Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2023.29440

290 (2023) 1–11
April

Study on the start-up operation and sludge characteristics of organic wastewater treated by the EGSB–two-stage anaerobic/aerobic biofilm process

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Received 11 November 2022; Accepted 8 March 2023

ABSTRACT

Most wastewater treatment reactors have problems such as slow start-up and a poor treatment effect. This study investigates the rapid start-up and optimal conditions of the expanded granular sludge bed (EGSB)–two-stage anaerobic/aerobic (A/O) biofilm process. The combined process was successfully started by gradually increasing the organic load for approximately 50 d, during which the organic loading rate increased from 4.6 to 46 kg·COD/(m^3·d). By investigating the operation effect of the EGSB reactor at hydraulic retention times (HRTs) of 4, 6, 8, and 10 h, reflux ratios of 6:1, 7:1, 8:1, and 9:1, and ammonia concentrations of 200–800 mg/L, the optimal operation conditions were determined. In the two-stage A/O process, hollow cylindrical fillers and hollow spherical fibrous fillers were used to observe the growth of biofilms on different fillers. The sludge structure and microorganisms were analyzed by scanning electron microscopy and high-throughput sequencing. The results show that when the combined process was successfully started, the chemical oxygen demand removal rate was 97%, and the ammonia nitrogen removal rate was 73%. The EGSB reactor had the best operation effect under the conditions of a low ammonia concentration, HRT = 8 h and reflux ratio of 8:1. In the two-stage A/O process, the biofilm growth condition of the hollow column filler with a high specific surface area was better than that of the hollow spherical filler containing flocculent fibers. The microstructure of the sludge was conducive to the metabolism of microorganisms. The primary microorganisms at the phylum level mainly included Proteobacteria, Firmicutes, Synergistetes, Actinobacteria, and Chloroflexi. These findings provide a new idea for the treatment of high-concentration organic wastewater and reactor start-up.

Keywords: Expanded granular sludge bed–two-stage anaerobic/aerobic biofilm process; Highly concentrated organic wastewater; Chemical oxygen demand; Ammonia nitrogen

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1. Introduction

With the development of society, the problem of water pollution has been widely studied. The discharge of organic sewage containing a large number of organic pollutants and a high-concentration of ammonia nitrogen is one of the main causes of water pollution through eutrophication [1–3]. The microorganisms in organic sewage also cause a decrease in dissolved oxygen (DO) content in water and destroy the water environment [4]. It is very important to remove organic and biological contents in organic sewage. It is of practical significance to explore more effective organic wastewater treatment technologies and to study the associated scientific problems to improve and protect the environment [5,6]. Conventional treatment technologies, such as filtration, air flotation, flocculation, adsorption and other processes, can reduce the concentration of pollutants, but the effect of a single treatment process is not ideal; therefore, many processes are often combined in actual treatment processes [7].

For organic wastewater with high chemical oxygen demand (COD) anaerobic bioreactors have the advantages of a high removal efficiency and low energy consumption [8,9]. The expanded granular sludge bed (EGSB) was improved from the up-flow anaerobic sludge bed (UASB) reactor and retains the latter's advantages of a small footprint and low energy consumption [10]. In addition, effluent reflux technology has been adopted to reduce the concentration of toxic substances in the reactor and increase the up-flow rate, thus promoting mass transfer between the sludge and wastewater [11]. This technology has a good effect on the treatment of high-concentration organic wastewater. Due to the poor removal effect of nitrogen and phosphorus in anaerobic treatment, it is necessary to combine anaerobic with aerobic treatment [12]. The multistage anaerobic/aerobic (A/O) biofilm process has both anoxic and aerobic zones; this configuration has a high oxygen utilization rate, can degrade organic pollutants quickly, and makes better use of microbial characteristics to treat pollutants [13,14]. Biofilms are formed on the filler, and anoxic and anaerobic layers can form inside it, which easily achieves denitrification. The oxygen concentration gradient on the biofilms can better complete nitrification and denitrification and strengthens the denitrification ability of the system [15,16].

Previous studies have shown that when removing pollutants by biological methods, Proteobacteria, Actinobacteria, Synergistetes, Firmicutes and so on are the main components of the microbial community, and the types and diversity of microorganisms involved directly affect the treatment effect of the reactor. Therefore, it is necessary to explore the characteristics of sludge in each stage of the combined process and clarify the removal mechanisms of pollutants [17].

The treatment effect of ammonia nitrogen in EGSB reactors is not good. In this work, an EGSB reactor and a two-stage A/O biofilm process were combined to treat simulated organic wastewater. This approach gave full play to the excellent performance of the EGSB reactor in the treatment of organic pollution, and the two-stage A/O process was used to compensate for the poor treatment effect of nitrogen and phosphorus in the EGSB reactor. Biological wastewater treatment reactors have the problem of long start-up times, and start-up conditions for combined processes are often more complicated than those for single processes. The troubleshooting method for the start-up stage of the combined process is provided, which can provide a reference for rapid reactor start-up. The operation effect of the combined process under different conditions was tested, and the optimum operation conditions were confirmed after the successful start-up of the combined process The sludge situation in the EGSB reactor and two-stage A/O process was analyzed, which provided a method for reducing the reactor start-up time and improving the pollutant removal rate in the actual wastewater treatment process.

2. Materials and methods

2.1. Experimental equipment

2.1.1. EGSB reactor

The reactor used in this test was made of Plexiglas, the interface was sealed with flanges, the inner diameter of the reaction zone was 50 mm, the height was 900 mm, and the effective volume was approximately 9 L. The temperature was controlled to 32°C ± 2°C using a heating rod and thermometer.

Under the action of a peristaltic pump, the water entered the heating device and the water inlet in turn. A porous plate was arranged at the bottom of the water inlet, and the device was equipped with 5 sampling locations. A three-phase separator was arranged in the precipitation area to separate the solid, liquid and gas phases, and the wastewater overflowed after reaching the top of the reactor.

2.1.2. Two-stage A/O biofilm process

The two-stage A/O biofilm system was equipped with 4 tanks, and the size of each tank was 20 cm × 20 cm × 50 cm (with an overflow height of 10 cm). The tanks were connected by a diagonal flow of water from top to bottom. Aeration was carried out in the aerobic tank through the aeration pump, and the aeration volume was controlled by the rotor gas flowmeter. A reflux pump was provided in the two-stage A/O process to ensure sludge reflux. A stirrer was placed in the anoxic tank to ensure that an anoxic environment was maintained in the tank (DO < 0.5 mg/L) and to improve the mass transfer of wastewater. A certain amount of filler was placed in the tank to increase the sludge residence time and reduce sludge loss. The fillers consisted of multisided hollow balls with an outer diameter of 50 mm, and the weight of approximately 2.93 g. Filler A was spherical, the outside of which consisted of a network structure, and the inside was wound with fiber. The diameter was 100 mm, the height was 100 mm, and the weight was approximately 31.29 g. The outer diameter of filler B was approximately 40 mm, the height was approximately 30 mm, and the weight was approximately 4.70 g. There were 8 holes in both the inner ring and the outer ring. The filler shapes are shown in Fig. 1.

After the wastewater passed through the EGSB reactor, it enteed the multistage A/O process, and the two stages operated synergistically. A schematic diagram of the EGSB–two-stage A/O biofilm process is shown in Fig. 2.

2.2. Wastewater quality

In this experiment, domestic sewage from the sewage plant at Shandong Jianzhu University was used. Sodium acetate was added as the carbon source, ammonium chloride was added as the nitrogen source, and potassium dihydrogen phosphate was added as the phosphorus source. The sodium acetate, ammonium chloride, potassium dihydrogen phosphate, etc. were all analytically pure and purchased from Sinopharm Chemical Reagent Co. Carbon, nitrogen and phosphorus sources were added to the sewage according to the ratio C:N:P = 200–300:5:1. A heating rod was used to keep the water warm. All trace elements in the sewage could meet the needs of microbial growth to a certain extent. The specific water quality is shown in Table 1.

2.3. Sludge inoculation

To enable the anaerobic reactor to start up quickly and meet the requirements of the treatment of high-concentration organic load wastewater, anaerobic granular sludge from the anaerobic section of the sewage treatment plant was used for inoculation. The volatile suspended solids/suspended solids (VSS/SS) ratio of the granular sludge was 0.578. After a period of cold storage, the bioactivity of the granular sludge in the EGSB reactor was low, and the diameter of most granular sludge particles was less than 1 mm. The amount of sludge inoculated was approximately 2.5 L, accounting for approximately 1/3 of the effective volume. The activated sludge in the two-stage A/O biofilm process was obtained from the biochemical tank of the sewage plant at Shandong Jianzhu University. The sludge mixed-liquor suspended solids (MLSS) was approximately 3,400 mg/L, mixed-liquor volatile suspended solids (MLVSS) was approximately 1,900 mg/L, and VSS/SS was 0.559. The inoculated amount of sludge was 25 L, accounting for approximately 1/3 of the effective volume.

2.4. Exploring influencing factors

The influencing factors of the combined process were investigated under the conditions of influent COD concentrations of 8,000–9,000 mg/L and temperatures of 32°C ± 2°C.

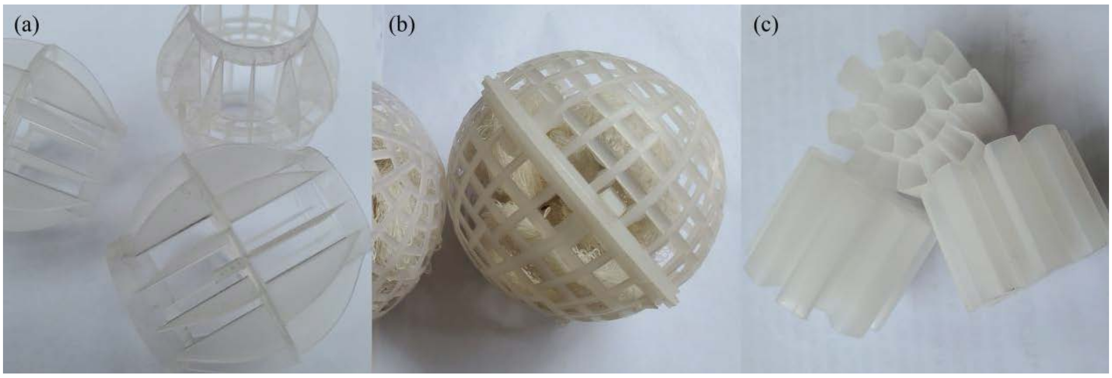

Fig. 1. Photographs of packing balls (a) filler balls in the start-up stage, (b) filler A and (c) filler B.

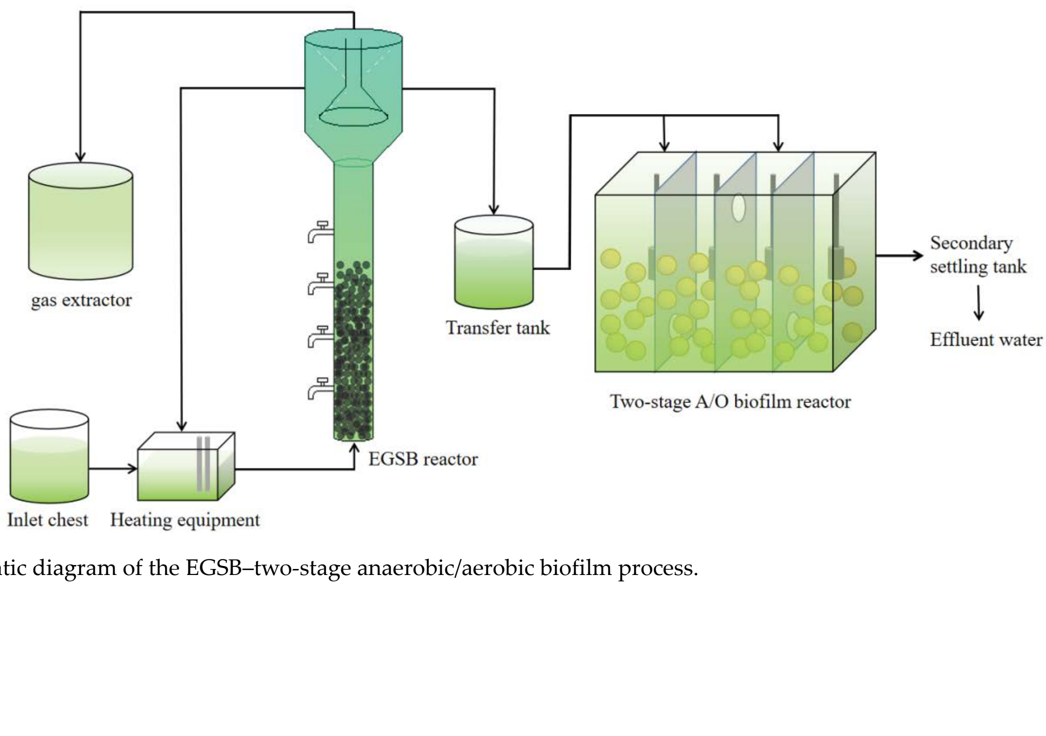

Fig. 2. Schematic diagram of the EGSB–two-stage anaerobic/aerobic biofilm process.

2.4.1. Hydraulic retention time

The reflux ratio was set to 8:1, and the hydraulic retention time (HRT) was adjusted to 4, 6, 8, and 10 h; each operating condition was applied for 7 d to explore the operation effect of the EGSB reactor under different HRTs.

2.4.2. Reflux ratio

The HRT was set to 8 h, and the reflux ratio was adjusted to 6:1, 7:1, 8:1, and 9:1. Each operating condition was applied for 7 d to explore the operation effect of the reactor under different reflux ratios.

2.4.3. Ammonia nitrogen concentration

The HRT of the EGSB reactor was set to 8 h, the reflux ratio was set to 8:1, and the influent ammonia nitrogen concentration was gradually increased from 200 to 800 mg/L. The removal effect of COD in the EGSB reactor with different concentrations of ammonia nitrogen was observed.

2.4.4. Filler type

Two different types of fillers were selected for biofilm growth to observe the film-hanging effect of different fillers and the removal effect of organic matter.

2.5. Detection and analysis methods

Parameters such as COD, NH_3–N, NO_2–N, NO_3–N, MLSS, and MLVSS were determined according to the Standard Methods for the Examination of Water and Wastewater (SEPA, 2002) [18]. DO and pH values were measured by a digital precision meter (Multi3620IDS). The microscopic morphology of the granular sludge was observed and recorded by field-emission scanning electron microscopy (SEM), and the pretreatment method was described by Liu [19]. High-throughput sequencing was performed using the dada2 method of Callahan et al. [20]. The sequencing region was the standard 16S V3-V4 bacterial region (a). PCR was used for sample amplification with the sequencing strategy NovaSeq-PE250. The upstream primer was 338 F, and the sequence was ACTCCTACGGGAGGCAGCA; the downstream primer was 806 R, and the sequence was GGACTACHVGGGTWTCTAAT. The dominant flora in different environments were identified through analysis and research on the Personalbiol platform.

3. Results and discussion

3.1. Initiation of the EGSB–two-stage A/O biofilm process

3.1.1. Start-up troubleshooting

When treating organic wastewater, the initial organic load should be controlled during start-up, and the process can be started by gradually increasing the organic loading rate (OLR) [21]. In the experiment, the start-up strategy of the combined process was based on continuous flow; the organic load and reflux ratio were constantly increased, and the parameters of the EGSB reactor were changed to control the influent of the A/O process to achieve the goal of rapid start-up of the combined process.

The granular sludge in the EGSB reactor was removed from the cold room at the initial stage of start-up and was

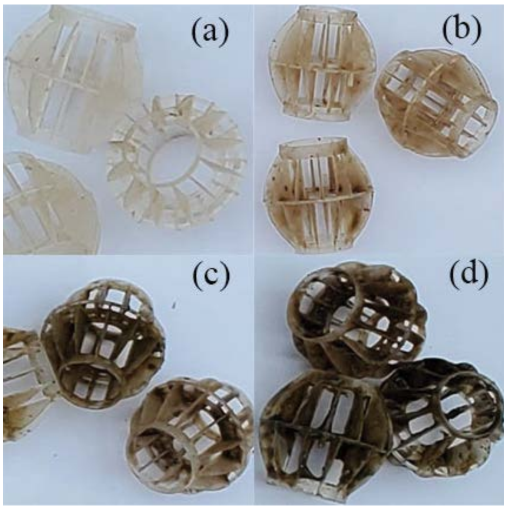

Fig. 4. Biofilm growth condition of the anaerobic/aerobic fillers (a) 20 d, (b) 30 d, (c) 40 d and (d) 45 d.

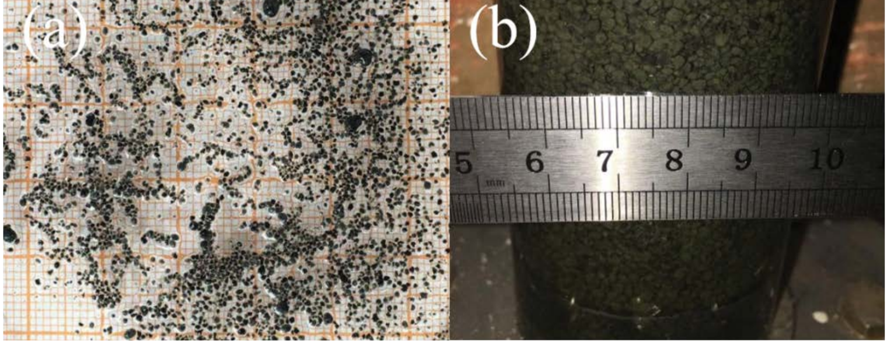

Fig. 3. Particle size comparison of the expanded granular sludge bed reactor granular sludge at the initial stage of inoculation and after successful start-up (a) initial stage of inoculation and (b) after successful start-up.

still in the phase of activity recovery. In the two-stage A/O process, only activated sludge from the aeration tank was inoculated, and filler was not added. The OLR was set to 4.6 kg·COD/(m³·d), the HRT was 6 h, and the reflux ratio was 3:1. On the 15th day, filler pellets were added to the two-stage A/O process, and the OLR was adjusted to 11.6 kg·COD/(m³·d). The HRT was increased to 7.2 h, and the reflux ratio was 3:1 to reduce the impact of organic load on the biofilm. The height of the expanded sludge bed was less than two-thirds of the reaction zone. At 20 d, the OLR was approximately 18 kg·COD/(m³·d), the HRT was 6 h, the reflux ratio was 3:1, and the two-stage A/O inlet organic load was increased to promote biofilm growth. At 30 d, with the increasing concentration of pollutants in the inlet water, the OLR was adjusted to 25.2 kg·COD/(m³·d), and the reflux ratio was adjusted to 5:1. During this period, the particle size of the granular sludge gradually increased, and the quantity of sludge also increased to a certain degree. At 40 d, the OLR was adjusted to 30.3 kg·COD/(m³·d), and the reflux ratio was 7:1. At 50 d, the particle size of the granular sludge at the bottom of the reactor reached 3–4 mm, and the effluent effect was stable. At this time, the reactor was started. The height of the expanded sludge bed had reached one-third of the height of the settling zone. When the EGSB reactor was successfully started, the OLR was approximately 46 kg·COD/(m³·d), the HRT was 6.35 h, the reflux ratio was 8:1, the MLSS of the granular sludge was 35.576 g/L, the MLVSS was 28.556 g/L, and the VSS/SS ratio was 0.8026.

In the A/O start-up stage, because of the flocculent sludge inoculated in the A/O process, the growth rate of biofilm on the filler was greatly improved. The activated sludge was inoculated into the reactor, and sewage from the influent of the biochemical tank at the Shandong Jianzhu University sewage plant was poured into the reactor at the beginning of start-up. The sludge structure was loose during the period of aeration. At this time, to avoid flocculent sludge being washed away, the DO in the aerobic zone was controlled to approximately 1.5 mg/L, and the DO in the anoxic zone was controlled to be less than 0.5 mg/L. The DO concentration in the aerobic zone was increased to 2 mg/L when the flocs became compact, and the reflux ratio was controlled within approximately 120%; 15 d later, the filler balls were added, and the dosage rate was 40%–60%. The reflux ratio was controlled to 100%, and the HRT was between 10 and 16 h. When the filler and sludge in the tank maintained contact, the biofilm covered the filler more densely, and the effluent effect was stable. It was considered that the A/O biofilm process was successfully started. The biofilm content of the filler was measured to be 92 mg/g.

In the start-up phase of the combined process, the sludge was cultivated with a low initial organic load. The OLR of the reactor was gradually increased, and the effect of the organic load on the sludge was reduced by increasing the reflux ratio. According to the removal effect of organic matter, the HRT should be regularly adjusted to reduce fluctuations in the organic matter content of the influent in the A/O process. When increasing the influent organic concentration, it was necessary to limit the impact on the granular sludge and to meet the sludge biofilm demand for organic matter in the two-stage A/O process to improve the start-up speed. After approximately 50 d, the particle size of the granular sludge at the bottom of the EGSB reactor reached 3–4 cm, the biofilm on the A/O filler was compact, the system effluent was stable, and the reactor was successfully started. The changes in particle size of granular sludge and growth of biofilm are shown in Figs. 3 and 4.

3.1.2. Contaminant changes during start-up

As shown in Fig. 5, the influent COD concentration was 1,150 mg/L, and the ammonia nitrogen concentration was 75 mg/L. The concentration of COD and ammonia nitrogen increased over time. The granular sludge in the EGSB reactor was removed from cold storage and was still recovering its activity in the first 10 d. In the two-stage A/O process, only activated sludge from the aeration tank was inoculated, and filler was not added. The removal rate of COD was approximately 80%, and the removal rate of ammonia nitrogen fluctuated between 50% and 70%. Activated sludge was lost along with the water flow.

When the process ran for 15–20 d, the removal rate began to rise with the increase in granular sludge activity in the EGSB reactor and the addition of fillers in the two-stage A/O process. However, the sludge could not adapt to

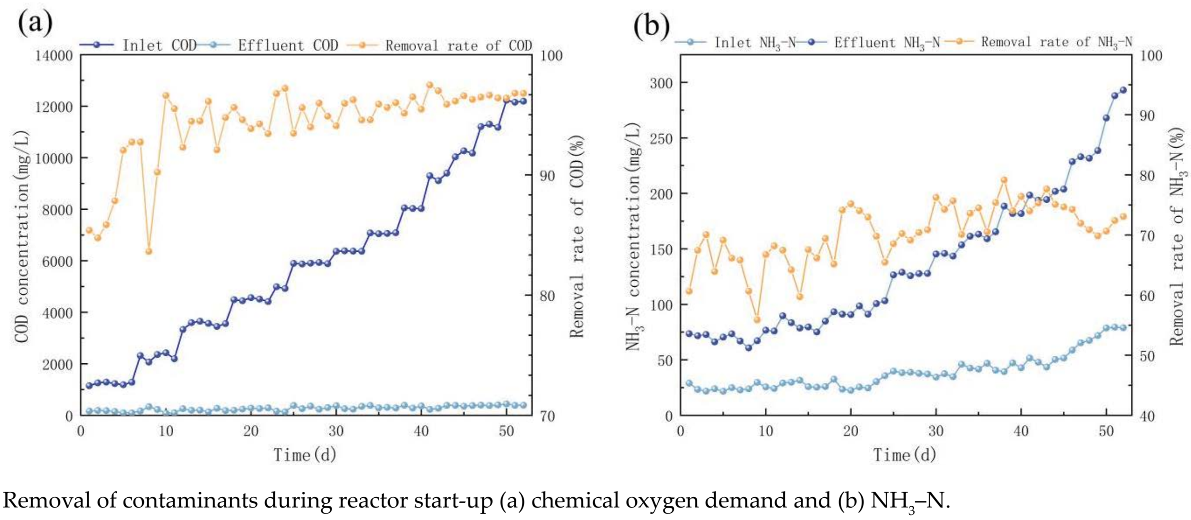

Fig. 5. Removal of contaminants during reactor start-up (a) chemical oxygen demand and (b) NH_3–N.

the sudden increase in organic load, the mass transfer effect of the EGSB reactor was insufficient, and the wastewater did not react adequately with the microorganisms in the granular sludge. Therefore, the removal effect of pollutants was unstable and still fluctuated substantially.

At 20–30 d, the concentrations of COD and ammonia nitrogen in the reactor influent continued to increase, and the pollution removal performance of the granular sludge in the EGSB reactor gradually stabilized. At the same time, the biofilm in the two-stage A/O process grew well, enabling the system to better cope with the impact of influent water quality changes. At this time, the COD removal rate was stable above 90%. In the two-stage A/O process, the biofilm microbial condition was stable, and the average sludge retention time in the reactor was longer than that of ordinary suspended sludge. Due to the slow growth and propagation of nitrifying bacteria, a greater sludge age promotes their dominance, so the combined process with biofilms has a better nitrification capacity [22]. At this time, the removal rate of ammonia nitrogen in water was stable above 65%.

At 30–50 d, the gas content of the EGSB reactor increased, and a large amount of gas existed in the crevices of the sludge; this gas expanded the sludge A/O biofilm, and material exchange between the formed biofilm and the wastewater improved. After continuous acclimation to the environment, the ability of the biofilm to absorb and transform nitrogen was also improved. At the same time, the effluent COD concentration of the anaerobic reactor increased and met the C/N ratio required for the normal growth, reproduction and development of microorganisms. At this time, the removal rate of ammonia nitrogen was between 70% and 80%.

When the process was successfully started, the influent COD and ammonia nitrogen concentrations were approximately 12,195 and 287 mg/L, respectively, and the effluent COD and ammonia nitrogen concentrations were approximately 392 and 76 mg/L, respectively. The removal efficiency reached 97% and 74%, respectively.

3.2. Operation influencing factors

3.2.1. Influence of HRT in the EGSB reactor

The HRT is the average time of mixing between wastewater and sludge microorganisms. A reasonable HRT can achieve a balance between nutrients and organic load in the system so that the microorganisms can grow and reproduce to the maximum extent and thus support a higher reactor load [23].

The experimental results are shown in Fig. 6; the initial HRT was 10 h. The up-flow rate of the reactor was reduced, and the mass transfer effect between the granular sludge and wastewater was weakened, which affected the treatment effect to a certain extent. Under these conditions, the average COD removal rate of the EGSB reactor was approximately 85.8%. After the HRT had been adjusted for 8 h, the up-flow rate of the reactor was increased, the granular sludge could settle, the COD removal rate was improved, and the gas production effect was better. The generation of bubbles promoted contact between the granular sludge and wastewater, and the mass transfer effect was improved. Under this condition, the average COD removal rate was approximately 88.7%. When the HRT was adjusted to 6 h, the upflow rate of the reactor further increased, which resulted in the loss of part of the granular sludge, incomplete mixing of sludge and water, and a poor mass transfer effect. Under this condition, the average COD removal rate was approximately 72%. When the HRT was set to 4 h, because the HRT was too short, the granular sludge could not quickly adapt to the high-concentration of organic pollutants. The fast upflow rate led to an insufficient contact time between sludge and water, and anaerobic microorganisms could not effectively degrade the high-concentration of organic matter in the wastewater. Part of the sludge was lost in the current. Under this condition, the average COD removal rate was approximately 48.6%.

When the HRT was 8 h, it could not only ensure enough contact time between the sludge and wastewater and support the growth, reproduction and metabolism of microorganisms but also reduce sludge loss and reduce the cost of the reactor.

3.2.2. Influence of the reflux ratio in the EGSB reactor

A low reflux ratio cannot effectively alleviate the problem of reactor acidification, while a high reflux ratio will increase the cost input and even cause some sludge loss with water flow [24]. This experiment explored the operation of the reactor under different reflux ratios, and the results were as follows.

As shown in Fig. 7, when the reflux ratio was set to 9:1, the inflow of water increased, the concentration of pollutants was diluted, and the up-flow rate also increased. Some of the small granular sludge was lost with the flow. At the same time, the sludge bed expanded and rose to the settling area, which aggravated sludge loss. Under these conditions, the average COD removal rate was 82.4%. When the reflux ratio was adjusted to 8:1, the swelling phenomenon of the sludge bed was alleviated, and the reaction zone was filled, which provided good conditions for mass transfer between

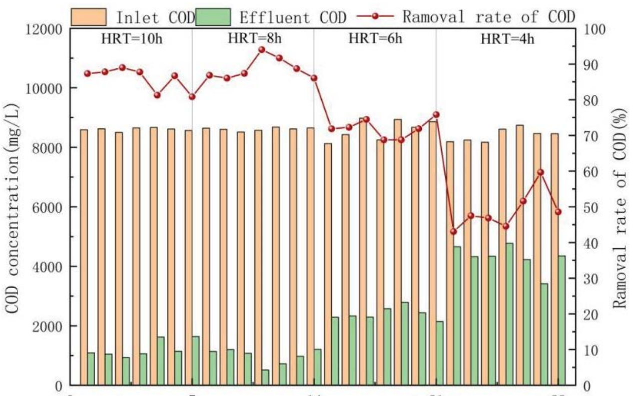

Fig. 6. Chemical oxygen demand removal effect of the expanded granular sludge bed reactor under different hydraulic retention times.

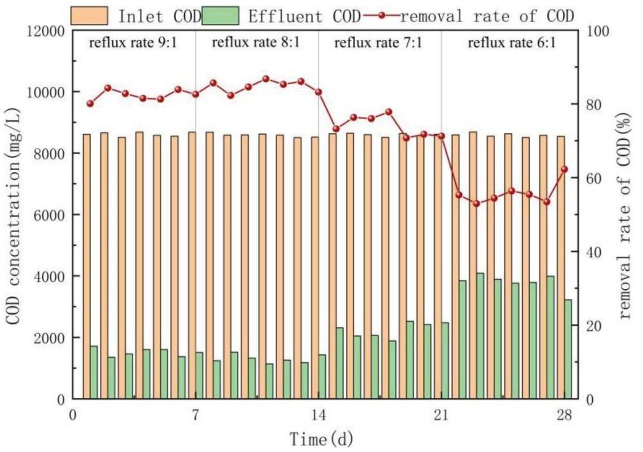

Fig. 7. Chemical oxygen demand removal effect of the expanded granular sludge bed reactor under different reflux ratios.

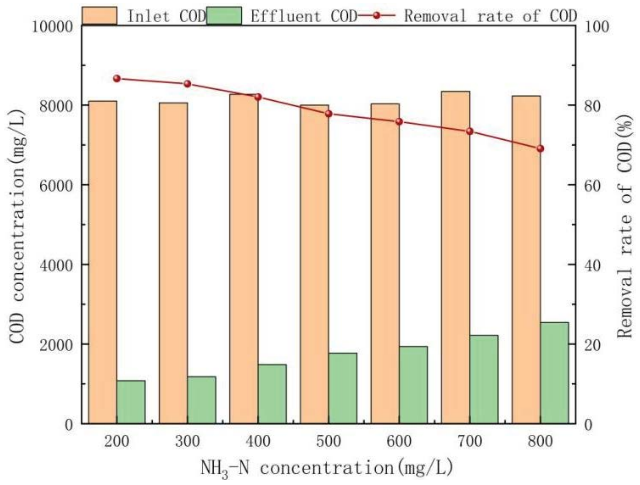

Fig. 8. Chemical oxygen demand treatment effect of the expanded granular sludge bed reactor under different ammonia nitrogen concentrations.

sludge and water. Under this condition, the average COD removal rate was approximately 84.86%. When the reflux ratio was adjusted to 7:1, the swelling height of the sludge bed was further reduced, and the influent organic load of the reactor was increased, which had a certain impact on the granular sludge and affected the removal of pollutants. Under this condition, the COD removal rate was approximately 73.9% on average. When the reflux ratio was adjusted to 6:1, the concentration of influent pollutants was further increased, which had a great impact on the granular sludge. At the same time, the swelling height of the sludge bed was low, and sludge–water mass transfer could not be fully carried out, influencing the removal effect of pollutants. Under this condition, the COD removal rate was approximately 55.8% on average.

According to comprehensive analysis of the operation conditions under different reflux ratios, a reflux ratio of 8:1 was more suitable for the operation conditions process requirements and treatment effect of the reactor.

3.2.3. Influence of ammonia concentration in the EGSB reactor

Most organic sewage is characterized by high ammonia nitrogen. Generally, a high concentration of ammonia nitrogen has an adverse impact on anaerobic biological treatment [25]. This experiment tested whether the process could maintain a good pollutant degradation effect of under high ammonia nitrogen.

As shown in Fig. 8, at first, with the increase in ammonia nitrogen concentration, the COD removal rate remained above 85%, and the pollutant removal capacity of the reactor did not change much. When the concentration of ammonia nitrogen increased to 400 mg/L, the COD removal effect of the reactor began to show a significant downward trend. When the concentration of ammonia nitrogen increased to 800 mg/L, the COD removal rate decreased to approximately 70%, and the effluent COD concentration was high, which had a great influence on the subsequent A/O operation effect. A large amount of ammonia nitrogen would have affected the growth and reproduction of anaerobic microorganisms to a certain extent and reduced the pollutant removal effect. Limited by the volume of the reactor, the amount of sludge and the number of microorganisms, the resistance to a high-ammonia-nitrogen environment was weak. In addition, high ammonia nitrogen inhibits the activity of anaerobic microorganisms and further aggravates the accumulation of ammonia nitrogen. Under these water quality conditions, anaerobic microorganisms could adapt to the higher-ammonia-nitrogen operating environment to a certain extent and weaken its inhibitory effect.

3.2.4. Influence of different fillers in the two-stage A/O process

Adding filler to the reactor can increase the residence time of microorganisms in the sludge and increase the amount of microorganisms, resulting in better pollutant removal. The material, surface area and size of the filler will affect the time and effect of film-hanging and the ability to remove pollutants. filler A containing fiber and Filler B with high specific surface area were used as fillers in the two-stage A/O process to observe the growth condition of biofilm on the filler surface.

Filler A was almost completely covered by activated sludge on the 22nd day. The effluent of the reactor was clean and clear, there was a large amount of flocculated sludge in the tank, the overall precipitation ability was enhanced, and the yellow-brown sludge was full of filler. The biofilm content of the filler was determined to be 118 mg/g. On the 21st day, film-hanging on filler B was basically completed. The thickness of the biofilm was relatively high, and anaerobic and anoxic gradients existed. At this time, the removal effect of organic matter in the reactor tended to be stable, the biomass in the reactor was relatively rich, and the biofilm amount was 129 mg/g.

As shown in Fig. 9, in the early stage of biofilm incubation, the influent COD concentration was relatively high. Due to the lack of biofilm attachment on the filler in the

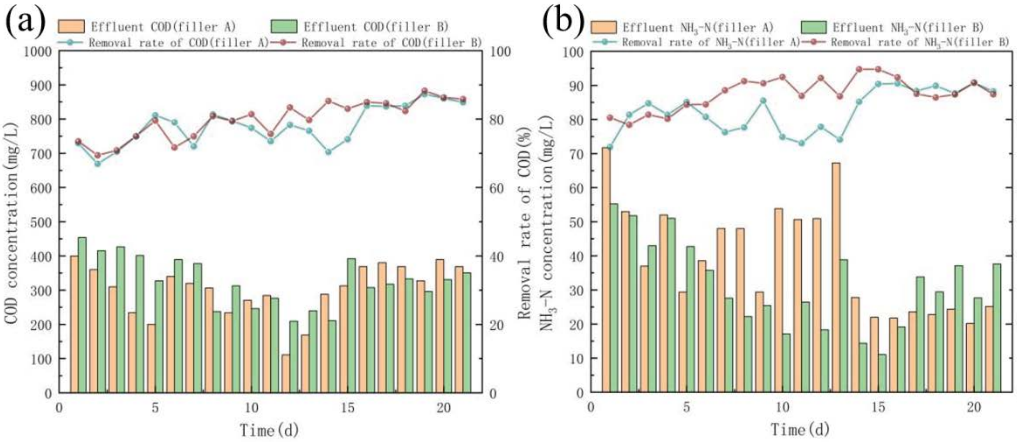

Fig. 9. Influence of different fillers on the removal rate of conventional pollutants (a) chemical oxygen demand and (b) NH_3–H).

early stage, some sludge was lost; the COD removal rates with both filler types were maintained at approximately 70%, and the ammonia nitrogen removal rates were lower than 80%. With increasing biofilm culture time, microorganisms gradually adapted to the conditions, the removal rates of COD and ammonia nitrogen gradually increased, and the removal rates of both COD and ammonia nitrogen fluctuated between 70% and 80%. However, the removal rate of ammonia nitrogen in B rose steadily, while that in A fluctuated greatly. Finally, the removal rates of COD and ammonia nitrogen both exceeded 85%, but the stability of filler B was greater than that of filler A. The comprehensive performance of filler B with a high specific surface area was better than that of filler A.

3.3. Analysis of sludge characteristics

Fig. 10 shows that after the successful start-up of the EGSB granular sludge, the sludge was in good condition, with a tight and thick structure. The internal voids were conducive to the exchange of gases and nutrients inside and outside the sludge, which strengthened sludge mass transfer, promoted the transfer of nutrients between different bacteria and improved the removal effect of pollutants. The sludge in the A/O aerobic zone and anaerobic zone contained large amounts of bacteria and sludge debris, which intertwined with each other to form a framework and form a biofilm together with the attached microorganisms. Gaps in this framework enhance material transfer inside and outside the biofilm to a certain extent, which makes the sewage treatment effect good.

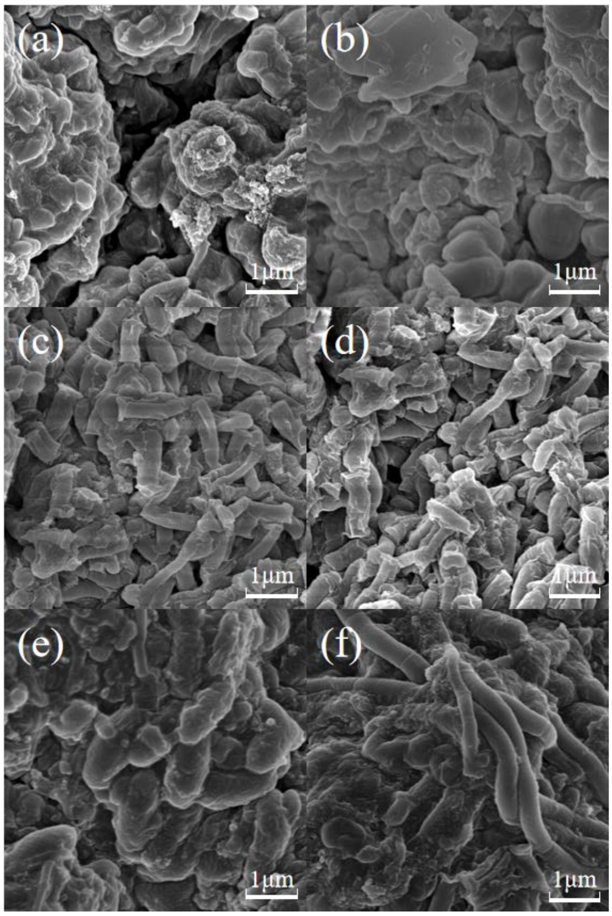

Fig. 10. Microbiological surface characteristics of sludge (a, b) expanded granular sludge bed, (c, d) aerobic-zone sludge in the anaerobic/aerobic process and (e, f) anaerobic-zone sludge in the anaerobic/aerobic process.

3.3.2. Richness and structural analysis of the sludge microbial population

The sludge microbial diversity index is shown in Table 2. A denotes the anaerobic granular sludge microorganisms after the initiation of EGSB reactor culture and acclimation; B denotes the microorganisms in the anoxic zone of the two-stage A/O biofilm process; and C denotes the microorganisms in the aerobic zone of the two-stage A/O biofilm process. Chao1 is a species richness index; the larger the value is, the larger the population [26]. The richness decreased from the anaerobic to anoxic to aerobic stage, and the lowest value was 1090, indicating that the species richness of the sludge was very high. Pielou's evenness index was approximately

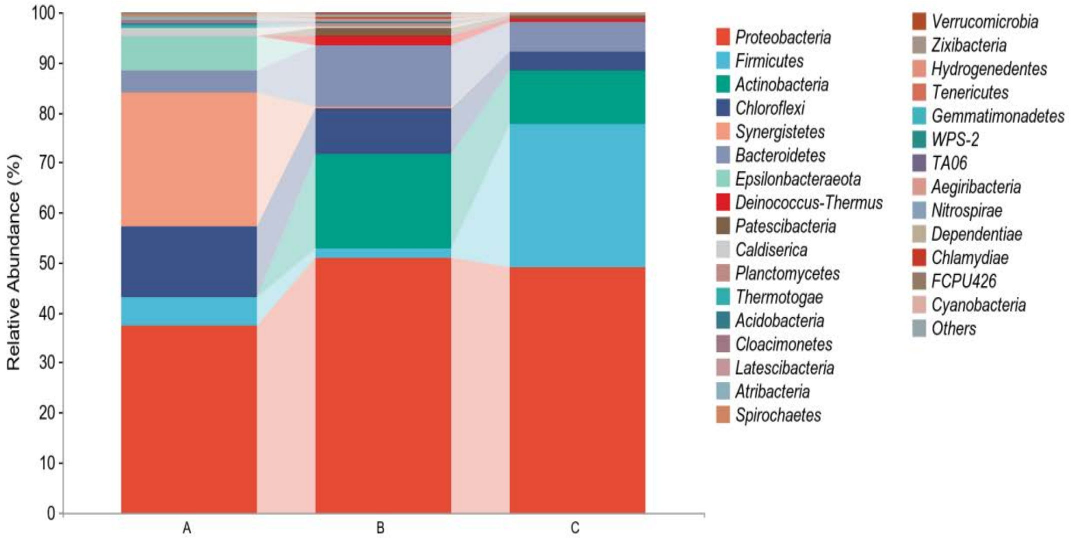

Fig. 11. Composition of primary microbial phyla (A) granular sludge microorganisms after expanded granular sludge bed initiation, (B) granular sludge microorganisms in the anoxic zone after biofilm establishment and (C) microbial condition of granular sludge in the aerobic zone after biofilm establishment.

Table 1
Water quality of wastewater during reactor start-up

Parameter	Value
pH	7.5–8.5
Temperature (°C)	32 ± 2
Chemical oxygen demand (mg/L)	1,150–12,195
NH_3–N (mg/L)	59–287
Total phosphorus (mg/L)	15–57

Table 2
Sludge microbial diversity index

Sample	Chao1	Pielou_e	Shannon	Simpson
A	2,504.61	0.706618	7.91392	0.977016
B	1,814.28	0.691788	7.34379	0.965715
C	1,090.36	0.616131	6.15318	0.948816

0.7, indicating uniform species and community composition [27]. The Shannon and Simpson indices were 6–8 and above 0.9, respectively, indicating that the community structure in the sludge was complex and evenly distributed.

As shown in Fig. 11, when the sludge was successfully cultured, the microorganism taxa mainly consisted of Proteobacteria, Actinobacteria, Synergistetes, Chloroflexi, Epsilonbacteraeota, Firmicutes, Bacteroidetes, Caldiserica, Deinococcus-Thermus, Nitrospirae, etc. In the anaerobic stage, the dominant bacteria were Proteobacteria (37.38%), Synergistetes (26.86%) and Chloroflexi (13.93%). Among them, Proteobacteria can change its form and has strong adaptability. It is the main population in activated sludge systems in sewage treatment and contains a variety of metabolic species [28]. It can remove nitrogen and phosphorus while degrading organic matter. This phylum is extremely diverse and includes a variety of anaerobic, aerobic or facultative bacteria; therefore, it is also the most dominant phylum in the anoxic and aerobic stages and plays an important role in the treatment of organic sewage. Synergistetes is a rod-like obligate anaerobic bacterium that can remove organic pollutants in water during metabolism [29]. It is one of the main phyla for removing organic pollutants in the anaerobic stage. Chloroflexi is the main bacterial taxon involved in the process of microbial denitrification and nitrogen removal [30]. The presence of Chloroflexi greatly promotes the removal of nitrogen compounds in water. In the anoxic stage, the dominant bacteria were Proteobacteria (50.90%), Actinobacteria (18.66%) and Bacteroidetes (12.23%). Among them, Actinobacteria provides a necessary matrix for the formation of activated sludge flocs, which plays a positive role in sewage treatment. Bacteroidetes can promote the flocculation of activated sludge filaments [31]. Both these taxa, as well as Proteobacteria, are involved in the obligate anaerobic and nitrogen fixation of chemoorganic nutrients. Both are the main bacterial groups for nitrogen removal in the anoxic stage. The existence of these two phyla is of great significance for nitrogen removal in the two-stage A/O process. In the aerobic stage, the dominant bacteria were Proteobacteria (49.09%), Firmicutes (28.59%) and Actinobacteria (10.65%). Firmicutes are the main bacteria involved in the process of microbial nitrogen removal, as commonly seen in experiments at various scales and in actual sewage treatment [32].

In general, the dominant strains in the combined process verified that the system had a good ability to remove organic matter and ammonia nitrogen. The rich microbial population, complex structure and uniform distribution of sludge in different stages laid a solid foundation for the high efficiency and stability of organic sewage treatment.

4. Conclusions

At the beginning of the EGSB reactor, the OLR was 4.6 kg·COD/(m^3·d), the HRT was 6 h, the reflux ratio was 3:1, the DO concentration in the two-stage A/O aerobic zone was approximately 2 mg/L, and the DO concentration in the anoxic zone was less than 0.5 mg/L. The reflux ratio was 100%. The HRT remained between 10 and 16 h, and the initial sludge inoculum volume of both stages was 1/3 of the effective volume. The operation parameters were adjusted according to the change in the pollutant removal rate. Finally, the OLR of the EGSB reactor was 46 kg·COD/(m^3·d), the HRT was 6.35 h, the reflux ratio was 8:1, the HRT of the two-stage A/O process was 14 h, and the biofilm volume was 92 mg/g. After successful start-up, the influent COD concentration of the combined process was 12,195 mg/L, the effluent concentration was 392 mg/L, and the removal rate reached 97%. The ammonia nitrogen concentration of the inlet water was 290 mg/L, and the ammonia nitrogen concentration of the outlet water was 78 mg/L. The removal rate reached 73%. Under the conditions of 32°C ± 2°C, HRT = 8 h and reflux ratio of 8:1, the combined process had a high efficiency. In subsequent experiments, the removal effect of organic matter gradually decreased with increasing ammonia concentrations. SEM analysis showed that the structure of the sludge was tight and thick, and the internal pores strengthened the mass transfer between sludge and water. Gene sequencing results showed that the species richness and uniformity of sludge microorganisms were high, and the complex species composition improved the resilience of the system. The microorganism phyla mainly included Proteobacteria, Synergistetes, Actinobacteria, Firmicutes, etc., which are the main phyla in the degradation of pollutants; therefore, the system could withstand high concentrations of pollutants. By adjusting the parameters of the EGSB reactor, the influent organic concentration of the two-stage A/O process could be controlled to achieve fast start-up and operation. The findings of this study will provide a new idea for the treatment of high-concentration organic wastewater.

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