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Comparison of in-situ sludge reduction in a sequencing batch biofilm reactor (SBBR) under different carriers: operation parameter optimizations

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ABSTRACT

Two SBBR systems, designated $_{FSC}$SBBR (floating spherical carrier) and $_{SC}$SBBR (multi-faceted polyethylene suspension carrier), were constructed for in-situ sludge reduction. The effects of the operating temperature (*T*), hydraulic retention time (HRT) and dissolved oxygen (DO) concentration on in-situ sludge reduction in the two SBBR systems were investigated, and the optimum carrier and operating parameters were determined. The results showed that an FSC carrier exhibited a better in-situ sludge reduction efficiency and contaminant removal efficiency than an SC carrier. The optimal operating parameters were a temperature of 25°C, HRT of 12 h and DO concentration of 4–5 mg/L, and the observed sludge yield was 0.21 ± 0.10 g MLSS/g COD. The analysis of biofilm samples showed that $_{FSC}$SBBR biofilms were richer in microbial species and exhibited higher diversity than $_{SC}$SBBR biofilms. Biofilm sludge hydrolysis and cytolysis promoted in-situ sludge reduction.

Keywords: In-situ sludge reduction; SBBR; Operating parameter; Biotic community

1. Introduction

The activated sludge process is a mature and stable wastewater treatment technology with high performance-cost ratio, and has been widely used in municipal wastewater treatment in recent decades. However, mass generation of excess sludge is the drawbacks of this process [1–3]. Relevant studies show that the costs of excess sludge treatment can account for 20%–65% of the total operation and management costs of sewage treatment plants [4]. In-situ sludge reduction technology refers to methodology in which sludge reduction occurs throughout the sewage treatment process, which can reduce excess sludge production without any external intervention [5]. This approach can solve the problem of excess sludge from the source [2,6,7]. Many scholars have confirmed that in-situ sludge reduction technology presents additional engineering advantages and application prospects.

The biofilm process involves the addition of a carrier with a relatively large specific surface area to an activated sludge reactor, and microorganisms then grow on the

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surface of the carrier to form an attached biofilm with a certain thickness [8,9]. Synchronized nitrogen and phosphorus removal and organic matter removal are accomplished through the combined action of the carrier, biofilm nutrient levels, food chain and local micro-environment [10,11]. Related studies also show that the biofilm process can not only achieve better in-situ sludge reduction but simultaneously achieves nitrification and denitrification, to enhance biological nitrogen removal [10,12,13]. The biofilm process can be flexibly combined with other wastewater treatment processes or operated separately, which can effectively promote in-situ sludge reduction while maintaining efficient contaminants removal.

At present, research on the in-situ sludge reduction process is mostly focused on the micro-pollutant removal effect, and this research is mainly being conducted in biofilms constructed with a single carrier [14–16]. However, due to the variety of potential carriers, the physical and chemical properties of the carrier can affect the formation of biofilms and, further affect in-situ sludge reduction. Therefore, there is an urgent need to explore the effects and mechanisms of different carriers in in-situ sludge reduction. Moreover, the biofilm morphology and microbial community attached to different carriers also need to be clarified.

In this paper, two SBRs with different carrier materials for in-situ sludge reduction were constructed. These carrier materials were a floating spherical carrier (FSC) with polyurethane cubes and fibre balls and a multi-faceted polyethylene suspension carrier (SC) with polyethylene as the skeleton. The purpose of this research was to compare the in-situ sludge reduction effects of two SBBR systems with two types of biological carriers under different working conditions and obtain the best in-situ sludge reduction operation conditions. The morphology of biofilm growing on the carriers and the diversity of microbial communities were analyzed to explore the mechanism of in-situ sludge reduction.

2. Materials and methods

2.1. Experimental setup and operating conditions

The effective volumes of the two SBBR reactors were 10 L (inner diameter 19 cm × height 50 cm), with a packing ratio of 60% (vol/vol). The FSC was a spherical structure with a diameter of 8 cm. The porosity of the porous polyethylene shell was 85%; the carrier was filled with a 20 mm polyurethane cube and two fibre balls with the diameter being 20 mm. The suspended carrier was a polyethylene polygonal prism (40 mm [basal diameter] × 60 mm [height]). The specific parameters of the two kinds of packing are shown in Table 1. The two reactors were fed water at 6 L/h through a peristaltic pump in the intake stage. The intake water was continuously stirred and manually mixed, and the reactor was drained to a volume of 3 L within 30 min. The test device is shown in Fig. 1.

Experiments at a single factor (the operating temperature [T], dissolved oxygen concentration [DO] and hydraulic retention time (HRT) of the $_{FSC}$SBBR and $_{SC}$SBBR systems) were controlled to observe the sludge yield (Y_{obs}) of the sludge remaining in the two systems under different operating conditions. First, the systems were controlled at DO = 5–6 mg/L and HRT = 12 h, and the operating temperatures of the two systems were regulated to remain stable at 15°C, 20°C, 25°C or 30°C. After stable operation under each condition, centralized monitoring was carried out for

Table 1
Specific parameters of biofilm carriers

Biofilm carriers	Density (kg m^3)	Specific surface (m^2 g)	Water retention
FSC	1.21–1.23	2.15–2.21	452.38%
SC	0.96–0.98	1.23–1.25	5.41%

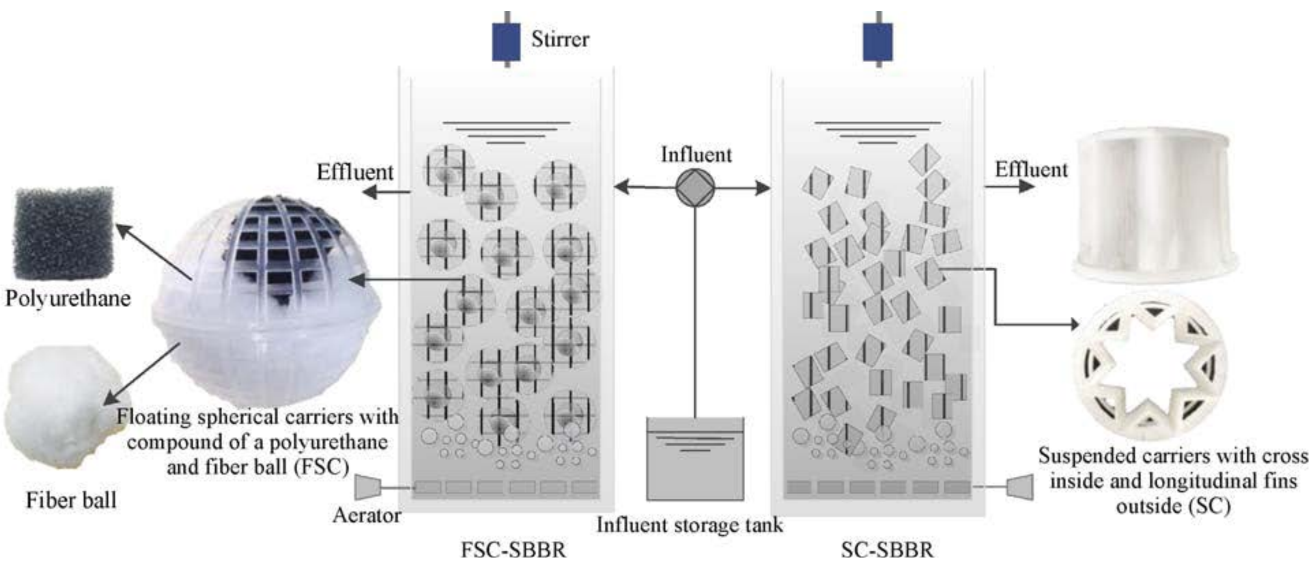

Fig. 1. Schematic diagram of the reactor.

15 d. Then, the temperature was held stable at 25°C and DO = 5–6 mg/L while HRT was regulated to 12 h (anoxic/anaerobic, aerobic), 10 h (anoxic/anaerobic, aerobic), 8 h (anoxic/anaerobic, aerobic) and 6 h (anoxic/anaerobic, aerobic), and centralized monitoring was conducted 15 d after stable operation under each anaerobic HRT condition. Finally, the systems were controlled so that HRT = 12 h, T = 25°C, and DO was stabilized with a flow meter at 2–3 mg/L, 5–6 mg/L or 7–8 mg/L, and monitoring were monitored for 15 d after stable operation under each working condition. The indicators for centralized monitoring included the change in chemical oxygen demand (COD), Y_{obs}, MLSS, ammonia nitrogen (NH_4^+–N), total nitrogen (TN) and total phosphorus (TP) concentrations.

To study the effect of DOM released through biofilm hydrolysis and cytolysis on in-situ sludge reduction performance, DOM release and degradation mechanisms, the supernatants of the $_{FSC}$SBBR and $_{SC}$SBBR systems were collected at different reaction stages and pretreated through microporous (pore size 0.45 μm) cellulose acetate membrane filtration.

2.2. Characteristics of wastewater

Activated sludge was collected from the high-concentration mud-water mixture in the sludge return pumping chamber of the Everbright Water secondary sedimentation tank (Ji'nan) and was used in this experiment after artificial domestication. The wastewater characteristics and detailed chemical composition of the influent wastewater were shown in Table 2.

2.3. Analytical methods

2.3.1. Physico-chemical analysis

The water samples were pretreated via centrifugation to remove suspended large particles, and the supernatant was collected. The analysis methods of conventional indexes were shown in Table 3 [17,18]. A portable meter (HACH, USA) was used for the monitoring of DO, pH and the oxidation–reduction potential (ORP). The sludge biofacies were observed through a microscope, and the statistics of sludge size was measured by laser particle sizer (Dandong, BT-9300S, China), where the sample was first spread evenly in ultrapure water and then measured three times to avoid multiple scattering. The water-soluble polysaccharide was determined by phenol–sulphuric acid method. The calculation method of observed sludge yield was based on Ni et al. [19]. IBM SPSS Statistics 22 software was used for significance test, and the F-value, the critical value F-crit and the probability value p-value were calculated, the test level was selected as 0.05.

Table 2
Characteristics of wastewater

Temperature (°C)	pH	COD (mg/L)	NH_4^+–N (mg/L)	TN (mg/L)	TP (mg/L)
15–20	7.5	200–250	45–55	60–65	8–12

Table 3
Analysis methods of conventional indexes

Number	Detection index	Determination method
1	COD	Potassium dichromate method
2	NH_4^+–N	Nessler's reagents spectrophotometer
3	TN	Alkaline potassium persulfate digestion-UV spectrophotometric method
4	TP	Potassium persulfate digestion-UV spectrophotometric method
5	MLSS/MLVSS	Filter paper weight method

2.3.2. SEM analysis

Biofilm samples were collected from the reactor packings that operated steadily under optimum conditions. The sampling positions were 0, 1, 2 and 4 cm from the surface to the interior ($_{FSC}$SBBR reactor) or 0 and 2 cm ($_{SC}$SBBR reactor).

The sludge samples were first fixed for 12 h with fresh 2.5% glutaraldehyde solution at 4°C and then rinsed three times with phosphate buffer solution. Each time, the samples were dehydrated through a concentration gradient of 30%, 50%, 70%, 90% and 100% ethanol solutions for 15 min and dried for 10 h in a drying dish. The samples were observed via SEM (Zeiss Sigma 300, Germany) after gilding treatment.

2.3.3. Analysis of microbial communities

Sludge samples were collected from the surface of $_{FSC}$SBBR and $_{SC}$SBBR biofilms after the systems has been running steadily for 120 d under DO = 4–5 mg/L and HRT = 12 h conditions. DNA extraction referred to the improved method of Yang et al. [20], and high-throughput sequencing referred to the method of Zheng [21]. Statistical analysis of community structure was performed via statistical methods based on the OTU distribution.

3. Results and discussion

3.1. Effect of technological parameters on in-situ sludge reduction

3.1.1. Temperature (T)

3.1.1.1. In-situ sludge reduction in $_{FSC}$SBBR and $_{SC}$SBBR at different temperatures

The daily observed sludge yield and cumulative sludge production of $_{FSC}$SBBR and $_{SC}$SBBR at different temperatures are shown in Fig. 2. The apparent sludge yield decreased slowly and then gradually increased throughout the operation period. The optimum in-situ operation temperature for the sludge in $_{FSC}$SBBR was 25°C, and the cumulative sludge production and observed sludge yield were 0.038 g MLSS/L d, and 0.21 ± 0.10 g MLSS/g COD, respectively.

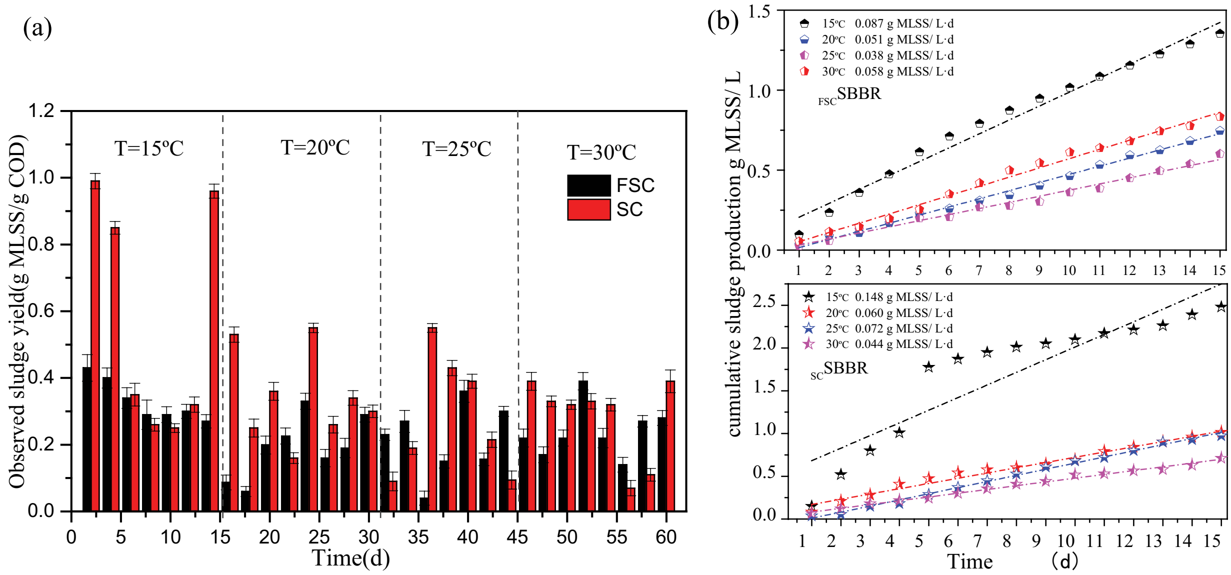

Fig. 2. Daily Y_{obs} (a) and cumulative sludge production and (b) of $_{FSC}$SBBR and s$_{C}$SBBR at different temperatures.

The minimum sludge yield and cumulative sludge production of the $_{SC}$SBBR system were observed at an operating temperature of 30°C and were 0.044 g MLSS/L and 0.28 ± 0.10 g MLSS g/COD, respectively. Comparison of the in-situ sludge reduction parameters of the two systems at the optimal operating temperature showed that the daily observed sludge yield of $_{FSC}$SBBR was 21.43% lower than that of $_{SC}$SBBR, and cumulative sludge production was reduced by 13.64%.

It could be seen that $_{FSC}$SBBR exhibited better in-situ sludge reduction effects when the temperature was regarded as the operation control factor. The efficiency of in-situ sludge reduction was positively correlated with temperature under certain conditions, with a higher operating temperature effectively promoting in-situ sludge reduction, similar to the results of Tian et al. [22]. However, it can be seen from Fig. 2 that the effect of in-situ sludge reduction increased slightly and the apparent observed sludge yield could even be increased with an increase in temperature. The optimal operating temperature in the sludge reduction system was based on the biofilm, and the optimal operating temperature of the $_{FSC}$SBBR system was 25°C.

To further explore the influence mechanism of temperature on the excess sludge production, temperatures of 15°C and 25°C, which were the two conditions resulting in the most significant difference in the apparent sludge yield, were selected to determine the change of ORP in the two reactors in one reaction cycle (Fig. 3). ORP can reflect microbial activity in the liquid phase of the system [12]. When ORP rises rapidly, microbial activity increases and consumes large amounts of substrates through microbial metabolism. However, the limited concentration of substrates will restrict the synthesis and metabolism of microorganisms in a high-ORP environment and induce in-situ sludge reduction [23]. The analysis of changes in ORP showed that the anoxic/anaerobic/aerobic environment in the reactor alternates continuously during a reaction cycle, and the environmental changes will affect microbial metabolism; thus, the ATP

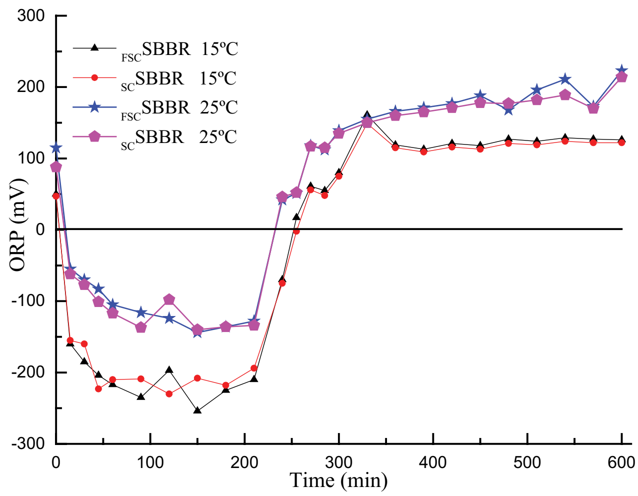

Fig. 3. Changes in ORP in $_{FSC}$SBBR and $_{SC}$SBBR at different temperatures.

synthesized by microorganisms during aerobic phase cannot be used for new cell synthesis, so metabolic decoupling is carried out. Moreover, microbial metabolic activity is higher at 25°C than at 15°C, and the energy demand is greater, which causes the decoupling phenomenon, and sludge reduction is achieved. The anoxic environment has a short duration at 15°C, and the environment changes alternately, which may mean that the decoupling phenomenon is not obvious, so the sludge reduction effect is not as good as that at 25°C.

3.1.1.2. Effect of temperature on the microbial community in the system

Temperature may affect the sludge reduction efficiency by changing the biofilm microbial community structure and richness. Sludge samples with the size of 1 cm^2 from the surface of a biofilm were selected for microscopic observation. The results are shown in Fig. 4 (from left to right, 15°C, 20°C and 25°C, respectively). It can be seen that the genus *Aspidisca* existing on the biofilm surface in two systems at 15°C, and the species of protozoa were relatively single. When the temperature was raised to 20°C, the genus *Vorticella* and the genus aphid, related to sludge disintegration, appeared at the surface of the $_{FSC}$SBBR biofilm, while only the genus *Spirostomum* was observed on the $_{SC}$SBBR biofilm. These findings indicate that protozoan species were further enriched, and more species that promote sludge disintegration have emerged [24]. When the temperature rose to 25°C, visible Tubificidae appeared in the biofilm in the $_{FSC}$SBBR system. Tubificidae are micro-animals that are useful for promoting the in-situ reduction of sludge [25], while only Beggiatoa can be observed in $_{SC}$SBBR. These results indicated that species of protozoa and metazoa were very abundant in the systems, especially in $_{FSC}$SBBR, and form a long food chain and a stable microbial population structure. A summary of protozoa and metazoans in the two systems at different temperatures is shown in Table 4. Rich micro-animals prey on microorganisms in the system, which can promote in-situ sludge reduction. Studies have shown that excess sludge production in activated sludge process is 10%–20% lower in summer than in winter due to the higher concentration of protozoa [26].

3.1.2. DO concentration

The daily observed sludge yield and cumulative sludge production of $_{FSC}$SBBR and $_{SC}$SBBR at different dissolved oxygen concentrations (DO) are shown in Fig. 5. With an increase in the DO concentration, the in-situ sludge reduction effect of $_{FSC}$SBBR first increased and then decreased rapidly. The minimum observed sludge yield (0.21 ± 0.10 g MLSS/g COD) decreased by 8.70% (DO = 4–5 mg/L) and 22.22% (DO = 2–3 mg/L) compared with the other two operating conditions. The increase in the DO concentration in the $_{SC}$SBBR system promoted in-situ sludge reduction, and the DO concentration was positively correlated with sludge reduction. The minimum observed sludge yield (0.27 ± 0.10 g MLSS/g COD) decreased by 3.57% (DO = 4–5 mg/L) and 30.77% (DO = 2–3 mg/L) compared with the other two operating conditions. The overall analysis showed that an increasing DO concentration effectively improves the in-situ sludge reduction effect, but an excessive DO concentration cannot significantly improve the sludge reduction effect and may even increase excess sludge production. The optimum DO condition for in-situ sludge reduction in the two systems is 4–5 mg/L, and the observed sludge yield is 0.21 ± 0.09 g MLSS/g COD.

To further analyse the effect of DO on in-situ sludge reduction, the changes in the ORP of mixed liquids over two operating cycles (HRT = 12 h) under different DO conditions were measured (Fig. 6). The figure indicated that the ORP of the two systems under different DO control conditions in the initial anoxic/anaerobic reaction stage showed a rapid downward trend. The influence of DO on

Table 4
Summary of protozoans and metazoans in $_{FSC}$SBBR and $_{SC}$SBBR

	15°C	20°C	25°C
$_{FSC}$SBBR	*Aspidisca*	*Vorticella* aphid *Litonotus*	Tubificidae
$_{SC}$SBBR	*Aspidisca*	*Spirostomum*	Beggiatoa

Note: This table only reflects the biofacies statistics on biofilms in typical areas of two systems.

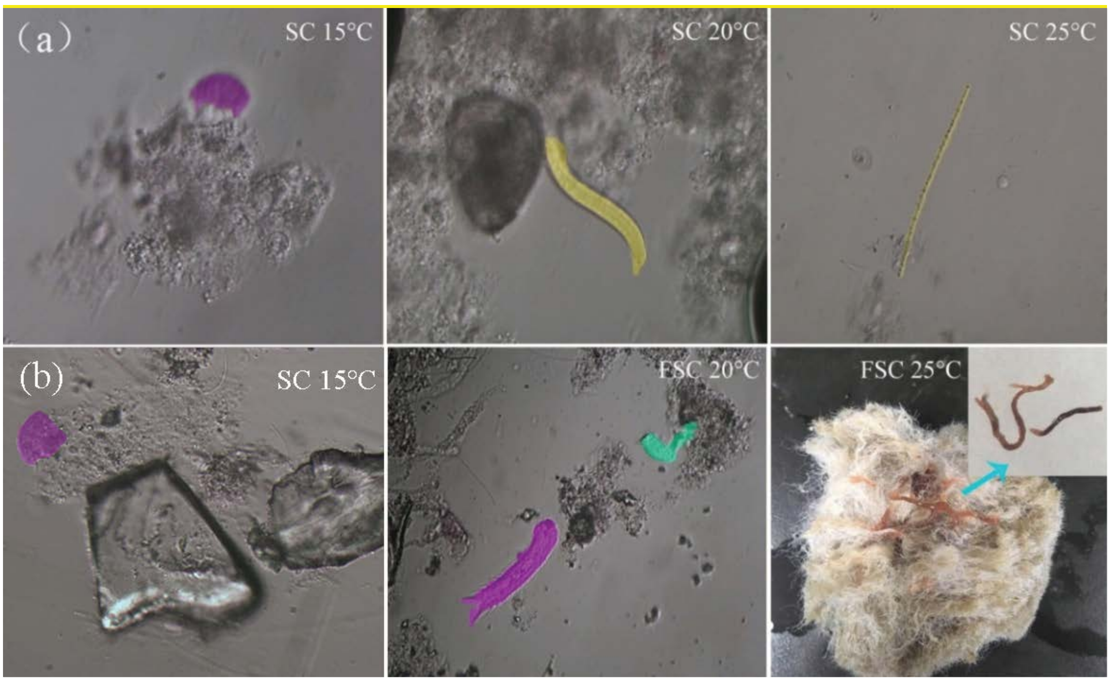

Fig. 4. Evolution of Protozoa and Metazoa in biofilms in (a) $_{SC}$SBBR and (b) $_{FSC}$SBBR at different temperatures.

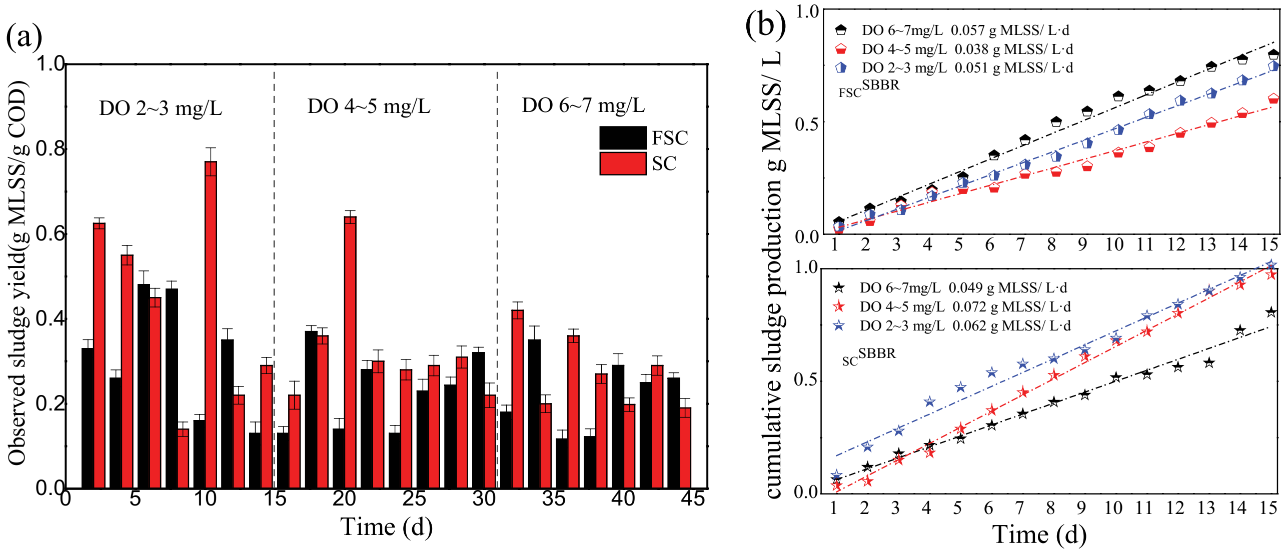

Fig. 5. Daily Y_{obs} (a) and cumulative sludge production (b) of $_{FSC}$SBBR and $_{SC}$SBBR at different DO concentrations.

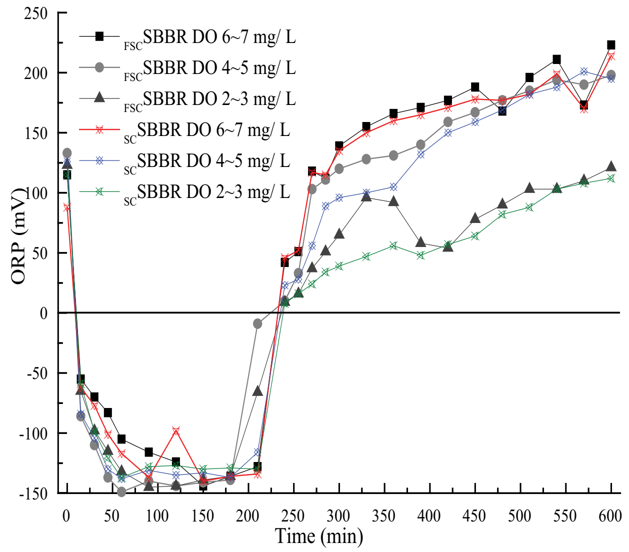

Fig. 6. Changes in ORP in $_{FSC}$SBBR and $_{SC}$SBBR under different DO conditions.

the ORP of the two systems was mainly reflected in the aeration stage, where a lower DO concentration resulted in a smaller increase in ORP. When DO was 4–5 or 6–7 mg/L, the increase in the ORP value was similar, indicating that an excessive DO concentration cannot promote an increase in the ORP value. When DO was 4–5 or 6–7 mg/L, the increase in ORP was similar. An excessive DO concentration could not promote an increase in the ORP value, and the DO concentration in the system reached saturation. An excessive DO concentration did not increase microbial activity but adversely affected biofilm robustness, causing biofilm shedding. The above analysis showed that a suitable DO concentration can increase the liquid-phase ORP of the system and enhance sludge reduction.

3.1.3. Hydraulic retention time

The daily observed sludge yield and cumulative sludge production of $_{FSC}$SBBR and $_{SC}$SBBR at different HRTs are shown in Fig. 7. The observed sludge yield of the $_{FSC}$SBBR system decreased by 25.00% (HRT 12 h), 26.67% (HRT 8 h) and 3.57% (HRT 6 h) than that of $_{SC}$SBBR. It can be seen that the $_{FSC}$SBBR system exhibits better in-situ sludge reduction performance than $_{SC}$SBBR under different HRT control factors. The overall analysis showed that extending HRT can effectively reduce excess sludge production, which were similar to the findings of Yang et al. [20], and Eusebi and Battistoni [27]. By comparing the observed sludge yield of FSC under three HRT conditions, we can see that the observed sludge yield (0.231 g MLSS/g COD) at HRT = 8 h was 26% lower than that at HRT = 6 h (0.311 g MLSS/g COD), while the observed sludge yield (0.23 g MLSS/g COD) at HRT = 12 h was not significantly lower than that at HRT = 8 h, which was only 6.7%, Similar conclusions can be drawn from the analysis of cumulative sludge production and pollutant removal efficiency. This indicating that although prolonging HRT is effective to improve sludge reduction effect, excessive HRT has limited effect on improving the sludge reduction, in practical applications, HRT should be flexibly determined by integrating various factors. It was also found in this experiment that the change of HRT had no obvious effect on the sludge reduction effect of the $_{SC}$SBBR system, which was suspected to be related to the unstable formation of SC carrier biofilm.

To further analyse the mechanism whereby HRT affected in-situ sludge reduction, the changes in the ammonia nitrogen concentration over two operating cycles (HRT = 12 h) under different DO conditions were measured (Fig. 8). These figures indicated that the NH_4^+–N concentration in the anoxic/anaerobic zone was significantly increased under the three conditions. Yang et al [20] also found that ammonia nitrogen and COD were increased in the anoxic zone, which was related to anaerobic hydrolysis and cytolysis

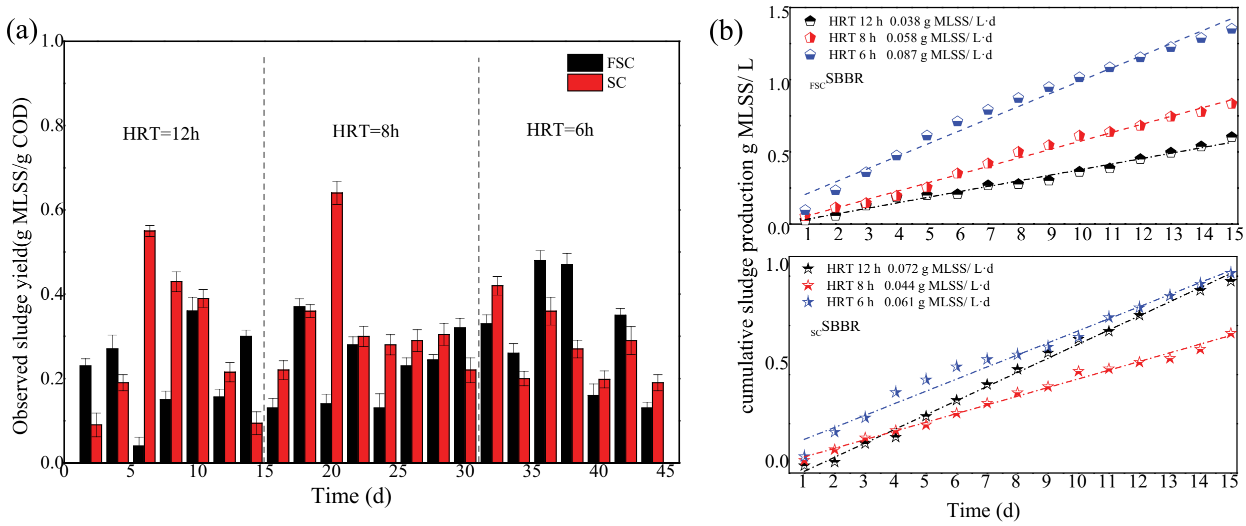

Fig. 7. Daily Y_{obs} (a) and cumulative sludge production (b) of $_{FSC}$SBBR and $_{SC}$SBBR at different HRTs.

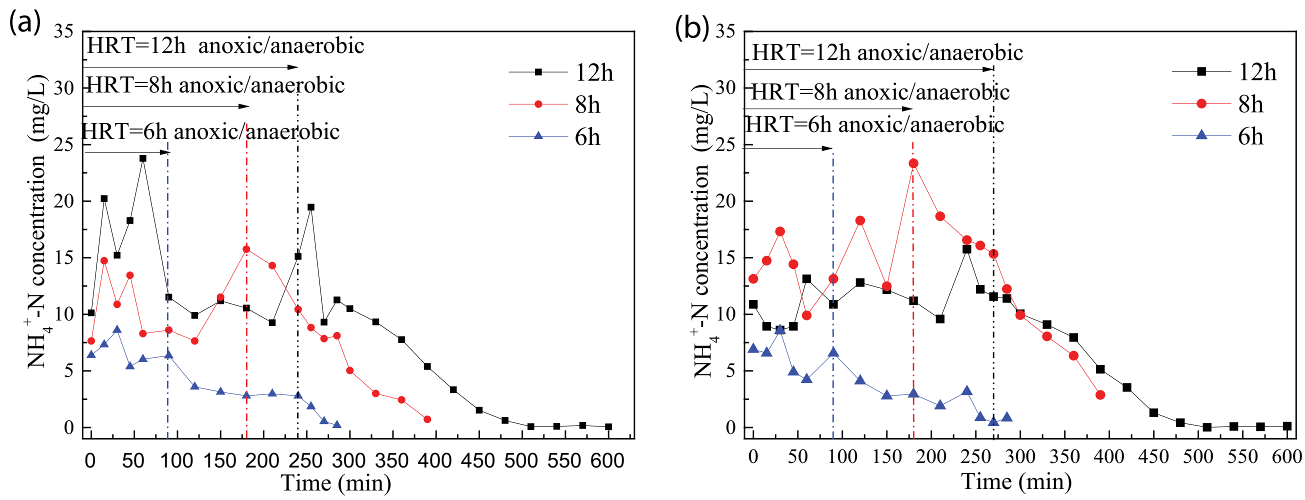

Fig. 8. Periodic variation of ammonia nitrogen contents in the supernatants of $_{FSC}$SBBR and $_{SC}$SBBR at different HRTs.

of microorganisms. Decaying microorganisms lyse the cell wall under anaerobic conditions, releasing dissolved organic matter such as ammonia nitrogen and polysaccharides, which are then ingested by other microorganisms for their own metabolism [28]. Therefore, the concentration of ammonia nitrogen first rises and then drops rapidly at this stage, which is referred to as cytosolic-recessive growth by many scholars [29].

The cytolysis efficiency was the highest under the condition of HRT = 12 h in $_{FSC}$SBBR system, and the sludge reduction effect was better than that under other HRT conditions. The longer HRT also allows the microorganisms in the system to have a more complete contact time with the substrate, and allows more soluble degradable organics to dissolve. Sun et al. [30] also showed that HRT could affect sludge reduction by affecting microbial cytolysis; and with the prolongation of HRT, the soluble organic matter content in the supernatant first increased and then decreased. In summary, $_{FSC}$SBBR showed a better sludge reduction effect when the HRT is 12 h.

3.1.4. Significant test

According to the above analysis, $_{FSC}$SBBR showed a better in-situ sludge reduction efficiency than $_{SC}$SBBR. The optimal operating conditions of the $_{FSC}$SBBR are a temperature of 25°C, HRT of 12 h, and DO concentration of 4–5 mg/L; and in order to more intuitively reflect the difference of sludge reduction effect between the two systems under the optimal operating conditions, the SPSS22 software was used to carry out the significance test, and the results were shown in Table 5.

Significant test showed that *F* values were higher than *F*-crit under three conditions, indicating that there was a difference between the observed sludge yields of $_{FSC}$SBBR and $_{SC}$SBBR in each conditions; further analysis was performed in combination with *p*-value, and the selected test level was 0.05, it can be seen that the *p*-values obtained under three conditions are all less than 0.05, which indicates that there is a significant difference between the apparent sludge yield of $_{FSC}$SBBR and $_{SC}$SBBR under each three optimal conditions.

Table 5
Significance test results of the two systems under the optimal operating conditions

	25°C		4–5 mg/L		12 h	
	$_{FSC}$SBBR	$_{SC}$SBBR	$_{FSC}$SBBR	$_{SC}$SBBR	$_{FSC}$SBBR	$_{SC}$SBBR
Average Y_{obs} (g MLSS/g COD)	0.22 ± 0.10	0.39 ± 0.07	0.21 ± 0.10	0.31 ± 0.04	0.22 ± 0.10	0.31 ± 0.03
F	11.652		4.804		4.591	
p-value	0.002		0.037		0.042	
F-crit	4.225		4.196		4.225	

In addition, the *p*-value (0.002) at 25°C is less than 0.01, which indicated that there is a very significant difference between the two samples under this condition, and in other words, the observed sludge yields of the two systems are quite different at 25°C.

3.1.5. Pollutant removal efficiency

From the perspective of pollutant removal efficiency, the average removal rates of pollutants (COD, NH_4^+–N, TN and TP) in the $_{FSC}$SBBR system operating under optimal conditions were 93.39%, 96.66%, 82.13% and 60.23%, respectively, which was better than $_{SC}$SBBR removal. The effects were increased by 5.22%, 1.43%, 6.57% and 7.47%, respectively. The efficiency of denitrification in the two reactors was maintained at a high level, and the effluent reached the A grade of the first level standard of the Discharge Standard of Pollutants for Municipal Wastewater Treatment Plant of China (GB18918−2002) [31].

A change in operating conditions can affect the denitrification efficiency of the system. Temperature can change the denitrification efficiency by affecting enzyme activity; a high or low temperature will have a negative impact on the denitrification effect of the system. The denitrification efficiency of the two systems was positively correlated with the HRT and the concentration of DO, where prolonging the HRT and increasing the concentration of DO improved the denitrification efficiency of the system. However, an excessive DO concentration (6–7 mg/L) inhibited denitrification to some extent because when the DO concentration is relatively high, the dissolved oxygen in the solution will resist the transfer resistance and penetrate through the biofilm to the inside of the biofilm. The anaerobic/anoxic microenvironment inside the biofilm is then destroyed, which affects the efficiency of simultaneous nitrification and denitrification. Compared with $_{SC}$SBBR, $_{FSC}$SBBR exhibited a better nitrogen and phosphorus removal effect, but the phosphorus removal effects of the two systems were not ideal because the SBBR system exhibits a long sludge age and low surplus sludge production, which restricts the phosphorus removal effect of the system.

3.2. Community analysis

3.2.1. Biofilm surface morphology analysis

Fig. 9 shows the surface and internal biofilm morphology of the two carriers. It can be seen from the figure that the biofilm microorganisms on the surface of the FSC and SC carriers mainly consist of rod bacteria, that microbial clusters grow, and that the structure is stable, mainly because EPS secreted by microorganisms can change the chemical properties of the carrier surface and increase the microorganisms adhesiveness of the carrier [32]. As the surface penetrates deeper into the interior, the FSC microenvironment gradually transitions from aerobic to anaerobic, eventually becoming hypoxic, and the morphology of the biofilm also changes. At a distance of 1 cm from the surface, the microbial structure began to loosen, and a small amount of microbial debris was formed. With an increase in the thickness of the biofilm, increasing amounts of bacterial debris appeared in the samples, and the microbial morphology gradually changed from rod-shaped bacteria to the *Sphaerophorus*. However, the thickness of the SC carrier biofilm was insufficient, and there was no environmental difference between the surface and the interior of the SC carrier. Therefore, the difference in microbial morphology was small, and no obvious bacterial debris appeared. However, the porous morphology that formed inside indicated that the microbial structure had also become loose.

It has been found that the concentration of ammonia nitrogen in the supernatant of a biomorphic debris area is higher than in other areas. It can be inferred that ruptured microbial cells release dissolved organic matter (DOM) and cause cytolysis. Microorganisms can degrade the intracellular substances released via cytolysis and use them for their own growth, to produce an in-situ sludge reduction in an anaerobic environment [5,33,34]. We analysed the organic matter content of the supernatant of $_{FSC}$SBBR samples in different stages of the system's operation cycle to verify this conclusion (Fig. 10).

It can be seen that the SUVA value of the supernatant in $_{FSC}$SBBR at different period of the was higher than that of the influent and that it showed an increasing trend. The increase in the SUVA value is considered to be related to the occurrence of lysis in the carriers. The lysis-cryptic growth phenomenon of the biofilm system increases the content of soluble microbial products (SMP). The main component of SMP is humic acid hydrophobic organic matter, whose SUVA value is higher than that of hydrophilic acid, which results in an increase in the SUVA value [35,36]. As the reaction proceeds, the COD content of the supernatant gradually decreases, while the polysaccharide concentration remains basically constant, indicating that the DOM concentration in the water is increasing and, thus, that lysis has occurred.

The morphological changes in the biofilms at different carrier positions in the two systems were further analysed. Sludge samples were selected at the same position as the SEM samples to measure sludge size, and the change in sludge size can indirectly reflect the change in the degree

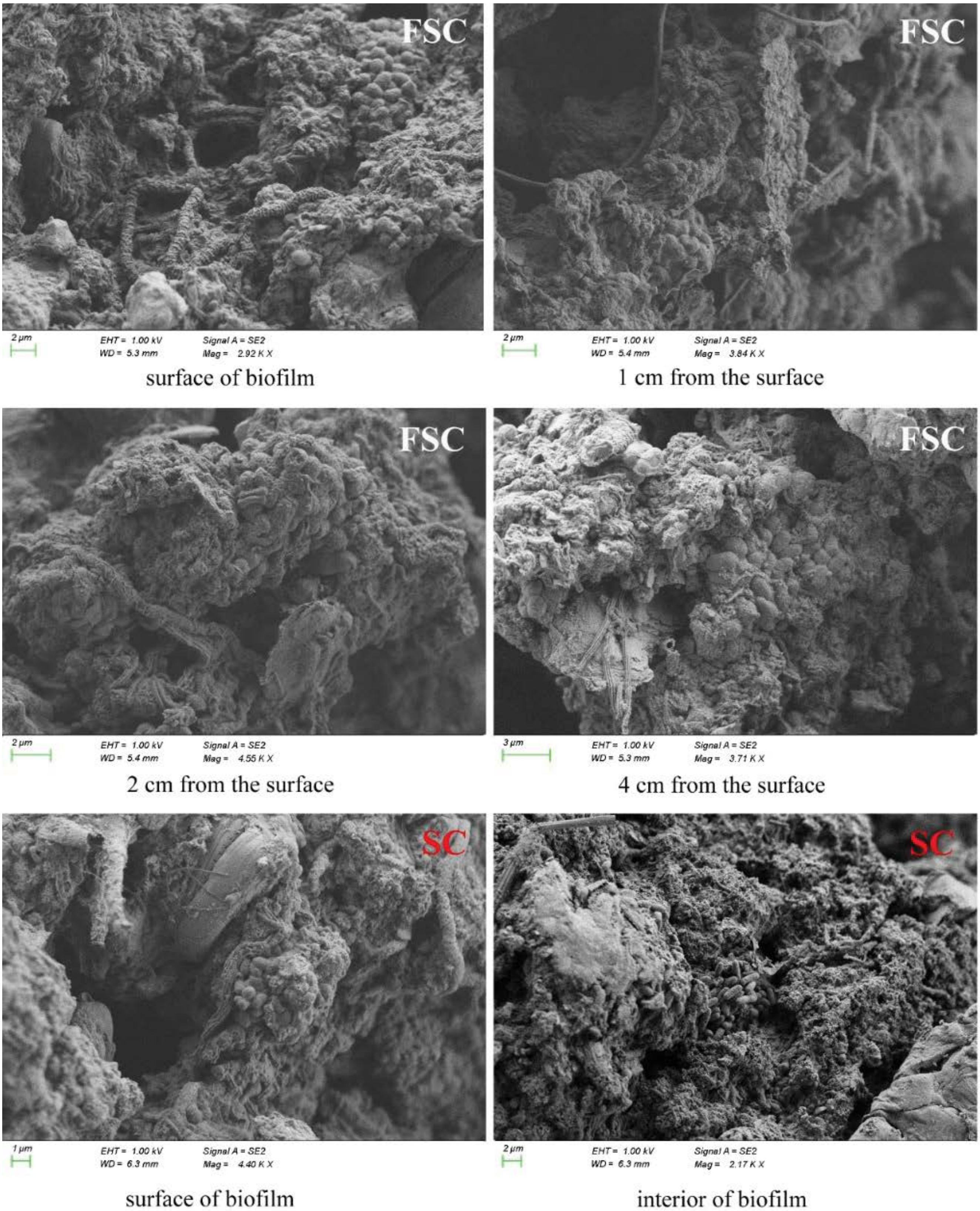

Fig. 9. SEM of biofilms in different regions of (a) $_{FSC}$SBBR and (b) $_{SC}$SBBR (from the surface to the bottom of the carrier).

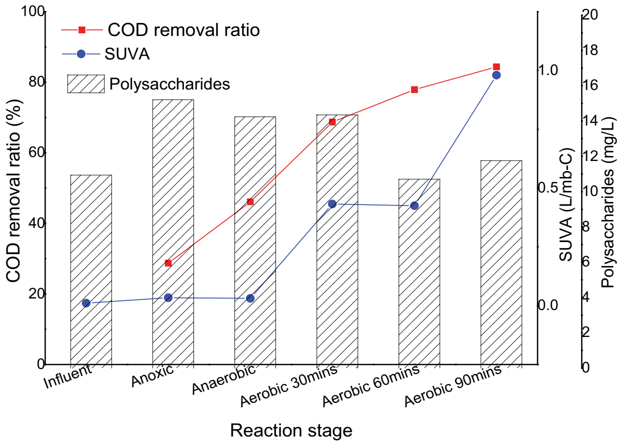

Fig. 10. Analysis of organic compounds in the supernatant of $_{FSC}$SBBR samples at different stages of the system's operation cycle.

of sludge hydrolysis. It can be seen from Table 6 that sludge size in the $_{FSC}$SBBR system was smaller than that in the $_{SC}$SBBR system, which indicates that the FSC packing biofilm exhibits a better hydrolysis and cytolysis efficiency than the SC packing biofilm, and its in-situ sludge reduction effect is better. For the $_{FSC}$SBBR system, the average particle size from the biofilm surface to the bottom of the sludge decreases slowly, and this effect is more pronounced in the $_{SC}$SBBR system. It can be seen that the size of biofilm particle attached to the bottom of the carrier becomes smaller with the increase of the biofilm thickness, which confirms the increase in fragmented sludge observed in the SEM image.

3.2.2. Analysis of microbial communities richness and diversity estimators

Sludge samples collected on the surface of the biofilms of the $_{FSC}$SBBR and $_{SC}$SBBRR systems after steady operation under optimal conditions were subjected to high-throughput sequencing. The results were shown in Table 7. A total of 44,481 ($_{FSC}$SBBR) and 47,288 ($_{SC}$SBBR) valid sequences were obtained via 454 pyrophosphate sequencing, with an average length of 423 bp. The sequences were divided by 3%, and 2,118 OTUs ($_{FSC}$SBBR) and 2,074 OTUs ($_{SC}$SBBR) were obtained for the two systems. The theoretical maximum OTU values calculated using the species richness index following the Chao theory were 2,526.63 ($_{FSC}$SBBR) and 2,494.43 ($_{SC}$SBBR), respectively. It can be seen that the population richness of the biofilm in the $_{FSC}$SBBR system was higher than that of $_{SC}$SBBR. The species distribution in the community was analysed according to the Shannon diversity index. The Shannon index of the $_{FSC}$SBBR system was 5.97, while that of the $_{SC}$SBBR system was 5.87, indicating that the diversity of microbial communities in the biofilm of the $_{FSC}$SBBR system was higher, and the OTUs in FSC carriers were distributed more evenly than those in SC carriers [37]. The results show that the sludge trapped by the spherical porous composite packing can promote the accumulation of sludge inside the packing to form a complex micro-environment and enrich the species of microorganisms in the biofilm. Compared with $_{SC}$SBBR, certain special microorganisms enriched in the $_{FSC}$SBBR biofilm can promote in-situ sludge reduction and enhance the sewage treatment effect, and the microbial population with high abundance can form a more stable ecological environment in the biofilm.

4. Conclusions

$_{FSC}$SBBR showed a better in-situ sludge reduction efficiency than $_{SC}$SBBR. The optimal operating conditions include a temperature of 25°C, HRT of 12 h and DO concentration of 4–5 mg/L, and the observed sludge yield is 0.21 + 0.10 g MLSS/g COD. $_{FSC}$SBBR can effectively reduce the yield of excess sludge based on the premise of ensuring good effluent quality. Scanning electron microscopy (SEM) and microbial population structure analysis showed that the

Table 6
Statistics of sludge size in two different carriers

	Sample position	Average size (μm)	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)
FSC	Surface	95.28	23.78	80.78	183.5
	1 cm from the surface	87.54	27.47	79.17	159.3
	3 cm from the surface	90	23.78	77.86	161.9
	Inside of the biofilm	86.59	23.91	82.88	167.4
SC	Surface	142.6	31.21	115	288.9
	Inside of the biofilm	95.37	14.93	79.92	198.2

Note: D_{10} is the maximum diameter of the particles when the volume cumulative percentage in the sample is 10%.

Table 7
Richness and diversity estimators of microbial communities in $_{FSC}$SBBR and $_{SC}$SBBR (α = 0.03)

Sample	Sequence number	OUT number	Shannon index	ACE index	Chaol index	Coverage
FSC	44,481	2,074	5.97	2,560.46	2,529.63	0.99
SC	47,288	2,118	5.87	2,556.54	2,499.43	0.99

$_{FSC}$SBBR carrier showed richer microbial species and higher diversity than the $_{SC}$SBBR carrier. Analysis of DOM and sludge particle size showed that the hydrolysis and cytolysis of biofilm sludge in the FSC carrier promoted in-situ sludge reduction.

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