Effects of Rice Husk Biochar Attachment Biofilm with Microorganisms on Nitrogen Removal of Digested Swine Wastewater

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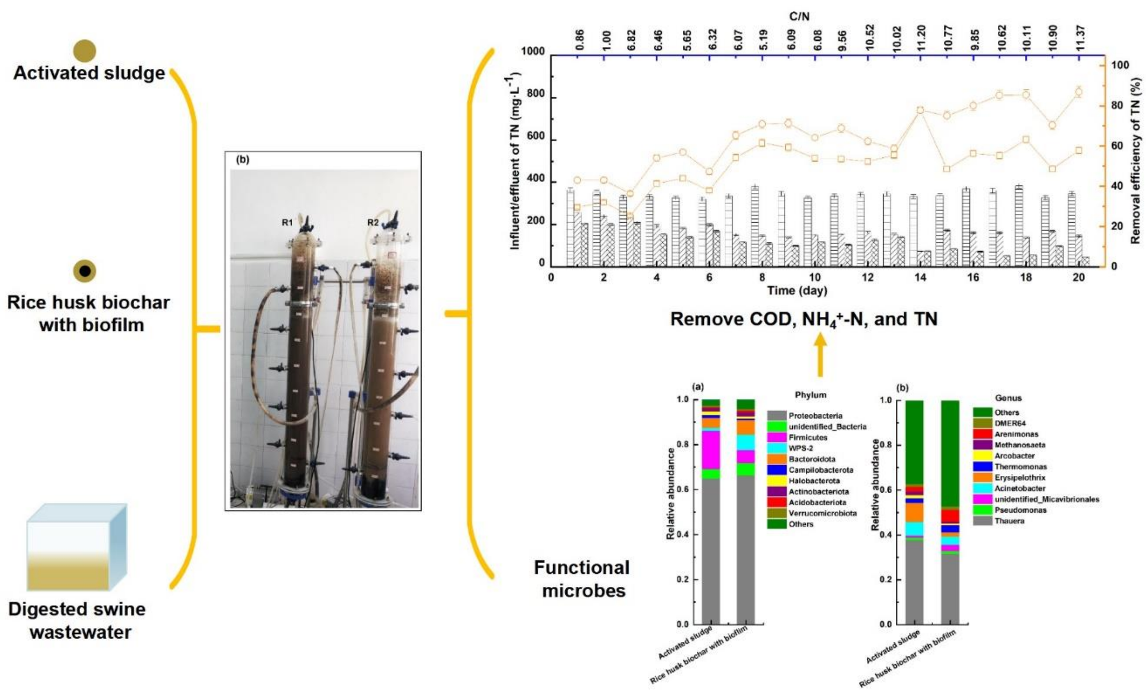
GRAPHICAL ABSTRACT



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Fanghui Pan,[a,b] Fei Huang,[b] Youbao Wang,[b,]* and Hongguang Zhu [a,]*

Nitrogen in digested swine wastewater is currently difficult to directly degrade by an activated sludge process in a sequencing batch reactor (SBR), with resulting failure of the effluent to meet emission standards. In this study, rice husk biochar was optionally added into SBR to enhance biochemical properties for digested swine wastewater, especially for nitrogen degradation. The relative nitrogen removal mechanism for microbial community was probed by means of high-throughput sequencing. The results indicated that chemical oxygen demand (COD), ammonia (NH_4^+-N), and total nitrogen (TN) removal efficiency of digested swine wastewater was separately at 85.3%, 81.3%, and 65.2% using rice husk biochar with biofilm, which was 3.5%, 24.4%, and 14.7% higher than that of activated sludge, under influent of 2609 mg·L^{-1} COD, 337.0 mg·L^{-1} NH_4^+-N, 344 mg/L TN, and 7.77 C/N. High-throughput sequencing revealed that rice husk biochar with biofilm contained *Proteobacteria*, *Thauera*, *Comamonas*, *Acinetobacter*, *Pseudomonas*, *Flavobacterium*, and *Corynebacterium* to enhance nitrogen removal of digested swine wastewater. The results not only provide theoretical support for biochar with biofilm to improve digested piggery wastewater treatment, but also have great significance in resource utilization of agricultural waste and eco-environmental protection.

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Keywords: Rice husk biochar; Biofilm; Digested piggery wastewater; Nitrogen removal; Microorganisms

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INTRODUCTION

The rapid development of the large-scale and intensive piggery industry has become an important and large source of pollutants in China (Kaufmann 2015; Chen *et al.* 2019; Wu *et al.* 2019). Wastewater from the piggery industry contains high chemical oxygen demand (COD), nitrogen, phosphorus, pathogenic microorganisms, and suspended solids (He *et al.* 2014a; Kizito *et al.* 2015), which easily cause pollution of the surrounding environment. It is worth noting that ammonia (NH_4^+) in piggery wastewater could be converted into nitrite (NO_2^-) or nitrate (NO_3^-) under the action of ammonia-oxidizing bacteria (AOB) or nitrite oxidizing bacteria (NOB) (Hooda *et al.* 2000; Rahimi *et al.* 2020). The NO_2^- and NO_3^- are capable of causing groundwater pollution due to surface runoff and leaching (Ji *et al.* 2018; Luo *et al.* 2018; Rahimi *et al.* 2020). Therefore, the piggery wastewater treatment faces a huge challenge (Luo *et al.* 2019; Zuo *et al.* 2020).

Biological treatment technology such as sequencing batch activated sludge method are usually used to treat the swine wastewater; this is because it is a cost-effective method compared with physical-chemical methods (Zheng *et al.* 2018; Dan *et al.* 2020). Although the biological treatment is accepted to handle the swine wastewater, it is difficult to satisfy the requirement of the increasingly stringent discharge standard, due to the effluent with low efficiency, high concentration, and scattered flocs (Jenni *et al.* 2014; Wei *et al.* 2014). The traditional activated sludge in sequencing batch reactor (SBR) for nitrogen removal requires a large amount of external carbon as electron donors for denitrification due to a low C/N ratio of piggery wastewater (Zuo *et al*. 2020). In order to improve biochemical performance of microorganisms in SBR, aerobic granular sludge (AGS) is routinely cultivated to facilitate nitrification, denitrification, and phosphorus removal as well as organic degradation (Arumugham *et al*. 2024). It was found that phylum *Proteobacteria* in AGS contributed to the pollutant removal process (Arumugham *et al*. 2024). However, the cultivation periods of AGS are long and the preservation methods of AGS are complex (Tanavarotai *et al*. 2022). Another way of enhancing the microbiological activity is to add biochar in SBR for the pollutant removal of the digested swine wastewater. Biochar could serve as a core for microbial aggregation and growth to resist the shock of high concentration organic wastewater (Zhang *et al.* 2017; Adams *et al.* 2020), and its large specific area (*e.g.* 1365 $m^2 \cdot g^{-1}$) enhances adhesion for bacteria hence supporting the growth of biofilms for pollutant removal of piggery wastewater (Alvarez *et al*. 2014; Adams *et al.* 2020).

Biochar from biomass pyrolysis as a microbial carrier for wastewater treatment has attracted widespread attention, including rice husk biochar. Agricultural activities have produced a great deal of rice husks every year (Fareed *et al.* 2020). According to the United Nations Food and Agriculture Organization (FAO), approximately 7.82×10^8 tons of rice is produced every year in the world, while each rice produces about 200 kilograms of husks, which means that nearly 1.56×10^8 tons of rice husk are produced each year (Alvarez *et al.* 2014). As the world's largest rice producer, China has generated a large amount of rice husks (Gao *et al.* 2015; Ren *et al.* 2019). However, the comprehensive utilization percentage of agricultural wastes containing rice husk is not high, only at 75% (Guo *et al.* 2015). The remaining agricultural wastes are always discarded, causing soil, water, and air pollution (Alvarez *et al.* 2014). In fact, those discarded agricultural wastes including rice husk can be reused as energy, fodder, fertilizer, and material (Kung *et al.* 2015; Zhang *et al.* 2015). Biochar prepared from rice husks can be used to replace the expensive active carbon to specifically remove inorganic or organic pollutants from wastewater. Li *et al.* (2020) utilized rice husk biochar with surface oxygenic functional groups as an activator for persulfate to govern textile dye wastewater and aniline wastewater through advanced oxidation treatment. Mandal *et al.* (2017) studied the rice husk biochar as an adsorbing material to remove atrazine herbicide and imidacloprid insecticide. Zhang *et al.* (2017) confirmed that the rice husk biochar with appropriate 0.2 to 1.2 mm particle size could serve as a biocarrier to improve AGS to treat pyridine wastewater. It is noteworthy that the investigation about rice husk biochar as biocarrier for microorganisms forming mature biofilm to treat digested swine wastewater is rarely reported.

The main objectives of this study were to: (1) explore COD, NH_4^+-N and TN removal efficiency of digested swine wastewater using biofilm attached to rice husk biochar, as a contrast of activated sludge; (2) consider the mechanisms of rice husk biochar with mature biofilm attachment to treat digested swine wastewater from the perspective of microbial community structure. This study provides a theoretical reference for the development of biochar from agricultural wastes for SBR-enhanced organic wastewater treatment similar to swine manure digestate.

EXPERIMENTAL

The Experimental section has two parts, namely a Materials and Methods section and a Determination method section. The goal is to evaluate the effects of rice husk biochar with adsorbed biofilm for enhancing biochemical reaction for contaminant removal of piggery wastewater. The activated sludge method served as a control.

Materials and Methods

Experimental materials and devices

The initial digested piggery wastewater was taken from a methane tank and secondary sedimentation tank in Mingsong Ecological Breeding Farmer Professional Cooperative (118°10′14.52″E, 30°59′36.79″N), Fanchang County, Wuhu City, Anhui Province, China. The water quality of original wastewater is given in Table 1. The concentration of NH_4^+-N and TN in digested piggery wastewater was high, and the range of C/N from 0.92 to 1.44 belonged to typical low-carbon ammonia-rich wastewater. During the experiment, the biogas tank wastewater and the secondary sedimentation tank wastewater were mixed based on a volume ratio of 1:1 as influent. In a different experiment phase, the influent was added into different concentrations of sodium acetate (CH_3COONa) (Analytic purity, Sinopharm Group) (Table 2).

Table 1. Water Quality of Digested Piggery Wastewater

Water Quality Index	Effluent From Biogas Digester	Effluent From Secondary Settling Tank
COD ($mg·L^{-1}$)	695 to 1078	196 to 316
NH_4^+-N ($mg·L^{-1}$)	749 to 752	57.5 to 60.2
NO_2^- ($mg·L^{-1}$)	0.22 to 0.35	0.60 to 1.64
NO_3^- ($mg·L^{-1}$)	0.00	13.6 to 18.0
TN ($mg·L^{-1}$)	769 to 793	75.4 to 76.2
pH	7.73 to 7.74	7.46 to 7.48

Table 2. Influent Concentration of Digest Wastewater During Different Periods in Lab

Laboratory Phases (Days)	Influent in SBR	Supplementation of Sodium Acetate ($mg·L^{-1}$)	C/N	Aeration Rate ($L·min^{-1}$)	pH
1-2	$V_{biogas\ tank}$:$V_{secondary\ clarifer}$ (1:1)	-	0.86 to 1.00	2.9	7.80 to 7.83
3-10	$V_{biogas\ tank}$:$V_{secondary\ clarifer}$ (1:1)	2500	5.19 to 6.82	2.9	7.51 to 8.02
11-20	$V_{biogas\ tank}$:$V_{secondary\ clarifer}$ (1:1)	4000	9.56 to 11.37	2.9	7.10

Activated sludge and rice husk biochar attached to mature biofilm were obtained from SBR in Restoration Ecology Laboratory, School of Ecology and Environment, Anhui

Normal University. The activated sludge in SBR was originally derived from a secondary clarifier (118º21′12.19"E, 31º16′42.58"N), in Chengnan Sewage Treatment Plant, Wuhu City, Anhui Province, China, with mixed liquid suspension substance (MLSS) of 6350 ± 324 mg·L^{-1}, sludge volume (SV) of 25 ± 1% and sludge volume index (SVI) of 39 ± 2 mg·L^{-1}.

Rice husk biochar specimens were prepared in the lab. The feedstock of rice husk was cleaned with tap water to wash out dust and impurities. Then the specimens were dried naturally, and put into an oven (101-2A, Hunan Electric Furnace & Oven Co.) at 105 °C for 12 h. After cooling, they were wrapped in aluminium foil and placed in a muffle furnace (SX2-4-10, Hunan Electric Furnace & Oven Co.) at 400 °C for 4 h without oxygen. After cooling, they were submerged with 15% wt of Na_2CO_3 (Analytic purity, Sinopharm Group) solution at 80 °C for 2 h. They were washed with distilled water and then submerged with 1 mol·L^{-1} HCl (Standard solution, Sinopharm Group) for 12 h. Subsequently, specimens were washed with distilled water and put into the oven (101-2A, Hunan Electric Furnace & Oven Co.) at 105 °C for 12 h. After cooling, they were ground with a mortar and pestle, then sieved using a 100-mesh screen to obtain the biochar with size of 45.3 to 439.7 μm. The rice husk biochar was used to cultivate biofilm in SBR as a carrier.

A self-made SBR with a plexiglass cylinder was installed for digested swine wastewater treatment in the laboratory. The SBR was configured with a diameter of 100 mm and a height of 1200 mm, giving an effective volume of 7.0 L and a ratio of height to diameter (*H*/*D*) of 12.0. The top of the SBR was closed with caps. The exhaust port of the air pump was connected with an intake tube that was linked to the inlet of the bottom head from the SBR. In addition, a nano-aeration disc was installed at the bottom of SBR. The intake tube was equipped with an air flow meter. The gas outlet in the upper part of the SBR was connected to an outlet pipe that was linked to the inlet of the air pump (see Fig. 1).

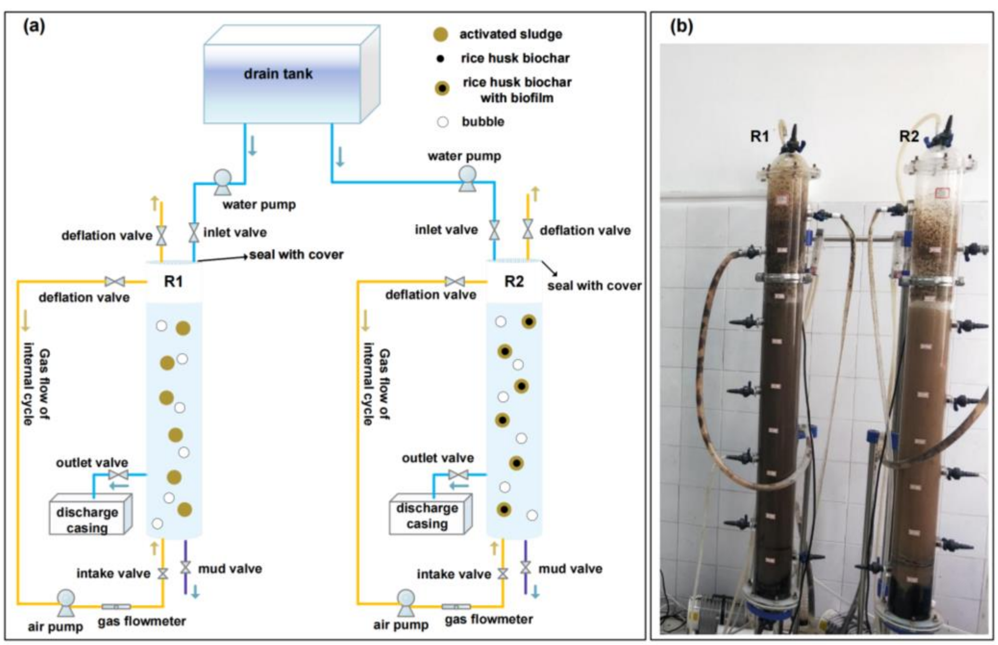

Fig. 1. Reaction equipment about SBR (R1: activated sludge; R2: rice husk biochar with biofilm) (a: sketch map; b: real image)

Thus, the internal circulation of air flow could be realized in the SBR. The vent valve of the cover from the top of the SBR was used to regulate the gas pressure and collect tail gas inside the reactor (Fig. 1). The aerate rate was set to 2.9 $L \cdot min^{-1}$. The operating period of SBR is displayed in Table 3.

Table 3. Cycle Period of Reaction Process in SBR

Operation Phase	Operation Content	Running Time
8:00 to 9:00	Sampling, drainage, influent	1 h
9:00 to 14:00	Aeration	5 h
14:00 to 17:00	Idle state	3 h
17:00 to 22:00	Aeration	5 h
22:00 to 1:00	Idle state	3 h
1:00 to 6:00	Aeration	5 h
6:00 to 8:00	Idle state	2 h

Determination Method

Monitoring of digested swine wastewater

The COD of influent or effluent water quality was measured by rapid catalytic process, according to the dichromate method according to HJ-828 (2017) to prepare the standard solution, then a COD rapid tester (5B-3A, Beijing Lianhua Yongxing Science and Technology Development Co., Ltd.) was used for analysis. NH_4^+-N concentration was assayed through sodium reagent spectrophotometry method in the light of HJ-525 (2009). Ultraviolet spectroscopy based on HJ/T 346 (2007) was used to determine NO_3^- and spectrophotometric method on the basis of GB 7498 (1987) was devoted to analysis NO_2^- through ultraviolet-visible spectrophotometer (Model number: 752, Shanghai Jinghua Technology Instrument Co., Ltd.).

Previous studies indicated that total nitrogen (TN) concentration could be calculated based on Eq. 1 (He *et al.* 2017a,b),

$$C_{TN}=C_{NH_4^+}+C_{NO_2^-}+C_{NO_3^-} \quad (1)$$

where C_{TN} is represented TN concentration, $mg \cdot L^{-1}$; $C_{NH_4^+}$ is denoted NH_4^+-N concentration, $mg \cdot L^{-1}$; $C_{NO_2^-}$ signifies NO_2^- concentration, $mg \cdot L^{-1}$; and $C_{NO_3^-}$ is expressed NO_3^- concentration, $mg \cdot L^{-1}$.

High-throughput sequencing of biofilm and activated sludge samples

The samples of activated sludge and rice husk biochar attached to biofilm were collected from SBR during the final phase of the experiment. The 16Sr DNA amplicon sequencing (Agilent 5400) and Illumina NovaSeq (NovaSeq PE250) sequencing was conducted at Novogene Co., Ltd. (Tianjin, China). Species annotation and abundance analysis were made through reads splicing and filtering as well as operational taxonomic units (OTUs) clustering.

RESULTS AND DISCUSSION

Removal Efficiency of Digested Swine Wastewater

COD degradation

The comparative result of COD degradation for digested swine wastewater between rice husk biochar attachment biofilm and activated sludge are shown in Fig. 2. In the reaction phase, within 1 to 2 days, when influent COD of digested swine wastewater was in the range of 307 and 348 mg·L^{-1}, as well as C/N in range of 0.86 and 1.00 (Table 2), the effluent COD decreased to 132 mg·L^{-1} with a removal efficiency of 62.1% using rice husk biochar attachment biofilm in SBR. Compared with contrasting group of activated sludge in SBR, the effluent COD was 41.4 mg·L^{-1} lower and the removal rate was 11.9% higher.

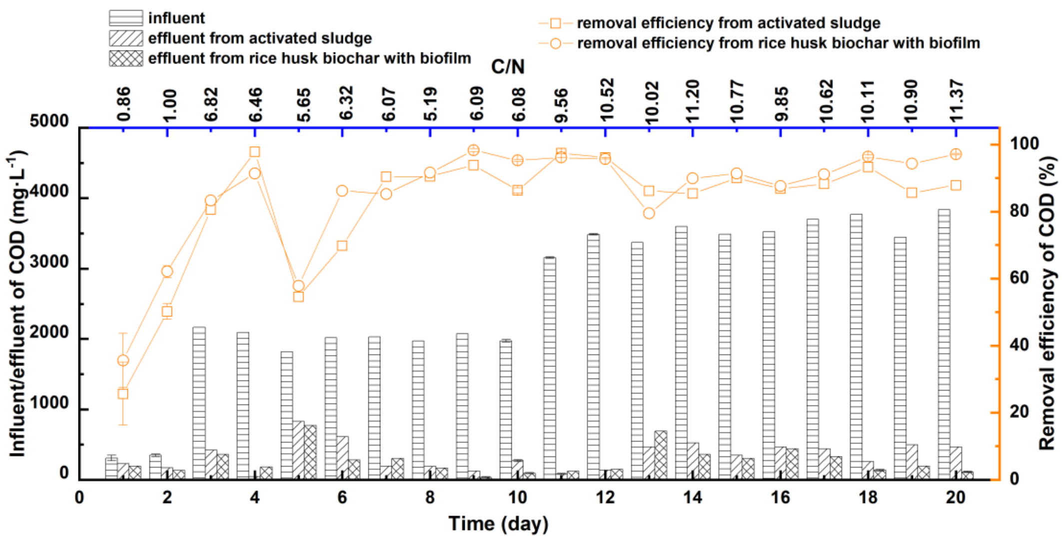

Fig. 2. COD removal efficiency of digested piggery wastewater

During the reaction period of 3 to 10 days, average influent of COD was at 2020 mg·L^{-1} with C/N of 6.08, after 2500 mg·L^{-1} sodium acetate supplement into the digested piggery wastewater (Table 2), the average removal efficiency of COD was at 86.1% with mean effluent COD of 273 mg·L^{-1}, under the condition of rice husk biochar attachment biofilm in SBR. The effluent COD from biochar with biofilm was lower (63.3 mg·L^{-1}) than that from activated sludge, as well as 3.18% higher removal efficiency. In the reaction phase of 11 to 20 days, the average influent COD was 3540 mg·L^{-1} and C/N was 10.5, after 4000 mg·L^{-1} sodium acetate addition into the digested swine wastewater (Table 2). The average effluent of COD was at 284 mg·L^{-1} and the mean removal efficiency of 91.9% was achieved through rice husk biochar attachment biofilm treatment in SBR. The effluent COD was lower 84.2 mg·L^{-1} with 2.24% higher removal efficiency in comparison with contrast group of activated sludge.

During the whole reaction period, the average influent of COD was 2610 mg·L^{-1}, as well as C/N of 7.77, the average effluent of COD was at 267 mg·L^{-1} and the removal efficiency of COD reached to 85.3%, under the condition of biochar with biofilm in SBR. In comparison to activated sludge, the effluent COD was lower, 70.9 mg·L^{-1}, as well as 3.5% higher removal efficiency. The effluent COD fulfilled the requirement of discharge standard from GB 18596 (2003) in China. It has been reported that the specific surface areas of rice husk biochar are as high as 1365 m^{2}·g^{-1} (Alvarez *et al*. 2014). The rice husk biochar with large specific area could improve adhesion for bacteria hence supporting the growth of biofilms (Adams *et al*. 2020). The biofilms with various microbes secrete Extracellular Polymeric Substances (EPS) to metabolize organic contaminants (COD) *via* several biochemical and/or bioelectrochemical reactions involved in respiration and cell growth (Jayakumar *et al.* 2021).

NH_4^+-N degradation

Figure 3 displays the NH_4^+-N degradation of digested swine wastewater in SBR with rice husk biochar attachment biofilm, considering activated sludge as contrast group in another SBR. More precisely, the influent C/N of digested swine wastewater ranged from 0.86 to 1.00, and the influent NH_4^+-N was between 346 and 356 mg·L^{-1}, during reaction phase of 1 to 2 days using biochar with biofilm in SBR to treat wastewater, the effluent NH_4^+-N declined to 141 mg·L^{-1} and the removal efficiency of NH_4^+-N reached to 59.3%. Compared with activated sludge group, the effluent of NH_4^+-N was lower, 57.5 mg·L^{-1} with 16.6% higher NH_4^+-N removal efficiency in biochar with biofilm group.

When the average influent C/N was at 6.08 after addition 2500 mg·L^{-1} sodium acetate into digested swine wastewater in reaction phase of 3 to 10 days, the mean effluent of NH_4^+-N was 39.1 mg·L^{-1} through biochar with biofilm treatment in SBR and the average removal efficiency of NH_4^+-N was 88.0%. The effluent of NH_4^+-N (84.2 mg·L^{-1}), was lower than that through activated sludge in SBR, and the removal rate of NH_4^+-N was 25.3% higher than that of contrast group. In reaction period of 11 to 20 days, after supplementing 4000 mg·L^{-1} sodium acetate into digested swine wastewater, the average influent of C/N was 10.5, and the average effluent of NH_4^+-N was reduced to 62.5 mg·L^{-1}. Meanwhile, the even removal rate of NH_4^+-N increased to 81.2% in SBR with rice husk biochar attachment with biofilm. Compared to the activated sludge group, the effluent of NH_4^+-N concentration was lower by 85.8 mg·L^{-1} from the rice husk biochar attachment biofilm group; simultaneously, the removal efficiency of NH_4^+-N was higher by 25.2%.

During reaction period of 1 to 20 days, the average influent of NH_4^+-N was 337 mg·L^{-1} and the mean value of C/N was 7.77 for digested swine wastewater. After rice husk biochar attachment biofilm treatment in SBR, the average effluent concentration of NH_4^+-N was 62.7 mg·L^{-1}. Meanwhile, the removal percentage of NH_4^+-N reached 81.3%. Compared with the control group, the effluent concentration of NH_4^+-N was lower by 82.4 mg·L^{-1}, while the removal efficiency of NH_4^+-N was 24.4% higher. Moreover, the effluent concentration of NH_4^+-N from rice husk biochar with attachment biofilm group realized the demand of discharge standard from GB 18596 (2003). Kizito *et al* (2015) has shown that rice husk biochar has the potential to adsorb NH_4^+-N from piggery manure anaerobic digestate slurry. Zhang *et al* (2017) has verified that the sludge formed in the reactor with the addition of biochar possessed excellent settling property, high biomass retention, and excellent degradation ability because EPS contents in the sludge increased to facilitate the treatment of high-strength refractory wastewater. The previous studies (Kizito *et al.* 2015; Zhang *et al.* 2017) could well support the result of rice husk biochar with biofilm for NH_4^+-N removal in this study.

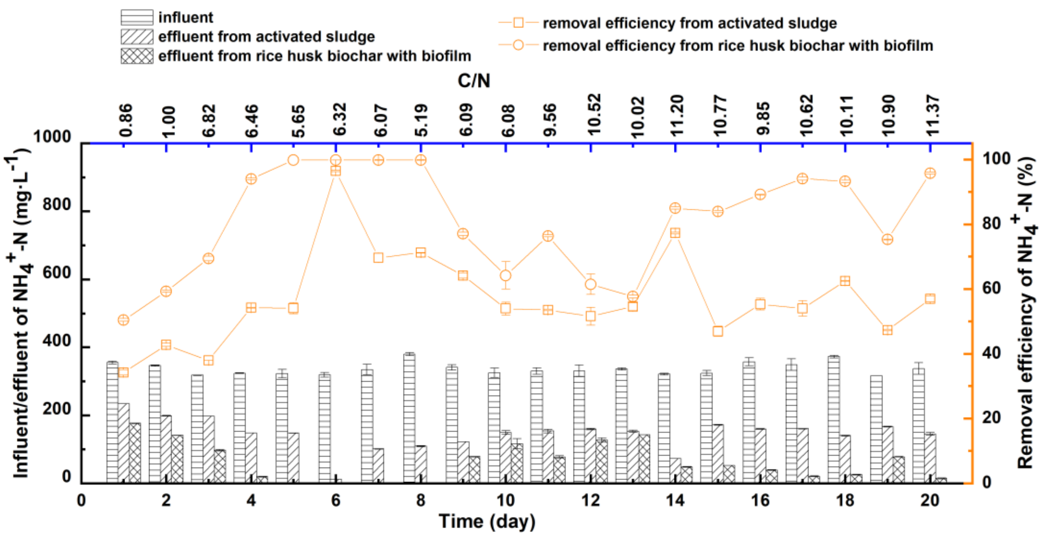

Fig. 3. NH_4^+-N removal efficiency of digested piggery wastewater

TN degradation

Figure 4 shows TN degradation of digested swine wastewater utilizing rice husk biochar in SBR in contrast to activated sludge group. In the reaction period of 1 to 2 days, the influent TN of digested swine wastewater was in the range of 353 to 362 mg·L^{-1} with a C/N of 0.86 to 1.00. The effluent of TN was maintained at 201 mg·L^{-1} as well as TN removal of 43.0%. The effluent TN was lower, 38.9 mg·L^{-1}, accompanied by an 11.0% higher removal efficiency, in comparison to control group of activated sludge.

During the experimental phase of 3 to 10 days, when the influent of digested swine wastewater was added into 2500 mg·L^{-1} sodium acetate, the average TN concentration was at 337 mg·L^{-1} with a mean C/N of 6.08. The average effluent TN of digested swine wastewater was maintained at 139 mg·L^{-1} and the even removal of TN was 58.3%, after biochar with biofilm treatment in SBR. The effluent TN was lower, 37.3 mg·L^{-1}, against that of activated sludge group, that is to say, the removal rate of TN was 11.1% higher against that of the control group. In the reaction period of 11 to 20 days, the average C/N was 10.5 and the mean TN concentration was 347 mg·L^{-1}, when 4000 mg·L^{-1} sodium acetate was supplemented into the influent of digested swine wastewater. The effluent of TN averaged at 85.7 mg·L^{-1}, and simultaneously the removal percentage of TN was 75.1%. The TN effluent was lower, 63.6 mg·L^{-1}, against that of the activated sludge group, meaning that the removal rate of TN was 18.1% higher than that of the contrast group.

In the whole reaction period, the influent digested swine wastewater of TN reached 344 mg·L^{-1} with an average C/N of 7.77. When the wastewater was treated by rice husk biochar attachment biofilm in SBR, the effluent of TN reached 119 mg·L^{-1} and the average removal percentage of TN was 65.2%. Compared with the activated sludge group, the effluent concentration of TN was lower by 51.07 mg·L^{-1}, with the removal percentage being higher by 14.72%. However, the effluent TN using biochar with biofilm was still high, which could be reduced by coupling with other effective technologies such as bio-electrochemical method and constructed wetland (Huang *et al.* 2013; Liu and Zhu 2020). It was confirmed that biochar increased TN removal and decreased N_2O accumulation because of biochar achieved the highest electron transfer efficiency and denitrifying enzyme activities, and the abundance of denitrifiers such as *Pseudomonas* increased (Wu *et al.* 2019). The biofim with denitrifiers attach themselves to rice husk biochar to produce EPS to metabolise inorganic contaminants (TN) *via* denitrification (Jayakumar *et al.* 2021).

That is why rice husk biochar with biofilm could remove nitrogen of the digested swine wastewater, based on previous studies (Wu *et al.* 2019; Jayakumar *et al.* 2021).

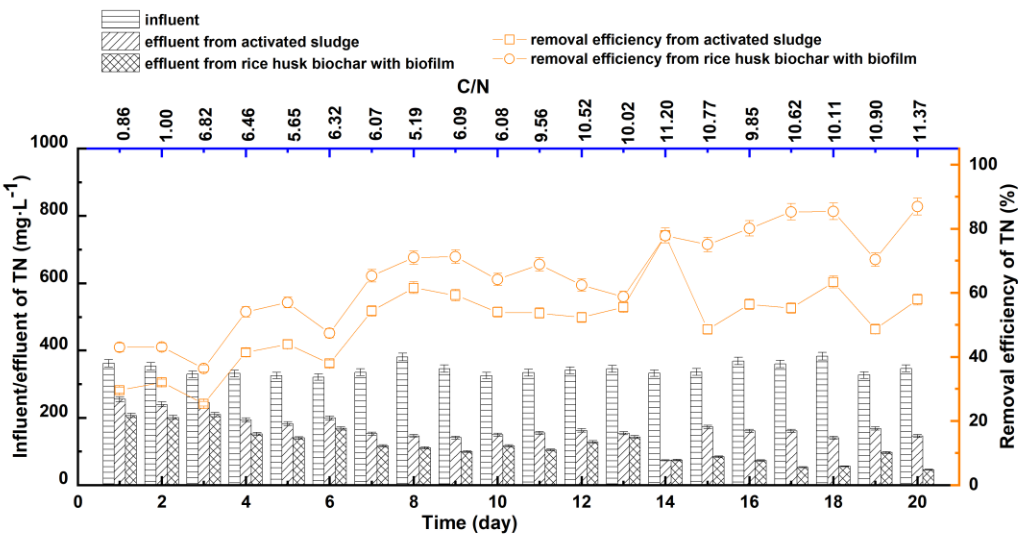

Fig. 4. TN removal efficiency of digested piggery wastewater

Influence of C/N on removal efficacy

The removal efficiencies of COD, NH_4^+-N, and TN were low in SBR with rice husk biochar attachment biofilm, and the average C/N of the digested swine wastewater was 0.93. However, the removal efficiency of COD, NH_4^+-N, and TN were extremely improved after the influent of C/N reached 7.77, when the wastewater was stepwise complemented with sodium acetate of 2500 mg·L^{-1} and then 4000 mg·L^{-1}. The organic carbon through adding sodium acetate not only provided nutrients for microorganisms but also supplied an electron donor for heterotrophic bacteria to denitrification.

He *et al.* (2014b) showed that the best removal efficiency of COD, NH_4^+-N, and TN could be obtained under condition of 21.3 C/N to gain simultaneous methanation denitrification (SMD) and partial nitrification and denitrification (PND) for piggery wastewater treatment in an anaerobic reactor. Dan *et al.* (2020) found that digested swine wastewater containing nitrogen and phosphorus was capable of being removed when C/N reached 6, while the nitrogen and phosphorus was hard to be eliminated due to deficient carbon source from C/N lower than 4. Wei *et al.* (2014) found that simultaneous N and P removal achieved best at influent C/N ratio of 9 and NH_4^+-N of 200 mg·L^{-1}. The C/N of 7.77 was concerned in this study, which was a bit higher than that of Dan *et al.* (2020) mentioned, which improved nitrogen removal efficiency. The C/N of 7.77 was quite a bit lower than He *et al.* (2014b)'s C/N of 21.26, and that would mean that this kind of carbon source intake was at least a reduction of 63.4%, namely the investment of additional carbon source could be saved. Despite the fact that the existing carbon source offers electron for denitrification to improve TN removal, the extra carbon addition increases the running cost (Dan *et al*. 2020; He *et al*. 2014b; Wei *et al.* 2014). From the view of long-term development, the relevant bio-denitrification technologies should be further advanced with no extra carbon source supplement to improve nitrogen removal efficiency. It is worth mentioning that biochar such as rice husk biochar is a possible candidate for use in cost-effective and sustainable biological water treatment, especially in agrarian economies with easy access to abundant biomass in the form of crop residues and organic wastes (Jayakumar *et al*. 2021).

Microbial Functionalities

Relative abundance analysis of microbial communities

Figure 5 shows the microbial community structure and relative abundance of species originating from the samples of rice husk biochar attachment biofilm and activated sludge. There was an apparent discrepancy of microorganism relative abundance in the phylum level between biochar with biofilm group and activated sludge group (Fig. 5a). The top 10 phyla in relative abundance were *Proteobacteria*, *Firmicutes*, *Bacteroidota*, *Actinobacteriota*, *Verrucomicrobiota*, *Acidobacteriota*, *Campylobacterota*, *Halobacterota*, unidentified bacteria, and WPS-2. The relative abundance of *Proteobacteria* showed the highest value of 0.66 and 0.65 in biochar with biofilm sample and activated sludge sample, respectively. Previous studies indicated that *Proteobacteria* belongs to facultative heterotrophic bacteria, with performance of respiratory and fermentation metabolism, and are the main functional bacteria to degrade organic matter and remove pollutants (He *et al.* 2017a,b). The relative abundance of *Firmicutes* was separately 0.06 and 0.17 in biochar with biofilm sample and activated sludge sample. It was reported that *Firmicutes* are common Gram-positive bacteria with a thick cell wall of 10 to 50 nm (Shu *et al.* 2015; He *et al.* 2017b). The *Bacillus* under *Firmicutes* is capable of decomposing organic matter and eliminating pollutants as important bacteria in sewage treatment (Mori *et al.* 2004; Wang *et al.* 2019). The relative abundance of *Bacteroidota* in biochar with biofilm sample and activated sludge sample was 0.06 and 0.04, respectively. It is known that *Bacteroidetes* are Gram-negative bacteria and obligate anaerobes with the function of decomposing organic matter (Ferrera and Sanchez 2016; Rahimi *et al.* 2020). The *Bacteroidetes* were discovered in an aerobic environment, which was associated with internal anoxic circumstances of biochar with biofilm and activated sludge to realize simultaneous nitrification and denitrification. The relative abundance of *Actinobacteriota* from rice husk biochar attachment biofilm sample and activated sludge sample was 0.018 and 0.013, respectively. Researchers have shown that *Actinobacteriota* is crucial for nitrification and denitrification in wastewater treatment (Ferrera and Sanchez 2016). Moreover, the relative abundance of *Acidobacteriota* was 0.009 and 0.008 in rice husk biochar attachment biofilm sample and activated sludge sample, respectively. It has been confirmed that the *Acidobacteriota* has the feature of decomposing and mineralizing organic matter (Kristensen *et al.* 2021).

The differences in relative abundance of species between rice husk biochar attachment biofilm sample and activated sludge sample are shown in Fig. 5b. The top 10 genera in relative abundance are *Thauera*, *Pseodomonas*, *Acinetobacter*, *Erysipelothrix*, *Thermomonas*, *Acobacter*, *Methanosaeta*, *Arenimonas*, unidentified_*Micavibrionales*, and DMER64. The relative abundance of *Thauera*, *Pseudomonas*, *Acinetobacter*, and *Arenimonas* were 0.32, 0.013, 0.04, and 0.05 in rice husk biochar attachment biofilm sample, at the same time, 0.38, 0.011, 0.06, and 0.02 in activated sludge sample, respectively. Those bacteria genera belong to phylum *Proteobacteria*. It is worth mentioning that *Thauera* has been the most dominant bacteria for aromatic hydrocarbon degradation in sewage disposal system (Mao *et al.* 2010). The relative abundance of *Thermomonas* and *Erysipelothrix* was 0.03 and 0.02 in rice husk biochar attachment biofilm sample, while 0.02 and 0.08 in activated sludge sample, respectively. Both *Thermomonas* and *Erysipelothrix* are classified as phylum *Firmicutes*. In addition, the relative abundance of *Acobacter* and *Methanosaeta* was 0.007 and 0.009 in rice husk biochar attachment biofilm sample, while it was 0.013 and 0.015 in activated sludge sample, respectively.

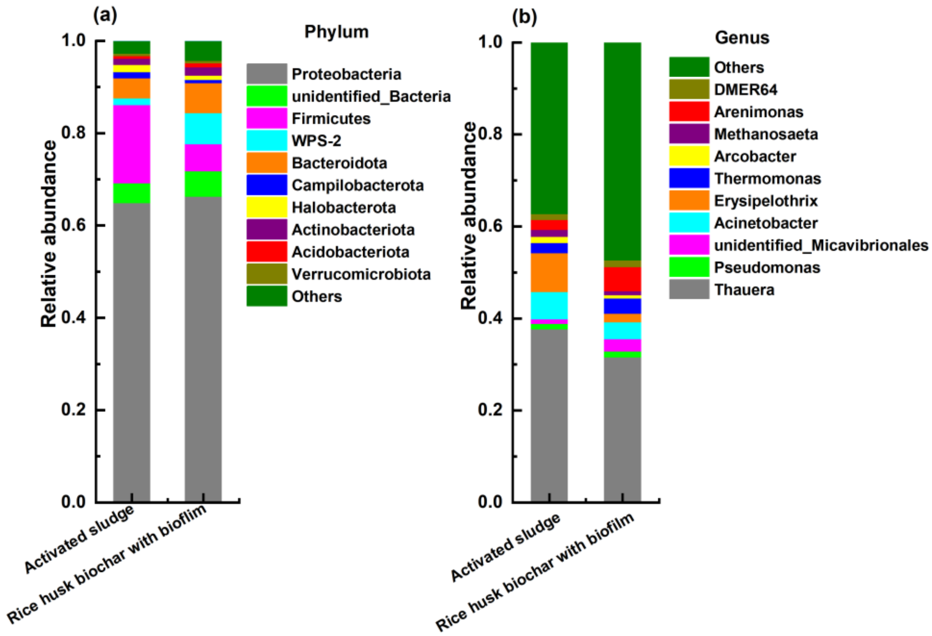

Fig. 5. Relative abundance of species at the phylum and genus level

Clustering analysis of species abundance

Figure 6 shows the result of microbial species abundance clustering with respect to rice husk biochar attachment biofilm sample and activated sludge sample. The main bacterial genera in rice husk biochar attachment biofilm were *Hydrogenophaga*, *Arenimonas*, *Novosphingobium*, *Paracoccus*, *Rhodobacter*, *Acinetobacter*, and *Sphingomonas*, which were grouped into phylum *Proteobacteria* (Ferrera and Sanchez 2016; Xie *et al.* 2021). There were also *Ferruginibacter*, OLB8, *Flavobacterium*, and *Leadbetterella* in rice husk biochar attachment sample, which belong to phylum *Bacteroidota*. *Lactobacillus*, *Thermomonas*, and *Christensenellaceae*_R-7_group were categorized as phylum *Firmicutes*, unidentified_*Micavibrionales* belongs to phylum unidentified_bacteria, and *Pirellula* belongs to phylum *Planctomycetes*, and appeared in rice husk biochar attachment sample. *Hydrogenophaga*, *Comamonas*, *Acinetobacter*, and *Sphingomonas*, sorted into phylum *Protebacteria*, were the primary bacterial genera discovered in the activated sludge sample. *Christensenellaceas*_R-7_group, *Proteiniclasticum*, *Erysipelothrix*, and *Tissierella* also appeared in the activated sludge sample, belonging to the phylum *Firmicutes*. The results indicated that there are extreme differences in the dominant genus between rice husk biochar attachment biofilm sample and activated sludge sample. Moreover, *Acinetobacter*, *Aomamonas*, *Pseudomonas*, *Flavobacterium*, and *Corynebacterium* were found in samples. Those species have characteristics of nitrification and denitrification with strong nitrogen removal performance (Liu and Zhu 2020; Rahimi *et al.* 2020).

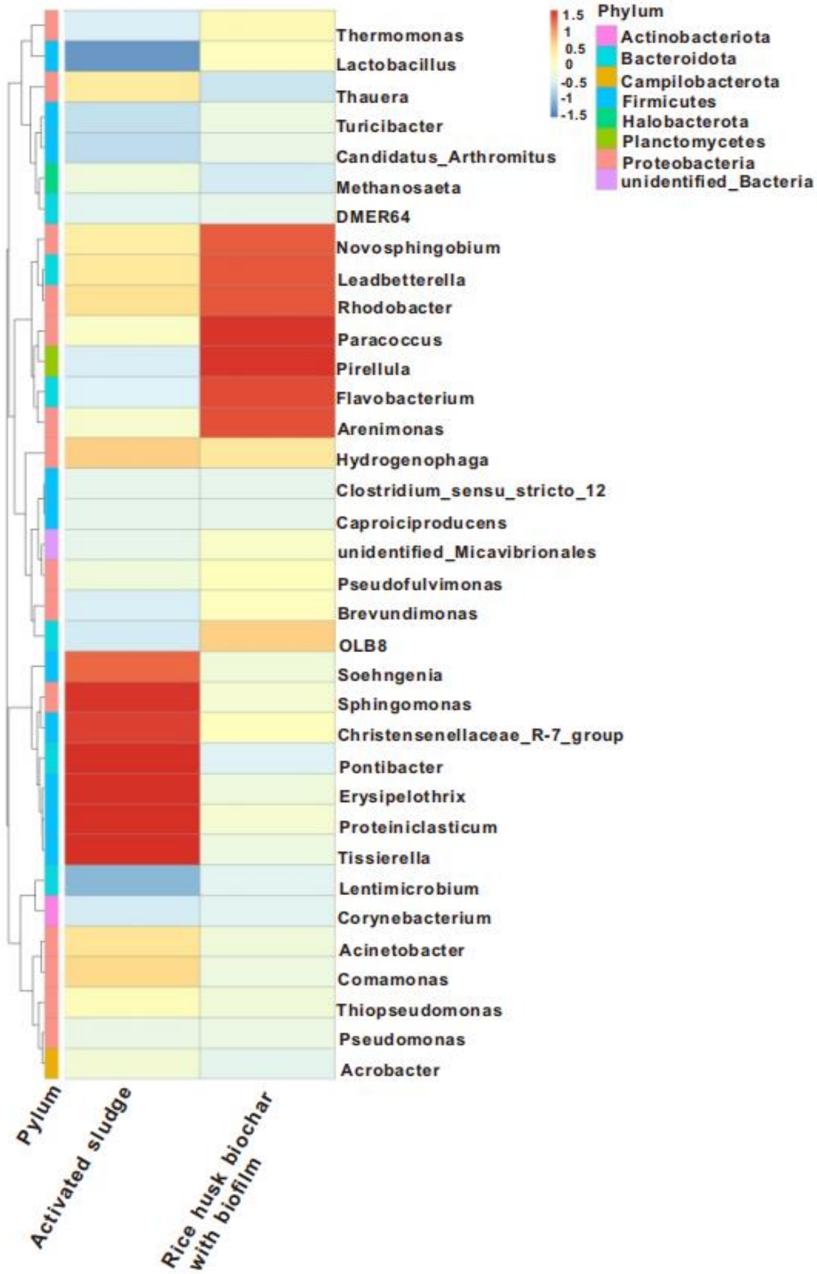

Fig. 6. Clustering map of species abundance

CONCLUSIONS

Rice husk biochar attachment biofilm was capable of improving treatment efficiency for digested swine wastewater.

1. The COD, NH_4^+-N, and TN removal efficiencies were 85.3%, 81.3%, and 65.2%, respectively, using rice husk biochar attachment biofilm treatment in SBR, when influent COD, NH_4^+-N, and TN of digested swine wastewater were 2610, 337, and 344 mg·L^{-1}, respectively, with an influent C/N of 7.77. In contrast to activated sludge, the removal efficiency of COD, NH_4^+-N, and TN were improved 3.5%, 24.4%, and 14.7%, respectively. Additionally, the effluent of COD and NH_4^+-N fulfilled the requirement of discharge standard from GB 18596 (2001) in China. That's contributed to rice husk biochar with large specific surface area to improve the growth biofilms to secrete EPS to metabolise organic or inorganic contaminants *via* biochemical and/or bioelectrochemical reactions including respiration, cell growth, nitrification, and denitrification.
2. High-throughput sequencing analysis indicated that microorganism community structure and relative abundance exhibited a distinct discrepancy between rice husk biochar attachment biofilm sample and activated sludge sample. The plentiful phylum *Proteobacteria* and *Thauera* genus that appeared in biochar with biofilm sample are critical for decomposing COD, NH_4^+-N, and TN in digested swine wastewater. Microbial species clustering revealed that *Acinetobacter*, *Comamonas*, *Pseudomonas*, *Flavobacterium*, and *Corynebacterium*, with functionality of heterotrophic nitrification and aerobic denitrification, occurred in biochar attachment biofilm sample.
3. Rice husk biochar was demonstrated to be a cost-effective and sustainable carrier for biological water treatment. The results provide a reference for the development of biochar from agricultural wastes for improving organic wastewater treatment. But sodium acetate has to be added into digested swine wastewater to improve denitrification, resulting in an increased cost. Advanced bio-denitrification technology should be offered in future research, with no extra carbon source addition to promote nitrogen removal of organic wastewater.

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