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Biological denitrification using starch/polycaprolactone blends as carbon source and biofilm support

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ABSTRACT

A novel kind of biodegradable polymer, cross-linked starch/polycaprolactone (PCL) blends was prepared, characterized and applied for biological denitrification. The results showed that the blends could be used as carbon source and biofilm carrier for denitrifying bacteria attachment. The temperature had a significant influence on denitrification rate, and the temperature constant was determined to be 0.0151 between 15 and 30°C in batch tests. The continuous fixed-bed experiments indicated that the average removal efficiency of NO_3 was 92.51% at 25°C, while it was only 68.69% at 15°C. The formation of NH_3-N was observed at both 15 and 25°C, but it kept below 1.0 mg/L. The excess release of dissolved organic compound was much lower at 15°C compared with that at 25°C. The scanning electron microscope results confirmed that both cross-linked starch and PCL could be utilized by denitrifying bacteria as carbon source for nitrate removal.

Keywords: Biodegradable polymer; Denitrification; Solid carbon source; Biofilm; Nitrate

1. Introduction

Nitrate pollution is one of the most important environmental problems in China. Nitrate itself is not toxic, but it can be reduced to nitrite which may cause methemoglobinaemia in infants, also called a blue-baby syndrome. Nitrate removal processes include physical, chemical, physico-chemical, and biological ones. Among these, heterotrophic biological denitrification seems to be one of the most promising processes [1]. In biological denitrification process, heterotrophic bacteria convert nitrate or nitrite to nitrogen gas. The denitrifying bacteria require sufficient organic carbon as an electron donor for nitrate removal [2]. Usually, methanol, ethanol, acetic acid, or glucose are used as carbon source for organic carbon-limited water and wastewater [3]. Recently, Strong et al. [4] found that a liquor derived as a by-product from the wet oxidation of waste activated sludge could be used as a carbon supplement for enhancing denitrification. However, in liquid carbon source-supported denitrification system, there is a risk of overdosing which would cause deterioration of effluent quality. In addition, it also requires a

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sophisticated and costly process control. A new type of denitrification method has been designed and called "solid-phase denitrification" in recent years, in order to overcome these problems, in which biodegradable polymers were used as carbon source and microbial attachment carrier [5–8]. There are two kinds of solid carbon sources, i.e. natural cellulose materials, including wheat straw [9,10], cotton [11], and newspaper [12]; and synthetic polymers, such as polyhydroxyalkanoates (PHAs) [13], polycaprolactone (PCL) [5,14–16]. However, synthetic polymers are expensive, although natural materials are much cheaper, they may bring excess release of dissolved organic compound (DOC) and color problems, especially at the start-up stage of the operation [5,17]. Therefore, the research and development of new biodegradable polymers becomes one of most important issues for extensive application of this new denitrification process. Blending with some cheap organic materials can reduce the cost of the synthetic polymers. For example, starch, as an abundant renewable polysaccharide with better biodegradability, is a most potential blending material for biodegradable plastics production [18]. The feasibility and efficiency of using cross-linked starch/PCL blends [19], starch/PCL blends [20], starch/PLA blends [21] as both solid carbon sources and biofilm carrier were studied in the past.

No matter in liquid carbon source (such as methanol and ethanol) or solid carbon source-supported denitrification systems, the denitrification rate would decrease when temperature is beyond the suitable range. However, there should be difference in the effect of temperature on denitrification between in liquid carbon source and solid carbon source-supported systems. In liquid carbon source-supported denitrification system, temperature mainly affect the activity of enzymes participated in nitrate reduction processes. However, the activity of enzymes in nitrate reduction processes and solid carbon source biodegradation processes are both affected by temperature in solid carbon source-supported denitrification system. Furthermore, the effect of temperature on solid carbon source biodegradation processes probably depends on the inherent characteristics of solid carbon source. Therefore, a novel kind of biodegradable polymer, cross-linked starch/PCL blends (THE BLENDS) was prepared, characterized, and used as solid carbon source and biofilm attachment carrier for denitrifying bacteria; the effect of temperature on denitrification was studied in batch test and column test, and the performance of the blends before and after use was characterized using scanning electron microscope (SEM).

2. Materials and methods

2.1. Materials

The molecular weight of PCL was 60,000 g/mol (Dalton). Cross-linked starch was produced by using epichlorohydrin as cross-linking agent. The mixture of cornstarch (10 g), distilled water (30 g), and NaCl (0.7 g) was added into a flask and stirred mechanically. The pH was adjusted to be 9.50 using NaOH solution (0.5 M). Then epichlorohydrin (0.8 mL) was added. The cross-linking reaction was carried out at 50℃ for 5 h under continuous agitation. Then it was poured into 1 L distilled water. The cross-linked starch was centrifuged, collected, washed, dried, and ground into powders. Cross-linked starch/PCL blends (THE BLENDS) were prepared by twin-screw extruder (SHL-35). The main characteristics of THE BLENDS are listed in Table 1.

Synthetic wastewater was used, containing about 50 mg NO_3-N/L and 10 mg PO_4-P/L by adding $NaNO_3$ and KH_2PO_4. The DO and pH value of synthetic water were not controlled during the tests.

2.2. Experimental apparatus

Batch experiments were conducted using 500 mL Erlenmeyer flasks as reactors. Triplicate tests were carried out. Continuous experiments were carried out in a fixed-bed reactor, a cylindrical Plexiglas with 50 mm inner diameter by 500 mm long [20]. THE BLENDS granules were used as carrier for bacterial attachment, and packing the column up to a height of 250 mm (282.34 g).

2.3. Experiment procedures

In the batch experiment, the synthetic wastewater (300 mL) was mixed with seeding activated sludge

Table 1
Main characteristics of THE BLENDS

Items	Values
Cross-linked starch (%)	46
PCL (%)	28
Cellulosine (%)	5
Additives (%)	21
Carbon* (%)	44.65
Diameter (mm)	3.0
Height (mm)	3.6–4
Dry weight (g) per piece	0.0292
Specific surface area (cm^2/g)	17.127

*Detected by TOC analyzer (HACH, IL530 TOC-TN).

(with the final concentration of 800 mg/L MLSS) collected from a local municipal wastewater treatment plant. Then it was added into a 500 mL Erlenmeyer flask which contained 80 g THE BLENDS granules. Nitrate concentration was kept at about 50 mg/L by addition of $NaNO_3$. Flasks were placed on a shaking incubator at 70 rpm and 25 ± 1℃. The synthetic wastewater (300 mL) was replaced every day. After operated for 6 months, the shaking incubator was changed to 100 rpm at 15, 20, 25, and 30℃ to study the effect of temperature on denitrification rate (each temperature value was operated for about two weeks).

In the continuous experiment, the synthetic wastewater was mixed with activated sludge (with the final concentration of 800 mg/L MLSS) and then added into the column up to a height of 350 mm. The synthetic wastewater was pumped into the reactor at a flow rate of 4.1 mL/min (HRT = 2 h) from the bottom of column. When denitrification rate was stable, the flow rate was changed to 8.2 mL/min (HRT = 1 h) to study the effect of temperature (25 and 15℃) on denitrification performance.

2.4. Water absorption and leaching experiment

2.4.1. Water absorption experiment

THE BLENDS granules were dried in a vacuum oven at 50℃ to a constant weight. Then, 10 g granules were immersed in water at room temperature (20–28℃). The granules were taken out at certain intervals, removing the excess water on the surface with tissue paper, and weighed [23]. Absorption was calculated as the percentage of weight change.

2.4.2. Leaching experiment

THE BLENDS granules were dried in a vacuum oven at 50℃ until a constant weight was achieved. Then, 10 g granules were immersed in 100 mL distilled water in a 250 mL Erlenmeyer flask and stored at room temperature (20–28℃) in the dark. Samples were taken periodically from the Erlenmeyer flask to monitor DOC, and then 100 mL distilled water was changed [19].

2.5. Analytical methods

Water samples were taken and filtered through 0.45 μm membrane before analysis. The concentration of NO_3-N was determined by UV-spectrophotometer (Shimadzu UV-3100) at 220 and 275 nm. The concentration of NO_2-N and NH_3-N were assayed using hydrochloric acid naphthyl ethylenediamine spectrophotometry method and hypochlorite-salicylic acid spectrophotometry method, respectively [22]. DOC was measured using a total organic carbon (TOC) analyzer (HACH, IL530 TOC-TN). The morphology of the samples was observed using a SEM (Fei Quanta 200).

The denitrification rate in batch tests were calculated by the slope of total soluble oxidized nitrogen (NO_3-N + NO_2-N) over time. The denitrification rate R_{vd} of the continuous reactor is given by the Eq. (1):

$$R_{vd} = Q_D \times (C_{in} - C_{ef})/V \qquad (1)$$

where C_{in} is the initial NO_3-N + NO_2-N concentration (mg/L); C_{ef} is the effluent NO_3-N + NO_2-N concentration (mg/L); Q_D is the flow rate (L/h); and V is the reactor volume (L).

3. Results and discussion

3.1. Water absorption and leaching characteristics of the blends

The water absorption and leaching characteristics of THE BLENDS are shown in Figs. 1 and 2. There was a sharp uptake of water and the equilibrium absorption rate of 24.95% was reached in 1 d, which was much large than PCL (data not shown). The hydroxyl group in starch can form a hydrogen bond with water and thus starch/PCL blends absorbed more water. It was reported that water absorption of starch/PCL blends ranged from 9 to 16% with different starch contents [23], while di Franco [24] found

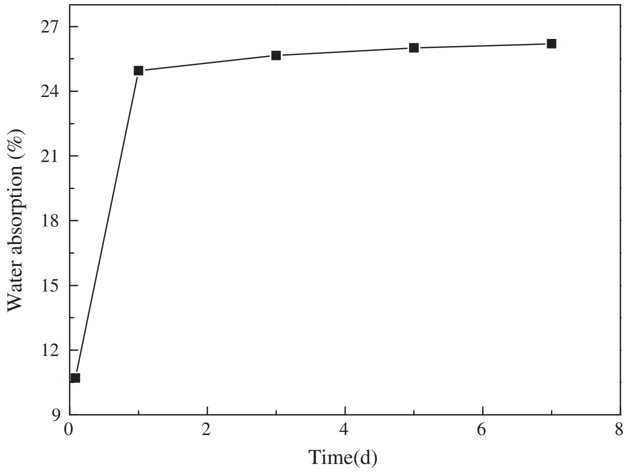

Fig. 1. Changes in water absorption of THE BLENDS over time.

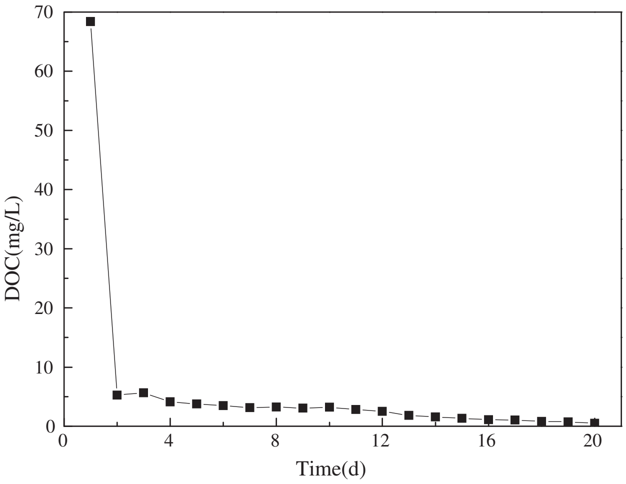

Fig. 2. Changes of DOC over time.

that PCL/starch blends (19.7 × 9.8 × 0.3 cm plate with 16% starch and 9% additives) absorbed around 21.5% water. This difference might be due to the specimen dimension and the amount of starch and additives in blends.

When THE BLENDS were immersed in distilled water, PCL was reported to be stable [24,25]. Thus, the main resource of DOC expected should be the leakage of the starch (Fig. 2). The DOC concentration was as high as 68.40 mg/L after 1 d leaching. This could be attributed to the release of starch attached on the surface of THE BLENDS. Then, DOC decreased sharply and became relatively stable.

3.2. Effect of temperature on denitrification performance

The effect of temperature on denitrification rate was carried out in both batch and continuous experiments after the biofilms on THE BLENDS were maturated. The batch experimental results were calculated and are shown in Table 2.

It was obvious that the denitrification rate increased with increasing temperature at the range of 15–30℃.

Table 2
Effect of temperature on denitrification rate in batch experiment

Temperature (℃)	Denitrification rate (mg N/L h)
15	5.79
20	7.47
25	8.67
30	9.81

The effect of temperature on denitrification rate can be described by Arrhenius equation as follows:

$$R_t = R_{20} 10^{K(t-20)} \tag{2}$$

where R_t is denitrifcation rate at t℃, R_{20} is denitrifcation rate at 20℃, K is temperature constant, and t is temperature.

Eq. (1) can be changed into the following form:

$$\log R_t = (t-20)K + \log R_{20} \tag{3}$$

The fitting results of denitrification rates and temperature are shown in Fig. 3. Temperature constant K was 0.0151 using THE BLENDS as solid carbon source, which was much smaller than K_{PCL} (0.068) in a batch denitrification experiment supported by PCL [14]. This result indicated that less significant influence of temperature on denitrification was observed for THE BLENDS than PCL, and it was probably due to the differences of biofilm and the inherent characteristics between THE BLENDS and PCL.

Fig. 4 shows the denitrification performance using THE BLENDS as carbon source and biofilm supporter in column experiment under different temperatures. It can be seen that the average NO_3-N removal efficiency reached 92.51% at 25℃, and below 1 mg/L NO_2-N concentration was observed. At 185 d, the temperature was switched to 15℃, then the average NO_3-N removal efficiency (68.69%) decreased sharply, meanwhile the average denitrification rate also decreased from 13.54 ± 0.90 (25℃) to 9.84 ± 1.19 mg N/(L h) (mean ± standard deviation),

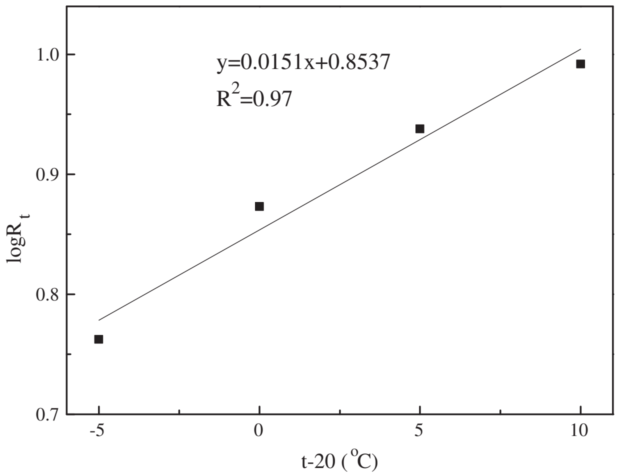

Fig. 3. Temperature constant of denitrification supported by THE BLENDS.

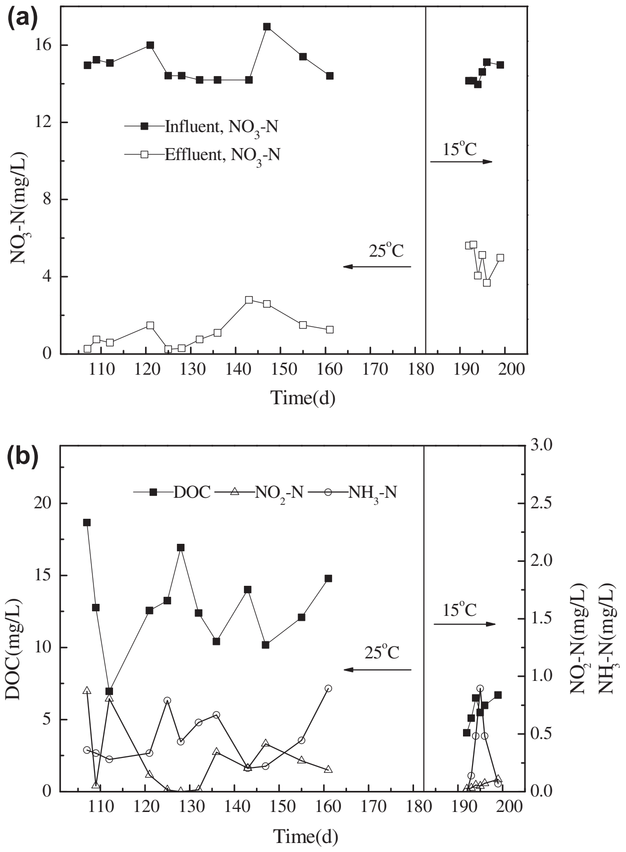

Fig. 4. Changes in (a) NO_3-N, (b) effluent NO_2-N, NH_3-N, and DOC concentrations over time with continuous column reactor system.

which was calculated using the concentrations of total soluble oxidized nitrogen species (nitrate + nitrite). Temperature is an important parameter influencing the denitrification performance. Denitrification rate would decrease when temperature is beyond the suitable range. In an upflow reactor packed with wheat straw as carbon source and carrier, nitrate removal efficiency decreased from 100 to 10% when temperature changed from higher than 20℃ to below that value [26]. The denitrification rate of 14℃ was approximately half of the rate observed at 30℃ in a cotton (cellulose)-dependent denitrification system [11]. The value of NO_2-N concentration was below 0.20 mg/L even at low temperature (15℃). There was a phenomenon of NH_3-N formation (below ca. 0.90 mg/L) during the whole 104 d operation period, which may be due to the dissimilatory nitrate reduction to ammonia (DNRA) process in which THE BLENDS acted as both e-donor and C-source, nitrate was e-acceptor, and ammonium, CO_2, and H_2O were the final products. The phenomenon of DNRA was also found in anaerobic sediments by Kelso [27]. Honda and Osawa [28] also found that a 0.1 mg/L NH_3-N increased in denitrification system using PCL as substrate.

Compared to 15℃, a higher accumulation of DOC was observed at 25℃ (Fig. 4). Under higher DOC, the denitrifiers can easily obtain carbon source for denitrification. Thus, a higher denitrification rate was obtained at 25℃ than at 15℃. The amount of accumulated DOC depends on the amount of released dissolved organic carbon and the need of microbes for both growth and denitrification. The enzymatic degradation process of starch/PCL blends should be affected by temperature. Usually, the biodegradation rate of polymer increased with increasing temperature under a suitable extent. Under a constant flow rate, the amount of released dissolved organic carbon at 25℃ should be higher than at 15℃. Though the need of organic carbon for both microbes growth and denitrification also increased with the temperature that increased from 15 to 25℃, the DOC in effluent was elevated approximately 100% (Fig. 4), indicating that the performance of the reactor was not fully utilized at 25℃.

3.3. SEM observation

THE BLENDS carriers used in this study were cylindrical granules Fig. 5(a). Biofilm attached on the surface of THE BLENDS granules comprised predominately of rod bacteria from SEM observation Fig. 5(b). Fig. 5(c) showed that fresh THE BLENDS display an irregular surface, which favored the attachment of bacterial cells on the surfaces of THE BLENDS granules. Generally, without deformed starch, granules were homogeneously dispersed throughout the PCL/starch blends as droplet-like particles [18]. The starch particles present in THE BLENDS showed a thermoplastic nature indicating that it underwent significant melting. Comparing the SEM images of fresh and used THE BLENDS, the later one Fig. 5(d) showed that its surface covered with pits and pores, the biodegradation was visible.

4. Conclusions

The cross-linked starch/PCL blends could serve as solid carbon source and biofilm support for denitrification, and the denitrification rate reached 13.54 ± 0.90 mg N/(L h) at 25℃. Nitrate removal efficiency depended on temperature. In batch tests, the effect of temperature on denitrification rate can be described by Arrhenius equation, and the temperature

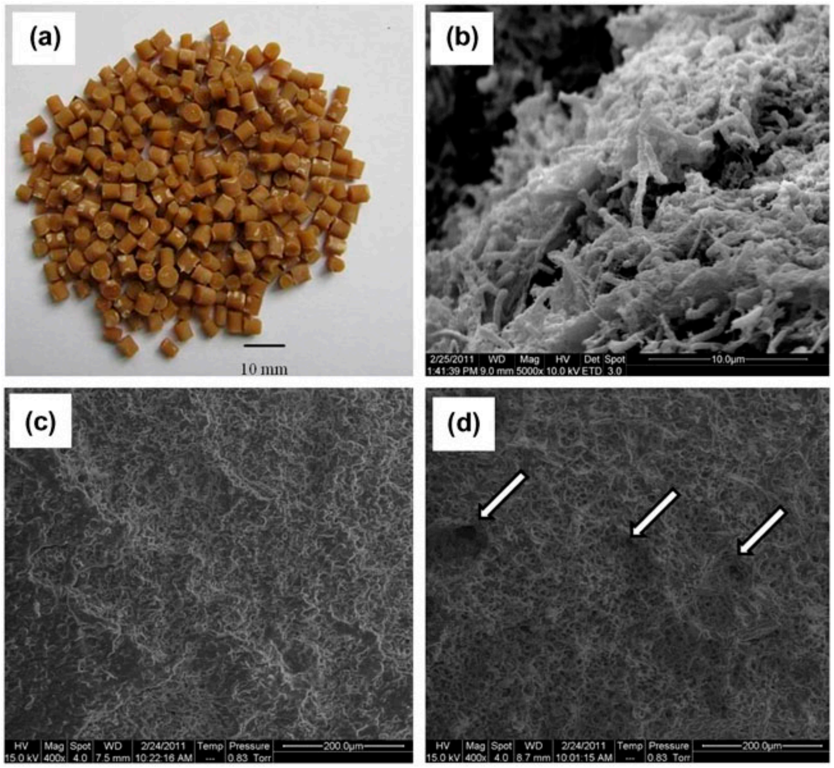

Fig. 5. Photograph of the fresh THE BLENDS (a), SEM images of biofilm attached on THE BLENDS (b), fresh THE BLENDS (c), and used THE BLENDS (d).

constant was 0.0151 at 15–30℃. However, nitrite accumulation was unconspicuous even under low temperature when nitrate reduction decreased. Besides, NH_3-N formation was observed during the whole column experiment, but below 1.0 mg/L. The main cost of "solid-phase denitrification" process depends on the price of solid carbon source. At present, this process is not a low-cost process due to the high price of synthetic polymers (such as PCL), blending PCL with cheap starch is a meaningful try to solve this drawback.

Acknowledgments

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