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Removal efficacy and mechanism of nitrogen and phosphorus by biological aluminum-based P-inactivation agent (BA-PIA)

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ABSTRACT

In this study, aluminum-based P-inactivation agent (Al-PIA) was used as a high-efficiency microbial carrier, and the biological Al-PIA (BA-PIA) was prepared by artificial aeration. Laboratory static experiments were conducted to study the effect of BA-PIA on reducing nitrogen and phosphorus contents in water. Physicochemical characterization and isotope tracing method were applied to analyze the removal mechanism of nitrogen and phosphorus. High-throughput techniques were used to analyze the characteristic bacterial genus in the BA-PIA system. The nitrogen and phosphorus removal experiment was conducted for 30 days, and the removal rates of $\text{NH}_4^+\text{-N}$, TN and TP by BA-PIA were 81.87%, 66.08% and 87.97%, respectively. The nitrogen removal pathways of BA-PIA were as follows: the nitrification reaction accounted for 59.0% (of which denitrification reaction accounted for 56.4%), microbial assimilation accounted for 18.1%, and the unreacted part accounted for 22.9%. The characteristic bacteria in the BA-PIA system were *Streptomyces*, *Nocardioidea*, *Saccharopolyspora*, *Nitrosomonas*, and *Marinobacter*. The loading of microorganisms only changed the surface physical properties of Al-PIA (such as specific surface area, pore volume and pore size), without changing its surface chemical properties. The removal mechanism of nitrogen by BA-PIA is the conversion of $\text{NH}_4^+\text{-N}$ into $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ by nitrifying bacteria, which are then reduced to nitrogen-containing gas by aerobic denitrifying bacteria. The phosphorus removal mechanism is that metal compounds (such as Al) on the surface of BA-PIA fix phosphorus through chemisorption processes, such as ligand exchange. Therefore, BA-PIA overcomes the deficiency of Al-PIA with only phosphorus removal ability, and has better application prospects.

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Introduction

Since the 1990s, the use of industrial products containing nitrogen and phosphorus has been increasing in daily life. As a result, the concentration of nitrogen and phosphorus in

natural lakes has increased, leading to the formation of eutrophic lakes, which has attracted much attention (Llab et al., 2020; Yan et al., 2021). Therefore, many technologies have been developed and applied to solve such problems, including in-situ capping method (Wang et al., 2021; Zhu et al., 2021), advanced oxidation processes, photocatalysis, etc. With the progress of material science, more new materials have been researched and prepared, including bio-zeolite (Zhou et al., 2016a, 2016b), modified zeolite (Wang et al., 2017;

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Wu et al., 2017), Phoslock® (Zhou et al., 2021; Ding et al., 2018; Wang et al., 2022a) aluminum-based P-inactivation agent (Al-PIA) (Lin et al., 2021a; Zhou et al., 2018; Li et al., 2019), etc. Among them, Phoslock® is very effective for adsorbing phosphorus, and is widely used in European and American countries (Guido et al., 2016). However, it is difficult to be promoted and applied in China due to its high cost. Ammonia nitrogen in water can be adsorbed by zeolite (Ali et al., 2020), which is often used in practical water environment restoration projects. However, it cannot adsorb phosphorus, so it cannot be used on a large scale. Nitrogen and phosphorus can both be adsorbed by iron-aluminum modified zeolite (Zhan et al., 2020), but the process of zeolite modification is complicated, so this study is still in the laboratory stage. Al-PIA, also known as calcined modified water purification plant sludge, is a new capping material developed by our research group (Liu et al., 2019). Phosphorus can be well adsorbed by this material due to the abundant aluminum-containing groups on its surface (Wang et al., 2013; Ichihara and Nishio, 2013). Meanwhile, Al-PIA also has good pore volume and pore size, so it can absorb more phosphorus.

As more new capping materials are studied, researchers are increasingly using active materials loaded with microorganisms to restore eutrophic water bodies. For example, Li et al. (2021) combined Phoslock® with aerobic denitrifying bacteria to restore eutrophic water bodies, and achieved remarkable removal effect. Yin et al. (2021) used polyvinyl alcohol (PVA), sodium alginate (SA) and rice husk powder as immobilized carriers for an aerobic denitrifier. The results showed that the removal rate of TN in water by immobilized particles reached 90%. Al-PIA has good pore volume and pore size. Therefore, using Al-PIA as a microbial carrier to prepare biological aluminum-based P-inactivation agent (BA-PIA) for removal of nitrogen and phosphorus in water is a novel and practical research.

In this paper, Al-PIA was mixed with the common microbial agents on the market and aerated to prepare BA-PIA. The effect of BA-PIA on the removal of nitrogen and phosphorus was studied through laboratory beaker experiments. ¹⁵N isotope tracer, high-throughput sequencing analysis and physicochemical characterization were used to analyze the mechanism of denitrification and phosphorus removal of materials. This work provides theoretical technical support for the application of BA-PIA in practical engineering.

1. Materials and methods

1.1. Experimental materials

Al-PIA was prepared by calcining sludge from a water purification plant at high temperature. Before use, the burnt material was crushed, sieved, and the material of 1–2 mm size was selected for use. Before the experiment, the stock material was washed with deionized water and dried. The specific preparation process of the material can be found by Liu's (2019) report. Moreover, Zhou et al. (2018) reported the changes in physicochemical properties of water purification plant sludge before and after calcination.

The microbial agent used in the experiment was provided by Beijing I&Earth Eco-Environmental Engineering Co., Ltd. The main bacteria in the bacterial agent were nitrifying bacteria and aerobic denitrifying bacteria.

BA-PIA preparation steps are as follows: (1) Cultivation of microbial solutions: According to the requirements of the experiment, 5 mL of the dissolved microbial broth was added to a 150 mL Erlenmeyer flask, and the flask was filled with 100 mL of liquid culture medium. The medium was autoclaved before use (sterilization conditions: temperature 120–126°C, pressure 0.10–0.14 MPa, time 30 min). The entire operation process was carried out on a sterile workbench. The medium was shaken for 1 day at 25–30°C and 120 r/min constant temperature. (2) Preparation of the mixed solutions: First, the raw water was sterilized in an autoclave (sterilization conditions: 120–126°C, pressure 0.10–0.14 MPa, time 30 min). Then, the microbial solution and sterilized raw water were mixed in a volume ratio of 1:9 to obtain the mixed solution. (3) Immobilization of microorganisms: The mixed solution was poured into a container containing Al-PIA, and its level was kept 3–5 cm higher than the surface of the material. The concentration of dissolved oxygen (DO) was controlled above 2–3 mg/L by aeration device to speed up the process of immobilization of microorganisms. After continuous cultivation for 3 days, the adsorbed microorganisms on the surface of Al-PIA were washed away by continuous water flow to obtain BA-PIA.

1.2. Experimental methods

1.2.1. Nitrogen and phosphorus load reduction experiment

The control system, Al-PIA system and BA-PIA system were set up in this experiment, with sixteen parallel samples for each system. The initial C/N/P ratio was about 200:5:2.5 in this experiment. KH₂PO₄ solution with phosphorus concentration of 2.5 mg/L, NH₄Cl solution with ammonia nitrogen concentration of 5 mg/L, and glucose solution with carbon concentration (calculated as C element) of 200 mg/L were prepared and mixed. HCl and NaOH solutions (0.1 mol/L) were used to adjust the pH of the mixed solution. Then, 100 mL of mixed solution was poured into a 150 mL Erlenmeyer flask, and 10 g/L of Al-PIA and BA-PIA were added to the Al-PIA and BA-PIA systems, respectively. No materials were added to the control system. These Erlenmeyer flasks were sealed with a vented sealing film to prevent dust in the air from falling into the flask, and then placed in a constant temperature incubator at 25°C for constant temperature cultivation. All the above steps were operated under aseptic conditions. The experiment lasted 30 days, and the experimental sampling method was destructive sampling. Two sets of parallel samples from different systems were taken at the same time for analysis each time. The concentrations of total phosphorus (TP), total nitrogen (TN), ammonia nitrogen (NH₄⁺-N), nitrite nitrogen (NO₂⁻-N), and nitrate nitrogen (NO₃⁻-N) in the water of different systems were detected. The culture test was not continued in the Erlenmeyer flask after sampling, and the experimental results were averaged for analysis. The original microbial solution and water sample in the BA-PIA system after the experiment were taken and analyzed by Illumina Genome Analyzer Iix.

1.2.2. Nitrogen isotope tracing experiment

In this experiment, 15 mg/L of $^{15}\text{NH}_4\text{Cl}$ solution was prepared, in which 100 mL solution contained 1.5 mg $^{15}\text{NH}_4\text{Cl}$ (0.526 mg $^{15}\text{NH}_4^+\text{-N}$ or 0.415 mg ^{15}N). The $^{15}\text{NH}_4^+\text{-N}$ concentration of the water sample was 5.26 mg/L. Glucose solution with a carbon concentration (calculated as C element) of 200 mg/L was mixed with the prepared $^{15}\text{NH}_4\text{Cl}$ solution for use. HCl and NaOH solutions (0.1 mol/L) were used to adjust the pH of the mixed solution. The remaining steps were the same as in Section 1.2.1. The experiment lasted for 30 days. At the end of the experiment, the ^{15}N concentration of the solution and the solid of the material surface were analyzed. Two parallel samples were taken, and the experimental results were averaged for analysis.

1.3. Analysis method

1.3.1. Measurement method of water related indicators

Portable dissolved oxygen (DO) meter was used to measure water temperature and DO (HQ30d type, HACH, China), and portable pH meter was used to measure pH (STARTER3100 type, Shanghai Ohaus Instrument Co., Ltd., China). Molybdenum and antimony anti-spectrophotometry method was used to detect TP concentration in water. The concentration of TN in water was analyzed by potassium persulfate oxidation-ultraviolet (UV) spectrophotometry, the concentration of $\text{NH}_4^+\text{-N}$ in water was analyzed using Nessler's reagent photometric method, the $\text{NO}_2^-\text{-N}$ concentration in water was analyzed by N-(1-na base)-ethylenediamine photometric method and the concentration of $\text{NO}_3^-\text{-N}$ in water was analyzed by UV spectrophotometry (SEPA of China, 2002).

1.3.2. Measurement method of solid sample

Physical and chemical characterization: morphology and elemental composition were analyzed by scanning electron microscope and energy dispersive spectrometer (SEM-EDS, SEM, Sigma 500, Zeiss company, Germany; EDS, X-Max, OXFORD instrument company, UK). The specific surface area, pore volume and pore sizes were measured by specific surface area and micropore analyzer (ASAP20220, Mike Instrument Company, USA). The crystal phases were analyzed by X-ray diffractometer (XRD, Rigaku Ultima IV, Rigaku Corporation, Japan).

1.3.3. Determination of isotope samples

The abundance of $^{15}\text{NH}_4^+\text{-N}$ in the sample was determined by isotope mass spectrometry after NH_3 was produced by MgO distillation and absorbed by boric acid solution and concentrated (Stevens and Laughlin, 1994). The abundance of $^{15}\text{NO}_3^-\text{-N}$ was determined by converting $\text{NO}_3^-\text{-N}$ to N_2O and then using isotope mass spectrometry (Stevens and Laughlin, 1994).

1.3.4. High-throughput sequencing methods

The high-throughput sequencing steps include DNA extraction, PCR amplification, library construction, and computer sequencing. The specific steps are described in previous research (Wang et al., 2022b).

1.4. Data analysis

The pollutant reduction rate P in the overlying water is calculated according to Formula (1):

$$P = \frac{C_{\text{NCi}} - C_{\text{Ci}}}{C_{\text{NCi}}} \times 100\% \quad (1)$$

where: C_{Ci} (mg/L) is the pollutant concentration in the different systems of i th time sampling; C_{NCi} (mg/L) is the pollutant concentration in the control system of i th time sampling; and i is the times of samplings.

The measurement result of the mass spectrometer is expressed as $\delta^{15}\text{N}(\text{‰})$, calculated according to Formula (2):

$$\delta^{15}\text{N}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000\text{‰} \quad (2)$$

where: R_{sample} and R_{standard} are $^{15}\text{N}/^{14}\text{N}$ values of sample and standard, respectively.

The ^{15}N isotope accounts for the atomic percentage of N (atom%) in the sample according to Formula (3):

$$\text{atom}\% = 100\% \times R_{\text{sample}}/(R_{\text{sample}} + 1) \quad (3)$$

where: R_{sample} is $^{15}\text{N} / ^{14}\text{N}$ of the sample.

The ^{15}N content in the water sample is calculated according to Formula (4):

$$\delta^{15}\text{N}(\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000\text{‰} \quad (4)$$

where: V_{sample} (L) is the sample volume; atom% is the ^{15}N atomic percentage in the water sample; atom%_{back} is the natural abundance of N: 0.365%; and N_{content} (mg/L) is the concentration of N in the water sample.

The ^{15}N content in solid sample is calculated according to Formula (5):

$$^{15}\text{N}_{\text{sample}} = m_{\text{sample}} \times (\text{atom}\% - \text{atom}\%_{\text{back}}) \times N_{\text{content}}\% \quad (5)$$

where: m_{sample} (mg) is the mass of the sample; atom% is the ^{15}N atomic percentage in the solid sample; atom%_{back} is the natural abundance of N: 0.365%; and $N_{\text{content}}\%$ is the mass fraction of N in the solid sample.

Origin2019 software was used for the analysis of variance.

2. Results and discussion

2.1. Ability to reduce ammonia nitrogen and TN load in water

The nitrogen and phosphorus removal experiments with Al-PIA and BA-PIA systems lasted for 30 days. The water temperature in each system was maintained at about 25°C. The pH range of the three systems was 6.5–7.5, and the DO concentration was 4.56–7.08 mg/L. The change in $\text{NH}_4^+\text{-N}$ concentration of different systems is shown in Fig. 1.

As can be seen from Fig. 1, the initial $\text{NH}_4^+\text{-N}$ concentration of the experiment was 5.2 mg/L, and the $\text{NH}_4^+\text{-N}$ concentration in the control system remained almost unchanged. In the Al-PIA system, when the dosage of the material was 10 g/L, the average reduction rate of $\text{NH}_4^+\text{-N}$ in the water by Al-PIA was about 0.22% compared to the control. The analysis of

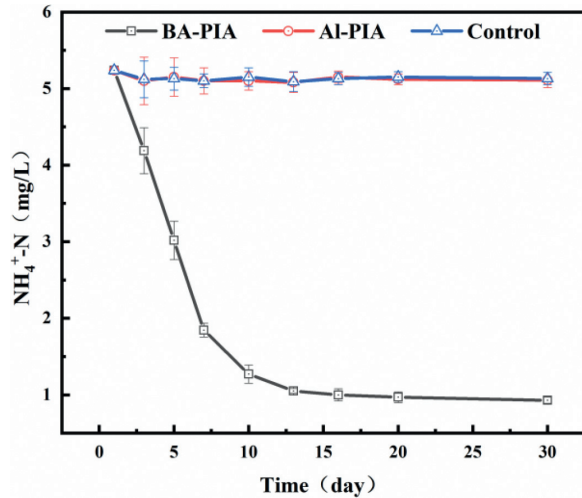


Fig. 1 – Changes of NH₄⁺-N in water of different systems (BA-PIA: biological aluminum-based P-inactivation agent; Al-PIA: aluminum-based P-inactivation agent).

variance showed that there was no significant difference between the Al-PIA system and the control system ($P > 0.05$). This shows that under the constant temperature and sterile environment of the incubator, Al-PIA cannot adsorb ammonia nitrogen, which is similar to the previous research results of our group (Zhou et al., 2018). However, in the static simulation experiment of Lin et al. (2021b), the active capping plate made of Al-PIA as raw material could effectively inhibit the migration of nitrogen and phosphorus from the sediment. It is more likely due to the physical barrier and the action of microorganisms. In the BA-PIA system, the dosage of the material was also 10 g/L. On the 30th day of the experiment, compared to the control, the removal rate of BA-PIA on NH₄⁺-N was 81.87%. The BA-PIA system lowered the ammonia nitrogen concentration to reach the class V standard of surface water (NH₄⁺-N < 2 mg/L) on the 7th day. On the 16th day, the concentration reached the class III standard of surface water (NH₄⁺-N < 1 mg/L). The system water continuously maintained a low ammonia nitrogen concentration. The analysis of variance showed that there was a very significant difference between the BA-PIA system and the control system ($P < 0.01$). This shows that Al-PIA was a good microbial carrier, and Al-PIA loaded with microorganisms had a better ability to reduce the ammonia nitrogen load in the water. Under the conditions of suitable temperature, pH and DO, BA-PIA can effectively remove ammonia nitrogen from water. The transfer and transformation mechanism of nitrogen in the BA-PIA system are further discussed in Sections 2.3, 2.4 and 2.5.

The effect of BA-PIA system on TN removal is shown in Fig. 2. The experiment lasted for 30 days and the TN concentration in the control system remained almost unchanged. In the Al-PIA system with the material dosage of 10 g/L, the TN concentration was basically the same as the control system. Compared with the experimental results in Fig. 1, it can be seen that the concentrations of TN and NH₄⁺-N in these two systems were almost the same, and the change trends were similar. The variance analysis showed that there was no sig-

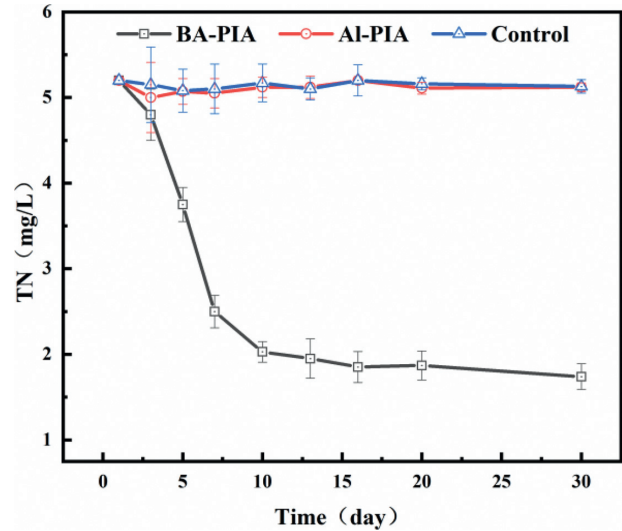


Fig. 2 – Changes of TN in water of different systems.

nificant difference between Al-PIA system and control system ($P > 0.05$). This is consistent with the above experimental results. In the BA-PIA system, the dosage of the material was 10 g/L. Compared to the control, the removal rate of TN in the water by BA-PIA was 66.08% on the 30th day of the experiment. The BA-PIA system reduced the TN concentration to the surface water class V (TN < 2 mg/L) on the 13th day, and the water continued to maintain a low TN concentration in this system. The analysis of variance showed that there was a very significant difference between the BA-PIA system and the control system ($P < 0.01$). Therefore, the BA-PIA system not only effectively reduced the ammonia nitrogen load in the water, but also had a better control effect on other forms of nitrogen in the water.

2.2. Ability to reduce phosphorus load in water

The phosphorus removal experiment lasted 30 days with the dosage of materials at 10 g/L, and the change in TP concentration of each system is shown in Fig. 3.

As seen from Fig. 3, the initial phosphorus concentration of each system was 2.39 mg/L, and the TP concentration in the control system remained almost unchanged. Compared to the control, BA-PIA and Al-PIA systems reduced TP concentration by 87.97% and 88.82%, respectively, on the 30th day. The BA-PIA and Al-PIA systems reduced the phosphorus concentration to the class V standard of surface water (TP < 0.4 mg/L) on the 16th day. The phosphorus concentration continued to decline, and reached surface water class IV (TP < 0.3 mg/L) on the 20th day. According to the ANOVA results, BA-PIA, Al-PIA and control systems had extremely significant differences ($P < 0.01$). Moreover, there was a non-significant difference between the BA-PIA system and the Al-PIA system ($P > 0.05$). The results showed that when Al-PIA was used as a microbial carrier, its ability to adsorb phosphorus was not affected. The phosphorus removal effects of BA-PIA and Al-PIA were equivalent at low concentrations. The possible reason is that the removal mechanism of phosphorus by Al-

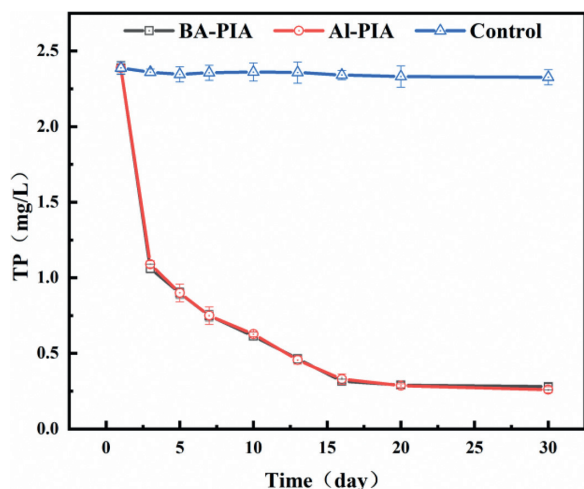


Fig. 3 – Changes of TP in water of different systems.

PIA mainly involves the fixing of the phosphorus in the water by iron-aluminum compound in the material to more stable iron-phosphorus or aluminum-phosphorus through ligand exchange, etc. (Toor et al., 2019). The loading of microorganisms does not affect the chemical reaction between the iron and aluminum compounds on the surface of the material and the phosphorus ions in the solution (Li et al., 2021). This is evident from the physical and chemical characterization of these materials in Sections 2.6 and 2.7. According to the above experimental results, the BA-PIA can effectively remove ammonia nitrogen in water while having a good phosphorus removal effect. Therefore, the use of BA-PIA to reduce nitrogen and phosphorus load in water has a good application prospect.

2.3. Changes in concentrations of NO_2^- -N and NO_3^- -N in water

In order to reveal the migration and transformation of nitrogen in different systems, this study focused on the changes in the concentration of NO_2^- -N and NO_3^- -N in different systems. The nitrogen sources used by the aerobic denitrifying bacteria in the experiment were all from the oxidation of nitrifying bacteria because no external nitrogen sources were added. The experimental results are shown in Fig. 4.

It can be found from Fig. 4 that the concentrations of NO_2^- -N and NO_3^- -N in the control system and the Al-PIA system were almost unchanged. This shows that under constant temperature and sterile environment in the laboratory and suitable pH and DO conditions, there was no conversion and migration of nitrogen in these two systems. This is consistent with the above experimental results. Fig. 4a shows that in the BA-PIA system, the NO_2^- -N concentration increased slightly on the 3rd day of the experiment, and began to decrease on the 5th day. Then it dropped to the level of the control system on the 10th day, and maintained the low concentration continuously. In particular, the highest concentration of NO_2^- -N in this system was only 0.031 mg/L. The analysis of variance showed that there was no significant difference between the BA-PIA system and the control system ($P > 0.05$). The small increase in nitrite nitrogen was mainly due to the fact that

the nitrifying bacteria on the surface of the material converts NH_4^+ -N into NO_2^- -N under aerobic conditions. At the same time, NO_2^- -N can also be easily oxidized under the condition of sufficient DO (Yue et al., 2018). It is extremely unstable in the water environment. Therefore, the nitrite nitrogen in the BA-PIA system only showed a short-term and small increase. The change in NO_3^- -N concentration of each system is shown in Fig. 4b) In the BA-PIA system, on the 3rd day of the experiment, the concentration of NO_3^- -N increased sharply and then decreased rapidly. This was likely due to the conversion of NH_4^+ -N to NO_2^- -N by aerobic nitrifying bacteria. The oxidized NO_2^- -N was converted into nitrogen-containing gas through denitrification (Tengxia et al., 2016). At the same time, some NO_2^- -N was oxidized to NO_3^- -N by DO in the water, which led to the rise in content of nitrate nitrogen in the water in a short time (Le et al., 2020). During the 3rd to 20th days, the NO_3^- -N concentration fluctuated between 0.6–0.9 mg/L, and there was a slow rising trend. This was mainly due to the fact that the NH_4^+ -N concentration in the system still maintained a relatively high concentration during this period, and the conversion process from NH_4^+ -N to NO_2^- -N to NO_3^- -N was constantly occurring in the system. At the same time, the aerobic denitrifying bacteria on the surface of the material reduced NO_3^- -N to nitrogen-containing gas, and there was assimilation by microorganisms (Li et al., 2021). Therefore, the NO_3^- -N concentration in the system maintained a dynamic equilibrium. With the increase in the number of experimental days, this dynamic balance was destroyed when the concentration of NH_4^+ -N in the water was low. Hence, the concentration of NO_3^- -N in the system displayed a downward trend. In combination with the results in Fig. 1, it can be inferred that the migration and transformation of nitrogen in the BA-PIA system mainly occurred through the conversion of NH_4^+ -N to NO_2^- -N and NO_3^- -N by nitrifying bacteria, and NO_3^- -N served as nitrogen source for aerobic denitrifying bacteria to produce nitrogen-containing gas. This process is similar to the biological denitrification process in conventional water treatment.

2.4. The migration and transformation of ^{15}N in the BA-PIA system

The migration and transformation of ^{15}N in the BA-PIA system is shown in Table 1. In this experiment, the unreacted part was replaced by the content of $^{15}\text{NH}_4^+$ -N in the water after the experiment. The nitrification part was replaced by the sum of the denitrification part and the content of $^{15}\text{NO}_3^-$ -N in the water after the experiment. The denitrification part was represented by the amount of nitrogen gas. Since it is impossible to directly collect the amount of gas containing ^{15}N , the proportion of denitrification was calculated indirectly through the material balance of ^{15}N (Harrison et al., 2012). There was no loss of ^{15}N added in this experiment, so the calculation method for the proportion of denitrification used ^{15}N exogenous addition amount to subtract unreacted part, the $^{15}\text{NO}_3^-$ -N content in the water after the experiment and the microbial assimilation part. This method is widely used in water treatment research (Chen et al., 2017). The part of microbial assimilation was replaced by the content of ^{15}N in the microorganisms after the experiment.

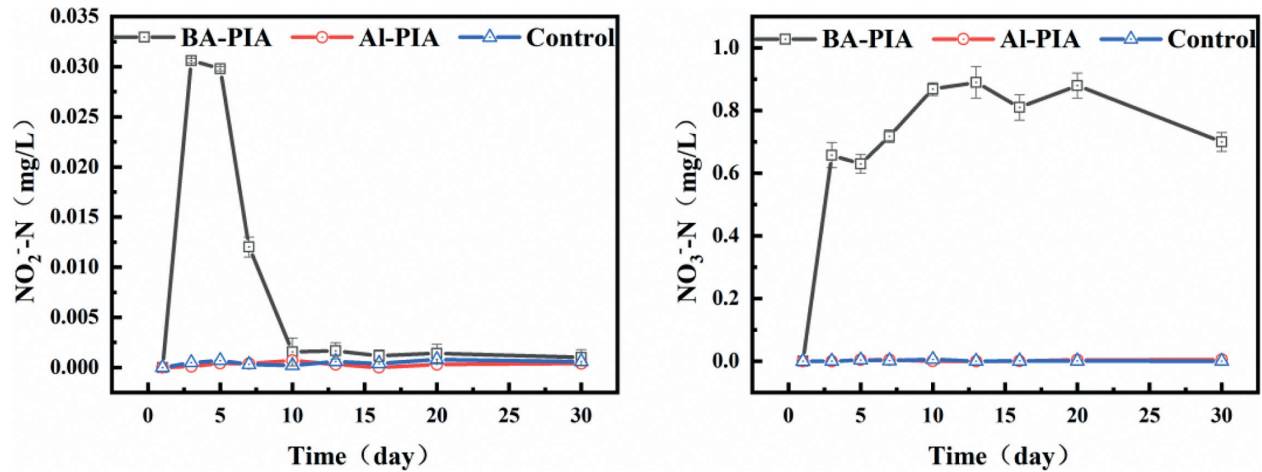


Fig. 4 – Changes of (a) NO_2^- -N and (b) NO_3^- -N in water of different systems.

Table 1 – The transfer and transformation of ^{15}N in the BA-PIA system

	^{15}N addition amount	No reaction	Nitrification	Denitrification	Microbial assimilation
^{15}N content (mg)	0.415±0.02	0.095±0.01	0.301±0.10	0.290±0.10	0.075±0.08
The proportion (%)	-	22.9	59.0	56.4	18.1

As shown in Table 1, in the BA-PIA system, after adding ^{15}N , the unreacted part of the system accounted for 22.9%, which is similar to the result in Section 2.1. The nitrification part accounted for 59.0%, the denitrification part accounted for 56.4%, and the proportion of microbial assimilation was 18.1%. The results show that in the BA-PIA system, the removal of ammonia nitrogen mainly depended on nitrification and denitrification. The microbial agents used in this experiment were mainly aerobic denitrifying bacteria and nitrifying bacteria, and aerobic denitrifying bacteria have both nitrification and denitrification functions (Chen et al., 2017). In the BA-PIA system, the specific process of nitrogen transformation was that nitrospira and aerobic denitrifying bacteria used NH_4^+ -N as the nitrogen source and converted it into NO_2^- -N with sufficient carbon and nitrogen sources. Then some aerobic denitrifying bacteria used NO_2^- -N as the nitrogen source, and reduced it to a nitrogen-containing gas (He et al., 2016; Yin et al., 2021). At the same time, part of NO_2^- -N was oxidized to NO_3^- -N by DO in water. When NO_3^- -N is used as the nitrogen source, aerobic denitrifying bacteria play an important role, which can quickly grow and remove NO_3^- -N (Yang et al., 2019). However, due to the limited amount of inoculation and the fact that the microbial agent was not a single flora, there were only small amounts of NH_4^+ -N and NO_3^- -N in the water after the experiment. Moreover, the proportion of nitrogen assimilated by the microorganisms in the BA-PIA system was 18.1%. This indicates that most of the ammonia nitrogen in the water was removed by denitrification, and the nitrogen assimilated by the microorganisms accounted for only a small part. This is consistent with the research results of Li et al. (2021).

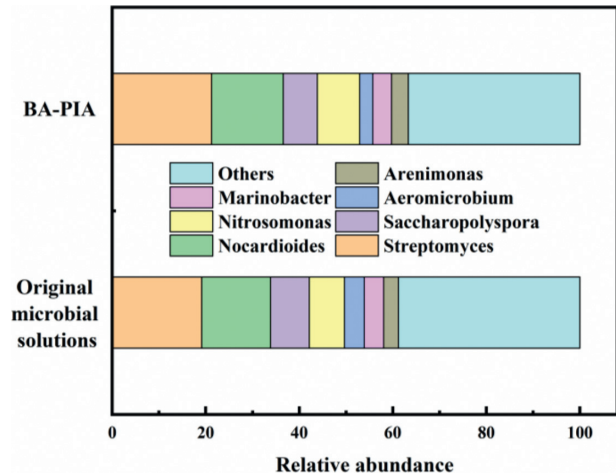


Fig. 5 – Genus microbial community structure in BA-PIA system.

2.5. Analysis of the composition of the microbial community in the BA-PIA system

The high-throughput analysis technology was used to analyze the composition of the microbial community in the BA-PIA system. The experimental results are shown in Fig. 5.

The relatively high-abundance bacterial genera are displayed in Fig. 5, and the unnamed bacterial genera and other relatively low-abundance bacterial genera are called others. The results show that the characteristic bacterial genera in the

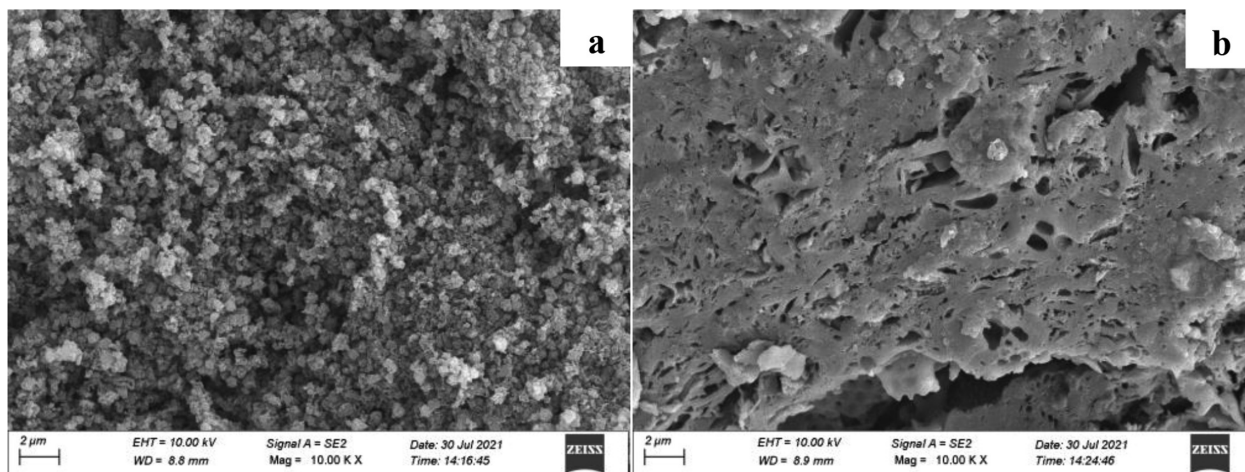


Fig. 6 – SEM micrographs of BA-PIA (a) before and (b) after the adsorption.

BA-PIA system are *Streptomyces*, *Nocardioides*, *Saccharopolyspora*, *Nitrosomonas*, *Aeromicrobium*, *Marinobacter*, and *Arenimonas*. *Streptomyces* can participate in the reduction of nitrite, producing nitrite and nitrate reductase (Amin et al., 2012). Symbiosis network analysis showed that *Nocardioides* are positively correlated with genes related to nitrate/nitrite reduction and nitrogen assimilation (Yi et al., 2021). *Saccharopolyspora* has the *glnR* gene, which is a wide-area transcriptional regulator that regulates nitrogen metabolism. It has a regulatory effect on many genes involved in nitrogen metabolism (Bodour and Miller-Maier, 1998). *Nitrosomonas* is a kind of Ammonia Oxidizing Bacteria (AOB), which can effectively reduce the concentration of ammonia nitrogen in water (Vadivelu et al., 2006). *Marinobacter* is a new type of halophilic denitrifying bacteria with rapid denitrification ability (Li et al., 2013). Comparing the microbial community in the original microbial solutions and the BA-PIA system, it can be seen that the similarity between the two was very high. The analysis of variance shows that there was no significant difference between the two sets of data ($P > 0.05$). At the same time, most of the characteristic bacterial genera in the BA-PIA system have nitrification, nitrosation and denitrification capabilities. This also proves that the denitrification mechanism of the BA-PIA is mainly oxidation of ammonia nitrogen by nitrosating bacteria and nitrifying bacteria, which is then reduced to nitrogen-containing gas by denitrifying bacteria.

2.6. Changes in physical characteristics of BA-PIA before and after adsorption

The changes in the surface morphology of BA-PIA before and after adsorption were characterized by SEM and BET. The results are as follows.

The SEM images of BA-PIA before and after adsorption are shown in Fig. 6. The surface of the Al-PIA after filming was rough and the shape was uniform as shown in Fig. 6a, and the material was loaded with a large number of bacilli. It can be seen from Fig. 6b that the surface of the BA-PIA was uniform after adsorption, and the surface shape was mostly blocky. At the same time, there were a large number of pores. A small number of bacilli were attached to some of

the pores (Li et al., 2021). The possible reason for this phenomenon is that the pore volume and pore size on the surface of the material increased due to the load of microorganisms on the material. This is conducive for the iron-aluminum compound on the surface of the material to fix phosphorus on the surface of the material. Comparing the two figures, it can be concluded that a large number of microorganisms in Fig. 6a are not reflected in Fig. 6b, indicating that a large number of microorganisms have migrated and diffused into the water.

The specific surface area, pore volume and pore diameter of BA-PIA before and after adsorption are shown in Table 2. The specific surface area, pore volume, and pore diameter of the material after adsorption were increased compared with that before adsorption. The specific surface area increased by $14.48 \text{ m}^2/\text{g}$, the pore volume increased by $0.225 \text{ cm}^3/\text{g}$, and the pore diameter increased by 10.53 nm . Compared with the previous research results of our research group, the specific surface area, pore volume and pore diameter of BA-PIA were lower than those of Al-PIA. However, after adsorption, these parameters of BA-PIA increased compared with those of Al-PIA. The specific surface area increased by $4.85 \text{ m}^2/\text{g}$, the pore volume increased by $0.049 \text{ cm}^3/\text{g}$, and the pore diameter increased by 3.12 nm . This is because a large number of microorganisms were loaded on the surface of BA-PIA, which occupied the pores on the surface of the material. Hence, the specific surface area, pore volume and pore size all reduced. At the same time, a large number of microorganisms on the surface of the material migrated to water after the experiment, leaving pores of different sizes on the surface of the material. This indicates that the loading of microorganisms caused a large change in the specific surface area, pore volume and pore diameter of the material, which is consistent with the results of the SEM. This also proves that the Al-PIA is a good microbial carrier and provides theoretical technical support for the large-scale application of BA-PIA.

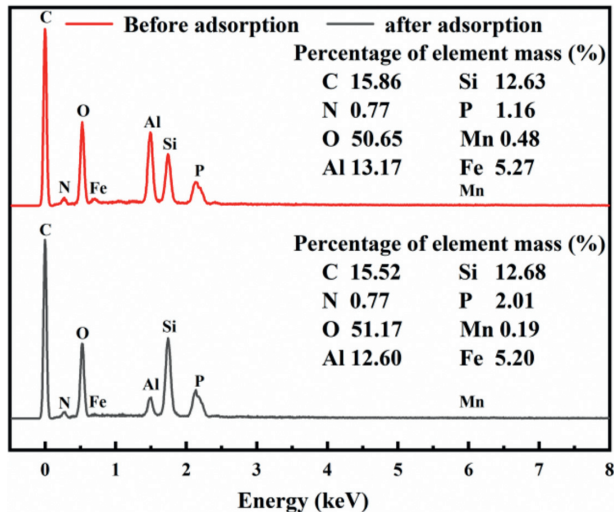
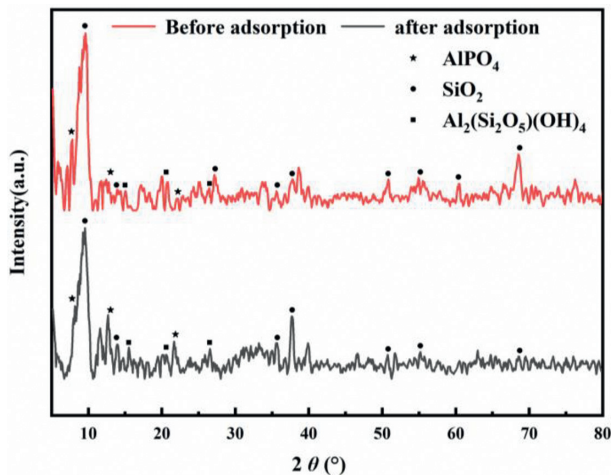
2.7. EDS and XRD analyses of BA-PIA

In order to reveal the adsorption mechanism of BA-PIA for phosphate in water, EDS and XRD analyses were used to char-

Table 2 – The specific surface area, pore volume and pore size of BA-PIA before and after the adsorption

	Specific surface area (m ² /g)	Pore volume (cm ³ /g)	Pore size (nm)
Before BA-PIA adsorption	62.28	0.105	8.12
After BA-PIA adsorption	76.76	0.310	18.65
Al-PIA	71.91	0.261	15.53

BA-PIA: biological aluminum-based P-inactivation agent; Al-PIA: aluminum-based P-inactivation agent

**Fig. 7 – The analysis results of surface element content of BA-PIA before and after the adsorption.****Fig. 8 – XRD pattern of BA-PIA before and after the adsorption.**

acterize BA-PIA before and after adsorption. The results are presented in Figs. 7 and 8.

Figure 7 shows the EDS analysis results of the surface elements before and after BA-PIA adsorption. The main elements on the surface of BA-PIA included C, O, Si, Al, and Fe. Among them, oxygen accounted for up to 50%, and metal el-

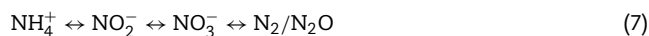
ements such as Al and Fe all came from the flocculant input during the flocculation process of the water purification plant. Compared with the previous research results of our research group, the proportions of metal elements such as Al and Fe increased, while the proportions of C and O elements decreased. This may be caused by different dosages of flocculants in water purification plants in different periods (Zhao et al., 2020). It may also be caused by more adequate calcination in the preparation process (Liu et al., 2019). The difference in content of each element before and after the material adsorption was compared. The proportions of C and Al after adsorption were 0.34% and 0.57% less than those before adsorption, respectively. In contrast, the proportions of metal elements Si and Fe remained almost unchanged, while the proportions of O and P increased by 0.52% and 0.85%, respectively. This is because the iron-aluminum oxides, iron-aluminum complexes and certain metal ions on the surface of the material fixed phosphorus on the surface of the material through ligand (ion) exchange, surface precipitation or complexation (Yang et al., 2006; Al-Tahmazi and Babatunde, 2016). Consequently, the O and P elements on the material surface increased, but Al, Fe and other metal elements were on the surface of the material. Therefore, the increase in the proportion of O and P led to a decrease in the proportion of Al, Fe and other metal elements (Liu et al., 2019).

The XRD patterns of BA-PIA before and after adsorption are shown in Fig. 8. The characteristic peaks of silicon dioxide (SiO₂) were detected at 2θ of 9.6°, 13.8°, 27.3°, 35.7°, 37.7°, 50.8°, 55.2°, 60.4° and 68.7°. Moreover, the characteristic peaks of kaolinite (Al₂(Si₂O₅)(OH)₄) were observed at 2θ of 15.5°, 20.6° and 26.5°. The results showed that the main components of BA-PIA were silica and kaolinite, which is consistent with the composition of the Al-PIA previously reported by our research group. This shows that the loading of microorganisms did not change the chemical properties of the surface of the material. The characteristic peaks of aluminum phosphate (AlPO₄) were detected at 2θ of 7.7°, 13.0° and 22.2°, which were higher after adsorption compared to those before adsorption. This shows that the aluminum compound and aluminum hydroxide compound on the surface of the material undergo a ligand exchange reaction with the phosphate in the water (Toor et al., 2019).

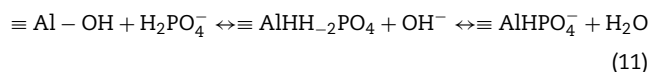
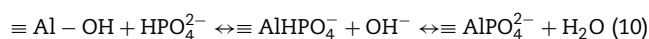
2.8. Mechanism of BA-PIA removal of nitrogen and phosphorus

The nitrogen removal mechanism of BA-PIA was elucidated through comprehensive results of isotope tracing and nitrogen removal experiment. The proposed mechanism is as fol-

lows: nitrifying bacteria first oxidize $\text{NH}_4^+\text{-N}$ into $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$; then, the aerobic denitrification bacteria use $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ as nitrogen sources to produce nitrogen-containing gas, thereby removing nitrogen from water. The specific reaction route is as follows:



Based on the above comprehensive analysis results of BA-PIA, it can be concluded that the phosphorus removal mechanism of BA-PIA is similar to that of Al-PIA. They both fix phosphorus in water through chemical adsorption. Specifically, Al and other metal compounds or hydroxides on the surface of the material fix phosphorus through ligand exchange, surface precipitation, complexation and other chemical adsorption processes. The specific reaction process equations are as follows:



3. Conclusion

- 1) Under the optimized process conditions (initial $\text{NH}_4^+\text{-N}$ concentration is 5.2 mg/L, initial TP concentration is 2.39 mg/L, and BA-PIA dosage is 10 g/L, the removal rate of ammonia nitrogen by BA-PIA was 81.87%, the removal rate of TP reached 87.97%, and the removal rate of TN reached 66.08%. This proves that BA-PIA can effectively reduce the nitrogen and phosphorus load, and provides theoretical technical support for the large-scale application of BA-PIA.
- 2) The migration and conversion of nitrogen in the BA-PIA system were mainly through the conversion of $\text{NH}_4^+\text{-N}$ into $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ by nitrifying bacteria. The oxidized $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ were then used as nitrogen sources by aerobic denitrifying bacteria to produce nitrogen-containing gas.
- 3) The loading of microorganisms only changed the physical properties of the material surface such as specific surface area, pore volume and pore size. In the phosphorus removal mechanism of BA-PIA, Al-containing compounds on the surface of the material fixed phosphorus in the water through ligand exchange, surface precipitation, and other chemical adsorption processes.

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 Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.06.018.

REFERENCES

- Ali, M.I., Fatima, B., Liaquat, R., Farooq, U., Liu, F.J., 2020. Enhanced biogas production at mesophilic and thermophilic temperatures from a slaughterhouse waste with zeolite as ammonia adsorbent. *Int. J. Environ. Sci. Te.* 18, 1–10.
- Al-Tahmazi, T., Babatunde, A.O., 2016. Mechanistic study of P retention by dewatered waterworks sludges. *Environ. Technol. Inno.* 6, 38–48.
- Amin, R., Reuther, J., Bera, A., Wohlleben, W., Mast, Y., 2012. A novel GlnR target gene, nnaR, is involved in nitrate/nitrite assimilation in *Streptomyces coelicolor*. *Microbiology* 158, 1172–1182.
- Bodour, A.A., Miller-Maier, R.M., 1998. Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. *J. Microbiol. Meth.* 32, 273–280.
- Chen, T., Zou, Z., Li, J., 2017. Transfer and transformation of $^{15}\text{NO}_3^-$ in bioretention and effect of Danish grass in the process. *Environ. Eng.* 35, 60–64.
- Ding, S., Sun, Q., Chen, X., Liu, Q., Wang, D., Lin, J., et al., 2018. Synergistic adsorption of phosphorus by iron in lanthanum modified bentonite (Phoslock): new insight into sediment phosphorus immobilization. *Water Res.* 134, 32–43.
- Guido, W., Frank, Oosterhout V., Grant, D., 2016. Management of eutrophication in Lake De Kuil (The Netherlands) using combined flocculant - Lanthanum modified bentonite treatment. *Water Res.* 97, 83–95.
- Harrison, M.D., Groffman, P.M., Mayer, P.M., Kaushal, S.S., 2012. Nitrate removal in two relict oxbow urban wetlands: a ^{15}N mass-balance approach. *Biogeochemistry* 111, 647–660.
- Ichihara, M., Nishio, T., 2013. Suppression of phosphorus release from sediments using water clarifier sludge as capping material. *Environ. Technol.* 34, 2291–2299.
- Le, L.T., Lee, S., Bui, X.T., Jahng, D., 2020. Suppression of nitrite-oxidizing bacteria under the combined conditions of high free ammonia and low dissolved oxygen concentrations for mainstream partial nitrification. *Environ. Technol. Inno.* 20, 101135–201146.
- Li, R., Zi, X., Wang, X., Zhang, X., Gao, H., Hu, N., 2013. *Marinobacter hydrocarbonoclasticus* NY-4, a novel denitrifying, moderately halophilic marine bacterium. *Springerplus* 2, 346–355.

- Li, S.W., Zhou, Z.M., Yang, S.M., Liu, S.P., Li, F., Yuan, B.L., 2019. The efficiency of controlling the phosphorus release from the sediment using calcined modified water purification plant sludge (C-WTPS). *J. Lake Sci.* 31, 961–968.
- Li, B., Zhou, Z., Ravi, N., Hu, Z., Guo, D., Chen, J., 2021. Combined remediation of eutrophic water by phoslock® and aerobic denitrifying bacteria. *Environ. Sci.* 42, 1861–1869.
- Liu, Q., Zhou, Z., Zhang, H., Li, F., Xie, J., Li, S., et al., 2019. Phosphorus removal characteristics of calcined water treatment plant sludge. *Environ. Chem.* 38, 325–333.
- Lin, C., Li, S., Zhu, B., Liu, S., Li, T., 2021a. Al-PHOSLOCK thin-layer capping to control phosphorus release from sediment: effect of hydraulic retention time and phosphorus migration/transformation mechanism. *J. Soils Sediments* 21, 1–9.
- Lin, C., Tian, Y., Ye, P., Tian, Y., Wu, X., Li, F., et al., 2021b. Preparation of active capping plate for sediment in urban river and its effect on nitrogen and phosphorus reduction. *Chin. J. Environ. Eng.* 15, 1927–1936.
- Llab, C., Swab, C., Jjab, C., Yxab, C., Xsab, C., Jcab, C., 2020. Characteristics of microbial eukaryotic community recovery in eutrophic water by using ecological floating beds. *Sci. Total Environ.* 711, 134551.
- SEPA of China, 2002. Monitoring and analyzing methods of water and wastewater, 4th edn China Environmental Science Press, Beijing.
- Stevens, R.J., Laughlin, R.J., 1994. Determining nitrogen-15 in nitrite or nitrate by producing nitrous oxide. *Soil Sci. Soc. Am. J.* 58, 1108–1116.
- He, T., Li, Z., Sun, Q., Xu, Y., Ye, Q., 2016. Heterotrophic nitrification and aerobic denitrification by *Pseudomonas tolaasii* Y-11 without nitrite accumulation during nitrogen conversion. *Bio. Technol.* 200, 493–499.
- Toor, U.A., Shin, H., Kim, D.J., 2019. Mechanistic insights into nature of complexation between aluminum and phosphates in polyaluminum chloride treated sludge for sustainable phosphorus recovery. *J. Ind. Eng. Chem.* 71, 425–434.
- Vadivelu, V.M., Keller, J., Yuan, Z., 2006. Effect of free ammonia and free nitrous acid concentration on the anabolic and catabolic processes of an enriched *Nitrosomonas* culture. *Biotechnol. Bioeng.* 95, 830–839.
- Wang, C., Liang, J., Pei, Y., Wendling, L.A., 2013. A method for determining the treatment dosage of drinking water treatment residuals for effective phosphorus immobilization in sediments. *Ecol. Eng.* 60, 421–427.
- Wang, X., Li, J., Li, S., Zheng, X., 2017. A study on removing nitrogen from paddy field rainfall runoff by an ecological ditch-zeolite barrier system. *Environ. Sci. Pollut. R.* 24, 1–14.
- Wang, Y., Li, S., Liu, S., Li, F., Zhou, Z., 2022a. Three kinds of active thin-layer capping materials for reducing the phosphorus load in eutrophic water body: comparison in dynamic experiment. *Environ. Sci. Pollut. R.* 29 (11), 16427–16435.
- Wang, Y., Yuan, S., Liu, S., Li, F., Zhou, Z., 2022b. Effect of aluminum-based P-inactivation agent (Al-PIA) capping on bacterial community in sediment. *J. Soils Sediments* 22 (4), 1344–1354.
- Wu, P., Lu, S., Xu, L., Liang, Q., Shen, Y., 2017. Efficiency and Mechanism of Nitrogen and Phosphorus removal in modified zeolite wetland. *Environ. Sci.* 38, 580–588.
- Yan, Q., Cheng, T., Song, J., Zhou, J., Cai, Z., 2021. Internal nutrient loading is a potential source of eutrophication in Shenzhen Bay. *China. Ecol. Indic.* 127, 107736.
- Yang, J.R., Wang, Y., Chen, H., Lyu, Y.K., 2019. Ammonium removal characteristics of an acid-resistant bacterium *Acinetobacter* sp. JR1 from pharmaceutical wastewater capable of heterotrophic nitrification-aerobic denitrification. *Bio. Technol.* 274, 56–64.
- Yang, Y., Zhao, Y.Q., Babatunde, A.O., Wang, L., Ren, Y.X., Han, Y., 2006. Characteristics and mechanisms of phosphate adsorption on dewatered alum sludge. *Sep. Purif. Technol.* 51, 193–200.
- Yin, C., Li, Y., Zhang, T., Liu, J., Chen, T., Cui, D., et al., 2021. Immobilization and efficacy of an aerobic denitrifier. *J. East Chin. Norm. Univ. (Natural Sci.)* 04, 1–7.
- Li, Y., Zhang, M., Xu, R., Lin, H., Sun, X., Xu, F., et al., 2021. Arsenic and antimony co-contamination influences on soil microbial community composition and functions: Relevance to arsenic resistance and carbon, nitrogen, and sulfur cycling. *ScienceDirect. Environ. Int.* 153, 106522–106531.
- Yue, X., Yu, G., Lu, Y., Liu, Z., Li, Q., Tang, J., et al., 2018. Effect of dissolved oxygen on nitrogen removal and the microbial community of the completely autotrophic nitrogen removal over nitrite process in a submerged aerated biological filter. *Bio. Technol.* 254, 67–74.
- Zhan, Y., Yu, Y., Lin, J., Wu, X., Zhao, Y., 2020. Assessment of iron-modified calcite/zeolite mixture as a capping material to control sedimentary phosphorus and nitrogen liberation. *Environ. Sci. Pollut. R.* 27, 1–17.
- Zhao, L., Liao, X., Zhou, Z., Li, F., 2020. Seasonal changes of water quality of a reservoir in quanzhou city and countermeasures. *Chin. Water Waste.* 36, 36–42.
- Zhou, Z., Huang, T., Yuan, B., 2016a. Nitrogen reduction using bioactive thin-layer capping (BTC) with biozeolite: A field experiment in a eutrophic river. *J. Environ. Sci.* 42, 119–125.
- Zhou, Z., Huang, T., Yuan, B., 2016b. Biozeolite thin-layer capping for reducing the phosphorus load in eutrophic water body. *J. Lake Sci.* 28, 726–733.
- Zhou, Z., Liu, Q., Li, S., Li, F., Zou, J., Liao, X., 2018. Characterizing the correlation between dephosphorization and solution pH in a calcined water treatment plant sludge. *Environ. Sci. Pollut. R.* 25, 18510–18518.
- Zhou, Z., Lin, C., Li, S., Liu, S., Li, F., Yuan, B., 2021. Four kinds of capping materials for controlling the phosphorus and nitrogen release from contaminated sediment: comparison in static simulation experiment. *Front. Environ. Sci. Eng.* 16, 29–39.
- Zhu, B., Li, S., Lin, C., Liu, S., Li, F., Zhou, Z., 2021. The effect of secondary capping on the control of phosphorus release from sediment by activated thin-layer capping with Al-PIA. *Environ. Sci. Pollut. R.* 28, 18062–18069.