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Exploring potential impact(s) of cerium in mining wastewater on the performance of partial-nitrification process and nitrogen conversion microflora

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ABSTRACT

Cerium Ce(III) is one of the major pollutants contained in wastewater generated during Ce(III) mining. However, the effect(s) of Ce(III) on the functional genera responsible for removing nitrogen biologically from wastewater has not been studied and reported. In this study, the effects of Ce(III) on aspects of partial-nitritation-(PN) process including ammonia oxidation rate (AOR), process kinetics, and microbial activities were investigated. It was found that the effect of dosing Ce(III) in the PN system correlated strongly with the AOR. Compared to the control, batch assays dosed with 5 mg/L Ce(III) showed elevated PN efficiency of about 121%, an indication that maximum biological response was feasible upon Ce(III) dose. It was also found that, PN performance was not adversely affected, given that Ce(III) dose was ≤20 mg/L. Process kinetics investigated also suggested that the maximum Ce(III) dose without any visible inhibition to the activities of ammonium oxidizing bacteria was 1.37 mg/L, but demonstrated otherwise when Ce(III) dose exceeded 5.63 mg/L. Compared to the control, microbes conducted efficient Ce(III) removal (averaged 98.66%) via biosorption using extracellular polymeric substances (EPS). Notably, significant deposits of Ce(III) was found within the EPS produced as revealed by SEM, EDX, CLSM and FTIR. 2-dimensional correlation infrared-(2DCOS-IR) revealed ester group (uronic acid) as a major organic functional group that promoted Ce(III) removal. Excitation-emission matrix-(EEM) spectrum and 2DCOS-IR suggested the dominance of Fulvic acid, hypothesized to have promoted the performance of the PN process under Ce(III) dosage.

1. Introduction

Rare earth elements (REE) are a group of 17 elements including lanthanides (from the lightest lanthanum (La) to the heaviest lutetium (Lu)), scandium (Sc), and yttrium (Y) (Brito et al., 2018). These elements are ordinarily categorized into two groups based on their atomic weight (AW), i.e., light rare earth elements (LREEs) and heavy rare earth elements (HREEs). LREEs include elements from La (AW of 138.91) to europium (AW of 151.96) whereas HREEs includes elements from gadolinium (AW of 157.25) through lutetium (AW of 174.97) with Y (Battsengel et al., 2018; Huang et al., 2020). In LREEs, La and cerium (Ce) are the major components of all REEs, accounting for about 68% of the average REEs concentration (Ahn et al., 2014). Usually, LREEs are trivalent, except for Ce, which have multiple valences (Ce(III) and Ce (IV)) in natural environments (Brito et al., 2018).

Recent reports have established that REE deposit is currently estimated at some 130 million tons in the world (Antwi et al., 2019), out of which 90% of the estimated deposits is obtained from China (Battsengel et al., 2018). These elements are mostly extracted using ammonium sulfate (Yan et al., 2018). However, this ammonium sulfate, though cheap is largely retained in REE mine tailings. Consequently, the ammonium left in the tailing is often leached into groundwater, and

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surrounding water bodies anytime rains comes into contact with the mine tailings. Several reports have established that high-strength nitrogen-rich mine wastewater (NMW) is mostly produced from mining sites even years after the mines are decommissioned (Zhang et al., 2019). Against this backdrop, several biological nitrogen removal processes have been proposed. Anammox process for instance has demonstrated as a promising technology for treating such wastewater due to its significant advantages, including no oxygen demand, low sludge production and no organic carbon demand (Dong et al., 2017). Compared with traditional biological nitrogen removal (nitrification/denitrification), the aeration cost could be reduced by 25%, and the demands on the carbon source are decreased by 40% (Antwi et al., 2019). Based on these advantages, partial nitritation/anammox process has been widely used to treat a low C/N ratio wastewater (Yao et al., 2017). However, partial nitritation (PN) plays an essential role in the application of anammox (Dong et al., 2017).

Ce is the most abundant rare earth element which makes up about 0.0046% wt of the earth's crust (Zhou et al., 2019) and this has motivated researchers to explore the effects of Ce on plants and animals (Huang et al., 2010; Jiang et al., 2017; Majumdar et al., 2016). However, almost no studies have so far been reported on the effect of Ce on microorganisms, especially in wastewater treatment. Some reports have shown that Ce(III) is the main valence of Ce in solution, and its toxicity is significantly higher than that of Ce(IV) (Milani et al., 2017). Moreover, according to existing report, the effect of Ce(III) on different organisms (plants, animals, and microorganisms) is different. For example, some reports could reveal that Ce(III) (0-71.4 µM) has acute and chronic toxicity to freshwater crustaceans (Daphnia magna) (Ma et al., 2016b). However, other studies revealed that Ce(III) (0–90 μ M) increased the vase life of Dianthus caryophyllus cut flower (Zheng and Guo, 2018). Therefore, as Ce(III) is significantly contained in NMW, it is necessary to explore its effect(s) on PN process performance and its responsible biological mechanism.

In this study, (1) the effects of various doses of Ce(III) on the nitrogen conversion process and kinetics during a PN process were explored and evaluated for the first time at lab scale, (2) the effects of Ce(III) on the sludge morphology and evolution of extracellular polymeric substance (EPS) was well investigated by scanning electron microscopy- energydispersive X-ray spectroscopy (SEM-EDX), confocal laser scanning microscopy (CLSM) and excitation-emission matrix (EEM) spectrum, and (3) Changes in the macroscopic composition of sludge at typical Ce(III) doses and organic functional groups were determined using Fourier transform infrared spectroscopy (FTIR) and 2-dimensional correlation infrared (2DCOS-IR), respectively.

2. Materials and methods

2.1. Inoculum and feeding

The inoculum was obtained in a PN reactor treating synthetic nitrogen-rich wastewater that had attained steady-state after 240 days of operation. In our previous study, it was found that the inoculum had *Nitrosomonas* (relative abundance of 49%) as the dominant genera, with mixed liquid volatile suspended solid (MLVSS) ranging 2.4–2.5 g/L (Zhang et al., 2019). The synthetic wastewater employed in this study was adapted from (Antwi et al., 2019; Su et al., 2020), as shown in Table S1.

2.2. Batch experiment setup and operation

Ten batch scale reactors, each with a total working volume of 300 mL and an effective working volume of 250 mL, were set up for the batch experiment (Fig. S1) and operated for 12 h as a complete cycle. Besides the control reactor (C0), all reactors including C1, C2, C3, C4, C5, C6, C7, C8, and C9 were dosed with 0.1, 1, 5, 10, 20, 50, 100, 300, and 500 mg/L of Ce(III), respectively. The pH value in each reactor was

maintained at 8.9 \pm 0.1 through a pH-adjusting solution (20 g/L NaOH). Dissolved oxygen (DO) was adjusted to 0.2 mg/L via DO monitor and intermittently aerating in every other 30 min. Details of these control methods can be found in previous studies (Zhang et al., 2019). The inoculum was washed at least three times with deionized water before use. Water sampling was conducted when the inoculum was thoroughly mixed with feeding after a minute and every two hours of operation. Samples were filtered with a 0.45 μ m filter. Sludge were obtained and stored at 4 °C after experiment.

2.3. Analytical methods

NH⁴₄-N, NO₂-N, NO₃-N and MLVSS of obtained in batch experiment were measured by lab scale UV/VIS spectrophotometer (SQ2800, Unico, Italy) according to standard methods of APHA (APHA, 2005). Ce (III) concentration before and after operation were analyzed by an inductively coupled plasma spectrometer (OES Optima 2000DV, Perkin Elmer, USA).

2.4. Performance indicator analysis and kinetic modeling of experimental data

Some performance analysis, kinetics modeling and linear modeling (y = mx + c) were employed to estimate classical parameters to help describe the performance of the PN process (Zhang et al., 2019). As presented in Table 1, performance indicators including AOR (Eq. (1)), nitrite oxidation rate (NOR) (Eq. (2)), SNPR (Eq. (3)), SOUR (Eq. (4)), and change in SOUR (\triangle SOUR) (Eq. (5)) were estimated and their corresponding values were used as basis to conclude on the efficacy of the PN process. Change in SOUR of dosed reactors and that of the control reactor was employed as intermediate parameter to establish kinetic model between PN performance and Ce(III) dose. The corresponding kinetic models employed in the evaluation of kinetic parameters were Giddings equation (Eq. (6)), Michaelis-Menten equation (Eq. (7)), Edwards equation (Eq. (8)) and Haldan equation (Eq. (9)). Details of these parameters and models were shown in Table 1, where Δ [NH⁺_{4 eff}], average ammonium change in effluent every two hours (mg/L); Δ [NO₃⁻-N_{eff}], average nitrate change in effluent every two hours (mg/L); Δ [NO₂⁻-N_{eff}], average nitrite change in effluent every two hours (mg/L);

Expressions used in statistical analysis and kinetic modeling.

Number	Name	Expression	Units
Eq. (1)	AOR	$AOR = \Delta [NH^+_{4eff}] / \Delta t$	mgN/ L/h
Eq.	NOR	$NOR = \Delta [NO_{3eff}] / \Delta t$	mgN/
Eq. (3)	SNPR	$SNPR = \Delta [NO_{2eff}^{\text{-}}] / \Delta t$	mgN/ L/h
Eq. (4)	SOUR	$\text{SOUR} = \frac{\frac{SNPR}{M(N)}M(O)}{\frac{MLVSS}{MLVSS}}$	mgO/ gVSS/ h
Eq. (5)	∆SOUR	$\Delta SOUR = SOUR_{(other \ reactors)}\text{-}SOUR_{(control}$ reactor)	mgO/ gVSS/ h
Eq. (6)	Giddings	$y = y0 + \frac{A}{w} * \sqrt{\frac{xc}{x}} * besseli\left(\frac{2 * \sqrt{xc * x}}{14.5}, 1\right) * \frac{-x - xc}{e} \frac{-x - xc}{w}$	mgO/ gVSS/ h
Eq. (7)	Michaelis- Menten	$V = \frac{Vmax \cdot S}{K_m + S}$	mgO/ gVSS/ h
Eq. (8)	Haldan	$V = rac{Vmax}{1+rac{K_m}{S}+\left(1+rac{S}{K_i} ight)}$	mgO/ gVSS/ h
Eq. (9)	Edwards	$\mathbf{V} = \mathbf{V}\max\left(\exp\left(-\frac{S}{Ki}\right) - \exp[\frac{f_0}{Km}]\left(-\frac{S}{Km}\right)\right)$	mgO/ gVSS/ h

 Δ t, sampling interval (hour); M(N), molar mass of nitrogen (g/mol); M (O), molar mass of oxygen (g/mol); SOUR_(other reactors), SOUR of other reactors; SOUR_(control reactor), SOUR of control reactor; y, Δ SOUR; x, Ce (III) does; *A*, *w*, *yO* and *xc* are fitting parameters; *V* is Δ SOUR, (mgO/gVSS/h); *Vmax* is the maximum *V* in modal, (mgO/gVSS/h); *Km* is half-rate saturation constant, Ce(III) dose under *V* = *V max* /2, S is the dose of Ce(III), inhibition constant (*Ki*) is the dose when inhibition of AOR occur.

2.5. Studies on sludge morphology by SEM-EDS

The obtained sludge samples were dried in a desiccator for a week after pretreatment (Zhang et al., 2019). The thoroughly dried samples were sprayed with gold by sputter coating machine (Q150R S, Quorum, England), to improve the resolution of the SEM image. Morphology of the samples was then observed via field emission scanning electron microscopy (FE-SEM) (MIRA3 LMH, Testcan-orsay, Czech Republic).

2.6. Identification of typical EPS using CLSM

FITC, Con A, and calcofluor white were used as stains to stain proteins, α -polysaccharides, and β -polysaccharides in sludge samples. A detailed procedure for staining was consistent with our previous study (Su et al., 2020). The stained samples were observed by confocal laser scanning microscopy (Leica TCS SP5, Leica, Germany).

2.7. Extraction of EPS and mapping of EEM fluorescence spectra

Sludge samples were centrifuged at 2000 rpm for 5 min after which supernatant was extracted and replaced with same volume of NaCl solution (0.05%). Sample and NaCl solution mixtures were subsequently centrifuged at 9000 r/min for 10 min, and the supernatant was collected for analysis for loosely bound extracellular polymeric substance (LB-EPS). EEM data were generated via a fluorescence spectrometer (FLS-980, Edinburgh Instruments, UK) by scanning over-excitation wavelengths of 250–450 nm (interval of 5 nm) and emission wavelengths of 260–550 nm (interval of 1 nm). Slit widths of excitation and emission were set at 2.3 nm, and scanning speed was set to 300 nm/min. Finally, EEM fluorescence spectra were plotted by Origin 9.5 using this data.

2.8. Macroscopic components analysis by FTIR

Sludge samples were washed three times by deionized water and dried for a week long. The dried samples were ground and passed through a 200 mesh sieve and mixed with spectrally pure Potassium bromide to form a disk. FTIR spectrum with wavelength in 4000–400 cm⁻¹ was measured via FTIR spectrophotometer (BRUKER TENSOR2, Bruker, Germany) at 1.5 cm⁻¹ resolution.

2.9. Regional correlation analysis through 2DCOS-IR spectroscopy

To study the effect of Ce(III) on the structure of protein and polysaccharides, and to determine the main functional groups of Ce(III) removal, a set of dose-dependent FTIR data were obtained with adding Ce(III) dose as exogenous perturbation, which was used to plot the 2DCOS-IR spectrum via Origin 9.5. The positive/negative correlation between the changes of peak and dose is indicated by the color of the positive cross peak (red/blue), and the darker the color, the more significant the correlation (Ma et al., 2016a). The full-region 2DCOS-IR map was drawn and found that the tortuous change area (1800–900 cm⁻¹) was the area with the most significant changes (Fig. S2). The obtained area was further cut into four regions of interest: amide I region, 1700–1600 cm⁻¹; amide II region, 1600–1500 cm⁻¹; amide III region, 1310–1175 cm⁻¹; glycoside region (contains furanoid ring, pyranoid ring, etc.), 1100–1000 cm⁻¹ (Chiaramaria et al., 2020).

3. Results and discussion

3.1. Profile of nitrogen conversion efficiency and Ce(III) removal within reactors

3.1.1. Effect of Ce(III) on the nitrogen conversion rate

The concentration of NH⁺₄ and NO⁻₂ within batch assays was analyzed every two hours after inoculation and feeding (Fig. 1a and b). AOR and specific nitrite production rate (SNPR) under different Ce(III) dosages were obtained by using a linear model (y = mx + c) to fit the experimental nitrogen (NH⁺₄ and NO⁻₂) data. The fitting parameters and coefficient of determination (R²) obtained from the linear model was presented in Table S2. Notably, all estimated R² were above 0.96, indicating experimental data had a good linear relationship with time. As shown in Fig. 1b and Table S2, it was found that the slope of the fitted line to the data obtained from reactors dosed with Ce(III) ranging 0.1–20 mg/L was higher than that of the control.

Conversely, reactors dosed with Ce(III) ranging 50-500 mg/L was lower than the control, an indication that AOR and SNPR increased at low dose (0.1–20 mg/L), and decreased significantly at high dose (50–500 mg/L). Therefore, it could be deduced that there was a correlation between Ce(III) dose and the performance of the PN process. The related study revealed that the oxidation resistance of sweet potato was slightly improved at a low dose (0-10 mg/L). In comparison, its development and photosynthesis were negatively affected at a high dose (20-80 mg/L) (Jiang et al., 2017). The reported observation in the latter study revealed that dosing Ce(III) could have a significant effect on or determine the trend of biological processes (Zhang et al., 2010). Again, biphasic dose response was also reported when long-term research was conducted to study the effect of Ce on plants. In that study, it was found that the effect of Ce on plants was characterized by stimulatory low doses and inhibitory (toxic) doses (Agathokleous and Calabrese, 2019). The reported literature coupled to the observation made in this present research suggested that the effect of Ce(III) on AOR is the hormetic dose response.

3.1.2. Effect of Ce(III) dose on AOB and NOB enrichment/inhibition

Due to the negligible total nitrogen loss ($4.94 \pm 3.16 \text{ mgN/L}$), SNPR, AOR, and NOR represented the actual NO₂ production rate of PN process, the apparent nitrite yield of ammonia oxidizing bacteria (AOB), the activity of the main competitor of AOB (NOB), respectively. The estimated values of AOR and SNPR were observably close (Fig. 1c) suggesting most of the NH⁴₄ was converted into NO₂ by AOB. NOR was maintained at a low level ($0.31 \pm 0.06 \text{ mgN/L/h}$) indicating NOB activity was significantly inhibited. Amazingly, the observable inhibition was not weakened or affected by the introduction of Ce(III) but instead was highly dependent on the combined pH-DO control strategy proposed in our previous studies (Zhang et al., 2019).

3.1.3. Response of SNPR to various dosages of Ce(III)

The parameter that characterizes the promotion or inhibition of REEs on plants and animals is usually its yield change, such as root or leaf quality of plant, milk, egg or meat yield of animals (Abdelnour et al., 2019; Agathokleous et al., 2019a). Correspondingly, for AOB, SNPR is a vital indicator to characterize their work efficiency (Tang et al., 2018a). Therefore, the SNPR of reactor (C1-C9) were compared to that of the control reactor (C0) to determine the degree of promotion/inhibition on the PN performance either under low or high dose of Ce(III) dose (Fig. 1d). In this study, with 5 mg/L Ce(III) dosed in reactor, the maximum PN performance (SNPR) was 121% as opposed to the control. Similarly, other studies reported increase in crop yield from 5% to 15% for numerous crop species after plant seed was treated with lower doses (<0.5 mg REE /kg soil) (Thomas et al., 2014). Notably, the key dose of no-observed-adverse-effect-level (NOAEL) (Agathokleous et al., 2019b) was found around 20 mg/L. Thus, at this dose, the promotion effect and the inhibitory effect was almost balanced. In this study, 500 mg/L of Ce



Fig. 1. Changes of nitrogen conversion and Ce(III) removal under different Ce(III) dose: (a) effluent ammonia $(NH_{4\,eff}^+)$; (b) effluent nitrite (NO_{2eff}) ; (c) ammonia oxidation rate (AOR), specific nitrite production rate (SNPR) and nitrite oxidation rate (NOR); (d) dose-biological response between Ce(III) dose and SNPR and (e) removal of Ce(III) in ammonia oxidation process.

(III) was the highest dose and its toxicity led to a biological response of only 75% compared to the control. Three key dose of Ce(III) with effect on SNPR were found and its corresponding effect was more evident with the morphological studies, EPS production and organic function group of AOB.

3.1.4. Removal of Ce(III) during ammonia oxidation process

After 12 h of batch experiment, the concentration of Ce(III) in effluent decreased significantly to 0.0026–0.224 mg/L and the Ce(III) removal of 97.37–99.96% was observed (Fig. 1e). Some reports have indicated that the biomass (EPS) contains many organic functional groups with strong binding ability to REEs (Josso et al., 2018; Martinez et al., 2018). Previous research has shown that AOB is wrapped in EPS in the PN sludge (Zhang et al., 2019). Therefore, most of Ce(III) may be captured in EPS. And it is necessary to further explore the changes in EPS yield and species, which will help identify the biological removal mechanism of Ce(III).

3.2. Kinetic modeling between PN performance and Ce(III) dose

In order to further understand the specific Ce(III) dose that affects ammonia oxidation performance, a suitable kinetic modeling was conducted to establish relationship between Ce(III) dose and ammonia oxidation performance. The proposed models employed in this study was also exploited to evaluate the applicability of the PN process under different water quality (different Ce(III) concentrations). As an excellent parameter to establish the existence of AOB metabolism (Ma et al., 2019), SOUR was used to show the variation of microbial activity at various Ce(III) dosage. It was adapted from the literature that the corresponding parameter value should be zero when Ce(III) dosage is zero when using enzyme kinetic model (Su et al., 2020). Therefore, Δ SOUR was used as an intermediate parameter to construct a kinetic model between PN performance and the various Ce(III) dosages.

In classic peak function model and enzyme kinetic model, Gidding model was the maximum correlation coefficient (R^2) modal to describe the correlation of \triangle SOUR and Ce(III) dose (R² of 0.748) (Fig. 2a). For the purposes of clarity, the illustration was divided into two regions of 0-20 mg/L and 20-500 mg/L with respect to the plus and minus of Δ SOUR. First, the data was first fitted by with a linear model when Ce (III) ranged 20-500 mg/L (Fig. 2b) and 50-500 mg/L (Fig. 2c). However, the coefficient of determination of the model was not satisfactory (R² of 0.854 and 0.908, respectively). Consequently, other inhibitionkinetics models, such as Edwards and Haldane models were subsequently employed (Fig. 2d and e), and their results (R^2 of 0.922 and 0.931, respectively) revealed greater efficiency. Finally, the data was fitted to the Michaelis-Menten model (Fig. 2f), which is the classic model in biological wastewater treatment technologies (Meng et al., 2018). The Michaelis-Menten model demonstrated good correlation as R² could reach 0.954. The maximum - Δ SOUR (Vmax) and half-rate constant (Km) obtained by this model were 10.93 mgO/gVSS/h and 266.73 mg/L,



Fig. 2. Kinetic modeling between Δ SOUR and Ce(III) dose: (a) identified Δ SOUR value at different Ce(III) dose; (b) linear fit at dose of 20–500 mg/L; (c) linear fit at dose of 50–500 mg/L; fitting of (d) Edwards, (e) Haldane and (f) Michaelis-Menten at dose of 20–500 mg/L and fitting of (g) Michaelis-Menten, (h) Haldane and (i) Edwards at dose of 0–20 mg/L.

respectively (Table S3), indicating that Δ SOUR will approach -10.93 mgO/gVSS/h with an increasing dosage. The SOUR of the control was 29.61 mgO/gVSS/h, hence, SOUR eventually stabilized at 18.68 mgO/gVSS/h (500 mg/L) with increasing dose, an indication that Ce (III) does ranging 0–500 mg/L appear not to have any acute toxicity to AOB.

By contrast, the obtained R^2 value (0.150) reached by the Michaelis-Menten model was very unsatisfactory (Fig. 2g). However, two inhibition kinetics models (Haldane and Edwards) agreed well with the experimental data (Fig. 2h and l). Notably, it was obvious that the Edwards model demonstrated significant efficiency ($R^2 = 0.993$) over the Haldane model ($R^2 = 0.898$). The *Vmax*, *Km*, and inhibition constant (*Ki*) calculated using the Edwards model was 16.32 mgO/gVSS/h, 1.37 mg/L, and 5.63 mg/L, respectively, suggesting the maximum boundary value (Kang et al., 2016) of Ce(III) is 1.37 mg/L and that the PN performance would be inhibited when Ce(III) dose exceeds 5.63 mg/L. As reported in other studies, the concentration of REEs in NMW wastewater is about 0–14.6 mg/L (Liu et al., 2019). Combined with the pretreatment cost, the Ce(III) dose in the feed of this process should be kept below 1.37 mg/L as much as possible, and need not exceed 5.63 mg/L.

3.3. The evolution of sludge morphology with typical Ce(III) dose

The of biological response of Ce(III) dose is as illustrated in Fig. 1d and presented in Table S2. It could be seen that three specific dose of Ce (III) manifested significant effects on SNPR, thus (1) the most significant dose for SNPR promotion (5 mg/L); (2) the most significant dose for SNPR inhibition (500 mg/L) and; (3) the dose which simultaneously demonstrated both SNPR promotion and inhibition at equal magnitude (20 mg/L). Therefore, the sludge morphology and distribution of elements at these three dose are worth determining to facilitate in the establishing of mechanism(s) leading to the effect of Ce(III) on AOB as well as the defense mechanisms put up by AOB to protect their existence from the toxic effect of the rare earth elements.

The EDS spectrum revealed that change of sludge composition was significant but gradual with the increase of Ce(III) dosage, especially the weight of Ce, which increased significantly (Fig. S3). The sludge samples of C0, C3, C5 and C9 observed under SEM-EDS showed that sludge of C0 comprised of round-shape cell wrapped in EPS (Fig. 3A,i), however, the profile of these cell was quite clear (Fig. 3A,ii). Similar to a previous research, it corroborated with the typical feature of partial nitrification sludge, implying this round-shape cell may be attributed to be AOB (Zhang et al., 2019). Under 5 mg/L Ce(III) dose, the sludge surface in some areas looks very smooth (Fig. 3B,i) but outline of cell was invisible, indicating EPS production was significantly increased (Su et al., 2020).



Fig. 3. The evolution of sludge morphology with typical Ce(III) dose: (A) control; (B) dose of 5 mg/L; (C) dose of 20 mg/L; (D) dose of 500 mg/L (NOTE:[i] full sludge micrographs at 20 μ m or 2 μ m Bar; [ii] enlarged view at 1 μ m Bar).

An interconnecting filamentous EPS matrices were also observed in an enlarged SEM micrographs (Fig. 3B,ii). Identical EPS structures were also observed in some previous reports (Salim et al., 2014; Vu et al., 2019), which provided a better attachment for the associated bacteria, and seemed to adhere on the cell surfaces (Vu et al., 2019). This structure only appeared on the surface of the sludge treated with Ce(III) at 5 mg/L , meaning the filamentous EPS may be produced by either the associated bacterial community or the AOB itself induced by its bacterial partners (Vu et al., 2019), and may causes the excellent SNPR. Moreover, the element composition of the sludge after treatment at this dose was small (Figs. S4 and S5) due to the low dose, hence Ce was not detected in C3 (Fig. S5).

The sludge morphology changed significantly at a Ce(III) dose of 20 mg/L, and many crisscrosses "ridges" appear on the surface (Fig. 3C, i). In the enlarged micrographs, many clusters of different sizes could be seen on these ridges (Fig. 3C,ii). Large clusters, small clusters, smooth surfaces and ridges were examined by EDS, which revealed that the weight of Ce in these areas were 1.33%, 0.74%, 1.34% and 1.71%, respectively (Fig. S6). These results indicates that the highly effective Ce-removing substances was produced, and resulted in the excellent Ce (III) removal (>99%).

After operating the batch reactors dosed with 500 mg/L Ce(III) for 12 h, the morphology of sludge changed greatly, and many clusters and flakes (Fig. S7) adhered to the surface of the sludge (Fig. 3D,i). It was observed that the surface of these substances is relatively rough in the enlarged view (Fig. 3D,ii) indicating they not being crystals but looks more of EPS. The EDS results showed that the weight of Ce at smooth surface, the cluster, small flakes and large flakes average 2.23%, 4.25%, 17.75% and 36.32%, respectively (Fig. S7). The formation process of flakes of observed in this study was attributable to the AOB or its coexisting microorganism secreting the corresponding EPS under the stress of Ce(III), and subsequently employ the EPS to protect the cells from damage as a result of the Ce(III) effect. The Ce-bound EPS gradually changed into small clusters, large clusters, small flakes, and large flakes as the binding amount increased, and the original functional groups were gradually destroyed. This phenomenon affected its ability to adhere to the sludge floc and eventually caused it to fall off the sludge surface (Fig. 3D,i).

3.4. Variation in EPS yield and specific components under Ce(III) influence

3.4.1. Evolution and distribution of EPS as revealed by CLSM

As discussed in Section 3.1, reactors dosed with Ce(III) of 500 mg/L had the most significant inhibitory effect on SNPR compared to the control. To further comprehend the resulting phenomenon, CLSM was exploited to characterize the differences in EPS production and species as well as the distribution of different EPS in sludge (Louvet et al., 2017). Therefore, C0 (Ce(III) of 0 mg/L) and C9 (Ce(III) of 500 mg/L) were selected as the main research objects, and their respective EPS type and distribution were investigated. Observably, CLSM images illustrated in Fig. 4A showed uniform pattern and distribution of sludge indicating that the β -polysaccharides, α -polysaccharides, and proteins in C0 was relatively evenly distributed. This further suggested the normal state of PN sludge (Yao et al., 2017). The content of substance of the collected sludge as revealed by fluorescence intensity was in the order of α -polysaccharide > protein (Fig. 4A,i–iii).

Green was the most dominant fluorescence in Fig. 4A,iv, followed by red and blue, indicating that α -polysaccharides was widely distributed on the outer sludge whereas β -polysaccharides and proteins were mostly distributed in the interior. However, the red and blue fluorescence became more visible in Fig. 4B,iv, whilst green was almost diminished, suggesting that the main components of EPS in the sludge were converted to β -polysaccharides and proteins after exposure to the Ce(III) dose (500 mg/L). Many studies have reported that EPS plays an important role in resisting the effects of heavy metal ion (Cao et al., 2018) but the various types of EPS have different heavy metal adsorption capabilities (Wang et al., 2014).

Polysaccharides and proteins are the main EPS (accounting for 75–90%) produced by microorganisms (Basuvaraj et al., 2015; Cao et al., 2018). Therefore, the release of some special polysaccharides or proteins may be an important means for AOB to respond to Ce(III) effect and subsequently protect itself from cell destruction. Many studies have shown that β -polysaccharides have excellent bonding ability with metal ions (Nadar et al., 2019; Nowak et al., 2019), and the content of Ce, Fe, Si, Al, Mg and other metal elements in C9 increased significantly as revealed by EDS (Fig. S7), suggesting that a material with strong metal ion bonding ability is produced.

3.4.2. LB-EPS transformations under Ce(III) dosage

Although the three main EPS viz. S-EPS, LB-EPS and TB-EPS have the



Fig. 4. Differences in EPS yield and components under typical Ce(III) stress: CLSM images (including fluorescence image of (i) β -polysaccharides, (ii) α -polysaccharides, (iii) proteins and (iv) a combination of above) of (A) control sample and (B) 500 mg/L Ce(III) treatment sample; EEM fluorescence spectra under Ce(III) dose of (C) 0 mg/L, (D) 5 mg/L, (E) 20 mg/L and (F) 500 mg/L.

same source, they play different functions namely fixation, flocculation, and structure maintenance, respectively (Wang et al., 2019). As the outermost structure of the sludge comprises of LB-EPS (Zhao et al., 2015), it therefore serves as the first barrier to protect cells from Ce(III) damage. Ideally, it's also been affirmed that content of LB-EPS is significantly higher than that of TB-EPS in sludge flocs (Basuvaraj et al., 2015). Therefore, the study of LB-EPS transformation under various doses of Ce(III) was studied using EEM to elucidate the mechanism of Ce (III) during promote or inhibit of SNPR.

Only one characteristic fluorescence peak (peak C) was found in the EEM fluorescence spectra of the control sample (CO) (Fig. 4C). The Excitation/emission wavelengths (Ex/Em) of Peak C were 275/321 nm, and these peak had been described as Tryptophan-like protein (Wang

et al., 2019). Four characteristic fluorescence peaks were present in spectra of sample C3, where the count and intensity of the peak had increased tremendously (Fig. 4D). Peaks A and B are located at 410/474 nm and 335/379 nm (Ex/Em), respectively, and are classified Fulvic acid-like substance (Shao et al., 2019; Wang et al., 2019). The maximum intensity of peak D did not appear in the spectrum, but according to the shape of the peak, it is known that Ex/Em is about 250/478 nm, which was preliminary judged as Fulvic acid-like substance (Park and Snyder, 2018). Peak C displayed a small red shift compared to control, and its fluorescence intensity increased to 3.34 times of the control (Table S4). In the spectra of C5, peak A disappears completely, and the maximum intensity of peak C and peak D were reduced by approximately 37.78% and 83.32%, respectively (Fig. 4E).

Peak B was replaced by peak E (Ex/Em of 325/415 nm), although they were closer in distribution. Consequently, peak E demonstrated to be a humic acid-like substance (Zhu et al., 2015). The number of characteristic fluorescence peak in C9 was further reduced, such that peak D and peak E disappeared (Fig. 4F). A new peak (Peak F) was generated at the Ex/Em of 305/389 nm which as well revealed to be Humic acid-like substance (Zhu et al., 2012).

According to the EEM spectrum, peak A (strongest peak) was only observable when 5 mg/L of Ce(III) was dosed (Fig. 4D), indicating fulvic acid which was represented by peak A, may had strong correlation to the promotion effect of Ce(III) on SNPR. As widely reported, Fulvic acid is one kind of humic acid which contains various reactive functional groups, such as hydroxyls, phenols, quinones and carboxyls (Liu et al., 2020). Therefore, the release of N-acylated-L-homoserine lactones (AHLs) may be stimulated by the addition of fulvic acid (Li et al., 2019), which improved quorum sensing and stimulated bacterial activity, and thereby improving nitrogen conversion rate (Liu et al., 2020). Tryptophan (peak C) was widely present in four samples, and its function in the PN sludge enhanced aggregation (Lin et al., 2018). Therefore, the granulation of PN sludge may be related to the dose of Ce(III). The alternating appearance of peak B, peak E, peak F indicated that the humic acid changed along with increasing dose of as Ce(III) (Tang et al., 2018b). The location of peak C and peak D demonstrated a blue/red shift in the emission and/or excitation scale indicating the change in the organic functional groups (Zhu et al., 2015).

3.5. Macroscopic components of sludge under typical Ce(III) stress

FTIR spectroscopy is a versatile technique that can distinguish or quantify different functional groups and offers a comprehensive insight



Fig. 5. Macroscopic components of sludge under typical Ce(III) stress: (A) comparison of mean spectra under Ce(III) dose of 0 mg/L (C0), 5 mg/L (C3), 20 mg/L (C5) and 500 mg/L (C9); single spectra comparison of (B[i]) C3&C0, (C[i]) C5&C0, (D[i]) C5&C3, (E[i]) C9&C0 and (F[i]) C9&C5; difference spectrum of (B[ii]) C3-C0, (C[ii]) C5-C0, (D[ii]) C5-C3, (E[ii]) C9-C0 and (F[ii]) C9-C5.

into the molecular structure of EPS, including both fluorescent and nonfluorescent substances (Huang et al., 2017; Li et al., 2014). Furthermore, functional substances and groups that played a role in the defense of Ce (III) toxicity was investigated using FTIR spectra with CLSM and EEM. In this study, the dominant peaks of four samples (C0, C3, C5 and C9) were identified using FTIR spectroscope (Fig. 5A), and their wavenumber, absorbance, primary assignment, possible compounds and reference were determined and presented (Table S5). Although the C9 spectrum was significantly different from the other spectra, the differences between C0, C3, and C5 spectra were relatively difficult to identify (Fig. 5A). Consequently, the difference in spectrum was used to compare the spectra visually and highlight the recognizable changes in peaks (Comparato Filho et al., 2019).

Compared with the control, the values of five peaks in C3 decreased $(990, 1025, 1546, 1643, and 3286 \text{ cm}^{-1})$ with the exception of one peak (470 cm⁻¹) which experience an increasing trend (Fig. 5B). Reportedly, peaks near 470 cm⁻¹ are caused by the stretching motion of the Ce(IV)-O bond indicating the production of CeO_2 (Verma et al., 2015). Another study also affirmed that peaks around 990, 1025 and 3286 cm⁻¹ are assigned to the stretching motion of C-C, C-O and O-H bond (Ma et al., 2016a; Peng et al., 2019) whereas peaks near 1546 and 1643 cm^{-1} are attributed to the stretching motion of C⁼N (or C⁼C) bond and the bending motion of C-O bond (Comparato Filho et al., 2019; Xu et al., 2018). Based on the above reports and supporting theories, the observable reduction suggested less polysaccharide and protein content were present in C3 (Table S5). C5 was much similar to C0 in terms of FTIR spectrum and ammonia oxidation performance (SNPR) (Figs. 1d and 5C, i). This similarity suggested that there was a correlation between functional groups and SNPR. Some differences were however manifested between spectra of C5-C0 and C3-C0 (Fig. 5B,ii and C,ii), which was attributed to the disappearing of the promoting effect. The absorbance of the four peaks (617, 1025, 1413 and 1546 cm^{-1}) in C5 was significantly higher than that in C3 (Fig. 5D,i,ii). Literature has established that peak around 617 cm⁻¹ is caused by the stretching motion of the Ce(III)-O bond, implying the production of Ce₂O₃ (Verma et al., 2015). Therefore, Ce was not expected be present in the control (C0) sample as peaks around 470 and 617 cm^{-1} are mainly attributable to the motion of skeletal mode in glucan (Ma et al., 2016a). Again, Peng and coworkers discussed that peak near 1413 cm^{-1} is caused by C-H bond's bending motion (Peng et al., 2019). A a result, the observed increased of polysaccharides was revealed by these peaks and the peak of 1025 cm^{-1} . Moreover, the increase peak at 1546 cm^{-1} indicated the protein content in C5 was higher than in C3.

The spectra of CO and C5 showed higher similarity (Fig. 5E and F). Therefore, only differences between C5 and C9 samples were analyzed. As depicted in Fig. 5F, 7 peaks (697, 721, 855, 1413, 1482, 2940 and 3286 cm^{-1}) were observed increasing whilst 4 other (990, 1025, 1145 and 1726 cm^{-1}) decreased. Peaks around 697 cm^{-1} are assigned to stretching vibrations of skeletal mode, representing an increase in glucan. Literature has established that peaks around 855, 2940 and 3286 cm⁻¹ are assigned to stretching vibrations of C-C bond, CH₂ groups and O-H bond (Ma et al., 2016a; Xu et al., 2018; Zhao et al., 2017). Consequently, peaks including 990 cm^{-1} , 1025 cm^{-1} and 1145 cm^{-1} demonstrated that bonds such as the bending motion of C-O bond, C-C bond and C-H bond (Ma et al., 2016a), respectively, were existing within the sludge biomass. This further suggested that the structure and type of the polysaccharide changed over time. In line with CLSM results, peak (1482 cm⁻¹) which suggested the stretching motion of C⁼N or C⁼C bond in protein was strengthened (Comparato Filho et al., 2019). Peak (1726 cm^{-1}) shows the stretching motion of C-O bond in lipids (Xu et al., 2018). However its sharp decrease of about 90.15% (Table S5) confirmed massive lipid reduction, an indication of the toxic effect from the Ce(III) dosage. Other reports have also shown that peaks near 721 cm⁻¹ are caused by the stretching motion of Ce(IV)-O bond and has the potential to combined with metal ions (abbreviated as CeO2-X) (Balamurugan et al., 2019). Coincidentally, the weight of metal (Fe, Al,

Mg, etc.) has a significant increase in C9 sample as revealed by EDS (Fig. S7). The absorbance of peak near 697 cm⁻¹ in C9 increased 195 times than C5 (Table S5), indicating a significant increase in glucan production (Ma et al., 2016a).

As presented in Fig. 5A-F and Table S5, the possible compound changes are shown in Fig. S8. At the dose of 5 mg/L, the organic functional groups of proteins and polysaccharides has decreased, and there are hardly any changes in other compounds (Fig. S8). In batch assay C3, a significant fraction of Ce(IV) (Fig. S9) and small molecular substance including fulvic acid, tryptophan and humic acid were found. The evolution of these substance suggested that they may had been produced by the polysaccharides and proteins as result of the effect of Ce(III). Undoubtedly, Qiu and coworkers had reported that, fulvic acid could serve as a growth regulator of plant, animal and microorganism (Qin et al., 2019) and thereby increasing cell membrane permeability, enhance secondary metabolites, and control hormone levels (Wang et al., 2020). As Ce(III) dose was increased from 5 mg/L to 20 mg/L, the relative abundance of these compounds observed was mainly polysaccharides and proteins with no significant change (Fig. S9). However, Ce₂O₃ significantly increased and glucan reduced to almost zero (0.05%) (Fig. S9), indicating that glucan may had played an important role in Ce conversion. Interestingly, CeO₂-X became almost the only form of Ce in C9 (Fig. S8). Simultaneously, the Ce(III) removal rate reached 99.96% and metal element content increased sharply in C9, suggesting some glucan that specifically adsorbs metal ions were produced in large quantities. The β -polysaccharide was revealed to be the main component of the polysaccharide according to the CLSM images. Therefore, β-glucan may be the main organic substance for efficient removal of Ce (III).

3.6. Special region and correlation change with Ce(III) dose

Due to the extreme heterogeneity of sludge sample, the resolution of FTIR spectrum is limited leading to the omission of some useful information (Li et al., 2014). Therefore, 2DCOS-IR spectrum was used to resolve the overlapped peak problems that may arise from FTIR spectroscopy. Thus Ce(III) dose was introduced as a perturbation sequence, and its correlation to structural variations was provided (Huang et al., 2017; Li et al., 2014). It has been reported that (1) amide-I region is the best-known vibration of peptide linkage (stretching of C=O and C-H, bending of N-H) (Chiaramaria et al., 2020; Lin et al., 2018), (2) amide-II region shows stretching of C-N bond and bending of N-H in polypeptide helices (Barth and Zscherp, 2002) and, (3) the absorbance of amide-III region was caused by stretching of C-N & C-C bond and bending of N-H and C-H bond (Chiaramaria et al., 2020).

As shown in Fig. 6A, only amide III region increased under Ce(III) dose of 5 mg/L, indicating decrease in peptide linkage and polypeptide helices, and the production of amino acids and peptides. According to the classic theory explaining humic acid formation (Lehmann and Kleber, 2015), humic acid can be produced via the combination of amino acids/peptides and polyphenols. Tanase et al. (2001) revealed that Ce(III) can change the structure of amino organics (diamine, monoamine, amino alcohol) by combining with them. In addition, the isomerization of glycoside-like matter by Ce(III) may lead to the production of cleavage of aromatic glycosides and polyphenols (Tanase et al., 2001). Therefore, the fulvic acid-like matter that appears in C3 may be derived from the combination of peptides and polyphenols produced by Ce(III). The presence of methylene was confirmed by the peak around 1290 cm^{-1} in amide III region (Wang and Guo, 2020). Positive correlation was always between this peak value and dose of Ce (III) (Fig. 6A,i-C,i), indicating that methylene increased with the dose. Some reports confirm that the ring oxygen (C-O-C bond of furanoid or pyranoid) can be replaced by methylene, which improves the structural stability of sugar derivatives (Soengas et al., 2011). That means ring oxygen was consumed at a dose of 5 mg/L, resulting in a decrease in absorbance in the glycoside region (Fig. 6A,ii). Although ring oxygen



Fig. 6. Special region and correlation change with Ce(III) dose: 2DCOS-IR spectra of (A[i]) C3&C0, (B[i]) C5&C3 and (C[i]) C9&C5; difference spectrum of (A[ii]) C3-C0, (B[ii]) C5-C3 and (C[ii]) C9-C5 (NOTE: A. I, amide I region; A. II, amide II region; A. III, amide III region; Glycosidic, glycosidic region).

was consumed more along with increasing dose (20 mg/L) of Ce(III), the increase in the absorbance of the glycoside region meant that the bacterial cell produced more glycosides under this pressure to maintain its normal life activities (Fig. 6B,ii). The absorbance of glycoside region decreased at the highest dose of 500 mg/L, suggesting that the dose of Ce(III) has exceeded the limit of microbial tolerance, and caused toxic effects. In particular, the peak value at 1726 cm⁻¹ was always negatively correlated with the dose (Fig. 6), and the uronic acid group was easy to bind with metal cations (Wang et al., 2018), which represented that the ester group (uronic acid) may be the key functional group to remove Ce(III) (Wang and Guo, 2020).

4. Conclusions

In this study, the effect of Ce(III) on PN performance at different dosages was determined via ammonium oxidation rate, process kinetics and molecular studies (SEM-EDS, CLSM, EEM, FTIR and 2DCOS-IR). This study revealed that whereas hormesis effect was existent between Ce(III) dose and AOR, Ce(III) had less influence on the NOR. Maximum Ce(III) dosage with no observable adverse effect on the PN process was Ce < 20 mg/L. Process kinetics revealed that, the inhibition of Ce(III) on AOB was not feasible when Ce(III) dosage was <1.37 mg/L whereas contrary when Ce(III) dosage exceeded 5.63 mg/L, suggested that pretreatment of NMW to the barest minimum is a prerequisite requires pretreatment to obtain efficient PN process. It was also found that biosorption was the main Ce(III) removal (97.37-99.96%) pathway as revealed by SEM-EDS micrographs and β -glucan determined with FTIR. Markedly, Ce(III) promoted the cleavage of protein and aromatic glycosides which subsequently produced peptides and polyphenols which combine to yield fulvic acid. Although fulvic acid supported the PN performance, the ester group (uronic acid group) served as key functional group that was responsible for the Ce(III) removal.

CRediT authorship contribution statement

Hao Su: Writing - original draft, Investigation, Formal analysis, Visualization, Data curation. Dachao Zhang: Project administration, Funding acquisition, Investigation, Formal analysis. Philip Antwi: Writing - original draft, Writing - review & editing, Investigation, Formal analysis, Visualization, Data curation. Longwen Xiao: Investigation, Formal analysis, Visualization. Xiaoyu Deng: Investigation, Zuwen Liu: Project administration, funding acquisition. Bei Long: Investigation. Miao Shi: Project administration, funding acquisition. Michael J. Manefield: Writing - review & editing. Huu Hao Ngo: Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111796.

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