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Aerobic Sludge Granulation Process in a 4-h Sequencing Batch Reactor for Treating Low Strength Wastewater

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The aim of this study is to determine design and process parameters for the development of aerobic granules using actual low-strength domestic wastewater by further investigate the efficiency of anaerobic-aerobic-anoxic (AN/O/AO) in a 2.5 L lab-scale column bioreactor, fed with influent from local sewerage treatment plant and seeding with fresh inoculums (seed sludge) from activated sludge tank. The operation of the bioreactor complies with the 4 h wastewater treatment cycle and it's specifically designed to be operated for 24 h continuously. Intermittent aeration setting mode is applied to run the sequencing batch reactor (SBR) to accelerate the development of the granules and enhance removal performances. An excellent average removal of Total Suspended Solids (~90 %), Chemical Oxygen Demand (~90 %), Ammoniacal Nitrogen (~80% with successfully converted to nitrate) and Phosphate (~100 %) was achieved in this aerobic granular sludge system. Matured, dense and compact granules with average size (1.80 ± 0.03 mm) in diameters were also successfully developed with a good settling velocity (40 m h^{-1}) and 30 minutes Sludge Volume Index 30 (60 mL g^{-1}).

1. Introduction

"The aerobic granular sludge (AGS) process is a promising technology for wastewater treatment. However, a long start-up period for granulation and instability during long-term operation still hinder the application of AGS technology, especially for low-strength wastewater" (Yu et al., 2021). To solve the problem without adding additives, addition of support material or carriers Intermittent Aeration is proposed. By alternating aerobic and anaerobic stages, it contributed to higher removal efficiencies of organics, nitrogen and phosphorus. Besides, it also accelerate aerobic granular sludge development with larger particle size, and more microbial communities. This intermittent aeration strategy also will shortens the time of the granulation process which favours a faster development of phosphorus accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) than continuous aeration (Carrera et al., 2019).

Recent study reported that Liang et.al (2022) have solved these two problems by developing AGS in anaerobic-aerobic-anoxic sequencing batch reactor (AN/O/AO_SBR) with addition of carriers. However, this study aimed to solve the problem by further investigate the efficiency of (AN/O/AO_SBR) without a carrier. The aim and objectives of this study is to determine design and process parameters of the aerobic granular sludge system to treat domestic wastewater in Malaysia. Hence, the specific objectives of the study: (i) To develop AGS using low-strength domestic wastewater (COD value ≤ 200 ±100 mg L^{-1}) via optimization of aeration time within a 4h-SBR process, (ii) To characterize the developed AGS and evaluate the performance in term of Total Suspended Solids (TSS), Chemical Oxygen Demand (COD), Ammoniacal Nitrogen (AN) and Phosphate (P) removal and (iii) To examine AGS microbial morphology by using FESEM analysis.

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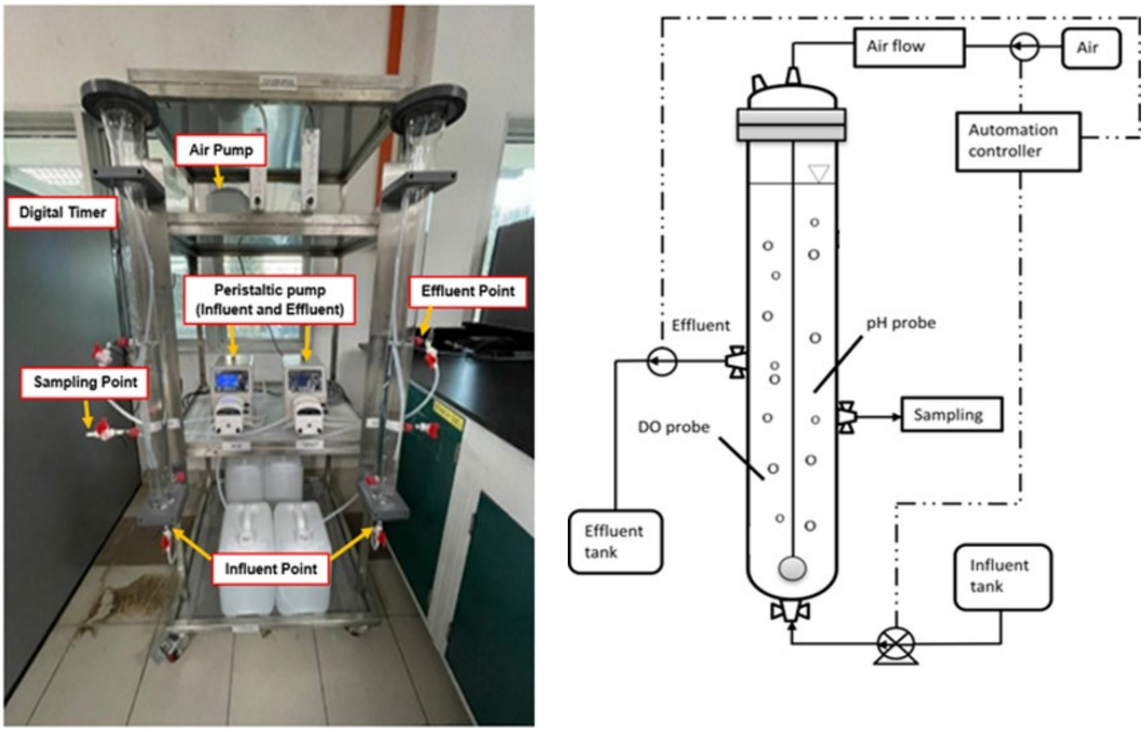
2. Methods and materials

2.1 Designed and setting up bioreactor

A 2.5 lab-scale single-walled cylindrical glass column prototype bioreactor was set-up at Indah Water Research Centre to be operated for 24 h continuously with 4 h cycle at ambient temperature in order to suit the actual treatment process. The average daily temperature across Malaysia is between 21°C and 32°C.The basis of this reactor set-up was developed according to the reactor configuration built in the Kluyver Laboratory for Biotechnology, Delft University of Technology, the Netherlands (Anuar, 2008). Apparatus used to run the bioreactor were digital timer, air pump, and peristaltic pump. Digital timer is used to set the flow of the treatment cycle as shown in Figure 1. Air pump is to supply air at the bottom of the bioreactor at a volumetric flow rate of m^3 h^{-1} (with superficial air flow velocity cm s^{-1}) using porous air stones to promote the formation of small air bubbles. While, peristaltic pump used for pumping influent and effluent through influent point and effluent point. The schematic diagram of the reactor set-up also shown in Figure 1. The system will be fed with actual domestic wastewater from a black tank connected from Bunus Regional Sewage Treatment Plant (STP) which is located nearby Indah Water Research Centre through a pipeline. Set value for operational parameters is shown in Table 1.



(a) (b)

Figure 1: (a) 2500 mL laboratory-scale SBR and (b) Schematic diagram of reactor

Table 1: Bioreactor set value

Parameter	Value during start-up
Working volume	2.5 L
Feeding	Low-strength domestic wastewater (COD 200 mg L^{-1})
Seeding	Fresh activated sludge from aeration tank of EA plant
MLSS volume (mL)	1,500 mg L^{-1}
MLSS (mgL^{-1})	> 3,000
Temperature (°C)	25
pH	6-8
DO(mgL^{-1})	< 2.0 mg L^{-1}
VER	1/2.5 = 0.4@40%
HRT	2.5 L / 6 L day^{-1} = 10 h
SRT	typical AGS system have SRT > 30 days

2.2 Seeding sludge

A fresh activated sludge taken from a full-scale Sequencing Batch Reactor Treatment Plant was used as seed sludge. At the beginning of reactor start-up, approximately 1500 mL of seed sludge was used, resulting in initial mixed liquor suspended solids (MLSS) of 3000 mg L^{-1} in the reactor. The characterization study is essential to provide physical and morphological information for the initialization of the granulation process where at the end of the study, the final characteristics of granulated seed sludge could be figured out. The physical characteristics were determined in terms of mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), size and settling velocity (v).

2.3 Influent feeding

The influent for the experiments with actual wastewater were collected from a black tank connected to full scale SBR wastewater treatment plant located at. For every cycle, 1000 mL was dosed to the reactor.

2.4 Chemical and apparatus

Chemical reagent is used to measure the aerobic granular sludge (AGS) removal performance. Composition of influent and effluent such as chemical oxygen demand (COD), ammoniacal nitrogen (AN), and phosphate (P) will be measured by mixing these samples with the chemical reagent. The vial contained reagent mix with sample will be placed in DR 3900 Spectrophotometer (HACH) to measure the value of the removal performance. While, for total suspended solid (TSS) filter paper, filter funnel and oven are used to carry out the experiment.

2.5 Analytical procedures

Different analytical methods will be conducted to determine and measure related parameters throughout the study. Both the sludge and water (influent and effluent) will be a crucial component for this work. Therefore, each of them must be analyzed according to standard procedures present in this chapter. The analytical methods for this study consist of three different parts: a) granules characteristics, b) bioreactor removal performance and c) microbial community in AGS based on scanning electron microscope (SEM) observation. The influent and effluent samples will be collected from the sampling port of the bioreactor while biomass samples will be obtained through the outlet port. All analytical measurements performed in this study were conducted according to Standard Methods for the examination of water and wastewater (APHA, 2012).

3. Results and discussion

3.1 Bioreactor time setting

The SBR system consists of four processes, and it was carried out in sequence feeding, aeration, settling and decanting. Starting from day 1 until day 43 intermittent aeration is applied during aeration phase. By reducing DO concentration, it will benefit simultaneous nitrification and denitrification process which solve the problem of low-strength municipal wastewater (COD value $\leq 200 \pm 100$ mg L^{-1}) in AGS development besides low sludge loading rate favour stable long-term granule stability (Liang et al, 2022). The Dissolved Oxygen (DO) values were below 3 mg L^{-1} during the process, and it took 15 min the DO to drop to 0 mg L^{-1} every time the blower stops for intermittent aeration and settling.

3.2 Formation, size, settling velocity and sludge volume index of granules

AGS was harvested using 0.3 mm, 0.6 mm and 1.0 mm sieve on day 43. The diameter of the granules was obtained under microscope observation (irregular shapes of granules were developed). The developed granules at 43 days are shown in Figure 2 and seed sludge in Figure 3. The diameter of AGS is between 1.04 mm to 4.25 mm, settling velocity 40 m hr^{-1} and sludge volume index 30 60 mL g^{-1} compared to seed sludge diameter < 0.20 mm, settling velocity < 10 m hr^{-1} sludge volume index 30 150 mL g^{-1}. Cocci bacteria are shown in Figure 4. The cocci bacteria were tightly linked to each other forming a cluster of spherical cells. One of the main biological factors that lead to the formation of compact and dense structure granules, Figure 5 shows filamentous bacteria. Filamentous bacteria was the initiator for the biomass aggregation process by forming mycelial pellets which settle very well. Act as an architectural backbone to increase the strength of the AGS structure. Figure 6 shows microalgae bacteria. Microalgae bacteria is commonly found in hot climate wastewater (exposed to sunlight). Micro colonies bacteria (Cocci-shaped, Rod-shaped, Filamentous, Algae etc.) acted as a supporting consortia during the formation of granular sludge. Figure 7 shows extracellular polysaccharide substances (EPS). EPS acts as a sticky glue between the microbial cells and strengthens the structure of the granules. Figure 8 shows cavities. Cavities act as a passage for the transportation of substrate, oxygen, and nutrients into the inner cores of the granules and ensure the stability within the granule's composition.

3.3 Removal efficiency of granules

During the 43 days development parameters such as TSS, COD, AN, and P were monitored daily and weekly. An excellent average removal of TSS (~90 %). By alternating anaerobic and aerobic conditions, it allows more organic uptakes eg. by phosphorus accumulating organisms (PAO). This is why TSS uptakes (removal) is high. COD (~90 %) indicate the removal performance of COD due to the transformation of the flocculent sludge into granular sludge. This result is comparable to the previous study by Ab Halim et al. (2018) who also conducted experiment by using actual domestic wastewater for the biological activity of granules. In the study conducted, when reached steady state, the removal rates were in the range of 85.37 % – 98.17 % for COD by matured granules at three high temperatures (30, 40 and 50 °C). AN (~80 % with successfully converted to nitrate). This is due to complete nitrification process (oxidation of ammonium ions to nitrate) and P (~100 %) was achieved in this aerobic granular sludge system. The basic principle of biological phosphorus removal is to expose bacteria to alternating anaerobic and aerobic conditions to promote "luxury uptake" of phosphorus. Under anoxic to anaerobic conditions, phosphorus accumulating organisms (PAO) have the ability to take in organic substrate.

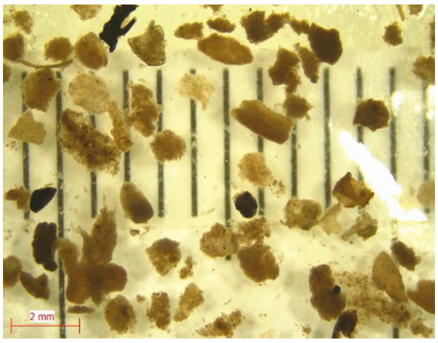


Figure 2: Physical characteristic of AGS

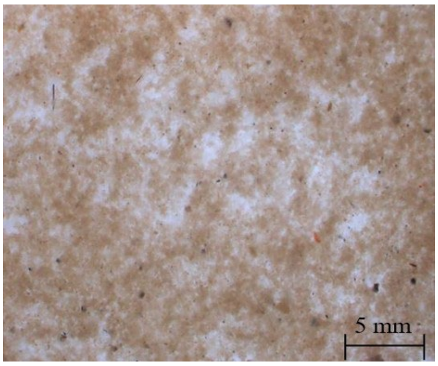


Figure 3: Physical characteristic of seed sludge

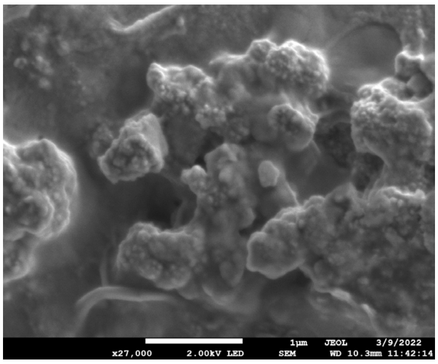


Figure 4: Microbial morphology of AGS – Cocci bacteria

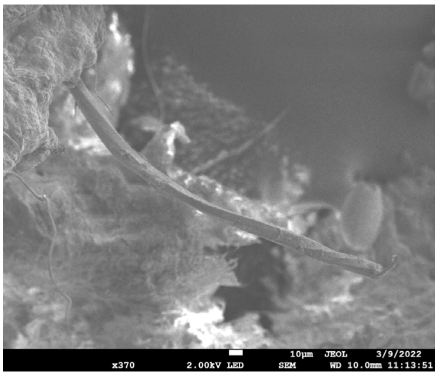


Figure 5: Microbial morphology of AGS – Filamentous bacteria

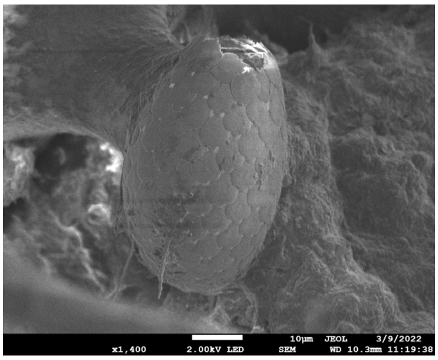


Figure 6: Microbial morphology of AGS – Microalgae bacteria

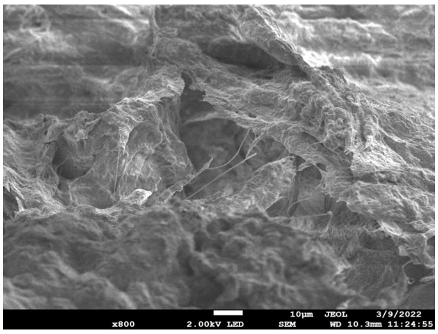


Figure 7: Microbial morphology of AGS – Extracellular Polysaccharide substance (EPS)

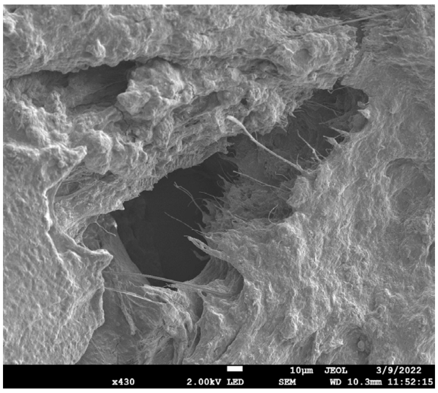


Figure 8: Microbial morphology of AGS - Cavities

4. Conclusions

AGS is successfully developed using low strength domestic wastewater within a 4-h SBR process with high removal efficiencies. It also has an excellent settling ability with settling velocity (40 m hr^{-1}) and SVI_{30} (60 mL g^{-1}). Many types of micro colonies observed within the granular structure of AGS such as cocci-shaped bacteria, filamentous bacteria, microalgae bacteria and spherical-shaped bacteria except rod-shaped bacteria (denitrifiers bacteria). EPS and cavities of AGS also can be seen clearly under SEM investigation. The limitation of this study is inability of nitrate removal performance due to the absence of denitrifiers bacteria. Future recommendations are to further investigate denitrifies existence in the inner layer of granules via SEM image analysis by optimizing aeration time within 3-h SBR process and to scale-up the AGS study from lab to pilot bioreactor.

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