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Characterizations of dissolved organic matter and bacterial community structures in rice washing drainage (RWD)-based synthetic groundwater denitrification

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HIGHLIGHTS

- Denitrification rate reached 64.1 mg NO_3^--N·(gVSS·h)$^{-1}$ in RWD-based system.
- FEEM results indicate sludge inoculation increased DOM generation.
- Tryptophan protein, aromatic protein and humic acid-like substances were main DOMs.
- Minimization of DOM was achieved with RWD as sole carbon and microbe source.
- Microbial community in both inoculated and un-inoculated systems was unveiled by Illumina sequencing.

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ABSTRACT

In this study, characteristics of dissolved organic matter (DOM) and bacterial community structure in rice washing drainage (RWD)-based groundwater denitrification systems inoculated with and without seeding sludge were investigated. Complete nitrate removal was achieved with a maximum denitrification rate of 64.1 mg NO_3^--N·(gVSS·h)$^{-1}$. Analysis of three-dimension fluorescence excitation-emission matrix (FEEM) identified three main compositions of DOM associated with tryptophan protein-like, aromatic protein-like, and polycarboxylate humic acid-like substances in the inoculated system, while one composition associated with tryptophan protein-like substance in the un-inoculated system. Illumina sequencing analysis revealed a distinguished bacterial community structure in two systems over time. Notably, the microbial diversity was significant lower in the un-inoculated system than that in the system inoculated with seeding sludge. The predominant phyla shifted from Proteobacteria (49.2%), Bacteroidetes (20.5%) and Chloroflexi (14.8%) in the seeding sludge to Bacteroidetes (56.3%) and Proteobacteria (37.7%) after the RWD addition in the inoculated system. With RWD as sole microbe source, temporal changes in the bacterial structure from Proteobacteria (99.4%) and Bacteroidetes (5.3%) to Proteobacteria (88.8%) and Bacteroidetes (10.3%) were observed in the un-inoculated system. Specific comparison down to the genus level showed the dominant denitrifying bacteria of *Thiobacillus*, *Anaerolineaceae* and *Methylophilaceae* in the seeding sludge. *Ideonella*, *Cloacibacterium* and *Enterobacter* were dominant after the RWD addition in the inoculated system, while *Stenotrophomonas* and *Enterobacter* were dominant genera when RWD as sole bacteria source in the un-inoculated system. This finding indicates that both RWD addition and inoculation had strong impacts on bacterial community structure.

1. Introduction

Nitrate pollution is a profound ongoing groundwater problem in many parts of the world (Hosono et al., 2014). Biological denitrification with high efficiency and cost-effectiveness is regarded as an

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appropriate technology for the nitrate elimination (Moussavi et al., 2015). Heterotrophic denitrification can reduce nitrate to harmless nitrogen gas using organic carbon as electron donors (Fernández-Nava et al., 2010; Hosono et al., 2014). However, organic carbon in groundwater is often stoichiometrically insufficient to achieve complete denitrification (Zhang et al., 2012). Therefore, a wide range of carbon substrates have been trialed to improve stability and efficiency of groundwater denitrification, such as methanol (Wang et al., 2009a), ethanol (Salminen et al., 2014), glucose (Carrey et al., 2014), biodegradable snack (Wang and Wang, 2013), wheat straw, sawdust, biodegradable plastic (Zhang et al., 2012) and polycaprolactone (Chu and Wang, 2013). Recently, some by-products discharged from agro-industrial plants, such as Brewer's spent grain (Benyoucef et al., 2013), cassava distiller's dried grains (CDDG) (Wan et al., 2015) and rice washing drainage (RWD) (He et al., 2016a), have also been studied as carbon source for enhancing denitrification in nitrate-contaminated groundwater. Particularly, RWD that generated from food processing using rice as raw material has been regarded as an alternative carbon source for denitrification due to its rich organic matter content (He et al., 2016a).

In addition to carbon source, denitrifying bacteria play a significant role in nitrate removal process. Activated sludge collected from wastewater treatment plants (WWTP) has been considered as seeding sludge for nitrate-contaminated groundwater treatment (Chu and Wang, 2013; Wang and Wang, 2013; Wan et al., 2015). However, a long acclimation period is often required for the seeding sludge adapting to different carbon sources, e.g. 30 days for Brewer's spent grain (Benyoucef et al., 2013). Nevertheless, certain substrates have been reported to be capable of serving as both carbon and microbe sources for groundwater denitrification processes, leading to avoidance of sludge acclimatization period (Wan et al., 2015; He et al., 2016b). Wan et al. (2015) investigated nitrate removal from synthetic and real groundwater using CDDG that served as sole carbon source and microbe seed, and reported remarkably high total nitrogen (TN) removal efficiency of 96.8% on Day 12. RWD's proper treatment involves high operation costs and often involves highly energy-intensive facilities (Jo et al., 2015). Therefore, from the viewpoint of energy-saving and environmental protection, RWD has been considered as both carbon and microbe sources for denitrification (He et al., 2016a, 2016b). He et al. (2016b) investigated denitrification efficiency in an upflow zeolite-based biofilm reactor with RWD as both carbon and microbial sources, and reported that nitrate removal efficiency reached 71.0% on the first day and steady removal efficiency of 98.2% was achieved after 5 days.

Soluble microbial products (SMP), the majority of dissolved organic matter (DOM) in biological treatment processes, are biologically derived from substrate metabolism during biomass growth and released from cell lysis during biomass decay (Barker and Stuckey, 1999). The presence of SMP may lower down the treatment efficiency of biological processes and impair the discharge quality, and even causes possible environmental hazard to the receiving water (Kunacheva and Stuckey, 2014). Moreover, the generation and characterization of SMP may differ largely in various biological treatment processes with different substrates and operational conditions, since the formation of SMP is closely related to the mechanisms of microbial defense, acclimation and survival strategies (Wang and Zhang, 2010). In addition, DOM sources in groundwater have substantial impacts on the evolution of groundwater chemistry, receiving increasing attention (Huang et al., 2015). Therefore, it is important to identify the primary components of DOM in groundwater denitrification processes not only for understanding the fundamental mechanisms of biological activity but also for minimizing the impacts of DOM generated from bio-treatment on groundwater quality. To date, few information is available and there is considerably more work needs to be done to fully understand the chemical characteristics of DOM in groundwater denitrification process.

In addition, since the characterization of groundwater microbiological quality is a research task for groundwater reuse to ensure acceptable human health risk (USEPA, 2012), it is significantly important to reveal bacterial community structures (BCS) in a RWD-based denitrification process. On the other hand, BCS would give insights into the denitrification mechanisms, as denitrification performance has a close relationship with microorganism groups in a groundwater-restoring denitrification (Zhang et al., 2016a). Furthermore, different denitrification microbiotas serve different primary roles and function with various activities in response to environmental changes (Monballiu et al., 2013). Recently, dominant bacteria in denitrification system using different carbon sources have been widely determined (Osaka et al., 2006; Lu and Chandran, 2010; Wan et al., 2015). For instance, *Methylophilaceae* and *Hyphomicrobiaceae* are methanol assimilating denitrifying bacteria, while *Comamonadaceae* and *Rhodocyclaceae* are dominant in acetate-feeding denitrification systems (Osaka et al., 2006). Moreover, in addition to denitrifying bacteria, a large number of non-denitrification bacteria also survived in the activated sludge as a complex microbial community (Hagman et al., 2008). Thus, the fierce competition for carbon source would occur between denitrifying and non-denitrifying bacteria, especially in a carbon-limited condition (Wan et al., 2015). Although dominant denitrifying bacteria have been mentioned in our previous study (He et al., 2016b), the effects of RWD addition on initial BCS in seeding sludge and the succession of BCS along the whole denitrification process were not investigated. To our best knowledge, this is the first study on the effect of seeding sludge and RWD addition on BCS in the RWD-based groundwater denitrification processes.

In this study, RWD was used as both carbon and microbe sources for efficient groundwater denitrification. The main objectives were to: i) investigate the RWD-based groundwater denitrification performance with and without inoculation of seeding sludge; ii) reveal the changes in DOM during the RWD-based denitrification process; iii) assess the effect of RWD addition and seeding sludge on BCS in the denitrification process; and iv) compare the BCS in inoculated and un-inoculated systems.

2. Material and methods

2.1. RWD and synthetic groundwater

RWD was obtained by washing Chinese Northeast rice with deionized water. Rice was washed in triplicate and all of the washed water was mixed, and the washing procedure has been reported in our previous study (He et al., 2016a). The characterization of RWD is shown in Table 1. The pH for the RWD was 7.35. The concentrations

Table 1
Characteristics of RWD used in the experiments.

Parameter	Average (±SD)
pH	7.35 ± 0.13
NO_3^--N (mg L^{-1})	0.63 ± 0.51
NO_2^--N (mg L^{-1})	0.04 ± 0.26
NH_4^+-N (mg L^{-1})	0.17 ± 0.09
Total Nitrogen (TN) (mg L^{-1})	66.82 ± 5.53
Total Phosphorus (P) (mg L^{-1})	33.96 ± 5.70
TOC (mg L^{-1})	495 ± 23
MLSS (mg L^{-1})	1463 ± 36