Study on rapid formation of aerobic granular sludge promoted by addition of Fe\(^{2+}\)

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Abstract. The aerobic granular sludge (AGS) process has been regarded as a promising sewage treatment technology. Compared with the activated sludge process, AGS process has the advantages of high efficiency and saving space. However, AGS process has several limitations in its application such as long cultivation cycle and easy disintegration. In this study, Fe\(^{2+}\) was added to the wastewater to cultivate AGS. The improvement on the cultivation of AGS with the addition of Fe\(^{2+}\) was investigated by analyzing the characteristics of AGS and the removal efficiency of pollutants. The results showed that adding Fe\(^{2+}\) (3~5 mg/L) to the reactor could effectively promote the formation of AGS. The sludge particles could be granulated within 7 days with the average particle size of more than 0.35 mm. In addition, the SEM showed that the dominant microorganisms were coryneform bacteria inside of the AGS. The XRD results indicates that the Fe\(^{2+}\) was converted into Fe\(_2\)O\(_3\) presenting in AGS. The mixed liquid suspended solids (MLSS) and the sludge volume index at 30 min (SVI\(_{30}\)) was 3.1 g/L and 40 mL/g, respectively, at the 40th day. The COD and NH\(_4^+\)-N were effectively removed by AGS. This study could provide an effective approach for rapid formation of AGS.

1. Introduction

Aerobic granular sludge (AGS) is a granular microbial formed by spontaneous aggregation of activated sludge microorganisms under aerobic conditions. AGS process has many advantages including high hydraulic loading, simultaneous nitrogen and phosphorus removal, and strong toxicity tolerance. Moreover, it could eliminate secondary sedimentation tanks to simplify the treating process and reduce the land occupying of the sewage treatment system due to its excellent settlement performance and compact structure. Therefore, it is more practically significant to study AGS\(^{[1-3]}\). The cultivation method, sludge characteristics, treatment efficiency and formation mechanism of AGS have been extensively studied over the last decades\(^{[4-5]}\). Several researchers have investigated its potential for treating refractory wastewater and wastewater with high concentrations of organic matters\(^{[6-7]}\). However, there are also many drawbacks of AGS such as difficult to cultivate and easy to disintegrate when the wastewater has low concentration of organic matters, such as municipal wastewater. In contrast, it is much easier to cultivate AGS with the wastewater with high organic loading. Long et al.\(^{[8]}\) have successfully cultivated AGS by the wastewater with COD concentration of 500~800 mg/L. Zhang et al.\(^{[9]}\) found that AGS could be achieved within 35 days under the condition of high organic loading (5.5 kg/m\(^3\)-d). Wang et al.\(^{[10]}\) reported that the AGS cultivated in the wastewater with 2000\(\pm\)200 mg/L COD
had effectively removed COD, NH$_4^+$-N and TP with the removal efficiency of 98.0%, 90.0% and 97.9%, respectively. Hamza et al.\textsuperscript{[11]} have achieved complete granulation of aerobic sludge after 4 weeks in SBR reactor with an influent COD concentration of 4500 mg/L. However, AGS process still faces the problems of difficult to cultivate and easy to disintegrate of the granular sludge, which has greatly inhibited its widely application.

Adding metal ion, especially the divalent metal ions, is a valid method to improve the stability of AGS. For instance, Ca$^{2+}$ affects the physical characteristics of the aerobic granules, while the addition of Mg$^{2+}$ increases microbial diversity in mature granules\textsuperscript{[12]}. Iron, a crucial element for nearly all organisms, participates in the most important metabolic reactions. Guo et al.\textsuperscript{[13]} reported that the iron can promote the growth and reproduction of microorganisms. Yu et al.\textsuperscript{[14]} found that Fe$^{2+}$ increases the granulation of anaerobic granular sludge in UASB reactors.

In this study, Fe$^{2+}$ was added to the low-strength organic wastewater to promote the cultivation of AGS. The characteristics of aerobic granular sludge and the pollutant removal capability of the AGS process were investigated. The rapid way for cultivation of aerobic granular sludge was obtained. The method is expected to provide theoretical support for the application of aerobic granular sludge for treating municipal wastewater.

2. Materials and methods

2.1 Simulated wastewater

The wastewater used in this study was synthetic wastewater. It was prepared according to the municipal wastewater quality of southern China. The main parameters were as follows: COD 250–300 mg/L, TP 4–5 mg/L, NH$_4^+$-N 35–45 mg/L, TN 40–50 mg/L, Fe$^{2+}$ 3–5 mg/L, and pH 7.0–8.5.

2.2 Seed sludge

The inoculated sludge was taken from the secondary sedimentation tank of a WWTP located in Shenzhen, China. The initial sludge concentration of mixed liquid suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were 3.2 g/L and 1.6 g/L, respectively, and the sludge volume index at 30 min (SVI$_{30}$) was 86.2 mL/g.

2.3 Reactor and operation

The study was conducted in SBR (Sequencing Batch Reactor) reactor (Fig.1.). The reactor is in circular column shape with working volume of 2.5 L. The effective height and inner diameter were 80 cm and 5 cm, respectively. The exchange ratio is 50%, and the reactor was operated sequentially in 120 min cycles, with 5 min of substrate filling, 103–112 min of aeration, 10–1 of settling and 2 min of discharging. The simulated wastewater in the intake tank was pumped to the reactor from the bottom. The airflow rate was 2.2 L/min supplied by an air pump and dispersed by a porous distributor, which created an up-flow air velocity of 1.87 cm/s.

Fig.1. The experimental device
2.4 Analytical methods
The morphology of the aerobic granule sludge was observed under a digital camera, and the microstructure was observed with scanning electron microscopy (SEM). Inorganic composition of the AGS was tested by X-ray diffraction (XRD). The COD, TP, NH$_4^+$-N, TN, MLSS, MLVSS and SVI$_{30}$ were measured according to the standard methods for the Examination of Water and Wastewater[15].

3. Results and discussion
3.1 Variation of the sludge morphology
The morphology of sludge particles in different periods was shown in Fig.2. The sludge particles were granulated on the 7th day, and 90% of the sludge particles have a particle size of more than 0.35 mm (Fig.3). Meanwhile, sludge particle size distributions are: 6.34% was at range of 0 to 0.35 mm, 52.64% was at range of 0.35 to 1 mm, 36.34% was at range of 1 to 2 mm, and 4.70% was at range of 2 to 3 mm. It can be seen that the sludge with a particle size greater than 0.34 mm accounts for more than 50%, which suggests that the sludge was successfully granulated[16]. With the operation of the reactor, the granular sludge had a more regular form, mostly spherical particles. The particle size of the sludge has showed increasing trend with operation time increasing according to the particle size results. It was found that the sludge with a particle size of 1~3 mm was 65.8% at 25th day, which was 51.9% on the 50th day. But the granular sludge with a particle size of 3~4 mm was 19.1% at the 50th day, which was only 4.3% on the 25th day. It suggests that the granular has grown with the operation proceeding. The SEM image of the sludge particles in the reactor on the 50th day was shown in Fig.1d. It could be seen that the inside of the AGS is mainly coryneform bacteria.

![Fig.2. Variations in the morphology of AGS(a. 7th day; b. 25th day; c. 45th day; d. SEM 50th day)](image)

3.2 Variation of the sludge characteristics
The concentration of MLSS and MLVSS can reflect the biomass in the reactor. The concentration of MLSS and MLVSS in the reactor was shown in Fig.4. In the first 20 days of the operation, the MLSS has greatly fluctuated, which was mainly caused by gradually reducing the settling time. Due to the poor settling performance of flocculent sludge, a large amount of sludge in the reactor was eluted, and the MLSS dropped rapidly from 2.0 g/L to 1.0 g/L in the reactor at the first 5 days. Similarly, the MLVSS also dropped from 1.0 g/L to 0.7 g/L. This is the basic selection phenomenon to finally retain granular sludge in the reactor as the sludge with poor settling properties will be eliminated, and the sludge with
good settling properties will naturally be maintained in the reactor. During the operation of the reactor, the settling time was continuously shortened, which was shortened to 1 min from the 20th day. The MLSS showed a decreasing trend. After 20 days, the sludge with excellent sedimentation performance was left to continue to grow and multiply. In addition, the MLSS began to show an increasing trend gradually, and stabilized at 3.1 g/L on the 40th day. The SVI30 reflects the settling performance of the sludge, the smaller is the SVI30 value, the better is the settling performance of the sludge. It could be seen that SVI30 showed a trend of increasing and then gradually decreasing with the operation proceeding. The SVI30 of the granular sludge rapidly increased from 60 mL/g to 110 mL/g within 5 days, then gradually decreased and remained stable at 40 mL/g.

EDS of the AGS was shown in Fig.5a. It can be seen that AGS contains Fe and accounts for 3.47%. XRD results of the AGS on the 50th day was given in Fig.5b. It could be seen that the granular sludge contains a large amount of NaCl and Fe$_2$O$_3$, which indicated that the addition of Fe$^{2+}$ finally exists with the form of Fe$_2$O$_3$ in granular sludge. The crystal nucleus hypothesis believes that Fe$_2$O$_3$ can be used as the nucleus of granular sludge and contribute to the formation of AGS$^{[17]}$. 
3.3 Pollutant removal performance

The concentrations of COD, TP, NH$_4^+$-N and TN in the influent and effluent were regularly sampled and analyzed as shown in Fig.6. The influent COD was 250~310 mg/L. In the early stage of the operation, the removal of COD by AGS process was ineffective, the effluent COD was 70~80 mg/L in the first 15 days. The effluent COD remained stable at 20~40 mg/L after 17 days, which complies to the GB18918-2002 1A discharge standard. The TP concentrations were between 4.5 and 6.0 mg/L in the influent, and was 1.5~3.0 mg/L in the effluent in the first 30 days. Thereafter, TP concentration in the effluent was stable and kept at 2.0~3.0 mg/L. The concentration of NH$_4^+$-N was 35~50 mg/L in the influent, and was 11.5 mg/L in the effluent on the 1$^{st}$ day. With the continuous operation of the reactor, the effluent NH$_4^+$-N concentration remains stable within 2 mg/L after 7 days and was less than 1 mg/L after 35 days. The TN of the influent was 40~50 mg/L. The TN concentration of the effluent fluctuates greatly, ranging from 20 to 35 mg/L during the first 13 days. After that, the effluent TN was stable at 20~25 mg/L. It can be seen that COD and NH$_4^+$-N could be effectively removed by AGS process with the concentration of 40 mg/L and less than 1 mg/L in the effluent, respectively. However, it was ineffectively of AGS to remove TP and TN with the effluent concentration of 2.0~3.0 mg/L and 20~25 mg/L, respectively.
Fig. 6. Variations of the COD, TP, NH\(_4^+\)-N and TN in the influent and effluent (a. COD and TP; b. NH\(_4^+\)-N and TN)

### 4. Conclusion

The addition of Fe\(^{2+}\) (3–5 mg/L) has effectively promoted the formation of AGS. The sludge particles can be granulated on day 7 (the control group without the addition of Fe\(^{2+}\) didn’t form AGS even on the 45th day), and 90% of the sludge particles have a particle size of more than 0.35 mm. Most of the particle size of the granular sludge was increased to 1–3 mm after 50 days, which was accounted for 51.9%. The pollutants in wastewater such as COD and NH\(_4^+\)-N could be removed effectively by AGS. AGS process has been mainly used to treat wastewater with high organic concentration in foreign countries, and its application for treating municipal wastewater is few. The fundamental reason for restricting its application in municipal wastewater treatment is difficult to cultivate and easy to disintegrate. In this study, AGS could be rapidly granulated and operated stably by addition of Fe\(^{2+}\), which could provide a way to cultivate AGS rapidly and proposed a theoretical basis for AGS to treat municipal wastewater.

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