

## ARTICLE

# Influence of Pyrolytic Biochar on Settleability and Denitrification of Activated Sludge Process

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Biochar is a massively produced by-product of biomass pyrolysis to obtain renewable energy and has not been fully used. Incomplete separation of sludge and effluent and insufficient denitrification of sewage are two of main factors that influence the efficiency of activated sludge process. In this work, we proposed a new utilization of biochar and investigated the effect of biochar addition on the performance of settleability and denitrification of activated sludge. Results show that the addition of biochar can improve the settleability of activated sludge by changing the physicochemical characteristics of sludge (*e.g.*, flocculating ability, zeta-potential, hydrophobicity, and extracellular polymeric substances constituents). Moreover, the dissolved organic carbon released from biochar obtained at lower pyrolysis temperature can improve the nitrate removal efficiency to a certain extent.

**Key words:** Biochar, Activated sludge, Settleability, Biological denitrification, Carbon source

## I. INTRODUCTION

As a cost-effective method, activated sludge process has been widely adopted in most wastewater treatment plants (WWTPs). However, the poor settling of activated sludge biomass remains a common problem, which results in increased effluent solid concentrations, decreased disinfection efficiencies, and increased treatment costs [1].

Some materials, such as diatomite and powdered activated carbon, have been used as additives to improve sludge settleability [2]. However, the high cost of powdered activated carbon (1.65–9.90 dollar per kilogram [3, 4]) limits its wide application in activated sludge process. Biochar is the carbonaceous by-product of biomass pyrolysis for preparation of renewable liquid fuels [5, 6], and its production cost is no more than one-tenth of the cost of powdered activated carbon [7, 8]. Adding biochar into activated sludge process may change the sludge properties. For instance, Oleszczuk *et al.* [9] dosed biochar into activated sludge and found that biochar decreased the phytotoxicity of sludge and positively affected seed germination. In addition, the variations of sludge properties caused by biochar addition may facilitate the separation and post-treatment of excess sludge by improving sludge settling property and increasing the heating value of dehydrated sludge

[10, 11]. Although the investigations on the improvement of sludge settling property by addition of activated carbon have been reported [2, 12], the improvement of settleability of sludge by biochar addition has not been found.

No sufficient carbon sources in urban sewages to act as electron donors for complete denitrification is another problem in WWTPs [13, 14], and nitrogen in the effluents is an important factor that causes eutrophication of water bodies [15] which may threaten the aquatic ecosystems and the living environment of human beings [16, 17]. Liquid carbon sources (*e.g.*, methanol, ethanol, and acetic acid) are widely added into the activated sludge process as an external carbon source to promote denitrification, however, they are expensive and potentially hazardous chemicals [18, 19]. In addition to the stable carbon in the skeleton, a certain amount of dissolved organic matter is present in the biochar [20, 21], which may be bioavailable and act as a carbon source for denitrification. Jamieson *et al.* [22] characterized dissolved organic matter in birch and maple biochars using optical analyses, and their findings suggest that pyrolytic temperature can influence the composition of biochar-derived dissolved organic matter and further influence the bioavailability of the dissolved organic matter. However, to our knowledge, most studies on biochar are in the field of soil remediation, few focus on the utilization of biochar-derived dissolved organic matter as a carbon source of WWTPs.

In this work, biochar was used as an additive to improve the settleability of sludge and the denitrification in activated sludge process. Three kinds of biochars by

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fast pyrolysis of rice husk waste at different temperatures were prepared. The effects of biochar addition on the settleability and physicochemical characteristics of sludge were evaluated. The effects of pyrolytic temperature on dissolved organic carbon (DOC) released from biochar and denitrification of sludge were investigated; and the potential influence mechanism of biochar addition on settleability and denitrification were discussed.

## II. MATERIALS AND METHODS

### A. Materials

The rice husk was obtained from Anhui Yineng Bioenergy Co. Ltd., Hefei, China. The samples were dried in an oven at 105 °C for 12 h, and then pulverized by a rotary cutting mill and screened to collect particles with sizes below 150  $\mu\text{m}$  (100 mesh). Finally, they were stored in a glass desiccator for further use. All reagents used in this study are of analytical grade purity and purchased from Sinopharm Chemical Reagent Co., Ltd. Shanghai, China.

### B. Preparation and characterization of biochar

The biochar was prepared by fast pyrolysis of rice husk at three different temperatures. Char-573, char-773, and char-973 denoted the biochar produced at temperatures of 573, 773, and 973 K, respectively. The pyrolysis experiment was conducted in a vertical fixed-bed reactor under a nitrogen atmosphere, which was described in our previous work [23].

Elemental compositions (C, H, N, and O) of rice husk and biochars were determined using an elemental analyzer (vario EL III, Elementar, Germany). Raman experiments were conducted with a LabRAM HR spectrometer using the green line of an argon laser ( $\lambda=514.5\text{ nm}$ ) as excitation source. Dissolved organic carbon (DOC) of samples was determined on a TOC analyzer (TOC-VCPN, Shimadzu Co.) using a sequencing extraction method. Specifically, 0.5 g of sample was added to 30 mL of deionized water and shaken using the reciprocating shaker (180 r/min) in the dark for 1 d. The mixture was then separated at 8000 r/min on a centrifuge. The separated solid was added to 30 mL of deionized water for another shaking of 2 d, and the mixture was separated again. The solid was added to 30 mL of deionized water for another shaking of 4 d and then separated. All the supernatant was collected and filtered through a 0.22  $\mu\text{m}$  membrane, and DOC was determined. All extraction experiments were conducted in duplication.

### C. Effects of biochar on sludge settleability

Ten of 500 mL graduated cylinders were used as laboratory-scale sequencing batch reactors (SBRs), and

each reactor was inoculated with 500 mL of activated sludge taken from an aeration tank from the Zhuzhuanjing WWTP, Hefei, China. The seeding sludge had a sludge age of 10 d, a mixed liquor suspended solids (MLSS) concentration of 8 g/L, and a sludge volume index (SVI) of 68.1 mL/g. After inoculation, the samples (rice husk, char-573, and char-773) were added into the reactors with sample/sludge mass ratio of 20%, 10%, and 5% (*w/w*), respectively. The control experiments were conducted under the same conditions without adding rice husk or chars into the reactor.

After that, the SBRs were maintained at  $28\pm 1\text{ }^\circ\text{C}$  and operated in successive each cycle of 12 h. One cycle consists of 5 min of influent addition, 10 h of aeration, 100 min of settling, and 15 min of effluent withdrawal. In each cycle, 200 mL of effluent was lightly sucked out by siphoning. The SBRs were then fed with synthetic wastewater, which had average COD,  $\text{NH}_4^+\text{-N}$ , and  $\text{PO}_4^{3-}\text{-P}$  concentrations of 400, 20, and 4 mg/L, respectively.  $\text{SV}_{30}$  was measured during the settling stage of each cycle for indication. The physicochemical properties of sludge were measured at the end of the experiment, including MLSS, zeta potential ( $\zeta$ -potential), flocculating ability, hydrophobicity, and the composition of extracellular polymeric substances (EPS).

### D. Denitrification batch experiments

Batch microcosm experiments were conducted to evaluate the capacity of biochar as a carbon source to enhance denitrification. The batch experiments were conducted in ten of 250 mL Erlenmeyer flasks. Then, 3 g of samples (rice husk, char-573, char-773, and char-973) and 150 mL of synthetic waste-water that contains 100 mg/L  $\text{NO}_3^-\text{-N}$  and 10 mg/L  $\text{PO}_4^{3-}\text{-P}$  were added into the Erlenmeyer flasks. Afterwards, 5 mL of anaerobic granular sludge (the seed sludge was taken from an anaerobic digester in a citrate-processing WWTP) was inoculated. The flasks were then purged with high purity nitrogen for 20 min to lower dissolved oxygen concentration. The flasks were sealed with glass stoppers and then incubated in a constant-temperature reciprocating shaker and agitated at 150 r/min and  $298\pm 3\text{ K}$ . The control experiment was conducted under the same conditions without adding rice husk or chars into the flasks.

Two duplicated experiments were conducted on each sample. The mixture (30 mL) was taken from each flask on day 2, 3, 5, 7, 11, 13, and 15, and the extracted volume was replaced with 30 mL of fresh synthetic wastewater. The flasks were again purged with high purity grade of nitrogen for 20 min, then sealed with glass stoppers, and incubated in the shaker. The mixture was centrifuged at 8000 r/min for 20 min, and the supernatant was collected and further filtered with a 0.22  $\mu\text{m}$  membrane for  $\text{NO}_3^-\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ , and  $\text{NH}_4^+\text{-N}$  analyses.

### E. Microbial community analysis

At the end of batch experiments, the suspension samples in control, char-573 group, and the inoculated sludge were collected and centrifuged at 6000 r/min for 10 min. The supernatant was decanted and the pellet was stored at  $-20\text{ }^{\circ}\text{C}$  until DNA extraction. Upon thawing, the total genomic DNA was extracted from the samples using the Power Soil DNA Kit (Mo Bio Laboratories, Carlsbad, CA, USA). Total DNA extraction as well as PCR-DGGE and 16S rRNA analysis were performed as described in supplementary materials). Nucleotide sequences were compared to sequences in the NCBI GenBank database using the BLASTn search program.

### F. Nitrogen release

Nitrogen release of samples was determined using the similar sequencing extraction method used in the determination of DOC. The concentrations of  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N, and  $\text{NH}_4^+$ -N in filtrate were also determined.

### G. Analytical methods

The concentrations of  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N,  $\text{NH}_4^+$ -N, and MLSS were analyzed following the standard methods for the examination of water and waste water [24]. The  $\zeta$ -potential of sludge suspension was determined by a  $\zeta$ -potential analyzer (Zetasizer Nano ZS, Malvern Co., UK). The flocculating ability was evaluated by the reflocculation ability of sludge flocs after disruption [25].

The hydrophobicity of sludge was estimated by measuring contact angle using axisymmetric drop shape analysis, which was described previously [26]. The sludge suspension was deposited to form a smooth thin layer on  $0.45\text{ }\mu\text{m}$  acetate cellulose membranes, which were washed twice with double-distilled water and then placed on a 1% agar plate. Prior to the measurement, the membranes were mounted on glass slides and air dried for 10 min, and the advancing contact angles were directly measured using the sessile drop technique with a drop of liquid water. The shape of a sessile distilled water droplet placed on the layer of biomass was determined using a contact angle analyzer (JC2000A, Powereach Co., Shanghai, China). All contact angle values were based on arithmetic means of at least 10 independent measurements.

Heat extraction methods were used to extract EPS from sludge [27]. The extracted solution was analyzed for total carbohydrates, protein, and humic substances. The total amount of EPS was expressed by the sum of carbohydrate, protein, and humic substances, which are the dominant components in EPS [28, 29]. Carbohydrates in EPS were determined according to the phenol-sulfuric acid method with glucose as standard [30]. Pro-

teins were determined by the Folin method with bovine serum albumin as standard [31]. Humic substances were determined by the improved Folin method [32].

### H. Statistical analysis

All statistical analyses were performed using the IBM SPSS 17.0 software (SPSS Inc., USA) for Windows. The Pearson product-moment correlation coefficient ( $r_p$ ) was used for linear estimations. Correlations were considered statistically significant if the correlation coefficient was more than the critical coefficient for  $P < 0.05$ .

## III. RESULTS AND DISCUSSION

### A. Characterization

The elemental compositions of rice husk and biochars are listed in Table S1 (supplementary materials). The C content in biochar slightly increases from 40.5% to 43.0%, whereas the H and O contents significantly decrease with the increase of pyrolytic temperature. The ash, mainly consisting of inorganic salts, is difficult to be volatilized and retained in biochar, and its content increases with the pyrolytic temperature increase.

### B. Effect of biochar addition on the settleability of activated sludge

#### 1. Settleability of activated sludge

The  $\text{SV}_{30}$  is an important index of the settleability of activated sludge. The results indicated that the settleability improved with the addition of rice husk and biochars. To evaluate the improvement of settleability, the decreased percentage (DP) of  $\text{SV}_{30}$  was applied and calculated using Eq.(1):

$$\text{DP}/\% = \frac{\text{SV}_{30\text{control}} - \text{SV}_{30x}}{\text{SV}_{30\text{control}}} \times 100\% \quad (1)$$

where  $\text{SV}_{30\text{control}}$  is the  $\text{SV}_{30}$  of the sludge in control reactor, and  $\text{SV}_{30x}$  is the  $\text{SV}_{30}$  of the sludge in other reactors with the addition of biochars or rice husk.

The DPs of  $\text{SV}_{30}$  with the addition of different dosages of rice husk, char-573, and char-773 are shown in FIG. 1. Overall, the addition of rice husk and chars can improve the settleability of sludge (DPs of  $\text{SV}_{30}$  ranged from 1.5% to 9.5% when rice husk and chars were added). After one cycle (12 h), DPs of  $\text{SV}_{30}$  increased dramatically to the range of 5.8%–23.0%, which indicates that the sludge is more easily to be separated during this period. DPs decreased in the following two cycles and gradually increased again in the last cycles. Given that the optimum sludge retention time for good bioflocculation and low effluent COD ranged from 2 d to 8 d [33], char-573 with 5% dosage may be

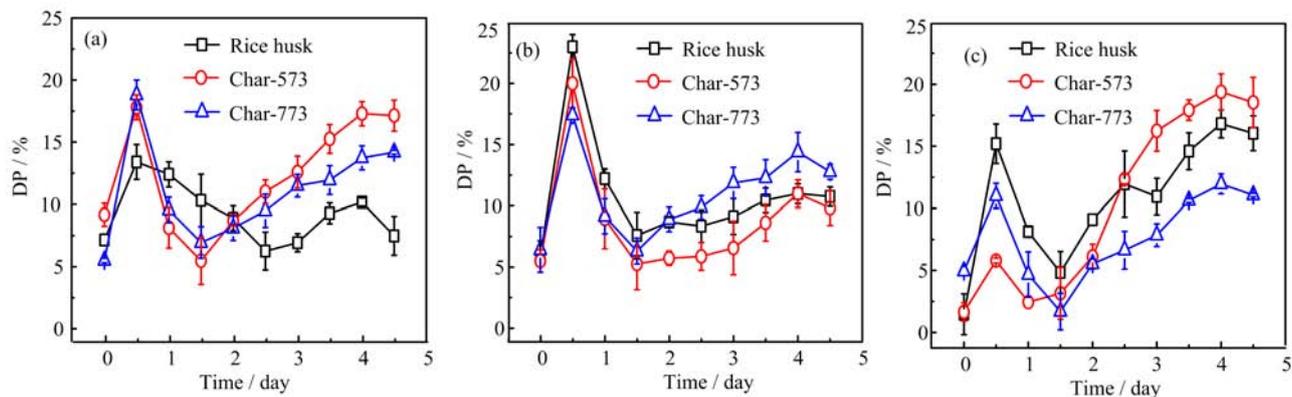


FIG. 1 The decreased percentage (DP) of  $SV_{30}$  in each reactor with different dosage of char-573, char-773, and rice husk. (a) 20%, (b) 10%, (c) 5%.

more suitable for practical usage in WWTP, in which the DP is maintained at 16.2%–19.3%.

## 2. Physicochemical characteristics of sludge

The physicochemical characteristics of sludge have an important influence on sludge settleability [27, 34]. The improvement of the settleability of sludge by the addition of chars or rice husk may be closely related to the variations of sludge properties. Therefore, some physicochemical characteristics like contact angle, flocculating ability, and zeta potential of sludge in each reactor were measured at the end of the experiment and shown in FIG. 2. The contact angle of sludge in the control experiment was  $84.1^\circ$ , while it ranged from  $71.8^\circ$  to  $81.3^\circ$  in the reactors containing chars and rice husk. The decrease of contact angle indicated that the hydrophobicity of sludge decreased after the addition of chars or rice husk. This result was probably due to the high porosity and high surface area of chars or rice husk, which enhanced the adsorption to water and hydrophilic sludge flocs [35]. With the addition of chars or rice husk, the zeta potential of sludge decreased from  $-16.7$  mV to the range between  $-20.0$  and  $-21.5$  mV. Furthermore, flocculating ability was 16.4% in the control experiment and increased to 25.2% or 52.9% after the addition of chars or rice husk, suggesting that the compactability of sludge is significantly improved [36].

It has been reported that EPS, mainly consisting of protein, humic substances, and carbohydrate, has a stabilizing effect on the sludge floc [37, 38]. The concentrations of protein, humic substances, and carbohydrate in sludge with and without chars/rice husk are shown in FIG. 3. After the addition of chars or rice husk, the concentration of humic substances decreased from 11.56 mg/g MLSS to 9.35–11.11 mg/g MLSS, and the concentration of carbohydrate decreased from 2.52 mg/g MLSS to 2.09–2.49 mg/g MLSS, whereas the concentration of protein kept around 8.5 mg/g MLSS. The improved flocculating ability of sludge by the ad-

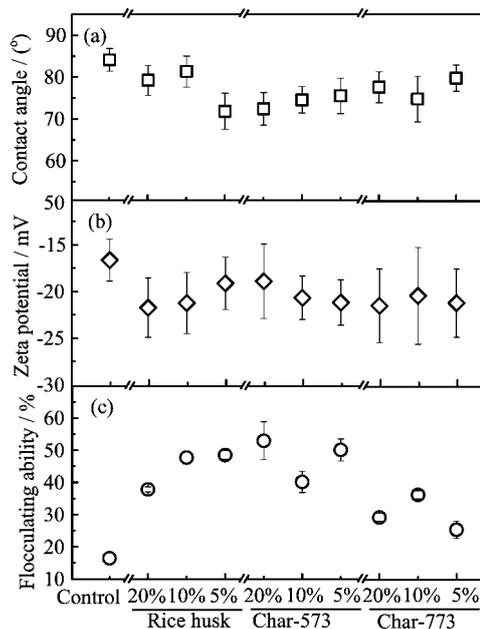


FIG. 2 (a) Contact angle, (b) zeta potential, and (c) flocculating ability of sludge at the end of the experiment.

dition of chars or rice husk may be attributed to the significant decrease in the concentration of humic substances [27, 38].

## 3. Correlation analysis

The correlation between the settleability and the properties of sludge was evaluated by Pearson product-moment correlation coefficient, and the results are summarized in Table I. The results indicated that the contact angle, flocculating ability, zeta potential, humic substances, and total EPS are significantly correlated with SVI ( $P < 0.05$ ). The contact angle, humic substances, and total EPS have a positive effect on SVI, whereas the flocculating abilities of sludge and SVI have

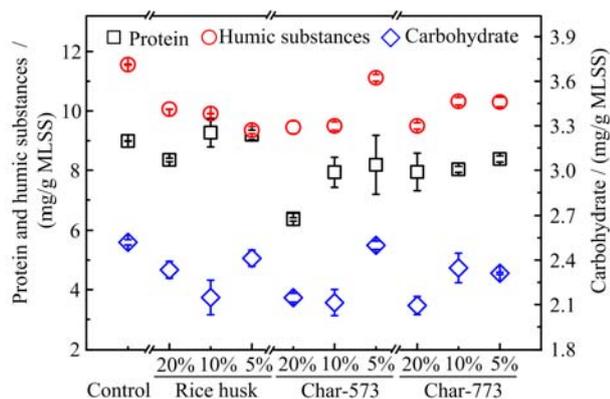


FIG. 3 Dominant components of EPS in each reactor at the end of experiment: protein, humic substances, and carbohydrate.

TABLE I Person's correlation coefficient ( $r_p$ ) and  $P$ -value between the characteristics and settleability of sludge.

| Parameter                    | SVI     |            |
|------------------------------|---------|------------|
|                              | $r_p$   | $P$ -value |
| Contact angle/(°)            | 0.7088  | 0.0217     |
| Flocculating ability/%       | -0.6350 | 0.0486     |
| Zeta potential/mV            | 0.7698  | 0.0092     |
| Protein/(mg/g MLSS)          | 0.5783  | 0.1029     |
| Humic substances/(mg/g MLSS) | 0.7303  | 0.0165     |
| Carbohydrate/(mg/g MLSS)     | 0.5410  | 0.1325     |
| Total EPS/(mg/g MLSS)        | 0.7990  | 0.0056     |

a negative correlation. These results are in accordance with previous studies [36, 39], which reveal that the physicochemical characteristics of sludge are changed by biochar addition, and the settleability of activated sludge is improved.

### C. Effect of biochar on denitrification

#### 1. DOC released from biochar

The DOC released from biochar may be used as a potential carbon source for microbes and is an important factor for enhanced denitrification [40]. FIG. S1 (in supplementary material) shows that the amount of DOC released from biochar decreases significantly with the increasing pyrolytic temperature, which suggests that few DOC of biochar obtained at high temperature can be utilized by microorganisms in activated sludge. Nevertheless, biochars with high DOC contents are not necessarily favorable for denitrification, because the organic compounds in biochar usually varied with the pyrolytic temperature, and some of them are nocuous to microorganisms. For instance, pinewood-derived biochar water extracts contain carboxyl and hydroxyl homologous series, which have been found to have inhibitory effects

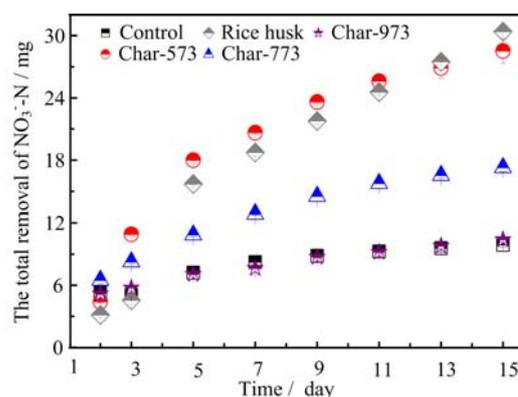


FIG. 4 The total removal amount of  $\text{NO}_3^-$ -N in denitrification batch experiment.

on the growth of aquatic photosynthetic microorganism [41]. Therefore, to elucidate the role of biochar as a potential carbon source, denitrification performance should be evaluated.

#### 2. Removal of nitrate

Denitrification by microorganisms is the main removal pathway of nitrate in sewage, during which a certain amount of carbon source is consumed. Rice husk, char-573, char-773, and char-973 were added into the synthetic waste water as a potential carbon source for denitrification. The total removal amount of nitrate in the flasks during the 15 d run is presented in FIG. 4. The removal efficiencies of nitrate were obviously different with dosing of different solid materials. In control group, the removal amount of nitrate was 5.3 mg on second day and rose slowly to 9.9 mg until the end of the experiment. Due to the absence of external carbon source, the soluble organic carbon released from the hydrolysis process of sludge is responsible for the denitrification in the control group [42]. In the reactor with the addition of rice husk, the removal amount of nitrate was 3.1 mg on second day, drastically rose to 15.7 mg on 5th day, and continuously rose to 30.4 mg during the later experiment time. For the group with char-573, the removal amount of nitrate was only 4.4 mg on second day, but quickly rose to 10.9 mg on third day and 18.0 mg on 5th day. Afterwards, it gradually rose to 28.5 mg during the later experiment time. For the group with char-773, the removal amount of nitrate quickly rose to 6.5 mg on second day and slowly rose to 17.3 mg at the end of the experiment.

Considering that nitrate is difficult to be removed by adsorption, thus the significant increase of denitrification by dosing rice husk and biochars should be attributed to the complementary carbon source. However, the types of carbon source supplied by different solid materials are discrepant. Cellulose and semicellulose are the dominant carbon source in rice husk that

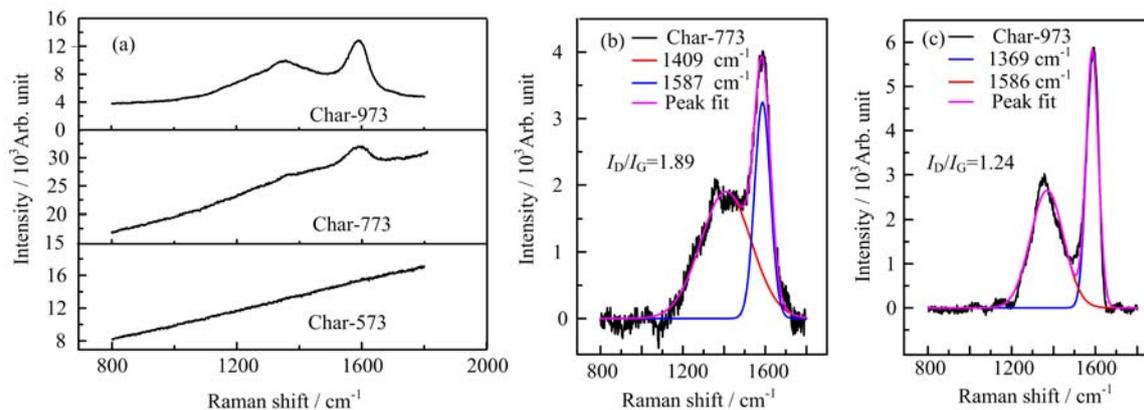


FIG. 5 (a) Raman spectra of biochars obtained at different temperatures. Raman spectra of (b) char-773, and (c) char-973 with Gaussian peak fit.

are beneficial to the denitrification [18]. In pyrolysis, cellulosic compounds are thermochemically decomposed to produce volatiles, and some bioavailable organic compounds are deposited or adsorbed on the carbon skeleton. The carbon source in biochars obtained at low temperature are easily-decomposed organic compounds, whereas with the increasing pyrolytic temperature, fewer bioavailable organic compounds remained. Thus, sludge with the addition of rice husk and char-573 exhibited good denitrification. To verify this hypothesis, we conducted a Raman analysis. The Raman spectra obviously indicate that the degree of aromatic condensation in the biochar increases with increasing pyrolytic temperature (FIG. 5(a)). D and G bands in Raman spectra are evidence of polyaromatic hydrocarbons and graphitic material, which are typically found at 1350 and 1530 cm<sup>-1</sup>, respectively [43]. FIG. 5(a) shows that the two peaks are absent in char-573, but appear in char-773 and char-973. At the same time, the results of peak fit show that intensities ratio of D to G band  $I_D/I_G$  of char-773 (1.89) is higher than that of char-973 (1.24) (FIG. 5 (b) and (c)), which indicates that the degree of aromatic condensation in the biochar increases with increasing pyrolysis temperature [44].

### 3. Accumulation of nitrite and ammonium

Given the biochars often contain some contents of N-containing species, N release experiments of biochars were performed. Ammonium was released from each sample and nitrate was released from rice husk only, and no nitrite was detected in leachate (FIG. 6 (a) and (b)). The total amounts of nitrate and ammonium released from rice husk are 0.07 and 0.79 mg/g, while those total amounts of ammonium released from char-573, char-773, and char-973 are 1.35, 0.59, and 0.28 mg/g, respectively. Although more ammonium is released by char-573 than other chars, its denitrification boost is still the best, which suggests that the effect of the N

release from biochar on denitrification is negligible.

During denitrification process, the product nitrite may accumulate in the system. FIG. 6(d) shows that nitrite accumulated significantly in first 5 d of the experiment. In the reactor containing rice husk, the nitrite concentration increased to 12.30 mg/L on second day, rose to 35.65 mg/L on third day, decreased on 5th day, and then disappeared during the rest of the experiment time. For char-573, the nitrite concentration was 8.91 mg/L on second day, and rose quickly to 46.16 mg/L on 5th day. Afterwards, an abrupt decrease of the nitrite concentration to 2.10 mg/L occurred on 7th day and the nitrite disappeared at the end of the experiment time. It was indicated that the competition for limited carbon source existed in nitrate and nitrite respirations, and nitrite accumulation showed that the available carbon was insufficient during the denitrification process [45, 46]. As for reactors containing char-773 and char-973, the accumulation of nitrite is negligible because little carbon source was provided.

Ammonium accumulation was observed in control and experiment groups (FIG. 6(c)). In control group, the ammonium concentration was 5.04 mg/L on second day, increased slowly to 11.92 mg/L on 7th day, and remained between 11 to 13 mg/L until the end of the experiments. This result further suggests the occurrence of sludge hydrolysis, during which the concentration of ammonium kept increasing [42, 47]. The change of ammonium concentration with time in reactors with biochar showed a similar trend.

### 4. Microbial community changes

The DGGE profiles show that the microbial community composition in the sludge changed at the end of the batch experiments (FIG. S2 in supplementary materials). The main bands in each sample are obviously different. Bands 1–3 are observed and extremely enriched in char-573 sample, whereas bands 4–6 are dominant

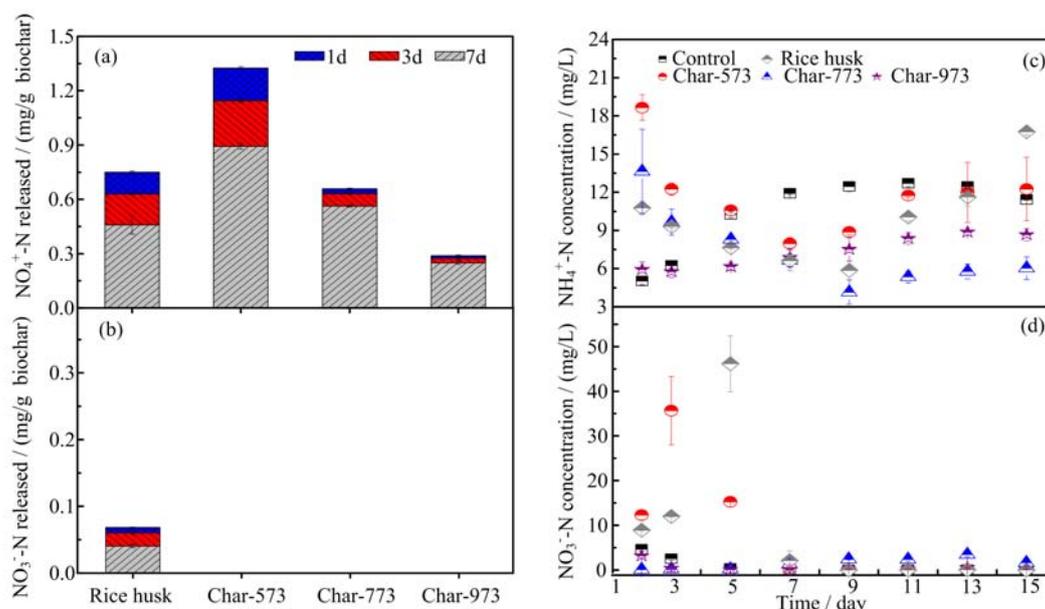


FIG. 6 The amount of  $\text{NH}_4^+\text{-N}$  (a) and  $\text{NO}_3^-\text{-N}$  (b) released from rice husk and biochars by leaching experiments; the concentration of  $\text{NH}_4^+\text{-N}$  (c) and  $\text{NO}_2^-\text{-N}$  (d) of supernatants during denitrification batch experiments.

in the inoculated sludge sample, and bands 7 and 8 are enriched in control sample. DNA sequences were compared with the NCBI GenBank database using the BLASTn search. Bands 1–3 have a high similarity to *Parabacteroides chartae* sp., which is usually detected in bacterial community in anaerobic bioreactors [48]. The gene sequences from band 4–6 closely belong to the genus of *Candidatus Cloacamonas acidaminovorans* sp., *Treponema caldaria* sp., and *Thermosiphon atlanticus* sp., respectively, all of which commonly present in many anaerobic digesters [49–51]. By contrast, band 7 affiliates with the genus *Sulfuritalea hydrogenivorans* sp. and band 8 affiliates with the genus *Sulfurisoma sediminicola* sp., two facultative autotrophs [52, 53], which indicates the microbial community in control group were gradually changed to facultative autotrophs because of the lack of carbon source.

#### D. Application potential

From the above results, dosing biochar can significantly improve the settleability of sludge by changing contact angle, zeta potential, and the flocculating ability of sludge. Therefore, the addition of biochar to improve the settleability of activated sludge can find practical usage in WWTP. However, although the biochar, particularly the biochar obtained at low pyrolytic temperature, was demonstrated to be useful to act as a carbon source for nitrate removal in denitrification phase, it should be taken into account that the release rate of DOC was very slow and the release period was more than 15 days. In many types of waste water configuration (e.g.,  $\text{A}^2\text{O}$ , SBR or their modified revision),

activated sludge is internally partially recycled (spatially or temporally) from denitrifying phase to anaerobic/aerobic phase. In such modes, the DOC gradually released from biochar may be consumed in the aerobic phase, thus DOC released from biochar could not be fully used for denitrification. In addition, the biochar has been reported to increase plant growth and crop yields when used into soil [54], the excess activated sludge with the addition of biochar is more valuable for soil application.

#### IV. CONCLUSION

In this work, we introduce a new application of pyrolytic biochar in waste water treatment, the biochar which was added into the reactor can improve the settling performance of sludge in the secondary sedimentation tank or in the setting process. Biochar obtained at low pyrolytic temperature was also demonstrated to be a potential carbon source for denitrification.

**Supplementary materials:** Microbial community analysis method is shown in Text S1. Elemental compositions of rice husk and biochars (Table S1), the amount of DOC released from rice husk and biochars (FIG. S1), and DGGE analysis of samples (FIG. S2) are also shown.

#### V. ACKNOWLEDGMENTS

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