The feasibility of aerobic granular sludge formation without inoculated sludge

Tao Song¹, Ji Li¹*, Xiaolei Zhang¹ and Peibing Shao²

¹School of Civil and Environmental Engineering, Shenzhen Key Laboratory of Water Resource Application and Environmental Pollution Control, Harbin Institute of Technology, Shenzhen, Shenzhen, Guangdong, P.R. China, 518055.
²Shenzhen Huichuangyuan Environment Protection Technology Co., Ltd.

*Corresponding author: Tel: 86-(755) 2603-2692; Fax: 86-(755) 2670-3651. Email: liji99@hit.edu.cn

Abstract. In this study, the feasibility of aerobic granular sludge formation without inoculated sludge was detected. After 10 days operation, AGSs were discovered in reactor and the MLSS and settling ability of AGS enhanced gradually. After 21 days operation, the effluent NH₄⁺-N and COD keep low than 10 and 100 mg/L, respectively. During the formation of AGS, the content of EPS gradually increased and remained stable. Microbial community showed that EPS secretion genera (such as Paracoccus, Flavobacterium and Thauera) were main genera in AGS.

1. Introduction
With the development of society, water quality problems have become increasingly serious problems, and there is an urgent need for wastewater treatment technologies that efficiently remove nutrients and organic pollutants. The aerobic granular sludge (AGS) process is an attractive wastewater treatment technology because it has many incomparable advantages compared with activated sludge, such as compact structure, high sedimentation capacity, resistance to high organic loads and Toxicity, the possibility of degrading organic carbon and nutrients at the same time, etc. However, the formation and cultivation of AGS still needs further research [1]. In many studies, it has been found that after the formation of AGS, there are obvious differences in sludge characteristics and microbial communities between inoculated sludge and AGS [2]. Therefore, the role of inoculated sludge in the formation of AGS requires attention. Many studies have found that when different sludge types are inoculated, the formation time and characteristics of AGS are different [3]. The inoculated sludge determines the microbial community of the granules formed during the cultivation process, thereby affecting the efficiency of the application and treatment process of the cultivation granules [4, 5]. Activated sludge was the most commonly inoculated sludge [4, 6-8]. Mature aerobic granules were dehydrated, air-dried, and inoculated to a different reactor to cultivated AGS [10]. Although some studies have used different inoculated sludge, whether the role of seed sludge is necessary to be studied. This study attempts to explore whether AGS can be formed without adding seed sludge.
2. Materials and Methods

2.1. Experimental equipment and operational conditions
AGS were cultivated by SBR, which were operated at room temperature (25 ± 3 °C). The total volume and working volume of the SBR was 1.7 L. The volumetric exchange ratio (VER) of three SBR was 50%, and the hydraulic retention time (HRT) was 6 hours (cycle is 3 hours). The reactor was operated sequentially cycles, with 5 min of anaerobic feeding, 165 min of aeration, 5 min of settling and 5 min of decanting.

![SBR cycle of AGS](image)

Figure 1 The SBR cycle of AGS

2.2. DNA extraction and PCR amplification
In order to analyze the bacterial community structure during granulation, the composition of the bacterial community in the inoculated sludge and the AGSs samples were studied employ high-throughput sequencing.

2.3. Synthetic wastewater
The low-concentration domestic sewage used in this study. The composition of synthetic wastewater is as follows: 300 mg/L COD (CH₃COONa), 45 mg/L NH₄⁺ (NH₄Cl), 5 mg/L P (KH₂PO₄), Sodium bicarbonate was added to synthetic wastewater at the concentration of 320 mg/L alkalinity (calculated in CaCO₃)

2.4. Analytical methods
The wastewater parameters, COD, NH₄⁺-N, TP, total nitrogen (TN), nitrate nitrogen (NO₃⁻-N), nitrite nitrogen (NO₂⁻-N), MLSS, and SVI were measured according to the Standard Methods (SEPA, 2002). The morphology of the sludge sample was recorded with a digital camera. EPS extraction was performed by heat treatment based on a referenced procedure, and the determinations of protein (PN) and polysaccharide (PS) were based on the Lowry method [11] and phenol-sulphuric acid method [12], respectively.

3. Results and discussion

3.1. Process of granular sludge formation
The sludge morphology of reactor was showed in Fig 2. As shown in Fig. 2a, after operation, white flocculent sludge occurred in reactor. AGS can be discovered in reactor after 10 days operation and kept stable. Indicated that AGS can form without inoculated sludge, and active sludge is not necessary to AGS formation.
3.2. Cultivation of AGS
The MLSS and SVI were shown in Fig 3, the MLSS of reactor increased from 0g/L to 4.21g/L gradually with operation. The SVI increased from 0 to 100 in first 7 day and then decreased. When reactor operated to 50th day, the SVI decreased to 23.2ml/g. It indicated that flocculent sludge with poor settling performance was formed first, and replaced by AGS during the granulation process.

3.3. The performance of AGS
At the beginning of the reaction, the ammonia nitrogen effluent of reactor was higher than 30mg/L in the first 11th day, which may due to low MLSS. With operation, ammonia nitrogen decreased gradually and ammonia nitrogen was completely removed at 21th day. Nitrate nitrogen became the major nitrogen product after 31th day. After 5 days operation, COD concentration kept low than 100mg/L. AGS still showed high efficient removal performance without inoculated sludge.
3.4. Extracellular polymeric substances (EPS) of AGS
EPS plays an important role in the formation and stability of AGS [13]. It can be seen from the Fig. 5 that during the formation of AGS, a large amount of EPS content was secreted, and the content of PN and PS both increased significantly. The content of PN is the main component in EPS, the increased proteins (PN) concentration facilitated the formation of microbial aggregates and the granulation of sludge [14]. When reactor ran to 30 days, the content of EPS kept stable.

3.5. Bacterial community of AGS
It can be seen from Fig. 6a that the genera diversity of AGS is significantly lower than that of activated sludge. With the formation of AGS, the diversity of microorganisms gradually increased. It can be seen...
from Fig 6b that the same genera in AGS and activated sludge accounts for more than 60% of AGS, while the same genera only accounts for about 30% in AS, indicating that when activated sludge is used as inoculation sludge 70% of the genera are removed, and the remaining species may account for more than 60% of the AGS. When AGS is formed, only a small amount of species in the activated sludge can provide more original species for the formation of AGS, but when inoculated sludge is not provided, AGS can still form and complete the enrichment of genera. It can be seen from the Fig 6c that the bacterial genera related to EPS secretion are abundantly enriched, such as Paracoccus, Flavobacterium, Brevundimonas, Comamonas, Diaphorobacter, chryseobacterium, Acinetobacter, Rhodobacter, etc [15]. Thauera may play an important role in granulation [16]. It shows that under the condition of no activated sludge, aerobic granule’s formation is positively correlated with the EPS-forming bacteria.

4. Conclusion
It is feasible to cultivate AGS without adding any inoculated sludge. AGS can be formed stably, can quickly form larger biomass and good settling performance. After AGS formation, AGS can maintain
greatly removal performance. The community structure and diversity in the sludge gradually changed with the operation.

Acknowledgements
This work was supported by Shenzhen Science and Technology Innovation Commission (Grant number JCYJ20180306172051662, KQJSCX20180328165658476, JCYJ20200109113006046, KCXFZ20201221173602008 and KCXFZ20200201006362).

References
[1] A M T. Mata, H M. Pinheiro, N D. Lourenço, Biochem Eng J. 2015, 104: 106-114.
[2] A. Li, X. Li, H. Yu, Process Biochemistry. 2011, 46(12): 2269-2276.
[3] Z. Song, Y. Pan, K. Zhang, J Environ Sci. 2010, 22(9): 1312-1318.
[4] Chen Y, Lee D. Effective aerobic granulation: Role of seed sludge[J]. Journal of the Taiwan Institute of Chemical Engineers. 2015, 52: 118-119.
[5] O T. Iorhemen, M S. Zaghloul, R A. Hamza, J Environ Chem Eng. 2020, 8(2): 103681.
[6] X. Zhao, Z. Chen, J. Shen, Environ Technol. 2014, 35(5-8): 938-944.
[7] J. Liu, J. Li, S. Piché-Choquette, J Water Process Eng. 2020, 36: 101269.
[8] S L D S. Rollemberg, L Q. de Oliveira, A R M. Barros, Bioresource Technol. 2019, 278: 195-204.
[9] W. Cai, M. Jin, Z. Zhao, Bioresource Technology Reports. 2018, 2: 7-14.
[10] S. Ogura, R A. Hamza, J H. Tay, J Water Process Eng. 2020, 36: 101298.
[11] G L. Peterson, Anal Biochem. 1977, 83(2): 346-356.
[12] M. Dubois, K A. Gilles, J K. Hamilton, Anal Chem. 1956, 28(3): 350-356.
[13] Z. Li, C. Wan, X. Liu, Sci Total Environ. 2020, 756: 144054.
[14] A. Xi, L A. Jie, B. Dda, J Water Process Eng. 40.
[15] A M S. Paulo, C L. Amorim, J. Costa, Sci Total Environ. 2021, 756: 144007.
[16] J. Zou, J. Pan, S. Wu, Sci Total Environ. 2019, 674: 105-113.