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A sustainable integrated anoxic/aerobic bio-contactor process for simultaneously in-situ deodorization and pollutants removal from decentralized domestic sewage

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ABSTRACT

The integration of anoxic filter and aerobic rotating biological contactor shows promise in treating rural domestic sewage. It offers high efficiency, low sludge production, and strong shock resistance. However, further optimization is needed for odor control, pollutant removal, and power consumption. In this study, the investigation on a one-pump-drive lab-scale device of retention anoxic filter (RAF) integrated with hydraulic rotating bio-contactor (HRBC) and its optimal operation mode were conducted. During the 50-day operation, optimal operation parameters were investigated. These parameters included a 175 % reflux ratio (RR), 5-h hydraulic retention time in the RAF (HRT_{RAF}), and 2.5-h hydraulic retention time in the HRBC ($HRT_{HRBC)}$. Those conditions characterized a micro-aerobic environment (DO: 0.6–0.8 mg/L) in RAF, inducing improved deodorization (89.3 % sulfide removal) and denitrification (85.9 % nitrate removal) simultaneously. During the operation period, 84.79 ± 3.87 % COD, 82.71± 2.06 % NH_4^+-N, 74.83 ± 2.06 % TN, 91.68± 2.12 % S^{2-}, and 89.04 ± 1.68 % TON were removed in RAF-HRBC. Based on large amount of operational data, organic loading rate curves of RAF-HRBC were validated and calibrated as a crucial reference to aid in full-scale designs and applications. The richness of microbial community was improved in both RAF and HRBC. In the RAF, the autotrophic sulfide-oxidizing nitrate-reducing bacteria (a-son) and heterotrophic sulfide-oxidizing nitrate-reducing bacteria (h-son) were selectively enriched, which intensified the sulfide removal and denitrification process. In the two-stage HRBC system, the 1st stage RBC was primarily composed of organics degraders, while the 2nd stage RBC consisted mainly of ammonium oxidizers. Overall, the integrated RAF-HRBC process holds significant potential for simultaneously improving pollutant removal and in-situ odor mitigation in decentralized domestic sewage treatment. This process specifically contributes to enhancing environmental sustainability and operational efficiency.

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1. Introduction

The uncontrolled discharge of untreated sewage, along with associated odor emissions, significantly impacts the rural water environment and poses risks to public health and hygiene [1]. Recent findings indicate that 67.8 % of rural domestic sewage treatment facilities in China are currently facing operational challenges. Furthermore, the deodorization performance of decentralized sewage treatment process has been rarely reported. The rotating bio-contactor (RBC), originated in Germany in the 1902s, is a type of biological treatment device composed of a treatment tank, driving device, rotating shaft, and several discs submerged in the wastewater [2]. The characteristics of flexibility, reliability, low sludge yields and strong shock resistance are feasible for rural sewage treatment [3]. Significant endeavors have been undertaken to improve energy efficiency and optimize the functionality of RBCs. Zha et al. [4] integrated rotating biological contactor with anoxic filter performing nearly 90 % removal efficiency for both COD and NH_4^+-N. Wang et al. [5] combined a vertical-flow constructed wetland with RBCs to improve the removal efficiency of phosphorus in rural sewage. Han et al. [6] developed a self-refluxing RBCs performing well in removing COD, SS, and NH_4^+-N. However, these devices have unsatisfactory denitrification efficiency. Moreover, there is a lack of research on odor control methods specifically designed for decentralized rural sewage treatment. Deodorization is particularly important for the small-scale, decentralized aerobic biological treatment processes relying on natural ventilation and cascade aeration around living areas.

Abbreviations

RAF	Retention anoxic filter
HRBC	Hydraulic rotating bio-contactor
RR	Reflux ratio
HRT	Hydraulic retention time
RS	Rotational speed
COD	Chemical Oxygen Demand
NH_4^+-N	Ammonium Nitrogen
TN	Total Nitrogen
TP	Total phosphorus
TON	threshold odor number
DO	Dissolved oxygen
WWTP	Wastewater Treatment Plant
NRB	nitrate-reducing bacteria
AOB	Ammonia oxidizing bacteria
h-bacteria	Heterotrophic bacteria
a-soNRB	autotrophic sulfide-oxidizing nitrate-reducing bacteria
h-soNRB	heterotrophic sulfide-oxidizing nitrate-reducing bacteria
SOAD	sulfide-oxidizing autotrophic denitrification

Odor emissions during the wastewater treatment process result in a wide list of detrimental impacts to public health and living environments. Hydrogen sulfide stands out as a prevalent odorant and is considered a reliable indicator of odor control performance [7,8]. Conventional gas-phase based methods for deodorization of urban WWTPs (e.g., adsorption, thermal oxidation, and bio-scrubbers) are not suitable for decentralized rural sewage treatment, because those require complex and expensive gas collection and treatment systems, and there may also be risks like gas short-circuiting, gas diffusion, and accumulation of toxic and harmful substances [9]. Emerging in-situ biological odor mitigation methods within activated sludge (AS) systems [10,11] addressed these limitations. However, the AS systems rely on units for sludge recirculation, sedimentation, and disposal, which pose challenges in terms of high energy consumption and complex maintenance, rendering it still impractical for decentralized rural sewage treatment facilities.

Therefore, this study developed a novel micro-powered self-reflux anoxic-aerobic biofilm system consisting of retention anoxic filter (RAF) and hydraulic rotating bio-contactor (HRBC) based on the requirements of decentralized rural sewage treatment. The process of simultaneous odor and pollutants removal in the RAF-HRBC is as follows: The aerobic unit (HRBC) degrades organics and oxidizes ammonium into nitrate. The effluent from HRBC containing dissolved oxygen (DO) and nitrate flows by gravity back to the anaerobic unit RAF packed with linen felts, creating a specific microaerobic environment preferring the growth of sulfide-oxidizing autotrophic denitrifying bacteria (a-soNRB) [12,13]. The microaerobic environment promotes the utilization of sulfide by a-soNRBs as electron donor to undergo sulfide-oxidizing autotrophic denitrification (SOAD), as described in Eqs. (1) and (2) [10,14]. This process not only achieved in-situ control of odor emission, but also enhanced denitrification efficiency. In the laboratory-scale, the stable operation of the system can be achieved with only one water pump and gravity flows.

$$S^{2-} + 0.4NO_3^- + 2.4H^+ \rightarrow S^0 + 0.2N_2 + 1.2H_2O, \Delta G^0 = -191.0\text{kJ} \tag{1}$$

$$S^0 + 1.2NO_3^- + 0.4H_2O \rightarrow SO_4^{2-} + 0.6N_2 + 0.8H^+, \Delta G^0 = -547.6\text{kJ} \tag{2}$$

In previous studies, the optimal operational parameters including reflux ratio (RR) and hydraulic retention time (HRT) were determined by simply comparing the pollutant removal efficiencies of the reactors at different default conditions [4,5]. However, the explanations regarding the mechanisms behind these findings in those studies were relatively unclear. Based on lab-scale system and batch tests, this study investigated the influence of the RR on the DO and the mass ratio of organic carbon to nitrate (C/N) levels in the RAF. Subsequently, the time evolutions of sulfide and main pollutants were analyzed under different DO, C/N, and HRBC rotational speed (RS) levels to determine the optimal RR, HRT_{RAF}, and HRT_{HRBC}.

This study introduces a novel in-situ odor emission abatement method for decentralized rural sewage treatment. The method also enhances the pollutants removal efficiency. The integrated RAF-HRBC offers a sustainable process for treating rural domestic wastewater in a decentralized manner. This approach holds promising potential for widespread application.

2. Material and methods

2.1. Experimental apparatus setup

The RAF-HRBC experimental scheme as well as the flow illustration are shown in Fig. 1. The device mainly consists of the hydraulic rotating bio-contactor (HRBC) and the retention anoxic filter (RAF). The HRBC contained two stages of RBC, each stage consisted of 14 plastic discs (diameter of 20 cm each) with non-woven fabric attached. The immersion rate of RBC was 45 %, and the total effective area for two stages of HRBC reactor was 1.58 m^2. The RAF was made of material PP with the retention zone (working volume 10 L) and the anoxic zone (working volume 10 L). The anoxic zone was packed approximately 80 % of working volume with numerous sheets of linen felt providing attachment space for microorganisms to grow. The felts were fixed and suspended by an iron frame with intervals of 20 mm. The retention zone was designed to mix the effluent and reflux flow, also to avoid water quality and quantity shock in the anoxic zone.

The effluent of RAF was divided into treating water flow and kinetic flow. The kinetic flow drove the waterwheel connected with the RBC, and the rotating speed of RBC can be adjusted by regulating kinetic flow rate through the adjustable valve on the rotameter. The effluent of HRBC was divided into reflux flow and the system effluent by adjustable divider in lower regulating tank.

2.2. Reflux ratio and DO control in the RAF

The DO level in the RAF was controlled by adjusting the reflux flow rate (indicated by the orange arrow in Fig. 1) from the HRBC to the RAF. This reflux flow was the treated water containing nitrate and DO from the HRBC. The DO concentration in the RAF could be altered by changing the amount of reflux flow that entered the RAF, which is referred to as the Reflux Ratio (RR = volume of reflux flow/volume of effluent) in the system. The volume of reflux flow was controlled by an adjustable divider in the lower regulating tank.

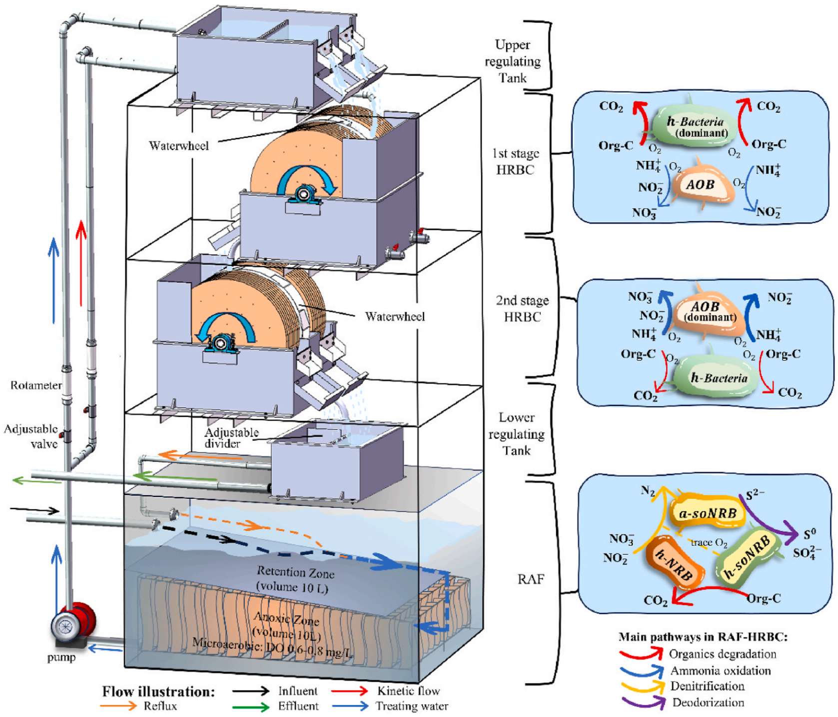

Fig. 1. Diagram of RAF-HRBC scheme and degradation pathways in the system.

This reflux flow was then mixed with the system influent (indicated by the black arrow in Fig. 1) for the denitrification and sulfide oxidation processes in the Anoxic Zone within the RAF.

2.3. Experimental conditions and operation

The paddings of RAF were inoculated with sludge from the secondary sedimentation tank of the Xishan Municipal Wastewater Treatment Plant (Wuxi, China). The HRBC biofilm was developed by 1-month continuous operation without inoculation. During the period of biofilm growth and operational parameters adjustment tests, synthetic wastewater (Table S1.) with trace element solution (Table S2.) based on real rural domestic sewage monitoring data was used to eliminate the influence of unstable influent water quality. During the 50-day stability monitoring period, the influent of the system was real domestic sewage (Table S3.) of rural households in Yixing, Wuxi, consisted of pretreated black water from septic tanks and grey water from bathroom and kitchen. The influent flow rate of the system was 45 L per day.

The drop height of two stages HRBC was set as 0.6 m according to the previous study [5]. HRBC RSs of 2, 4, 6, 8, 10 and 12 rpm were set to monitor the COD and NH_4^+-N removal time course in the HRBC. Twelve RRs from 25 % to 200 % with interval of 25 % were performed in RAF-HRBC to analyze the DO and C/N variations in the RAF. And the influence of DO and C/N levels on the pollutant removal and deodorization in RAF was investigated through batch assays.

2.4. Batch assays

The batch tests were conducted to avoid the continues influent impacts on analyzing odor/pollutants removal time courses under different DO, C/N levels in RAF. The customized set for the batch tests is shown in Fig. S1. The sulfide, COD and nitrate removal time courses were performed under six DO levels (0, 0.1–0.3, 0.6–0.8, 1.2–1.5, 1.8–1.9 and 2.1–2.4 mg/L) and five C/Ns (0.86, 2:1, 3:1, 4:1 and 6:1) in well-sealed Erlenmeyer flasks (250 ml, Shuniu, Chengdu). Numerous sheets of linen felt (VSS: 12.7 g/L) were incubated with 200 ml prepared medium with filling fraction of 80 % to simulating the RAF reactor configuration. Argon gas was passed through the Erlenmeyer flasks for 15 min to remove oxygen from the solutions and the headspaces. Prescribed DO levels were maintained by micro air pump (F16H, Xinweicheng, Chengdu), and were monitored by the DO meter (YSI-DO200, YSI, Yellow Springs, OH, USA) simultaneously during the tests. The tests spanned 20 h during which samples were collected and tested for measurements of COD, nitrate, and sulfide concentrations hourly. All measurements were conducted in triplicate. The media recipe for batch tests is shown in Tables S4–S6.

2.5. Microbial community analysis

The biofilm samples were collected from the filter biofilm in RAF (named A), the biofilm of the discs in the 1st stage of HRBC reactor (named O1) and the 2nd stage of HRBC reactor (named O2) on the 50th day of the stable operation period. Three parallel samples were taken from each zone. The nine samples were sent in dry ice to Shanghai Sangon Biotech Co., Ltd. For a high throughput sequencing test.

The genomic DNA extraction was performed using a E. Z.N.A™ Mag-Bind Soil DNA Kit (Omega, M5635-02, USA). The 16 S rRNA V3–V4 amplicon was amplified using 2 × Hieff® Robust PCR Master Mix (Yeasen, 10105ES03, China). Two universals bacterial 16 S rRNA gene amplicon PCR primers (PAGE purified) were used: the amplicon PCR forward primer (CCTACGGGNGGCWGCAG) and amplicon PCR reverse primer (GACTACHVGGGTATCTAATCC). The PCR amplification and measurement details can be referred to Zha et al. [15].

2.6. Analysis methods

All samples were filtered through 0.22 μm membrane (PES, PALL, USA). Standard methods (Rice, Bridgewater, and Association 2012) were employed to measure the COD, ammonium, nitrate, nitrite, total nitrogen, and total phosphorus. The determination of sulfide in a liquid, specifically referring to H_2S(aq), S^{2-}, and HS-, under alkaline conditions, was carried out using the methylene blue method. The threshold odor number (TON) were measured by Chinese SEPA standard methods.

Statistical analysis was performed using SPSS 24 software (IBM Corporation, USA) and OriginPro 2018b software (OriginLab Corporation, USA). To determine statistically significant differences, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc analysis ($p < 0.05$) was conducted using OriginPro 2018b software (OriginLab Corporation, USA). The operational taxonomic units (OTUs) resulting from the sequencing of microbial samples were classified using Mothur software (version 1.30.1). The obtained sequences were phylogenetically assigned at various taxonomic levels based on a 97 % similarity threshold for further analysis. Representative sequences from each OTU were compared to the Ribosomal Database Project (RDP) classifier (version 2.12) and the National Center for Biotechnology Information (NCBI) Blast+ (version 2.28) for microbial community analysis.

3. Results and discussion

3.1. RAF deodorization and pollutant removal efficiency

3.1.1. RR influence on DO and C/N in RAF

In the RAF-HRBC system, the reflux of oxygenated nitrification effluent from HRBC significantly affects the deodorization and denitrification efficiency by altering DO, nitrate concentration, and C/N levels in RAF. Among these, C/N and DO levels are the two most direct influencing factors to the deodorization and denitrification effectiveness in the RAF [12,16]. As the RR increased in increments of 25 % from 0 % to 300 %, the DO level increased from 0 mg/L to 2.36 mg/L (Fig. 2a), The fitted linear relationship between the RR and the DO levels in the RAF was $y = 0.1707x^2 + 0.267x - 0.0311$, $R^2 = 0.9899$. The C/N decreased from 20.75 to a minimum value of 1.78 (Fig. 2b) as the RR increased from 25 % to 300 %, The fitted linear relationship between the RR and the C/N in the RAF was: $y = 5.7379x^{-0.938}$, $R^2 = 0.9993$. The concentration of nitrate variations over reflux ratios are shown in supplementary materials (Fig. S2.).

The variations in C/N and DO resulting from different RRs had subsequent effects on the degradation rates of COD, nitrate, and sulfide in RAF. The following discussions focus on these influences.

3.1.2. DO dependence

At a temperature of 25 °C, with a C/N of 4:1, the time courses of sulfide, nitrate, and COD concentrations at different DO levels are shown in Fig. 3a, b, and 3c, respectively (the time course trends were similar at other C/N ratios and temperatures).

As shown in Fig. 3a, the sulfide removal process was limited under anaerobic conditions (DO: 0 mg/L). When the DO was 0.1–0.3 mg/L, the "rebound" of sulfide concentration disappeared, and a removal rate of 98.3 % was achieved by the 13th hour. After the DO exceeded 0.5 mg/L, the removal speed of sulfide significantly increased, when the DO levels were 0.6–0.8, 1.2–1.5, 1.8–1.9 and 2.1–2.4 mg/L, all dosed sulfide was oxidized in 6, 4, 3, 1.5 h, respectively. It is obvious that sulfide rebound can be eliminated by trace DO dose. Moreover, the sulfide removal was significantly improved by increasing the DO level in RAF. In practical applications, adjusting the reflux ratio to maintain an appropriate DO level of 0.6–0.8 mg/L can be a feasible method to improve the deodorization efficiency of RAF.

The removal efficiency of nitrate was also significantly affected by DO levels (Fig. 3b). For DO levels of 0.1–0.3 mg/L and 0.6–0.8 mg/L, the nitrate removal rate remained similar in the first 2 h and reached a removal rate of 50 %. However, after 2 h, the denitrification efficiency at the DO level of 0.6–0.8 mg/L was significantly higher than that at 0.1–0.3 mg/L. The nitrate removal efficiencies in the 5th hour at DO levels of 0.1–0.3 mg/L and 0.6–0.8 mg/L were 65.8 % and 85.9 %, respectively. This might be due to the enhanced metabolism of autotrophic denitrifying bacteria, like a-soNRBs, in environments with a DO level of 0.6–0.8 mg/L. When DO level exceeds 1 mg/L, the efficiency of denitrification significantly decreased. This might be because denitrifying bacteria utilized the oxygen in the water instead of nitrate or nitrite for respiration and metabolism [17].

In contrast, the changes in DO levels had a lesser impact on the removal rates of COD (Fig. 3c). Generally, the removal rate of COD was slightly lower at higher DO levels compared to lower DO levels. This could be attributed to the reduced activity of heterotrophic denitrifying bacteria in organic degradation processes when there is an excess supply of DO. Based on the DO levels, the optimal COD removal efficiency of 40 % was achieved between 4 and 7 h.

According to the above, the optimal dissolved oxygen in RAF should be regulated to 0.6–0.8 mg/L through a 175 % RR from HRBC to achieve optimal removal rates of sulfide (87.93 %), nitrate (85.91 %) and COD (39.29 %) in 5 h, and the optimal HRT of RAF was set as 5 h.

3.1.3. C/N dependence

The stoichiometric molar ratios of organic carbon to nitrate in SOAD and heterotrophic denitrification are 3:4 and 3:1, respectively. Considering the carbon sources required for biological growth and other loss of carbon sources, a molar ratio greater than 1:1 (mass ratio greater than 0.86) is considered as high C/N for SOAD. For heterotrophic denitrification, a mole ratio greater than 3 (mass ratio

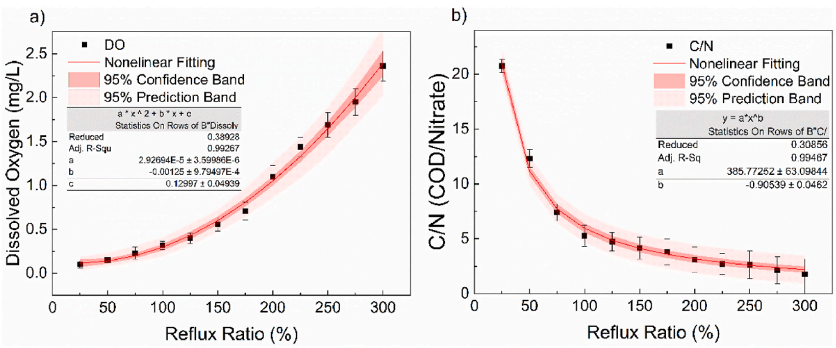

Fig. 2. The influence of RR on DO (a) and C/N (b) in RAF.

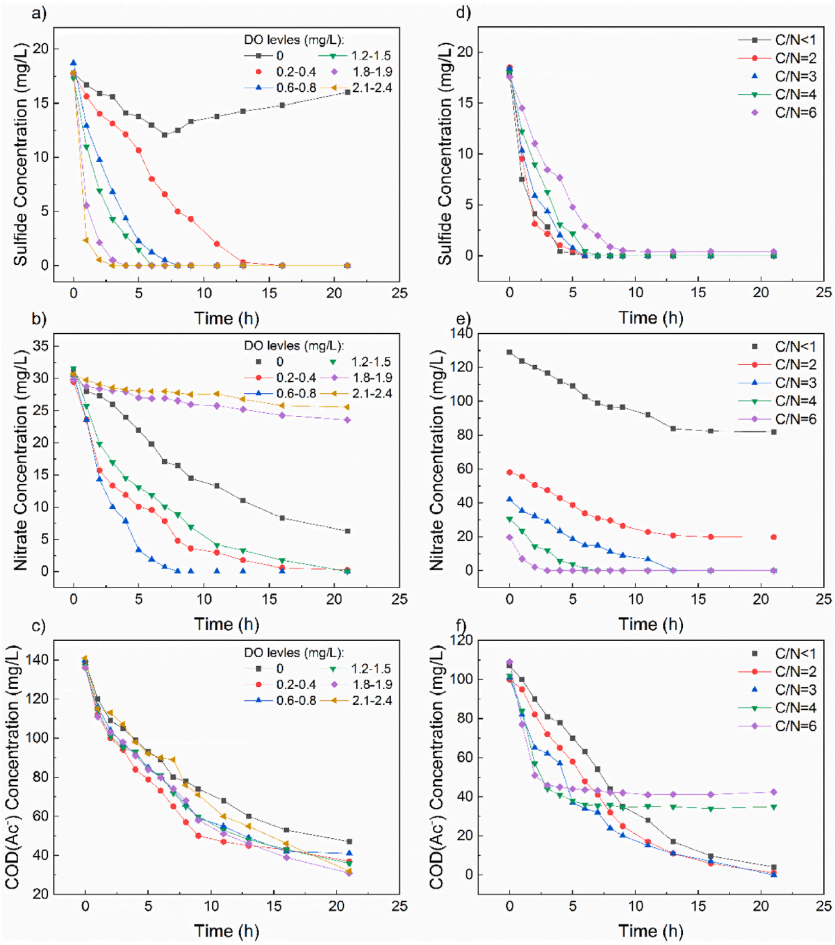

Fig. 3. C–N–S removal time courses under different DO and C/N levels.

greater than 2.58) is considered sufficient for carbon source supply [18]. Consequently, experiments were conducted to investigate the removal time courses of COD, nitrate, and sulfide in RAF under different C/N mass ratios of 0.86, 2, 3, 4, and 6.

At a temperature of 25 °C and a DO level of 0.6–0.8 mg/L, the time evolution of sulfide, nitrate, and COD under different C/Ns are shown in Fig. 3d, e, and 3f, respectively. Chen et al. [12] found that the sulfide "rebound" intensified as C/N increased. However, in this study, in a micro-aerobic environment (DO 0.6–0.8 mg/L), no sulfide concentration increase was observed under all 5 C/N (Fig. 3d). When the C/N was <1, 2, 3, 4, and 6, it took approximately 3, 3, 4, 5 and 7 h, respectively, to achieve a 90 % sulfide removal rate.

The speed of nitrate removal increased as the C/N increased (Fig. 3e). When the C/N was 0.86 and 2, nitrate reductions yielded at 13 h and 16 h, respectively. However, as the C/N increased, the nitrate reduction process significantly accelerated. At C/N of 3, 4, and

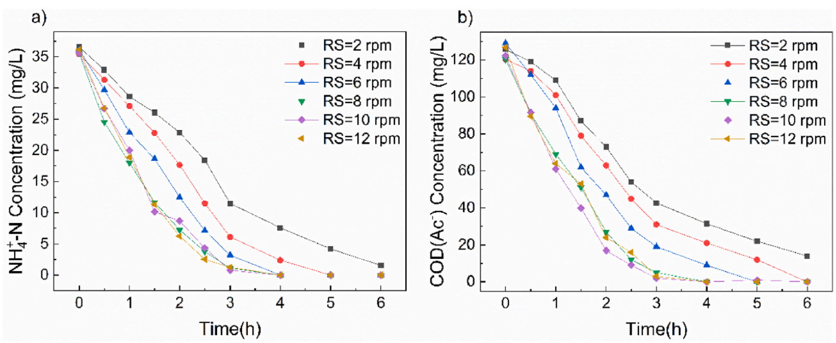

Fig. 4. The time evolution of NH_4^+-N and COD removal under different RSs.

6, the nitrate was completely removed at approximately the 13th, 7th, and 3rd hour, respectively.

The removal speed of COD increased as C/N increased (Fig. 3f). At C/N of 0.86, 2, and 3, it took approximately 7, 5.5, and 4 h, respectively, to achieve a 40 % COD removal rate. In an anoxic-aerobic system, COD removal primarily occurs in the aerobic section and 40 % removal rate of COD in the RAF is acceptable. When C/N exceeded 3, the removal efficiency of COD significantly increased. However, at C/N of 4 and 6, the COD removal speeds dropped at the 5th hour and the 3rd hour, respectively. The unchanged concentration of COD could be attributed to the limited availability of nitrate, which serves as a reaction substrate. In anaerobic or anoxic conditions, heterotrophic denitrification is a primary pathway for removing organic matter.

Based on the discussion above, when the RR was set at 175 %, the DO and C/N were 0.6–0.8 mg/L and 3.82, respectively. Considering the time evolutions of pollutants, it is recommended to set the HRT for the RAF at 5 h. Under these conditions, the removal rates for sulfide, nitrate, and COD are approximately 89 %, 87.3 %, and 39.1 %, respectively.

3.2. RS influence on NH_4^+-N and COD removal in HRBC

The RS of the RBCs is a crucial factor that influences the efficiency of pollutant removal in HRBC. Fig. 4b illustrates the variation of COD concentration over time at six RSs (2, 4, 6, 8, 10, and 12 rpm). When the RSs were below 8 rpm, there was a notable increase in the COD removal rate in HRBC with the increase in RSs. However, once the speed reached 8 rpm, there were no significant changes observed in the COD removal rate. Based on the reactor influent quality (COD: 160–180 mg/L) and the comparison with similar processes [4,5], a COD removal rate greater than 90 % can be considered as optimal COD removal efficiency. The approximate time required to achieve a 90 % COD removal rate at rotating speeds of 2, 4, 6, 8, 10, and 12 rpm were 6.5, 5, 4, 2.5, 2.5, and 2.5 h, respectively. Increasing RS of RBCs enhanced the mass transfer frequency among the biofilm, wastewater, and atmosphere, resulting in a significant improvement of oxygenation efficiency and aerobic heterotrophic bacterial metabolism in the reactor [2]. As the RS increased to 8 rpm, the enhanced oxygenation performance of the RBCs resulted in a shift towards saturation in the DO concentration within the reactor. At this juncture, the limiting factor for the speed of organic matter removal ceased to be DO and instead became the biomass within the system. Since the biomass metabolism remained stable during operation, there were no significant changes

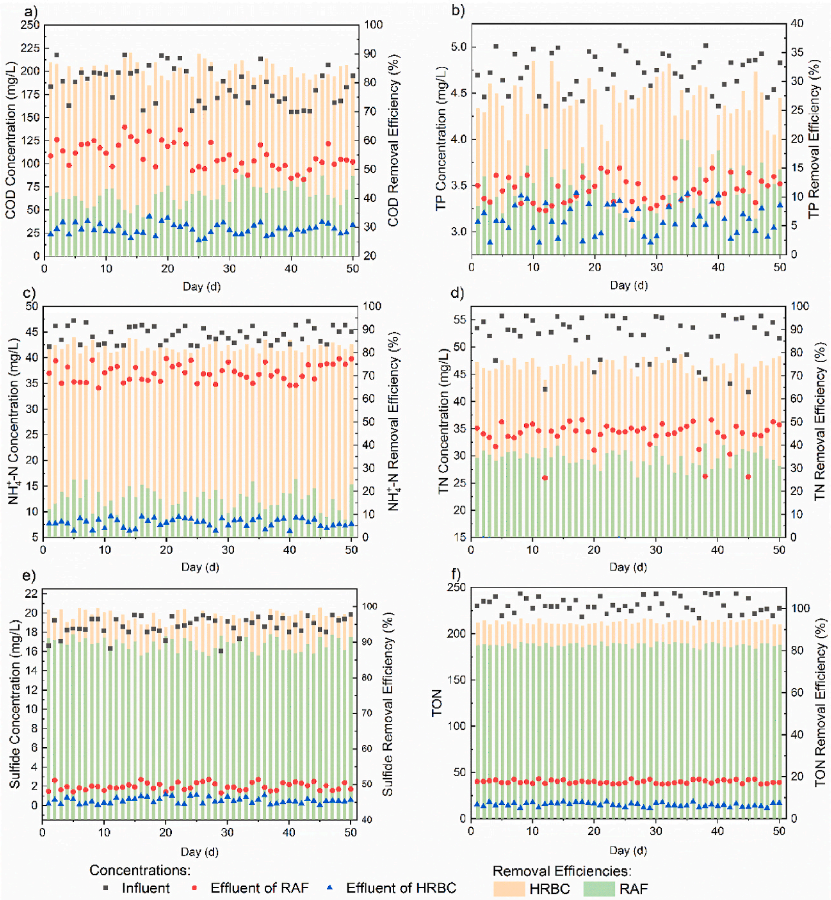

Fig. 5. The performance of RAF-HRBC during the 50-day operation. The removal of COD (a), TP (b), NH_4^+-N (c), TN (d), and the deodorization efficiency (e), (f).

observed in the COD removal rate as the RSs continued to increase.

Like the trend observed in COD removal with RS, the removal process of NH_4^+-N in the HRBC also exhibited a noticeable acceleration followed by stabilization as RSs increased (Fig. 4a). When the RSs were 2, 4, 6, 8, 10, and 12 rpm, the time required to achieve a 90 % removal rate of NH_4^+-N were approximately 5.5, 4, 3, 2.5, 2.6, and 2.5 h, respectively. The initial increase in RSs (from 2 to 8 rpm) resulted in an enhanced hydraulic shear effect on the disc surface. This, in turn, led to a faster rate of biofilm detachment and renewal. A thinner biofilm promoted the mass transfer process of DO, thereby significantly enhancing nitrification. As the RSs continued to exceed 8 rpm, there were no significant changes observed in the NH_4^+-N removal rate.

Thus, it is recommended to set the optimal operational RS of the HRBC at 8 rpm and the hydraulic retention time at 2.5 h. These settings provide a balance between efficient COD and NH_4^+-N removal while also considering energy consumption.

3.3. RAF-HRBC performance at stable running phase

Based on the preliminary conclusions, the optimal operating parameters for the RAF-HRBC combined system have been determined as follows: HRT_{RAF} of 5 h, HRT_{HRBC} of 2.5 h, RR of 175 %, and RS of 8 rpm. These parameters were utilized for stability performance monitoring over a duration of 50 days. The removal performance of COD, NH_4^+-N, TN, TP, sulfides, and TON in the combined system is depicted in Fig. 5. A comparison of this study and other similar processes regarding pollutants removal, deodorization, and energy consumption is shown in Table 1.

The overall average COD removal rate of the combined process reached 84.79 ± 3.87 % in which the RAF accounted for 42.06 ± 4.38 % of the total COD removal rate (Fig. 5a). In RAF, organic degradation was mainly conducted through heterotrophic denitrification. The COD removal performance of the process was good, with an average effluent COD concentration of 33.4 ± 3.08 mg/L. This study achieved a higher COD removal efficiency compared with other bio-contactor processes (Table 1). This can be attributed to the implementation of source separation in the system influent, which effectively reduced the organic load and improved the biodegradability of the influent. Furthermore, the excellent biomass enrichment capability and reoxygenation efficiency induced by the non-woven fabric attached on the HRBC discs also contributed to a good organic degradation performance.

The average removal rate of NH_4^+-N was 82.71 ± 2.06 %. The anaerobic reactor accounted for 17.46 ± 2.12 % of the total NH_4^+-N removal. The effluent average concentration of NH_4^+-N in RAF-HRBC was 7.72 ± 0.96 mg/L (Fig. 5c).

The average TN removal rate of the RAF was 34.64 ± 2.63 %, while the overall TN removal rate of the process is 66.70 ± 2.06 %. The average TN concentration in the effluent of the combined process was 17.82 ± 1.17 mg/L (Fig. 5d). Wang et al. [5] and Zha et al. [4] set RR of 200 % for the ANF-WDRBC process. During the stable operation of their processes, the TN removal rates were 48.04 ± 4.77 % and 52.33 % ± 3.80 % respectively, which were significantly lower than the TN removal rate achieved in this study using the RAF-HRBC process. This is because a retention zone was designed for the RAF in this study to avoid the impact of influent and reflux on the anaerobic zone, which enhanced the denitrification efficiency in the anaerobic zone. At the same time, this study reduced the RR (175 %) and strictly controls the RAF reactor to be in a micro-aerobic environment (DO = 0.6–0.8 mg/L), which simultaneously improved the autotrophic and heterotrophic denitrification efficiency in the RAF. A trace amount of oxygen can stimulate the synthesis of certain enzyme system components (e.g., nitrate reductase) within heterotrophic denitrifying bacteria, without causing these bacteria to solely rely on dissolved oxygen for respiration, which would inhibit nitrate reduction [17]. Moreover, micro-aerobic also enhanced the metabolism of a-soNRB. Therefore, the denitrification efficiency in RAF is significantly improved, leading to an increase in the overall TN removal rate in the combined process.

In this study, the focus of investigation was not on the removal efficiency of TP since the system effluent was designed to

Table 1
A comparison of this study and previous studies regarding pollutants removal, deodorization, and energy consumption.

Treating process	Pollutants in effluent (mg/L) (Removal efficiency)			Deodorization Effluent quality (removal efficiency)		Energy consumption & maintenance problem	References
	COD	NH_4^+-N	TN	S^{2-}	TON		
RAF-HRBC	33.4 ± 3.08 (84.79 ± 3.87 %)	7.72 ± 0.96 (82.71 ± 2.06 %)	12.82 ± 1.17 (74.83 ± 2.06 %)	1.57 ± 0.47 (91.68 ± 2.12 %)	25.23 ± 1.82 (89.04 ± 1.68 %)	One pump only	This study
DP-TFCW	66 ± 11 (72.8 ± 5.0 %)	16.8 ± 1.1 (73.5 ± 1.2 %)	N/A	3.0 ± 4.6 (85.8 ± 22.2 %)	43 ± 33 (73.5 ± 21.4 %)	Two pumps (Potential plugged problem)	[19]
AS based A/O system	47.7 (82.1 %)	2.84 (92.8 %)	7.79 (96.4 %)	N/A	N/A	One blower (AS based, potential filamentous bulking)	[20]
ANF-RBC	12.97 ± 1.46 (88.4 ± 2.16 %)	2.81 ± 0.18 (89.6 ± 13.63 %)	15.86 ± 1.45 (52.33 ± 3.80 %)	N/A	N/A	Two pumps	[15]
ANF/ts-WDRBC	36.55 ± 4.73 (79.36 ± 3.43 %)	6.38 ± 0.85 (83.18 ± 2.50 %)	22.07 ± 1.69 (48.04 ± 4.77 %)	N/A	N/A	Two pumps	[5]
NISRRBC	37.41 ± 9.40 (90 %)	9.27 ± 1.28 (93 %)	18.67 ± 1.46 (50 %)	N/A	N/A	One pump and motor drive RBC	[6]

subsequently be introduced in the ecological unit (e.g., constructive wetland) for further phosphorus related treatment. Based on the stable operation data, the average TP concentration in the effluent was 3.16 ± 0.16 mg/L. The average TP removal rate in the anaerobic unit was 26.48 ± 3.78 %, and the overall TP removal rate of the process was 32.83 ± 4.01 % (Fig. 5b).

The average removal rate of sulfides in the effluent from RAF is 91.68 ± 2.12 % (Fig. 5e), and the average removal rate of TON in the RAF effluent is 89.04 ± 1.68 % (Fig. 5f). The deodorization efficiency and the power consumption of the RAF-HRBC process were better compared to previous studies (Table 1).

During the operation of RAF-HRBC, the accumulated biofilm sludge is discharged by opening the valve at the bottom of the two reactors every 30 days. In comparison to the activated sludge method used for treating domestic wastewater, the biofilm process generates less surplus sludge, resulting in a longer sludge discharge cycle and easier maintenance.

RAF-HRBC demonstrates favorable performance in terms of organics, nutrients, and deodorization. Additionally, the RAF-HRBC process only requires one single water pump as its power unit. This results in low energy consumption and easy operation and maintenance. Therefore, it has significant advantages for widespread adoption in economically challenged and professionally understaffed rural areas in China.

3.4. *Microbial community*

A total of eleven bacterial phyla were identified from RAF(A), the 1st stage of HRBC (O1), and the 2nd stage of HRBC (O2) after 50 days of stable operation., and the abundance of bacterial phylum in each zone was statistically analyzed (Fig. 6b).

In RAF, the phyla with the highest abundance were Proteobacteria (49.68 %), Bacteroidetes (19.50 %), Firmicutes (8.17 %), and Chloroflexi (6.89 %) (Fig. 6b). Proteobacteria has a wide distribution in nature and includes various genera, such as Thauera, Arcobacter, Sphaerotilus, involved in denitrification, sulfide oxidation, and organics degradation [21]. Bacteroidetes contribute significantly to the denitrification process, and its abundance shows a positive correlation with biomass hydrolysis activities [22]. Firmicutes are essential bacterial phyla in the metabolism of ammonium, nitrate, and nitrite. This phylum also encompasses genera with sulfate reduction capabilities, like Desulfosporosinus and Desulfotomaculum [23]. Chloroflexi phyla not only includes complex organic compounds degraders like Herpetosiphon and Ornatilinea [24], but also encompasses sulfide oxidizers such as Chloroflexus aggregans and Oscillochloris trichoides [25]. In the two-stage HRBC, Proteobacteria and Bacteroidetes remained the top two dominant phyla. Nevertheless, a substantial rise in the abundance of Proteobacteria was observed, with abundances of 71.58 % in O1 and 58.28 % in O2. On the other hand, the abundance of Bacteroidetes decreased, with abundances of 8.74 % in O1 and 15.36 % in O2. Nitrospirae is a dominant phylum specific to HRBC, with abundances of 5.26 % in O1 and 10.12 % in O2. Nitrospirae is closely related to the nitrification process [26], and its abundance also supported the high ammonium removal efficiency in HRBC.

A total of 35 bacterial genera and their respective abundances were also determined (Fig. 6a). The dominant genera with the top five abundances in RAF, O1, and O2 are shown in Table S7. In RAF, Thauera is a major functional bacterial genus involved in the denitrification process. It is classified as a heterotrophic nitrate reducing bacteria (hNRB) due to its excellent ability to degrade aromatic compounds [27]. Acinetobacter can utilize nitrate or nitrite as electron acceptors to accomplish simultaneous denitrification and phosphorus removal processes [28]. Arcobacter and Pseudomonas have been reported to possess the ability to oxidize sulfide. Wirsen et al. [29] isolated an Arcobacter strain, A. sulfidicus, from the ocean, which can oxidize sulfide to filamentous elemental sulfur

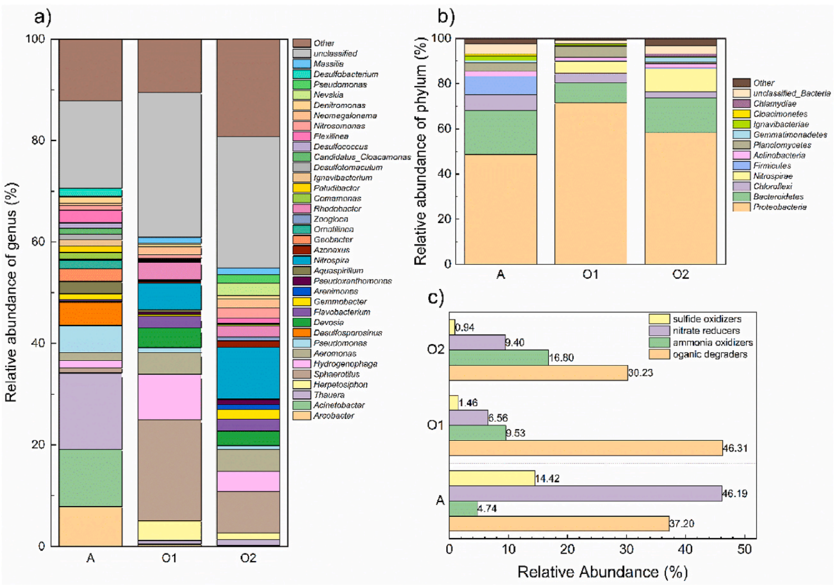

Fig. 6. Relative abundance of microbial community at the (a) genus and (b) phylum levels; and (c) relative abundance of functional groups of bacteria in different zones.

under autotrophic conditions using nitrate as an electron acceptor. Liu et al. [30] also observed the enrichment of Arcobacter in their autotrophic denitrification reactor during operation. Pseudomonas is a common heterotrophic denitrifying bacterium. Researchers have identified specific species (e.g., Pseudomonas CRS1 isolated from hot springs [31] within this genus that exhibit sulfur oxidation capabilities. These species can simultaneously metabolize organics and nitrates while oxidizing sulfides. However, limited reports suggest its predominance in low sulfide concentration environments. The 5.37 % abundance of Pseudomonas in RAF is believed to be associated with the specific microaerobic conditions and ample carbon source supply in the RAF-HRBC system. Desulfosporosinus is also one of the dominant bacterial genera in RAF. Its main function in anaerobic environments is the reduction of sulfate [23]. The abundance of these dominant bacterial genera was closely related to the efficient denitrification, deodorization, and organics removal in RAF.

The dominant bacterial genera in the two-stage RBCs are similar, but the proportions of each functional bacterial genus varied. In O1, Sphaerotilus (19.91 %) and Hydrogenophaga (8.90 %) are the two dominant bacterial genera. Both of these genera are known for their organic compound degradation functions. However, an excessive presence of Sphaerotilus in activated sludge can lead to a risk of filamentous bulking risk [32]. Nitrospira is the most dominant bacterial genus in O2, with an abundance of 10.12 % in the O2 biofilm, which is significantly higher compared to O1 with an abundance of 5.26 %. Nitrospira is widely distributed in conventional biological denitrification processes and is a key bacterial genus involved in the aerobic nitrification of ammonium [26].

To explain the functions of different zones in RAF-HRBC, statistical analysis was conducted on the abundance distribution of various functional groups of genera (Fig. 6c). The abundance of ammonium oxidizers (e.g., Nitrospira) was higher in O2 than in O1, while organic degraders (involving Sphaerotilus, Herpetosiphon, and Hydrogenophaga) showed the opposite distribution. This pattern may be due to DO content and competition between autotrophic and heterotrophic bacteria. And this indicated that Organic degradation was primarily facilitated in O1 while nitrification mainly occurred in O2. The denitrification group was significantly more abundant in RAF (46.19 %) compared to O1 (6.56 %) and O2 (9.40 %), corresponding to nitrate removal efficiency. Sulfide oxidizers, such as Arcobacter, Ignavibacterium, and Pseudomonas, were primarily found in RAF (14.42 %), showing increased abundance compared to previous studies [19]. This aligns with the synchronized denitrification and deodorization efficiency of the RAF system.

The above statistics and analysis provide further confirmation that the optimization of the RAF-HRBC biofilm system has significantly improved the structure and distribution of the functional microbial community. The microbial community also illustrated the interaction of simultaneous carbon, nitrogen, and sulfide removal within the system. Organic load affected the removal efficiency of sulfide, nitrate, and organics in the system. The main reason behind this is that the supply of carbon sources can alter the structure of autotrophic/heterotrophic microbial populations within system. A higher organic load resulted in higher sulfide removal efficiency but lower nitrate and carbon removal efficiencies. The DO also impacted the growth and metabolism of microbes within the system. Under micro aerobic condition (0.6–0.8 mg/L), the removal efficiency of sulfide is noticeably higher compared to anaerobic conditions (0–0.2 mg/L). However, excessive dissolved oxygen also reduced the efficiency of heterotrophic denitrification, thereby affecting the removal of nitrate and carbon.

4. Conclusion

In this study, the RAF-HRBC system was operated to examine its effectiveness in odor abatement and pollutant removal. The optimal operation parameters were determined. The RR of 175 % and HRT_{RAF} of 5 h were determined through batch tests, the discs RS of 8 rpm and the HRT_{HRBC} of 2.5 h were obtained through lab-scale HRBC tests. The average removal efficiencies of 84.79 ± 3.87 % COD, 82.71± 2.06 % NH_4^+-N, 66.70 ± 2.06 % TN, 91.68± 2.12 % S^{2-} and 89.04 ± 1.68 % TON were detected during 50 days under the optimal operation parameters. Based on large amount of operational data, an organic loading rate analysis of RAF-HRBC was also conducted to determine the relationship between desired effluent COD concentrations and the COD loading rates. The curves were critical references for the HRBC effective surface area calculation in full-scale applications. RAF-HRBC system exhibited an improvement in the richness of the microbial community. In RAF, there was a selective enrichment of a-soNRB and h-soNRB such as Arcobacter and Pseudomonas. This enrichment amplified the effectiveness of sulfide removal and denitrification processes in RAF. In the two-stage HRBC, the first stage RBC was dominated by organics degraders, while the second stage RBC was dominated by ammonium oxidizers. This differentiation in microbial communities contributed to varying removal efficiencies of COD and NH_4^+-N in the respective stages of the system. The integration of RAF-HRBC biological process provides a stable, easy-maintained, cost-effective, energy-saving, and high-efficient alternative for decentralized rural sewage treatment.

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Data availability statement

The authors do not have permission to share data.

CRediT authorship contribution statement

Helai Cheng: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Wenhua Lee:** Writing – review & editing, Investigation, Formal analysis. **Cangxiang Wen:** Software, Data curation. **Hongliang Dai:** Writing – review & editing. **Fangkui Cheng:** Investigation. **Xiwu Lu:** Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Helai Cheng, Xiwu Lu reports financial support was provided by Department of Ecology and Environment of Jiangsu Province. Helai Cheng, Xiwu Lu reports a relationship with Souteast University that includes: employment.

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Appendix A. Supplementary data

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