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Nitrogenous compounds removal from recalcitrant wastewaters using biofilms on filamentous bamboo

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ABSTRACT

Two biofilm reactors, one based on the use of filamentous bamboo (bioreactor with filamentous bamboo, (BFB)) and the other on the use of filamentous plastics (bioreactor with filamentous plastics, (BFP)), were used to remove nitrogenous compounds from recalcitrant wastewater that was characterized by a high organic load and a low C/N ratio. The BFP was used as a control bioreactor. Experimental results indicated that when influent COD (chemical oxygen demand associated with a $K_2Cr_2O_4$ oxidizer) concentrations were in the range of 804–5100 mg/L and BOD_5 maintained at 45.0–1100 mg/L, a total nitrogen (TN) removal rate of 43.06–96.70% and 28.6–82.66% for BFB corresponded to COD levels of 98–251 mg/L and a BOD/TN value of 0.51–1.87, but the corresponding TN rate was only 4.86–39.43% and 3.46–48.72% for BFP. The removal efficiency of nitrogenous compounds of the BFB was thus much higher than that of the BFP. Experimental results indicated that effective denitrification had been achieved, due to the use of filamentous bamboo as a means for removing biological nitrogenous compounds, a system that is suitable for the treatment of high-concentration recalcitrant wastewater in which the C/N ratio is low. The distribution characteristics of the main bacteria indicated that the total bacterial count at the inlet and outlet was roughly the same during the wastewater treatment. Bacteria and nitrifying bacteria were distributed near the outlet, while denitrifying bacteria was evenly distributed in the reactor.

Keywords: Filamentous bamboo; Biocarriers; Removal of nitrogenous compounds; Biodegradability; Most probable number; Nitrifying bacteria; Denitrifying bacteria

1. Introduction

Simultaneous nitrification and denitrification (SND) processes are widely used for the large-scale transformation of ammonia nitrogen (NH_4^+-N) into gaseous nitrogen within a single reactor [1,2]. SND applications have also been used for the removal of nitrogenous compounds. This process, which encompasses SND procedures, is dependent on the establishment of simultaneous aerobic and anoxic environments within a reaction system [3]. To obtain aerobic and anoxic environments under almost identical conditions, many processes have been utilized, including oxidation ditch processes [4], granular sludge processes [5], and biofilm processes [6]. Due to the cost-effective and high efficacy nature of some of these biofilm processes, many have been widely

investigated for the purpose of developing SND [7]. Because of their capacity to form a richer nitrifying bacterial community, natural biocarriers are potentially more beneficial than inert biocarriers [6,7]. Moreover, the natural biocarriers can decompose during water treatment, resulting in a thicker biofilm, which provides anaerobic conditions for the enriching of denitrifying bacteria.

Natural biocarriers can enhance the nitrifying bacteria biomass and they also have the capacity to effectively improve the ammonification process [8,9]. Due to the requirement of SND systems for denitrification, which requires sufficient carbon as an energy source—measured in terms of chemical oxygen demand (COD)—and a supply of electrons, the development of such SND systems has previously been limited. For this reason, the addition of external carbon-containing substrates, to facilitate the removal of nitrate nitrogen (NO_3^--N) by heterotrophs, will enable the continued operation of SND systems [10,11]. Certain disadvantages are associated with the use of liquid carbon sources for such a system, since this would require a close and sophisticated process control (to avoid the risk of overdosing), which would result in a deterioration of effluent water quality [12,13]. To overcome the above-mentioned problems, solid carbon sources are preferred as alternatives for use in denitrification systems [10–13].

Filamentous bamboo was chosen as a novel material for improving the efficiency of removing nitrogenous compounds. Several potential advantages are associated with this choice, since such a system represents an immobilized biocarrier for nitrifying bacteria and denitrifying microbes, and because it can function as a supplementary solid carbon source for denitrifying microbes [4,9]. Inert biocarriers, for example, plastic biocarriers and light ceramsite, have previously been considered for the purpose of bioremediation of polluted water bodies; but, to the best of our knowledge, there have been no reports on the use of biodegradable materials as biocarriers.

The objectives of our study were threefold. First, to assess the removal efficiency of total nitrogen (TN), NH_4^+-N, NO_3^--N, and nitrite nitrogen (NO_2^--N) using filamentous bamboo as a biocarrier, and, secondly, to investigate the characteristics of biodegradable filamentous bamboo in terms of its role in removing nitrogenous compounds from recalcitrant wastewater with a high organic load and low C/N ratio. Our final aim was to examine the biological mechanisms associated with the use of filamentous bamboo as a biocarrier during in the removal of nitrogenous compounds.

2. Materials and methods

2.1. Filamentous bamboo and filamentous plastics

Filamentous bamboo, composed of cellulose and lignin, was sampled by cutting into 10 × 1 × 1 mm pieces. The physical characteristics of the filamentous bamboo were as follows: porosity: 85%; specific surface area: 158 m^2/m^3; and bulk density: 1.1 kg/L. To remove water-soluble bamboo juice from the tissue, 100-g filamentous bamboo was soaked, at room temperature (28˚C), in 2 L aqueous solution of NaOH for 7 d prior to use, after which the filamentous bamboo was washed with pure water to eliminate the NaOH residue. Finally, the filamentous bamboo was oven-dried at 60˚C for 12 h and then cooled in a desiccator. Filamentous plastics were purchased from an online retailer. Some filamentous bamboo was also added to the bioreactor every 10 d, to supplement the filamentous bamboo that had been consumed during the biodenitrification and bio-synthesis processes.

2.2. Experimental apparatus

Experiments were carried out in two identical continuous-flow biofilm reactors, each with a working volume of 2.33 L, an inner diameter of 10 cm, and a height of 45 cm. Both reactors were made of

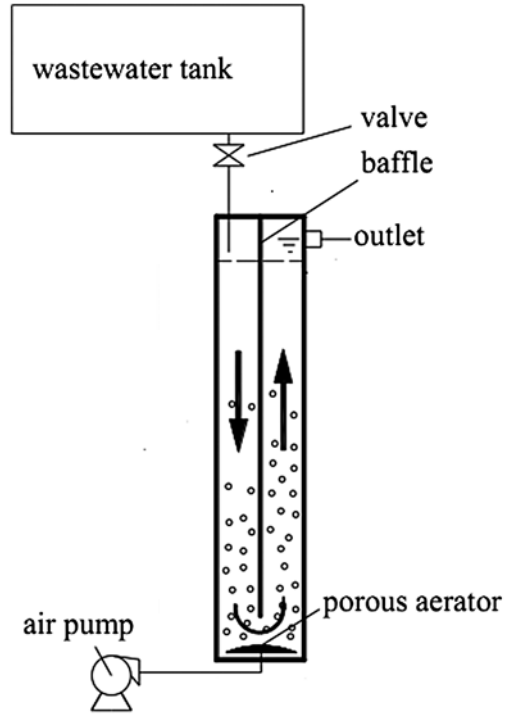

Fig. 1. Schematic diagram of experimental setup.

polymethyl methacrylate (Fig. 1). Each bioreactor was divided by a baffle into two separated areas, a downflow area and an upflow area. This system enabled the effective evaluation of mass transport advantages in the bioreactor [14]. The bioreactors (Fig. 1), consisting of filamentous bamboo and filamentous plastics, were filled into two biofilm reactors at a filling ratio of about 90%. The two bioreactors—one with filamentous bamboo (BFB) and the other with filamentous plastics (BFP)—were set up, with the BFP acting as a control.

The two reactors were inoculated with 0.5-L activated sludge seed and operated at a hydraulic retention time of 4 h (for biofilm formation and activated sludge domestication) and cultured on a continuous basis until steady-state biomass loading on the filamentous bamboo and filamentous plastics was achieved. Air was supplied from the bottom of the reactors and the experimental study was carried out at a water temperature of 19.0 ± 1.5°C. Wastewater was supplied to the top of the reactor and gravitated downwards.

2.3. Wastewaters

Two kinds of wastewater were utilized. Wastewater 1 (W_1) inflow consisted of Chinese traditional medicine wastewater and septic-tank effluent, with the following water-quality characteristics: COD: 804–5,100 mg/L; BOD: 45–1,100 mg/L; NH_4^+-N: 19–76.2 mg/L; NO_2^--N:<0.14 mg/L; NO_3^--N: 0.22–4.17 mg/L; and BOD/TN: 1.23–6.35.

Wastewater 2 (W_2) inflow was obtained from septic-tank effluent, with the following water-quality characteristics: COD: 98–251 mg/L; BOD: 37.5–77.5 mg/L; NH_4^+-N: 27.6–105 mg/L; NO_2^--N:<0.46 mg/L; NO_3^--N: 0.01–0.36 mg/L; and BOD/TN value: 0.51–1.87.

2.4. Analytical methods

2.4.1. Water quality analysis

Water samples were collected once 2 d at the end of one cycle and all water samples were filtered through a 0.45-μm membrane. During the total operation cycle, samples were collected at regular intervals and tested within 2 h of collection. All compositional analyses were performed in triplicate. The content of nitrogenous compounds, including NH_4^+-N, NO_2^--N, and NO_3^--N, were determined with a ion chromatograph analyzer (Model: PIC-10A, Instrument Co., Ltd. Puren, Qingdao, China), the mobile phase and the flow rate as well as were methylsulfonic acid, 0.5 mL/min and 20°C. TN was measured by an UV–Vis spectrophotometer (Shimadzu UV2450, Japan), and COD and BOD were analyzed according to standard methods [15].

2.4.2. Analytical methods for assessing total bacteria, nitrifying bacteria, and denitrifying bacteria

Concentration of bacteria was determined as follows: a section of filamentous bamboo of known area was collected from the reactor and transferred to a sterile Erlenmeyer flask to which 10 glass beads were added, to disperse the biofilm from the bamboo. The flask was shaken 10–15 min at 200–250 rpm after which 1 mL of turbid liquid was withdrawn, for determining the abundance of bacteria by the dilution-plate method and the most probable number.

2.4.3. Biomass weight analysis

Biomass weight was determined as follows: a section of filamentous bamboo of known area with a mature biofilm was collected from the reactor, placed on a glass, dried for 12 h at 60°C, and weighed after cooling. The weight of filamentous bamboo and glass was recorded and termed as "G_1". Next, the bamboo was soaked in 10% (m/v) NaOH for 6 h, to disperse the biofilm, washed with sterile water, dried for 12 h at 60°C, and weighed after cooling. The weight of the bamboo and glass at this stage was termed as"G_2". The biomass weight on the filamentous bamboo was calculated as G_2–G_1.

2.4.4. Biofilm thickness

Biofilm thickness was measured according to the methods of Tanyolaç and Beyenal [16], and the infrared spectrum analysis was conducted by infrared spectrometer (ALPHA, Germany).

2.5. Experimental procedure

W_2 was pressed into the top of the downflow area of the reactor. At a hydraulic retain time (HRT) of 6 h, the nitrogenous compounds removal efficiency was very low for the first 8 d of acclimatization start-up time (data not shown). When the biofilm began to grow at a steady state on filamentous bamboo, the reactor was fed with W_1 for 10 d. This stage represented the beginning of the experimental procedure, which consisted of two steps: an investigation of the removal efficiency of nitrogenous compounds of W_1, for 61 d (1); and of W_2, for 61 d (2). Three replicate reactors were designed and mean data were obtained.

3. Results

3.1. Nitrogenous compound removal experiment (W_1)

Fig. 2(a–d) illustrates the changes in concentrations of NH_4^+-N, NO_2^--N, NO_3^--N, and TN for measured in W_1.

Fig. 2(a) indicates that the NH_4^+-N and TN removal rates were, respectively, 42.44–100% and 43.06–96.70% for BFB. The BFP removal rates for NH_4^+-N and TN were in the ranges of 25.59–81.85% and 4.86–39.43%, respectively. The influent concentrations of NH_4^+-N and TN were in the ranges of 19–76.2 mg/L and 22.13–80.41 mg/L, respectively, during conditions when DO ranged from 0.90 to 2.30 mg/L. In the BFB, the effluent concentrations of NH_4^+-N and TN were 0–34.6 mg/L and 0.73–35.15 mg/L, respectively, while the DO ranged from 1.70 to 3.30 mg/L. In the BFP, the effluent concentrations of NH_4^+-N and TN were in the ranges of 5.7–30.4 mg/L and 17–60 mg/L, respectively, while the DO ranged from 1.90 to 3.50 mg/L. Significant downward trends were noted as shown in Fig. 2(a) and (d), representing concentrations of NH_4^+-N and TN when the influent COD and BOD concentrations were 74.8–843 mg/L and 8–198 mg/L, respectively, and regular reductions in the concentrations of NH_4^+-N and TN were observed. During the study, the BOD/COD ratio ranged from 0.01 to 0.08 of influent, and the effluent BOD/COD ratio ranged from 0.07 to 0.75. In the BFB, the influent NO_2^--N and NO_3^--N concentrations fell within the ranges of 0.01–0.2 mg/L and 0.91–5.51 mg/L, respectively, and the effluent concentrations were, respectively, 0.01–0.12 mg/L and 0.2–3.21 mg/L for BFB, and 1.6–17.5 mg/L and 2.4–28.3 mg/L for BFP. Compared to the control bioreactor (BFP), the concentrations of NO_2^--N and NO_3^--N remained lower in the BFB, and a higher TN removal rate was realized.

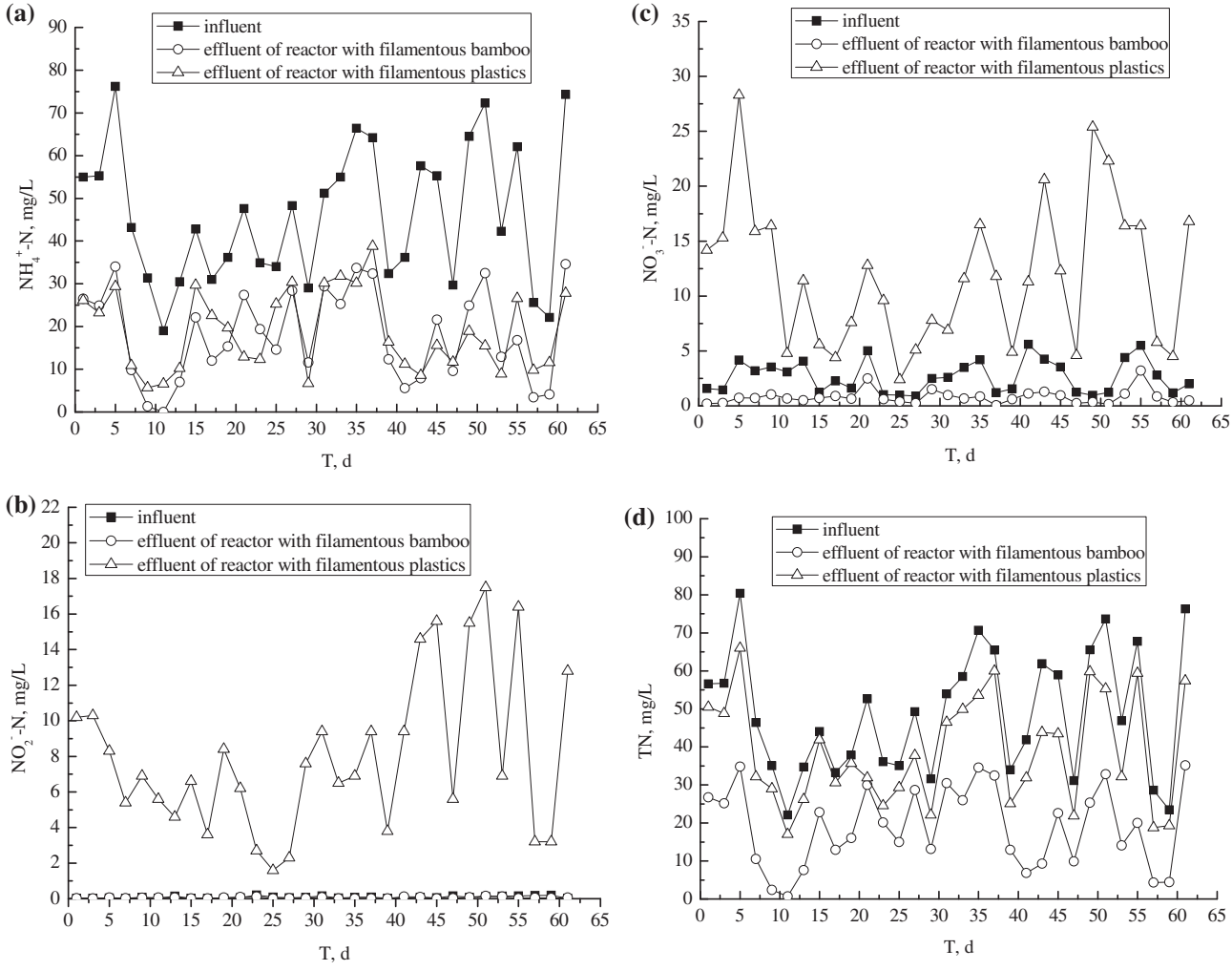

Fig. 2. Concentrations of nitrogenous compounds: changes in influents and effluents under high organic load conditions.

Removal of NH_4^+-N was obvious during the experiment, but no significant accumulation of NO_2^--N and NO_3^--N was noted, which suggests that NH_4^+-N was oxidized into NO_2^--N and NO_3^--N, and the NO_2^--N and NO_3^--N were oxidized continuously into gaseous nitrogen under aerobic conditions. Some studies have indicated that the mechanism involved in the removal of nitrogenous compounds is SND [17,18]. As noted by Rahimi et al. [19], the thicker biofilm is advantageous for SND. This occurred during the present experiment and, therefore, the concentration of TN decreased rapidly. This appeared to be influenced by the characteristics of filamentous bamboo.

Compared to other biocarriers that cannot be degraded by micro-organisms, filamentous bamboo can be decomposed by micro-organisms during water treatment, resulting in a thicker biofilm on the bamboo. During the experiment, the filamentous bamboo surface attained the greatest biofilm depth, with an average thickness of 450–670 μm. A thicker biofilm is beneficial for the removal of nitrogenous compounds [20], since it supports anaerobic conditions, which facilitate the conversion of refractory compounds into simple compounds, resulting in an improvement in the concentrations of BOD/COD and SND of raw water [21]. The nitrification process also took place in the external layer of biofilm because of the abundance of DO, which enabled vigorous growth of nitrifying bacteria which facilitated the nitrification process, resulting in a rapid decline in the concentration of NH_4^+-N during the experiment and the transformation of NH_4^+-N into NO_2^--N and NO_3^--N. Denitrification took place within the internal layer of biofilm, where anoxic conditions prevailed in the micro-environment. Thus, the NO_2^--N and NO_3^--N represented the products of oxidation products of NH_4^+-N in the external of the biofilm. An ample carbon source was also made available from the oxidation of organic compounds in the wastewater, and the decomposition products from filamentous bamboo.

The biological uptake of nitrogenous nutrients, from biological tissues, was also a component that facilitated the removal of nitrogenous compounds. For example, a BOD removal in the range of 37–902 mg/L was obtained, which represents the consumption of about 3–60 mg/L of nitrogenous compounds (assuming that $Y = 0.6$ mg-VSS (volatile suspended solid)/mg-BOD, and that the cell formula is $C_5H_7O_2N$). This explains the observed removal of nitrogenous compounds, as evidenced by the small amount of nitrogenous compounds present at the end of the experiment. The fraction of nitrogenous compounds consumed by biosynthesis was therefore significant when BOD concentrations were high.

3.2. Removal of nitrogenous compounds in W_2

The removal efficiency of nitrogenous compounds removal in W_2 is illustrated in Fig. 3.

Fig. 3 shows that the removal efficiency of nitrogenous compounds removal in W_2 was similar to that of W_1 during the experiment, and that changes in NH_4^+-N and TN indicated a strictly positive linear relationship.

Graphs illustrating changes in nitrogenous compounds (Fig. 3) indicate that the NH_4^+-N and TN removal rates were, respectively, 27.63–78.88% and 28.60–82.66% in the BFB, and 15.64–86.70% and 3.46–48.72% in the BFP. Influent concentrations of NH_4^+-N and TN were in the ranges of 32.5–104.7 mg/L and 33.6–105.93 mg/L, respectively, and the effluent concentrations of BFB for NH_4^+-N and TN were in the ranges of 9.78–53.6 mg/L and 10.04–53.64 mg/L, respectively, at DO concentrations ranging from 2.45 to 3.52 mg/L. In the BFP, the effluent concentrations of NH_4^+-N and TN were in the ranges of 10.3–55.1 mg/L and 23.3–73.4 mg/L, respectively, at DO levels that ranged from 2.38 to 4.02 mg/L. Downward trends in the concentrations of NH_4^+-N and TN were noted when the influent COD and BOD concentrations were in the ranges of 98–251 mg/L and 34–82.5 mg/L, respectively, and DO fell within the range of 1.73–3.27 mg/L. When the BOD/TN (ab.C/N) was in the range of 0.8–2.3, the NO_2^--N concentration did not exceed 1.34 mg/L and the concentration of NO_3^--N in the BFB effluent was 0.86 mg/L. Compared to BFP, the BFB obtained a higher TN removal rate and a lower level of NO_2^--N and NO_3^--N accumulation.

Traditional theories consider that an adequate available carbon source is necessary for denitrification because carbon is used as an electron donor and as an energy source for denitrifying bacteria [22]. Certain studies also consider that the lowest C/N ratio should be in the range of 3–4 during denitrification [23]. Nevertheless, during our experiment, we observed denitrification when the C/N ratio ranged from 0.8 to 2.3.

It can be assumed that filamentous bamboo releases carbon fractions, some of which are not biodegradable and some of which are available for denitrification. During the removal of nitrogenous compounds under low-ratio C/N conditions, the consumable fraction of the DOC enhanced the activities of denitrifying bacteria and nitrifying bacteria. When filamentous bamboo was used as a carbon source for the removal of nitrogenous compounds, all of the water-soluble component, and a significant portion of the cellulose and hemicelluloses, were lost during the experiment, but lignin and mineral components remained unchanged. But the easily soluble fraction of

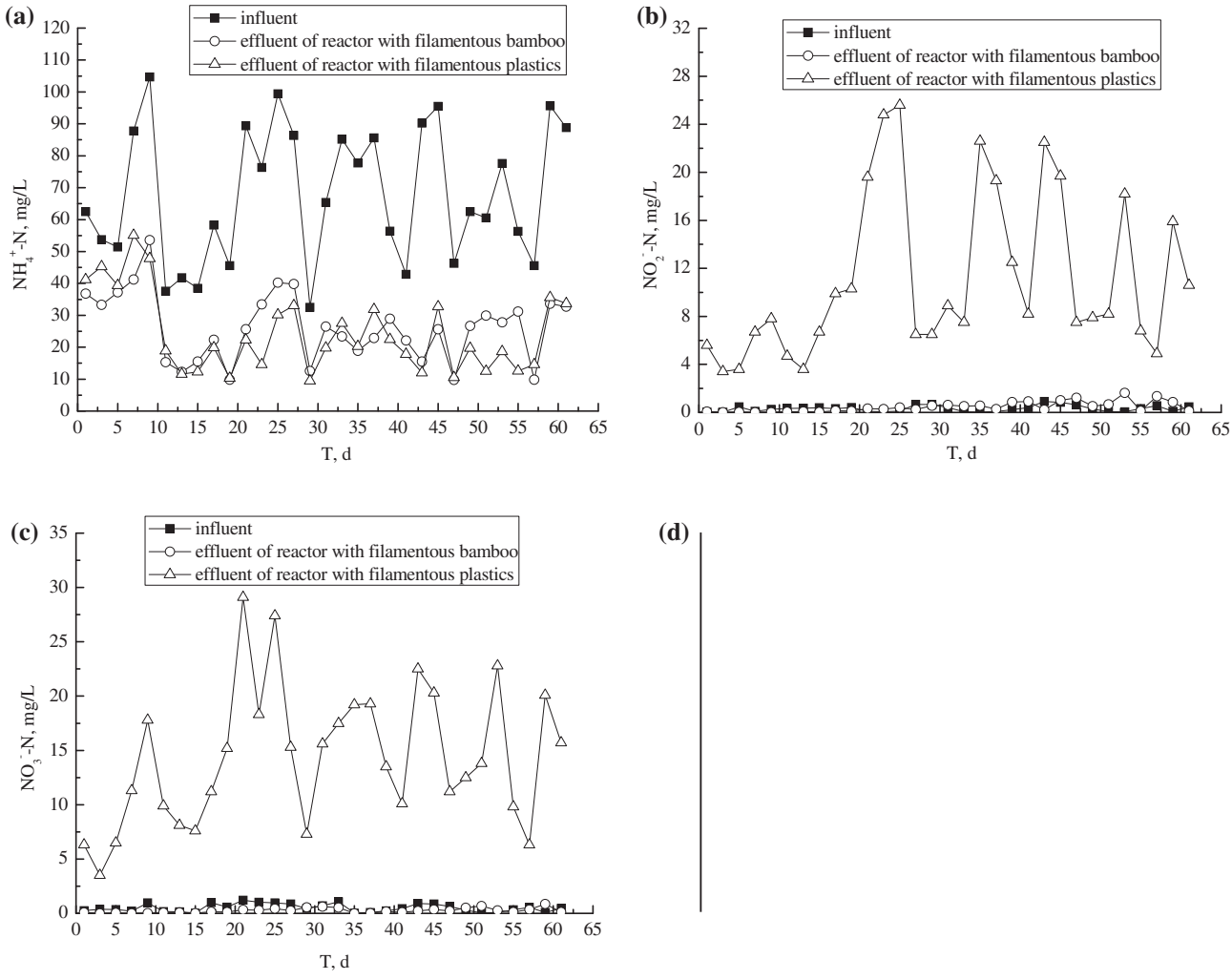

Fig. 3. Changes to nitrogenous compounds in the influent and effluent, under low C/N ratio conditions.

carbon would have contributed toward an increase in the total carbon source.

3.3. Infrared spectrum analysis

Changes in the functional groups on the filamentous bamboo were investigated by means of infrared spectroscopy during the experiments, to analyze the biodegradable performance of filamentous bamboo and to assess its use as a solid-phase carbon source. Compared to raw filamentous bamboo which disposed with sodium hydroxide, the changed performance of the functional groups on the employed filamentous bamboo indicated the following: the intensity of the main functional groups [–OH (~3,400 cm^{-1}, ~1,049 cm^{-1})], [$–CH_2$ (~2,920 cm^{-1})], and [$–NH_2$ (~1,650 cm^{-1})] on the filamentous bamboo was significantly reduced due to cellulose, fat, protein, and polysaccharide in the tissue of the filamentous bamboo that had been biodegraded.

3.4. Distribution of the bacterial communities in the bioreactor, under two different conditions

Distribution results of different bacteria communities in the bioreactor under two different conditions, in terms of organic loading and the C/N ratio, are shown in Table 1.

Results summarized in Table 1 indicate that the bacterial concentrations in different parts of the bioreactor were almost equal under high organic load conditions, suggesting that the high organic load caused a vigorous growth of bacteria throughout the bioreactor.

Under low C/N ratio conditions, there was an obvious decline in the total bacteria number along the

Table 1
Distribution of the different bacteria communities in the inlet and the outlet of the reactor

	High organic load			Low C/N ratio		
Bacterial concentration (cfu/mL)	Inlet	Bottom	Outlet	Inlet	Bottom	Outlet
Total bacteria number	6×10^{10}	4.7×10^{10}	4.3×10^{10}	8.9×10^{10}	1.8×10^{10}	9×10^{9}
Nitrifying bacteria number				1.4×10^{9}	9.5×10^{7}	2.9×10^{7}
Denitrifying bacteria number				1.6×10^{7}	1.1×10^{7}	0.97×10^{7}

water flow direction in the reactor, which may have been due to a gradual decrease in COD from inlet to the outlet. The nitrifying bacteria number around the inlet area was 48.3 times that in the outlet, and 14.7 times that at the bottom of the reactor. Nevertheless, the denitrifying bacteria number near the inlet was 1.65 times that near the outlet, and 1.45 times that at the bottom of the reactor. The distribution density of denitrifying bacteria was evenly distributed along the direction of water flow and nitrifying bacteria decreased rapidly from the inlet to the outlet areas, possibly because the high organic load of NH_4^+-N. The low organic load at the inlet of the bioreactor allowed for the rapid growth of nitrifying bacteria, while the growth of denitrifying bacteria was limited by high NH_4^+-N concentration and a low concentration of organic matter. The concentration of denitrifying bacteria increased slowly when the NH_4^+-N was oxidized to NO_2^--N and NO_3^--N, whereas the level of nitrifying bacteria decreased with a reduction in the NH_4^+-N concentration.

On the second, fourth, and sixth days, under low C/N ratio conditions, the biomass weights of the filamentous bamboo were, respectively, 9.55, 18.7, and 12.2 g/m^2, at the inlet of the bioreactor, while those at the outlet were 4.99, 6.40, and 3.80 g/m^2, respectively. The ratios, on the second, fourth, and sixth days, of biomass weight at the inlet and those at outlet were 1.89, 2.92, and 3.20, respectively, which was much lower than the ratio (of 9.89) of the total bacteria number at the inlet and that at the outlet in the same water sample. This was mainly attributed to the nature of the septic-tank effluent, which was comprised of high levels of bacteria and very little humus, leading to a situation where the total bacteria number was high while the biomass was low. Nevertheless, a low total number of bacteria associated with a high level of bamboo putrefaction, was observed at the outlet of the bioreactor. In this context, it should also be noted that NH_4^+-N and COD were oxidized at the inlet, and the denitrification process mainly took place in the upflow area of the bioreactor.

3.5. Discussion

In contrast to the situation relating to inert biocarriers, filamentous bamboo was used as a medium for micro-organism growth, resulting in an increase in the number, variety, and functional diversity of micro-organisms present in the system [22]. Moreover, the micro-organisms attached to the bamboo caused decomposition of the filamentous bamboo into organic matter, and the filamentous bamboo biocarrier was gradually reduced [4].

With the biofilm growth and maturation on filamentous bamboo, the biofilm thickness continued to increase and formed aerobic and anoxic layers on the filamentous bamboo, due to limitations in the dissolved oxygen (DO) mass transport process [20]. In contrast to the situation relating to non-biodegradable matter, the presence of anoxic micro-organisms resulted in a decay of filamentous bamboo, resulting in the conversion of high molecular weight cellulose to compounds with a lower molecular weight, which became available for anoxic micro-organisms associated with the denitrification of bacteria. Moreover, the biofilm on the filamentous bamboo was significantly firmer, compared to other inert biocarriers, and the internal layer of biofilm had the capacity to obtain adequate nutrients from the filamentous bamboo, so remained active. Thus, the adhesion ability of the biofilm on filamentous bamboo was not reduced because the biofilm on the filamentous bamboo remained steady. In addition, a significant level of consumption of nitrogenous compounds by bio-synthesis occurred when the BOD level increased.

Steady and high-performance biofilm on filamentous bamboo is a decisive factor for the effective removal of nitrogenous compounds from recalcitrant wastewater with a high organic load and a low C/N ratio. When the content of refractory organic matter in raw water is high, the refractory compound matter is unavailable for the removal of nitrogenous compounds, since the carbon source can be translated into simple organic matter, available for denitrifying

bacteria in the internal layer of the biofilm. When the C/N ratio in raw water is low and the carbon source is too low for denitrification, extra organic matter can therefore be supplemented by the decay products of filamentous bamboo.

4. Conclusions

We investigated the removal efficiency of nitrogenous compounds from recalcitrant wastewater with a high organic load and a low C/N ratio, using filamentous bamboo as a biocarrier. We also compared the composition of the influent and the effluent and investigated improved biodegradability. The properties of filamentous bamboo are a critical factor to consider when studying the removal of nitrogenous compounds, when using filamentous bamboo as biocarrier for the treatment of recalcitrant wastewater with a high organic load and a low C/N ratio.

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