Effect of low-intensity ultrasound on partial nitrification: Performance, sludge characteristics, and properties of extracellular polymeric substances

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ARTICLE INFO

ABSTRACT

Ultrasound technology, which is environment-friendly and economical, has emerged as a novel strategy that can be used to enhance the partial nitrification process. However, its effect on this process remains unclear. Therefore, in this study, partial nitrification sludge was subjected to low-intensity (0.15 W/mL) ultrasound treatment for 10 min, and the effect of ultrasonic treatment on the partial nitrification process was evaluated based on changes in reactor performance, sludge characteristics, and the properties of extracellular polymeric substances (EPS). The results obtained showed that the ultrasonic treatment enhanced nitrite accumulation performance as well as the activity of ammonia-oxidizing bacteria from 3.3 to 16.6 mg O_2/g VSS,·while inhibiting the activity of nitrite-oxidizing bacteria. Further analysis showed that owing to the ultrasonic treatment, there was an increase in EPS contents. Particularly, there was a significant increase in loosely bound polysaccharide (PS) contents, indicating the occurrence of intracellular PS anabolics as well as PS secretion. Additionally, ultrasonic treatment induced a significant increase in carbonyl, hydroxyl, and amine functional group contents, and EPS analysis results revealed that it had a positive effect on mass transfer efficiency; thus, it enhanced the partial nitrification process. Overall, this study describes the effect of intermittent low-intensity ultrasound on the partial nitrification process as well as the associated enhancement mechanism.

1. Introduction

Partial nitrification, which offers the possibility of saving aeration energy and lowering excessive sludge production, is a novel biological nitrogen removal process that has attracted a lot of attention recently [1–3]. It can be realized and maintained by controlling dissolved oxygen (DO) concentration, free ammonia and free nitrite acid inhibitor concentration, temperature, additive agent concentration, and magnetic field strength [4–8]. However, it is still associated with drawbacks, including low sludge activity, slow start-up, low nitrite accumulation capacity, and instability [9,10]. Therefore, studies on how it can be rendered more efficient are necessary.

Generally, ultrasound refers to sound waves with frequency above 20 kHz [11], and they can be divided into high-intensity ultrasound (>100 W/cm^2) and low-intensity ultrasound (<10 W/cm^2), depending on their intensities [12]. High-intensity ultrasound can cause sludge lysis-cryptic growth, which is predominantly used for sludge

disintegration to achieve sludge reduction [13,14]. Reportedly, low-intensity ultrasound has diverse effects on target sludge, including mechanical, cavitation, and thermal effects [15]. It has also been demonstrated that it can significantly enhance the activity of anaerobic ammonia-oxidizing bacteria (AAOB) and ammonia-oxidizing bacteria (AOB) [16,17]. Further, it can promote the oxidation of ammonia nitrogen by AOB, while inhibiting the oxidation of nitrite by nitrite-oxidizing bacteria (NOB), and realizing nitrite accumulation [18,19]. Furthermore, it has been reported that it can enhance the abundance of AOB, while washing out NOB [20], and in terms of the microbial genus level, it promotes the relative abundance of *Nitrosomonas,* while decreasing *Nitrobacter* population [21]. Overall, it represents an effective strategy by which the efficiency of the partial nitrification process can be enhanced. However, its effects on this process as well as the associated mechanism still need further exploration.

Thus far, it has been demonstrated that suitable ultrasonic treatment can promote cell wall penetration and strengthen transfer efficiency,

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thereby promoting the progress of cell anabolics [22]. Additionally, a previous study showed that low-intensity ultrasound has different effects on the biological treatment of sewage, and is associated with the promotion and inhibition of co-existing microorganisms [17]. In a previous study, it was observed that ultrasonic treatment resulted in a decrease in sludge particle size and also brought about changes in the spatial distribution and composition of extracellular polymeric substances (EPS) [23]. It has also been reported that ultrasonic treatment can destroy cell wall and sludge structure, leading to a decrease in sludge particle size [24]. Overall, ultrasonic treatment significantly influences sludge EPS properties. Several factors, including pore formation, cell wall thickness, cell membrane integrity, and cytoplasmic content release, affect microbial activity [25]. To date, most studies focused on the impact of ultrasonic treatment on sludge activity and community structure [19,26], and the improvement of the efficiency on the partial nitrification process. However, the effect of ultrasonic treatment on the sludge EPS properties during the partial nitrification process is less studied, and the enhancement mechanism of ultrasonic treatment on sludge require further investigation. Thus, it is urgent to understand the correlation between ultrasonic treatment and the properties of sludge EPS during the partial nitrification process.

In this study, to evaluate the effect of low-intensity ultrasound on the efficiency of the partial nitrification process, reactor performance, sludge characteristics, and EPS properties were investigated. Further, the nitrogen conversion performance and nitrite accumulation ratio (NAR) of the reactors were analyzed, and differences in the sludge bioactivity and Zeta potentials of the reactors were also assessed, along with the variations in EPS content and composition. Furthermore, the correlation between ultrasonic treatment and sludge EPS properties during the partial nitrification process was investigated, and the mechanism by which ultrasonic treatment enhances sludge bioactivity during the partial nitrification process was also clarified.

2. Materials and methods

2.1. Inocula and feeding media

The seed sludge was obtained from a UNITANK bioreactor of a local wastewater treatment plant (WWTP) in Ganzhou, China, which mainly treats municipal sewage with design capacity of 60000 m^3/d. The synthetic sewage used in this study was prepared with chemical oxygen demand and ammonia nitrogen concentrations of approximately 150 and 60 mg/L, respectively. Its detailed composition was as follows: CH_3COONa, 208 mg/L; NH_4Cl, 229 mg/L; KH_2PO_4, 20 mg/L; $MgSO_4·7H_2O$, 14 mg/L; $NaHCO_3$, 900 mg/L; $CaCl_2$, 12.5 mg/L; and $Fe_2(SO_4)_3$, 3 mg/L.

2.2. Reactor operation

The partial nitrification experiments were performed in parallel sequencing batch reactors (SBRs) with an effective working volume of 1 L. The SBRs had a volumetric exchange ratio of 50%. The mixed liquor volatile suspended solids (MLVSS) concentration was 2200 mg/L, the organic loading rate (OLR) was 0.05 kg COD/(kg MLVSS·d) and the nitrogen loading was 0.02 kg N/(kg MLVSS·d). The sludge retention time (SRT) was maintained at approximately 20 days, and the hydraulic retention time (HRT) was 16 h. Each cycle was performed for 8 h, and comprised: feeding (10 min), reacting (300 min), setting (60 min), decanting (10 min), and idling (100 min) phases. The intermittent aeration operation mode (30 min aeration every hour) was used during the reacting phase, and the total aeration time was 150 min at an aeration rate of 1.0 ± 0.2 L/min. The pH of the reactors was controlled at 8.0 using a solution of $NaHCO_3$. The control reactor (without ultrasonic treatment) and the experimental reactor (ultrasonic treatment was performed each 24 h) were operated under the same operation parameters.

During the idling phase of the experimental reactor, sludge samples were collected for ultrasonic treatment. The device used for the ultrasonic treatment was a probe-type generator (JY88-IIN, Ningbo, Xinzhi, China) with a frequency of 20 kHz and a probe diameter of 6 mm. The partial nitrification sludge was placed in a beaker, with the probe immersed 10 mm below the liquid surface for intermittent ultrasonic treatment. The intermittent irradiation was 24 h with reference to Liu et al. [27].

In the early stage, the effect of ultrasonic irradiation time was explored with the same other parameters. The results showed that the AOB activity was significantly improved while the NOB activity was inhibited with ultrasonic irradiation time of 10 min (Fig. S1). The effect of ultrasonic intensity was also explored with the same other parameters. The results showed that the AOB activity was increased by 101.3% while the NOB activity was inhibited with ultrasonic intensity of 0.15 W/mL (Fig. S2). Considering the promotion effect and low energy consumption, the appropriate ultrasonic intensity and irradiation time was 0.15 W/mL, 10 min, respectively.

2.3. Analysis methods

To determine the ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, and MLVSS contents of the effluent, standard methods [28] were used. During the reactor setting phase, i.e., after some sludge had been collected for ultrasonic treatment, the sludge was analyzed, and its characteristics were determined. The sludge was collected three times from the reactor when the reactor was running stably, and mixed together for analysis. OUR, DHA and SVI were all measured three times. To measure dehydrogenase activity (DHA), 2,3,5-triphenyl tetrazolium chloride (TTC) spectrophotometry was used [29], and the sludge volume index (SVI) was measured as previously described [30]. Additionally, the Zeta potential of the sludge was measured using a Zetasizer Nano S90 (Malvern, UK) instrument, and the contact angle with water was measured using a contact-angle analyzer (Dataphysics DCAT, Germany). The nitrite accumulation ratio (NAR) of the sludge was calculated using Equation (1).

$$NAR = \frac{NO_2^- \text{-} N}{NO_2^- \text{-} N + NO_3^- \text{-} N} \times 100 \tag{1}$$

where, NO_2^--N and NO_3^--N represent effluent nitrite and nitrate concentrations in mg/L, respectively.

2.4. Analysis of the specific oxygen uptake rate of AOB and NOB

The method used for the determination of the specific oxygen uptake rate (SOUR) of AOB and NOB was based on the addition of allylthiorea (ATU) and $NaClO_3$, which are selective AOB and NOB inhibitors, respectively [31]. To conduct the tests, which were performed in triplicates, breathing bottles were used as reactors. Specifically, to determine the oxygen consumption rate of AOB (OUR_{AOB}), 20 mg/L of ammonia nitrogen and 2.13 g/L of $NaClO_3$ (NOB activity inhibitor) were placed in each reactor bottle, and distilled water saturated with dissolved oxygen was added. Changes in DO concentration were monitored and recorded, OUR_{AOB} was calculated. Similarly, to determine the oxygen consumption rate of NOB (OUR_{NOB}), 20 mg/L of nitrite and 2.13 g/L of ATU (AOB activity inhibitor) were placed in each reactor bottle, and dissolved oxygen-saturated distilled water was added. Thereafter, changes in DO concentration were monitored and recorded, and OUR_{NOB} was calculated. The specific oxygen uptake rate (SOUR) was then calculated from the OURs per unit mass of sludge, using Equations (2) and (3).

$$SOUR_{AOB} = \frac{OUR_{AOB} \times 1000 \times 3600}{MLVSS \times 60} \tag{2}$$

$$SOUR_{NOB} = \frac{OUR_{NOB} \times 1000 \times 3600}{MLVSS \times 60} \qquad (3)$$

2.5. EPS analysis

EPS were extracted from sludge using ultrasonic-thermal extraction. Specifically, 10 mL of mixed sludge was transferred to a centrifuge tube, and centrifugation was performed at 4,000 r/min for 10 min. Thereafter, the supernatant was removed, and the pellet in the centrifuge tube was diluted to 10 mL with phosphate buffered saline (PBS), and the operation was repeated thrice. The supernatant thus collected was considered as loosely bound EPS (LB-EPS). The temperature of the residual pellet was controlled at 80 °C for 60 min in a constant temperature water bath. PBS was again added and centrifugation was further performed at 10,000 r/min for 10 min. The supernatant thus collected was considered as tightly bound EPS (TB-EPS), which was subsequently filtered using a 0.45-μm membrane.

The polysaccharide (PS) contents of the supernatant samples were determined using the phenol–sulfuric acid method [32], while the modified Lowry method was used for the determination of their protein (PN) contents [33].

The 3-D excitation emission matrix fluorescence (3D-EEM) of the supernatant was determined using a fluorescence photometer (Cary Eclipse, VARIAN, USA). The excitation wavelength was varied in the range 200–400 nm at intervals of 5 nm, and the scanning was performed continuously from 220 to 500 nm at a scanning rate of 3000 nm·min^{-1}.

The EPS extract was freeze-dried at − 80 °C, and the resulting powder sample was mixed with powdered KBr, and to determine the chemical composition of the extract, Fourier-transform infrared (FTIR) spectrometry was performed using an ALPHA spectrophotometer (Bruker, Germany).

2.6. Statistical analysis

All assays were conducted in triplicate, and results were presented as the mean ± standard deviation. Statistical tests were performed with the statistical program SPSS 25.0 (SPSS Inc., Chicago, USA). Analysis of variance (ANOVA) was used to compare results by group, the difference was considered significant if $p < 0.05$.

3. Results and discussion

3.1. Effect of ultrasonic treatment on the efficiency of the partial nitrification process

The effect of ultrasonic treatment on the efficiency of the partial nitrification process is shown in Fig. 1. Throughout the operation process, the effluent ammonia nitrogen in both the control and the
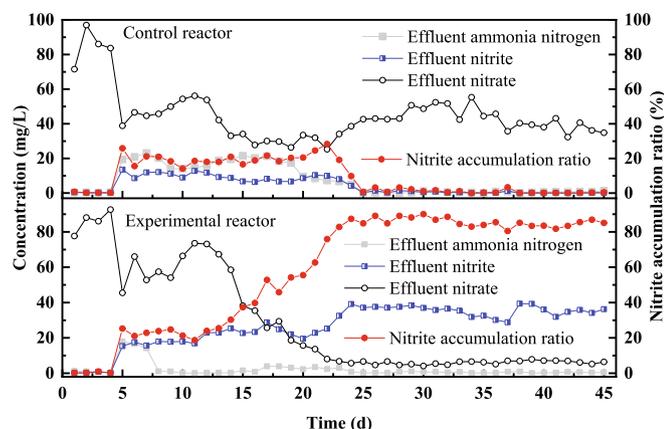

Fig. 1. Effect of ultrasonic treatment on partial nitrification performance.

experimental reactors was practically oxidized. However, compared with the control reactor, the experimental reactor showed a higher effluent nitrite concentration as well as a higher NAR value, up to 85%. Additionally, the effluent nitrate concentration was maintained at 5 mg/L, and the main species in the effluent was nitrite. Overall, the results obtained showed that via ultrasonic treatment, a partial nitrification process with a higher NAR was achieved. Studies have shown that ultrasonic treatment can enhance AOB activity, while inhibiting NOB activity [18,19]. Low-intensity ultrasound acts on microorganisms mainly in three aspects: promoting mass transfer, increasing enzyme activity, and accelerating cell metabolism, thereby promoting biological activity [34]. Ultrasonic cavitation of appropriate intensity can promote the reversible penetration of cells, strengthen the transmission of intracellular and extracellular substances, reduce the metabolic inhibition caused by the accumulation of secondary metabolites, and promote the progress of cell synthesis reactions. In addition, ultrasonic treatment disperses the sludge flocs and increases the mass transfer area, thereby enhancing the mass transfer rate and increasing enzyme activity [35]. Therefore, consistent with the results of these previous studies, this study shows that enhanced partial nitrification can be realized via intermittent ultrasonic treatment.

3.2. Effect of ultrasonic treatment on partial nitrification sludge characteristics

To further determine the effects of ultrasonic treatment on sludge characteristics, analyses were performed on day 30, the characteristics of sludge samples collected from the two reactors were compared (Table 1). Owing to ultrasonic treatment, there was a significant increase in SOUR$_{AOB}$ from 3.3 to 16.6 mg O$_2$/g VSS·min, a decrease in SOUR$_{NOB}$ from 4.1 to 3.6 mg O$_2$/g VSS·min, and an increase in DHA from 2.1 to 3.0 mg μg/mg VSS·h. Additionally, the SVI value of the sludge from the experimental reactor (176 mL/g) was higher than that from the control reactor (105 mL/g), indicating that ultrasonic treatment resulted in an increase in SVI values, and a slight deterioration in the sludge settling performance. However, AOB bioactivity, which favors the partial nitrification process, was significantly enhanced.

As previously reported, microorganisms have different tolerances to the stimulating effects of ultrasound [36], and it has also been reported that ultrasonic treatment can damage cell growth and reduce cell activity [37]. In this study, it was observed that the damaging effect of ultrasonic treatment inhibited NOB bioactivity. Overall, the results obtained showed that in the experimental reactor, AOB activity was enhanced, while NOB activity was inhibited. This implies that ultrasonic treatment altered sludge characteristics such that the partial nitrification process was enhanced.

The Zeta potential and contact angle were tested to determine the ultrasonic effect on the surface properties of the partial nitrification sludge. The effects of ultrasonic treatment on the Zeta potential and contact angel of the sludge were analyzed on day 30 (Fig. 2). After the partial nitrification sludge was subjected to low-intensity ultrasonication, there was an increase in its Zeta potential from −24.5 to −34.6 mV. Additionally, there was an increase in the magnitude of the mutual repulsive forces between the negative charges on the surface of the sludge particles, which enhanced the electronegativity of the sludge. This implies that the stability of the colloid was increased, as sludge aggregation was inhibited by the increasing electrostatic repulsion resulting from the ultrasonic treatment. Thus, the sludge settling

Table 1
Effect of ultrasonic treatment on partial nitrification sludge.

Reactor	DHA(μg/mg VSS·h)	SOUR (mg O$_2$/g VSS·min)		SVI (mL/g)
		AOB	NOB	
Control	2.1 ± 0.8	3.3 ± 0.5	4.1 ± 0.6	105 ± 7.1
Experimental	3.0 ± 0.3	16.6 ± 2.4	3.6 ± 0.5	176 ± 14.7
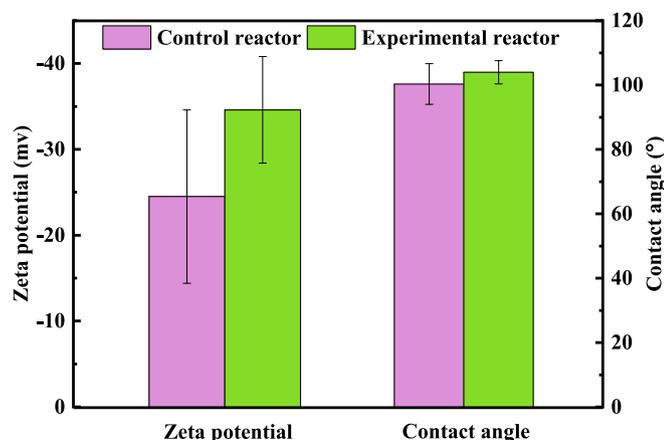
Fig. 2. Effect of ultrasonic treatment on Zeta potential and contact angle.

Table 2
Effect of ultrasonic treatment on EPS content and components.

Reactor	LB-PN	LB-PS	TB-PN	TB-PS	T-PN	T-PS
Control	12.2 ± 1.0	8.3 ± 0.2	11.4 ± 2.2	1.8 ± 0.6	23.6 ± 3.3	10.0 ± 0.8
Experimental	11.4 ± 0.7	18.0 ± 0.5	17.7 ± 0.2	3.2 ± 0.6	29.1 ± 0.8	21.2 ± 1.1

performance deteriorated as shown in Table 1. Wang et al [38] also found that a higher Zeta potential inhibited sludge accumulation. Additionally, there were no significant changes in the contact angle of the sludge after ultrasonic treatment ($p > 0.05$), implying that the correlation between ultrasonic treatment and the sludge contact angle is weak. Li et al [24] found that ultrasonic treatment changed the cell wall and sludge structure, which will affect the surface characteristics of the sludge. In this study, it was found that the Zeta potential and contact angle of the experimental group increased, indicating that the ultrasonic treatment changed the sludge characteristics.

3.3. Effect of ultrasonic treatment on EPS properties

3.3.1. Effect of ultrasonic treatment on EPS components and contents

To determine the effect of ultrasonic treatment on the EPS properties in the two reactors, sludge samples were collected for analysis on day 30. The main components and contents of the sludge EPS are shown in Fig. 3, which revealed that EPS contents, especially LB-PS, was significantly affected by the ultrasonic treatment. The LB- and TB-EPS contents of the sludge from the experimental reactor (29.4 and 20.9 mg/g VSS, respectively) were higher than those of the sludge from the control reactor (20.5 and 13.2 mg/g VSS, respectively). Additionally, ultrasonic treatment resulted in an increase in total EPS contents. Compared with the control reactor (10.0 mg/g VSS), there was a significant increase in the T-PS content of the sludge from the experimental reactor (21.2 mg/g VSS), while the differences in the other EPS contents were insignificant (Table 2). The sludge from the experimental reactor also showed an
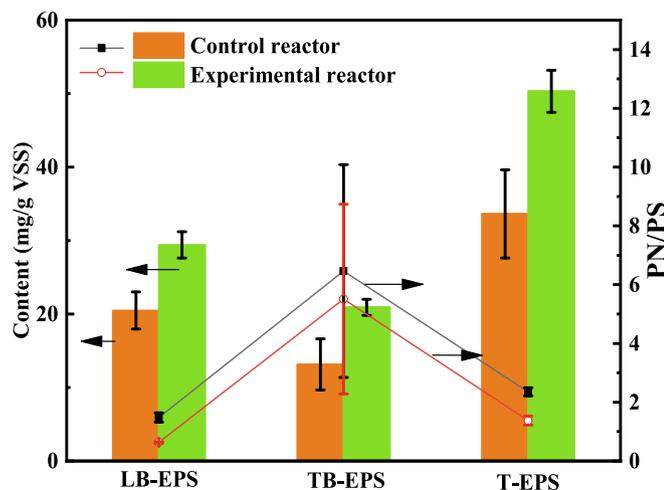
increase in the PN/PS ratio, suggesting that PS is more vulnerable to ultrasonic treatment than PN.

In this study, when ultrasonic treatment was used to stimulate sludge, the sludge cells secreted EPS to protect themselves, and it was observed that excess EPS in the form of LB-EPS weakened the cell adhesion and flocculation structure of the sludge, resulting in poor biological flocculation. In addition, Ye et al [39] also found that the sludge flocculation performance was inversely related to the EPS content in the sludge. Related research pointed out that the increase of EPS content exerted a negative impact on the binding of sludge cells, led to the worsened sludge floc density, the expanded porosity, and the poor flocculation performance [40,41]. Thus, the cells were more susceptible to erosion, and the mud-water separation performance of the sludge was poor [42], leading to a slight deterioration of sludge settlement performance (Table 1). Meanwhile, studies have shown that cavitation induced by low-intensity ultrasound irradiation stretches cell surface, thereby enhancing cell permeability, and accelerating the rate at which organic substrates and metabolites enter and exit cells [34]. This resulted in a further increase in EPS content. Ultrasonic treatment has a greater effect on the synthesis of intracellular PS and on the PS secretion process. Therefore, EPS components and contents were altered by ultrasonic treatment, such that sludge bioactivity was enhanced.

3.3.2. Effect of ultrasonic treatment on EPS composition and structure

The chemical composition of EPS was investigated on day 30. Based on the results of the 3D-EEM analysis (Fig. 4), the main fluorescence characteristic peaks observed included: the 225/332.03–350, 275–280/330–354, 270–275/453.93–460, and 355–360/453.93–456.06 peak corresponded to tryptophan amino acid, tryptophan protein, polyaromatic-type humic acid, and polycarboxylate-type humic acid, respectively [43].

Owing to the ultrasonic treatment, there was a slight change in the tryptophan amino acid and tryptophan protein contents of LB-EPS (Fig. 4(a) and (b)), and compared with LB-EPS, there was an obviously increase in the tryptophan amino acid and tryptophan protein contents of TB-EPS from the experimental reactor (Fig. 4(d)). Additionally, there was a slight decrease in the polyaromatic-type humic acid and polycarboxylate-type humic acid contents of TB-EPS owing to the ultrasonic treatment. The results of the 3D-EEM analysis of EPS are showed in Table 3. The emission wavelength corresponding to peak A in the control group and the peak A in the ultrasound group showed a red-shift, while the emission wavelength corresponding to peak B in the control group and peak B in the ultrasound group also showed a red-shift. The blue-shift of a fluorescence peak indicates a decrease in aromatic ring and conjugated bond functional group contents, while the red shift indicates an increase in carbonyl, hydroxyl, and amine functional group contents [44]. Therefore, the results obtained showed that there was a significant increase in carbonyl, hydroxyl, and amine functional group contents owing to ultrasonic treatment. This affected the sludge surface properties, and favored biochemical reactions on cell surfaces, thereby improved the activity of partial nitrification sludge.

FTIR was used to analyze the main functional groups in the sludge EPS, and the results obtained are shown in Fig. 5. The peak at 3433–3478 cm^{-1} corresponded to O–H stretching vibrations, and the peak at 2369–2414 cm^{-1} corresponded to the C–H stretching vibrations in alkanes and polysaccharides. The peak at 1638–1655 cm^{-1} could be attributed to the secondary structure of the EPS protein, which was

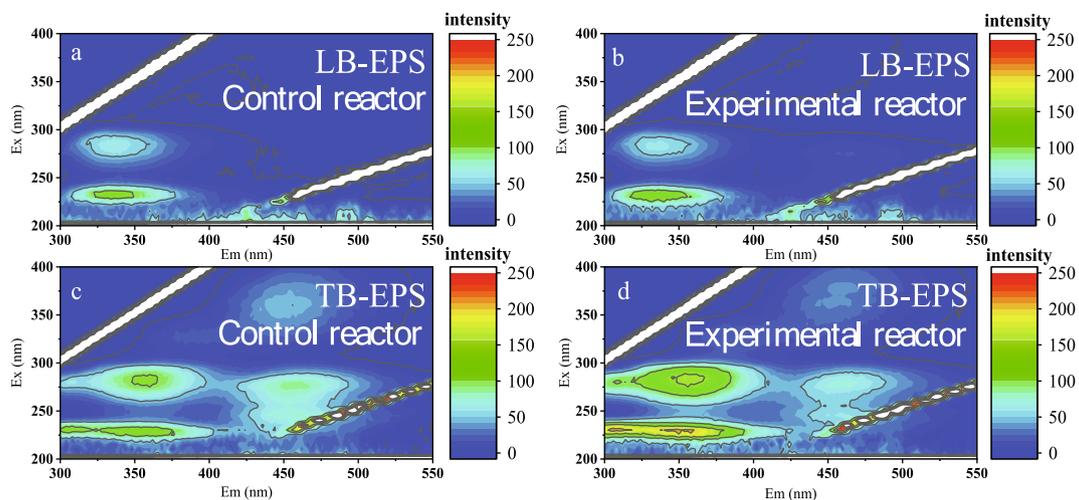**Fig. 3.** Effect of ultrasonic treatment on EPS content and PN/PS.

Fig. 4. 3D-EEM analysis of EPS.

Table 3
Results of the 3D-EEM analysis of EPS.

EPS	Reactor	Peak A (E_x/E_m)/nm	Intensity	Peak B (E_x/E_m)/nm	Intensity	Peak C (E_x/E_m)/nm	Intensity	Peak D (E_x/E_m)/nm	Intensity
LB-EPS	Control	225/332.03	122.16	280/330	68.85	—	—	—	—
	Experimental	225/332.03	133.12	280/335.93	65.72	—	—	—	—
TB-EPS	Control	225/350	136.50	275/354	106.68	270/453.93	89.78	355/453.93	50.08
	Experimental	225/346	212.80	280/352	161.39	275/460	72.66	360/456.06	37.27

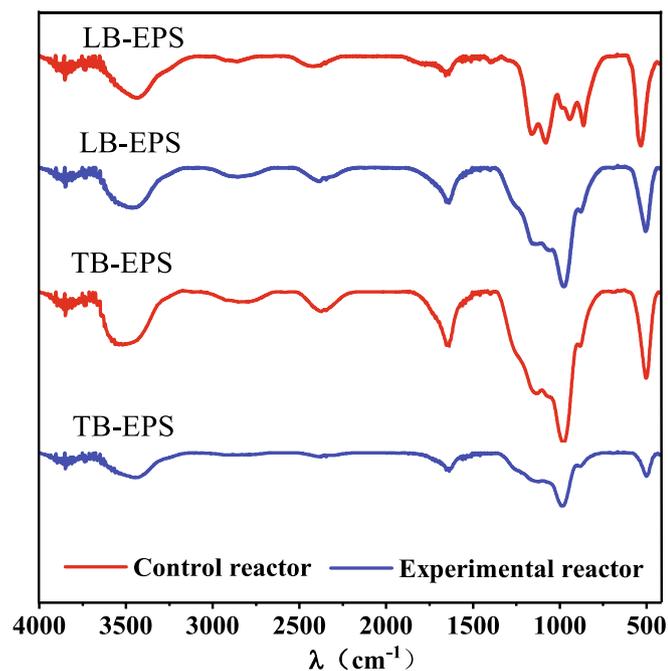**Fig. 5.** FTIR analysis of EPS.

induced by the C = O stretching vibration and NH$_2$ bending vibration in the amide compound. The peak in the range 975–1078 cm^{-1} corresponded to the C—O stretching vibration in carbohydrates and polysaccharides, and the peaks in the range 496–530 cm^{-1} were induced by unsaturated bonds. These results indicate that the types of functional groups in EPS are similar to those caused by ultrasonic treatment, which also resulted in an increase in the functional group contents. Therefore, low-intensity ultrasound favored partial nitrification sludge activity via

changes in the chemical composition of sludge EPS.

The variation of sludge performance, sludge characteristic, and EPS properties revealed the effects of intermittent low-intensity ultrasound on partial nitrification sludge. The stimulating effect of ultrasound on microorganisms resulted in an increase in EPS secretion (Fig. 4), especially the LB-PS content, suggesting that there was an increase in the efficiency of mass transfer through the cell wall, resulting in an increase in DHA (Table 1). It has been demonstrated that ultrasonic treatment enhances ammonia monooxygenase and AOB activity [21]. Additionally, ultrasonic treatment stimulates microorganisms to different extends. Therefore, owing ultrasonic treatment, AOB activity was enhanced, while NOB activity was inhabited. In summary, ultrasonic treatment has different effects on sludge activity and EPS properties.

4. Conclusion

In this study, the low-intensity (0.15 W/mL) intermittent ultrasonic treatment of partial nitrification sludge for 10 min resulted in an increase in the efficiency of the partial nitrification process, and an 85% increase in the NAR was observed. Additionally, SOUR$_{AOB}$ increased significantly from 3.3 to 16.6 mg O$_2$/g VSS·min, while SOUR$_{NOB}$ decreased from 4.1 to 3.6 mg O$_2$/g VSS·min. The Zeta potential of the ultrasonication-treated sludge increased from -24.5 to -34.6 mV, while its settlement performance decreased slightly. The ultrasonic treatment also had significant effects on EPS properties, especially on LB-PS content, which increased from 8.3 to 18.0 mg/g VSS, and an increase in carbonyl, hydroxyl, and amine functional group contents was also observed, suggesting that ultrasonic treatment had a positive effect on the mass transfer efficiency of microorganisms, thereby enhancing partial nitrification.

CRediT authorship contribution statement

Shuai Tian: Data curation, Writing - original draft. **Shuchang Huang:** Methodology. **Yichun Zhu:** Conceptualization, Supervision,

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2021.105527.

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