



Biological denitrification in simulated groundwater using polybutylene succinate or polylactic acid-based composites as carbon source

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ABSTRACT

Two novel multiple component carbon sources, mainly polybutylene succinate (PBS) and polylactic acid, were prepared by twin-screw extrusion to treat simulated groundwater. Both of the composites also contained high-density polyethylene and walnut shell (WS) to adjust the denitrification rate. The composites could release soluble organic carbon and be carriers for bacteria. The addition of foaming agent in the composite enriched the porous channels which increased the surface area for biofilm loading. The results indicated that the average N-NO₃⁻ removal efficiency in reactor 1 (filled with WS/PBS) was 83%. When the inflow concentration of nitrate changed from 50 to 55 mg L⁻¹, the reactor 1 had an acclimation time of 13 d to accept the high concentration. However, the N-NO₃⁻ removal efficiency in reactor 2 (filled with WS/PLA) decreased from 86 to 78% because of the propagation of blue-green algae which also led to a high COD value in effluent. WS/PBS was a stable carbon source releaser and could adapt to the environmental change easily. Therefore, it has the potential of using as carbon source of biological denitrification of groundwater.

Keywords: Denitrification; N-NO₃⁻ removal; Biodegradable polymer; Solid carbon source

1. Introduction

Groundwater is an important part of fresh water, which is also an essential water supply source for domestic water. However, the concentration of nitrate in the groundwater is in the trend of increase due to the excessive use of fertilizers in intensive agriculture and the irrigation with ammonia-rich effluents discharged by wastewater treatment plants. Too much nitrogen compounds discharged into

water bodies would not only cause great environmental problems, such as eutrophication and algal blooms [1], but also lead to health risk of cancer [2]. For this reason, nitrate removal is of great concern in water treatment.

Biological denitrification is an efficiency and low-power consumption method operated by heterotrophic bacteria communities, in which the nitrate is converted to nitrogen gas. In groundwater, there is no sufficient organic matter acting as an electron donor to raise the heterotrophic bacteria communities, and the microbial denitrification may not be possible under

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natural conditions [3]. Therefore, supplying electron donors is the primary way to improve the efficacy of biological denitrification in groundwater. Many kinds of carbon sources, such as soluble organic carbons (e.g. ethanol, methanol, and acetate) and natural solid carbon sources (e.g. cotton, wheat straw) [4–7], served as electron donors have been utilized in biological denitrification. However, as large amounts of soluble organic carbon supply for *in situ* purifying nitrate contaminated groundwater might lead to a risk of overdosing and an increase of COD in effluent. On the other hand, the natural plants show an efficient nitrate removal degree in a couple months [8], but could not be a steady carbon source and microbial carriers in a long run.

On this basis, biodegradable polymers (BDPs), which are insoluble but could degrade into simple organic carbons at a low rate, are extensively studied [9–15]. They can serve as both biofilm carriers and carbon sources for microbes. BDPs could decompose into small molecular compounds, further to soluble organic substances by bacterial enzymatic degradation or chemical hydrolysis, which could be used as electron donors [16,17]. Polybutylene succinate (PBS) and polylactic acid (PLA) are widely utilized BDPs, and have been used as electron donors for biological denitrification [18,19]. However, pure BDPs still present serious shortages in biological denitrification. With the degradation of BDPs, the volume of the carbon sources becomes smaller and the area of biofilm decreases which might weaken the efficiency of denitrification rate [20].

According to our previous work, the walnut shell (WS) particles (2–4 mm in diameter) could be a good biofilm supporter and maintained a good N-NO_3^- removal degree in the same reactor for few months. The main parts of WS are xylogen, pentosan, and fiber. As a part of plant, the WS is non-toxic, non-perishable, and could be better degraded by microbia than PBS and PLA. In this paper, two kinds of multiple component composites containing non-degradable high-density polyethylene (HDPE) as skeleton, WS as reaction rate regulator, and PBS or PLA mainly as carbon source releaser were prepared by twin-screw extrusion. Most importantly, the skeleton structure could avoid the decrease of biofilm area when the fixed bed bioreactors (FBBRs) were operated for a long time. Moreover, foaming agent was added in preparation of the composites to form inside holes and channels for increasing the granules' surface area. The FBBRs filled with the composites can operated for a long time with relatively high denitrification efficiency.

2. Materials and methods

2.1. Materials

The PBS was purchased from Anqing He Xing Chemical Co., Ltd. (HX-E101) with the molecular weight of 300,000. The PLA was purchased from Nanjing Gaoxin Biochemical Co., Ltd. (GX-101A) with the molecular weight of 200,000. The HDPE was purchased from China Petrochemical Corporation. The WSs were purchased from Nanyang, Henan Province, China. Before use, the WS was grinded to below 100 mesh (the particle size is about 150 μm). The other chemical reagents were purchased from Chemical Reagent Corporation, Beijing, China.

2.2. Preparation of PBS or PLA based composites

The composites were manufactured using a twin-screw extruder (TSE-30A, Nanjing Ruiya Polymer Processing Equipment Ltd.) and cut into cylindrical granules with a height of 3 mm, a diameter of 3 mm, and a special surface of $0.4 \text{ m}^2 \text{ g}^{-1}$. The extrusion temperatures are 170–185°C for PBS-based composite (WS/PBS) and 160–170°C for PLA-based composite (WS/PLA). The components of the composites are shown in Table 1.

2.3. Biological denitrification setup

Two laboratory-scale FBBRs were used to investigate the denitrification performance of the two kinds of composites. The first one (reactor 1) was filled with PBS-based composite (WS/PBS) and the other one (reactor 2) was filled with PLA-based composite (WS/PLA). The reactors were made by Plexiglas with the volume of 1,270 ml. They were filled with 600 ml of carbon source granules and 670 ml of simulated groundwater. The weight for WS/PBS and WS/PLA was approximate 720 and 708 g, respectively. The simulated groundwater was prepared by dissolving KNO_3 , NaH_2PO_3 , and trace elements in deionized water. The details were shown in Table 2. The simulated groundwater consisted of N-NO_3^- (50 mg L^{-1}), P-PO_4^{3-} (7.45 mg L^{-1}), Mg^{2+} (1.58 mg L^{-1}), Ca^{2+} (1.19 mg L^{-1}), Zn^{2+} (0.19 mg L^{-1}), Fe^{2+} (0.03 mg L^{-1}), SO_4^{2-} (0.32 mg L^{-1}). The COD of the simulated groundwater was very low ($\leq 3 \text{ mg L}^{-1}$) because of no dissolvable organic carbon in it and the pH was 7.35 ± 0.02 . There were one inlet on the bottom and two outlets separately in the middle and on the top. The simulated groundwater was pumped into the FBBRs by a peristaltic pump (Baoding Shenchen Precision

Table 1
Components of the composites filled in the reactors

Components		Mass fraction in %	Role
Reactor 1	Reactor 2		
PBS	PLA	38	Carbon source
Walnut shell	Walnut shell	30	
HDPE	HDPE	30	Skeleton body
Isoascorbic acid	Isoascorbic acid	0.5	Deoxidant
NaHCO ₃	NaHCO ₃	0.5	Foaming agent/pH value regulator
Maleic anhydride	Maleic anhydride	1	Cross-linking agent

Table 2
Components of simulated groundwater

Reagents	Concentration in mg L ⁻¹
KNO ₃	361 ^a
NaH ₂ PO ₃	25
MgCl ₂ ·6H ₂ O	13.39
CaCl ₂	3.29
ZnSO ₄ ·7H ₂ O	0.82
FeSO ₄ ·7H ₂ O	0.15

^aThe N-NO₃⁻ concentration was 50 mg L⁻¹.

Pump Co., Ltd., Hebei) at a constant rate. The scheme of the experimental setup was shown as Fig. 1.

2.4. Operation of FBBRs

The biological denitrification of simulated groundwater included two processes, the acclimation of denitrifying bacterial and the stable period. The acclimation of denitrifying bacterial was carried out by inoculating the activated sludge into FBBRs. The activated sludge was taken from an anoxic tank at the Fangzhuang wastewater treatment plant in Beijing. Two hundred milliliter of activated sludge with

1.2 g L⁻¹ concentration of total suspended solid was introduced into the reactor. The duration for acclimation was 15 d with the simulated groundwater inflow rate of 0.31 ml min⁻¹ [21]. When calculated with volume of the water in reactor, the hydraulic retention time (HRT) would be 36 h (Table 3).

Then, the FBBRs turned into a stable period with the HRT of 24 h. The stable period was also divided into two cycles. In the first cycle, the simulated groundwater was pumped into the reactor at a rate of 1.40 ml min⁻¹ for 8 h, then the overflowed water was collected by flask. In the second cycle, water in the reactor was pumped from the outlet to inlet to complete internal circulation at the rate of 1.40 ml min⁻¹ for 16 h.

2.5. Monitoring of the effluent

The denitrification performances of the two FBBRs were studied by monitoring the concentration of N-NO₃⁻, N-NO₂⁻ and the COD, pH of the effluent every 3 d. The soluble nitrate-N and nitrite-N concentrations in the effluent were determined by ultraviolet spectrophotometry (UV765, Shanghai Precision & Scientific Instrument Co., Ltd.) according to Chinese SEPA Industrial and National Standard Methods (SEPA, 2007 SEPA, 1987). The COD of the effluent was determined by COD speed measurement by potassium chromate method with spectrophotometer (5B-3C, Lianhua Tech. Co., Ltd.) according to Chinese

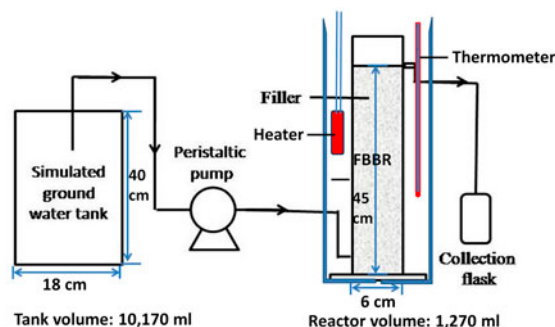


Fig. 1. Scheme of the experimental setup.

Table 3
Different modes of operation

	Acclimation period		Stable operation	
	Time (d)	HRT (h)	Time (d)	HRT (h)
Reactor 1	1–15	36	16–60	24
Reactor 2	1–15	36	16–60	24

SEPA National Standard (SEPA, 2007). And the pH value was monitored by pH meter (PH500A, CLEAN, US).

2.6. Characterization of the composites after utilization

The two kinds of carbon sources granules were randomly taken out when the FBBRs were stopped. Parts of granules were freeze-dried for 6 h to protect the biofilm on the surface and the rest were washed by deionized water to remove the biofilm. Afterwards, the two sets of samples were examined by scanning electron microscopy (SEM, S-4800, Hitachi, Japan) and optical microscope (BX60, Olympus, Japan).

3. Results and discussion

3.1. Denitrification performance in acclimation period

In the acclimation period, the biofilm had not yet completely attached onto the surface of granules. In order to identify the appropriate environment for cultivating the bacterial colony, a temperature control system was used to maintain the reactors with a constant temperature of $26 \pm 1^\circ\text{C}$. The N-NO_3^- removal performances of the two reactors are exhibited in Figs. 2 and 3. The nitrate removal degree decreased gradually and nitrite accumulation was not observed from the both two reactors. After cultivation for 1 d, the concentration of N-NO_3^- in effluent of reactor 1 filled with NS/PBS was 41.1 mg L^{-1} and the N-NO_3^- removal efficiency is 17.8%, while the N-NO_3^- removal efficiency of reactor 2 filled with WS/PLA was 19.8%.

Comparing with the two composites, WS/PLA possessed a shorter acclimation time of denitrifying

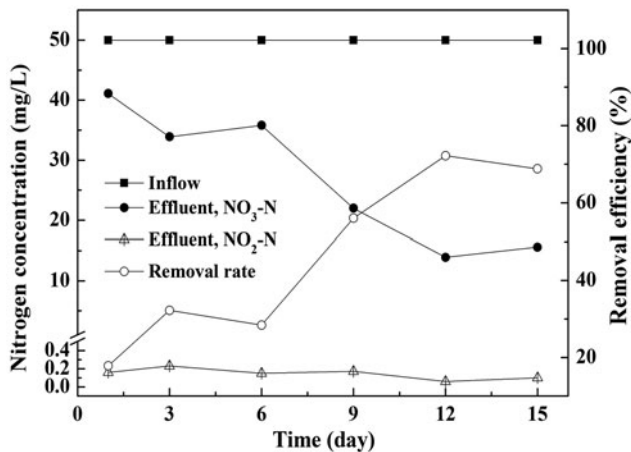


Fig. 2. N-NO_3^- removal performance in reactor 1 (1–16 d).

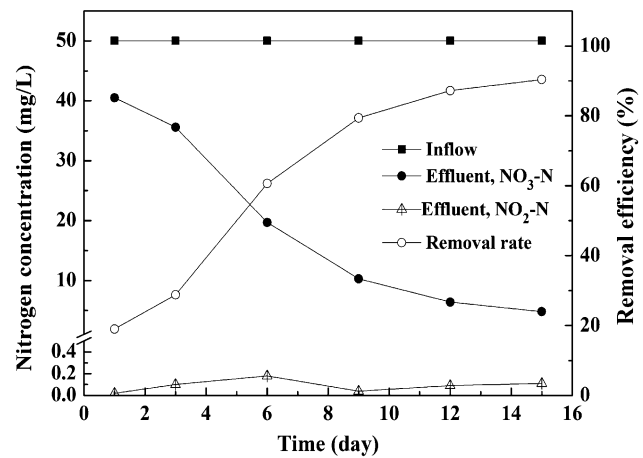


Fig. 3. N-NO_3^- removal performance in reactor 2 (1–16 d).

bacteria. The reason was that it was easier for microbial to attach on the WS/PLA granules probably because of its better biocompatible ability of PLA. Additionally, PLA could efficiently release organic carbon which could be readily taken up by denitrifying bacteria. As a result, in the last days of the acclimation, effluent nitrate concentration of reactor 2 was 4.8 mg L^{-1} with a N-NO_3^- removal efficiency of 90.4%. The nitrite concentration in both of reactors maintained at a low level and NO_2^- accumulation was not observed.

During the acclimation period, the COD and pH value were monitored and the results are shown in Fig. 4. At the initial several days, the effluent COD of the two reactors was very high, especially for reactor 2. That was mainly contributed by the activated sludge which contains a mass of organic matter. With

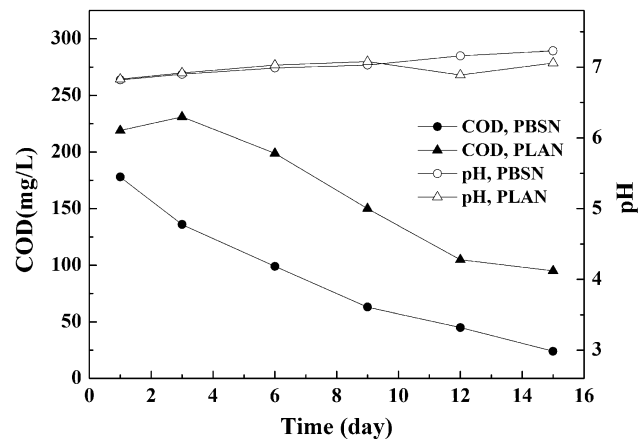


Fig. 4. Effluent COD and pH in the two reactors (1–16 d).

the growth of denitrifying bacteria, organic matter was consumed and then the effluent COD decreased gradually. Meanwhile, the bacteria in activated sludge might enzymatically catalyze the transformation of solid organic carbon to soluble organic carbon and further use it as nutrient for growth. Usually, before the bacteria attaching onto the granules to form a stable biofilm, the soluble organic carbon could not be used completely and further led to the effluent with a high COD [22]. Once the bacteria attached onto the granules, they proliferate gradually to form a functional biofilm which had an effective degradation of nitrate and a higher consumption of soluble organic carbon. The N-removal degree bores a close relationship with the biodegradability of the solid carbon source, and such a relationship would be reflected by the COD data [18]. At the same time, the pH of the effluent was 6.78 ± 0.02 in the beginning and then increased up to 7.23 in reactor 1 and 7.06 in reactor 2 (Fig. 4). The temperature control system was shut-down after acclimation period.

3.2. Denitrification performance in the stable operation period

The results of nitrate removal from the simulated groundwater in the two reactors are shown in Figs. 5 and 6. During 18–60 d, the average temperature of the FBBRs was 25.3°C (range of $21.3\text{--}27.9^\circ\text{C}$). In this period, there was a high N-NO_3^- removal degree in both reactors because the process of denitrification had been carried out stably in the two reactors. The effluent N-NO_3^- of reactor 1 was lower than 10 mg L^{-1} with the lowest of 5.9 mg L^{-1} which means the reactor 1 was operating effectively.

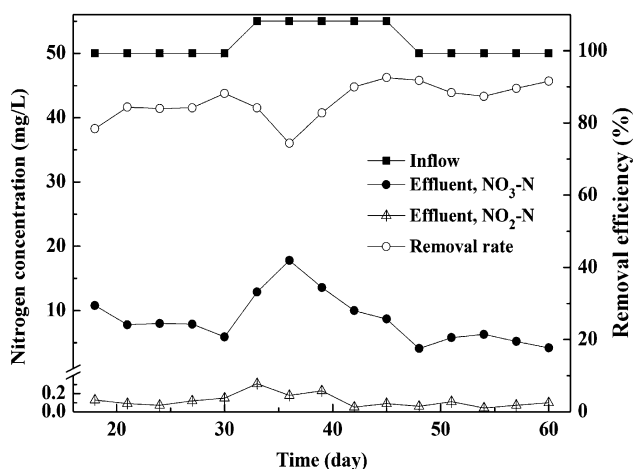


Fig. 5. N-NO_3^- removal performance in reactor 1 (18–60 d).

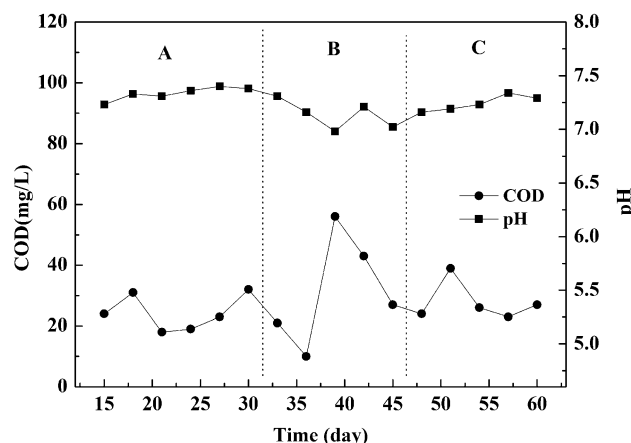


Fig. 6. Effluent COD and pH in reactor 1 (18–60 d).

For investigating the adaptability of reactor 1 to different quality of simulated groundwater, the influent nitrate concentration was changed from 50 to 55 mg L^{-1} at the 33rd day. The effluent nitrate concentration increased sharply from 5.9 to 12.9 mg L^{-1} correspondingly and continued for 10 d (Fig. 5). The highest effluent nitrate concentration was 17.8 mg L^{-1} with the N-NO_3^- removal efficiency of 74.4%. However, at the 45th day, the N-NO_3^- removal efficiency of reactor 1 raised up to 92.6% as the microbial had adapted the changed inflow, revealed a nitrogen removal efficiency of $215.78 \text{ mg (L d)}^{-1}$. The denitrification regained a high efficiency and even higher than that before the influent concentration was changed. From the 48th day, the influent nitrate concentration of FBBRs returned to 50 mg L^{-1} . The reactor 1 was operating steadily during the following days with effluent N-NO_3^- of $4\text{--}6 \text{ mg L}^{-1}$ and the removal efficiency of 87–92%, which indicated that the non-biodegradable component, HDPE, did not prevent carbon release.

The COD and pH value were monitored during the stable operation period and the results are shown in Fig. 6. In 15–30 d, the average COD in reactor 1 was 25.78 mg L^{-1} with a fluctuation range of $18.5\text{--}33.9 \text{ mg L}^{-1}$. Since the COD of the simulated groundwater was nearly zero, all the soluble organic carbon in effluent was released by the WS/PBS. The COD of effluent was about 20 mg L^{-1} which indicated that the amount of soluble organic carbon exceeded the requirement of the bacteria for growth and denitrifying. As aforementioned, since the bacteria could quickly adapt the increased N-NO_3^- in the simulated groundwater, the COD decreased as the influent N-NO_3^- was changed from 50 to 55 mg L^{-1} at the 33rd day, and the decreased COD was mainly due to the

consumption of denitrifying bacteria for a higher denitrification rate. van Rijn et al. [23] had reported that a COD/N-NO₃⁻ ratio between 3.0 and 6.0 enabled complete N-NO₃⁻ reduction for most readily available organic carbon sources. With regard to the higher COD and N-NO₃⁻ in the present work, the ratio was small (1.51). It might be because the high N-NO₃⁻ concentration (50 mg L⁻¹) stimulated the growth of denitrifying bacteria and improved the efficiency of denitrification. As the N-NO₃⁻ increased and then returned to the former concentration, the pH value of the effluent had no tremendous change and varied slightly in the range of 7.30–7.57.

For reactor 2, the denitrification was stable with the N-NO₃⁻ removal efficiency above 86% in the reaction time of 18–30 d, but lower than that in reactor 1. However, in the 33rd day, the blue-green algae occurred in reactor 2. Then the N-NO₃⁻ concentration in the effluent increased from 6.9 to 10.8 mg L⁻¹ (Fig. 7). In the following days, it fluctuated irregularly in the range of 9.1–13.6 mg L⁻¹. On the same time, the variation of effluent COD was significant. At the beginning of operation period, the COD was very high at 121 mg L⁻¹, then it dropped sharply to 58 mg L⁻¹ (Fig. 8) and the lowest was 24 mg L⁻¹. When the blue-green algae appeared in the reactor 2, the COD increased and fluctuated noticeably.

3.3. Morphology

The morphology of the WS/PBS and WS/PLA after denitrifying was observed by SEM and the images are depicted in Fig. 9. In the WS/PBS composite, HDPE formed the skeleton structure and the biodegradable components, PBS and WS, degraded

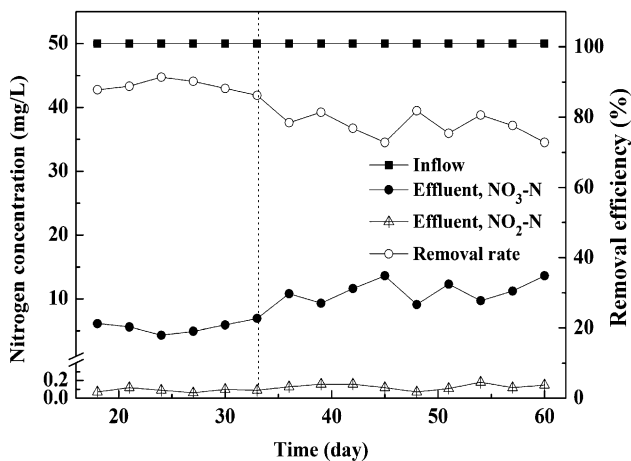


Fig. 7. N-NO₃⁻ removal performance in reactor 2 (18–60 d).

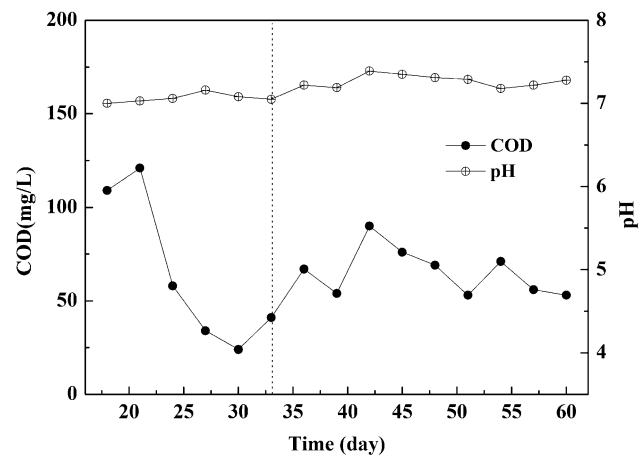


Fig. 8. Effluent COD and pH in reactor 2 (18–60 d).

into soluble organic carbon which acted as an electron donor [24] to denitrify heterotrophic bacteria. As the outer biodegradable components were converted into soluble organic carbon, the skeleton and porous channels were exposed which were clearly observed by SEM (Fig. 9(a)). The porous channels provided the paths for the soluble organic carbon to spreading from the inside to outside in the later period. The channels could even be found in the granules with biofilm (Fig. 9(b) and (e)). It was believed that the HDPE did not hinder soluble organic carbon release in the long term. The surface of WS/PBS was bacteria film (Fig. 9(b)), and the porous channel could be observed through the hole of film. The bacteria film also attached on the surface of WS/PLA, and we could find some coccus and rod bacteria on the film.

The optical microscope images of the bacteria in reactor 2 are shown in Fig. 10. The Fig. 10(a) confirmed that there are two kinds of denitrifying bacteria in reactor 2, coccus and rod bacteria. The spherical blue-green algae was shown in Fig. 10(b), it was green and could produce oxygen by photosynthesis [25]. So the increased and fluctuated COD of reactor 2 might stem from the blue-green algae which could product organic matter by photosynthesis.

On one hand, the blue-green algae produced oxygen and led to an aerobic environment in the reactor. Such an aerobic environment was negative for denitrification. On the other hand, we found that there was no denitrifying bacteria near the blue-green algae in reactor 2, which proved that the growth of blue-green algae occupied the room of denitrifying bacteria and broken the ecological balance of the denitrifying system. So the proliferation of blue-green algae was an important reason for the weakness of denitrification.

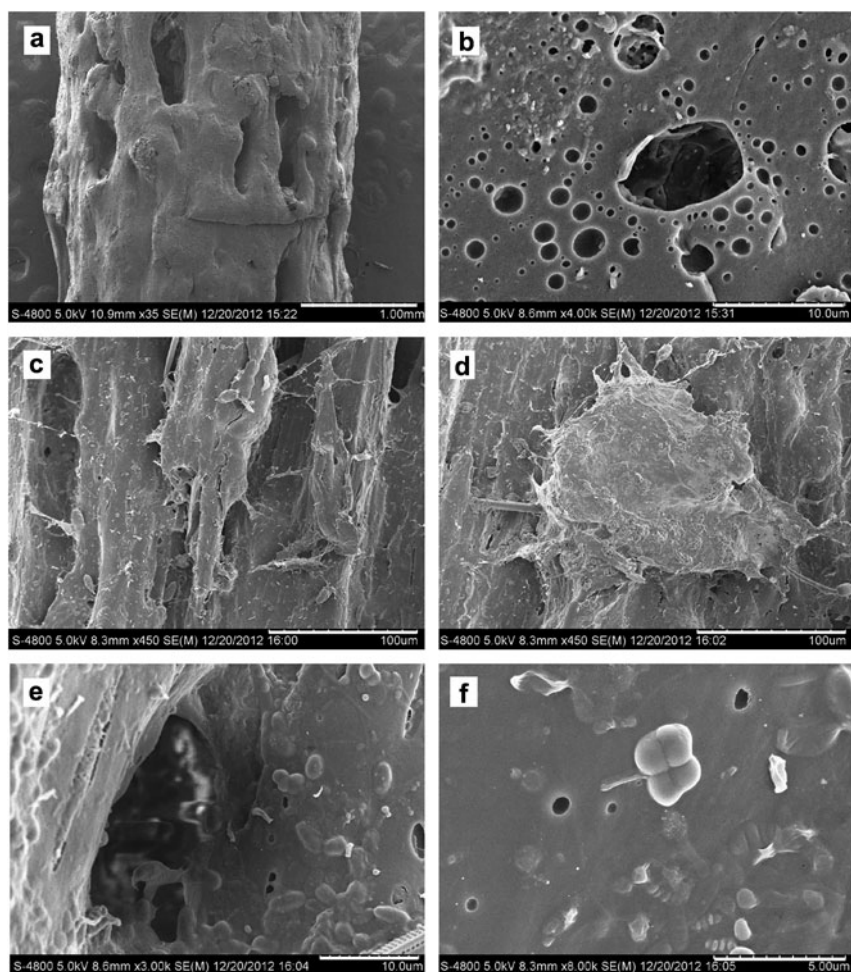


Fig. 9. SEM images of the composites after denitrifying ((a) WS/PBS without biofilm; (b) WS/PBS with biofilm; (c) WS/PLA without biofilm; (d–f) WS/PLA with biofilm).

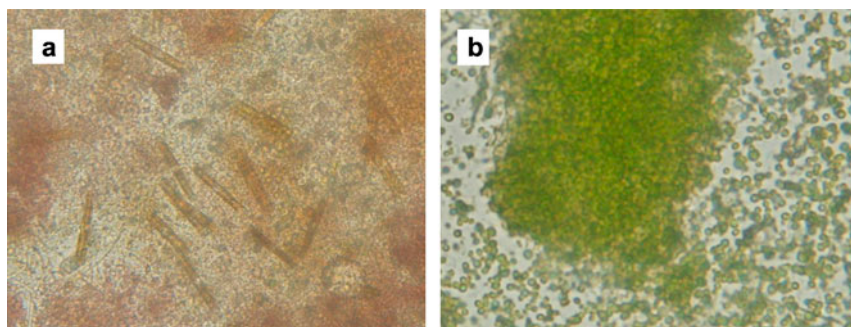


Fig. 10. Microscope images of microbial in reactor 2 ((a) denitrifying bacteria; (b) blue-green algae).

4. Conclusion

Two kinds of multiple component composites, containing non-degradable HDPE as skeleton, WS, and PBS or PLA as primary carbon source releasers, were

prepared by twin-screw extrusion. The denitrifying performance of FBBRs filled with WS/PBS or WS/PLA was studied by monitoring the $N-NO_3^-$ concentration, COD, and pH variation. FBBRs packed

with WS/PBS showed long-lasting and stable nitrate removal abilities. Throughout the whole experiment, the WS/PBS released appropriate soluble organic carbon to enable the denitrification process. In the stable operation period, the N-NO_3^- concentration in the effluent was 4.3 mg L^{-1} and the N-NO_3^- removal efficiency was 92.3%. Meanwhile as the nitrate concentration in the inflow was increased, the acclimation time of the bacteria was 13 d. However, the FBBR filled with WS/PLA was easily affected by green algae which would restrain the denitrification process.

Acknowledgements

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