Efficient nitrogen removal by simultaneous photoelectrocatalytic oxidation and electrochemically active biofilm denitrification

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ABSTRACT

A novel nitrogen removal strategy, the combined photoelectrocatalytic oxidation and autotrophic biofilm denitrification, was developed to achieve simultaneous NH_4^+ -N oxidation and NO_3^- -N reduction in a single reactor. AgI/TiO₂-NTs and autotrophic denitrifying biofilm were used as anode and cathode, respectively. NH_4^+ -N oxidation efficiency was enhanced by 3 times after the introduction of visible light. Moreover, NO_3^- -N and NO_2^- -N generated in the process of NH_4^+ -N oxidation could be further removed by biocathode. Applied voltage and pH have significant effects on nitrogen removal. The NO_3^- -N and NH_4^+ -N removal efficiencies gradually increased from 27.2% to 74.3% and 60.0% to 83.1%, respectively, when the voltage increased from 1.0 V to 3.0 V. Neutral or slightly alkaline condition was favorable on account of the autotrophic denitrifying biofilm. The optimum TN (TN = NH_4^+ -N + NO_3^- -N + NO_2^- -N) removal efficiency was up to 89.6% at pH 8 with 3.0 V voltage.

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1. Introduction

Wastewater discharge containing excessive nitrogenous pollutants and artificial fixed nitrogen have drastically altered the global nitrogen cycle, leading to serious impacts on environment and human health. Aqueous ammonium ($\text{NH}_4^+\text{-N}$), one of the major nitrogen-containing pollutants, was recognized as a potential source of eutrophication. Nitrate ($\text{NO}_3^-\text{-N}$), another type of nitrogen, was known to cause methemoglobinemia, “blue baby” syndrome [1], and other behavioral problems [2]. In many regions, the concentration of $\text{NO}_3^-\text{-N}$ in water is much higher than the guideline range ($< 10 \text{ mg N/L}$) proposed by World Health Organization for human consumption [3]. Therefore, it is crucial to develop technologies for the highly effective removal of nitrogen.

Biological technology is a traditional treatment method for nitrogen removal, which needs to carry out nitrification and denitrification in two separate reactors. In nitrification reactor, $\text{NH}_4^+\text{-N}$ is converted into $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ in aerobic conditions. In denitrification reactor, $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ are converted into N_2 in anaerobic conditions [4]. Not only different concentrations of dissolved oxygen should be provided to nitrification and denitrification respectively, but also extra organic carbon required to denitrification may cause secondary pollution [5]. Separate aerobic and anoxic reactors, and strict control of reaction condition increase the treatment costs and operating complexity.

Recently, bioelectrochemical technology has attracted considerable attentions for nitrogen removal [6,7]. Through proper acclimation, electrochemically active

autotrophic denitrifying bacteria immobilized on the cathode can utilize the electrons or hydrogen gas generated on the cathode as an electron donor to convert NO_3^- -N into harmless N_2 [8,9]. In this process, no extra organic carbon sources need to be added as electron donors. Therefore, the formation of secondary pollution can be avoided in bioelectrochemical treatment.

On the other hand, photocatalytic technology is appealing as a promising method for wastewater treatment since the photocatalysts can utilize solar energy to generate electrons (e^-) and holes (h^+) to enable redox reactions with aqueous contaminants [10]. Some studies reported that either NH_4^+ -N or NO_3^- -N can be removed from wastewater by TiO_2 -based photocatalysis through oxidative or reductive pathways, respectively [11-16]. However, the reported NH_4^+ -N or NO_3^- -N removal needs to be carried out under UV light irradiation, which only accounts for approximately 4% of solar energy. Therefore, it is of crucial importance to develop novel system which can utilize abundant visible light accounting for approximately 44% of solar radiation. The well-known photosensitive but unstable AgX (X: Br, I) were widely applied in photographic films. Interestingly, when AgX was loaded onto the surface of a semiconductor, such as TiO_2 , the decomposition of AgX was drastically inhibited since the photogenerated electrons can be ultimately transferred to O_2 rather than Ag^+ . In this way, the high visible-light-responsive performance of AgX can be utilized. Recently, a series of organic pollutants were reported to be efficiently degraded on AgX/ TiO_2 under visible light irradiation [17-19]. Since the valence band (VB) edge of AgI (2.22 V vs. NHE at pH 7) [20,21] is more positive than the standard redox

potential of NH_4^+ ($E_{\text{NO}_3^-/\text{NH}_4^+} = 0.363 \text{ V}$, $E_{\text{NO}_2^-/\text{NH}_4^+} = 0.343 \text{ V}$, $E_{\text{N}_2/\text{NH}_4^+} = -0.276 \text{ V}$) [22], it is thermodynamically feasible that NH_4^+ can be oxidatively degraded by photogenerated h^+ on AgI. Besides, it is generally accepted that more efficient separation of photogenerated electron-hole pairs can be achieved in photoelectrocatalytic process by applied bias voltage than single photocatalytic one. Thus, the photogenerated holes of AgX/TiO₂ can be more efficiently utilized in photoelectrocatalytic reactions.

For the reduction of NO_3^- -N, hole scavengers were always added in single photocatalytic procedure, which may cause secondary pollution and greatly affected the final product [23,24]. Since bioelectrochemical technology was already proved to have advantages in the aspect of avoiding secondary pollution and enhancing NO_3^- -N reduction, the ingenious combination of visible light photoelectrocatalytic oxidation and bioelectrochemical reduction seems to be a better choice for efficient and clean nitrogen removal within the scope of our knowledge.

In this work, a novel nitrogen removal strategy, the combined photoelectrocatalytic oxidation and autotrophic biofilm denitrification, was developed to achieve simultaneous NH_4^+ -N oxidation and NO_3^- -N reduction in a single reactor. Herein, a visible-light-active AgI/TiO₂ nanotubes (NTs) was chosen as anode for NH_4^+ -N removal by photoelectrocatalytic oxidization under visible light irradiation. The electrochemically active autotrophic denitrifying biofilm were acclimated as cathode for NO_3^- -N reduction. Intriguingly, different from previously reported photocatalytic oxidation or autotrophic denitrification in separate aerobic and anoxic

reactors, this system can achieve nitrogen removal from NH_4^+ -N and NO_3^- -N mixed N-sources wastewater simultaneously in a single reactor. The results provide a low-cost and energy-clean treatment process for efficient nitrogen removal in the field of nitrogenous pollutants elimination.

2. Methods

2.1. Preparation of AgI/TiO₂-NTs photoanode

AgI/TiO₂-NTs photoanode was prepared by anodic oxidation [25] and dissolution-precipitation method. Firstly, TiO₂-NTs were formed on the titanium surface by electrochemical anodization. Prior to anodization, a Ti sheet (90 mm×25 mm×0.35 mm, 99.5% purity) was repeatedly polished with different abrasive sandpapers to a mirror-like finish and ultrasonically cleaned in acetone, ethanol and deionized water for 15 min, respectively. The cleaned Ti sheet was anodized in an electrolyte (0.5 wt% NaF and 0.5 mol/L Na₂SO₄) at 20 V for 5 h in a two-electrode configuration with a same-sized copper sheet cathode. After anodization, the TiO₂-NTs electrode was washed with deionized water and dried in air. Secondly, AgI/TiO₂-NTs electrode was obtained via the following dissolution-precipitation procedure. Namely, the as-formed TiO₂-NTs electrode was dipped in KI solution (1×10^{-3} mol/L) and AgNO₃ solution (1×10^{-3} mol/L, pH=11) for 2 h, sequentially. Finally, the dipped sample was calcined at 350°C for 2 h to obtain AgI/TiO₂-NTs electrode (See digital photo in Fig. S1A).

2.2. Preparation of autotrophic denitrifying *biofilm* cathode

The autotrophic denitrifying *biofilm* cathode was prepared according to our previous report [7]. First, autotrophic denitrifying bacteria originated from Qige wastewater treatment plant (Hangzhou, China) were incubated in an anoxic bio-electrochemical reactor (BER) with two graphite sheets (120 mm×25 mm×4 mm, 90 mm effective length) as anode and cathode, respectively. A DC voltage was applied on the electrodes and gradually increased from 0 to 3.0 V to acclimatize the bacteria. The electrochemically active denitrifying bacteria were selectively enriched on graphite cathode. The BER was filled with 150 mL growth medium. 1 L growth medium contained the following components [26]: 0.3 g KH_2PO_4 , 0.4 g Na_2HPO_4 , 0.5 g NaCl, 3.0 g NaHCO_3 , 0.1 g MgSO_4 , 0.01 g CaCl_2 , 0.3 g KNO_3 and 1 mL trace metal solution (70 mg/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 30 mg/L $\text{Na}_2\text{MO}_4 \cdot 2\text{H}_2\text{O}$, 600 mg/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 30 mg/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 10 mg/L $\text{CuCl}_2 \cdot \text{H}_2\text{O}$, 300 mg/L H_3BO_3 , 20 mg/L $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and 1000 mg/L EDTA). The pH of growth medium was around 7.0. Hydraulic residence time was 24 h. The medium was boiled for 30 min to get rid of the dissolved oxygen ($\text{DO} < 0.5 \text{ mg/L}$) and cooled before being added to the BER. After 1~2 months, the autotrophic denitrification *biofilm* (2~3 mm thick) with good electrochemical activity was formed on the surface of cathode (See digital photo in Fig. S1B).

2.3 Reactor start-up

Fig. 1 showed the detailed schematic of the reactor used in this study. The reactor

was a hermetical cube which was made of perspex (120 mm×55 mm×55 mm) with an open gas vent for gas-out and an influent/effluent vent on the top and a couple of electrodes (120 mm × 25 mm × 4 mm, 90 mm effective length). To increase the electrical conductivity, AgI/TiO₂-NTs photoanode was stuck on a graphite sheet by graphite conducting resin (DY-50, Xilite adhesive Co., LTD, China). The structures of AgI/TiO₂-NTs electrodes mentioned hereinafter were all suchlike. The cathode was autotrophic denitrifying [biofilm](#) electrode or graphite electrode (control group). The two electrodes were connected to a DC power supply (WYL-302s, Silin Electronic Instrument Co., LTD, China) and the distance between the electrodes was 2 cm. The light was supplied by a 300 W xenon lamp (LanSheng electronics Co., LTD, China) placed at 10 cm distance from the AgI/TiO₂-NTs electrode. A 420 nm optical filter was installed on the output of the lamp to ensure that the light was completely visible light, which was harmless to biofilm. Artificial wastewater was boiled to ensure an anoxic condition in the reactor. The components of the artificial wastewater was the same as the growth medium except the components of NH₄⁺-N and NO₃⁻-N which were changed according to different studies. The net liquid volume was 150 mL. The study was operated at room temperature under constant magnetic stirring.

2.4. Batch experiment

After successful start-up, the performances of nitrogen removal from NH₄⁺-N and NO₃⁻-N mixed N-sources wastewater were investigated in different conditions by separate batch experiments. Liquid samples were taken every 1 h and the

concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ were measured according to standard methods for the examination of water and wastewater [27]. The effects of single photoelectrocatalysis on $\text{NH}_4^+\text{-N}$ removal and combined photoelectrocatalysis-autotrophic denitrifying biofilm on nitrogen removal were examined at different applied voltages under visible light irradiation. In addition, the nitrogen removal without visible light irradiation or applied voltage was also studied in the same reactor to further identify the role of electrocatalysis and photocatalysis, respectively. In all the above experiments, the values of pH were kept around 8.0. The nitrogenous concentration of simulated wastewater was 35.0 ± 2.0 mg/L $\text{NH}_4^+\text{-N}$ and 20.0 ± 2.0 mg/L $\text{NO}_3^-\text{-N}$.

2.5. Analytical methods

The concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ were calculated by five-point external standard calibration curves using a UV/Vis spectrophotometer (Unico UV-2102 PC). In detail, $\text{NO}_3^-\text{-N}$ concentrations were determined using corrected absorbance ($A_c = A_{220} - 2A_{275}$) by monitoring the absorbance at 220 nm (A_{220}) and 275 nm (A_{275}), respectively [7,27]. $\text{NO}_2^-\text{-N}$ concentrations were measured by sulfanilamide colorimetric method [7,27]. The corrected absorbance ($A_c = A_{540} - A_{\text{H}_2\text{O}}$) was estimated by monitoring the absorbance at 540 nm in the sample solution (A_{540}) and control water solution ($A_{\text{H}_2\text{O}}$) after adding colorimetric reagents. The method for NH_4^+ analysis was Nessler reagent-colorimetry [27]. NH_4^+ concentrations were estimated using corrected absorbance ($A_c = A_{420} - A_{\text{H}_2\text{O}}$) by monitoring the absorbance at 420 nm in the sample solution (A_{420}) and control water solution ($A_{\text{H}_2\text{O}}$) after adding Nessler reagent. The dehydrogenase activity of the autotrophic denitrification biofilm was determined by measuring 2,3,5-triphenyl-tetrazoliumchloride dehydrogenase

activity [7,32]. One enzyme unit was defined as the amount of enzyme producing 1 μg of triphenylformazan salt per hour.

3. Results and discussion

3.1. Characterization of AgI/TiO₂-NTs electrode

The structure and morphology of AgI/TiO₂-NTs photoanode were investigated by XRD and SEM, respectively. As depicted in Fig. S2, obvious diffraction peaks of anatase TiO₂ were observed, while the intensity of AgI was relatively very weak indicating low loading amount. In detail, AgI was identified as a mixture of β and γ phase. Moreover, TiO₂-NTs were successfully fabricated via the anodization method (Fig. S3A). Almost uniform and hollow TiO₂-NTs with diameter ranging from 80 nm to 100 nm could be observed. Small particles ascribing to AgI were observed to be deposited on TiO₂-NTs (Fig. S3B). By EDX analysis (Fig. S3C), the atomic ratio of Ag to Ti was calculated to be 1.25%, indicating that very low content of AgI was loaded on TiO₂-NTs.

3.2. NH₄⁺-N removal by *photoelectrocatalytic* oxidation

The role of *photoelectrocatalysis* in nitrogen removal was studied under the On/Off visible light irradiation. AgI/TiO₂-NTs electrode and graphite electrode were used as the anode and the cathode, respectively. A voltage of 3.0 V was applied on the two electrodes. Fig. 2a showed the effect of *photoelectrocatalysis* on NH₄⁺-N removal. Under dark condition, the concentration of NH₄⁺-N decreased from 36.9 mg/L to 28.3

mg/L after 12 h, and the removal efficiency was only 23.3%. However, under visible light irradiation, the concentration of $\text{NH}_4^+\text{-N}$ decreased from 36.0 mg/L to 10.4 mg/L. The removal efficiency reached 71.1%. The $\text{NH}_4^+\text{-N}$ removal efficiency under visible light irradiation was 3 times higher than that without light irradiation, indicating that photocatalysis had a significantly positive effect on $\text{NH}_4^+\text{-N}$ removal.

To identify the variation of $\text{NH}_4^+\text{-N}$ during the photoelectrocatalytic process, the concentrations of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ were also determined (Fig. 2b). Under dark condition, there were no observable accumulations of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ after 12 h, indicating that $\text{NH}_4^+\text{-N}$ was not converted to $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$. Thus, the $\text{NH}_4^+\text{-N}$ removal efficiency of 23.3% under dark condition (see Fig. 2a) was probably due to the volatilization of $\text{NH}_4^+\text{-N}$ into air or oxidation to N_2 (represented as Loss). Under visible light irradiation, the concentrations of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ after 12 h reaction were 14.7 mg/L and 1.0 mg/L, respectively, indicating that about 43.6% of $\text{NH}_4^+\text{-N}$ was oxidized to $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ by photoelectrocatalytic reaction. According to the nitrogen mass conservation, about 27.5% of $\text{NH}_4^+\text{-N}$ was oxidized to gaseous products or volatilized to air (Loss). Others were in the wastewater in the form of $\text{NH}_4^+\text{-N}$ (28.9%), $\text{NO}_3^-\text{-N}$ (40.8%) and $\text{NO}_2^-\text{-N}$ (2.8%) (Fig. 2c). To make clear the portion of volatilization, a blank experiment without any electric current was also performed in the dark. As shown in Fig. S4, the concentration of $\text{NH}_4^+\text{-N}$ decreased from 35.4 mg/L to 27.8 mg/L after 12 h constant stirring in the dark at 0 V applied potential. The formation of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ were negligible. Thus, the Loss (21.6%) in the blank experiment can be attributed to volatilization of ammonia.

However, under both visible light irradiation and 3.0 V applied potential, the value of Loss was about 27.5% (Fig. 2c), which is larger than blank experiment. Therefore, the oxidation of $\text{NH}_4^+\text{-N}$ to gaseous products can not be excluded, and the word “Loss” was still used to represent volatilization of $\text{NH}_4^+\text{-N}$ into air and oxidation to gaseous products. For convenience, the amount of $\text{NH}_4^+\text{-N}$ removal mentioned below includes the Loss.

3.3. Nitrogen removal by combined *photoelectrocatalysis* and autotrophic denitrifying biofilm system

In order to study the removal of $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$, an electrochemically active autotrophic denitrifying biofilm electrode was used instead of graphite electrode as the cathode in the experimental group. For comparison, a graphite electrode was also used as cathode in the blank group. The anodes were both AgI/TiO₂-NTs electrodes. The initial $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in their mixed wastewater were about 35.0 mg/L and 20.0 mg/L, respectively.

Under visible light irradiation for 12 h, the performances of nitrogen removal were shown in Fig. 3. It can be seen that the $\text{NH}_4^+\text{-N}$ removal efficiency in the autotrophic denitrifying biofilm cathode group was a little higher than that in the graphite cathode blank group, which were both more than 70% (Fig. 3a). However, the difference of $\text{NO}_3^-\text{-N}$ concentrations between the two groups was very obvious. In the autotrophic denitrifying biofilm electrode group, the concentration of $\text{NO}_3^-\text{-N}$ firstly increased and then decreased from about 28.4 mg/L to 11.9 mg/L. The increasing $\text{NO}_3^-\text{-N}$ came

from the [photoelectrocatalytic](#) process. The decreasing NO_3^- -N concentration was due to the denitrification action of electrochemically active autotrophic denitrifying [biofilm](#) electrode. In the blank group, the concentration of NO_3^- -N gradually increased to 30.4 mg/L [which was higher than the initial concentration](#) (Fig. 3b). It can be concluded that NH_4^+ -N was converted to NO_3^- -N by [photoelectrocatalytic](#) process under visible light irradiation, but NO_3^- -N was not effectively reduced by graphite cathode. In addition, it was found based on Fig. 3c that the application of autotrophic denitrifying [biofilm](#) cathode also inhibited the formation of NO_2^- -N. Therefore, [photoelectrocatalysis](#) and autotrophic denitrifying [biofilm](#) could be applied simultaneously in a single reactor to achieve efficient nitrogen removal from NH_4^+ -N and NO_3^- -N mixed N-sources wastewater.

3.4. Effect of different applied voltages on nitrogen removal

Since the promotion of nitrogen removal was demonstrated with the presences of both [photoelectrocatalysis](#) and autotrophic denitrifying [biofilm](#), four applied voltages (1.0, 2.0, 3.0 and 4.0 V) were applied to investigate the effect of different applied voltages on nitrogen removal from NH_4^+ -N and NO_3^- -N mixed N-sources wastewater. Similarly, AgI/TiO₂-NTs was used as anode and autotrophic denitrifying [biofilm](#) as cathode. Fig. 4a showed that the removal efficiency of NH_4^+ -N without applied voltage (0 V) was 36.6%. When the applied voltage increased from 1.0 V to 3.0 V, the removal efficiency of NH_4^+ -N increased from 60.0% to 83.1%. However, when the applied voltage increased to 4.0 V, the value decreased to 76.7%. These results

indicated that the photoelectrocatalytic activity was greatly enhanced with appropriate applied voltages. Since photogenerated electrons can be driven away from holes under an applied voltage, reduced recombination rate and improved utilization ratio of holes can be thus obtained. The optimal applied potential was estimated to be 3.0 V in the tested range.

Fig. 4b showed the denitrification efficiency and the accumulation of NO_2^- -N under different applied voltages. When the applied voltage increased from 1.0 to 4.0 V, the removal efficiency of NO_3^- -N were 27.2%, 50.6%, 74.3% and 52.3%, respectively. As a comparison, the removal efficiency of NO_3^- -N in blank test without applied voltage was 8.3%. Obviously, the denitrification was facilitated by an applied voltage. The accumulations of NO_2^- -N were 0.68 mg/L, 0.72 mg/L, 0.54 mg/L, 0.32 mg/L and 0.86 mg/L under different applied voltages (0 V, 1.0 V, 2.0 V, 3.0 V and 4.0 V), respectively. The minimum accumulation of NO_2^- -N (0.32 mg/L) was observed when the applied voltage was 3.0 V. Thus, applied voltages could facilitate the denitrification and decrease the accumulation of NO_2^- -N. In this work, the optimal applied voltage was 3.0 V. Continuous current is a main control factor for electrochemically active biofilm denitrification. Higher voltages are helpful to generate more hydrogen gas by electrolysis so that there are enough electron donors for denitrification. However, extravagant voltages would lead to so much hydrogen gas that the structure of the biofilm was destroyed. What is more, overmuch hydrogen gas caused “hydrogen inhibition” [28]. It was harmful to the growth and metabolism of biofilm. Therefore, it is reasonable that the removal efficiency decreased when the

applied voltage was 4.0 V.

Denitrification coupled to NH_4^+ -N photoelectrocatalytic removal was enhanced which can be demonstrated by the TN removal with the applied voltages. Fig. 4c showed the TN removal efficiency under four applied voltages. Maximum TN removal (89.6%) was observed at an applied voltage of 3.0 V in the present study.

3.5. Effect of pH on nitrogen removal

The performances of nitrogen removal at different pH values were examined in the reactors under visible light irradiation at 3.0 V applied voltage. AgI/TiO₂-NTs electrode and autotrophic denitrifying biofilm electrode were used as anode and cathode, respectively. The initial concentrations of NH_4^+ -N and NO_3^- -N were about 36.0 mg/L and 20.0 mg/L, respectively.

As shown in Fig. 5a, the optimal pH for denitrification was about 7.5 in this work. With an increase of pH value from 6.0 to 9.0, denitrification efficiency increased in the pH range of 6.0~7.5 and then decreased (pH 8.0~9.0). The maximum denitrification efficiency was 84.6% when the pH value was 7.5. Denitrification declination was observed at higher or lower pH range. When the pH increased to 9.0 or decreased to 6.0, the denitrification efficiencies declined significantly to 48.9% or 45.8%, respectively. Besides, the pH value was found to play an important role in NO_2^- -N accumulation. At pH 7.5 and 8.0, the accumulations of NO_2^- -N were 0.37 mg/L and 0.32 mg/L, respectively, which were lower than the NO_2^- -N accumulations at other pH values. NO_2^- -N accumulations were all more than 1.0 mg/L in higher or

lower pH range. It was reported that the optimum pH for denitrification was 7.5~8.0 [29,30]. And the pH value higher than 9.0 or lower than 6.0 would cause a significant recession or even a stop of denitrification [31]. The optimal pH in this work for denitrification was about 7.5~8.0 which was in accordance with the reported value.

From Fig. 5b, it can be seen that the removal efficiency of NH_4^+ -N was enhanced with increasing pH (from 6.0 to 9.0). Since the removal of NH_4^+ -N consists of two parts, including photoelectrocatalytic oxidation and volatilization, the promoting effect of pH on which part should be distinguished to verify the superiority of combined photoelectrocatalysis and bioelectrochemical technology in the present study.

Since pH value could affect the equilibrium between NH_4^+ and NH_3 , and higher pH would improve the volatilization of NH_3 from water, the effect of pH on the volatilization of NH_4^+ -N into air should be considered first. As illustrated in Fig. S5, the percentage of TN “Loss” (composed of the volatilization of NH_4^+ -N into air, NH_4^+ -N oxidation to N_2 , and NO_3^- -N reduction to N_2) was significantly increased at $\text{pH} > 7$. For example, the value of TN “Loss” was calculated to be 25.5% and 53.4% at pH 6 and pH 7.5, respectively. The largest value was observed at pH 8 (79.8%). However, when pH further increased to 9, the value of TN “Loss” decreased slightly to 77.6%. Since the volatilization of NH_4^+ -N was more likely to happen at pH 9 than pH 8, the contribution of volatilization to TN “Loss” should not be the dominant factor.

The above conclusion can also be verified by the control experiment using graphite

electrode as cathode for the [photoelectrocatalytic](#) oxidation of single $\text{NH}_4^+\text{-N}$ (Fig. 2c). Also at pH 8 and 3.0 V voltage, the value of Loss was 27.5% when 71.1% $\text{NH}_4^+\text{-N}$ was removed. Comparing with the removal of $\text{NH}_4^+\text{-N}$ in $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ mixture using electrochemically active autotrophic denitrifying [biofilm](#) as cathode, the value of TN “Loss” (79.8%) drastically increased while the change in removal efficiency of $\text{NH}_4^+\text{-N}$ (83.2%) was relatively very small. Besides, it has been verified in Fig. 5a that the denitrification efficiency was much high at pH 8.0 than pH 9.0. Therefore, the reduction of $\text{NO}_3^-\text{-N}$ to N_2 by electrons or hydrogen in the denitrification process may probably be the main contributor to the largest TN “Loss” at pH 8. Accordingly, the rapid electron-hole separation can be achieved which may meanwhile increase the oxidative removal dynamic of $\text{NH}_4^+\text{-N}$ by the photoanode.

Considering that the denitrification process would be restricted but the $\text{NH}_4^+\text{-N}$ removal would be improved when pH was higher than 7.5, the TN removal was measured to find out the optimum pH circumstance. As shown in Fig. 5c, the maximum TN removal efficiency was 89.6% when pH was 8.0. Therefore, the favorable pH value was 8.0 for the whole combined process of [photoelectrocatalysis](#) and electrochemically active autotrophic denitrifying [biofilm](#).

3.6. The stability of the AgI/TiO₂-NTs anode

In the present study, the stability of the AgI/TiO₂-NTs anode in long time experiments was investigated in two ways. First, cyclic runs were conducted for the removal of $\text{NH}_4^+\text{-N}$ using AgI/TiO₂-NTs anode and an autotrophic denitrifying

biofilm cathode under visible light irradiation at optimized condition. As shown in Fig. 6a, little change of activity was observed. Moreover, the cyclic photocurrent response of AgI/TiO₂-NTs anode was also tested in 0.1 M Na₂SO₄ solution and the results were shown in Fig. 6b. In the tested potential range from 0 V to 0.6 V (vs Ag/AgCl), negligible loss of photocurrent was observed after 8 repeated runs, indicating stable photoelectrocatalytic property. Overall, AgI/TiO₂-NTs anode exhibits good stability and cyclic performance.

3.7. The stability of the denitrifying biofilm cathode

In order to investigate the effect of oxidative species (photogenerated holes or free radicals) on the stability of autotrophic denitrification biofilm, dynamic study for removal of NH₄⁺-N and NO₃⁻-N mixture was firstly carried out under UV-A light irradiation which is much more powerful than visible light. Herein, pristine and stable TiO₂-NTs with more positive valence band (+2.7 V at pH 7) [33] was used as anode. It can be observed from Fig. S6 that the denitrification action of electrochemically active autotrophic denitrifying biofilm was greatly inhibited by UV-A light. Interestingly, although TiO₂-NTs can not be excited by visible light, high activity for NO₃⁻-N removal can still be observed using TiO₂-NTs anode and biofilm cathode under visible light irradiation. Thus, it can be inferred that UV-A light is harmful to the biofilm cathode, while visible light is harmless. Furthermore, the stability of the denitrifying biofilm cathode was also investigated when AgI/TiO₂-NTs was used as anode. By measuring dehydrogenase activity of the biofilm during visible light

irradiation, the stability of the denitrifying biofilm can be quantitatively assessed. As shown in Fig. 7, the dehydrogenase activity of the biofilm cathode after 12 h visible light irradiation was 13.0 $\mu\text{g}/(\text{mL}\cdot\text{h})$, which exhibited negligible difference to that before visible light irradiation.

3.8. Probable nitrogen removal pathway

For more information on the mechanism of ammonia oxidation, cyclic voltammetry (CV) measurements were conducted with the addition of NH_4^+ or Ag^+ using AgI/TiO₂-NTs anode and Pt cathode, respectively. To avoid the reduction of Ag^+ added or products formed from NH_4^+ oxidation on cathode, the CV experiments were carried out in two separate cells using salt bridge as connection. The effect of NH_4^+ addition on the CV of AgI/TiO₂-NTs was shown in Fig. S7a. An oxidative peak at 0.29 V vs Ag/AgCl (0.49 V vs NHE) appeared after addition of 20 mg/L NH_4^+ . When the concentration of NH_4^+ increased to 50 mg/L, the peak gradually shifted to 0.27 V vs Ag/AgCl (0.47 V vs NHE). To exclude the possible oxidation of AgI on the anode, control experiment using TiO₂-NTs was also performed. As shown in Fig. S7b, similar oxidative peak with much lower intensity was still observed. Moreover, the current response increased gradually with increasing concentration of NH_4^+ . Thus, the oxidative peak was ascribed to the oxidation of NH_4^+ not I⁻ in the lattice of AgI. In the same way, the effect of AgNO_3 addition on the CV of AgI/TiO₂-NTs anode was also investigated and the result was shown in Fig. S7c. No signal of oxidative peaks were observed in the tested potential range, which may be probably due to high redox

potential of $\text{Ag}^{3+}/\text{Ag}^+$ ($E^0=1.9\text{ V}$ vs NHE) and $\text{Ag}^{3+}/\text{Ag}^{2+}$ ($E^0=1.8\text{V}$ vs NHE). Above all, oxidation of NH_4^+ by the photogenerated holes was more easily than oxidation of Ag^+ or I^- in AgI lattice.

Based on the results above, the probable nitrogen removal pathway could be represented as Fig. 8a. In the photoelectrocatalytic oxidation process, AgI/TiO₂-NTs anode could generate electron-hole pairs under visible light irradiation. Its conduction band edge and valence band edge fully covers the redox position of NH_4^+ , indicating that photoelectrocatalytic oxidation of NH_4^+ -N was thermodynamically feasible on the AgI/TiO₂-NTs anode (Fig. 8b). When an artificial wastewater containing NH_4^+ -N and NO_3^- -N flowed into the reactor, a part of NH_4^+ -N was oxidized to N_2 (evidenced by MS spectra in Fig. S8) by photo-generated holes on the surface AgI/TiO₂-NTs electrode under visible light conditions. Meanwhile, some NH_4^+ -N was oxidized to NO_2^- -N and NO_3^- -N by holes. In this process, a small part of NH_4^+ -N volatilized into air because of continuous stirring. Some researchers also reported that NH_4^+ -N was oxidized by holes produced on the surface of TiO₂ photocatalyst [11,13] under UV light irradiation. Our work used the AgI sensitized TiO₂-NTs which could oxidize NH_4^+ -N under harmless visible light irradiation, so that the autotrophic denitrifying biofilm on the cathode would not be affected by harmful UV light.

In the denitrification process on the surface of autotrophic denitrifying biofilm cathode, NO_2^- -N and NO_3^- -N generated on anode moved to cathode, where they were reduced to N_2 by autotrophic denitrifying biofilm at an applied voltage condition. Hydrogen produced on cathode served as electron donors in the process of autotrophic

biofilm denitrification. Moreover, the constant production of H_2 on the surface of cathode created an anoxic condition around autotrophic biofilm. According to experimental results using graphite electrode as cathode, it was found that NO_2^- -N and NO_3^- -N could seldom be reduced by photo-generated electrons in this reaction condition. Therefore, the **removal** of NO_2^- -N and NO_3^- -N were attributed to autotrophic biofilm denitrification.

4. Conclusions

The present study provides a novel strategy for efficient nitrogen removal from NH_4^+ -N and NO_3^- -N mixed N-sources wastewater in a single reactor via simultaneous **photoelectrocatalytic** oxidation and autotrophic biofilm denitrification. About 27.5% NH_4^+ -N was oxidized to N_2 or volatilized to air, and 43.6% NH_4^+ -N was oxidized to NO_3^- -N and NO_2^- -N by **photoelectrocatalytic** reaction. The NO_2^- -N and NO_3^- -N were further reduced to N_2 by autotrophic denitrifying **biofilm**. In this way, complete nitrogen removal was achieved. Applied voltage and pH were critical factors affecting nitrogen removal, while the optimum values were 3.0 V and 8.0, respectively.

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Figure Captions

Fig. 1. A schematic diagram and the basic principle of the reactor.

Fig. 2. $\text{NH}_4^+\text{-N}$ removal (a), $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ formation (b) with and without visible light irradiation; Mass balances during $\text{NH}_4^+\text{-N}$ removal by photocatalytic oxidation (c). Reaction conditions: applied voltage = 3.0 V (2.71 mA/cm^2), pH 8.0, Initial $\text{NH}_4^+\text{-N}$ = 35.0 mg/L .

Fig. 3. Concentration variations of $\text{NH}_4^+\text{-N}$ (a), $\text{NO}_3^-\text{-N}$ (b), $\text{NO}_2^-\text{-N}$ (c) using AgI/ TiO_2 -NTs anode and an autotrophic denitrifying biofilms cathode or a graphite cathode under visible light irradiation. Reaction conditions: applied voltage = 3.0 V (2.71 mA/cm^2), pH 8.0, Initial $\text{NH}_4^+\text{-N}$ = 35.0 mg/L , Initial $\text{NO}_3^-\text{-N}$ = 20.0 mg/L .

Fig. 4. Effects of different applied voltages (0, 1.0, 2.0, 3.0 and 4.0 V) on $\text{NH}_4^+\text{-N}$ removal (a) $\text{NO}_3^-\text{-N}$ removal and $\text{NO}_2^-\text{-N}$ formation (b), TN removal (c) in the reactor at pH value of 8.0. Reaction conditions: Initial $\text{NH}_4^+\text{-N}$ = 35.0 mg/L , Initial $\text{NO}_3^-\text{-N}$ = 20.0 mg/L . The current densities at different applied potentials (from 1.0 V to 4.0 V) were 0.29 mA/cm^2 (1.0 V), 1.73 mA/cm^2 (2.0 V), 2.71 mA/cm^2 (3.0 V) and 3.8 mA/cm^2 (4.0 V), respectively.

Fig. 5. Effects of different pH (6.0, 7.0, 7.5, 8.0 and 9.0) on $\text{NO}_3^-\text{-N}$ removal (a), $\text{NH}_4^+\text{-N}$ removal (b), TN removal (c) in the reactor. Reaction conditions: applied voltage = 3.0 V (2.71 mA/cm^2), Initial $\text{NH}_4^+\text{-N}$ = 35.0 mg/L , Initial $\text{NO}_3^-\text{-N}$ = 20.0 mg/L .

Fig. 6. Cyclic removal of $\text{NH}_4^+\text{-N}$ using AgI/ TiO_2 -NTs anode and an autotrophic denitrifying biofilm cathode under visible light irradiation at optimized condition (a), and cyclic photocurrent response of AgI/ TiO_2 -NTs anode 0.1M Na_2SO_4 solution (b).

Fig. 7. Effect of visible light irradiation on dehydrogenase activity of the biofilm cathode at applied potential of 3.0 V (2.71 mA/cm^2).

Fig. 8. The probable nitrogen removal pathways in the process (a); Bandgap structure of AgI/ TiO_2 -NTs and the solution redox positions for NH_4^+ oxidation (b).

Figure(s)

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Fig. 5. Effects of different pH (6.0, 7.0, 7.5, 8.0 and 9.0) on NO₃⁻-N removal (a), NH₄⁺-N removal (b), TN removal (c) in the reactor. **Reaction conditions:** applied voltage = 3.0 V (2.71 mA/cm²), Initial NH₄⁺-N = 35.0 mg/L, Initial NO₃⁻-N = 20.0 mg/L.

Fig. 6. Cyclic removal of $\text{NH}_4^+\text{-N}$ using $\text{AgI/TiO}_2\text{-NTs}$ anode and an autotrophic denitrifying biofilm cathode under visible light irradiation at optimized condition (a); cyclic photocurrent response of $\text{AgI/TiO}_2\text{-NTs}$ anode in 0.1 M Na_2SO_4 solution (b).

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Graphical Abstract