bioflocculants [28–30]. As shown in Fig. 5,

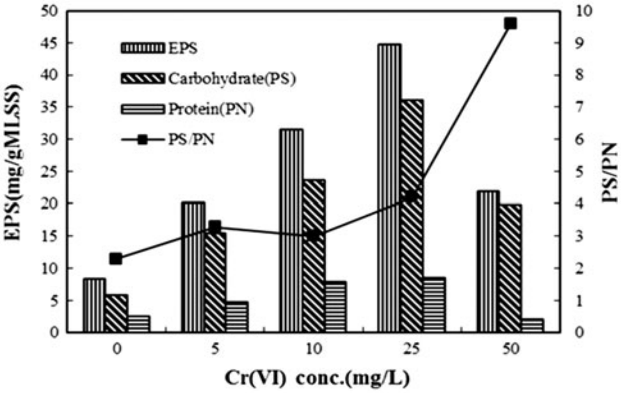

Fig. 5. Effects of Cr(VI) on the EPS of the sludge.

the minimum quantity of EPS was 8.42 mg/g MLSS, which was observed in the control system (SBR-A). In SBR-B, addition of 5 mg/L Cr(VI) simulated EPS formation, with the amount of EPS reaching 20.22 mg/g MLSS. Similar trends could also be observed in SBR-C and D, and the polysaccharide and protein contents increased with the increasing Cr(VI) concentration. However, addition of 50 mg/L Cr(VI) produced an obvious decrease in EPS secretion, indicating that the microbial metabolism and self-aggregation were seriously inhibited.

4. Discussion

The average COD removal efficiencies obviously decreased with the increasing Cr(VI) concentration. A previous study demonstrated that the substrate removal performance depends on the microbial metabolism in the activated sludge system, and that the ETS activity is an important parameter for the evaluation of the microbial metabolic level [22]. In this study, the TTC–ETS of the aerobic granular sludge increased by 20.2% with the increasing Cr(VI) concentration of up to 5 mg/L. The stimulation mechanism of heavy metal ions on aerobic granules is complex, and the reactions (including catabolism or anabolism) in which different metals play a role have not yet been determined. The addition of 25 and 50 mg/L Cr(VI) resulted in 32.0 and 42.3% inhibition of TTC-ETS of aerobic granules, respectively.

Cr(VI) significantly inhibited the NH_4^+-N removal rate of the aerobic granules, and its inhibitory effect gradually increased with the increasing Cr(VI) concentration (Fig. 2(b)). The decrease in the NH_4^+-N removal rate of the aerobic granules indicated that the nitrification process was inhibited [31]. As a result, the sAUR of the granules obviously decreased. The addition of 5, 10, 25, and 50 mg/L Cr(VI) resulted in 9.49, 31.66, 58.04, and 75.62% decreases in the sAUR, respectively, when compared with the control system (SBR-A), as shown in Fig. 3.

Aerobic granules revealed a high and stable removal efficiency at Cr(VI) concentrations < 10 mg/L. Cr(VI) removal rates were 72.09 and 68.59%, respectively, at the 3 and 5 mg/L Cr(VI). The mechanism of Cr(VI) removal was complex, including physical, chemical, and biological functions. Aerobic granules have high permeability, porosity, and surface area, all of which are beneficial to adsorption. Previous studies showed that after contacting metal ions, the surface of biomass became more compact and the coccoid bacteria appeared somewhat wrinkled [16,32]. This could be the result of metal complexation or chemical precipitation. Also, transformation of elemental composition on granular surface implied that ion exchange was involved [33]. However, with the concentration of Cr(VI) increasing, aerobic granular sludge exhibited an obviously inhibitory effect on the Cr(VI) removal rate. As 50 mg/L Cr(VI) (SBR-E) was highly toxic, the Cr(VI) removal rate dropped to 8.07%. These elevated levels of Cr(VI) might destroy the structure of aerobic granules as well as inactivate microbe.

The inhibitory effect of Cr(VI) on NH_4^+-N removal efficiency was much stronger than that on the COD removal rate of the aerobic granular sludge. A previous study demonstrated that Cr(VI) exerted a severe impact on the protozoan growth on the surface of the active sludge, causing damage to the protozoan cell membranes and proteins, which disrupted the predation of protozoans and further affected COD removal [34]. However, the compact structure of the aerobic granular sludge could improve the resistance of the bacteria at the inner layer of the sludge to external toxicity. In addition, the electrostatic absorption and chelation of EPS could convert the heavy metals from dissolved form to soluble form. These factors could decrease the toxicity of the heavy metals and improve the COD removal rate of the micro-organisms [35]. Nevertheless, it must be noted that excess amount of heavy metal ions could alter the osmotic balance of heterotrophic and autotrophic bacteria and hinder the circulation of minerals, thus inhibiting the normal growth and metabolism of the bacteria. Furthermore, it has been reported that the tolerance of the nitrifying bacteria to toxic substances is relatively lower than that of the organics-degrading heterotrophic bacteria [36], resulting in a decreased in nitrification. Previous studies have shown that many external factors may inhibit the biological nitrification process [37]. The enzyme reaction theory considers that the process of nitrification inhibition is either competing or noncompeting with the nitrification enzymes [38,39]. In recent years, research on molecular biology techniques has revealed that the possible reasons for nitrification inhibition could be gene transcription, synthesis, and long-term change in the flora, except the traditional enzyme reaction [40]. Furthermore, ammonia monooxygenase encoding mRNA (amoA mRNA) can be used as an indicator of ammonia oxidation activity [41]. Certain heavy metals, such as Cd^{2+} and Cu^{2+}, could affect amoA mRNA copies in the activated sludge, and higher concentrations of these metals could lead to greater inhibition of the amoA mRNA [42]. Moreover, studies have proven that nitrification tends to occur at the oxygen-containing outer part of the granular sludge, and are thus more easily affected by heavy metals. Conversely, organics-degrading heterotrophic bacteria are aerobic or facultative, and are more likely to occur at the inner layer of the granular sludge; as a result, the influence of Cr(VI) on the nitrification process is obvious [43,44].

5. Conclusion

When compared with the control system, addition of 5 mg/L Cr(VI) (SBR-B) decreased the average COD and NH_4^+-N removal rates of the aerobic granular sludge from 96.34 to 94.59% and from 84.57 to 73.24%, respectively. Similar results were also noted for other Cr(VI) concentrations in SBR-C, D, and E. These findings suggested that Cr(VI) could produce greater inhibitory effects on the nitrification process than on the organic substrate removal process. Further analysis of the microbial activity (including ETS and EPS) and nitrification process indicated that the following might be the possible reasons for the greater inhibitory effects of Cr(VI) on the nitrification process: (A) variation in the tolerance of the bacteria to toxic substances—The tolerance of the nitrifying bacteria to Cr(VI) was worse than that of the organics-degrading heterotrophic bacteria; (B) distribution of bacteria in the aerobic granular sludge—The nitrifying bacteria tended to occur at the outer layer of the granular sludge, whereas the heterotrophic bacteria were present throughout the granules, including the inner and outer; and (C) improvement in the COD removal rate of the micro-organisms as a result of EPS secretion.

List of symbols

C — NH_4^+-N concentration in mg/L

t — the reaction time in min

dc/dt — the variation in NH_4^+-N concentration vs. time in mg/(L min)

X — the concentration of the MLSS in g/L

k — the slope rate of the NH_4^+-N concentration decline curve in mg/(L h)

ETS_t — the activity of the electron transport system in μg/(mg h)

D_{485} — the absorbance at 485 nm

V — the volume of the extractant in mL

k' — the slope of standard curve in mL/μg

W — the dry weight of the activated sludge in mg

t' — the incubation time in h

R_{COD} — the average COD removal efficiencies during the period, when Cr(VI) was continuously fed

$NH_4^+ - N$ — the average NH_4^+-N removal efficiencies during the period, when Cr(VI) was continuously fed

[Cr(VI)] — the Cr(VI) concentration in the influent

C_0 — the sAUR of the control system fed with different concentrations of Cr(VI) in mg N/(g MLSS h)

C' — the sAUR of other reactors fed with different concentrations of Cr(VI) in mg N/(g MLSS h)

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