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Filamentous sludge bulking recovery to a limited state for pollutant removal improvement using a novel gravity selector

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ABSTRACT

A novel gravity selector was firstly introduced between the aerobic zone and clarifier in an anaerobic/anoxic/oxic (A^2/O) reactor to reduce severe filamentous sludge bulking to limited filamentous bulking (LFB). By regulating the aeration periods of operation cycles of the selector and other parameters, lighter filamentous bulking sludge was gradually washed out, reducing the sludge volume index (SVI) from 418 to 210 mL/g. A stable LFB state was achieved in 80 d with pollutant removal efficiencies of 90.1%, 93.7%, 87.9% and 88.5% for COD, NH_4^+-N, TP and TN, respectively. *Thiothrix* and *Beggiatoa*, with extended filaments, dominated the filamentous bacterial population of the first phase, whereas *Haliscomenobacter hydrossis*, with ingrown, thin, and short filaments, dominated the LFB state. Therefore, the bulking control of the gravity selector relied on the different settling velocities of bacteria with different filament morphologies during sedimentation periods in the gravity field. The bacteria with long, outstretched filaments settled slowly and were preferentially washed out via the upper outlet. The LFB state was realised at low DO contents of 0.5~1.0 mg/L and a long sludge retention time (SRT) of 28 d with considerable denitrifying dephosphatation, indicating that the gravity selector can improve pollutant removal and energy utilisation.

Keywords: Limited filamentous bulking; Domestic sewage; Biological nutrient removal; Nitrification; *Haliscomenobacter hydrossis*

1. Introduction

Several technologies are used for wastewater treatment, including adsorption [1,2], photocatalysis [3] and bioremediation technologies. The activated sludge process of bioremediation is the most commonly used method for nitrogen (N) and phosphorus (P) removal in municipal wastewater treatment worldwide. However, filamentous sludge bulking hinders the stable operation of wastewater treatment plants (WWTPs) that adopt this process, and various methods have been used to attempt to control this bulking [4]. The overgrown filamentous bacteria in sludge flocs are the main reason for filamentous bulking; their long filaments become attached to the many gas bubbles that form due to denitrification or digestion in the clarifier, causing the activated sludge to expand and float [5,6]. Severe filamentous bulking leads to massive loss of biomass and a dramatic reduction in pollutant treatment performance. There are two ways to prevent the excess proliferation of filamentous bacteria: specific (e.g., the adjustment of operating parameters and the installation of anaerobic, anoxic or oxicbio-selectors to create an unfavourable environment for specific filamentous bacteria; the accumulation of *Lecanein-*

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ermis rotifers to reduce *Haliscomenobacter hydrossis*) [7,8] and non-specific methods (the application of chlorination and ozonation to kill most types of filamentous bacteria and the addition of flocculants to promote sludge sedimentation) [9–11]. However, the effects of these strategies are not ideal due to the complexity and diversity of filamentous microorganisms.

Guo et al. [12] developed an innovative technique in the operation of a real WWTP that utilised the limited bulking of filamentous sludge to improve pollutant removal in sewage, providing a new understanding of filamentous sludge bulking. Limited filamentous bulking (LFB) was defined as a stable state with the limited growth of filamentous bacteria in activated sludge induced by decreasing the dissolved oxygen (DO) concentration. The sludge volume index (SVI) ranged from 150 to 250 mL/g, and the nutrient removal efficiencies increased the non-bulking condition [12]. The inflated LFB flocs facilitated the simultaneous nitrification-denitrification inside as well as the capture of pollutant particulates in the sedimentation basin. Therefore, effluent quality was improved at a lower energy cost. Concrete implementation methods of inducing LFB have been reported previously [12,13] and successfully achieved in laboratory-scale systems. Dominant filamentous bacteria, such as Eikelboom *Type 0041*, *Sphaerotilusnatans*, *Microthrixparvicella* and *Haliscomenobacter hydrossis*, have also been identified [13–16]. However, all the existing research started from non-bulking systems with the purposes of enhancing treatment effect and saving energy; no LFB system achievement induced from a serious filamentous bulking system was reported. Considering the negative consequences of bulking, it is desirable to develop an approach to treat a severe filamentous bulking system such that it achieves an LFB state.

The anaerobic/anoxic/oxic (A^2/O) process, a classic simultaneous biological N- and P-removal technology adopted in WWTPs, is at risk of filamentous sludge bulking frequently due to its function of nutrient removal [22]. This process has a high energy cost for carbon sources and aeration consumption. Therefore, an LFB process that could prevent or remedy filamentous bulking in A^2/O systems would have valuable applications. There is limited potential for the use of non-specific methods to induce a severe filamentous bulking A^2/O process into an LFB state because of economic factors and the difficulty of accurately controlling the dose of bactericides in WWTPs [17]. Specific methods are also impractical for an A^2/O process because 3 types of filamentous bacteria may adapt to and survive in the altered anaerobic/anoxic/oxic environment that are detrimental to common filamentous bacteria: (a) fermentative and polyphosphate (poly-P)-accumulating bacteria that use carbon sources in the anaerobic zone; (b) bacteria able to utilise nitrogen oxide (NO_x^-) as the final electron acceptor; and (c) bacteria that prefer low organic concentrations in the oxic zone, which is indispensable and large in A^2/O system used for nitrification [18]. These reasons increase the difficulty of limiting filamentous bacteria and inducing LFB in an A^2/O-like process.

Light weight and difficult sedimentation are common features of filamentous sludge because of the loose structure and long filaments of filamentous bacteria. Although relationships between operation conditions and the types of filamentous organisms they favour have been identified, solutions to correct the identified conditions are not always feasible [19]. However, it is possible that a gravity selector could be introduced to wash out the excessive bulking sludge from an A^2/O reactor. Abundant filamentous bacteria that cannot be easily settled could be discharged from the overflow port during sedimentation cycles, reducing the amount of bulking sludge retained in the system for LFB induction. To evaluate this concept, a post-gravity selector with an intermittent aeration cycle (IAC) was designed and installed between the oxic zones and the clarifier. Each IAC was 1 h, comprising an aeration period of a few minutes and a sedimentation period for the remaining time. The pathways of the recycling sludge and operation methods in the A^2/O reactor were altered for sludge selection and LFB induction. The intermittent aeration also served as agitation to remove the numerous gas bubbles attached to filaments, which might prevent the normal sludge-water separation.

The aims of this study were to investigate the following: 1) the feasibility and principle of eliminating severe filamentous bulking from an A^2/O system and creating an LFB state by introducing a gravity selector, 2) the effects of the LFB A^2/O process on pollutant removal, and 3) the variation in the dominant filamentous bacteria in the different operational phases of the experiment.

2. Materials and methods

2.1. Experimental A^2/O reactor with a gravity selector

The configuration of the experimental reactor is shown in Fig. 1. The reactor was made of plastic steel with a working volume of approximately 180 L. The main reaction chamber was divided by perforated flashboards to create 5 compartments along the flow direction: anaerobic zone, anoxic zone, oxic zone 1, oxic zone 2 and gravity selector. The volume ratio was $V_{anaerobic}:V_{anoxic}:V_{oxic1}:V_{oxic2}:V_{selector}$ = 1:2:1.5:1.5:3. Three diffuser discs were installed at the bottom of the oxic zones and gravity selector, and the aeration intensity could be adjusted by glass gas rotameters connected to an air compressor. One of these aeration apparatuses in the gravity selector was triggered accurately by an electromagnetic valve connected to a microcomputer timer to establish the IAC. The sludge was stirred during the aeration period of each IAC to promote the release of gas bubbles and then settled hierarchically; light excessive bulking sludge was discharged from the upper overflow, whereas non-bulking sludge and less-bulking sludge were recycled in the selector from the bottom. The reactor process was converted from an A^2/O process to an A^2/O-gravity selector process by opening and closing certain valves, as shown in Fig. 1 (the real reactor was demonstrated in Fig. S1).

2.2. Experimental procedures

The experimental reactor was processed for 130 d at room temperature (23±2°C) with 4 phases according to different IACs, including 7 runs. The operation parameters are provided in Table 1. In phase I, the reactor was operated as an A^2/O process for filamentous bacteria inoculation and

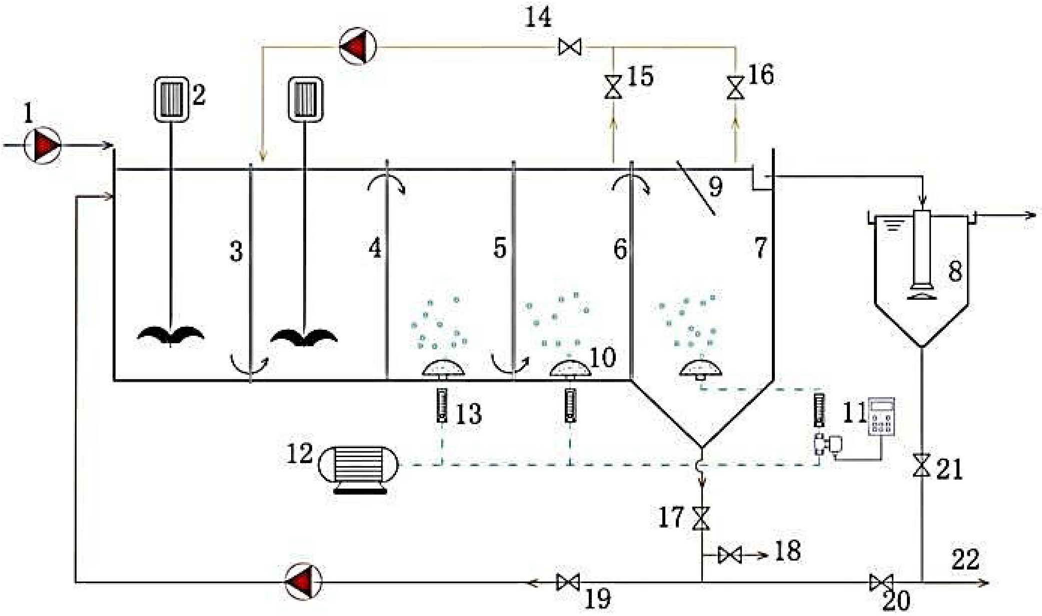

Fig. 1. Schematic diagram of the A2/O reactor with the gravity selector. (1: peristaltic pump; 2: agitator; 3: anaerobic zone; 4: anoxic zone; 5: oxic zone 1; 6: oxic zone 2; 7: gravity selector; 8: clarifier; 9: sludge dashboard; 10: diffuser disc; 11: microcomputer timer; 12: air compressor; 13: gas flowmeter; 14: nitrified liquid reflux valve 1 (NRV 1); 15: NRV 2; 16: NRV 3; 17: sludge return valve 1 (SRV1); 18: sludge outlet 2; 19: SRV 2; 20: SRV 4; 21: SRV 3; 22: sludge outlet 1).

Table 1
Experimental parameters, operations and objectives of the different phases

Phase	Run	Time (d)	OLR (kg COD/ kg MLSS·d)	DO (mg/L)		SRT (d)	IAC (aerationmin/ sedimentation min)	Operations and objectives
				Oxic zones	Gravity selector			
I	1	1–10	0.27–0.29	2.0	2.0	10	–	Induce severe filamentous bulking inA^{2}/O mode by inoculating filamentous bacteria without the gravity selector start-up
II	2	11–20	0.55–0.69	2.0	2.0[a]	10	2/58	Investigate the LFB control when OLR was increased under the A^{2}/O-gravity selector mode because of MLSS loss in phase I. SRV 4 was closed, and sludge was discharged from both outlet 1 and 2 to maintain SRT = 10 d.
	3	21–40	0.28–0.35	2.0	2.0[a]	16	2/58	Investigate the LFB control when MLSS and SRT were increased under the A^{2}/ O-gravity selector mode by discharging sludge only from outlet 1
III	4	41–60	0.26–0.28	2.0	2.0[a]	22	1/59	Investigate the LFB control when the aeration period of IAC was also decreased under the A^{2}/O-gravity selector mode. Excess sludge was discharged only from outlet 1.
	5	61–80	0.25–0.27	2.0	0.5[a]	22	1/59	Investigate the LFB control when the DO concentrations were also decreased, which occurred gradually. Excess sludge was discharged only from outlet 1.
	6	81–100	0.23–0.26	1.0	0.5[a]	22	1/59	
IV	7	101–130	0.21–0.23	1.0	0.5[a]	28	0.5/59.5	Investigate the LFB control when the aeration period of IAC was further decreased and the SRT was prolonged by returning a fraction of the settled sludge in the clarifier. SRV 4 was opened, and excess sludge was discharged only from outlet 1.

[a] DO concentration under intermittent aeration

severe bulking cultivation, and the diffuser disc in the gravity selector continued aerating without timing. The operation in this phase was achieved through opening NRV 1 (14), NRV 3 (16), SRV 2 (19), SRV 3 (21) and SRV 4 (20) and closing NRV 2 (15) and SRV 1 (17); sludge was discharged from outlet 1 (22) only (Fig. 1). The volume ratio of A^2/O in this phase was $V_{anaerobic}:V_{anoxic}:V_{oxic} = 1:2:6$. The large volume of the oxiczones stimulated the reproduction of filamentous bacteria. In phase II, the reactor was operated in A^2/O-gravity selector mode for filamentous bulking control, and the aeration in the selector occurred intermittently according to the IAC (see Table 1). The operation in phases II and III involved opening NRV 1 (14), NRV 2 (15), SRV 1 (17), SRV 2 (19) and SRV 3 (21) and closing NRV 3 (16) and SRV 4 (20); sludge was discharged from outlet 1 (22) and outlet 2 (18) (Fig. 1). In phase IV, SRV 4 (20) (Fig. 1) was also opened for additional sludge return. The influent flow rate was held constant at 15 L/h. The internal reflux (recycled nitrifying liquid) ratio and external reflux (recycled sludge) ratio were 300% and 90%, respectively.

2.3. Wastewater and sludge

Raw sewage from a residential septic tank was pumped and stored in a 2 m^3 water reservoir as experimental wastewater. The pollutant concentrations in the influent during the experiment were as follows: chemical oxygen demand (COD): 350±50 mg/L; ammonia nitrogen (NH_4^+-N): 78.5±8.2 mg/L; total Kjeldahl nitrogen (TKN): 103.2±7.6 mg/L; total nitrogen (TN): 109.6±6.83 mg/L; total phosphorus (TP): 5.2±2.1 mg/L; nitrite nitrogen (NO_2^--N): 0.5±0.2 mg/L; nitrate nitrogen (NO_3^--N): 1.3±0.5 mg/L; pH: 7.35±0.15; and alkalinity ($CaCO_3$): 500±25 mg/L.

Inoculated filamentous bulking sludge was obtained from another pilot A^2/O reactor in the same laboratory suffering from serious filamentous bulking because of the prolonged incorrect operation of a low sludge load and inhomogeneous aeration; the 30 min activated sludge volume (SV_{30}) and SVI were 95% and 450 mL/g, respectively. The reactor in A^2/O process mode before inoculation exhibited regular COD, TN and TP removal efficiencies using the same sewage, with values of approximately 90%, 90% and 85%, respectively. The SVI of the sludge was 130 mL/g.

2.4. Analytical methods

COD, NH_4^+-N and TP were detected using a 5B-6C multi-parameter water quality analyser (LianHua Environmental Instrument Institute, Langzhou, PR China). TKN was measured with an L6250-type automatic azotometer (Behr, Germany). The NO_2^--N, NO_3^--N, mixed liquor suspended solid (MLSS), SV_{30}, and SVI values were all determined according to standard Chinese NEPA methods (Chinese NEPA, 2002). TN was calculated as the sum of TKN, NO_2^--N and NO_3^--N. The pH, DO, and temperature were determined using a pH/Oxi 340i metre with pH and DO probes (WTW, Germany). The ratio of denitrifying phosphate-accumulating bacteria to phosphate-accumulating bacteria (DPAO/PAO) in the sludge was determined based on the method recommended by Wachtmeister et al. [20].

2.5. Examination and identification of filamentous microorganisms

The water-sludge mixed liquid was collected from oxic zone 2, and smears were made for observation using an Olympus BX51/52 (Japan) every two days. The dominant filamentous bacteria were identified by morphology observation, Gram and Neisser staining reactions and sulphur deposit tests according to the methods presented by Eikelboom [21] and Jenkins et al. [22]. The filament index (FI), a subjective assessment of the size of filamentous populations, was used to represent the abundance of filamentous bacteria on a scale ranging from FI = 0 (no filaments) to FI = 5 (very many filaments) [22]. The surface structure of the washed-out and LFB sludge was treated for scanning electron microscopy (SEM) (S-3400N, HITACHI Company, Japan) observation using the method described by Luongo and Zhang [23].

2.6. High-throughput pyrosequencing of LFB sludge

An LFB sludge sample from run 6 was centrifuged at 10,000 rpm for 15 min to concentrate it and then stored at –20°C before DNA extraction. A soil biological genomic extraction kit (Sangon Biotech Co., Ltd., Shanghai, China) was used for genomic DNA extraction according to the manufacturer's protocol. The product was purified using a DNA purification kit (Sangon). Then, the purified DNA was submitted to the commercial service provider Sangon Biotech Co. for high-throughput sequencing on an Illumina MiSeq platform (San Diego, CA, USA). Finally, non-target sequences and chimaeras were removed, and the RDP Classifier tool was used to assign the available sequences to different operational taxonomic units by matching the data to data in the Ribosomal Database Project at 97% identity.

3. Results and discussion

3.1. Settling ability variation of activated sludge

In run 1 of phase I (Table 1), 3 L of concentrated filamentous bulking sludge (MLSS = 5750 mg/L) from another A/O reactor in the filamentous bulking condition was added to the sewage reservoir and blended with influent to feed the experimental reactor for continual 3-d inoculation. During run 1, the reactor was operated in A^2/O mode by opening and closing certain valves (see 2.2) to cause severe filamentous bulking. Several important sludge properties, including the MLSS, SV_{30} and SVI, were determined over the entire experiment and are shown in Fig. 2. Both the SVI and SV_{30} of the sludge exhibited dramatic growth, from 102 mL/g to 410 mL/g and from 30% to 98%, respectively, by the end of this phase due to the intentional cultivation of filamentous bacteria in the A^2/O mode. The MLSS decreased from 2,800 mg/L to 2,250 mg/L; a considerable amount of biomass was lost, representing the magnitude of the filamentous bulking outbreak in this reactor.

In run 2 of phase II, due to NRV 3 (16) and SRV 4 (20) (Fig. 1) being the only closed values and the inflow rate being maintained at 15 L/h (Table 1), the aeration in the gravity selector changed from continuous to intermittent. This change caused the volume of the reaction zones to decrease to 120 L ($V_{anaerobic}:V_{anoxic}:V_{oxic} = 1:2:3$) in the

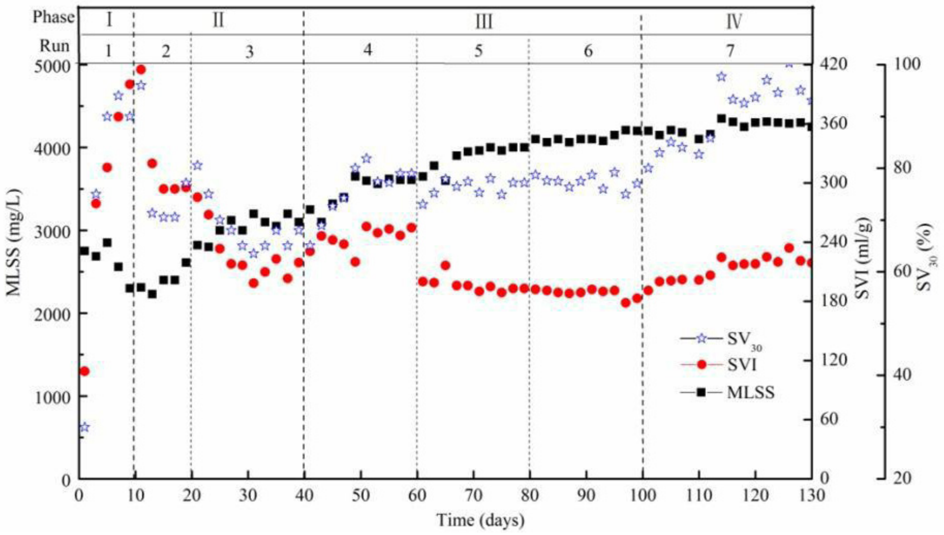

Fig. 2. Variation in sludge settling during the experiment.

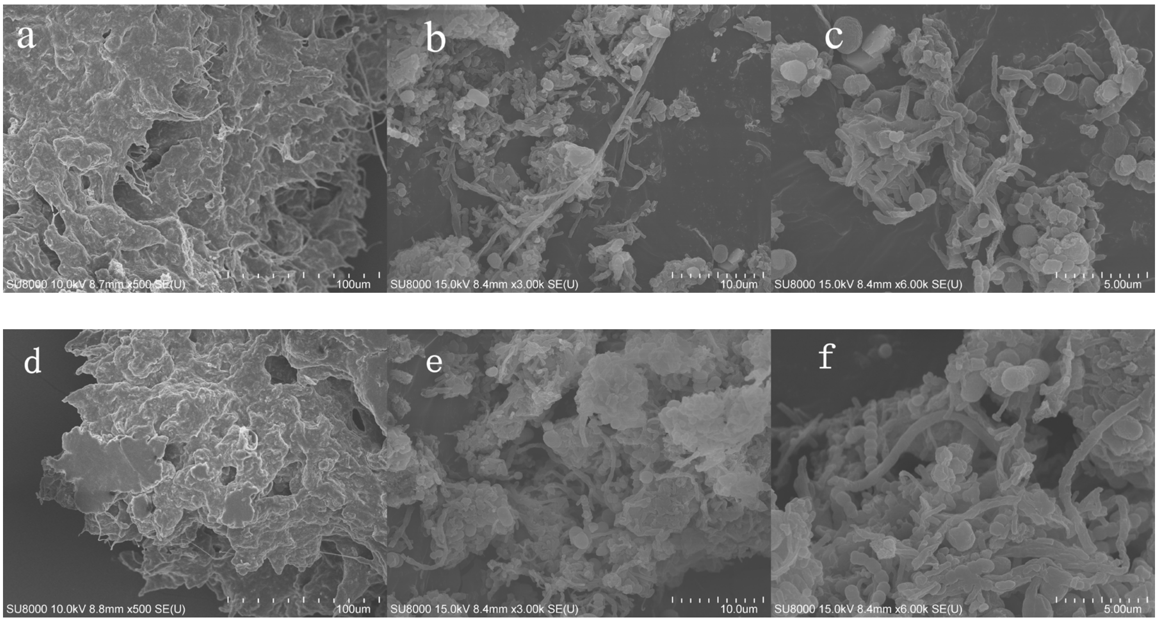

Fig. 3. SEM image of the sludge of the selector: sludge washed out from the upper outlet, 500× (a); 3000× (b) and 6000× (c); sludge returned from the bottom, 500× (d); 3000× (e) and 6000× (f).

A^2/O-gravity selector mode during run 2. Furthermore, the MLSS in this run was thin due to biomass loss in phase I. Therefore, the sludge organic loading rate (OLR) increased significantly from 0.28 to 0.65 kg COD/(kg MLSS·d). With the positive effects of the pollutant concentration gradient [24] and gravity selection on bulking control, the MLSS recovered to 2,700 mg/L by the end of run 2. The SVI and SV_{30} also decreased, with final values of 290 mL/g and 70%, respectively (Fig. 2). The sludge settling ability improved to some extent.

In phase II, the gravity selector was activated, and the sludge was blended and selected during the sedimentation periods of the IAC. The severe bulking sludge of light weight and slow sedimentation velocity settled on the surface of the sludge layer and was preferentially discharged from the upper outlet during each IAC. A considerable number of scattered sludge flocs were found floating on the water surface to be washed out. Figs. 3a, 3b and 3c illustrate that these floating flocs had more filaments extending out of them than did the settled flocs and a loose structure. Less-bulking sludge settled rapidly to the bottom with high sedimentation velocity relative to that of the severe bulking sludge and was returned back to the system from the bottom of the selector in run 3 once SRV4 (20) (Fig. 1) was closed. Figs. 3d, 3e and 3f show the appearance and interior of the bottom-settled sludge, which had a smoother surface than the severe bulking sludge, and filaments curved and grew inside of this space. During these processes, the sludge settling ability consistently improved.

The IAC of the selector was set at 2/58 (aeration min/sedimentation min) in phase II; consequently, the severe bulking sludge was eliminated through the upper outlet to the clarifier during and after 2 min of each aeration period. Therefore, the sludge retention time (SRT) could be controlled by discharging the settled sludge in the gravity selector (outlet 2) and/or the clarifier (outlet 1). In run 2, SRT was maintained at a low duration of 10 d through discharging excess sludge from both sludge outlets 1 and 2.

To accumulate more biomass and increase the treatment capacity of the system, the SRT was extended to 16 d in run 3 by discharging sludge only from outlet 1. In this run, NRV3, SRV 4 and outlet 2 were closed (see 2.2). The SRT and biomass of the system increased considerably, as shown in Fig. 2.

The MLSS increased to 3,200 mg/L by the middle of run 3 and then stabilised until the end. This phenomenon indicated that periodically intermittent aeration did not influence the stabilisation of MLSS in the main reaction zone, which might be due to the short aeration time and rapid recovery of the MLSS of the concentrated sludge settling to the bottom of the selector. A balance between the input of nutrients into the system and the sludge discharge was established gradually. SV_{30} and SVI showed significant drops to 65% and 210 mL/g, suggesting that the settling ability of the sludge improved continuously; this improvement is credited to the constant washout of filamentous sludge in the gravity selector.

When the experiment entered run 4 of phase III, which was similar to run 3 in that NRV3 and SRV4 were closed and excess sludge was discharged only from outlet 1, the aeration period of the IAC was decreased to 1 min, less excess sludge was discharged, the SRT was prolonged to 22 d, and the MLSS increased to 3,700 mg/L. The SV_{30} and SVI rose to 82% and 260 mL/g, respectively, and the settling ability of the sludge improved slightly due to the shortened intermittent aeration periods for filamentous sludge discharge and washout. Due to the suppressive effects of certain filamentous microorganisms on DO concentrations [25], the DO concentrations in the oxic zones and gravity selector (during aeration period) decreased from run 4 to run 5 of phase III from 2.0 and 2.0 mg/L, respectively, to 2.0 and 0.5 mg/L, respectively, and then decreased in run 6 to 1.0 and 0.5 mg/L, respectively (Table 1). The MLSS increased to 4,200 mg/L. The SV_{30} and SVI decreased from run 4 to run 5 and then stabilised in run 6 at mean values of 80% and 195 mL/g, respectively. The index of settling ability was in the LFB range of approximately 200 mL/g [12].

However, excessive bulking appeared again when the experiment entered run 7 of phase IV, during which the aeration period of the IAC was further reduced to 0.5 min (Table 1). The MLSS, SV_{30} and SVI all increased, and the values of SV_{30} and SVI increased to over 95% and 230 mL/g, respectively, by the end of this phase. These changes occurred due to the continuously reduced intensity of washout for filamentous sludge in the selector; the low OLR also stimulated filamentous bacteria. Thus, an excessively long SRT of over 28 d and a short intermittent aeration period of less than 1 min each hour were disadvantageous to filamentous bulking recovery and LFB achievement in this novel system.

3.2. Characteristics of pollutant removal

Fig. 4 presents the influent and effluent concentration variations of COD, TP and various N pollutants (NH_4^+-N, NO_2^--N, NO_3^--N and TN) in the experimental reactor during different phases. The removal of COD, TP and TN decreased considerably during phase I of filamentous bulking induction; the average removal efficiencies were only 74%, 35% and 80% with effluent COD, TP, and NH_4^+-N concentrations of 60, 2.5 and 20 mg/L, respectively.

With the start of the gravity selector and increased biomass in phase II, the COD and TN removal efficiencies of the A^2/O reactor began to recover in run 2, with their values increasing to 80% and 70%, respectively. There was no noticeable improvement in TP removal; the removal efficiency was still 40% during run 2. This lack of improvement

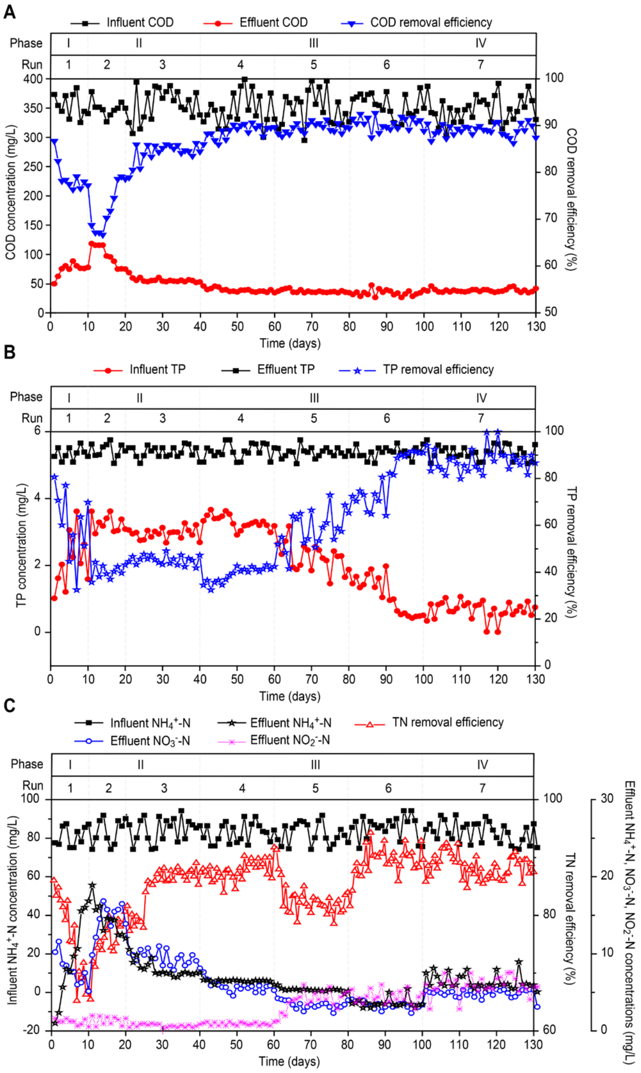

Fig. 4. Pollutant removal performance of COD, N and P.

might have occurred because phosphate-accumulating organisms (PAOs) comprise numerous complex communities, making them more difficult to recover than other functional microorganisms [26].

A continuous increase in the pollutant removal efficiencies was observed in run 3 for the MLSS accumulation and bulking control in the reactor; the COD, TP and TN removal efficiencies increased to 85%, 45% and 86%, respectively. However, the TP and NH_4^+-N concentrations in the effluent were still not sufficiently low to meet the discharge standard of pollutants for municipal WWTPs in China (TP ≤ 0.5 mg/L, NH_4^+-N ≤ 5 mg/L), although the sludge indexes of settling ability were within the range of LFB.

In run 4 of phase III, the slight decrease in sludge settling ability did not impact the treatment of COD, NH_4^+-N and TN; the effluent concentrations of COD, NH_4^+-N and TN were reduced to below 50, 8 and 15 mg/L, respectively. This lack of change in treatment efficiency is due to the constant maintenance of biomass in this system by reducing the sludge discharge to prolong the SRT. The selector with intermittent aeration could also be regarded as a supplemental reactor, which could purify the effluent from A^2/O.

The gradual decrease in DO concentration during runs 5 and 6 did not influence the removal of COD and TN; the removal efficiencies reached 90% and 89%, respectively, by the end of phase III. The effluent NH_4^+-N concentration decreased from 7.2 mg/L to 2.5 mg/L, which indicated the ideal nitrification effect of the system. However, the dominant component of effluent TN was NO_2^--N instead of NO_3^--N, which dominated in previous phases. The nitrosation rate (NO_2^--N/NO_3^--N) increased to approximately 60% because nitrifying bacteria are composed of two groups: ammonia-oxidising bacteria (AOB) and nitrite-oxidising bacteria (NOB). The latter could promote the transformation of oxide nitrite, the product of NH_4^+-N oxidised by AOB, into nitrate under sufficiently high aeration conditions. Under a low DO concentration, the stepwise nitrifying reaction stopped at AOB oxidisation with nitrite accumulation [27]. The involvement of short-cut denitrification in the system would benefit synchronous N and P removal with lower carbon sources and energy. Interestingly, the TP removal efficiency decreased considerably, from 42% to 30%, at the beginning of this phase and then grew to 88% by the end of run 6. Conventional PAOs favour short SRTs [28]; thus, the TP removal efficiency decreased initially. The subsequent recovery of the TP removal rate can be attributed to the proliferation of denitrifying phosphorus-accumulating organisms (DPAOs) in the system. DPAOs favour adequate NO_3^--N electron acceptors [29], long SRTs [30], and low OLR [31] and DO [32]; these conditions are in accordance with the operational conditions of the latter part of phase III. The DPAO/PAO ratio of the sludge in run 6 was calculated as the maximum anoxic P uptake rate to oxic P uptake rate. The resulting value was approximately 90%, which indicated that the TP removal relied mainly on DPAOs with a long SRT of 22 d. Thus, the A^2/O process was able to transform severe filamentous bulking into a stable LFB condition with an adequate SVI and sufficiently low effluent pollutant concentrations.

The deterioration of the sludge settling ability in run 7 led to turbid effluent and slightly decreased COD and TP removal efficiencies. The excessively long SRT of 28 d caused the sludge to age and disintegrate, and the DPAOs became inactivated because of competition from glycogen-accumulating organisms (GAOs) under the long SRT [33]. The nitrosation rate also decreased to approximately 50%, and the TN removal rate decreased slightly. This trend occurred because NOB with a long generation time had recovered at the SRT and outcompeted AOB, which led to the increase in the effluent NO_3^--N concentration. Increased NO_3^--N production would require a larger carbon source for N removal by denitrification; hence, the TN removal decreased under the same COD conditions in the influent.

There was no discernible sludge loss during the experiment even though the sludge was bulking, and the interface of sludge-water separation in the selector was consistently clear. These conditions occurred due to the periodic agitation by aeration for sludge reshuffling and the elimination of microbubbles attached to the filaments to prevent sludge caking and floating. The retention of adequate biomass in the reactor ensured the pollutant treatment effect.

3.3. Development of filamentous populations

Four main types of filamentous bacteria were dominant in the 4 different experimental phases. The population sizes of these bacteria are provided in Table 2, which indicates a clear development characteristic.

Thiothrix grew excessively in phase I (Fig. 5a), followed by *Beggiatoa* (Fig. 5b). No nocardioforms or *Haliscomenobacter hydrossis* were observed in the start phase. *Thiothrix* and *Beggiatoa* are both sulphur bacteria that favour low DO concentrations and abundant H_2S [21,34]. In this reactor, these two types of filamentous bacteria were inoculated from the bulking A/O reactor, which was operated with inadequate aeration and putrefactive sewage. Long extended filaments (100–500 µm) were the main component of these filamentous bacteria bodies [21], which easily attached to the gas bubbles from denitrification and led to sludge flotation. Sludge flotation was the main reason why the MLSS was lost and treatment decreased in this phase.

With the introduction of the gravity selector, *Thiothrix* and *Beggiatoa* began to disappear during phase II. *Beggiatoa* disappeared more rapidly than *Thiothrix* (Table 2). The final absence of these two types of filamentous bacteria is relevant to their common morphology of long filaments out of the flocs. Although the gas bubbles on the filaments were periodically excluded from the gravity selector, outstretched long filaments continued to slow the settling velocity of flocs and had to be eliminated. As shown in Table 2, *Thiothrix* was absent starting in phase IV, whereas *Beggiatoa* could not be observed by the end of phase II. This difference between the two types

Table 2
Population sizes (FI) of the dominant filamentous bacteria during the experiment

Bacteria	Day 10	Day 40	Day 100	Day 130
Thiothrix I	4	3	1	0
Nocardioforms	0	0	1	2
Beggiatoa spp.	3	0	0	0
Haliscomenobacter hydrossis	0	1	3	4

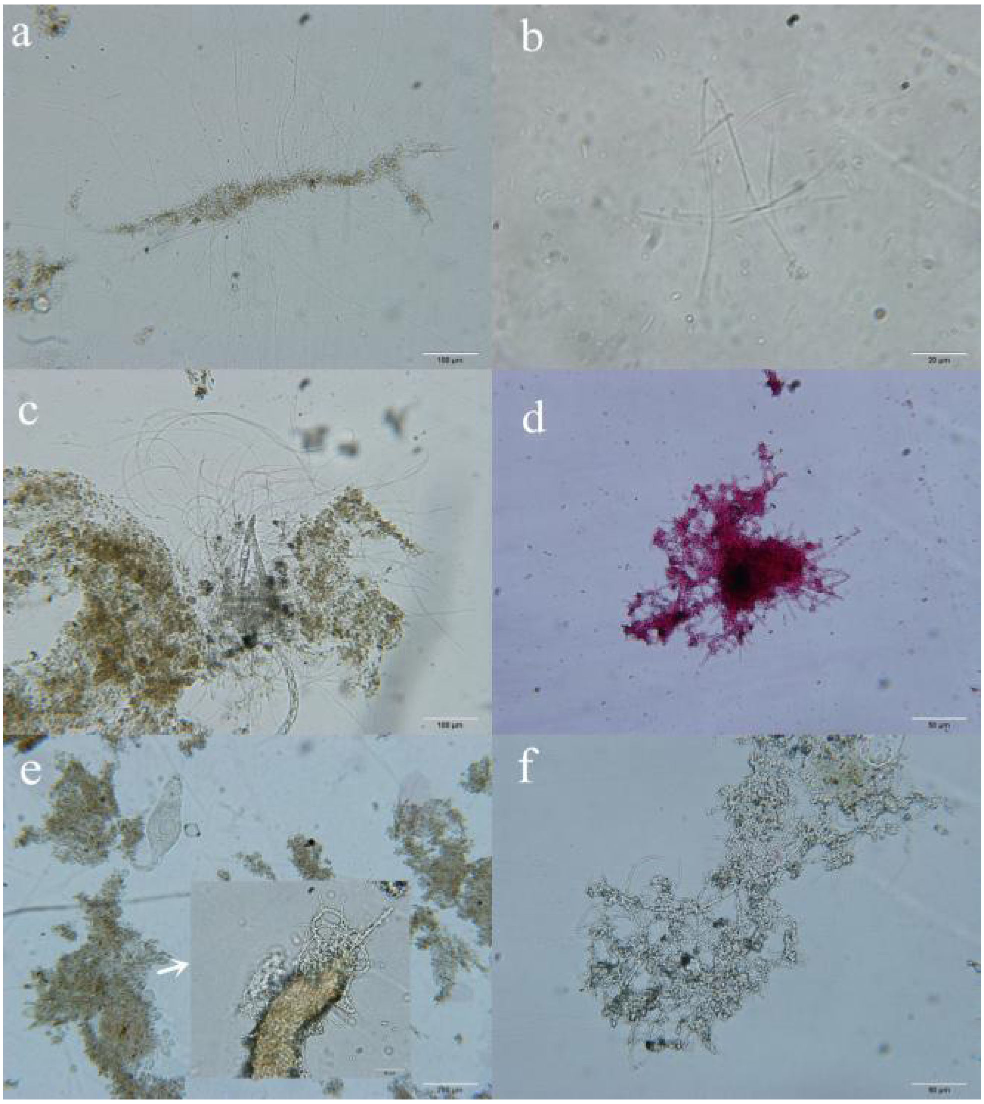

Fig. 5. Evolution of the morphology of filamentous flocs during filamentous bulking control: a) Thiothrix in phase I, b) Beggiatoa in phase I, c) sludge flocs on day 10, d) sludge flocs on day 40, e) sludge flocs on day 100, and f) sludge flocs on day 140.

of bacteria might be because *Beggiatoa* always glided freely in water (Fig. 5b), whereas *Thiothrix* was smoothly curved and frequently grew on the surface of thick and solid substances as a bouquet, which improved its settling velocity and prevented it from being washed out quickly.

In contrast to the case discussed above, the abundance of nocardioforms and *Haliscomenobacter hydrossis* increased gradually from phase II. *Haliscomenobacter hydrossis* was dominant in the LFB state (Figs. 5e and f and Fig. 6), which corresponds well to previous studies [13]. Its coiled ingrowth is in accordance with a previous report on the morphology of LFB bacteria [16]. These two types of filamentous bacteria favour long SRTs, low DO and low OLR [21], which were the operational conditions of runs 6 and 7. Furthermore, the filaments of these bacteria did not stretch out considerably. The nocardioforms were curved and grew within the flocs [21]. *Haliscomenobacter hydrossis* was thin (filament diameter = 0.5 µm) and short (10–100 µm), making it difficult to observe the extended filaments [21,22]. Therefore, the structures improved the tightness and settling ability of the sludge to protect it from being readily washed out. *Haliscomenobacter hydrossis* dominance has been reported to be correlated with enhanced nutrient removal [35]. The loose and swollen sludge flocs benefited from simultaneous nitrification and denitrification; therefore, the LFB state (run 6) exhibited high pollutant removal rates. The low level of nocardioforms in the LFB state might have been observed because these bacteria cause foaming [36]; once these bacteria grew to a certain extent, they would have been eliminated from the selector.

Overall, the balance between the washout for overproliferation and the growth of filamentous bacteria in the system guaranteed the stability of MLSS and SVI in the stable LFB phase. Through analysing the characteristics of the main genera (Table 3), it could be found that the LFB sludge contained abundant various bacteria responsible for nutrient removal except filamentous

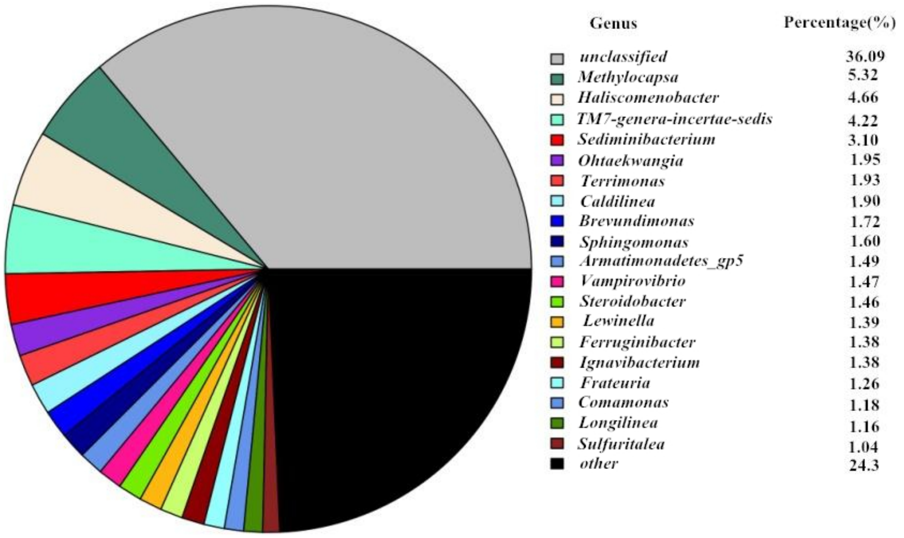

Fig. 6. Genus-level bacterial population distribution in the LFB sludge.

Table 3
The characteristics of important genera in LFB state

Genus of bacteria	Characteristics
Methylocapsa	Methane-oxidizing bacteria, they typically coupled with N and COD removal. [37]
Haliscomenobacter	Filamentous bacteria, they belong to *Bacteroidetes*, abundant in enhanced P removal reactors. [38]
TM7_genera_incertae_sedis	The genus contains some types of filamentous bacteria, familiar invarious activated-sludge treatment systems. [39]
Sediminibacterium, Ohtaekwangia, Terrimonas and Lewinella	These genera belonging to *Bacteroidete*s which is a phylum plays a key role in hydrolysis and acidification. [40]
Brevundimonas	The genus is potential PAOs within *Alphaproteobacteria*. [41]

bacteria, which ensure the favourable treatment performance of the system.

4. Conclusion

Severe filamentous bulking in an A^2/O reactor could be treated and recovered to a stable LFB state by installing a gravity selector after the oxic zones. The settling ability of sludge in the system was significantly improved. The pollutant treatments were also enhanced under the LFB state with long SRT and low aeration in the oxic zones; this nutrient removal involved with AOB and DPAOs. The principle of the gravity selector was persistent washout of overgrown filamentous bacteria with extended long filaments that settled slowly on the surface, while the filamentous bacteria with filaments curved inside were retained for LFB induction. *Haliscomenobacter hydrossis* dominated the LFB state.

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Supplementary

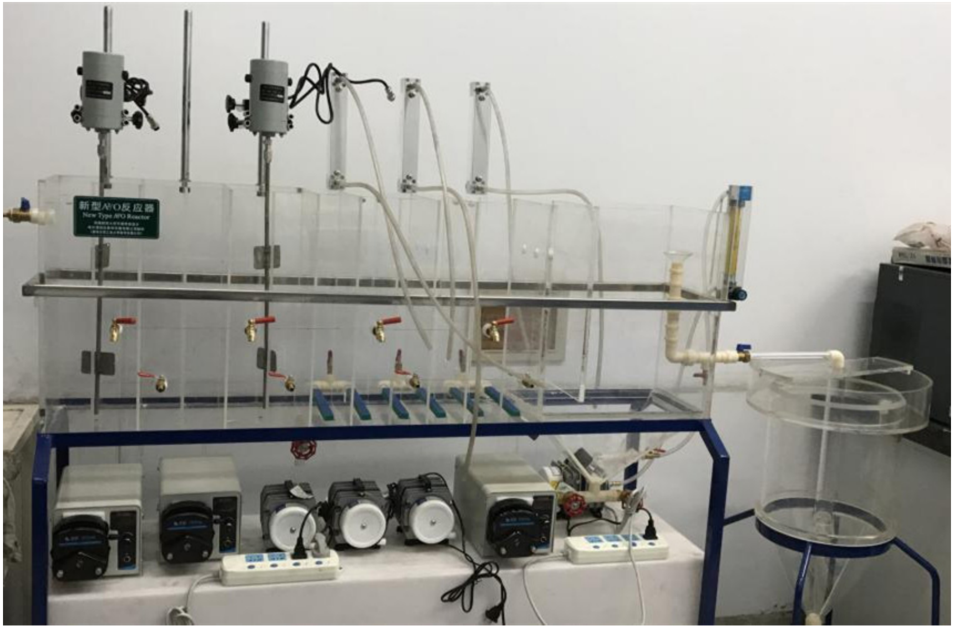

Fig. S1. The configuration of real experimental reactor.