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Removal of ammonia nitrogen from black and odorous water by macrophytes based on laboratory microcosm experiments

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In recent years, the removal mechanism of ammonia nitrogen in black and odorous water (BOW), especially in the process of phytoremediation, has been a research "hotspot". Here, the migration process of ammonia nitrogen in macrophytes (*Acorus calamus*, *Canna indica* and *Eichhornia crassipes*) was detected by Fourier transform infrared (FT-IR) spectroscopy. Experiments revealed that the concentration of ammonia nitrogen ($\text{NH}_4^+\text{-N}$) was reduced significantly. Maximum reduction in the $\text{NH}_4^+\text{-N}$ concentration was obtained in 75% BOW: the absorption of $\text{NH}_4^+\text{-N}$ was >90% in *A. calamus* and *C. indica*, and >80% in *E. crassipes*. After two 10 days cultivations, in the culture dishes of *A. calamus* and *C. indica*, absorption of $\text{NH}_4^+\text{-N}$ was >90% whereas, in the culture dishes of *E. crassipes*, absorption of $\text{NH}_4^+\text{-N}$ was ~50% and ~60%. FT-IR spectroscopy showed that $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ could be absorbed by the root and migrate to the stem and leaf of macrophytes. $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were transformed, and the direction was $\text{NH}_4^+\text{-N} \rightarrow \text{NO}_2^-\text{-N} \rightarrow \text{NO}_3^-\text{-N}$. The migration rate of $\text{NH}_4^+\text{-N}$ in *C. indica* was faster because of its regular and smooth capillaries according to scanning electron microscopy. Our study on the removal and transformation mechanism of ammonia nitrogen in BOW could be an important reference for other bodies of water.

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1 Introduction

Black and odorous water (BOW) is an extreme example of organic pollution in water.^{1–3} BOW may have a pungent smell, which some people may find unpleasant or disgusting.

There are two main mechanisms of water-blackening: one is insoluble substances in water in the form of solid/adsorbed particles; the other is colored organic compounds dissolved in water.⁴

There are four types of odorants: methane (CH_4), hydrogen sulfide (H_2S), ammonia (NH_3) and other small-molecule gases. If the water body is seriously polluted by organic matter, the aerobic decomposition of organic matter makes the oxygen consumption rate in the water body higher than the reoxygenation rate, thereby resulting in water hypoxia. In anoxic water, odor production is synchronized with blackening, and organic matter is decomposed anaerobically to produce CH_4 , H_2S , NH_3 and other odorous and volatile small molecular compounds, which overflow the water surface and enter the atmosphere,

thereby emitting odor. Ding *et al.*⁵ found that the odor of the water body was caused mainly by the release of H_2S and NH_3 during the decomposition of organic matter containing sulfur or nitrogen.

Aquatic plants (also known as "macrophytes") can take-up nitrogen compounds in water through absorption and adsorption,⁶ which can reduce the nitrogen content in eutrophic water effectively. William⁷ pointed out that the effect of a macrophyte-based ecological-treatment system is as good as that of typical chemical-treatment system. Macrophytes increase the concentration of dissolved oxygen, provide oxygen for aquatic animals and aerobic microorganisms in the water, restore the damaged aquatic ecosystem, and promote aerobic microorganisms to effectively degrade organic nutrients in the water through respiration; the growth of macrophytes consumes nitrogen in the water, thereby avoiding eutrophication effectively.

Macrophytes mainly absorb nitrogen compounds in the sediment through the roots, then distribute them to the branches and, finally, release them into the water through the living plant or the decay of a dead plant.⁸ In natural waters, large-root plants (*e.g.*, submerged macrophytes) have more competitive advantages than phytoplankton because they can absorb nutrients directly from sediment.⁹

Macrophytes can absorb nitrogen compounds directly from water or sediment.¹⁰ The storage of nitrogen compounds in submerged plants is more stable than that in algae.¹¹ Ammonia

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nitrogen ($\text{NH}_4^+\text{-N}$) can be removed by plant uptake, but nitrification and denitrification remain the main mechanisms of removal. The physiological and metabolic activities of submerged plants are directly related to the migration and transformation of nutrients.¹² Submerged macrophytes can control the growth of phytoplankton, provide shelter for zooplankton-feeding animals, repair the aquatic ecosystem as well as maintain the balance of the aquatic ecosystem by biological manipulation.¹³

Yao *et al.*¹⁴ studied the purification effect of six macrophytes (*Ceratophyllum demersum*, *Vallisneria* species, *Vallisneria macrocephala*, *Hydrilla verticillata*, *Sagittaria humilis*, and crown grass) in simulated wastewater. With an increase in treatment time, the proportion of ammonia nitrogen decreased and the proportion of nitrate nitrogen increased. *Vallisneria* species had a good purification effect on nitrogen compounds.

In the present study, three types of macrophytes, *Acorus calamus*, *Canna indica* and *Eichhornia crassipes*, were used to remove ammonia from BOW. The migration and transformation of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ were investigated by Fourier transform infrared (FT-IR) spectroscopy.

2 Experimental section

2.1 Absorption experiment

The macrophytes were *A. calamus*, *C. indica* and *E. crassipes* in their mature period. The water quality was simulated to be black and odorous. According to the average concentration of the water body on site and plant tolerance, the initial concentration of ammonia nitrogen was set at 24 mg L^{-1} for the first 10 days and 16 mg L^{-1} for the second 10 days.

The experiment was simulated in the laboratory. Each plant was placed into five BOW concentrations: 0%, 25%, 50%, 75% and 100% (BOW/pure water, vol%), respectively. Each treatment was repeated thrice. All treatments were carried out in a bucket with a volume of 20 L and duration of 20 days.

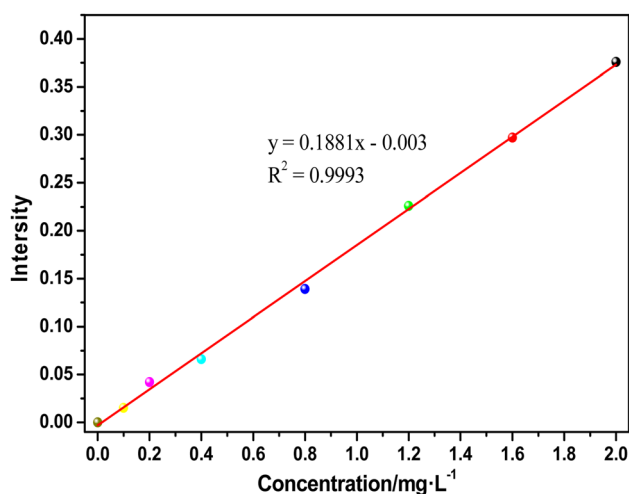


Fig. 1 Standard curve for testing the concentration of ammonia nitrogen.

At 8 am on the days 1, 3, 5, 10, 14, 16 and 20 of the experiment, samples were taken to determine the amount of ammonia nitrogen in treated water samples, and BOW of identical concentration was replenished on day-10.

The $\text{NH}_4^+\text{-N}$ concentration was tested using Nessler's reagent spectrophotometry method. The standard curve is shown in Fig. 1. The spectral intensity vs. concentration of ammonia nitrogen yielded the equation $y = 0.1881x - 0.003$ and the coefficient of association was 0.9993.

2.2 Characterization method

The transformation of nitrogen-containing compounds was characterized by FT-IR spectroscopy carried out on a vector 33 FT-IR spectrophotometer (Bruker, USA) with a DTGS detector at a resolution of 4 cm^{-1} . The microcapillary structure of aquatic plants was visualized on a Flex-SEM1000 electron microscope (Hitachi, Japan).

3 Results and discussion

The absorption performances of *A. calamus*, *C. indica* and *E. crassipes* for ammonia nitrogen in five types of BOW are shown in Fig. 2 and Fig. 3.

After 10 days of cultivation in BOW with an initial concentration of ammonia nitrogen of 24 mg L^{-1} , the concentration of ammonia nitrogen in the utensils employed for cultivating *A. calamus* and *C. indica* was $<2 \text{ mg L}^{-1}$ (Fig. 2). After 10 days, the concentration of ammonia nitrogen in the utensils employed for cultivating *A. calamus* and *C. indica* remained $<2 \text{ mg L}^{-1}$. These results showed that *A. calamus* and *C. indica* had a good absorption effect on ammonia nitrogen. For *E. crassipes*, the concentration of ammonia nitrogen in the culture dish was $\sim 8 \text{ mg L}^{-1}$ after two 10 days cultivations, which indicated that the absorption effect of *E. crassipes* was inferior to that of *A. calamus* and *C. indica*.

Fig. 3 shows that, after two 10 days cultivations, in the culture dishes of *A. calamus* and *C. indica*, absorption of ammonia nitrogen was $>90\%$ whereas, in the culture dishes of *E. crassipes*, absorption of ammonia nitrogen was $\sim 50\%$ and $\sim 60\%$. Among the five types of BOW, 75% BOW had the highest absorption efficiency of ammonia nitrogen, irrespective of whether *A. calamus*, *C. indica* or *E. crassipes* was the macrophyte.

FT-IR spectroscopy is an important means to study the absorption of ammonia nitrogen by plants and its transformation within plants. The characterization results are shown in Fig. 4–8. We focused on analyses of the changes in the nitrogen-containing compounds $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$. According to the literature,^{15,16} the characteristic peaks (in cm^{-1}) of $\text{NH}_4^+\text{-N}$ are 1738 and 1459, whereas they are 1511 and 1332 for $\text{NO}_2^-\text{-N}$, and 1414 cm^{-1} for $\text{NO}_3^-\text{-N}$.

The FT-IR spectra of the root, stem and leaf of *A. calamus* cultivated in BOW for 10 days are shown in Fig. 4. Fig. 4A reveals the FT-IR spectrum of the root of *A. calamus* cultivated in BOW for 10 days. Irrespective of the BOW concentration (0–100%), the characteristic peaks of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ appeared in the spectrum, thereby indicating that the root



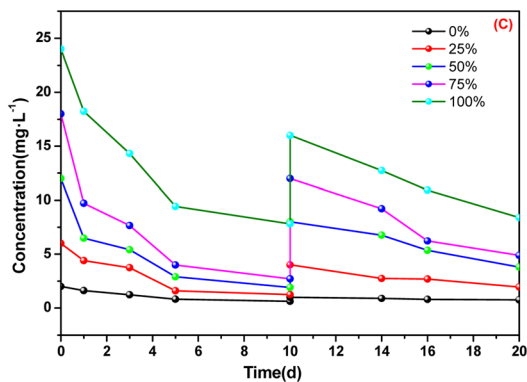
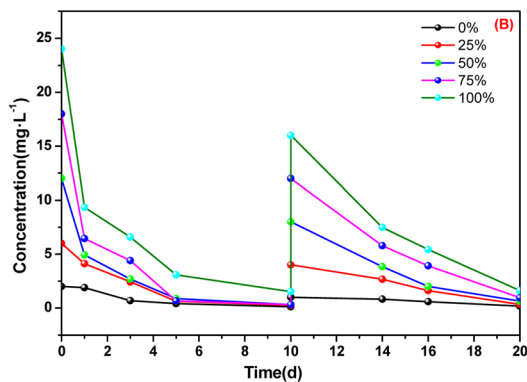
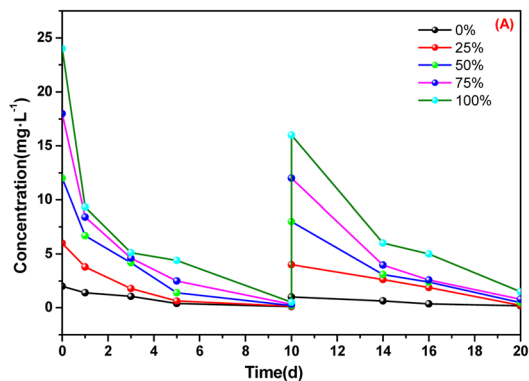


Fig. 2 Absorption of ammonia nitrogen for three macrophytes. (A) *Acorus calamus*; (B) *Canna indica*; (C) *Eichhornia crassipes*.

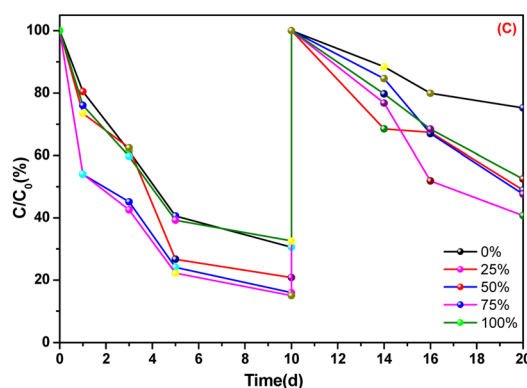
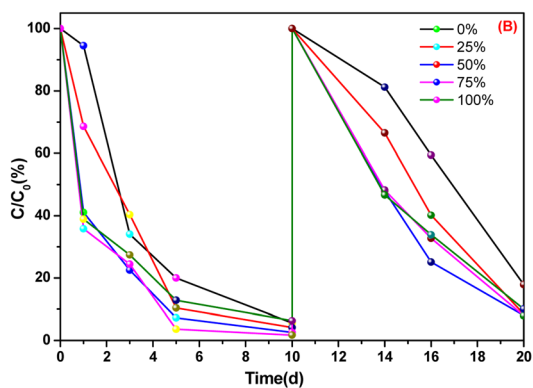
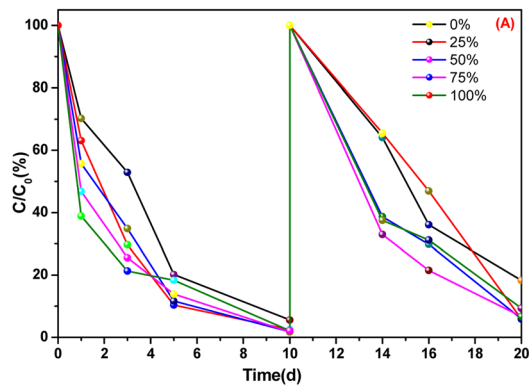


Fig. 3 Absorption efficiency of ammonia nitrogen in three macrophytes. (A) *Acorus calamus*; (B) *Canna indica*; (C) *Eichhornia crassipes*.



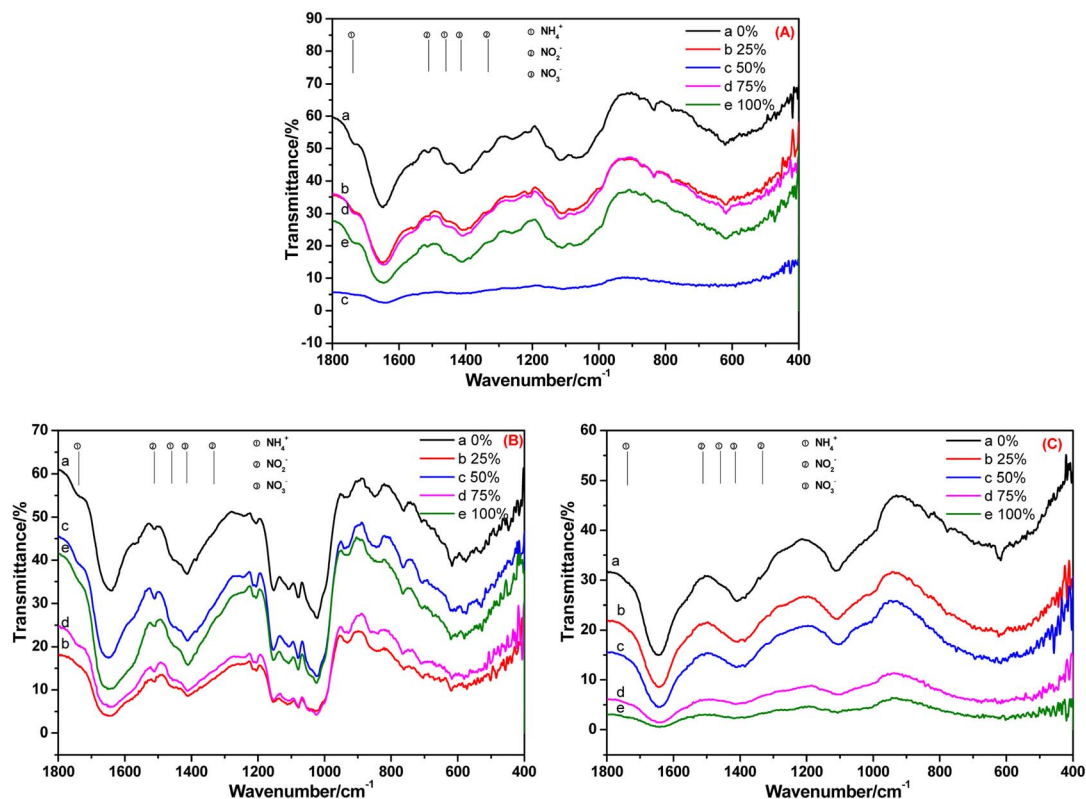


Fig. 4 FT-IR spectra of the (A) root (B) stem and (C) leaf of *Acorus calamus* cultivated in black and odorous water for 10 days.

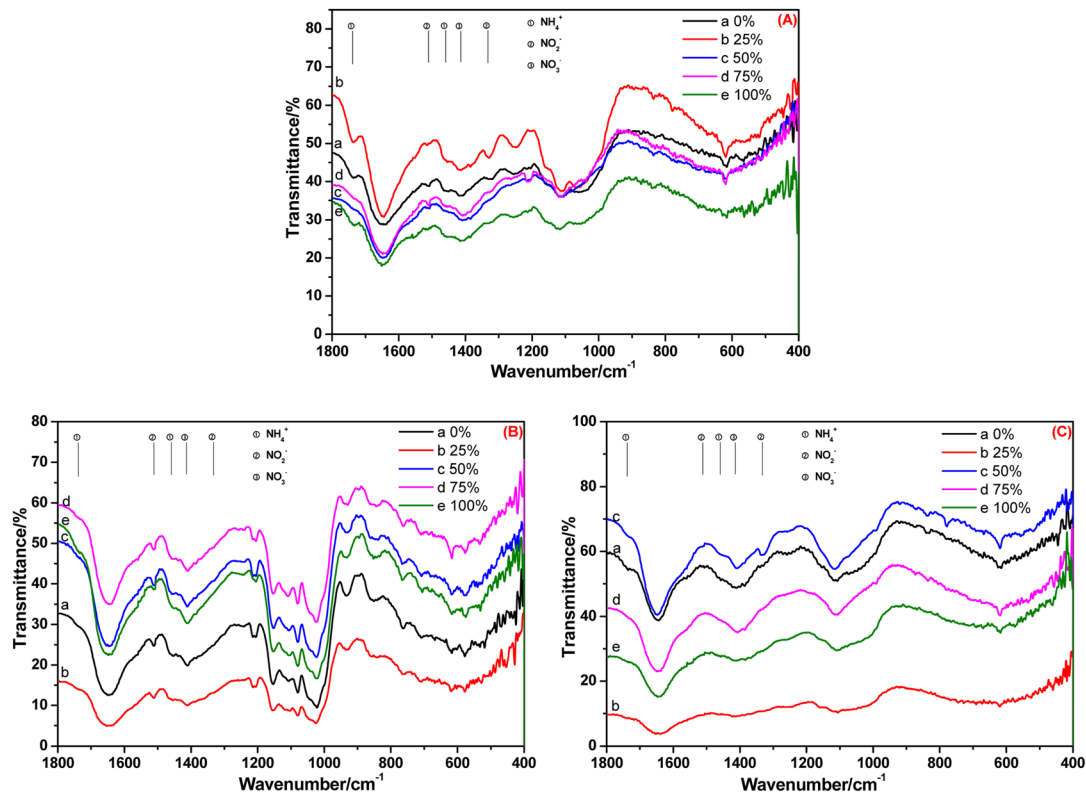


Fig. 5 FT-IR spectra of the root (A), stem (B) and leaf (C) of *A. calamus* cultivated in black and odorous water for 20 days.



absorbed $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in BOW. Fig. 4B reveals the FT-IR spectrum of the stem of *A. calamus* cultivated in BOW for 10 days. Irrespective of the BOW concentration (0–100%), the characteristic peaks of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ appeared in the spectrum, thereby indicating that $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the root migrated to the stem. However, compared with the root, the intensity of the characteristic peak of $\text{NH}_4^+\text{-N}$ at 1738 cm^{-1} and 1459 cm^{-1} in the FT-IR spectrum of the stem was weakened significantly, and the relative intensity of the peaks at 1511 cm^{-1} and 1414 cm^{-1} was enhanced. These findings indicated that the concentration of $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the stem was greater than that in the root. This phenomenon may have been caused by the transformation of $\text{NH}_4^+\text{-N}$ to $\text{NO}_2^-\text{-N}$, and a small amount of $\text{NO}_2^-\text{-N}$ being transformed to $\text{NO}_3^-\text{-N}$. Fig. 4C denotes the FT-IR spectrum of a leaf of *A. calamus* after 10 days of cultivation in BOW. Irrespective of the BOW concentration (0–100%), the characteristic peaks of $\text{NO}_3^-\text{-N}$ were the strongest, whereas the characteristic peaks of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were very weak. These observations suggested that $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in the root migrated to the leaf through the stem, $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were transformed, and the likely equation is: $\text{NH}_4^+\text{-N} \rightarrow \text{NO}_2^-\text{-N} \rightarrow \text{NO}_3^-\text{-N}$.

The FT-IR spectra of the root, stem and leaf of *A. calamus* cultivated in BOW for 20 days are shown in Fig. 5. Irrespective of the BOW concentration (0–100%), the characteristic peaks of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ were observed (Fig. 5A), thereby indicating that the root absorbed $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in BOW, but the $\text{NH}_4^+\text{-N}$ concentration was stronger compared

with that at 10 days. Compared with Fig. 5B, the relative intensity of the peak at 1738 cm^{-1} in Fig. 4B was reduced significantly. This observation indicated that, compared with 10 days previously, the $\text{NH}_4^+\text{-N}$ level in the stem had decreased significantly. In Fig. 5C, the peak at 1738 cm^{-1} is present, but the peaks at 1511 cm^{-1} and 1332 cm^{-1} are absent. A possible reason is that the conversion of $\text{NH}_4^+\text{-N}$ to $\text{NO}_2^-\text{-N}$ was slower than the conversion of $\text{NO}_2^-\text{-N}$ to $\text{NO}_3^-\text{-N}$ in the leaf.

The FT-IR spectra of the root, stem and leaf of *C. indica* cultivated in BOW for 10 days are shown in Fig. 6. Fig. 6A reveals that $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ were absorbed in the root irrespective of the BOW concentration. At 100% BOW, the $\text{NH}_4^+\text{-N}$ concentration in the root was the highest whereas, at the other BOW percentages, the $\text{NH}_4^+\text{-N}$ concentration was much lower than that observed in *A. calamus*. In the stem of *C. indica*, residual $\text{NH}_4^+\text{-N}$ was present, but very little $\text{NO}_2^-\text{-N}$ was present in BOW (except at 100% BOW). These data indicated that the speed of migration and transformation of $\text{NO}_2^-\text{-N}$ was rapid. In Fig. 6C, the peak at 1332 cm^{-1} (characteristic peak of $\text{NO}_2^-\text{-N}$) was present, but peaks at 1738 , 1459 and 1511 cm^{-1} (characteristic peaks of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$) were absent, which indicated that a small amount of $\text{NO}_2^-\text{-N}$ remained in the leaf during migration and transformation. Compared with *A. calamus*, residual amounts of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were present. However, inorganic nitrogen in *C. indica* can be transformed rapidly to $\text{NO}_3^-\text{-N}$ due to the straight-channel capillaries in *C. indica*.

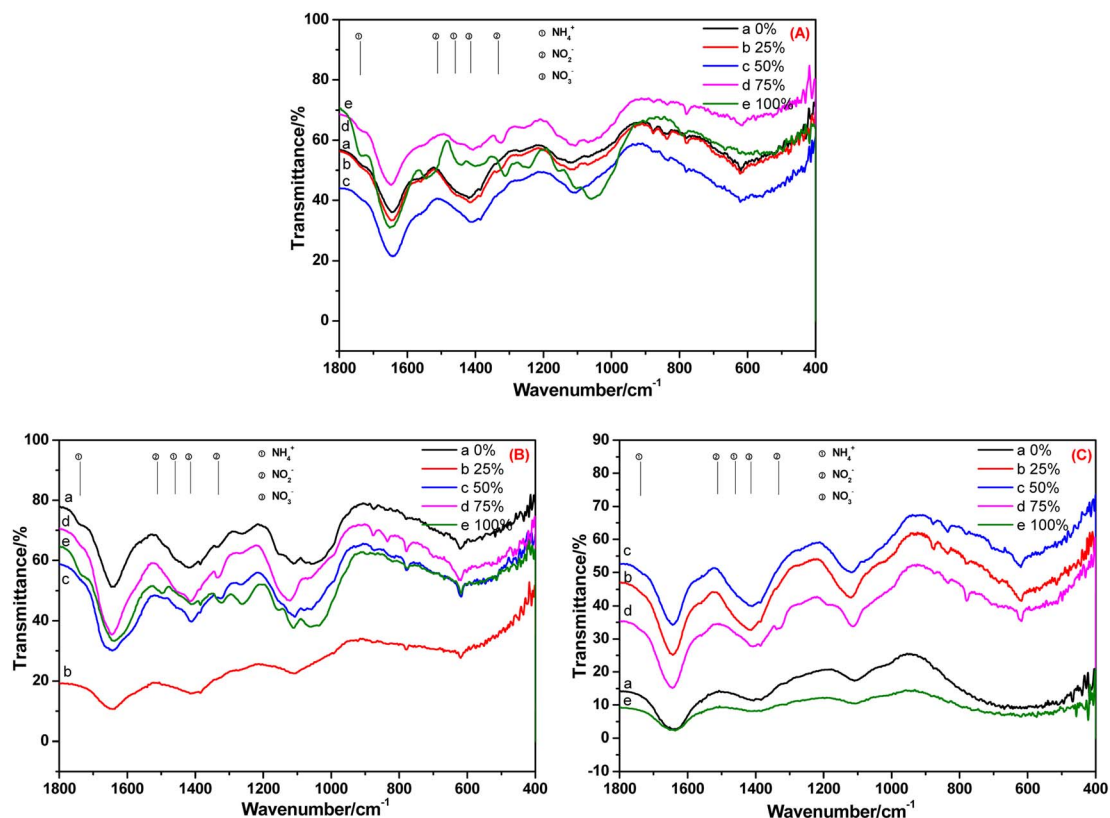


Fig. 6 FT-IR spectra of the root (A), stem (B) and leaf (C) of *Canna indica* cultivated in black and odorous water for 10 days.



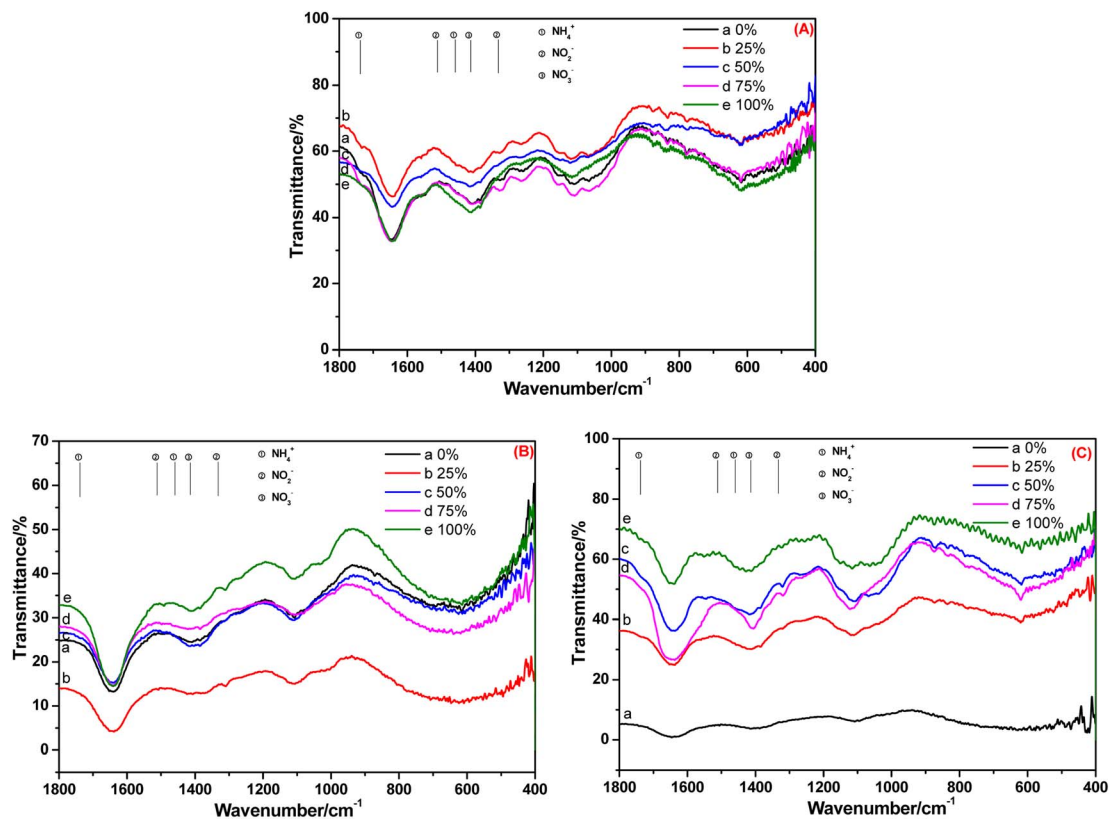


Fig. 7 FT-IR spectra of the (A) root, (B) stem and (C) leaf of *Canna indica* cultivated in black and odorous water for 20 days.

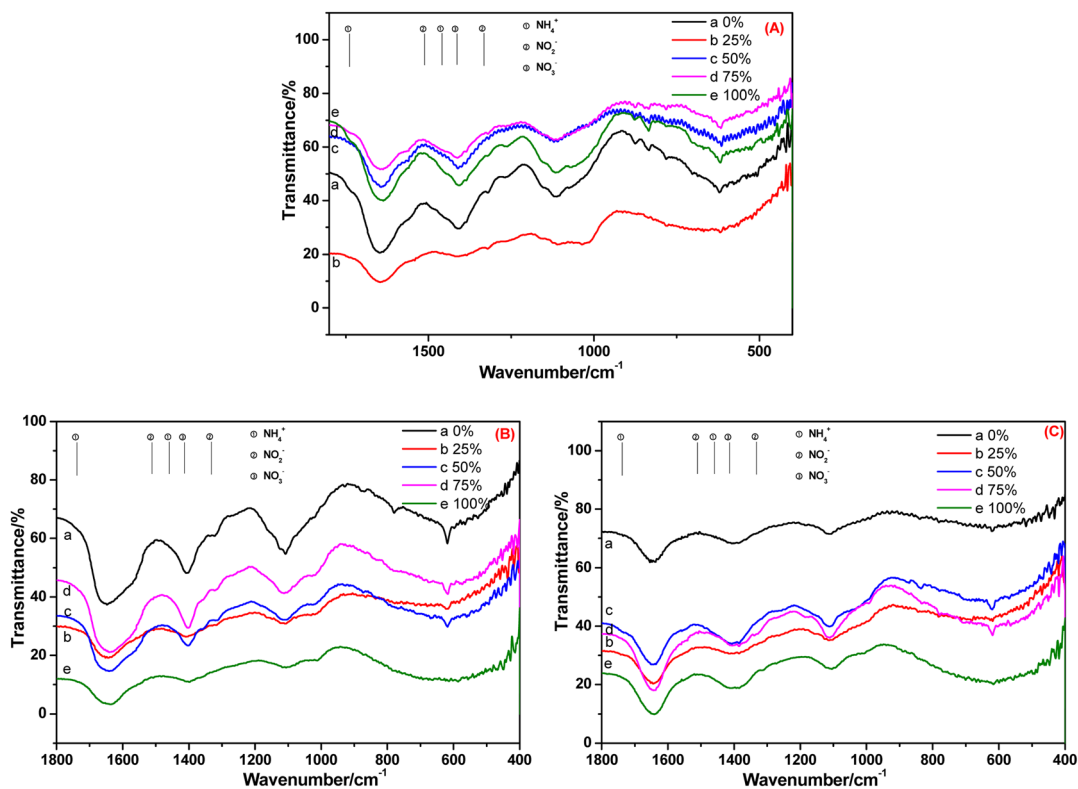


Fig. 8 FT-IR spectra of the root (A) and leaf (B) of *Eichhornia crassipes* cultivated in black and odorous water for 10 days and the leaf (C) cultivated for 20 days.



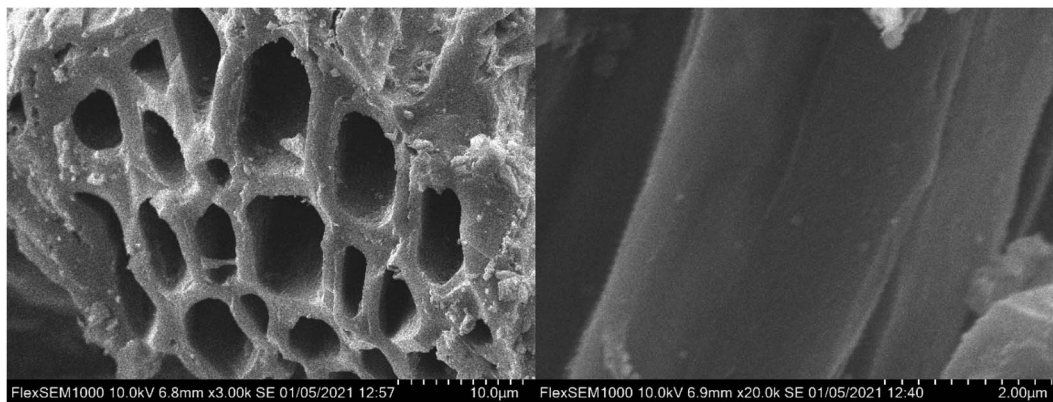


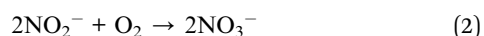
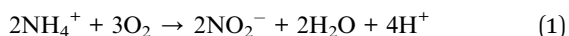
Fig. 9 Scanning electron micrographs of dehydrated *Canna indica*.

After 20 days of cultivation in BOW, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ appeared in the root of *C. indica* (Fig. 7A). However, only $\text{NO}_3^-\text{-N}$ was present in the stem and leaf: $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were absent (Fig. 7B and C). A possible reason was that because the cultivation time was longer, the absorption capacity was weakened, and $\text{NO}_2^-\text{-N}$ was transformed completely into $\text{NO}_3^-\text{-N}$, and the transformation rate of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ was greater than their migration rate.

The FT-IR spectra of root and leaf of *E. crassipes* cultivated in BOW for 10 days, and that of the leaf cultivated in BOW for 20 days, are shown in Fig. 8. The migration and transformation performance of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ in *E. crassipes* were analogous to those of *A. calamus* and *C. indica*. The difference was that the concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were very low, which may have been due to the weak absorption of *E. crassipes*.

The capillary structure of aquatic plants is important for the migration of ammonia nitrogen, nitrite and nitrate. Scanning electron micrographs (Fig. 9) revealed many capillary structures in *C. indica*, and the tube walls were smooth. Three types of inorganic nitrogen compounds migrate in macrophytes and are transformed in the order $\text{NH}_4^+\text{-N} \rightarrow \text{NO}_2^-\text{-N} \rightarrow \text{NO}_3^-\text{-N}$. There are many ammonia- and nitrite-oxidizing bacteria in macrophytes. They are responsible for the oxidation of ammonia (*i.e.*, ammonia nitrogen is oxidized to nitrite if sufficient oxygen is present) and nitrite (*i.e.*, nitrite is oxidized to nitrate by nitrite oxidoreductase). Both processes use oxygen as the electron-acceptor.

Our study is based on biological nitrification, and the conversion mechanism of ammonia nitrogen is shown in eqn (1) and (2).



The absorption performances of *A. calamus* and *C. indica* for $\text{NH}_4^+\text{-N}$ were more robust than those of *E. crassipes* because *C. indica* and *A. calamus* grew rapidly after they were moved into wastewater, their height increased significantly, and the root system extended swiftly. At the end of our experiment, some

roots extended into the bottom of the water tank. Also, the biomass accumulation of *C. indica* and *A. calamus* was greater than that of *E. crassipes*.

4 Conclusions

The migration process of $\text{NH}_4^+\text{-N}$ in *A. calamus*, *C. indica* and *E. crassipes* was detected *via* FT-IR spectroscopy. $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were transformed in the direction $\text{NH}_4^+\text{-N} \rightarrow \text{NO}_2^-\text{-N} \rightarrow \text{NO}_3^-\text{-N}$ from the root to stem and leaf. A multitude of ammonia- and nitrite-oxidizing bacteria are present in macrophytes, and they are responsible for ammonia oxidation and nitrite oxidation. The migration rate of $\text{NH}_4^+\text{-N}$ in *C. indica* was faster because of its regular and smooth capillaries. *A. calamus* and *C. indica* could absorb more $\text{NH}_4^+\text{-N}$ than *E. crassipes*. This was because *C. indica* and *A. calamus* grew rapidly upon transfer to wastewater, their height increased significantly, and the root system extended swiftly. The efficiency and quantity of transformation will be the focus of further research. Our study on the removal and transformation mechanism of ammonia nitrogen in BOW could be an important reference for other bodies of water.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest

There are no conflicts of interest to declare.

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