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# Comparison of nitrogen removal and microbial properties in solid-phase denitrification systems for water purification with various pretreated lignocellulosic carriers

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## HIGHLIGHTS

- Denitrification efficiency largely affected via various materials and pretreatments.
- Acid treatment favored sustainable denitrification and declined effluent $NH_4^+$-N.
- Higher abundant denitrifiers and fermentative bacteria in pretreated SPD system.

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## ABSTRACT

This study explored the water purification performances of solid-phase denitrification systems filled with either untreated, acid and alkali pretreated corncob, rice straw and rice hulls. Nitrate reduction improved via pretreatments was found in ascending order from corncob, rice straw and rice hulls systems due to their various chemical compositions, while the pretreated rice hull systems still had the lowest nitrate reduction efficiencies (<90%). Besides, nitrite accumulation only frequently detected (<0.5 mg $L^{-1}$) in rice hulls system especially in untreated system, while ammonium occurrences in effluent were much more prevalent in corncob and rice straw systems than those of rice hulls system, and could be impaired via acid pretreatment. Miseq sequencing analysis showed that the higher abundances of dominant denitrifiers (*Bosea*, *Acidovorax*, *Simplicispira*, *Dechloromonas*, etc.) and fermentative anaerobic bacteria (*Actinotalea*, *Cellulomonas*, *Opitutus*, etc.) co-existed in the pretreated systems than those of none pretreatment, which was vital for efficiently sustainable nitrogen removal.



## 1. Introduction

Nowadays, natural water contaminated by nitrate is one of the major environmental concerns in worldwide (Abdul-Rahman et al., 2002). Biological denitrification is still the most popular and cost-saving approach used for nitrate removal. Heterotrophic and autotrophic biological denitrification are the two distinct methods, the former relying on available carbon sources and the latter depending on iron, hydrogen or sulfur compounds (Karanasios et al., 2010). Thereinto, heterotrophic biological denitrification is commonly realized in biological systems treating polluted natural water, but the deficiency of available carbon source for heterotrophic denitrification is still a challenge (Wang and Chu, 2016). Thus, external carbon sources addition for enhancing heterotrophic denitrification has been considered to be achievable approach in recent years, especially the utilization of solid carbon sources (Warneke et al., 2011). It has been reported that solid carbon sources could be used as carbon sources for microorganisms growth and carrier for biofilm attachment in denitrification systems, which was commonly named as solid-phase denitrification (SPD) (Wang and Chu, 2016; Wu et al., 2012). In recent years, substantial biodegradable materials have been studied to enhance the operation performances of SPD systems, including natural plant materials (rice straw, rice hulls, wood chips, corncob, etc) (Rusznyak et al., 2010; Yang et al., 2015) and synthetic biodegradable polymers (Polycaprolactone, Polybutylene Succinate, etc) (Ruan et al., 2016; Zhang et al., 2016), and plant-based solid carbon sources are much more prevalent and cost-saving.

It is well known that biodegradable materials are usually used as carbon sources and biofilm carriers in SPD system. The former depends on the substantial carbon components of the materials

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and the slow release characteristics, and the latter relies on large surface structure for bacteria attachment. The plant-based solid carbon sources are lignocellulosic materials mainly contained biodegradable cellulose, branched hemicelluloses, and recalcitrant lignin network interacting closely with each other as well as posed as protective barrier of biodegradable cellulose and hemicelluloses, which satisfy the requirements of carbon sources and biofilm carriers in SPD systems (Falk et al., 2010; Ge et al., 2012). The nutrients releasing from lignocellulosic materials commonly appeared to release dissolved carbon sources rapidly at first from a portion of cellulose as well as hemicelluloses, then releasing rate gradually decreased and microbial degradation of the remaining mainly occurred (Feng et al., 2015; Gao et al., 2013).Thus, the operation risks including increasing effluent organics level in the initial operation days and organics deficiency in the latter operation days may be occurred in SPD systems. Since microbial degradation play an important role in acquiring carbon sources during long-term operation of SPD systems, reducing the recalcitrant characteristics of lignocellulosic materials to some extent seemed to be an attractive approach.

Plant biomass recalcitrance was mainly affected via accessible surface area and chemical compositions of lignin, hemicellulose and cellulose(Sun et al., 2016). Pretreatment of lignocellulosic materials might be an alternative method, which is well known in the field of the production of various biorefinery products from lignocellulosic biomass. Many pretreatment technologies have been developed including physical (size reduction, etc.), physico-chemical (liquid hot water, etc.), chemical (acids, alkalines, and oxidizing agents), and biological treatment, which obviously improved the digestibility and accessibility of lignocellulosic biomass (Brar et al., 2016; Elumalai et al., 2016; Sun et al., 2016; Zhang et al., 2015). However, there is not enough information to tell the effects of pretreatment technologies on denitrification performances and operation risks. In addition, microbial community in biofilms of SPD system was related on the biocarriers, which decided the operation performance. During the long-term operation of SPD process, it was suggested that some enriched denitrifiers could directly utilize biodegradable carriers as carbon sources, and others might utilize hydrolysates as electron donors from carriers degradation via other microorganisms (Wang and Chu, 2016; Ruan et al., 2016). However, few studies focused on the changes in microbial community structure in biofilms of SPD systems treating natural water using different pretreated biodegradable carriers.

In order to study the characteristics of nitrogen removal and variations in microbial community in SPD systems for the treatment of nitrate enriched water, three representative agricultural by-products including corncob, rice straw and rice hulls pretreated by different physico-chemical methods were selected as biodegradable carriers and solid organic carbon source. Thus, the objectives of this study were presented as follows: 1) observe the effects of three biodegradable carriers with various pretreatment on nitrogen removal performances, organics variations and denitrification pathways; 2) explore the mechanism of nitrogen and organics removal via further examining the characteristics of microbial community among various biodegradable carriers.

## 2. Materials and methods

### 2.1. Materials

Three lignocellulosic carriers including wilted corncob, rice straw and rice hulls were obtained from the countryside of eastern China. The dried corncob and rice straw were cut into pieces with approximately equal lengths of rice hulls. Each carrier with a weight of 12.5 ± 0.05 g was placed into mesh bag (9 cm × 11 cm) and then pretreated in dilute sulphuric acid (0.01 mol $L^{-1}$) or sodium hydroxide (0.01 mol $L^{-1}$). After that, all mesh bags with carriers were heated at 120 °C for 1 h in an autoclave, then washed by distilled water after another 2 h cooling time and dried in a drying oven. At the same time, dried carriers without pretreatment were used as control.

Synthetic contaminated raw water with water quality similar to those of surface water in eastern China was used as influent in the whole experiment. Sodium acetate, potassium nitrate, potassium dihydrogen orthophosphate were used as the carbon, nitrogen and phosphorus source, respectively. The composition of synthetic raw water is presented as follows (mg $L^{-1}$): 24.4 of $CH_3COONa{\cdot}3H_2O$ (4.3 as C), 16 of $KNO_3$ (4 as N), and 0.9 of $KH_2PO_4$ (0.2 as P).

### 2.2. Experimental set-up and operation procedures

Nine cuboid solid-phase denitrification reactors ($R_1$-$R_9$) were constructed by Plexiglas, and each reactor had a working volume of 4.2 L (12 cm × 12 cm × 30 cm). Reactors $R_1$, $R_4$ and $R_7$ filled with the same weight of untreated corncob, rice straw and rice hulls, respectively. The carriers of corncob, rice straw and rice pretreated by acid were fed into reactors $R_2$, $R_5$ and $R_8$, respectively. At the same time, the carriers of corncob, rice straw and rice pretreated by alkali were fed into reactors $R_3$, $R_6$ and $R_9$, respectively. All reactors filled with the same weight of carriers (100 g). River sediment with raw water obtained from the source water area located in Zhejiang Province was used as inoculums and fed into reactors in the startup period of 2 days under aeration conditions. Then the sediment in each reactor was discharged, and polluted raw water was treated at a hydraulic retention time (HRT) of 24 h. All reactors were placed in a room with a constant temperature of 25 ± 2 °C during this experiment. No aeration was supplied in reactors after discharge of sediment, and the dissolve oxygen (DO) levels in every reactor were always less than 0.3 mg $L^{-1}$. The detail information of each reactor is shown in Table 1.

### 2.3. Analytical techniques

#### 2.3.1. Regular index analysis

Chemical composition including lignin, cellulose and hemicellulose of various lignocellulosic carriers was analyzed according to the methods provided by Hill et al. (1998). Nitrate, nitrite and ammonia were analyzed according to standard analytical methods (Chinese Standard Methods for the Examination of Water, 2002). Dissolved organic carbon (DOC) was determined using a catalytic combustion TOC analyzer (TOC-V CPH, Shimadzu). DO was measured with a DO meter (YSI Model52, USA).

#### 2.3.2. DNA extraction and PCR amplification

Total DNA of biofilm samples obtained from operation reactors was extracted using a soil DNA kit (OMEGA). The extracted DNA was distinguished by 0.8% agarose gel electrophoresis and quantified by UV spectrophotometer. The V4 region of 16S rRNA genes were amplified by polymerase chain reaction (PCR) amplification using the specific primers 520F (5′-barcode + GCACCTAAYTGGGYD TAAAGNG-3′) and 802R (5′-TACNVGGGTATCTAATCC-3′). The oligonucleotide sequence 'barcode' in primer 520F was used to distinguish samples from same library. 25 μL reaction system was used for PCR amplification, including NEB Q5 DNA high-fidelity polymerase (0.25 μL), 10 mM dNTPs (0.5 μL), 5 × PCR reaction buffer (5 μL), 5 × high GC buffer (5 μL), DNA template (1 μL), forward primer (1 μL), reverse primer (1 μL) and sterilizing ultrapure water (11.25 μL) under the conditions including the successive procedures of pre-degeneration at 98 °C (30 s), 25–27 cycles of operation