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# Cultivation and Characteristics of Aerobic Granular Sludge in SBAR

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**Abstract.** By cultivating and observing the formation process of granular sludge and the characteristics of mature aerobic granular sludge, the formation mechanism of aerobic granular sludge and the structure of the granular sludge are studied. After 60 days, the removal rate of COD and NH3-N of aerobic granular sludge is much higher than that of traditional flocculation sludge, and both of them can be stabilized up to 90%. The removal rate of COD is about 90% and the removal rate of NH3-N at 45d is 95% in final reactor, which enhances the effect of wastewater purification greatly. Due to the compact structure of aerobic granular sludge, the density is much larger than that of traditional activated sludge, and its high activity and the short sedimentation time contribute to much higher sedimentation rate than that of flocculation sludge.

## 1. Introduction

The aerobic granular sludge is a kind of special biological membrane structure [1], which has the characteristics of biological density, high density, fast sedimentation [2-3]. It can maintain a high sludge concentration and reactor volume load so that secondary clarifier can be reduced or eliminated. TOH S K [4] thinks that it has high value, as well as the effective degradation of organics. The volume of suspended solids (SS) in the effluent is low and nitrogen and phosphorus removal effect is excellent. Yang Qi and Ruan Wenquan [5-6] say that it has high persistence. The technology of aerobic granular sludge is raised up in the mid-1990s and attracts increasing attention in the field of wastewater treatment. What is more, it is also a trend for the development of wastewater treatment plant in the future, so the further study of the cultivation process and the characteristics of granular sludge not only can improve the understanding of aerobic granular sludge, but also provides the basis for further study of aerobic granular sludge. However, the formation mechanism, the optimum cultivation conditions, microbiological characteristics and key process control measures of the aerobic granular sludge still need further study to achieve the best treatment effect [7].

This paper aims to cultivate aerobic granular sludge in laboratory environment. Under the condition of using SBAR as the reactor and simulating wastewater as water source, we try to transform ordinary floc sludge into aerobic granular sludge under specific conditions. It has a certain reference value for the further practical application of aerobic granular sludge.

## 2. Materials and Methods

### *2.1. Materials*

*2.1.1. SBAR.* The test device adopts Sequencing Batch Airlift Reactor with homemade organic glass tube. The diameter of the inner cylinder is 60mm, height is 900mm and the diameter of outer cylinder is 110mm, height is 1100mm. The total volume is 8L, the effective volume is 6L and the ratio of height to diameter (H/D) is 10. Aeration is arranged in the inner pipe at the bottom and the gas flow is controlled by the flowmeter. The sludge rises with the airflow in the inner tube, and then enters the outer tube to the top, then moves to the outer tube, and then moves to the bottom of the reactor to enter the inner tube again, so as to achieve the internal circulation. The operation period of the reactor is 6h, of which the influent period is 10 min, aeration period is 310 min, the settling time decreases progressively from 30 min and the time is compensated for aeration. The whole time is controlled by the time controller and the overflow device. The specific device is shown in Figure 1.

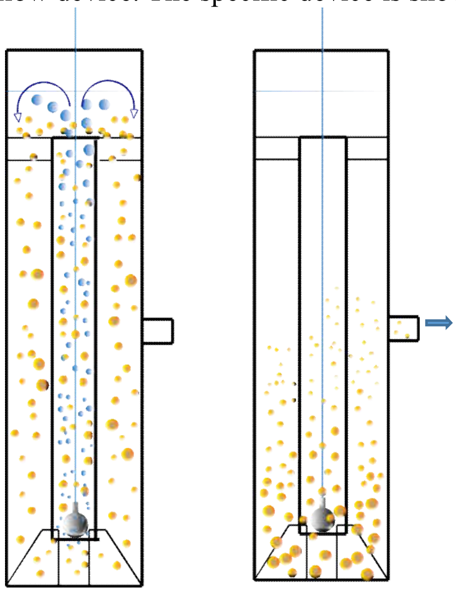

**Figure 1.** SBAR

*2.1.2. Test water and inoculating sludge.* Artificial simulated domestic sewage sucrose, beef extract peptone and potato starches were used as a carbon source and peptone and sucrose in water were added by COD 1:1. $NH_4Cl$ was used as a nitrogen source and $KH_2PO_4$ was used as a source of phosphorus for each 1L water to ensure COD: N: P=100:5:2. In addition, $CaCl_2$、$MgSO_4$、$FeSO_4 \cdot 7H_2O$、$NaHCO_3$ and $H_3BO_3$、$ZnCl_2$、$CuCl_2$、$MnSO_4 \cdot H_2O$、$(NH_4)\ 6Mo_7O_{24} \cdot 4H_2O$、$AlCl_3$、$CoCl_2 \cdot 6H_2O$、$NiCl_2$、$FeCl_3 \cdot 6H_2O$、KI and other mineral elements and trace elements were added. Also, proper amount of $NaHCO_3$ was added as a buffer. Adjust the pH of water distribution to 7 ~ 7.5.

The inoculated sludge was taken from reflux sludge in the secondary clarifier of the Songjiang wastewater treatment plant in Shanghai. The sludge is dark brown and floc. The mass concentration of the sludge is 3g/L after being put in the reactor, and SVI is 123.83mL/g.

### *2.2. Analysis Items and Methods*

*2.2.1. Routine Analysis Items.* COD, MLSS, MLVSS, $NH_4$-N and TN were all determined by standard method. The specific method is shown in Table 1.

**Table 1.** Standards for determination of water quality parameters

| Items | Methods |
|---|---|
| COD | Potassium dichromate method |
| MLSS、MLVSS | Gravimetric method |
| $NH_4$-N | Nessler's Reagent Spectrophotometry |
| TN | Alkaline potassium persulphate digestion-UV spectrophotometric method |

*2.2.2. Methods of observing the Morphology of Granular Sludge.* The OLYMPUS CX-31 microscope and the OLYMPUS E-620 digital camera were combined to take pictures. The microscopic surface observation was completed by the Quanta-250 scanning electron microscope. The granular sludge should be dehydrated and dried before the use of the electron microscope, and the specific method is: soaked at 2.5% glutaraldehyde for 30 min to fix, and then the fixed granular sludge is cleaned three times by phosphoric acid buffer solution, each time 10 min. Once again, the granular sludge was dehydrated for 10 min in 50%, 70%, 80%, 90%, 95%, and 100% ethanol solutions, and was cleaned three times by TERT butanol drying, each time 10 min. Then the aerobic granular sludge is frozen after freezing to make tertiary butanol sublimated. Finally, the granular sludge can be observed by ion sputtering on the metal film [8].

## 3. Results and Discussions

### *3.1. Formation Process and Structure of Aerobic Granular Sludge*

During the experiment, the morphology of the sludge was observed by the OLYMPUS optical microscope. As shown in Figure 2, the floc sludge form has not changed much at the initial stage of adding the core, and the floc is evenly distributed in the system. After 10 days of cultivation, the observation of the sludge became yellow brown, and the smaller size of the particles had been observed. For 30 days, under the function of hydraulic action (shear force and secondary flow) and Brown movement, there is an effective collision between the microorganism and the nucleus, so the floc begins to become dense and the kernel is gradually formed. It is observed that the existence of the nucleation accelerates the process [9]. For the $60^{th}$ day, the average particle size of the granular sludge in the reactor was about 0.4mm and according to the judgement basis of Bhunia [10] and so on, the granulation of the sludge is realized when more than half of the particle size is greater than 0.34mm. A large number of fixed ciliates and filamentous bacteria were attached to the surface of the mature particles (Figure 3), and the particles began to break after the temperature dropped.

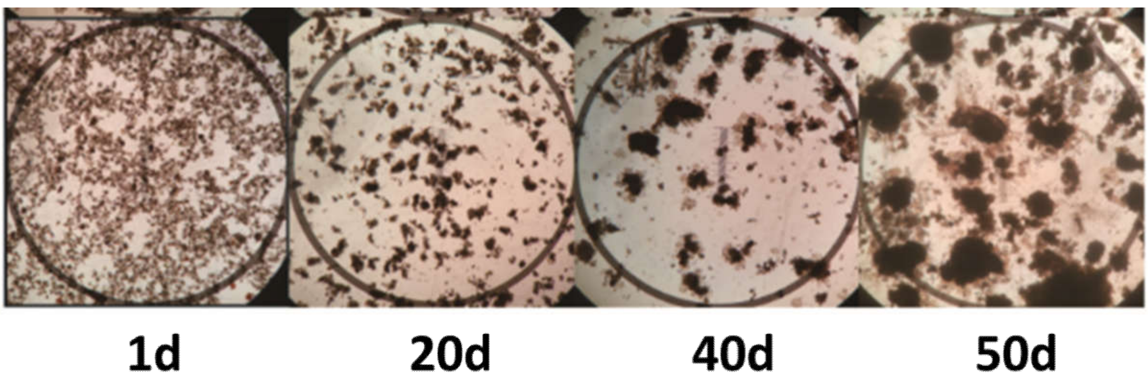


**Figure 2.** Formation process of aerobic granular sludge

a. Filamentous bacteria b. Particles with attached ciliates c. Broken aerobic granular sludge

**Figure 3.** Mature and broken aerobic granular sludge

### *3.2. Changes of Sludge Concentration and Sedimentation Index*

In the process of operation, the changes of the mixture concentration of the reactor and the change of the sedimentation index of the granular sludge are shown in Figure 4. The settling properties of sludge all gradually become better with the gradual granulating of floc sludge and SVI dropped by degrees.

Figure 4 shows a decline in MLSS during the early period of the training. It indicates that the sludge with the poor settlement performance is washed out by the gradual decrease of the settling time and with the increase of COD load to smooth, MLSS fluctuates under the selection of hydraulic pressure. When the growth is close to the washed sludge, the system is stable, and the MLSS begins to grow steadily and gradually. According to Lei [11], the reason may be that the calcium content in activated sludge increased obviously in the SBAR with the decrease of the precipitation time. The increase of calcium content resulted in the reduction of the ratio of the mass of volatile solid to the mass of the total solid from 0.88 to 0.53. The increase of the content of the inorganic matter in the sludge improves the settling performance of the sludge, so as to cope with the selection pressure formed by the shorter precipitation time.

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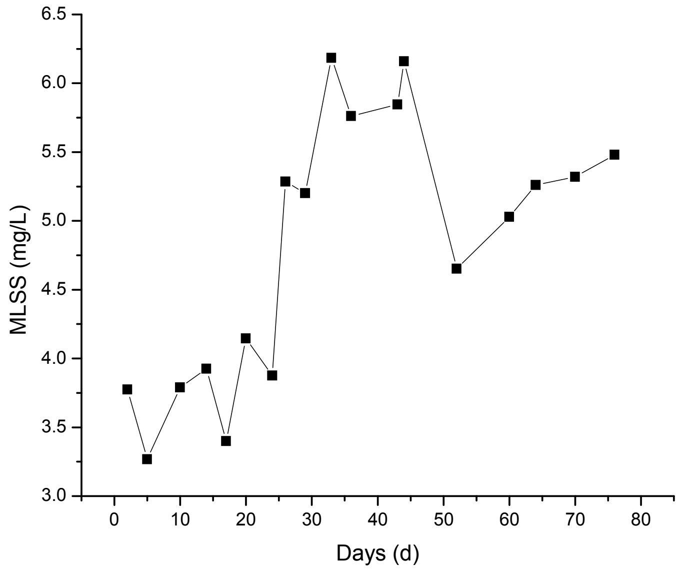
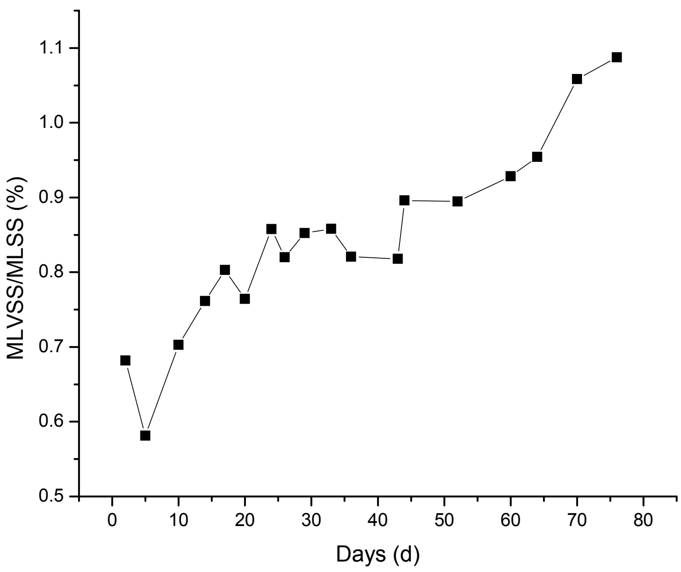


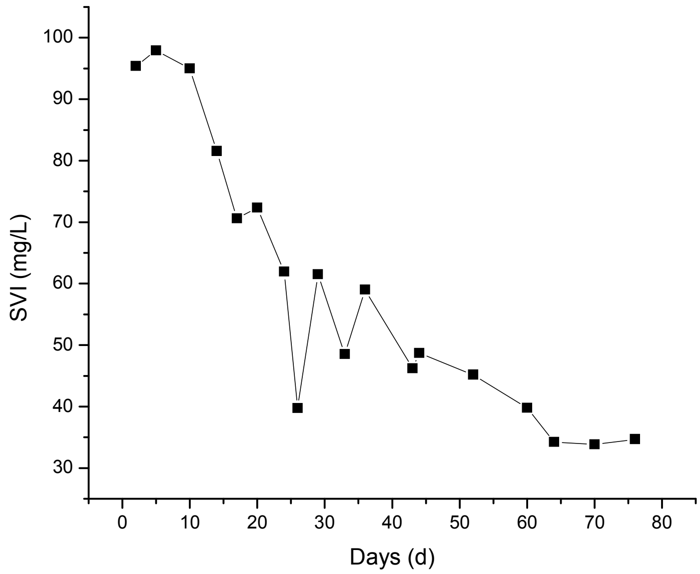



**Figure 4.** Changes of sludge characteristics during the formation of aerobic granular sludge

*3.3. Analysis of Wastewater Treatment Capacity*

The volume load gradually increased to 2.34kg COD/ ($m^3$·d) during the domestication stage. The removal rate of COD has a tendency to decrease first and then rise, that means the overall capacity of the domestication period is increasing. On the 20th day, the removal rate of COD reached 90%, and was stable in the subsequent cultivation. In general, the removal of nitrogen should be stable around 30 to 40 days under the premise of the conventional activated sludge adapted the system. In Figure 5, it can be seen that the removal rate of $NH_4$-N in the reactor is stable at 40 days, all of which can be close to complete nitrification. Finally, the removal rate of COD in the reactor is about 90%, and the removal rate of $NH_4$-N is up to 95% in 45 days. With the increase of particles and the formation of simultaneous nitrification and denitrification, the removal rate of total nitrogen eventually stabilized at 70%.

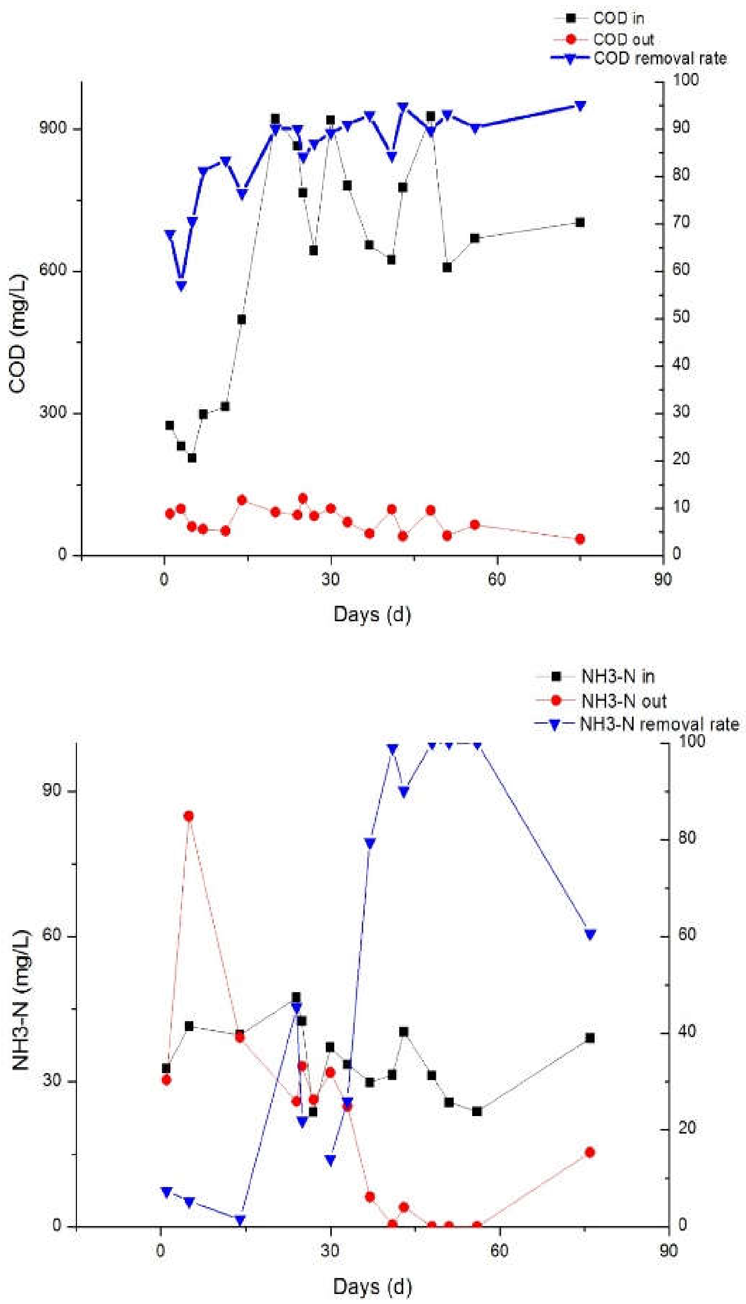
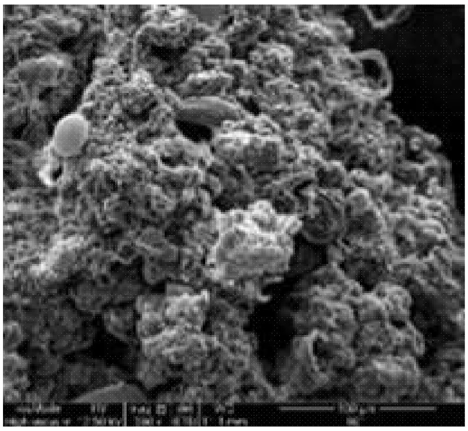


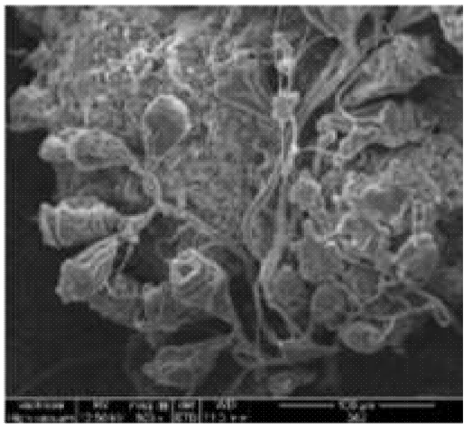

**Figure 5.** Removal effect of COD and $NH_4$-N during the formation of aerobic granular sludge

### *3.4. Discussion on the Characteristics of Mature Aerobic Granular Sludge*

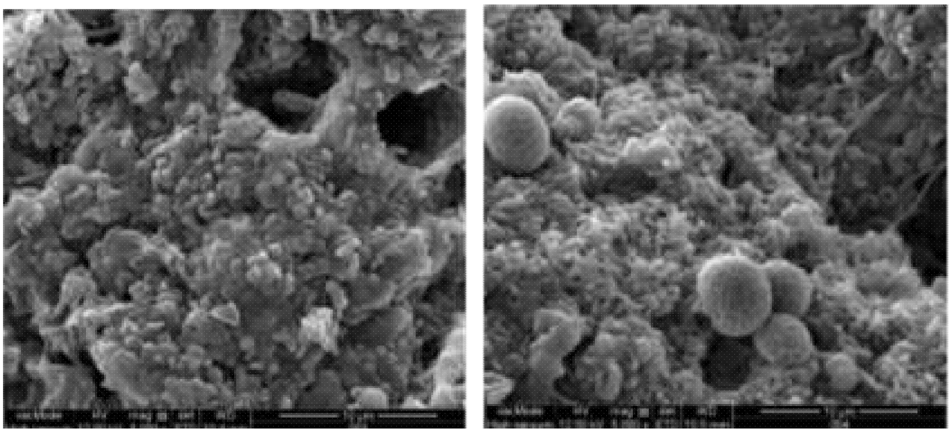
By scanning electron microscopy to observe, the mature granular sludge has a rough surface, and the particles (Fig. 6c, d) are composed of a large number of cocci and bacilli, and are composed of pores and filamentous bacteria skeletons, and pores and filamentous fungi and nymphs are present on the surface thereof. (Figures 6a, b), the presence of these pores and channels facilitates make the entry of organics and dissolved oxygen into the particles more easily. The attachment of ciliates, such as larvae, is like the plant's roots to the soil, favoring the stability of the granules and making the effluent clearer.



a. Sludge surface of granules ×500



b. Cockroaches attached to the surface of particles ×500



c. Cocci and Bacillus covered with granules ×5000

**Figure 6.** Mature aerobic granular sludge within a scanning electron micrograph of SBAR

With the maturation of the granules, the increase in density will increase the sedimentation rate and compressibility of the granule sludge. Compared with the inoculation of flocculent sludge, the proportion of aerobic granule sludge increases (Table 2), and the sedimentation rate is extremely high. Which is more convenient for mud separation. The respiration rate of granular sludge is greatly increased, showing a better biological activity, while a higher biological activity has a better effect on the removal of organic matter.

**Table 2.** Characteristics of Inoculated floc sludge and Granular sludge in SBAR

| Sludge index / Reactor | proportion of sludge | settlement rate (0.5mm)cm/s | SOUR g/(kg.h) | particle size /% | | |
|---|---|---|---|---|---|---|
| | | | | ≤0.1 | 0.1-0.3 | >0.3 |
| floc sludge | 1.006 | 1.035 | 25.36 | 100 | — | — |
| R1 | 1.022 | 4.886 | 45.62 | 23.23 | 54.5 | 22.27 |

The particle size distribution at the initial stage of formation was compared, and the particle size distribution at forty-fifth days was measured as shown in Table 2. The proportion, settlement rate and water content of granular sludge are much better than that of floc sludge. The proportion of the sludge affects the sedimentation rate to a certain extent, and the higher the proportion of particle, the faster the sedimentation rate is. The activity of microorganism in unit sludge concentration is also higher than that of floc sludge [12].

## 4. Conclusion

Compared to traditional floc structure sludge, aerobic granular sludge has a greater advantage in the field of water purification. Through the analysis of experimental results, the conclusions are as follows:

1) The maturity of aerobic granular sludge for COD and $NH_3$-N removal rate is much higher than the traditional floc sludge, which can be stabilized at more than 90%. The final reactor's COD removal rate remained at about 90% and $NH_4$-N removal rate reaches 95%. So the water purification effect is greatly enhanced.

2) Due to the compact particle structure of aerobic granular sludge, its density is much larger than ordinary activated sludge, and its activity is higher. The required settling time is short. The sedimentation rate is 4.886 cm/s which is much higher than the floc sludge which is 1.035 cm/s. While SVI value reducing from 120 ml/g to 40 ml/g during culture, MLSS can be maintained at about 6 g/L. Granulation increases the specific gravity of sludge, allowing microorganisms to be retained and reused, achieving separation of hydraulic retention time and solid residence time. It creates favorable conditions for the development and design of high-efficiency treatment processes.

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