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Study on culture of stable aerobic granular sludge

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Abstract: In this study, three reactors were established with anaerobic plug-flow times of 1.5 hours, 1 hour, and 0.5 hours. The results indicated that the anaerobic plug-flow time of 1 hour constructed favorable "feast-famine" period ratio, promoting the enrichment of microorganisms that stored and utilized the internal carbon source, such as Phosphate and Glycogen Accumulating Organisms (PAO and GAO). This resulted in the formation of granular sludge with both short granulation time and structural stability. In contrast, shortening the anaerobic feed time to 0.5 hours was detrimental to the growth of GAO, leading to slow granularization of aerobic sludge. The anaerobic influent time of 1.5 h leads to the shortening of starvation period and poor stability of particle structure.

1. Introduction

According to the Statistical Yearbook of Urban Construction and the Bulletin of Ecological Environment in China, the total amount of sewage discharged in China is increasing year by year. China is still facing severe water pollution problems. Sewage treatment plant is the main place to remove sewage pollutants, but the traditional activated sludge process has high energy consumption and large area, so it is difficult to meet the requirements of sewage treatment in the 14 th Five-Year Plan. Therefore, it is urgent to develop an efficient and low-consumption water treatment process.

1.1. Aerobic granular sludge

Aerobic granular sludge is a microbial aggregate formed through self-coagulation, and since Mishima and Nakamura first obtained aerobic granular sludge in 1991 in a continuous-flow reactor, the process has garnered significant attention. Aerobic granular sludge has the advantages of excellent sedimentation performance, high biological retention, and functional flora coupling[1]. It is an efficient and low-consumption water treatment process, which has great application potential in high-concentration organic wastewater treatment, nitrogen and phosphorus removal, and degradation of persistent and toxic organic pollutants[2]. However, the long granulation cycle and the instability of granular sludge under long-term operation are technical challenges that impede the widespread application of aerobic granular sludge. Existing research indicates that the stability of aerobic granular sludge is primarily influenced by factors such as carbon source type, organic load, hydraulic shear force, and water inflow mode. Among them, aerobic granular sludge cultivated by the anaerobic plug-flow water inflow method has a short start-up time and significantly enhanced stability, making it a hot research topic at present.

1.2. Bottom plug-flow mode

The bottom plug-flow mode is a new aerobic granular sludge cultivation method in which influent water enters the reactor in a parallel flow and does not completely mix with the liquid phase in the reactor[3]. Compared with the rapid water influent and rapid water influent aeration mode, the high local substrate concentration formed in this water influent mode is conducive to the utilization of organic substrate by microorganisms[4-5], promoting the enrichment of PAO and GAO and the formation of aerobic granular sludge with a dense structure and stable properties. This model offers a new approach to address the technical challenges of long start-up time and unstable granules in aerobic granular sludge. However, the optimal water inflow time condition remains unclear. To tackle these issues, this study aims to establish three groups of aerobic granular sludge sequencing batch reactors (SBR) with varying anaerobic plug flow times. The study will investigate the impact of anaerobic plug flow time on the formation and performance of granular sludge, optimize the anaerobic plug flow time of SBR, and provide a theoretical basis and technical support for the efficient and stable treatment of wastewater using aerobic granular sludge.

2. Materials and methods

The materials and methods used in the test are as follows.

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2.1. Reactor setup and operation

The aerobic granular sludge was cultured in three identical reactors, and the same nutrient solution was used as influent, and it was placed in the same environment to ensure that the experiment could minimize the influence of other factors. The aerobic granular sludge used in this study was cultured and operated in an SBR (Sequencing Batch Reactor) reactor (Fig.1.), constructed of acrylic with an inner diameter of 8 cm, a height of 100 cm, and an effective volume of 4 L. The reactor column was equipped with drain valves at 20 cm intervals for sampling.

Samples were sourced from the aeration tank of Sanya Creative New City Wastewater Treatment Co. Ltd. and inoculated with MLSS (Mixed Liquor Suspended Solids) of 4000±100 mg/L. The specific reactor operation cycle parameters are detailed in Table 1. The aeration head is connected with a gas flowmeter to ensure that the gas velocity on the inner surfaces of the three reactors is 1.2 cm/s. During the experiment, the reactor was always placed in a constant temperature room to keep the operating temperature of the reactor at 26±2℃. The influent pH is maintained at about 7.8. The influent water employed synthetic wastewater, with sodium acetate as the main carbon source, ammonium chloride as the nitrogen source, and potassium dihydrogen phosphate and dipotassium hydrogen phosphate as the phosphorus source. The influent water COD concentration was 700±50 mg/L, with the C/N ratio maintained at 20:1. Additionally, trace elements such as Zn^{2+}, Mn^{2+}, and $Mo_7O_{24}^{2-}$ were added to support normal microbial growth.

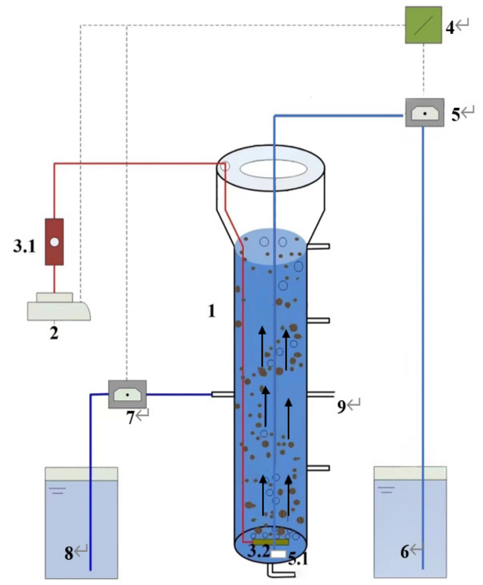

Fig. 1. Device diagram of aerobic granular sludge reactor
1-Reactor 2-Aeration pump 3.1-Flowmeter 3.2-Aetion head
4-Auto controller 5-Influent pump 5.1-Water distributor
6-Intfluent bucket 7-Solenoid valve 8-Water effluent bucket
9-Outfall

Table 1. The operation mode of aerobic granular sludge reactor.

Reac-tor	Cycle Time (h)	Influent time (min)	Aeration Time (min)	Settling time (min)	Effluent Time (min)
R1	4	90	140	5	5
R2	4	60	170	5	5
R3	4	30	200	5	5

2.2. Analytical methods

The changes of morphological characteristics during the formation of aerobic granular sludge were observed by stereomicroscope once a week.

Weigh the weighing bottle dried at 103℃ and weighed constantly, and record its mass as m_0(mg). Dry the filter paper and weigh it as m_1(mg). Take 10ml of evenly mixed water sample, filter it with previously dried filter paper, put it into weighing bottle, dry it at 103℃ for 2 h, then cool it, and weigh it as m_2(mg).

$$MLSS\ (mg/L) = (m_2-m_1-m_0)\ *100 \qquad (1)$$

The influent and effluent quality of the reactor are measured twice a week. The influent water sample is collected from the uniformly simulated artificial wastewater in the inlet bucket, while the effluent water sample is taken from the wastewater discharged at any stage of the day. The wastewater is only eligible for analysis after being filtered by a 0.45 μm microporous membrane. The testing method is detailed in Table 2.

Granular sludge DNA extraction was performed using the TIANamp Soil DNA kit following the manufacturer's instructions. The extracted DNA samples were sequenced and analyzed using the BioCorp platform.

Table 2. Conventional water quality analysis method.

Number	Index	Method
1	COD	COD rapid digestion method
2	NH_4^+-N NO_2^--N NO_3^--N	Continuous automatic nutrient analyzer
3	TN	Ultraviolet spectrophotometry

3. Results and disscussion

3.1. Effect of anaerobic feed time on aerobic granular sludge morphology

The morphology of aerobic granular sludge was studied under different anaerobic feeding times using a stereomicroscope(Fig.2.). The R1 reactor exhibited the fastest granulation speed. After 25 days of culture, the majority of the floc sludge appeared in the form of aggregates. Subsequently, the granular sludge in R1 matured gradually, achieving good granulation after 30 days. However, an increase in the proportion of flocculent sludge was observed, presumably due to the disintegration of some particles. In the R2 reactor, after 35 days of operation, granular sludge was initially formed and the properties of the particles formed were stable, with no disintegration observed during system operation. This suggests that an anaerobic influent time of 1 hour can achieve the best ratio of "satiety-starvation period," which is beneficial for the rapid formation of stable granular sludge. In the R3 reactor, particles appeared after 44 days of culture, presumably because the short influent time in the anoxic stage was not conducive to full contact between the influent substrate and sludge. Numerous research results indicate that the anaerobic plug flow water intake mode is helpful for larger particles to better absorb the matrix and shorten the sludge granulation time in the system. However, a decrease in the influent time of the plug flow makes it difficult for the particles to fully absorb the biodegradable organic matter (rbCOD) in water, resulting in the longest sludge granulation time and relatively poor sedimentation performance under the anaerobic influent condition of 0.5 hours.

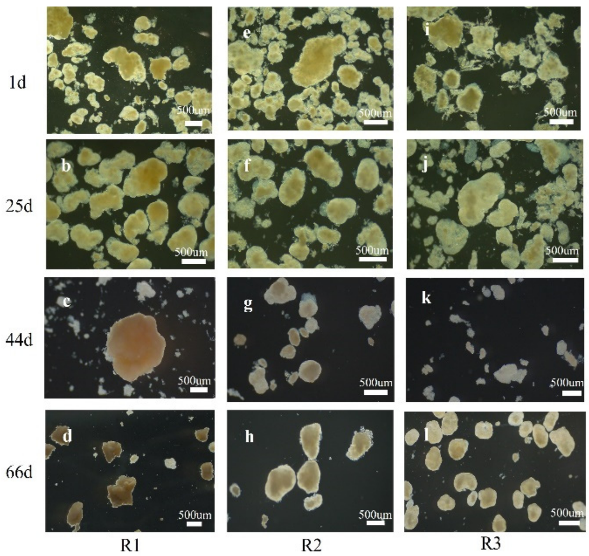

Fig. 2. Stereomicroscope of aerobic granular sludge under different anaerobic influent time.(a- d. R1; e-h. R2; i-l. R3)

3.2. Effect of anaerobic feed time on aerobic granular sludge MLSS

During the formation stage of aerobic granular sludge, the changes in sludge concentration in the reactor are illustrated in Fig.3. The Mixed Liquor Suspended Solids (MLSS) in the R1 reactor exhibited an initial increase followed by a subsequent decrease, while the MLSS in the R2 reactor remained stable, and the MLSS in the R3 reactor gradually decreased.

Forty days before the operation of the reactor, the MLSS in the R1 reactor rapidly increased, reaching a peak of 11870 mg/L, significantly higher than that in the other two reactors. This suggests that an extended anaerobic influent time is advantageous for the absorption of substrates by microorganisms in the sludge. However, after 40 days of operation, the MLSS of R1 noticeably decreased, with the sludge concentration in the reactor reaching only 6170 mg/L by the end of the operation. At the same time, it was observed that the proportion of flocculent sludge in sludge samples increased, and it was speculated that some particles disintegrated in the system, and the sedimentation performance deteriorated after particle disintegration, which led to a large amount of sludge discharge and a decrease in sludge concentration in the reactor. The MLSS of R2 reactor was kept at about 7000 mg/L, which indicated that the anaerobic water inflow time of 1 h was beneficial to keep the total sludge stable. Conversely, the average concentration of sludge in the R3 reactor remained consistently low throughout the entire culture process. The MLSS of the sludge in the reactor slowly increased to 7150 mg/L 17 days before the operation, and gradually decreased with the operation of the reactor. By the end of the operation, the MLSS of the sludge was less than 4000 mg/L.

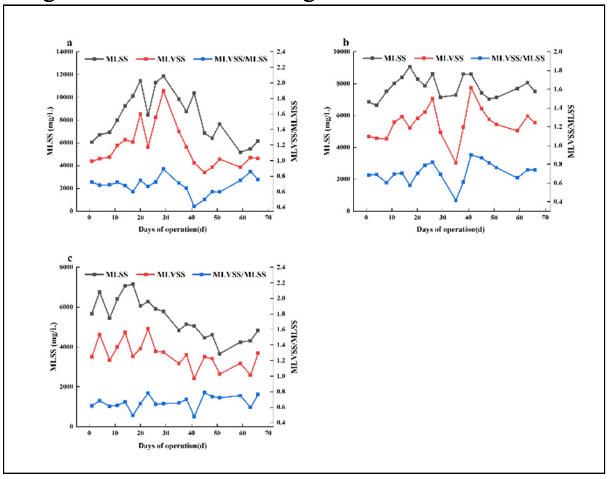

Fig. 3. Nitrogen removal performance of aerobic granular sludge under different anaerobic influent time.(a. R1; b. R2; c. R3)

3.3. Effect of anaerobic feed time on aerobic granular sludge COD and N removal efficiency

The nitrogen removal performance of aerobic granular sludge in the three groups of reactors is depicted in Fig.4. The average NH_4^+-N removal rates of the three reactors being 99.6% (R1), 99.54% (R2), and 98.79% (R3). Furthermore, the effluent NO_2^--N concentration was consistently maintained at ap-proximately 0.1 mg/L. There is no obvious accumulation of NO_2^--N, which indicates that the anaerobic influent time has little effect on the nitrification performance of aerobic granular sludge. The concentration of NO_3^--N in the effluent of R1 reactor first decreased and increased, while the concentration of NO_3^--N in the effluent of R2 and R3 reactors continued to decrease. After the sludge granules are completed, the denitrification performance is significantly improved[7]. It is speculated that Proteobacteria are gradually enriched during granulation[8]. This microorganism can secrete a large amount of EPS, which is also an important reason for the enhancement of particle stability[9].

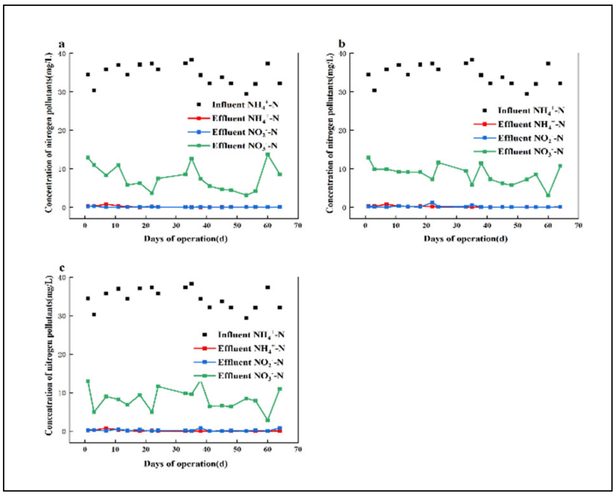

Fig. 4. Nitrogen removal efficient of aerobic granular sludge under different anaerobic influent time.(a. R1; b. R2; c. R3)

3.4. Effect of anaerobic feed time on aerobic granular sludge microbial community

As shown in Fig.5., the dominant bacteria in sludge are: *Candidatus Competitor*, *Candidatus Contendobacter*, *Candidatus Accumulibacter*, and *Defluviicoccus*. *Candidatus_Competibacter* and *Defluviicoccus* are common strains of GAO[10], while *Candidatus_Accumulibacter* is the primary type of PAO[11]. The relative abundance of *Defluviicoccus* in the three reactors gradually enriched with the culture, while the relative abundance of *Candidatus Accumulibacter* was always less than 1%. Towards the end of the culture period, the relative abundance of PAO and GAO bacteria, such as *Candidatus Competibacter*, was notably lower in the R3 reactor compared to the R1 and R2 reactors. This difference is attributed to the shorter influent time in the anoxic section of the R3 reactor. The relative abundance of GAO and PAO is positively correlated with the anaerobic influent time, indicating that the length of anaerobic influent time affects the enrichment of GAO and PAO. Throughout the entire culture process, the relative abundance of *Candidatus_Competibacter* in the R3 reactor is consistently the lowest, aligning with the observation that the granulation time in the R3 reactor is the longest.

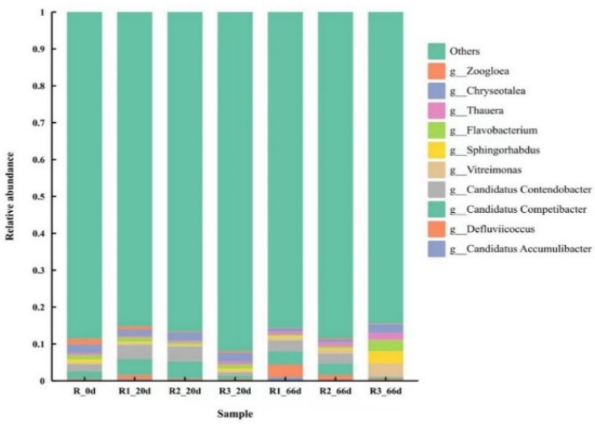

Fig. 5. Relative abundance of genus microbial community in the process of aerobic granular sludge formation.

4. Conclusions

(1) Under the condition of 1-hour anaerobic influent, particles emerged in the reactor 30 days after start-up, and no particle disintegration occurred during operation, demonstrating the characteristics of a short granulation time and stable structure of aerobic sludge.

(2) With an anaerobic influent time of 1 hour, aerobic granular sludge exhibited a strong anaerobic COD absorption capacity and enhanced denitrification capacity, contributing to efficient pollutant removal.

(3) The relative abundance of GAO and PAO is positively correlated with anaerobic influent time, and they are important microorganisms that affect sludge granulation and structural stability. In the reactor with anaerobic influent time of 0.5 h, the relative abundance of PAO and GAO bacteria is the lowest and the granulation time is the longest.

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