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Research review paper

Biological nitrate removal from water and wastewater by solid-phase denitrification process

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ABSTRACT

Nitrate pollution in receiving waters has become a serious issue worldwide. Solid-phase denitrification process is an emerging technology, which has received increasing attention in recent years. It uses biodegradable polymers as both the carbon source and biofilm carrier for denitrifying microorganisms. A vast array of natural and synthetic biopolymers, including woodchips, sawdust, straw, cotton, maize cobs, seaweed, bark, polyhydroxyalkanoate (PHA), polycaprolactone (PCL), polybutylene succinate (PBS) and polylactic acid (PLA), have been widely used for denitrification due to their good performance, low cost and large available quantities. This paper presents an overview on the application of solid-phase denitrification in nitrate removal from drinking water, groundwater, aquaculture wastewater, the secondary effluent and wastewater with low C/N ratio. The types of solid carbon source, the influencing factors, the microbial community of biofilm attached on the biodegradable carriers, the potential adverse effect, and the cost of denitrification process are introduced and evaluated. Woodchips and polycaprolactone are the popular and competitive natural plant-like and synthetic biodegradable polymers used for denitrification, respectively. Most of the denitrifiers reported in solid-phase denitrification affiliated to the family *Comamonadaceae* in the class *Betaproteobacteria*. The members of genera *Diaphorobacter*, *Acidovorax and Simplicispira* were mostly reported. In future study, more attention should be paid to the simultaneous removal of nitrate and toxic organic contaminants such as pesticide and PPCPs by solid-phase denitrification, to the elucidation of the metabolic and regulatory relationship between decomposition of solid carbon source and denitrification, and to the post-treatment of the municipal secondary effluent. Solid-phase denitrification process is a promising technology for the removal of nitrate from water and wastewater.

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1. Introduction

Increasing levels of nitrate in receiving waters have been becoming serious issues worldwide due to the intensive application of fertilizers and pesticides, and sewage irrigation. The high concentration of nitrate has the potential risks of eutrophication and toxic algal blooms in receiving waters (Ghafari et al., 2008). Nitrate is identified as one of the hazardous contaminants in drinking water because it can cause infantile methemoglobinemia (blue baby syndrome) and reduction of nitrate into nitrites in saliva might induce the formation of nitrosamines, which are known carcinogens (Matiju et al., 1992). The maximum admissible concentration limit of nitrate in drinking water is 10 mg L^{-1} as nitrate-nitrogen (NO_3-N) set by the US Environmental Protection Agency and 50 mg L^{-1} as nitrate by World Health Organization to reduce the risks to human health (Tsai et al., 2004).

Biological nitrification and denitrification is a very important topic in the field of water pollution control (Wang and Yang, 2004; Liu et al., 2005; Chen et al., 2006). Different technologies have been researched and developed for nitrate removal, including ion exchange, adsorption, membrane separation, electrodialysis, chemical denitrification and biological denitrification (Aslan and Turkman, 2003; Wang and Kang, 2005). Biological denitrification is conducted by denitrifying microbes which use nitrate as terminal electron acceptor, and organic and inorganic substances as electron donor and energy source for sustaining the microbial growth (Ines et al., 1998; Ghafari et al., 2008). There are two types of biological denitrification, heterotrophic and autotrophic. Autotrophic denitrifiers utilize hydrogen, iron or sulfur compounds as energy source and inorganic carbon compounds such as carbon dioxide and bicarbonate as carbon source (Karanasios et al., 2010). Heterotrophic denitrifiers which use organic carbon compounds as carbon source are the most common denitrifiers in nature (Van Rijn et al., 2006). Heterotrophic biological denitrification is considered to be more economically, practically on a large scale, and ultimately reduce nitrate to nitrogen gas with high selectivity (Ovez et al., 2006a; Schipper et al., 2010b). The traditional technique is to add the water soluble substances such as methanol, ethanol, acetic acid and glucose into the denitrification reactor (Modin et al., 2007; Bill et al., 2009). There are the risks of insufficient doses or overdosing that entails a deterioration of the effluent quality. A complex process control and continuous monitoring is demanded. Moreover, some liquid carbon sources such as methanol and ethanol have security risks during storage, transportation and operation owing to their toxicity and inflammability. Recently, solid-phase denitrification which uses solid substances involving natural plants-like materials and synthetic biodegradable polymers served as carbon source for denitrification and biofilm carriers, has proved to be a promising alternative to remove nitrate from water and wastewater (Hiraishi and Khan, 2003; Boley and Muller, 2005; Chu and Wang, 2016).

This paper presents overview of the application of solid-phase denitrification for nitrate removal from water and wastewater. The types of solid carbon sources commonly used, the parameters affecting denitrification rate such as temperature and dissolved oxygen (DO), the characteristics and microbial community of biofilm attached on the biodegradable carriers, the adverse effects of solid-phase denitrification and the cost of denitrification are introduced and evaluated. The challenge of this technique and future outlook are proposed. The present paper would be useful for researchers and engineers in the field of nitrogen removal from water and wastewater.

2. Solid-phase denitrification: principle and characterization

Fig. 1 illustrates the reaction mechanism involved in solid-phase denitrification process. The polymers used in this process are called solid carbon source, which can be natural or synthetic, they should be water insoluble and biodegradable. The solid carbon source was initially hydrolyzed by extracellular enzymes such as lipase excreted by the microbes in attached biofilm and then decomposed into soluble and small-molecular substrates. Most of the substrates are utilized by denitrifying microbes to act as electron donor to reduce nitrate to nitrite, nitric oxide, nitrous oxide and finally to nitrogen gas, which is the most likely and the favorite pathway. There is still another route of dissimilatory nitrate reduction to ammonium (DNRA). DNRA competes with denitrification and converts nitrate to ammonium rather than converting nitrate to N_2 (Van Rijn et al., 2006). It has been reported that DNRA is a minor process involved in nitrate removal and less than 4–10% of removed nitrate was attributed to DNRA (Gibert et al., 2008; Healy et al., 2012). In addition, there is possibility that some of substrates are anaerobically digested to produce methane instead of denitrification. In the presence of oxygen, a part of substrates might be degraded by aerobic biodegradation process (Boley and Muller, 2005), in which CO_2 and biomass are produced.

Since the solid carbon sources are accessible by denitrifying microbes only after decomposition, the amount of the released organic carbon is regulated by bacteria responding to nitrate levels in the aqueous phase. Therefore, the risks of overdosing or insufficient dose could be avoided. The control and supervision of the process is simple (Gutierrez-Wing et al., 2012). Nowadays, solid-phase denitrification has been applied for in-situ groundwater remediation, for the treatment of drinking water, groundwater and wastewater with a low C/N ratio, for the tertiary treatment of the secondary effluent and recirculating aquaculture system for nitrate removal.

3. Solid carbon source commonly used for denitrification

3.1. Types of solid carbon sources and their denitrification rate

There are two kinds of solid carbon sources available for solid-phase denitrification: the natural plant-like materials and synthetic biodegradable polymers. Table 1 presents the types of carbon sources, their advantages and drawbacks. The natural materials such as woodchips, straws and cottons are cheap and available, but the high release of dissolved organic carbon (DOC) and color was found in the effluent, especially during the start-up period (Volokita et al., 1996; Aslan and Turkman, 2004; Ovez, 2006; Xu et al., 2009; Robertson, 2010; Cameron and Schipper, 2012). The biodegradable polymers, including polyhydroxyalkanoate (PHA), poly-3-hydroxybutyric acid (PHB), poly-3-hydroxybutyrate-*co*-hyroxyvelate (PHBV), polycaprolactone (PCL), polybutylene succinate (PBS) and polylactic acid (PLA), are proved to be the suitable carbon sources for denitrification due to their low release of DOC, however their cost are relatively high (Honda and Osawa, 2002; Hiraishi and Khan, 2003; Walters et al., 2009; Zhao et al., 2009; Zhou et al., 2009; Shen and Wang, 2011; Takahashi et al., 2011; Wu et al., 2013b).

The carbon sources used as an electron donor for denitrification have influence on the conversion rate of nitrate to nitrogen. Table 2 illustrates the nitrate removal efficiency and denitrification rate using different

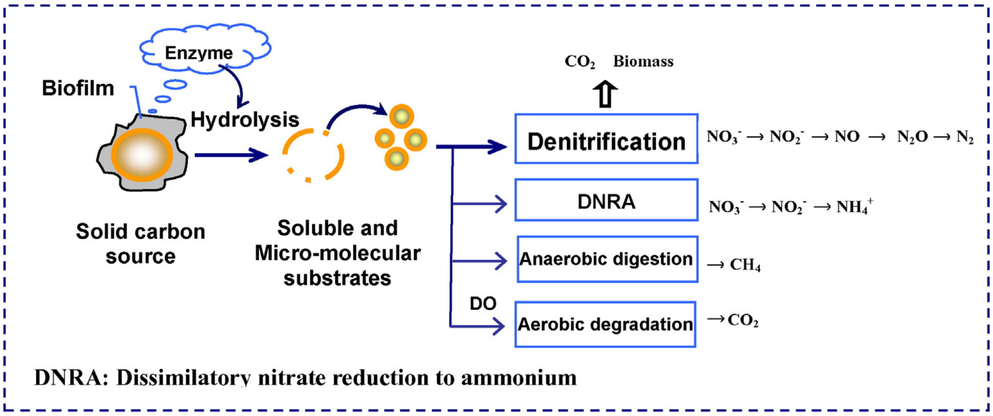

Fig. 1. Schematic diagram of solid-phase denitrification.

solid carbon sources. Denitrification rate (nitrate removal rate) was calculated as the difference between the nitrate concentrations in influent and effluent divided by hydraulic retention time (HRT). The wide range in the denitrification rate is most likely due to the differences in the types and bioavailability of carbon source, hydraulic conditions and operational parameters, such as the nitrate loading rate, reactor configuration and temperature (Ghafari et al., 2008). Generally, denitrification process using synthetic biodegradable polymers possess more consistent and higher nitrate removal rates than that using the natural organics substances.

Of the various natural plant-like materials used, woodchips are the most popular and considered to be attractive in practical application due to their lower cost, higher C/N ratio, longer duration of effectiveness and readily availability at moderate cost (Cameron and Schipper, 2012; Healy et al., 2012). Woodchips exhibited a long term (5–15 years) nitrate removal rate (1–20 g N m^{-3} d^{-1}) with minimal maintenance (Robertson, 2010). Warneke et al. (2011) demonstrated that wood materials had moderate and sustained nitrate removal with less adverse effects such as DOC and N_2O release, compared with other materials such as maize cobs and wheat straws.

Regarding the biodegradable polymers available, PHA and PHB are microbial storage materials and biodegradable by naturally occurring bacteria (Hiraishi and Khan, 2003). The synthetic polymers, such as PCL and PBS, have been used in agricultural film, package and the carriers for sustained-released drugs or pesticides, which were proved to be effective carbon source for denitrification. PCL is economically more attractive since the production cost of PCL is almost half of PHB (Hiraishi and Khan, 2003). The stoichiometric relationship describing the denitrification reaction using PHB and PCL as carbon source are as follows (Boley et al., 2000; Honda and Osawa, 2002):

$$0.494C_4H_6O_2 + NO_3^- \rightarrow 0.415N_2 + HCO_3^- + 0.130CO_2 + 0.169C_5H_7O_2N + 0.390H_2O \quad (1)$$

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

Table 1
Types of carbon sources commonly used in solid-phase denitrification and their characteristics.

	Solid carbon source	
	Natural plant-like materials	Synthetic biodegradable polymers
Carbon media	Woodchips (Robertson, 2010), wheat straws (Soares and Abeliovich, 1998; Fan et al., 2012), maize cobs (Cameron and Schipper, 2010), liquorices (Ovez et al., 2006b), pine needles (Healy et al., 2012), seaweed (Ovez et al., 2006a), newspaper (Volokita et al., 1996), sawdust, bark, leaf compost, barley straw (Healy et al., 2012)	PHAs (PHB, PHBV) (Khan et al., 2007; Gutierrez-Wing et al., 2012), PCL (Chu and Wang, 2013), PBS (Shen et al., 2016), PLA (Fan et al., 2012)
Advantages	Cheap, abundant in many countries	High and consistent denitrification efficiency and rate, low DOC release
Drawbacks	High release of DOC and color, low denitrification rate	High cost of denitrification

$C_4H_6O_2$ and $C_6H_{10}O_2$ represent the monomer unit of PHB and PCL. $C_5H_7O_2N$ is the general formula for the bacterial biomass with a yield coefficient of 0.45 g biomass g^{-1} PHB consumed. The theoretical amount of PHB and PCL consumed for removing 1 g NO_3-N is 3.03 and 1.36 g, respectively, which is comparable to the soluble carbon source. For methanol and ethanol commonly used, this value is 2.47 and 2.01 g g^{-1} NO_3-N, respectively (Matiju et al., 1992). The working amount of biopolymer consumed would be slightly higher than the stoichiometric values because of the consumption of residual oxygen in the water (Boley and Muller, 2005; Gutierrez-Wing et al., 2012). A part of biodegradable polymers might be decomposed by aerobic biodegradation instead of denitrification.

To reduce the cost and improve the bioavailability, blending biodegradable plastics with the cheap organic substances such as starch and bamboo powder has been developed (Tokiwa et al., 2009). Blending of starch with PCL, PBS, PLA and PHBV (Koenig and Huang, 1995; Tokiwa et al., 2009; Wu et al., 2015), and PCL/bamboo powder composite (Chen et al., 2009) were prepared to produce the environment-friendly biopolymer composite with low cost. Our research group studied the denitrification performance of PCL/starch blends (Shen et al., 2013b, 2015a, 2015b). The denitrification rate of PCL/starch blends was 2.1–3.0 times higher than that of PCL and a fast start-up of 2 days was observed for PCL/blends-packed reactor, compared to 16 d for PCL-packed reactor. But the initial DOC release was higher for PCL/blends. Zhang et al. (2012) found that the biodegradable plastic (60% starch and 30% polypropylene) showed higher nitrate removal efficiency and longer life-span than wheat straw and sawdust in groundwater remediation. Chu and Wang (2016) compared the denitrification performance of three kinds of biodegradable polymers PHBV, PHBV/starch and PHBV/bamboo powder (BP) blends in the packed-bed bioreactors to remove nitrate from groundwater. The experimental results showed that under the conditions without external inoculum, a fast start-up of 30–40 d was observed in bioreactors filled with both PHBV/starch and PHBV/BP blends and it took more than 3 months for PHBV reactor to reach the same loading rate. The PHBV/BP is considered to be the economically attractive carbon source with good denitrification performance, such as better nitrate removal efficiency and less adverse effects in nitrite accumulation and DOC release.

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Table 2
Denitrification performance of solid-phase denitrification system using different carbon sources.

Carbon sources	Influent	Influent nitrate conc. (mg L^{-1})	Temperature (°C)	Nitrate removal (%)	Denitrification rate (g N/L d)	References
Liquorices	Drinking water	100	20–4	40–100	0.167	Ovez et al. (2006b)
Giant reed			21–24	30–100	0.102	
Wheat straw	Drinking water	22.6		75–90	0.04–0.06	Soares and Abeliovich (1998)
Corncobs	Tap water with NO_3	24.5–25.5	27–33	56–90	0.203–0.125	Xu et al. (2009)
Soft wood	Groundwater	50		96–66	0.025–0.017	Gibert et al. (2008)
Woodchips	Drainage water	20–25	10		0.008–0.034	Moorman et al. (2010)
Woodchips	Synthetic water	3.1–48.8	21–23.5	60–100	0.009–0.023	Robertson (2010)
Woodchips	Groundwater	19.5–32.5	10	87	0.002–0.003	Healy et al. (2012)
Cardboard				95		
Pine needle				95		
Barley straw				75		
Woodchips	Drinking water	141–159	23.5		0.005–0.011	Cameron and Schipper (2010)
Wheat straw					0.0058–0.023	
Maize cobs					0.015–0.043	
Wood chips	Aquaculture wastewater	203.6 ± 10.6		99.7 ± 0.2	1.365 ± 0.039	Saliling et al. (2007)
Wheat straw				99.9 ± 0.05	1.361 ± 0.080	
PCL	Secondary effluent	25–35	18	88–99	1.23–3.80	Li et al. (2016a)
			8	20–31	1.23–1.67	
PCL	Synthetic water	55	25	70	0.64	Honda and Osawa (2002)
PCL	Groundwater	60–80	20–30	92–96	0.19–0.56	Chu and Wang (2013)
PCL	Tap water with NO_3	26–16	25	94	0.59–0.66	Wu et al. (2013a)
PCL/Starch	Synthetic water	50–15	25	90	0.54–0.64	Shen et al. (2013a)
PLA/Starch	Synthetic water	50	25	97	0.39	Wu et al. (2015)
PLA/PHBV	Synthetic water	50	30	96.8	0.16	Xu et al. (2011)
PBS	Synthetic water	15	25	95	1.71	Wu et al. (2013b)
			15	70	1.26	
PBS	Aquaculture wastewater	146	24	90	0.66	Zhu et al. (2015)
PBS	Synthetic water	50	25	98	0.60	Shen et al. (2016)
PHB	Aquaculture water	50			2.5	Gutierrez-Wing et al. (2012)
		220			8.0	
PHBV	Synthetic water	560	25		1.0	Khan et al. (2007)

3.2. Effect of physical and hydraulic properties

Studies have showed no significant difference in the nitrate removal rates using woodchips with different particle sizes. Also, no distinct difference was found between hardwood and softwood (Schipper et al., 2010b). Cameron and Schipper (2012) investigated the nitrate removal in denitrification beds filled with woodchips of four kinds of size: 4, 6, 15 and 61 mm, respectively. The denitrification rate ranged 0.004–0.005 g NO_3-N L^{-1} d^{-1} for all kinds of woodchips in 10–23 months of operation at 23.5 °C. They suggested that increasing temperature and carbon availability are more efficient ways to improve denitrification performance, rather than the hydraulic efficiency of the carbon source. The pore geometry of the solid carriers might affect the denitrification by capturing the initial degradation products in the biofilm. Gutierrez-Wing et al. (2012) reported that the more porous solid carbon materials might have harbored more fines in its pores and increase the surface availability for enzymatic degradation. It should be noted that the porosity of the carries is expected to change due to biodegradation. Robertson (2010) studied the nitrate removal using woodchips with varying age. The fresh chips had the highest denitrification rate of 15.4–23.0 mg N L^{-1} d^{-1}, while it declined to 12.1 and 9.1 mg N L^{-1} d^{-1}, respectively when using 2-year and 7-year old woodchips. The woodchips lost about 50% of their reactivity during the first year, but remained relatively stable degradation rates for a couple of years thereafter.

The denitrification rate almost doubled when the surface area of PHA granules used was doubled (Muller et al., 1992). Zhang et al. (2016) investigated the denitrification rate of PCL with different molecular weight (MW) of 60,000, 80,000 and 140,000 g mol^{-1}, and with different shape of pellet and cylinder. Generally, the biodegradability and denitrification rate increased with the decrease in MW. However, the PCL with cylinder shape, rough surface and highest MW exhibited the highest nitrate removal efficiency of 97.4%, compared to 96.7%, 75.1% and 63.2% with MW 60, 000, 80,000 and 140,000, respectively and pellet shape at a HRT of 2.0 h and influent nitrate concentration of 18.6 mg L^{-1}. They suggested that the irregular shape and rough surface which benefit the attachment of biofilm might play a more important role than MW in solid-phase denitrification.

Finally, it should be noted that since the solid substrates are degraded as carbon source, the usable organic compounds in the substrate will decrease with operation time. Therefore, renewal of the solid carbon source after a certain time becomes essential for the continuation of the solid-phase denitrification process. Saliling et al. (2007) evaluated the mass reduction rates of woodchips and wheat straw in denitrification process. The longevity of woodchips and straw was 1.0 and 0.5 year, respectively, if the organic carrier's life is 50% of its initial biomass.

4. Factors influencing denitrification performance

4.1. Temperature

Temperature is an important factor controlling the solid-phase denitrification by affecting the activity of the enzymes involved in both hydrolysis of the solid substrate and nitrate reduction. At a low temperature, both the efficiency of carbon source hydrolysis and the activity of the denitrifying bacteria decreased (Canziani et al., 1999), leading to a decrease in the denitrification rate.

Our previous study indicated that the denitrification rate declined sharply when temperature is below optimal. The average nitrate removal efficiency decreased from 92.5% (at 25 °C) to 68.7% (at 15 °C) when using PCL/starch blend as carbon source for denitrification (Shen et al., 2015a). We also found that the nitrate removal rate decreased by around 50% when temperature decreased by 5 °C when

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using PCL as carbon source for nitrate removal from groundwater. At the lower temperatures (10–15 °C), the TN removal was 82% on average even extending HRT to 13–17 h, compared to more than 92% of TN removal at HRT of 3–6 h at temperatures of higher than 20 °C (Chu and Wang, 2013). Cameron and Schipper (2010) reported that the nitrate removal rate increased by 1.7 times when temperature increased 10 °C in a denitrification bed filled with softwoods. The nitrate removal rate was 1.2–2.3 times higher at 23.5 °C than that at 14 °C during a long-term operation of 23 months when using different carbon substrates, including maize cobs, green wastes, wheat straw and softwood. However, a higher temperature might induce a faster microbial decomposition of solid carbon source, leading to higher release of DOC and ammonium (Cameron and Schipper, 2012).

4.2. Dissolved oxygen concentration

Most of the denitrifying bacteria are facultative anaerobes, which utilize nitrate as a terminal electron acceptor in the absence of oxygen (Van Rijn et al., 2006). Therefore, the presence of dissolved oxygen, which is a more energetically efficient electron acceptor, might suppress the denitrification process either by direct competition or by enzyme inhibition. In solid-phase denitrification system, there is anoxic microenvironment in the inner zones of biofilm due to the fact that oxygen might be consumed up by facultative bacteria during diffusion transportation. Many researches have shown that denitrification could occur when DO concentration was up to 4.0–5.0 mg L^{-1}, although the denitrification rate decreased with increase in DO levels (Gutierrez-Wing et al., 2012). Moreover, the presence of DO enables to increase the consumption of the carbon source since a portion of liable organic carbon was consumed by aerobic respiration rather than by denitrification (Boley and Muller, 2005; Gutierrez-Wing et al., 2012). Nitrite accumulation might occur because dissolved oxygen can inhibit the activity of the enzymes involved in nitrate reduction. Therefore, limiting the concentration of dissolved oxygen in the denitrification reactor seems to be unnecessary, but it could make the process more efficient.

Hiraishi and Khan (2003) reported that the denitrification rate of *Diaphorobacter nitroreducens* strain NA10B decreased with increase in DO concentration when using PHBV powders as carbon substrate, but it still maintained at more than 3 mg NO_3-N g^{-1} h^{-1} even under complete aerobic conditions. Gutierrez-Wing et al. (2012) found that the denitrification rate decreased from 5.5 to 0.5 g NO_3-N L^{-1} d when the DO levels increased from 0.5 to 4.0 mg L^{-1} in a recirculating aquaculture water system filled with PHB. When the DO levels were kept at 4–5 mg L^{-1}, a minimum denitrification rate of 0.18 g NO_3-N L^{-1} d^{-1} was observed for 6 days and then decreased to zero after that time. Xu et al. (2009) reported that the nitrate removal maintained at higher than 85% with increasing DO levels in the influent from 1.5 to 4.0 mg L^{-1}, but it decreased to 50% at higher than 4.0 mg DO L^{-1} in a solid-phase denitrification system using corncobs as carbon source.

4.3. HRT (superficial hydraulic velocity) and others

It is important to determine an appropriate HRT for a denitrification reactor because it is associated directly with the nitrate removal efficiency. The studies by Wang and Wang (2013) and Ovez et al. (2006b) showed that decreasing HRT to certain values led to an increase in effluent nitrate concentrations and nitrite accumulation. The studies by Gibert et al. (2008) revealed that the extent of nitrate removal was correlated to HRT in a denitrification permeable reactive barrier filled with woodchips for groundwater treatment. The removal efficiency of nitrate was more than 96% at a HRT of 6.6 d (a flow rate of 18 mL/h), whereas it decreased to 66% at a HRT of 1.6 d (a flow rate of 66 mL/h). Although increase in HRT might allow the bacterial population to degrade the organic substrate and lead to high nitrate removal efficiency, it is also responsible for high release of DOC and ammonium. During the design of a denitrification reactor, HRT should be optimized to get better denitrification performance.

Xu et al. (2009) investigated the effect of flow rate on denitrification in a reactor packed with corncobs. The denitrification rate increased when the flow rate increased from 8.5 to 153 L d^{-1}. However, the nitrate removal efficiency initially kept at more than 90% when the flow rate ranged 8.5–50 L d^{-1} and then declined sharply to 56% when the flow rate was higher than 50 L/d. Soares and Abeliovich (1998) reported that with the increase in water velocity from 0.03 to 0.12 m h^{-1}, the nitrate removal rate initially increased to the highest level of 0.053 g N L^{-1} d^{-1} at 0.05 m h^{-1} and then decreased gradually to 0.032 g N L^{-1} d^{-1}. Similar trend was observed in denitrification of drinking water using newspaper as carbon source (Volokita et al., 1996). The higher velocity might induce the detachment and washout of biofilm as well as washout of the solubilized substrate, leading to a decrease in nitrate removal.

Regarding other factors controlling solid-phase denitrification, high nitrate concentration in influent tends to increase the denitrification rate but reduce nitrate removal efficiency due to the lack of soluble carbon substrate compared to high nitrate loading, whereas low inputs of nitrate would result in denitrification being nitrate-limited (Xu et al., 2009; Schipper et al., 2010a; Ashok and Hait, 2015). Denitrification is positively related to pH with optimum values of 7.0–8.0 (Hiscock et al., 1991). Generally, pH in the range of 6.5 to 8.5 is suitable for solid-phase denitrification. In the case of the effect of influent salinity, Gutierrez-Wing et al. (2012) documented that the denitrification ability of PHB in a recirculating system was considered similar in all tested salinities varying between 0 and 30 g L^{-1}.

5. Application of solid-phase denitrification

5.1. Nitrate removal from drinking water and groundwater

As shown in Table 2, solid-phase denitrification using both the natural materials and the synthetic biodegradable polymers has been studied widely in nitrate reduction from drinking water and groundwater. The types of bioreactor commonly used include the packed bed (fixed bed), biofilter and fluidized bed. Most applications use packed bed bioreactor due to the operational simplicity and ease of control. The advantage of the fluidized bed reactor is that it could avoid the problems of clogging and channeling.

The solid-phase denitrification process for water treatment was initially developed from using the natural organic substances, such as straw, wood and cotton, etc. Soares and Abeliovich (1998) evaluated the biological denitrification of drinking water in an up-flow packed reactor using wheat straw as carbon source and biofilm carrier. The denitrification rate remained at 0.04–0.60 g N L^{-1} d^{-1} by replenishing straw periodically about 2 weeks. Healy et al. (2012) studied the denitrification of groundwater using different substrates, including pine woodchips, cardboard, pine needles and barley straw. The nitrate removal ranged 67–89% at steady-state period if pollution swapping was considered. The pine needle bioreactor showed the highest DOC release and carbon fluxes were highest for cardboard and straw bioreactors.

Recent researches demonstrated that synthetic biodegradable polymers including PHB, PCL, PBS and PCL/starch blends are good carriers and carbon source for denitrification. Mergaert et al. (2001) reported a PHBV-packed bed reactor for nitrate removal from drinking water, they found that the maximal surface-related denitrification rate was 14 mg N m^{-2} h^{-1} at 25 °C. We studied the nitrate removal from groundwater in a packed reactor filled with PCL, and found that the effluent nitrate concentrations were lower than 3.7 mg N L^{-1} at temperatures of higher than 24 °C and HRT of 3–6 h during a long-term operation of 561 d. Nitrite and ammonium remained at low levels (less than 0.32 and 0.78 mg N L^{-1}, respectively) (Chu and Wang, 2013). The studies by Wu et al. (2013b) showed that a nitrate removal

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of 95% was obtained using PBS as carbon source for denitrification of drinking water at a HRT of 0.5 h and temperature of 25 °C.

Furthermore, it is reported that nitrate and pesticides could be removed simultaneously from drinking water in the fixed bed reactor filled with PCL (Boley et al., 2003) and with wheat straw (Aslan and Turkman, 2004; Aslan and Turkman, 2005), respectively. The solid substrates could also act as adsorbent for the adsorption of pesticides, which were then biologically degraded in a co-metabolism under anoxic condition. The denitrification rate was not affected in a short-term operation of 1 month by adding pesticide endosulfan, but it decreased partly coupled with an inhibition of biomass production during a long-term operation of 6 months in PCL-packed reactor (Boley et al., 2003). When the concentrations of endosulfan ($\alpha + \beta$) increased from 1.0 to 1.5 mg L^{-1}, the nitrate removal decreased from around 100% to 70%, while the removal of endosulfan ($\alpha + \beta$) decreased from 65 to 70% to 14% in bioreactor filled with wheat straw at temperatures of higher than 20 °C. About 21.3% of endosulfan ($\alpha + \beta$) was removed by adsorption and 68.2% was removed by biodegradation (Aslan and Turkman, 2004). Pesticides trifluralin and fenitrothion were also reported to be removed effectively together with nitrate by solid-phase denitrification using wheat straw (Aslan and Turkman, 2005).

In addition to the application for ex situ treatment of groundwater in bioreactors, solid-phase denitrification has been also used for in situ groundwater remediation by direct treatment of groundwater into the aquifer without water extraction. In situ denitrification is a very attractive alternative due to the relative simplicity, low investment and operational costs, but it is difficult to control. The permeable reactive barrier is major technique for in situ groundwater treatment. The studies by Gibert et al. (2008) showed that softwood was the suitable carbon source for denitrification in a permeable reactive barrier, with a nitrate removal of more than 98% and a denitrification rate of 0.067 mg N L^{-1} d^{-1} g_{sub}^{-1}.

A coupling heterotrophic-autotrophic denitrification reactor (HAD) was developed for groundwater remediation, which were filled with cotton/zero-valent iron (ZVI) (Della Rocca et al., 2006; Della Rocca et al., 2007) and pine bark/sponge iron (Huang et al., 2015), respectively. ZVI was used to decrease DO level in water and produce cathodic hydrogen according to Eqs. (3) and (4). The low DO levels could favor the heterotrophic denitrification using cotton or pine bark as carbon source, and hydrogen production would allow the occurrence of autotrophic denitrification process. CO_2 generated by heterotrophic denitrification can be used as inorganic carbon source by the autotrophic denitrifiers. The nitrate removal rate by the HAD reactor filled with cotton/ZVI reached 0.235–0.275 g N L^{-1} d^{-1}, which was higher than that of the denitrification reactor using cotton alone (0.190 g N L^{-1} d^{-1}) (Della Rocca et al., 2006). Hydrogenotrophic denitrification contributed only 10%–20% of nitrate removal in HAD permeable reactive barrier to treat groundwater (Huang et al., 2015). In addition, Li et al. (2016b) reported that nitrate could be removed effectively in water by a HAD reactor filled with woodchips and sulfur.

$$2Fe(0) + O_2 + 2H_2O \rightarrow 4OH^- + 2Fe^{2+} \tag{3}$$

$$Fe(0) + 2H_2O \rightarrow H_2 + Fe^{2+} + 2OH^- \tag{4}$$

5.2. Denitrification for recirculating aquaculture system

In recirculating aquaculture system, ammonium is converted to nitrate by aerobic nitrification, while nitrate accumulation usually occurs, which limited the water reuse. The maximal concentration of nitrate was reported as high as 400–500 mg N L^{-1} in recirculating systems (Van Rijn et al., 2006). An aquaculture system coupled with a denitrification reactor packed with water insoluble carbon source could provide a stable water quality and potentially decreased the nitrogen output to natural water bodies (Boley et al., 2000; Gutierrez-Wing et al., 2012). Boley and Muller (2005) developed a fluidized bed reactor using PCL for denitrification in a recirculating aquaculture system breeding eels. Water is recirculated from the aquarium to the nitrification and denitrification reactors in series and returned to the aquarium. The nitrate concentration in the aquaria was low (3–10 mg N L^{-1}) by using this system, whereas it reached 200 mg N L^{-1} using the untreated control system. The concentrations of nitrite and phosphate also remained at low levels and the growth of fish was not affected. Saliling et al. (2007) evaluated the denitrification performance of the lab-scale packed reactors using woodchips and wheat straw respectively as carriers and carbon source to remove nitrate from synthetic aquaculture wastewater. Approximately 99% of nitrate removal was achieved when the influent nitrate concentration was 200 mg N L^{-1}. The concentration of effluent ammonium remained near zero and nitrite was around 2.0 mg N L^{-1}. Woodchips and wheat straw lost 16.2% and 37.7%, respectively during the 140 d of experiment. Zhu et al. (2015) studied the denitrification using PBS as carbon source for treating effluent from a recirculating aquaculture system (120–165 mg NO_3-N L^{-1}). The nitrate removal efficiency reached 70–99%, and nitrite concentration was maintained below 1 mg L^{-1}. In addition, the existence of salinity (2.5%) showed more stable denitrification performance, but caused adverse effects such as excessive effluent dissolved organic carbon (DOC) and dissimilation nitrate reduction to ammonia.

5.3. Treatment of wastewater with low C/N ratio and the secondary effluent

Solid-phase denitrification has been investigated as an alternative for treating wastewater with low C/N ratio such as reject water and biologically treated effluent. Walters et al. (2009) developed an airlift suspension reactor filled with PHB/PCL composite as carriers and external carbon source for the treatment of reject water with a low COD/N ratio. A nitrate removal of 75% was achieved through simultaneous nitrification and denitrification, in which denitrification took place in the pore structure of PHB/PCL composite by CLSM observation. Schipper et al. (2010a) investigated the nitrate removal from three kinds of effluent: dairy shed effluent (up to 350 mg NO_3-N L^{-1} with little organic N and ammonium), treated domestic effluent (up to 20 mg NO_3-N L^{-1}, 15 mg NH_4-N L^{-1} and 5.0 mg L^{-1} organic nitrogen) and glasshouse effluent (up to 225 mg NO_3-N L^{-1}, 80 mg NH_4-N L^{-1} and 25 mg L^{-1} organic nitrogen) using large-scale denitrification beds filled with woodchips. Nitrate was nearly completely removed for dairy shed effluent and domestic effluent, but nitrate removal reached only 40% for glasshouse effluent due to the high input nitrate concentration and high flow rate (150 m^3 d^{-1}). Denitrification was the major mechanism for nitrate removal and nitrate removal rates ranged 0.005–0.01 g N L^{-1} d^{-1} in the three denitrification beds, whereas the removal of ammonium, organic nitrogen, BOD and phosphorus was not observed in the systems. Li et al. (2016a) investigated nitrate removal from the secondary effluent of a municipal wastewater treatment plant using a biofilter packed with PCL. The influent nitrate concentration varied between 18 and 33 mg L^{-1}. The effluent nitrate concentration decreased to lower than 5.4 mg L^{-1} with the nitrate removal efficiency of 88–99% at 18 °C. The higher denitrification rate of 0.9–2.6 g N L^{-1} d^{-1} was attributed to the periodical backwash, thus clogging and channeling was avoided.

6. Microbial community of biofilm attached on biodegradable carriers

Biological denitrification is the reduction of nitrate to nitrogen gas through a sequence of enzymatic reactions. Many bacteria are capable of growing during this process. Analysis of the function and structure of the microbial community in the solid-phase denitrification system is necessary to provide a basis for their practical application to nitrate removal. It is interesting to note that the biofilm attached on the biodegradable carriers has a high activity with the VSS/TSS ratio of more than 90% (Chu and Wang, 2013, 2016).

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As shown in Fig. 1, in a solid-phase denitrification system two processes coexist, hydrolysis and denitrification. Hydrolysis of the solid carbon source by extracellular enzymes excreted by the degrading microorganisms is the first and important step and then the degradation products were utilized by denitrifying bacteria. Takahashi et al. (2011) revealed that the hydrolysis rate of PLA might be a key factor to affect nitrate removal since the denitrifying bacteria utilize the hydrolysates released from PLA as electron donor. Honda and Osawa (2002) reported that PCL can be decomposed almost completely by soil microbes such as *Penicillium* sp. 14–3 and *Penicillium* sp. 26-1 which could secrete a kind of lipase. Abou-Zeid et al. (2001) documented that some species isolated to degrade PHB, PHBV and PCL under anaerobic conditions belonged to genus *Clostridium* according to 16S rDNA analysis. Our recent study showed that under the conditions of without external inoculum, bacteria belonging to genus *Clostridium* in phylum *Firmicus*, which play the primary role in decomposing the biopolymers, are the most predominant in the packed reactors when using PHBV-based biopolymers as carbon source (Chu and Wang, 2016). Khan et al. (2007) constructed two solid-phase denitrification reactors, designated reactors A and B, for nitrogen removal by acclimating activated sludge with pellets and flakes of poly(3-hydoxybutyrate-*co*-3-hydroxyvalerate) (PHBV) as the sole added substrate under denitrifying conditions, respectively. Most of the predominant denitrifiers isolated quantitatively by the plate-counting method using non-selective agar medium were unable to degrade PHBV and were identified as members of genera of the class *Betaproteobacteria*. Most of the nirS and nosZ clones of the microbial community from reactor A were derived from members of the family *Comamonadaceae* and other phylogenetic groups of the *Betaproteobacteria*, suggesting that the efficiency of denitrification using PHBV was affected by the availability of intermediate metabolites as possible reducing-power sources as well as of the solid substrate, and that particular species of the *Betaproteobacteria* play the primary role in denitrification. Mergaert et al. (2001) studied the characteristics of the heterotrophic bacteria isolated from a fixed bed reactor treating well water using PHBV as biofilm carrier and electron donor for denitrification. Most of the isolates were able to reduce nitrate to nitrite or to denitrify using 3HB as sole carbon source. Only two groups, *Acidovorax facilis* and *Brevundimonas*-like strains, are capable of both degrading PHBV and denitrification.

Denitrifying bacteria belong to a genetically diverse and metabolically versatile group. Most of denitrifiers are facultative anaerobic bacteria, but denitrification could occur in the presence of dissolved oxygen for some species (Matiju et al., 1992). There have been a variety of studies characterizing the microbial ecology in solid-phase denitrification systems and some bacterial populations were isolated from the mixed cultures. Table 3 summarized the publications on the community of denitrifying bacteria using solid substrates as carbon source. It is clear that most of the denitrifiers reported in solid-phase denitrification specifically belong to family *Comamonadaceae* in the phylum *Proteobacteria*. The predominant genera are *Diaphorobacter*, *Acidovorax*, *Simplicispira* and *Comamonas*. In the denitrification process using soluble organic substances such methanol and ethanol, the genera *Pseudomonas*, *Paracoccus*, *Alcaligenes* and *Bacillus* comprise the important groups of denitrifiers (Blaszczyk, 1982; Lee and Welander, 1996; Neef et al., 1996).

Table 3
Publications on the major denitrifying bacteria in solid-phase denitrification system mostly at genus levels.

Carbon source	Denitrifying bacteria	References
PHBV/PHB	*Acidovorax facilis*, *Brevundimonas*, *Pseudomonas*, *Achromobacter*, *Agrobacterium*, *Phyllobacterium*, *Afipia*, *Stenotrophomonas*	Mergaert et al. (2001)
PHBV/PHB	*Diaphorobacter*	Khan and Hiraishi (2002)
PHBV/PHB	Family *Comamonadaceae*	Khan et al. (2002)
PHBV	*Acidovorax*, *Brachymonas*, *Comamonas*, *Diaphorobacter*, *Simplicispira*	Khan et al. (2007)
PHBV, PHBV/starch, PHBV/BP	*Comamonadaceae*, *Thiothrix*, *Microvirgula*, *Dechloromonas*, *Helicobacteraceae*	Chu and Wang (2016)
PHAs	*Acidovorax*, *Brevundimonas*, *Comamonas* sp., *Pseudomonas*	Hiraishi and Khan (2003)
PCL	*Alicycliphilus*, *Diaphorobacter*, *Simplicispira*, *Acidovorax*, *Dechloromonas*, *Azospira*, *Hydrogenophaga*, *Thauera*	Wu et al. (2013a)
PCL	*Diaphorobacter*, *Hydrogenophaga*, *Desulfovibrio*, *Simplicispira*, *Acidovorax*	Chu and Wang (2013)
PCL	*Acidovorax facilis*	Boley et al. (2003)
PCL, methanol, acetate, etc.	*Acidovorax avenae* subsp. *avenae* LMG 17238	Nalcaci et al. (2011)
PCL/Starch	*Diaphorobacter*, *Acidovorax Dechloromonas*, *Alicycliphilus*	Shen et al. (2013a)
PLA/Starch	*Alicycliphilus*, *Diaphorobacter*	Shen et al. (2013b)
PBS	*Diaphorobacter*, *Dechloromonas*, *Thauera*, *Alicycliphilus*, *Simplicispira*	Wu et al. (2013b)
PBS	*Simplicispira*, *Acidovorax*, *Azoarcus*, *Afipia*, *Comamonas*	Zhu et al. (2015)
Woodchips	*Thiobacillus denitrificans*, *Acidobacteriaceae* bacterium, *Hydrogenophaga* sp., *Comamonas* sp.	Li et al. (2016b)

Khan et al. (2002) reported that *Betaproteobacteria*, especially members of the family *Comamonadaceae* were the major PHBV-degrading denitrifiers isolated from activated sludge. Takahashi et al. (2011) documented that bacteria belonging to the family *Comamonadaceae* enriched highly and played a primary role in PLA-based denitrification reactor. Khan and Hiraishi (2002) isolated denitrifying strains belonging to genus *Diaphorobacter* in the family *Comamonadaceae* from activated sludge, which were capable of denitrification under aerobic and anaerobic conditions by using PHB and PHBV as carbon and energy source. Khardenavis et al. (2007) reported that both nitrate and nitrite reductase were present in the eight strains of *Diaphorobacter* isolated from the activated sludge for treating wastewater from chemical and dye industries. Nalcaci et al. (2011) observed that the bacterial strain *Acidovorax avenae* subsp. *avenae* LMG 17238 could utilize different carbon sources, such as ethanol, methanol, sodium acetate, glucose and PCL, to reduce nitrate to N_2 gas from drinking water. Our previous study revealed that bacteria of genera *Diaphorobacter Hydrogenophaga*, *Rhodocyclaceae uncultured and Desulfovibrio* were highly enriched in the PCL biofilm based on the pyrosequencing analysis (Chu and Wang, 2013).

The abundance of denitrifying community could be estimated by determining the copy numbers of nitrite reductase (*nirS* and *nirK*) and nitrous-oxide reductase (*nosZ*) functional genes. Warneke et al. (2011) studied the nitrate removal in denitrification reactor using different solid carbon source. Woodchips exhibited a high abundance of nitrite reductase genes as a proportion of total bacterial DNA (16S rRNA), while maize cobs had a low copy number of denitrification genes as a proportion of total bacteria. These results demonstrated that a large amount of carbon source released from woodchips was utilized by denitrifiers, whereas a substantial proportion of carbon source released from maize cob was likely consumed by non-denitrifying bacteria, fungi and/or yeasts. The ratio of *nirS*/*nirK* and *nirS*/*nosZ* varied with carbon sources, indicating that the compositions of denitrifiers might be distinct for different carbon substrates. Analysis of *nirS* and *nosZ* gene clone library of a PHBV-based denitrification process by Khan et al. (2007) revealed that most of the *nirS* and *nosZ* clones belonged to the members of family *Comamonadaceae* and other phylogenetic groups of the *Betaproteobacteria*.

7. The potential adverse effect of solid-phase denitrification and cost evaluation

The potential adverse effect, including the release of DOC and the ammonium formation, nitrite accumulation and emission of greenhouse gas such as N_2O and CH_4, should be taken into account for the

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practical application of solid-phase denitrification process (Ashok and Hait, 2015).

7.1. Leaching of DOC and ammonium and nitrite accumulation

The leaching of DOC from the biodegradable polymers is necessary for microbial growth and denitrification. However, if the release of DOC exceeded the amount needed by microorganisms for denitrification and growth, DOC would accumulate. In addition, the soluble microbial products (SMP) released during microbial metabolism also lead to an increase in the effluent DOC. We studied the nitrate removal from groundwater using PCL as carbon source for denitrification and observed that the effluent DOC remained at 1.7–5.2 mg L^{-1}, which was related to the protein-like and SMP-like substances (Chu and Wang, 2013). There are two known dissimilatory nitrate-reducing pathways: denitrification and DNRA which leads to increase in ammonium concentration in the effluent (Van Rijn et al., 2006). DNRA is a minor process involved in nitrate removal. Greenan et al. (2006) added 15N-labbed nitrate into the denitrification biofilter reactors filled with different woodchips and observed that DNRA accounted for less than 4% of total nitrate removal. Similarly, Gibert et al. (2008) concluded that less than 10% of nitrate removed was attributed to DNRA. Anaerobic environments with high ratio of organic carbon to nitrate-nitrogen tended to favor the DNRA organisms over denitrifiers (Van Rijn and Barak, 1998). Nitrite is an intermediate during nitrate reduction. Nitrite accumulation occurs due to the incomplete denitrification which might be induced by high influent nitrate and DO levels, low temperature and HRT (Shen et al., 2013b; Wu et al., 2013a).

The release of DOC and the ammonium formation were related to the types of carbon sources and the operation conditions, such as temperature, HRT and operation time. It is known that the concentration of effluent DOC was lower when using biodegradable polymers for denitrification than using natural plant-like materials. A small increase in DOC of 3.0 mg L^{-1} was observed in a packed reactor filled with PCL (Honda and Osawa, 2002). Cameron and Schipper (2010) documented that the leaching concentration of DOC and ammonium increased at start-up period and declined during 2–6 month of operation for all tested carriers, including woodchips, straws and maize cobs in denitrification beds, while generally leaching of DOC and ammonium was higher at 23.5 °C than at 14 °C. A higher release of DOC and ammonium during the start-up period of denitrification reactors was also observed in our previous research (Shen et al., 2013b). Typically, a higher HRT seems to favor a greater dissolution of carbon source and ammonium production (Robertson and Cherry, 1995; Healy et al., 2012). Our research showed that the effluent DOC decreased from 9.4–26.8 mg L^{-1} to 3.1–13.1 mg L^{-1} as the HRT decreased from 1.0 h to 0.5 h when using PCL as carbon source for denitrification (Wu et al., 2013a), suggesting that the selection of appropriate carbon source and critical design of HRT and the operational temperature are important to minimize the leaching concentration of DOC and ammonium formation. In general, the denitrification process, including using the soluble ethanol and methanol as extra carbon source, requires additional treatment to prevent the deterioration of the effluent quality. The treatment methods commonly used for drinking water, such as adsorption by granular activated carbon, are efficient to polish the effluent (Soares and Abeliovich, 1998; Karanasios et al., 2010).

7.2. Emission of greenhouse gas such as N_2O and CH_4

Ideally, nitrate was converted totally to N_2 in a denitrification bioreactor. In some cases incomplete denitrification resulted in the production of N_2O. The lower temperature, the carbon substrate with lower reaction rates and the presence of higher DO would lead to less complete nitrate removal, resulting in N_2O emission in the outflow (Elgood et al., 2010). Anaerobic degradation of the organic carbon source resulted in the production of CO_2 and possibly CH_4. It is likely that methanogenes could develop in the inner part of the biofilm attached on the solid substrate when the microbial consumption of nitrate exceeded its diffusion into the biofilm. The fluxes of CH_4 was low when nitrate remained sufficiently high to suppress methanogens, while the methanogenic bacteria enable to compete with denitrifiers for carbon source once nitrate was depleted in the bioreactor (Schipper et al., 2010b).

Elgood et al. (2010) reported that the dissolved N_2O and CH_4 production was 6.4 μg N L^{-1} (2.4 mg N m^{-2} d^{-1}) and 974 μg C L^{-1} (297 mg C m^{-2} d^{-1}), respectively in a stream of denitrifying bioreactor filled with woodchips for treating agricultural drainage. Dissolved N_2O production was observed during the winter and spring months and the overall production was only a small fraction (0.6%) of the nitrate amount consumed, whereas CH_4 production was higher during the warmer months when nitrate removal was relatively complete. One way to limit CH_4 production is to optimize the HRT to ensure that nitrate was removed only just as it exited in the bioreactor (Healy et al., 2012).

The types of carbon sources play an important role in the production of N_2O and CH_4. Warneke et al. (2011) investigated nitrate reduction in denitrification beds using a variety of carbon sources, including different kinds of woodchips, maize cods, sawdust and wheat straw etc. The results showed that the dissolved N_2O concentration in the effluent ranged from below detection limit for sawdust to 214.5–1472.5 μg L^{-1} for wheat straw at temperatures of 16.8–27.1 °C. Almost 10% of the removed nitrate was converted to the dissolved N_2O in the wheat straw bioreactor at warm temperature. Regarding to CH_4 production, there was little net dissolved CH_4 production in woodchips and sawdust bioreactors. For wheat straw and maize cobs bioreactors, the dissolved CH_4 concentration in the effluent ranged from 139 to 1201 L^{-1} to 7375–10,600 μg C L^{-1}, respectively at 16.8 °C, while less dissolved CH_4 was detected at 27.1 °C. Moorman et al. (2010) found that the dissolved N_2O concentration was 13.5–73.2 μg N L^{-1} in a woodchips denitrification bioreactor to treat drainage water during a 9-year study, it accounted for 0.62% of the nitrate removed. Healy et al. (2012) studied the nitrate removal using different organic carbon media in laboratory denitrification bioreactors, and found that greenhouse gas emissions were dominated by CO_2 and CH_4 fluxes with little N_2O in denitrifying bioreactors at 10 °C. The released N_2O in the gas fluxes was less than 0.6 mg N m^{-2} d^{-1} for bioreactors containing cardboard, pine needles and barley straw and 1.45–2.5 mg N m^{-2} d^{-1} for woodchips bioreactor at steady state of operation. The CH_4 emission was 3.5–6.5 g C m^{-2} d^{-1} for cardboard bioreactor and less than 0.36 g C m^{-2} d^{-1} for woodchips bioreactor, respectively. The proportion of CH_4 in the total C flux ranged 20–47%. There are not information on N_2O and CH_4 emission using biodegradable polymers as carbon source for denitrification.

7.3. Cost evaluation

The cost of denitrification is an important issue to be considered for the application of solid-phase denitrification process. The estimated cost on the basis of the publications for nitrate removal using different carbon sources was shown in Table 4. The cost of denitrification was calculated according to the required amount of substrate for denitrification and the unit price of the substrate. The consumption of substrate was lower for natural materials, which remained at the same order of magnitude for biodegradable polymers and soluble carbon sources. As shown in Table 4, among the natural materials, giant reed had the lowest cost. The cost of using PCL and PHBV was 2–7 times higher than that using methanol and ethanol, but it was as the same as using acetic acid. The PHBV/BP composite is more cost effective than other biodegradable polymers, which is attractive for practical application. It should be noted that the cost estimation is based on the production cost of substrates and does not cover other expense for the conventional system such as the cost of process control. Taking into account of the high efficiency of denitrification, the biodegradable polymers would become a competitive carbon source for solid-phase denitrification in practice if their

Table 4
Estimated costs of denitrification using different carbon sources.

Substrate	Price of substrate (€/kg)	Consumption of substrate (kg/kg NO_3-N)	Cost of denitrification (€/kg NO_3-N)	References
PCL	4.1–5.0	1.3–1.8	5.4–8.9	Boley et al. (2000); Chu and Wang (2016)
PHBV	4.6	1.49–1.65	6.9–7.6	Chu and Wang (2016)
PHBV/Starch	2.5	2.08–2.60	5.1–6.4	
PHBP/BP	2.4	1.69–1.86	4.0–4.4	
Liquorice	8.0	0.29	2.4	Nalcaci et al. (2011)
Giant reed	0.5	0.94	0.5	Nalcaci et al. (2011)
Methanol	0.4–1.0	2.08–3.98	1.0–4.0	Boley et al. (2000); Chu and Wang (2016)
Ethanol	1.2	2.0	2.4	Boley et al. (2000)
Acetic acid	1.4	3.5	8.0	Boley et al. (2000)

price could be reduced to a certain extent. Moreover, in some cases, such as the treatment of the sensitive aquaculture wastewater, the solid-phase denitrification with biodegradable polymers might be appropriate without the risk of the storage and toxicity of methanol.

8. Concluding remarks and future perspectives

Solid-phase denitrification is a promising technique to remove nitrate from a variety of water and wastewater, including drinking water, groundwater, aquaculture wastewater, secondary effluent and wastewater with low C/N ratio. The types of carbon sources, temperature, DO levels and HRT play important roles in determining the denitrification rate and effluent quality, including the release of DOC and ammonium formation, nitrite accumulation and production of N_2O and CH_4.

The selection of an appropriate carbon source is a tradeoff between their denitrification rate, availability and cost. Although a variety of natural materials were investigated for denitrification, woodchip would be attractive in practical application due to its low cost, long life-span of effectiveness and less adverse effects. Compared with the natural plant-like substrates, the synthetic biodegradable polymers exhibited better and stable denitrification performance. The availability of the cheap biodegradable polymers such as the wastes of the bioplastic industry is determinant for its application in commercial denitrification process. Studying the mass reduction rate of solid carbon materials to determine their renewal period is necessary in the continuation of the solid-phase denitrification. The post-treatment to remove the released DOC from the solid-phase denitrification needs to be further investigated for its practical application in drinking water. To date, solid-phase denitrification technique has been studied mostly on the laboratory scales except for in-situ groundwater remediation. The fact that a little research was reported on the performance of solid-phase denitrification in commercial systems is another drawback for its full-scale application.

Regarding the bacterial community, most of the denitrifiers reported in solid-phase denitrification process specifically affiliated to the family *Comamonadaceae* in the class *Betaproteobacteria*. The popular genera were *Diaphorobacter*, *Acidovorax*, *Simplicispira* and *Comamonas*. The bacteria belonging to genus *Clostridium* are capable of degrading the biodegradable polymers under denitrification conditions. Elucidating the metabolic and regulatory relationship between degradation of solid carbon source and denitrification, specifying the depolymerizing enzyme systems for degrading biodegradable polymers under denitrification conditions, and identifying the denitrifying bacteria using the natural plant-like carbon source are important subjects for the future studies.

Recently, the occurrence of the emerging contaminants in groundwater and the biological treated effluent, such as pharmaceutical and personal care products (PPCPs), has received increasing concerns. The simultaneous removal of nitrate and PPCPs form these waters by solid-phase denitrification should be paid more attention to guarantee the ecological safety and human health.

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