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Feasibility investigation of various leaves as carbon sources for biological denitrification

Bogo A-H. Orou Wagou[a], Junting Zhao[a], Gaigai Cao[a], Yunpeng Shen[b], Zhenjun Wu[a], Dongjin Wang[a], Qiaochong He[a,*]

[a]*School of Environmental Engineering, Henan University of Technology, Zhengzhou 450001, China, Tel. +86 10 67756039; email: qiaochonghe@haut.edu.cn (Q. He), Tel. +86 13027601750; email: haaqorouwagou@gmail.com (B. A-H. Orou Wagou), Tel. +86 18623717923; email: zjtyjk@126.com (J. Zhao), Tel. +86 18039571827; email: gaig2022@163.com (G. Cao), Tel. +86 13629841966; email: wuzhenjun@haut.edu.cn (Z. Wu), Tel. +86 18623718015; email: djwan@haut.edu.cn (D. Wan)*
[b]*School of Biological Engineering, Henan University of Technology, Zhengzhou 450001, China, Tel. +86 18623711885; email: shenswjs@haut.edu.cn (Y. Shen)*

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ABSTRACT

Nitrate (NO_3^-) pollution has a significant effect on aquatic ecology. This study aimed to remove NO_3^- from water using heterotrophic denitrification. Denitrification performance using three different leaves as carbon sources for heterotrophic denitrification were investigated in side-by side microcosm studies, including leaves of *Ginkgo biloba* (*Gb*, Gingko), *Firmiana simplex* (*Fs*, Sycamore), and *Cerasus serrulata* (*Cs*, Oriental cherry). Results showed that leaves can reduce and remove nitrate from water with various removal efficiencies at varying dosages for different species of leaves. Compared to gingko and sycamore, oriental cherry presented a higher denitrification rate. Furthermore, anaerobic digestion experiment for the leaves revealed that the biodegradability and bioavailable of oriental cherry was higher than those of gingko and sycamore. Therefore, oriental cherry was selected for a continuous experiment, in which the influence of hydraulic retention time (HRT) on NO_3^- removal was investigated. When the HRT was 6 h, NO_3^- was not completely removed, while NO_3^- was removed at HRT = 12 h, with no accumulation of NO_2^- or NH_4^+ even at low temperatures. Results demonstrated that oriental cherry can effectively reduce nitrate from water to the allowable range of water quality under the appropriate HRT. Therefore, oriental cherry leaf-mediated heterotrophic denitrification is a suitable denitrification process for NO_3^- removal from water.

Keywords: Nitrate removal; Leaves; Carbon sources; Heterotrophic denitrification; Feasibility investigation

1. Introduction

Nitrogen (N) is an essential component of living organisms [1,2]. Nitrate (NO_3^-) is one of the main elements, a stable and chemically unreactive species of nitrogen obtained through the natural nitrogen cycle. NO_3^- pollution of ground and surface water is a serious problem worldwide [3,4]. The main sources of NO_3^- in groundwater include industry, fertilizer, food processing, and human and various animal excreta. Excessive intake of NO_3^- can cause health problems in humans: NO_3^- converted into nitrite (NO_2^-), can lead to methemoglobin hematic disease [5]. In addition, long-term exposure to high NO_3^- levels in drinking water is a risk factor for several types of cancer including gastric, colorectal, bladder, urothelial, and brain tumors [6]. Therefore, a maximum level of 11.3 mg N/L

* Corresponding author.

in drinking water has been proposed by the World Health Organization [7]. The China National Standard for Drinking Water Quality (GB5749-2006) also limits the concentration of NO_3^- to below 10 mg N/L.

In residential area and farmland of China, the NO_3^- concentration in groundwater reached 162.83 and 75.71 mg/L, respectively [8]. The contamination of groundwater by NO_3^- is also a common phenomenon observed in some regions of the United States of America, Japan, and others developed countries [9,10]. Sharp increases in the NO_3^- concentration (59–215 mg N/L) were detected in groundwater in developing countries such as the Palestinian Authority and India [11,12]. Given the reported prevalence of NO_3^-–N pollution [13,14], its necessary to use effective processes to remove NO_3^-–N. Numerous advanced physicochemical and biological treatments have been recommended for extracting excessive NO_3^- from water. Compared with various physicochemical NO_3^- removal methods (e.g., adsorption, ion exchange, reverse osmosis), a biological method has the advantages of high efficiency, low cost, and transforming NO_3^- to a harmless substance (i.e., removal of organic and nitrogen rates), which is one of the most important application prospects of the method [15].

Microorganisms can reduce and degrade NO_3^- by using NO_3^- as an electron acceptor and adding inorganic or organic electron donors [16,17]. Heterotrophic denitrification, which requires an organic carbon source, is the most cost-effective method for treating NO_3^- pollution [18]. The denitrification rate is strongly affected by the type of carbon source. Natural materials such as oak woodchips and gravel [19], commercial plant-based compost and crushed palm tree leaves [20], baby-leaf lettuce, coffee wastes, tomato wastes and agro-industrial wastes [21,22], agri-food sludge: different fresh vegetable wastes (pepper waste, tomato waste and leek waste, and as bulking agents: vine shoot pruning, garlic stalks and avocado leaves) [23], wheat straw, cotton, poly (butylene succinate) and newspaper [24], banana peel [25], waste sludge [26], pine bark and spongy iron [27] have been developed as organic carbon sources for use in heterotrophic denitrification. The method was cost-effective; however, the pretreatment process was complicated and lengthy [25]. The concentration and nature of carbon source have an important impact on the rate, denitrification efficiency and development of biomass. The added carbon source must meet the conditions of low cost, non-toxic, easy storage and biomass production without microbial adaptation.

Leaves, a common agricultural waste product, usually contain lignocellulose compounds with high carbon nitrogen ratio or lignin content. These leaves wastes hinder and pollute the environment. On the other hand, because they are rich in carbon and secondary metabolites (lignin, polyphenols, saponins and anthraquinone) [28,29], they might be valuable resources for removing nitrate from water. The purpose of this study is to remove NO_3^- from polluted water by heterotrophic denitrification using leaves as carbon sources and electron donor. The objectives were as follows: (1) to test the leaching laws of chemical oxygen demand (COD), NO_3^-, NO_2^- and NH_4^+ of the selected solid carbon sources in the leaching experiment; (2) to assess the ability of these carbon sources to promote denitrification in microcosm studies; and (3) to investigate the denitrification performance supported by the optimal organic substrate in continuous study.

2. Materials and methods

Leaves are an important part of biosolid waste in the world. Among them, *Ginkgo biloba* (Gb, Gingko), *Firmiana simplex* (Fs, Sycamore), and *Cerasus serrulata* (Cs, Oriental cherry) are the most easily obtained. They are produced and readily available in schools, universities and public places, but they are not properly recycled. Therefore, these leaves were used as carbon sources in this study. The leaves were collected from the school yard of Henan University of Technology. The leaves were dried at 105°C for 30 min (ZXRD-A7230, Zhicheng, China), and then ground to powder using electric heating (SS-1022, Shengshun, Multi-Purpose High-Speed Disintegratop, Wuyi Haina Electric Co., Ltd. China) with constant temperature blast drying.

2.1. Synthetic NO_3^- polluted water

Synthetic water was prepared by adding 0.304 g/L $NaNO_3$ and 0.022 g/L KH_2PO_4 to tap water, obtaining NO_3^- polluted water with 50 mg N/L and an N/P of 10.

2.2. Activated sludge acclimation

Activated sludge for inoculation of denitrifying bacteria was collected from the Wulongkou Wastewater Treatment Plant (Zhengzhou, China) and acclimated in culture solution at (24°C ± 5°C) for one month. The culture solution was prepared by adding 0.1562 g CH_3COONa, 0.304 g $NaNO_3$, 0.022 g KH_2PO_4, and 1 mL trace element solution to 1 L tap water. The trace element solution contained: 0.05 g/L H_3BO_3, 2.72 g/L $FeCl_3{\cdot}6H_2O$, 0.05 g/L $ZnCl_2{\cdot}H_2O$, 0.11 g/L $CuCl_2{\cdot}6H_2O$, 0.49 g/L $MnCl_2{\cdot}4H_2O$, 0.11 g/L $NiCl_2{\cdot}6H_2O$, and 2 g/L $CoCl_2{\cdot}6H_2O$ in 1 L of pure water. The culture solution was replaced every 2 d. After acclimation, the mixed-liquid suspended solids (MLSS) and, mixed liquor volatile suspended solids (MLVSS) were 3,869.33 ± 132.32 g/mL and 1,582.67 ± 32.33 g/mL, respectively.

2.3. Experiment procedure

As listed in Table 1, five stages were carried out in this study, including leaching experiment, three microcosm studies and a continuous experiment.

2.3.1. Leaching experiment

A leaching experiment was conducted to reveal the COD leaching behavior of each selected leaf. Ginkgo, sycamore, and oriental cherry leaves were obtained by drying, grinding, and screening. Only particle size fractions of 1–2 mm (standard test sieve, GB/T6003.1-2012) were used in this study. The three leaves (1 g gingko, 1 g sycamore, and 1 g oriental cherry) were added into separate 1 L glass bottles containing 900 mL deionized water. Each leaf was tested in duplicate. After sterilization, all glass bottles were sealed and shaken manually. Each flask was sealed with a

Table 1
Experiment procedure in this study

Stages	Experiments		Aims
I	Leaching experiment		To reveal COD leaching behavior of each selected leaf
II	Microcosm studies	Denitrification performance at 1 g dosage	To assess denitrification performance of different leaves as carbon sources at different dosages
III		Denitrification performance at 2 g dosage	
IV		Denitrification performance for digested liquid and leaves	To evaluate the biodegradability and bioavailable of different leaves as carbon source for denitrification
V	Continuous experiment		To investigate the denitrification performance of oriental cherry leaves for treating NO_3^- polluted water

glass stopper, and the experiment was conducted at room temperature (28°C ± 7°C) for 2 weeks.

2.3.2. Microcosm studies

Leaves of 1–2 mm pieces were set up in a 1 L glass bottle containing 900 mL of synthetic NO_3^- polluted water. Gingko, sycamore, and oriental cherry leaves were individually used as the carbon source in the microcosm studies, and each leaf was tested in duplicate.

2.3.2.1. Dried leaves at 1 g

Each original leaf (1 g, in duplicate) was put in a 1 L glass bottle containing 900 mL of synthetic NO_3^- polluted water, and 5 mL of activated sludge was directly added to each of bottle. All glass bottles were sealed and shaken by hand. Each flask was sealed with a glass stopper, and the experiments were conducted at room temperature (26°C ± 8°C) for 25 d.

2.3.2.2. Dried leaves at 2 g

The experiments were conducted as above with, 2 g of leaf added to six glass bottles. The experiments were conducted at room temperature (25°C ± 6°C) for 10 d.

2.3.2.3. Digested liquid and leaves preparation

To investigate the degradation of the leaves, a digestion experiment was carried out to obtain digested liquid and leaves. Leaves (20 g) were added to a 1 L glass bottle containing 1 L deionized water, and the bottles were well sealed to start the digestion. After 4 months of anaerobic digestion, the liquid and digested leaves were separated by hand. The digested leaves were dried for the microcosm study. Based on the COD concentration in the digested liquid, different doses of liquid were collected to make the initial COD concentration the same in the denitrification microcosm study. For the liquid gingko and sycamore leaves, each glass bottle was filled with 400 mL digested solution and 500 mL synthetic NO_3^- polluted water. For the liquid oriental cherry leaves, the glass bottle was filled with 80 mL digested solution and 820 mL synthetic NO_3^- polluted water. In addition, three glass bottles were set up to investigate the denitrification performance using digested leaves. Each digested leaf (2 g) was added to a bottle containing 900 mL synthetic NO_3^- polluted water, and then 5 mL activated sludge was added. All glass bottles were sealed and shaken by hand. Each flask was sealed with a glass stopper, and the experiments were conducted at room temperature (19°C ± 7°C) for 10 d.

2.3.3. Continuous experiment

After a microcosm denitrification study using various leaves as carbon sources NO_3^- removal from water, oriental cherry leaf showed good denitrification performance and was selected to continuous experiment. A continuous study was conducted to investigate the denitrification performance of oriental cherry leaves for treating NO_3^- polluted water. Two glass columns with a volume of 650 mL (5 cm × 30 cm) were employed as bioreactors. Oriental cherry leaf (100 g), red volcanic stone (35 g), and activated sludge (250 mL) were directly added to both of the columns. The effective volume of each bioreactor was 350 mL. Each reactor was continuously fed with synthetic water with a NO_3^- concentration of 50 mg NO_3^-–N/L and an of N/P ratio of 10. The influence of hydraulic retention time (HRT) on the NO_3^- removal rate was also studied. A continuous experiment was carried out to establish and evaluate the system's feasibility and stability. Both experiments were conducted at room temperature (20°C ± 3°C and 21°C ± 7°C, respectively, in the day, below 10°C at night) for 18 d (HRT = 6 h) and 6 d (HRT = 12 h), respectively.

2.4. Analytical methods

Glass bottles were left on a stand for 30 min before sampling to allow the solution to settle. Supernatant (8 mL) was taken periodically removed from each glass bottle for analysis of NO_3^-–N, NO_2^-–N, NH_4^+–N, and COD. Before the analysis, water samples were directly filtered through the 0.45 μm membrane. HACH DRB 200 and HACH DR 900 were used to determine the COD concentration. NO_3^-–N, NO_2^-–N, and NH_4^+–N concentrations were measured by a UV/VIS spectrophotometer (UV-5900 PC METASH) using the Water and Wastewater Monitoring Analysis Method [30].

3. Results and discussion

3.1. Leaching experiment

The variation in COD concentration over time was of crucial for investigating the leaching behavior of carbon sources. As shown in Fig. 1a, the COD concentration in the gingko, sycamore, and oriental cherry leaf reactors increased over time. The initial COD concentration was the highest, at 1,055.1; 1,078.1 and 1,185.4 mg/L for the different reactors, respectively, and was observed for 0.5–2 d. The concentration (1,185.4 mg/L) increased in COD might be attributed to organic matter released from solid particles contained in the oriental cherry leaf reactor. Zhang et al. [31] also found that the COD release rates of solid carbon sources were higher at the beginning of leaching experiments. After the first day, COD concentrations in gingko, sycamore, and oriental cherry leaf reactors fluctuated over time. COD concentrations varied from 500.7 to 1,055.1 mg/L in gingko, 470.02 to 1,078.1 mg/L in sycamore and 625.3 to 1,185.4 mg/L in oriental cherry leaf reactors. The COD average concentration was 900 mg/L.

Fig. 1b shows that NO_3^- concentrations in gingko, sycamore, and oriental cherry leaf reactors increased on the first day. Furthermore, NO_3^- concentrations in different leaf reactors fluctuated during the experiment and they ranged from 0.06 to 0.27 mg N/L. Leaves contains nitrogen, and the nitrogen was transformed to NO_3^- by microorganism. Microorganism were inputted in the reactors with the addition of leaves due to the incomplete disinfection leaves in the leaching experiment. Subsequently, the NO_3^- was reduced by denitrifying bacteria, resulting in the decrease in NO_3^- concentration.

As shown in Fig. 1c, NO_2^- concentrations in gingko, sycamore, and oriental cherry leaf reactors decreased on the first day. NO_2^- concentrations in the different leaf reactors fluctuated over time because of NO_2^- accumulation. The concentration of NO_2^- ranged from 0.12 to 0.21 mg N/L. Fig. 1d shows that NH_4^+ concentrations in gingko, sycamore, and oriental cherry leaf reactors decreased on the first day. Furthermore, NH_4^+ concentrations in the different leaf reactors fluctuated over time because of NH_4^+ accumulation. The concentration of NH_4^+ ranged from 0.02 to 1.29 mg N/L. As mentioned before, the leaves added in the reactors were not completely sterilized. Thus, bacteria were also inputted in the reactors. Nitrogen was released from the leaves and transformed to NH_4^+, followed by NO_2^- and then NO_3^-. This was the reason why the NH_4^+ increased and decreased.

3.2. Microcosm studies

3.2.1. Denitrification performance at 1 g dosage

3.2.1.1. NO_3^- removal

As shown in Fig. 2a, NO_3^- concentrations using gingko, oriental cherry, and sycamore leaves as carbon sources decreased from 54.37 to 1.73 mg N/L, 50.42 to 8.88 mg N/L, and 55.47 to 42.28 mg N/L, respectively over time. The NO_3^- removal efficiency reached almost 100% after 25 d in the gingko leaf reactor, indicating that this reactor had a higher NO_3^- removal rate than oriental cherry (90%) and sycamore leaves (57%) reactors. Low removal performance using sycamore leaves was because the sycamore leaves contained high amounts of lignin, which has an adverse effect on its degradability and microbial activity [32,33]. A lack of an available carbon source for denitrification would lead to inadequate NO_3^- removal [26,34].

As shown in Fig. 2d, COD concentrations in gingko, sycamore, and oriental cherry leave reactors fluctuated during all experiments. Furthermore, the initial COD concentration was the highest in the gingko leaf reactor, at 1,124.06 mg/L, which was observed for 7 d. Even with high COD release for all type of leaves (~1,000 mg/L), the denitrification performance was different. This was due to that the bioavailability of organic matters released from different leaves, which was found in the below anerobic digestion experiment.

3.2.1.2. NO_2^- accumulation

The NO_2^- concentration was typically between 0.15 and 9.66 mg N/L, as shown in Fig. 2b. In all reactors, the NO_2^- concentrations increased in 2 d. In contrast, NO_2^- concentrations remained almost constant over time for 25 d. While gingko and oriental cherry leaf concentrations decreased after 2 d, at the end of the experiment, the NO_2^- concentration was 0.15 mg N/L for gingko, 0.1 mg N/L for sycamore, and 3.26 mg N/L for oriental cherry leaf reactors. NO_3^- was first reduced to NO_2^-, resulting in the coexistence of NO_3^- and NO_2^- in the system. Furthermore, bacteria preferentially used NO_3^- when NO_3^- and NO_2^- were both available, because NO_2^- reductase was enzymatically inhibited by high NO_3^- concentration, leading to rapid NO_2^- accumulation during the denitrification process [25,35].

3.2.1.3. NH_4^+ accumulation

Fig. 2c shows that NH_4^+ concentrations in gingko, sycamore, and oriental cherry leaf reactors fluctuated over time because of NH_4^+ accumulation. The accumulation of NH_4^+ in the system may be due to the reduced activity of microorganisms and the decrease in nutrients, which led to an increase in decaying cells, consistent with the slowing trend of denitrification. Moreover, it may also lead to the accumulation of intermediate NH_4^+ products, which are more toxic and highly reductive.

3.2.2. Denitrification performance at 2 g dosage

3.2.2.1. NO_3^- removal

As shown in Fig. 3a, NO_3^- concentrations using gingko, oriental cherry and sycamore leaves as carbon sources decreased from 58.88 mg N/L to below the detection limit, 56.13 to 2.5 mg N/L and 59.65 to 42.83 mg N/L, respectively over time. The NO_3^- removal efficiency for sycamore, oriental cherry, and gingko leave reactors reached 55%, 97%, and 100%, respectively, at the end of the experiment. The higher NO_3^- removal efficiency than previous microcosm study at 1 g dosage was due to the higher leaf dose, which guaranteed sufficient organic matter for denitrification.

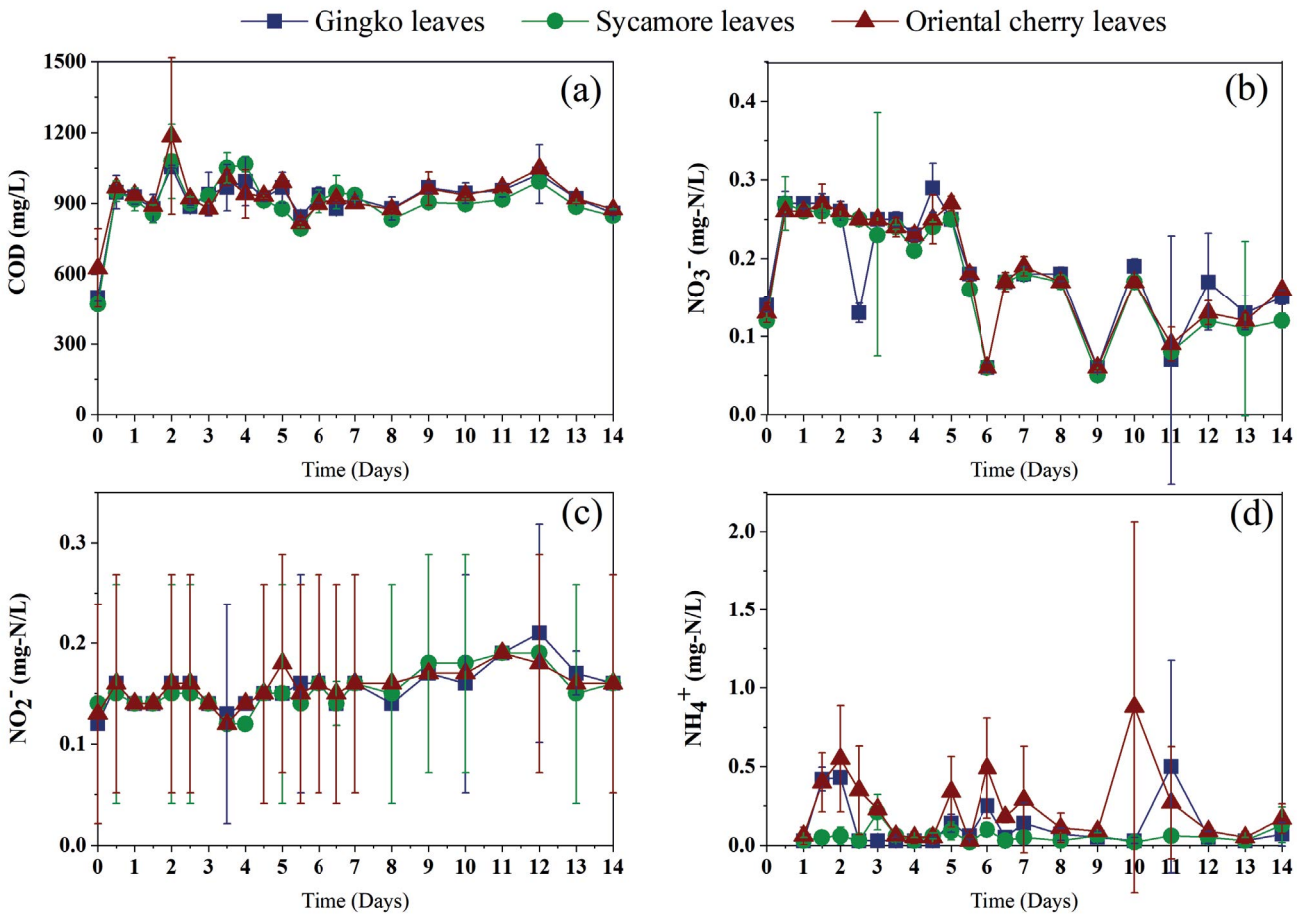

Fig. 1. Changes in (a) COD, (b) NO_3^-, (c) NO_2^- and (d) NH_4^+ concentrations in the leaching experiment.

3.2.2.2. Accumulations of NO_2^- and NH_4^+

As shown in Fig. 3b, NO_2^- concentrations increased in the gingko and oriental leaf reactors on the first day and 4 d, respectively. Furthermore, NO_2^- concentrations decreased over time in the gingko and oriental cherry leaf reactors. The NO_2^- concentration in the sycamore leaf reactor remained almost constant over time. At the end of the experiment, the NO_2^- concentration was 0.13 mg N/L for gingko, 0.82 mg N/L for sycamore, and 0.11 mg N/L for oriental cherry leaf reactors. Furthermore, Fig. 3b, shows that, the peak of gingko, sycamore, and oriental cherry leaves in NO_2^- was 8.82, 1.49, and 13.91 mg N/L, respectively. The reduction in NO_2^- concentration was due to the addition of leaves and exponential degradation. This was because NO_2^- is an intermediate product of denitrification [35]. Furthermore, a relatively high concentration of residual NO_3^- at the beginning of the experiment could inhibit the synthesis and activity of NO_2^- reductase. Therefore, NO_2^- concentration increased with the initial decrease in NO_3^- concentration [36]. Subsequently, a low concentration of residual NO_3^- reduced inhibition of NO_2^- reductase, and the NO_2^- concentration then decreased with time [37]. Brief NO_2^- accumulation was also observed in other denitrification processes. For instance, a visible accumulation of NO_2^- along with a decrease in NO_3^- occurred in the study by [38], because NO_2^- reductase was enzymatically inhibited by a high NO_3^- concentration.

As shown in Fig. 3c, NH_4^+ concentrations in gingko, sycamore, and oriental cherry leaf reactors fluctuated over time because of NH_4^+ accumulation and releasing from leaves. Interestingly, although the initial NH_4^+ concentration was higher in all reactors (5.01 mg N/L for gingko, 5.41 mg N/L for sycamore, and 15.74 mg N/L for oriental cherry) on the first day. The NH_4^+ concentration reached a lower level (1.9 mg N/L for gingko, 1.23 mg N/L for sycamore, and 5.94 mg N/L for oriental cherry) in reactors on the 10 d. The first increase in NH_4^+ was speculated to be the occurrence of slow leaf degradation in the solution. Protein degradation can produce NH_4^+ via ammonification [34]. The decrease in NH_4^+ concentration was mainly attributed to the bio-transformation by microorganisms in the reactors.

3.2.2.3. Change in COD

As shown in Fig. 3d, COD concentrations in gingko, sycamore, and oriental cherry leaf reactors fluctuated during all experiments. Furthermore, the initial COD concentration was the highest in the oriental cherry leaf reactors, at 2,359.68 mg/L, and was observed for 6 d. The increase in COD was due to organic matter releasing and exponential

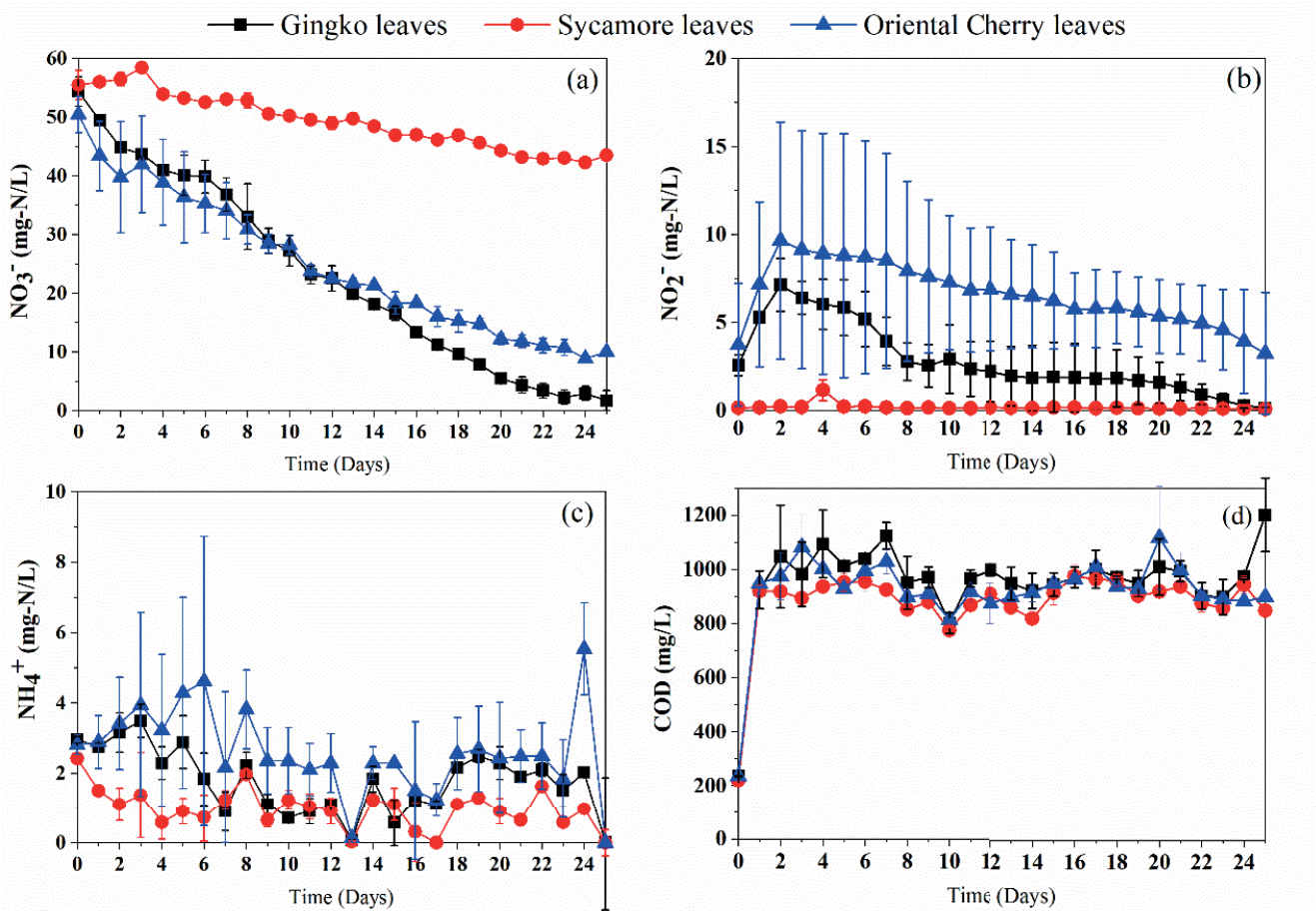

Fig. 2. Changes in (a) NO_3^-, (b) NO_2^-, (c) NH_4^+ and (d) COD concentrations in the microcosm study at 1 g dosage.

degradation. Furthermore, COD in the reactor was not only used for respiration, but also for cell growth and maintenance [17,26].

3.2.3. Denitrification performance for digested liquid and leaves

3.2.3.1. NO_3^- removal

The denitrification mechanism in different liquid leaf-based bioreactors was investigated to evaluated the bioavailability of organic matters released from leaves. As shown in Fig. 4a, NO_3^- concentration at each reactor decreased during the first day. In particular, NO_3^- concentration at liquid oriental cherry, gingko and sycamore leaf reactors decreased significantly and reached below detection limit, 53.27 and 61.85 mg N/L at digested liquid of oriental cherry, gingko and liquid sycamore leaves on day 1, respectively, while NO_3^- concentration at digested leaves of oriental cherry, gingko and sycamore decreased slightly and reached 42.28, 55.69 and 58.99 mg N/L at digested oriental cherry, gingko and sycamore leaves on day 1, respectively. The maximum NO_3^- removal efficiency was 100%, 38.15%, 46.73%, 57.72%, 44.31% and 41.01% for digested liquid – oriental cherry, sycamore, gingko leaves, digested leaves of oriental cherry, gingko and sycamore, respectively. The results indicated that oriental cherry leaves could be used as carbon source for denitrification.

3.2.3.2. Accumulations of NO_2^- and NH_4^+

Fig. 4b shows that NO_2^- concentrations for digested liquid of gingko, sycamore, and oriental cherry leaf reactors increased from 0.29 to 0.53 mg N/L in 5 d, 0.22 to 0.53 mg N/L on the first day, and 0.29 to 2.69 mg N/L in 6 d, respectively. In contrast, NO_2^- concentrations decreased for liquid gingko and oriental cherry leaf reactors from 2.75 to 2.6 mg N/L in 2 d and from 1.69 to 0.08 mg N/L in 3 d, respectively. Meanwhile, NO_2^- concentrations in digested gingko and sycamore leaf reactors increased from 0.19 to 0.22 mg N/L and 0.19 to 0.66 mg N/L on the first day, respectively. The NO_2^- concentration in digested oriental cherry leaf reactors increased from 0.32 to 2.08 mg N/L in just 5 d. Due to NO_2^- accumulation, NO_2^- concentrations using digested gingko and sycamore leaf reactors fluctuated over time, while, after 5 d, NO_2^- in digested oriental cherry leaf reactors decreased from 2.08 to 1.94 mg N/L over time. The initial high concentration in the digested liquid (2 mg N/L) was due to the digestion of leaves for 4 months before the denitrification experiment. Furthermore, compared with Fig. 4a, it could be seen that the peak in

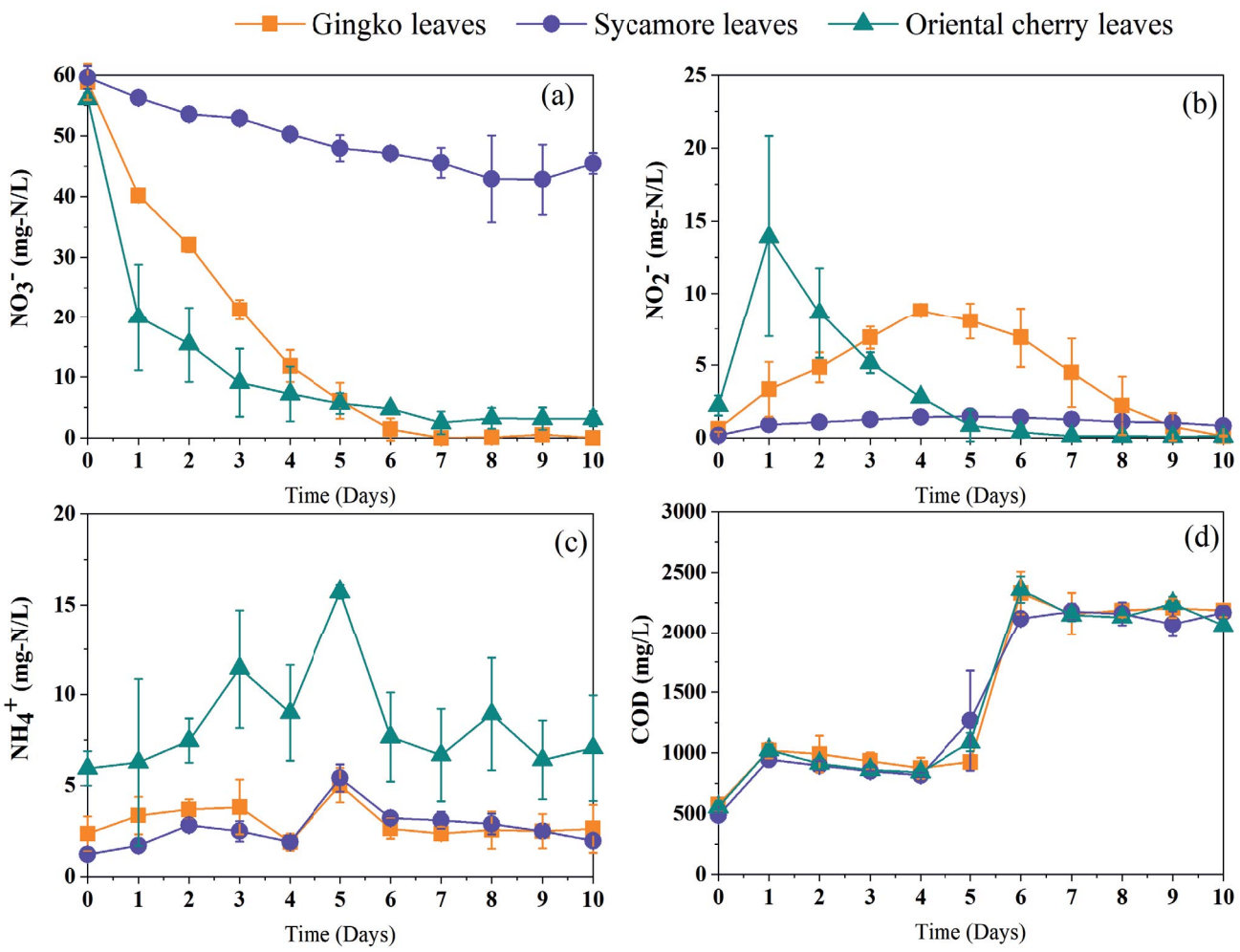

Fig. 3. Changes in (a) NO_3^-, (b) NO_2^-, (c) NH_4^+ and (d) COD concentrations in the microcosm study at 2 g dosage.

NO_2^- (digested sycamore leaves) almost corresponded with the minimum value of NO_3^-, which was due to that NO_2^- was an intermediate product of denitrification [35]. Afterward, NO_2^- concentration at liquid oriental cherry and digested sycamore leaf reactors decreased with time and then remained almost constant (below 0.11 mg N/L, respectively) at the end of experiments.

Fig. 4c shows that NH_4^+ concentrations increased for liquid gingko, sycamore, and oriental cherry leaf reactors, from 19.72 to 20.78 mg N/L in 2 d, 10.05 to 10.84 mg N/L, and 3.29 to 3.82 mg N/L on the first day, respectively. After the first day, NH_4^+ for liquid gingko, sycamore, and oriental cherry leaf reactors fluctuated over time. The NH_4^+ concentrations for digested gingko, oriental cherry, and sycamore leaf reactors decreased from 1.96 to 0 mg N/L and 2.36 to 0 mg N/L on the first day and from 1.83 to 0 mg N/L in 3 d, respectively. The results indicated that the more leaves contained, the higher the NH_4^+ concentration reached. The first increase in NH_4^+ concentration was speculated to be the occurrence of DNRA and ammonification of protein in the solution, and the decrease in NH_4^+ concentration was mainly attributed to ammonia oxidizing bacteria (AOB). The second increase in NH_4^+ concentration was ascribed to protein contained in leaves in the reactors.

3.2.3.3. Change in COD

Fig. 4d shows that COD concentrations in liquid gingko and sycamore leaf reactors increased from 362.21 to 374.68 mg/L and 53.36 to 87.67 mg/L in 2 d, respectively. In contrast, the COD concentration in oriental cherry leaf reactors decreased from 474.51 to 121.99 mg/L in 3 d. After 2 and 3 d, COD concentrations using liquid gingko sycamore and oriental cherry leaf reactors fluctuated over time. As shown in Fig. 4d, COD concentrations for digested gingko, sycamore, and oriental cherry leaf reactors increased from 0.32 to 187.5 mg/L, 0.32 to 112.63 mg/L, and 3.44 to 56.48 mg/L in 2 d, respectively. After 2 d, COD concentrations in digested gingko sycamore and oriental cherry leaf reactors fluctuated over time. The COD concentration decreased significantly at liquid oriental cherry leaf rectors during 3, 7 and 10 d, showing that organic matters in the solution were utilized by denitrifying bacteria during this period. COD concentration remained

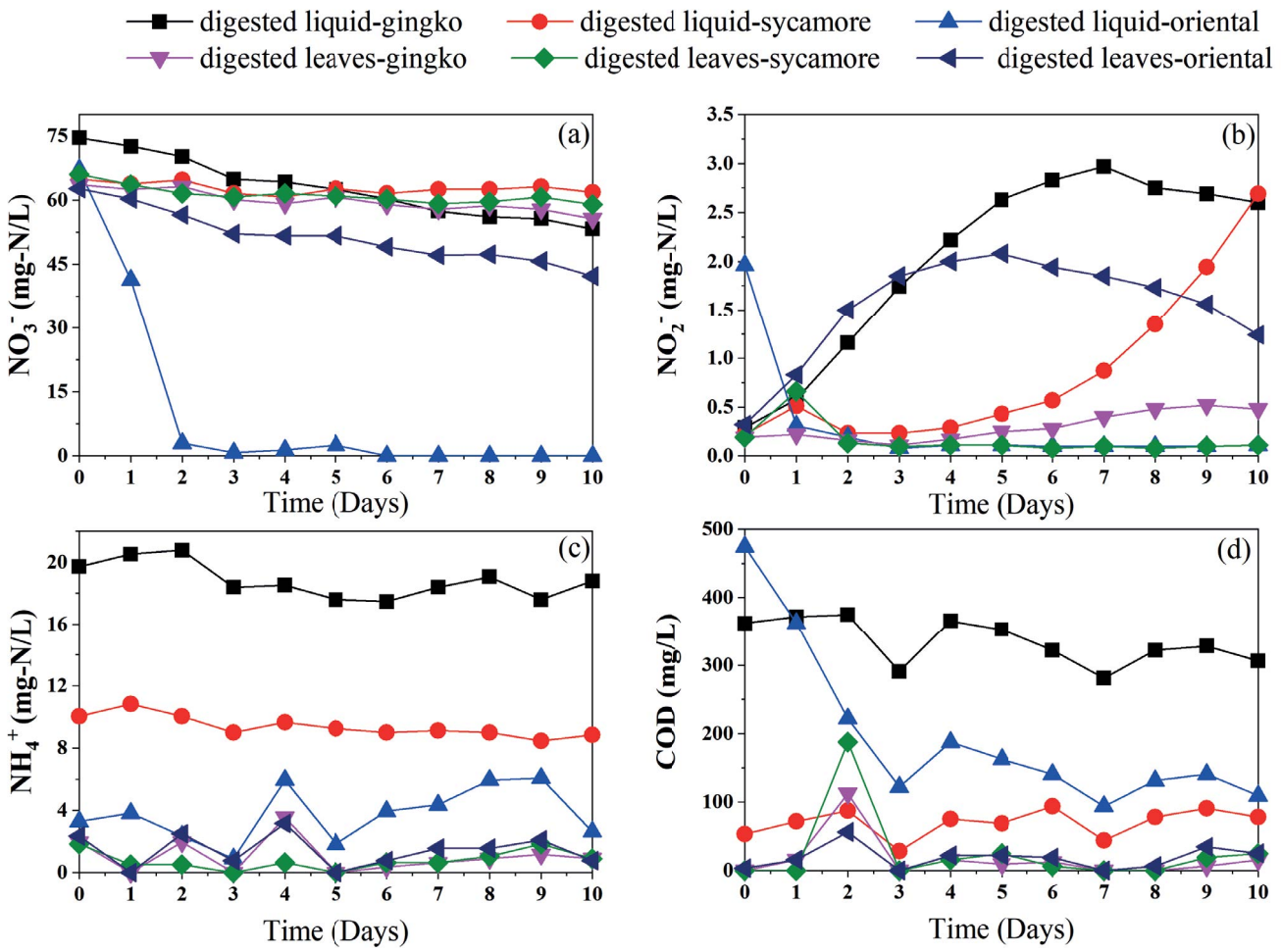

Fig. 4. Changes in (a) NO_3^-, (b) NO_2^-, (c) NH_4^+ and (d) COD concentrations in the microcosm study using digested liquid and leaves as carbon sources.

around 15.9 mg/L at digested gingko leaf reactors. And then, at respectively 25.3 mg/L digested sycamore and oriental leaf reactors, and 78.3 mg/L at liquid sycamore leaf reactors at the end experiments, suggesting that the organic matters were efficiently used by microorganisms. On the other reactors, COD concentration at liquid gingko leaves (306.1 mg/L) was high even at the end of experiments. Furthermore, although initial solution COD concentration in the reactor at liquid gingko leaves was similar to that at sycamore leaves, the denitrification performance of the former was more excellent than that of the later, implying that solid particles contained in reactors played an important role in biological denitrification, namely, they might act as bacteria carriers and solid carbon sources beneficial for the denitrifying bacteria growth.

3.3. Continuous study

Microcosm studies demonstrated that oriental cherry leaves could be an effective electron donor for denitrification. To investigate its NO_3^- removal performance in practical, a continuous study was carried at hydraulic retention times (HRT) of 6 and 12 h. As shown in Fig. 5a, oriental cherry leaves were degraded in two glass columns. The NO_3^- concentration in the influent was 64.04 mg N/L on the first day. NO_3^- concentrations in influent remained constant (10°C) over time at HRT = 6 and 12 h. Bacterial growth affected the denitrification performance. Bacterial growth was adapted to the environment during the experiment and NO_3^- concentration decreased from 52.5 to 11.4 mg N/L. After 6 d, NO_3^- concentrations in effluent increased from 14.26 to 29.32 mg N/L until day 15 because the temperature was too low.

NO_3^- concentrations in effluent remained constant (10°C) for days 15–18. To achieve complete NO_3^- removal at a low temperature, HRT was extended to 12 h, and the NO_3^- concentrations decreased from 0.3 to 0.08 mg N/L. There is a correlation between HRT and the amount of NO_3^- removed: the longer the HRT, the more NO_3^- is removed [15,17,19]. HRT and temperature are the main factors in determining NO_3^- removal efficiency, with a positive relationship observed between HRT and NO_3^- removal efficiency [39].

As shown in Fig. 5b, the NO_2^- concentration in the effluent was 21.38 mg N/L on the first day, and it decreased from 21.38 to 0.11 mg N/L during days 0–5. High NO_2^- accumulation has also been linked to short HRT [39,40]. NO_2^- was

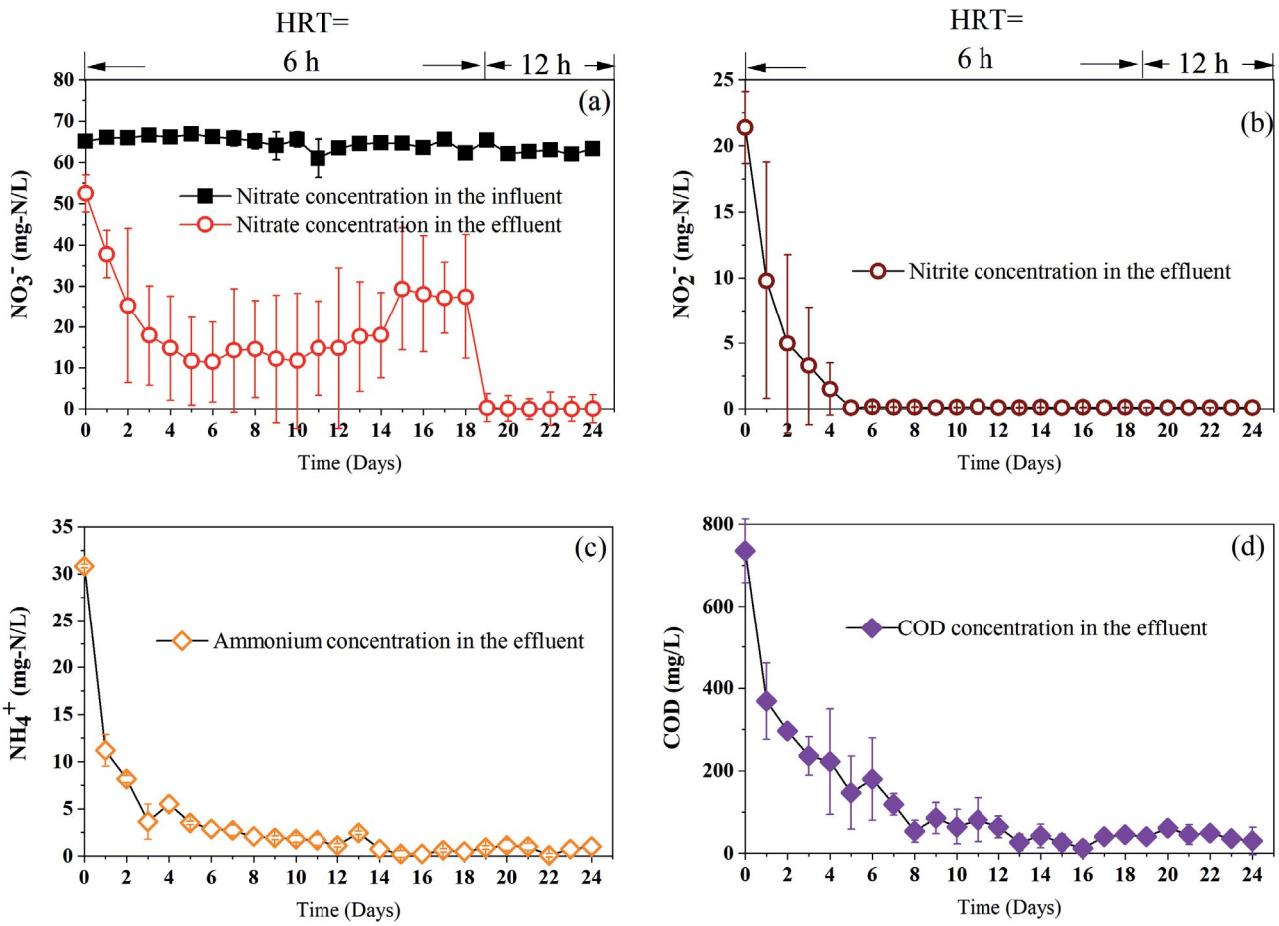

Fig. 5. Changes in (a) NO_3^-, (b) NO_2^-, (c) NH_4^+ and (d) COD concentrations in continuous study.

also detected in the effluent of other studies. The highest NO_2^- effluent concentration achieved by each media was 3.9, 1.94, 0.32, and 0.11 mg N/L for barley straw, wood chips, corn stover, and corn cobs, respectively [41]. After 5 d, NO_2^- concentrations remained constant (at 10°C) over time. When the HRT was changed to 12 h, the NO_2^- concentrations in the effluent remained almost constant over time. HRT and temperature had an impact on the denitrification performance during the acclimation period, determining NO_2^- accumulation [39,42].

Fig. 5c shows that the NH_4^+ concentration in the effluent was 30.83 mg N/L on the first day. The high concentration was due to nitrogen released from leaves. At 6 h HRT, NH_4^+ concentrations in effluent decreased from 30.83 to 3.66 mg N/L. After 4 d, NH_4^+ concentrations decreased from 5.52 to 1.14 mg N/L. NH_4^+ concentrations then decreased from 2.46 to 0.48 mg N/L over time due to the slow release of leaves. HRT was then changed to 12 h, and the NH_4^+ concentrations decreased from 1.28 mg N/L to below the detection limit over time. In the continuous study, NH_4^+ accumulation in the effluent in the leaves reactors is similar to in the rice washing drainage system [43,44] and the wheat straw and sawdust systems [31].

As shown in Fig. 5d, the COD concentration in the effluent was 735.01 mg/L on the first day. COD concentrations were around 50 mg/L after 8 d of operation at an HRT of 6 h. HRT was then changed to 12 h, and the COD concentrations ranged from 24.27 to 6.62 mg/L. COD concentration decreased significantly at each HRT showing that organic matters in the solution were utilized by denitrifying bacteria during the period. The results showed that oriental cherry leaves can be used as a carbon source for heterotrophic denitrification.

3.4. Applicability

Lignocellulosic substances, such as wood biomass, agricultural waste (barley/bagasse/banana peel/corn straw, and wheat), and various cellulose wastes (municipal solid waste, pulp, and plant wood waste), can be used to effectively reduce NO_3^-. The natural carbon sources of biomass are not only the "hotbed" for microbial growth in the denitrification process but can also produce energy and fertilizers as well as eliminate NO_3^- containing water for microbial growth [25,35].

In the leaves-filled bioreactor, organic matter degradation starts with a leaching process, followed by a hydrolysis phase, characterized by the breakdown of the released macromolecules into simpler compounds. Afterwards, the denitrifying bacteria use the simpler compounds as

electron donor to reduce NO_3^- to nitrogen gas, achieving effective NO_3^- removal from water. Dissolved organic carbon plays an important role in the denitrification process. There are two sources for the dissolution of organic carbon: being directly dissolved at the beginning and gradually hydrolyzed by bacteria as the reaction progresses [33]. Adding the appropriate dosage of leaves to the bioreactor increases the NO_3^- removal rate, which can improve the cost-effectiveness of the method. These three varieties of leaves are difficult for bacteria to degrade, which can lead to a decrease in denitrification over time. However, the organic matter released from leaves can be degraded and then used by heterotrophic bacteria for NO_3^- reduction when the contact time was long enough. In addition, the production and accumulation of NO_2^- are negligible due to the presence of major and trace elements.

4. Conclusions

This study evaluated the performance of different leaves as alternative organic carbon sources for denitrification bioreactors. Three denitrification carbon sources were studied: gingko, sycamore, and oriental cherry leaves. Using oriental cherry leaves completely removed NO_3^- from ~50 mg N/L to below the detection limit. At 12 h HRT, the oriental cherry leaf denitrification system had the advantages of stability, high efficiency, and few by-products. Leaves are a suitable carbon source as they are inexpensive, readily available, and can maintain a stable and high NO_3^- reduction rate (if the HRT/flow rate through the bioreactor is appropriate). To optimize the denitrifying bioreactor for the specific conditions, the particle size may be adjusted to obtain an appropriate HRT. More cost-effective leaves as carbon sources should be developed in the future.

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