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ORIGINAL PAPER

Comparison of polycaprolactone and starch/polycaprolactone blends as carbon source for biological denitrification

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Abstract The cross-linked starch/polycaprolactone (SPCL10) and starch/polycaprolactone (SPCL12) blends were prepared, characterized and used as carbon source and biofilm support for biological nitrate removal. The results showed that SPCL10 and SPCL12 had similar performance on water absorption (about 21 %) and leaching capacity. FTIR spectra confirmed the cross-linking reaction between starch and PCL. SEM displayed a thermoplastic nature of SPCL10 and SPCL12. These blends could serve as solid carbon source and biofilm support for biological denitrification, and the acclimation time of microbial biofilm on the surfaces of SPCL10, SPCL12 and PCL were about 2 days, 2 days and 16 days, respectively. The average denitrification rates were 0.0216, 0.0154 and 0.0071 mg NO_3-N/(g h) for SPCL10, SPCL12 and PCL, respectively, and the effluent NO_2-N concentration was below 1 mg/L at all cases. The phenomenon of ammonia formation was observed, but ammonia concentration was below 0.5 mg/L.

Keywords Biodegradable polymer · Starch · Polycaprolactone · Nitrate · Denitrification

Introduction

Nitrate pollution is an important environmental problem in most area of China, and the situation was getting worse and worse (SEPA SEPA 2007). Among various methods available for nitrate removal, heterotrophic denitrification seems to be the most promising process. In this process, nitrate was reduced to nitrogen gas usually according to the following sequence: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. Commonly, heterotrophic denitrifying bacteria use methanol, ethanol, acetic acid or glucose as carbon source when organic carbon-limited water or wastewater was treated; however, there is a risk of overdosing and requires a sophisticated and costly process control. To avoid these problems, a new type of denitrification method has been designed in recent years, using insoluble biodegradable polymers as carbon source and biofilm carrier simultaneously, which is accessible only by enzymatic attack (Boley et al. 2000). There are two kinds of solid carbon sources, namely natural materials, including wheat straw (Aslan and Türkman 2005; Fan et al. 2012; Soares and Abeliovich 1998), cotton (Volokita et al. 1996a), waste newspaper (Volokita et al. 1996b), pine bark (Trois et al. 2010a, b), crab-shell chitin (Robinson-Lora and Brennan 2009) and synthetic polymers, such as polyhydroxyalkanoates (PHAs) (Hiraishi and Khan 2003), polycaprolactone (PCL) (Boley et al. 2000; Boley and Müller 2005; Wang and Wang 2009; Zhou et al. 2009; Chu and Wang 2011a, b; Shen and Wang 2011; Chu and Wang 2013; Shen et al. 2013a; Wu et al. 2013a), PBS (Wu et al. 2013b) and polylactic acid (PLA) (Wang and Wang 2012; Shen et al. 2013b). However, synthetic polymers are expensive, while natural materials were much cheaper but may bring ammonia (Robinson-Lora and Brennan 2009), high dissolved organic carbon (DOC) release and color problems

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(Aslan and Türkman 2003). Therefore, developing suitable biodegradable polymers is a key issue for extensive application of this new denitrification method.

Starch is an abundant renewable polysaccharide with better biodegradability and low cost, and it is also a most potential blending material for biodegradable plastics production or medical application. Usually, aliphatic polyesters are perfect materials to blend with starch, since they are biodegradable and thermoplastic with thermal stability, excellent mechanical properties, good water resistance and dimensional stability (Gupta et al. 2007). Some aliphatic polymers/starch blends have been investigated, such as PCL (Avella et al. 2000; Matzinos et al. 2002; Vertuccio et al. 2009), poly(butylene succinate) (PBS) (Zeng et al. 2011), poly(hydroxybutyrate-co-valerate) (PHBV) (Kotnis et al. 1995) and poly(lactic acid) (PLA) (Mihai et al. 2007; Yokesahachart and Yoksan 2011). However, these studies mainly focus on increasing mechanical properties, decreasing water absorption or restraining retrogradation of these biodegradable blends.

In this study, starch/polycaprolactone (SPCL12) and cross-linked starch/PCL (SPCL10) blends were prepared, characterized and used for biological nitrate removal in order to reduce the cost of solid carbon source. The water absorption and leaching capacity, FTIR and morphology were analyzed, and the feasibility and efficiency of these blends used for nitrate removal were studied in solid-phase denitrification process.

Materials and methods

Preparation of blends

The polycaprolactone (PCL) used in this study has a molecular weight of 60,000 g/mol (Dalton). Cross-linked starch was produced by using epichlorohydrin as cross-linking agent. The mixture of corn starch (10 g), distilled water (30 g) and NaCl (0.7 g) was put into a flask and then stirred mechanically. The pH of the slurry was adjusted to 9.50 using sodium hydroxide solution (0.5 M). Then, epichlorohydrin (0.8 mL) was added. The cross-linking reaction was carried out at 50 °C for 5 h under mechanical agitation. After that, the slurry was poured into 1 L distilled water, and then the cross-linked starch was collected by centrifugation. The precipitated product was washed several times with distilled water, dried overnight at 60 °C, and then ground into the fine uniform powders. Starch/PCL (SPCL12) and cross-linked starch/PCL (SPCL10) blends were prepared by twin-screw extruder. The main characteristics of SPCL10, SPCL 12 and PCL are as follows: starch—49, 49, 0 %; PCL—30, 30, 100 %; additives—21, 21, 0 %; carbon—45.35, 45.16, 59.97 % (detected by TOC analyzer); diameter—3, 3, 3 mm; height—4, 4, 3.5 mm; dry weight—0.0317, 0.0430, 0.0269 g/piece; specific surface area—17.35, 12.06, 17.52 cm^2/g.

Natural groundwater used in the experiment was obtained from Changping Campus, Tsinghua University (containing 6.5–13.6 mg/L NO_3-N, 28–35 mg/L Cl^-, 0.0–1.1 mg/L DOC, 7–15 CFU/100 mL, pH 7.4–7.9, NO_2-N and NH_3-N concentrations were below the detectable limit). Synthetic nitrate-contaminated groundwater was prepared by adding $NaNO_3$ and KH_2PO_4 into the natural groundwater to provide NO_3-N and P concentrations of about 50 and 10 mg/L, respectively. The DO and pH of synthetic wastewater were not controlled during the tests.

Denitrification procedures

Eighty grams of SPCL10, SPCL12 and PCL was added into different flasks, followed by the addition of 300 mL synthetic wastewater. The denitrifiers were enriched by the proliferation of indigenous microbes in the synthetic wastewater. Flasks were placed on a shaking incubator at 70 rpm and 30 ± 1 °C. The synthetic wastewater was replaced every day, and samples were collected daily to monitor the concentration of NO_3-N, NO_2-N and NH_3-N.

Water absorption and leaching experiment

Water absorption experiment

The granules were dried in a vacuum oven at 50 °C to a constant weight. Then, 10 g was immersed in water at room temperature. The samples were taken at certain intervals, removing the excess water on the surface with tissue paper and weighing (Mani and Bhattacharya 2001). Absorption was calculated as the percentage of weight change. The DOC was also monitored periodically.

Leaching experiment

The granules were dried in a vacuum oven at 50 °C until a constant weight. Then, 10 g was immersed in 100 mL distilled water at 250-mL Erlenmeyer flask and stored at room temperature in the dark. Samples were taken periodically from the Erlenmeyer flask to monitor DOC, and then, 100 mL distilled water was changed.

Analytical methods

Samples were filtered through 0.45-μm membrane. NO_3-N was determined by UV spectrophotometer (Shimadzu UV-3100) at 220 and 275 nm, and NO_2-N and NH_3-N were assayed by hydrochloric acid naphthyl ethylenediamine spectrophotometry method and hypochlorite–salicylic acid spectrophotometry method, respectively (SEPA 2002). DOC was measured using a TOC analyzer (HACH, IL530 TOC-TN), and the samples were neither acidified nor sparged during analysis. IR spectra were measured using an attenuated total reflection (ATR) method with Spectrum GX FTIR system (Perkin Elmer). The morphology of the sample was observed using a SEM (Fei Quanta 200).

N removal efficiency (N_{re}) is defined by the Eq (1):

$$N_{re} = 100 \times (NO_3 - N_{in} - NO_3 - N_{ef} - NO_2 - N_{ef} - NH_3 - N_{ef})/NO_3 - N_{in} \quad (1)$$

where NO_3-N_{in} is the influent NO_3-N concentration, and NO_3-N_{ef}, NO_2-N_{ef} and NH_3-N_{ef} are the effluent NO_3-N, NO_2-N and NH_3-N concentrations, respectively.

Results and discussion

Water absorption and leaching characteristics

The water absorption and leaching characteristics are shown in Figs. 1 and 2. There were sharp uptakes of water, and the equilibrium absorption rate reached 21.6 and 21.98 % in the first day for SPCL10 and SPCL12, respectively, which were much higher than PCL (0.4 %). The hydroxyl group in starch can form a hydrogen bond with water; thus, starch/PCL blends could absorb more water. No significant difference in water absorption was observed between SPCL10 and SPCL12. Water absorption of starch/PCL blends varied from 9 to 16 % with different starch content (Mani and Bhattacharya 2001). di Franco et al. (2004)found that PCL/starch blends absorbed around 21.5 % water.

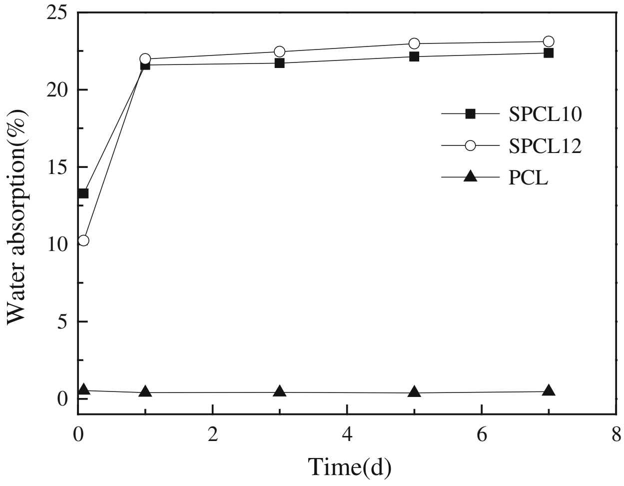

Fig. 1 Changes in water absorption of SPCL10, SPCL12 and PCL over time

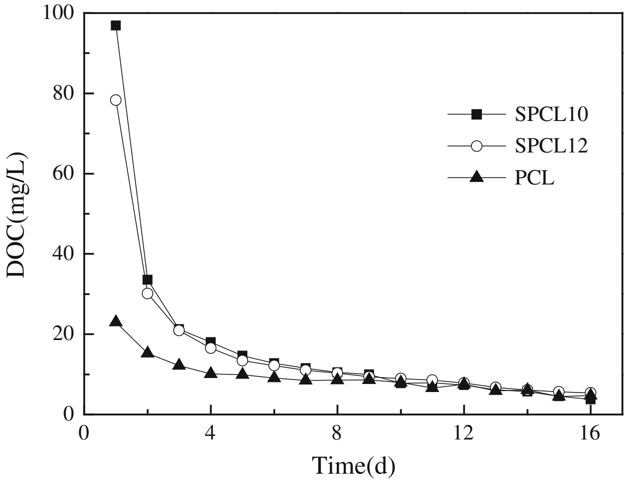

Fig. 2 Changes in DOC of SPCL10, SPCL12 and PCL over time

When starch/PCL blends were immersed in phosphate buffer (pH 7.2) and in the absence of microorganisms, PCL is reported to be stable (Bastioli 1998; di Franco et al. 2004). However, in this study, PCL also showed a release of DOC (Fig. 2), which might be related to the diffusion of low molecular weight compounds, such as additives or monomer. After 1-day leaching, a high release of DOC was observed for SPCL10, SPCL12 and PCL, and then the amount of released DOC decreased significantly. For starch/PCL blends, the DOC concentrations were higher than PCL until tenth day, after that the difference was negligible, indicating that besides the diffusion of low molecular weight compounds from PCL, the hydrolysis of the starch was also a main result for DOC release, especially in earlier period. The leaching performance of SPCL10 and SPCL12 was similar.

Characterization by FTIR and SEM

The FTIR spectra are presented in Fig. 3, which showed that the characteristic bands of the stretchings of CH groups and C = O groups were clearly present at 2,950 and 1,725 cm^{-1}, respectively. Furthermore, a band at 1,416 cm^{-1} is due to the –CH_2 scissoring vibration, and the bands at 1,463 and 1,368 cm^{-1} are due to the –CH bending of –CH_3 (symmetric) and OH in-place bending vibrations, respectively. In spectra for SPCL10 and SPCL12, the absorption peak at 3,340 cm^{-1} was assigned to the stretching of the OH groups in starch molecules. However, no adsorption peak was found in the 3,650–3,200 cm^{-1} region in PCL spectrum, implying that no OH group existed. In addition, the cross-linking reaction was characterized by comparing the FTIR spectra of both SPCL10 and SPCL12. In spectra of SPCL10, the characteristic absorption bands of starch at 1,020 and 1,160 cm^{-1}, which were due to C–O stretching vibrations (Jyothi et al. 2006), were increased in intensity than those of SPCL12. The band at about 1,022 cm^{-1} was sensitive to cross-linking, and its absorbance increased with the increase in cross-linking density (Ispas-Szabo et al. 2000).

A SEM morphological analysis is shown in Fig. 4. It is clear that PCL had a smooth surface, while the irregular nature of the starch granules was observed in SPCL10 and SPCL12, indicating that starch granules have been completely restructured, which suggested that the starch/PCL

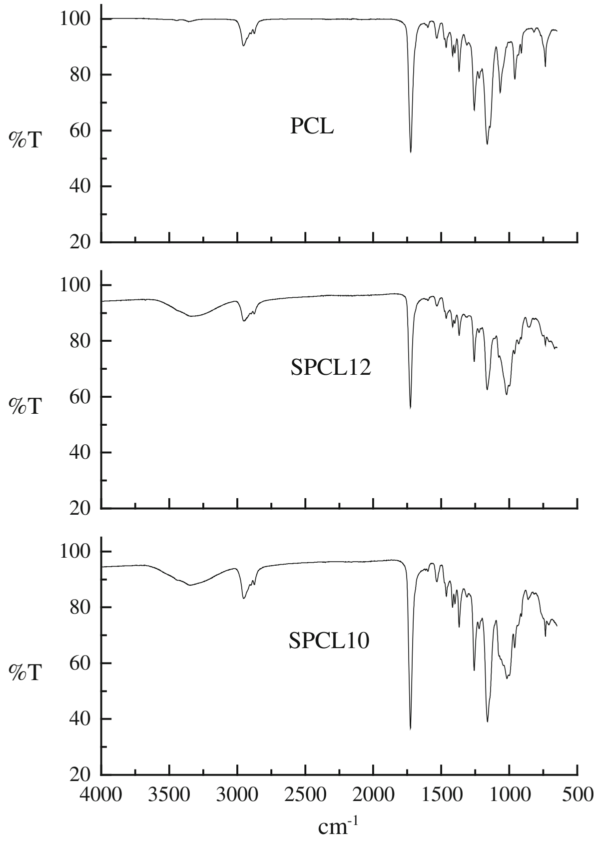

Fig. 3 FTIR spectra of SPCL10, SPCL12 and PCL

displayed thermoplastic nature. Generally, without deformation, starch granules are homogeneously dispersed throughout the PCL/starch blends as droplet-like particles (Avella et al. 2000; Ishiaku et al. 2002; Matzinos et al. 2002).

Denitrification performance during start-up period

The samples of SPCL10, SPCL12 and PCL were inoculated by the proliferation of indigenous microbes in groundwater, and the acclimation performances are depicted in Fig. 5, which showed the change in NO_3-N concentrations in effluent over time. The performances of SPCL10 and SPCL12 were similar, 50 mg/L NO_3-N could be completely removed on second day, and the removal efficiencies were above 97 %, indicating that starch PCL blends have a short acclimation time, and starch pretreated by cross-linking reaction has no significant influence on acclimation time. The acclimation time of SPCL10 and SPCL12 (both about 2 days) was shorter than that of a starch/PE (polyethylene) blends (15 days). PCL was an insoluble biodegradable polymer, which could not be utilized effectively by microorganism at the first several days of inoculation, so nitrate removal efficiencies were below 30 %. About 12 days later, the concentration of NO_3-N in effluent decreased rapidly (below 10 mg/L) due to the proliferation of denitrifiers, and the nitrate removal efficiency reached 87 % on the 16th day, indicating that the acclimation time of PCL was about 16 days, which was much longer than that of starch PCL blends. This was probably due to the difference of morphological characters and biodegradability between starch/PCL blends and PCL. It was clear that PCL blended with starch (SPCL10) could significantly increase the surface roughness (Fig. 4). Roughness of carrier is important for bacterial attachment. The number of attached cells increased with increase in the surface roughness. In addition, starch in starch/PCL blends is more biodegradable than PCL (Avella et al. 2000; di Franco et al. 2004), so the biodegradability of SPCL10 and SPCL12 was better than PCL. Therefore, the adhesion process of denitrifying bacteria on the surfaces of SPCL10 and SPCL12 was much easier than that on PCL, and the proliferation rate of the attached denitrifiers on SPCL10 and SPCL12 was much quicker than that on PCL. As a result, the acclimation time of SPCL10 and SPCL12 was shorter than PCL. The acclimation time of PCL in this study was similar to that obtained by Boley et al. (2000), but is shorter than that of PLA (40 days) (Fan et al. 2012). Surface hydrophobicity of carriers was also an influencing factor for cell attachment. Usually, bacterial cells are hydrophilic, and

Fig. 4 Scanning electron micrographs of surfaces of PCL, SPCL10 and SPCL12

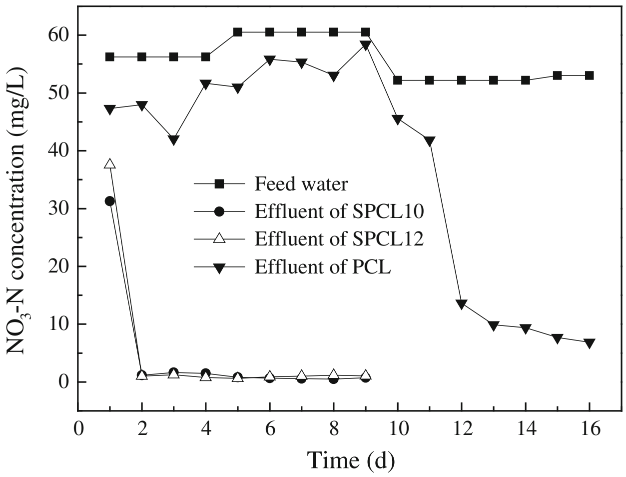

Fig. 5 Change in NO_3-N concentration as a function of time during the start-up period

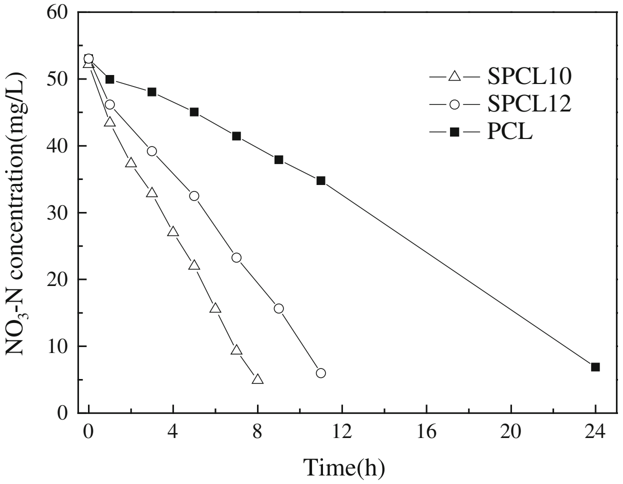

Fig. 6 Change in NO_3-N concentration as a function of time during denitrifacation

the number of attached cells increased with the increase in surface hydrophobicity of carriers (Lorite et al. 2011; Teixeira and Oliveira 1999). However, starch is hydrophilic material, and the presence of PCL led to a significant improvement of the material hydrophobicity (Averous et al. 2000), suggesting that the hydrophilic of SPCL10 and SPCL12 was better than that of PCL. In the present study, our results indicated that hydrophobicity plays a less important role than the morphological characteristics and biodegradability for denitrifying bacteria attachment and biofilm development during the start-up period.

Denitrification performance during stable period

By continuous acclimation of denitrifiers, the biofilms on SPCL10, SPCL12 and PCL were maturated. Figure 6 shows the change in NO_3-N concentration at stable period. It was found that there was a significant difference in denitrification rate among three solid carbon sources. The denitrification rate (weight-based) of SPCL10, SPCL12 and PCL was calculated to be 0.0216, 0.0154 and 0.0071 mg NO_3-N/(g h), respectively. It is clear that the denitrification rate of SPCL10 and SPCL12 was higher than that of PCL. The denitrification rate has a close relation with the biodegradability of solid carbon source (Hiraishi and Khan 2003). Starch is more biodegradable than PCL. Therefore, the biodegradability of SPCL10 and SPCL12 was better than PCL. Besides the biodegradability of solid carbon source, the specific surface area also affected the denitrification rate. The surface-based denitrification rate of SPCL10, SPCL12 and PCL was 13.21, 12.77 and 4.05 mg NO_3-N/(m^2 h), respectively, indicating that starch pretreated by cross-linking reaction has no significant effect on the surface-based denitrification rate. The volumetric denitrification rate almost doubles when the surface area of PHA granules used is doubled (Müller et al. 1992). It is clear that the difference of denitrification rate between SPCL10 and SPCL12 was probably due to the difference of their specific surface area, while the difference of denitrification rate between starch PCL blends and PCL was probably due to the difference of their biodegradability.

The comparison of denitrification rate of starch PCL blends, PCL and other solid carbon sources is summarized in Table 1. The weight-based rate of SPCL10 and SPCL12 was comparable to starch/PE blends.

After start-up period, the mature biofilm on the surface of SPCL10, SPCL12 and PCL was developed. The variation in NO_2-N and NH_3-N at stable period is listed in Table 2. In dissimilatory nitrate reduction process, nitrate is reduced at the sequence of $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$, which is defined as denitrification, while nitrate can also be reduced to ammonia by the process of dissimilatory nitrate reduction to ammonia (DNRA), which was conducted by fermentative bacteria. In the DNRA process, organic compounds were both electron donor and carbon source, nitrate was electron acceptor, and ammonium, CO_2 and H_2O were the final products. However, nitrate reduction to gaseous nitrogen was predominant over DNRA. The phenomenon of DNRA was also found in anaerobic sediments by Kelso et al. (1997). Honda and Osawa (2002) found that a 0.1 mg/L NH_3-N formed in denitrification system using PCL as substrate. In this study, the concentration of NH_3-N was <0.5 mg/L, which was the maximum contaminant level, set by "Standards for Drinking Water (GB 5749-2006)" of China. The NO_2-N concentration was also lower than the maximum contaminant level (1.0 mg/L) set by "Standards for Drinking Water (GB 5749-2006)" of China. NO_2-N is an intermediate of denitrification, and its formation and accumulation

Table 1 Denitrification rate for different solid carbon sources

Substances	Temperature (°C)	Weight-based rate [mg NO_3-N/(g h)]	Surface-based rate [mg NO_3-N/(m^2 h)]	References
SPCL10	30	0.022	13.456	This paper
SPCL12	30	0.016	13.267	This paper
PCL	30	0.007	3.995	This paper
PCL	30	0.038	42.77	Wang and Wang (2009)
PCL	13–30	0.0046–0.0283	N/A[a]	Zhou et al. (2009)
Biodegradable meal box	30	0.1584	52.80	Wang and Wang (2009)
PLA	30	0.0026	N/A	Fan et al. (2012)
Irradiated wheat straw	30	0.087	N/A	Fan et al. (2012)
Wheat straw	30	0.074	N/A	Fan et al. (2012)
Starch/PE blends	13–30	0.010–0.030	N/A	Zhou et al. (2009)
Liquorice	N/A	0.019	0.40	
Giant reed	N/A	0.018	0.415	
Crab-shell chitin	N/A	0.20	N/A	Robinson-Lora and Brennan (2009)

[a] *N/A* not available

Table 2 Change in NO_2-N and NH_3-N concentration during stable period

Time (h)	NO_2-N (mg/L)			NH_3-N (mg/L)		
	SPCL10	SPCL12	PCL	SPCL10	SPCL12	PCL
1	0.016	0.094	0.094	0.02	0.01	0.08
2	0.039	–	–	0.01	–	–
3	0.047	0.476	0.070	0.01	0.04	0.12
4	0.055	–	–	<0.01	–	–
5	0.070	0.593	0.016	0.02	0.04	0.07
6	0.102	–	–	0.03	–	–
7	0.008	0.515	0.062	<0.01	0.28	0.05
8	0.117	–	–	<0.01	–	–
9	–	0.374	0.593	–	0.02	<0.01[a]
11	–	0.008	0.445	–	<0.01	<0.01
13	–	0.008	–	–	<0.01	–
24	–	–	0.016	–	–	0.01

[a] The minimum detectable limits of NH_3-N is 0.01 mg/L

depended on many factors, such as types of carbon substrate, DO and pH (Gómez et al. 2002). For example, more nitrite was accumulated using sucrose as carbon substrate compared with methanol and ethanol.

Starch/PCL blends biodegradation and denitrification mechanisms

Biodegradation is the process by which organic substances are broken down by living organisms, polymer is firstly converted to its monomers, and then these monomers are mineralized (Shah et al. 2008). Starch/PCL blends are assumed to be completely biodegradable since each component in the blends is readily biodegradable. A number of studies have reported the biodegradation processes and mechanisms of starch, PCL and starch/PCL blends. PCL degradation is initiated with chain scission, followed by reduction in molecular weight and the liberation of low molecular weight organic acid, e.g., adipic acid (Benedict et al. 1983). When PCL immersed in enzyme lipase solution, the quantity of acid liberated was coincided with its biodegradability (Ishiaku et al. 2002). Microorganisms including bacteria and fungi secrete enzymes to biodegrade PCL. Tokiwa and Suzuki (1977) showed that PCL was hydrolyzed by lipases from various microorganisms. Oda et al. (1997)found that a strain B273 identified as *Alcaligenes faecalis* excreted lipases to hydrolyze PCL. Starch is a carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds. Amylase is hydrolase synthesized by microorganisms to hydrolyze starch into sugars.

PCL/starch blends were biodegraded almost completely both in compost and in activated sludge condition (Bastioli 1998). Though PCL undergoes a surface mechanism of degradation (Fukushima et al. 2010), the process of PCL/starch blends enzymatic degradation was bulk degradation, which is typically characterized by hydrolysis of chemical bonds in polymer chain at the center of the matrix, resulting in a highly porous material (Duarte et al. 2010). The enzymatic degradation process of starch/PCL blends was affected by different factors. Dry granular sago starch/PCL blends undergo higher weight loss compared to their thermoplastic starch/PCL counterparts after 18-h exposure to the enzyme glucoamylase, and the weight loss increased with increase in sago starch concentration because the enzyme selectively hydrolyzes the starch so enzyme activity increased with increase in starch content (Ishiaku et al. 2002). They believed that the tendency toward decreased hydrolysis of thermoplastic starch/PCL blends had been attributed to the de-structuring of the starch. However, Singh et al. (2003) reported that the inherent biodegradability of PCL/starch blends did not depend very significantly on the concentration of starch in the polyester matrix, but on the compatibilization efficiency. In the presence of compatibilizer, the starch was distributed well in the blends and covered by PCL, and also good interconnections between starch and PCL phases were observed (Avella et al. 2000). The biodegradability of starch is better than that of PCL. So, the inherent biodegradability of starch/PCL blends should be decreased when starch covered by compatibilizer. However, Avella et al. (2000) found that the addition of modified PCL as compatibilizer did not affect the biodegradability of PCL/starch blends. In this study, SPCL10 and SPCL12 are thermoplastic starch/PCL blends, while the denitrification rate of these blends was higher than PCL, indicating that the biodegradability of these blends probably better than PCL. Since the acclimation time and the surface-based denitrification rate of SPCL10 and SPCL12 were similar, starch pretreated by cross-linking reaction (SPCL10) has no significant influence on denitrification performance.

Since starch and PCL macromolecules are too large to pass through cellular membranes, so they had to firstly be hydrolyzed to small water soluble monomers, dimers, trimer, etc., which can be used as electron donors to reduce nitrate as well as carbon source for living cells. If the monomer unit of PCL (–$OCH_2CH_2CLH_2CH_2CH_2CO$–) was consumed by microbes, denitrification can be considered as follows (Honda and Osawa 2002):

$$6NO_3^- + C_6H_{10}O_2 \rightarrow 3N_2 + 6CO_2 + 2H_2O + 6OH^- \quad (2)$$

Theoretically, the amount of PCL required to remove 1 g nitrogen is 1.36 g.

As for starch, denitrification can be considered as follows (Shen et al. 2013a):

$$24NO_3^- + 5C_6H_{10}O_5 \rightarrow 12N_2 + 30CO_2 + 13H_2O + 24OH^- \quad (3)$$

Theoretically, the amount of starch required to remove 1 g nitrogen is 2.41 g. The contents of starch and PCL in SPCL10 (or in SPCL12) are 49 and 30 %, respectively. So the amount of SPCL10 or SPCL12 required to remove 1 g nitrogen is about 2.01 g.

Conclusion

Starch/PCL blends could be used as solid carbon source and biofilm support for nitrate removal from groundwater, and denitrifier can be enriched by the proliferation of indigenous microbes in the groundwater. The acclimation time of biofilm on the surfaces of SPCL10, SPCL12 and PCL was about 2 days, 2 days and 16 days, respectively. After start-up period, the average denitrification rate was 0.0216, 0.0154 and 0.0071 mg NO_3-N/(g h) for SPCL10, SPCL12 and PCL, respectively. PCL blend with starch or cross-linked starch can significantly shorten the acclimation time of biofilm and increase the denitrification rate. In the solid-phase denitrification system, the NH_3-N formation was observed, but the concentration was less than the maximum contaminant level (0.5 mg/L) set by "Standards for Drinking Water (GB 5749-2006)" of China. Starch pretreated by cross-linking reaction (SPCL10) has no significant influence on denitrification performance. Based on the start-up period, denitrification efficiency and cost, SPCL10 and SPCL12 are more suitable as carbon source for denitrification than PCL.

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