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DEVELOPMENT OF AEROBIC GRANULES IN SEQUENCING BATCH REACTOR SYSTEM FOR TREATING HIGH TEMPERATURE DOMESTIC WASTEWATER

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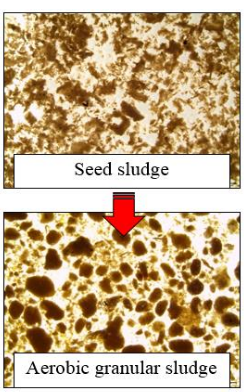
Graphical abstract



Abstract

The application of aerobic granular sludge (AGS) in treating real domestic wastewater at high temperature is still lacking. In this study, the microstructure and morphology of the granules, as well as bioreactor performance, were investigated during the treatment of real domestic wastewater at high temperature (50 °C). The experiment was executed in a sequencing batch reactor (SBR) with a complete cycle time of 3 hours for the treatment of low-strength domestic wastewater at an organic loading rate (OLR) of 0.6 kg COD m^{-3} d^{-1}. Stable mature granules with average diameters between 2.0 and 5.0 mm, and good biomass concentration of 5.8 g L−1 were observed in the bioreactor. AGS achieved promising results in the treatment of domestic wastewater with good removal rates of 84.4 %, 99.6 % and 81.7 % for chemical oxygen demand (COD), ammoniacal nitrogen (NH_3–N), and total phosphorus (TP), respectively. The study demonstrated the formation capabilities of AGS in a single, high and slender column type-bioreactor at high temperature which is suitable to be applied in hot climate condition areas especially countries with tropical and desert-like climates.

Keywords: Aerobic granular sludge, sequencing batch reactor, high temperature, hot climate, domestic wastewater, wastewater treatment

Abstrak

Aplikasi enapcemar granular aerobik (AGS) untuk merawat air kumbahan domestik pada suhu yang tinggi masih lagi kurang. Dalam kajian ini, mikrostruktur dan morfologi granular, serta prestasi bioreaktor diterokai semasa rawatan air kumbahan domestik pada suhu yang tinggi (50 °C). Eksperimen ini dijalankan menggunakan reaktor kelompok berjujukan (SBR) dengan kitaran masa yang lengkap selama 3 jam semasa merawat air kumbahan domestik yang rendah kekuatannya dengan kadar muatan organik (OLR) 0.6 kg COD m^{-3} d^{-1}. Granular matang yang stabil telah terbentuk dengan purata diameter antara 2.0 hingga 5.0 mm dan kepekatan biomas sebanyak 5.8 g L^{-1} direkodkan dalam bioreaktor. AGS juga mencapai hasil yang memuaskan dengan kadar kecekapan penyingkiran yang baik sebanyak 84.4 %, 99.6 % dan 81.7 % bagi permintaan oksigen kimia (COD), nitrogen ammonia (NH_3–N) dan jumlah fosforus (TP). Kajian ini membuktikan keupayaan pembentukan AGS dalam bioreaktor jenis kolum yang tunggal, tinggi dan langsing pada suhu tinggi yang sesuai untuk diaplikasikan di kawasan keadaan beriklim panas terutamanya di negara-negara seperti beriklim tropika dan padang pasir.

Kata kunci: Enapcemar granular aerobik, reaktor kelompok berjujukan, suhu tinggi, iklim panas, air kumbahan domestik, rawatan air kumbahan

1.0 INTRODUCTION

Due to rapid industrialisation, urbanisation and the enormous rise in human population since the mid-20th century, wastewater treatment and management have become an issue of major concern throughout the globe. If this issue is not properly handled, wastewater from domestic and industry sectors can cause serious pollution problems. Therefore, the main objective of wastewater treatment is to dispose of the wastewater without jeopardising public health or even the unacceptable impairing environment. Based on this principle, research efforts have recently been made in particular areas of wastewater technology all over the world to recycle, and even purify the quality of the treated wastewater that can be reused safely for different beneficial applications (e.g., irrigation). In this regard, the aerobic granular sludge (AGS) is recognized as a remarkable technology for wastewater treatment [1, 2].

Research on the development of AGS by using sequencing batch reactor (SBR) started in the late 1990s. Since then, most of the studies have been focusing on the investigation of the parameters affecting the growth of aerobic granular sludge during the start-up of SBR [3-5]. Usually, granular sludge consists of stronger aggregation architecture structure, higher biomass concentration, excellent solid-liquid separation, and better strength to tolerate shock loading rates [6, 7]. Previous research have covered areas of applying knowledge to micro- and macro-scale observations of the granular sludge with following deterministic features: both architecture and behaviour, permeability, surface hydrophobicity, settle-ability, morphology, mechanical stability, porosity, thermodynamics, and its practical functions in the removal of organic compounds and nutrients [8, 9].

In a special case, transformation of flocculent into granular sludge could be favoured by certain conditions such as a short settling period for the screening of particles with excellent settling velocity [10-12], anaerobic filling to encourage the propagation of slow growing microorganisms such as glycogen-accumulating organisms (GAO) or polyphosphate-accumulating organisms (PAO) [13], and high shear force caused by aeration [14,15]. Additionally, the evolution of flocculent into granular sludge has also been scientifically investigated within periods of two to three weeks under feasible conditions of laboratory-scale reactors filling with synthetic wastewater [16-19], industrial wastewater [20-23], as well as domestic wastewater [24,25].

An understanding of AGS, its physiochemical characteristics, and the technological applications using different types of wastewater at different temperatures have been steadily making progress to encourage the use of this technology. To date, most research works on AGS using SBR system (AGS-SBR) has generally focussed on treating synthetic wastewater at ambient temperature e.g., 20 – 25 °C or lower [26,27]. Although some literature reported studies on AGS cultivated at high temperature [28-33], detailed knowledge regarding the granulation practices are still lacking such as the application of aerobic granular sludge in treating real domestic wastewater at high temperature under mesophilic, and thermophilic conditions.

The aim of this research was to investigate the possibility of cultivating AGS by using SBR system in treating real domestic wastewater at high temperature (50 °C). The samples of seed sludge were obtained from the wastewater treatment plant of Madinah city in Saudi Arabia. Madinah has a desert-like climate with the temperature reaching close to 50°C in summer. This study focussed on granules microstructure and morphology throughout the granulation process, and discussed the reactor performance and biomass profile. In addition, this study also highlighted critical and crucial issues during the system set-up for discussions of some strategies for advance improvement. This research work is expected to contribute further knowledge on the application of AGS granulation mechanism which is essential for its practical implementation in the real domestic wastewater treatment in hot climate areas.

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2.0 METHODOLOGY

2.1 Experimental Procedures and Bioreactor Set-up

A double-walled cylindrical glass column bioreactor (internal diameter of 10 cm with a total height of 68.5 cm) consisting of a working volume of 4 L was used in this study (see Figure 1). Next, 2 L of activated sludge from the treatment plant was added into the bioreactor during the start-up period as inoculum. Feeding pump, discharge pump, and an aerator pump with the setting time for each phase in the bioreactor was controlled by a programmable logic controller (PLC). The bioreactor was operated through successive cycles of 3 hours. Each cycle consisted of a 60 minute-influent feeding stage from the bottom of the bioreactor without stirring, a 110 minute-aeration stage, a 5 minute-settling period and a 5 minute-effluent discharge period. Real domestic wastewater was fed into the system and discharged by a set of two peristaltic pumps. Furthermore, the bioreactor was aerated with an air pump that was operated at a constant flow rate of 0.6 m^3 h^{-1} (2.1 cm s^{-1} superficial air flow velocity). A porous diffuser located at the bottom of the bioreactor boosted the aeration process by making small air bubbles. The effluent withdrawal point was positioned at the middle height of the glass column, operating at volumetric exchange ratio (VER) of 50 % per cycle. The bioreactor was scheduled to run for 82 days (202 cycles) without excess sludge discharge, thus the effluent was the only passage for biomass wasting to be transported. The working temperature of the bioreactor was kept at 50 ± 1°C using water bath sleeves, and a thermostat without controlling the dissolved oxygen and pH level.

2.2 Real Domestic Wastewater Characteristics and Inoculation Sludge Sample Collection

The sample of real domestic wastewater was obtained from Madinah city Sewage Treatment Plant in Saudi Arabia, which was a local municipal wastewater treatment plant (WWTP). The raw wastewater sample was collected from the inlet point to the plant before any type of treatment and was then sieved with a mesh of 1.0 mm to eliminate large debri and solid materials which can cause clogging to the influent tubes. The collected sample of real domestic wastewater was stored in a cool storage room at a temperature of 4 °C until it was fed to the reactor system. The characteristics of the real domestic wastewater used throughout the experiment and real domestic wastewater as described in previous literature [34] are presented in Table 1. The real domestic wastewater characteristics which showed a typical variation of raw wastewater samples was collected from WWTP in terms of chemical oxygen demand (COD), ammoniacal nitrogen (NH_3–N), and total phosphorus (TP). The bioreactor was inoculated with fresh activated sludge collected from one of the aeration tanks of the same WWTP in Madinah city. The amount of inoculum was about 2 L, with a mixed liquor suspended solid (MLSS) concentration of 3.7 g L^{-1}, a mixed liquor volatile suspended solid (MLVSS) concentration of 3.2 g L^{-1}, and a sludge volume index (SVI_5) of 178.5 mL g^{-1}. The seed sludge was brown in colour with fluffy loose structure.

2.3 Analytical Methods

MLSS and MLVSS measurements were determined according to Standard Methods for the Examination of Water and Wastewater [35]. Chemical oxygen demand (COD), ammoniacal nitrogen (NH_3–N) and total phosphorus (TP) were determined using a spectrophotometer (DR 5000, Hach Co., USA). The value of pH and dissolved oxygen (DO) were monitored constantly by the pH and DO sensors introduced in the bioreactor and these values were recorded by a pH/DO meter (Orion 4-Star Benchtop pH/DO Meter). The sludge volume index (SVI_5) measurements were determined by following the method proposed by de Kreuk *et al*. [26]. The morphology and structure of the developed aerobic granular sludge were observed regularly by using a stereomicroscope equipped with a digital image analyser (I-Solution Premium). Scanning electron microscope (SEM) (SU8020, Hitachi, Japan) was used to further visualise microstructure compositions within the cultivated granules. The granules were prepared according to Dahalan *et al*. [1] and Rosman *et al*.'s [21] approach. Platinum sputter coating for 60 seconds (Q150R S, Quarum, UK) was chosen for the pre-treatment procedure for SEM image.

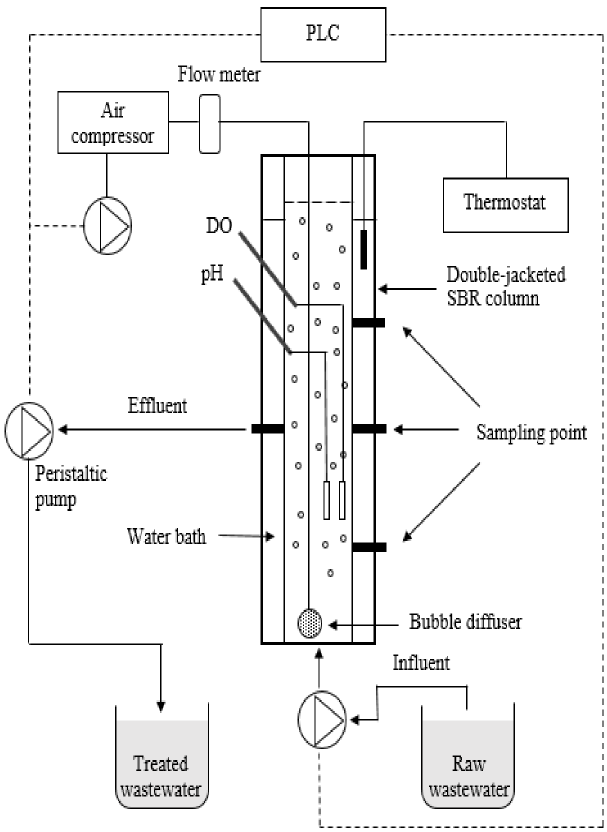

Figure 1 Schematic diagram of operational reactor setup

3.0 RESULTS AND DISCUSSION

3.1 Development of AGS in SBR Fed with Real Domestic Wastewater at High Temperature and Morphology Observation

Experiments were executed to demonstrate the development of aerobic granular sludge at high temperature in SBR system fed with real domestic wastewater and the findings are shown in Table 2. The 3-hour cycle time was preferably taken due to the convincing and promising results from previous studies of real domestic wastewater treatment [17, 24, 25]. At the beginning of experiments, the size of the aerobic granules was unevenly distributed by variable sizes between 0.5 – 1.0 mm, and the shapes looked like non-clear boundary under stereomicroscope examination (see Figure 2a). The suspended flocs then continuously disappeared and were replaced by small and brown granules with an average diameter between 1.0 and 1.5 mm. Under high shear forces, density of the suspended flocs increased probably as a result of the bridging process among microbial the cells, EPS and ion [36]. Eventually, the size of the granular sludge gradually increased at an average diameter of 2.0 – 3.0 mm. For mature granulation, the initial smooth granules exhibited a clearly defined outline boundary with the size increasing from 4.0 to 5.0 mm (see Figure 2b).

Theoretically the mechanism of aerobic granular sludge formation in thermophilic condition can be divided into four main stages. The mechanism involves (i) cell-to-cell interactions/attachment to initiate granulation process, (ii) microaggregates formation by these self-attached cells, (iii) biosynthesis of extracellular polymeric substances (EPS) by the microaggregates and (iv) maturation of granules which depends on the external hydrodynamic parameters applied by the configuration of bioreactor and its working conditions such as high temperature or thermophilic condition as in the case of this study [37-40].

Table 1 Characteristics of real domestic wastewater

Parameters[a]	Real domestic wastewater (this study)	Real domestic wastewater[b]
pH	7.33 ± 0.06	n.s.[c]
Chemical oxygen demand (COD)	148 ± 2	130.33
Biochemical oxygen demand (BOD)	127.67 ± 1.53	128.57
Ammoniacal nitrogen (NH_3–N)	38 ± 1	26.25
Total nitrogen (TN)	49.17 ± 1.99	28.46
Phosphate (PO_4^{3-})	18.30 ± 1.08	1.92
Total phosphorus (TP)	27.46 ± 0.74	n.s.
Total suspended solids (TSS)	111.33 ± 2.31	111.33
Volatile suspended solids (VSS)	60.67 ± 3.06	n.s.
Total dissolved solids (TDS)	685 ± 14.53	593.50

[a]All parameters unit in mg L^{-1} except pH.
[b]Source: Al-Jlil [34]
[c]n.s. = Not specified.

Table 2 Operational conditions of SBR and characteristics of AGS cultivated

Conditions and characteristics	AGS_{50} – real domestic wastewater
Organic loading rate (kg COD m^{-3} d^{-1})	0.6
Temperature (°C)	50 ± 1
Total cycle time (h)	3
Aeration time (min)	110
Air flow (m^3 h^{-1})	0.6
Highest average diameter at cycle-202, day-82 (mm)	5.55 ± 0.22
MLSS (g L^{-1})	5.8 ± 0.02
MLVSS (g L^{-1})	4.13 ± 0.03
SVI_5 (mL g^{-1})	73.37 ± 0.28
COD removal efficiency (%)	84.4
NH_3–N removal efficiency (%)	99.6
TP removal efficiency (%)	81.7

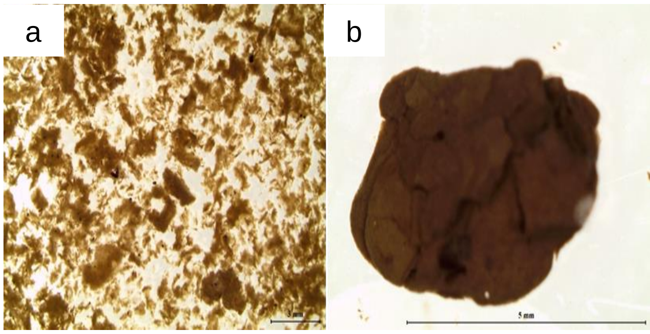

Figure 2 Images of the development of aerobic granular sludge in SBR fed with real domestic wastewater at 50 ± 1 °C. (a) Inoculum sludge during early stage of experiment (magnification: 6.7x); (b) Aerobic granular sludge cultivated after 202 cycles (82 days) of operation (magnification: 20x)

Observation of the seed sludge using SEM showed a regular flocculent activated sludge with feathery structure surrounded by fluffy edges. The sludge volume index (SVI_5) for the introductory seed was 178.5 mL g^{-1}, which indicated a poor settling ability. At the beginning of the experiment, the bioreactor underwent an almost complete washout of the suspended biomass. The system was incapable of maintaining an optimum concentration of biomass in the reactor because of the short settling period applied to the system, and the foaming conditions associated with real domestic wastewater. The remaining particles in the reactor also exposed clearly notable microcolonies for consortium on the surface of the granules (see Figure 3a).

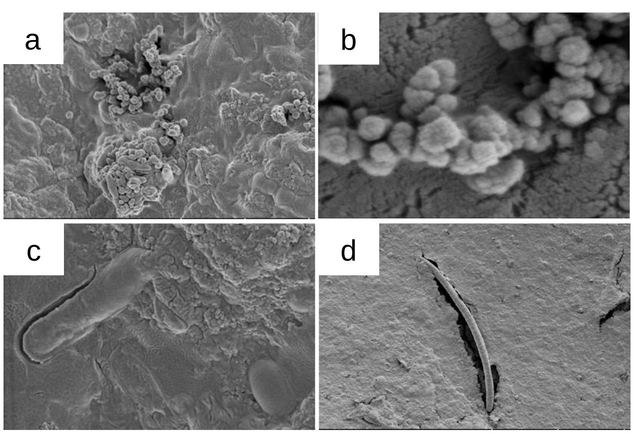

Figure 3 SEM images of the developed aerobic granular sludge. (a) Cluster of microorganisms tightly linked to one another (magnification: 13000x). (b) Cocci colonies on granule surface (magnification: 25000x). (c) Rod-shaped bacteria on AGS (magnification: 25000x). (d) Presence of filamentous bacteria as a backbone support to AGS structure (magnification: 180x)

The mature granules obtained in the system also revealed that the outer layer comprised of a dense mixture of bacteria. These results are possible because of the limited diffusion and mass transport of nutrients from outside towards both the inside layer and centre of the granule causing certain starved bacteria cells to die, and which was then consumed by the outer bacteria [20]. These bacteria occur in a large variety in terms of morphologies. The developed mature granule composed of the microstructure of cocci and rods microorganisms (see Figures 3 b and c).

The growth of aerobic granules on real domestic sewage at high temperature also exposed filamentous structures (see Figure 3d), which could be due to the presence of COD during the aeration phase. In general, aerobic granules cultivated in the bioreactor often received consistent influent organics concentration in terms of chemical oxygen demand, COD. Carbohydrates for instance acetate, citric acid, glucose, and other readily biodegradable organics can promote the growth of filamentous organisms. Even though readily degradable substrates such as acetate were taken up during the non-aerated feeding phase, yet complex substrates might still be available during the aerobic phase [24]. This could lead to a boost in the growth of filamentous heterotrophic organisms [41, 42]. Furthermore, in this study, low-strength domestic wastewater used as influent to feed the granules has low substrate concentration. Filamentous microorganisms are slow growing, which means they have very low Monod affinity constant (Ks), and maximum specific growth rate (μmax) [43]. Based on the kinetic selection theory, at low substrate concentration, filamentous microorganisms would reach higher substrate removal rate compared to the floc-forming microorganisms that prevail at high substrate concentration [43]. Additionally, at low substrate conditions, compact granules can transform to become more open and filamentous [43]. Under these circumstances, substrate concentration exerts a double-stress through a low level of substrate in the liquid phase and a steep gradient of substrate concentration within the granule [43]. These factors (substrate concentration and concentration gradient) possibly also encouraged filamentous growth in aerobic granules in the bioreactor. Even though filamentous growth normally occurred in aerobic granules in the bioreactor, low-levels and moderate-levels of filamentous growth do not cause operational problems, and may even stabilise the granules structure because they act as a backbone that strengthens the structure of aerobic granules [13].

3.2 Biomass Profile and Settling Characteristics

Figure 4 shows the profile of SVI_5 and biomass concentration for the experimental duration of 202 cycles (82 days). During the initial stage of the experiment, a large amount of inoculum sludge was washed out from the bioreactor due to the poor settling layouts, and the inoculum sludge was possibly in the acclimatization phase to its new condition (i.e., the high temperature and the mode of bioreactor system). Othman *et al*. [22] described a similar situation when cultivating aerobic granules using real livestock wastewater at room temperature (27 – 30 °C). Ebrahimi *et al*. [30] also reported a similar condition during the formation process of aerobic granules with synthetic wastewater at 20 – 30 °C. During the first few cycles, MLSS dropped from 3.7 to 2.2 g L^{-1} basically because of the short settling period and short effluent withdrawal from the bioreactor. After 24 cycles (20 days) of operation, the MLSS increased rapidly from 3.6 to 5.0 g L^{-1}, this data proved improvement of biomass concentration in the bioreactor. In addition, flocculent sludge attached to the wall of the bioreactor played an assisting role in preventing solid biomass washout. During this stage, the microorganisms reacted with real domestic wastewater at a higher rate, and sludge was seen to be foaming at the top of the reactor, probably due to young sludge age and high aeration rate. After 82 cycles (62 days) of operation, MLSS had gone through massive washout and consequently, MLSS decreased from 4.5 to 3.9 g L^{-1}, apparently as a result of evolution from flocculating to granular sludge. The MLSS started to increase gradually again after 106 cycles (67 days) of operation when small granules (2.0 – 3.0 mm) were identified in the bioreactor and MLSS reached to 5.8 g L^{-1} at the final stage of the experiment.

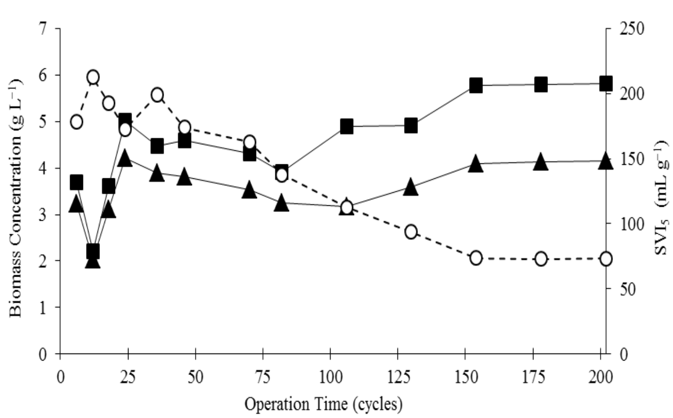

Figure 4 Profiles of biomass concentrations and SVI_5 in the SBR at 50 ± 1 °C for 202 cycles (82 days): MLSS concentration (■), MLVSS concentration (▲) and SVI_5 (○)

Normally a great number of microorganisms from biomass in the bioreactor activated the formation of aerobic granules. Based on this study, it could be seen in Figure 4 that the MLVSS also has a similar trend as those of MLSS (3.2 – 4.1 g L^{-1}). The findings of Val del Río *et al*. [44] indicate that biomass activity in the bioreactor could be sustained when stable granules were formed. Abdullah *et al*. [20] also stated that stable concentration of biomass meant that the excellent accumulation of biomass in the bioreactor. Excellent biomass concentrations during the experiment could have triggered high collisions of particles, and hence increased the shear stress on the aerobic granules [45] whereas lack of shear stress on granules could cause irregularities and low density [13, 46]. Additionally, irregularity was expected to be less common in the formation process of aerobic granules due to the occurrence of more collisions in the system [24].

The settling features could be expressed in terms of SVI_5. During 36 cycles (33 days) of inoculation, the SVI_5 values displayed some fluctuation probably because of microorganisms that were adapting slowly to their current condition (e.g., the mode of SBR operation). When the aerobic granules began to develop after 46 cycles (40 days), the SVI_5 gradually reduced from 173.8 to 73.4 mL g^{-1} during cycle 202 (on day 82). The same trend and finding were reported by Song *et al*. [29] in treating synthetic wastewater at 25 – 35 °C, in which SVI decreased steadily during the granules formation process. Previous literature reported that dense granules with great compactness could be attained at the end of the experimental period when SVI value was low [47]. Hence, the biological treatment of wastewater could be enhanced with the presence of aerobic granules with high settling attributes.

3.3 Removal Efficiencies of Aerobic Granular Sludge

3.3.1 Chemical Oxygen Demand Removal

The performance of AGS in the removal of organic matter (in terms of COD) from wastewater at high temperature is shown in Figure 5. At the beginning of the experiment, the effluent COD concentration increased from 82 mg L^{-1} to 117 mg L^{-1}, probably because of the adapting stage of the inoculum sludge to its new environment (e.g., the mode of SBR system and at high temperature). The effluent quality was not good as the removal performance was about 16.4 % during the first few cycles, and the COD removal performance was unstable. However, when the transition of flocculent sludge into granular sludge occurred in the bioreactor system, the removal increased steadily between 20.6 % and 77.1 %. Afterwards, the COD removal performance reached up to 84.4 % at the end of the experiment that demonstrated excellent biological activity during aerobic degradation process of real domestic wastewater at a high temperature of the microbes in the bioreactor. These results are comparable with previous research conducted by Song *et al*. [29] who also reported COD removal performance of above 80 % after 50 days of operation in the treatment of synthetic domestic wastewater using aerobic granular sludge at 25 – 35 °C.

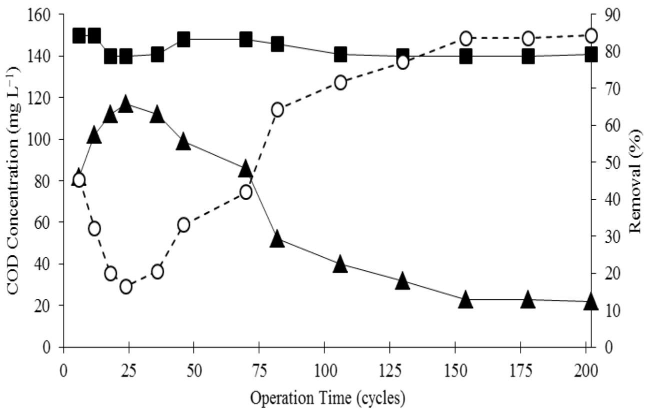

Figure 5 Profile of COD removal performance in the SBR at 50 ± 1 °C within 202 cycles (82 days): Influent (■), Effluent (▲) and Removal percentage (○)

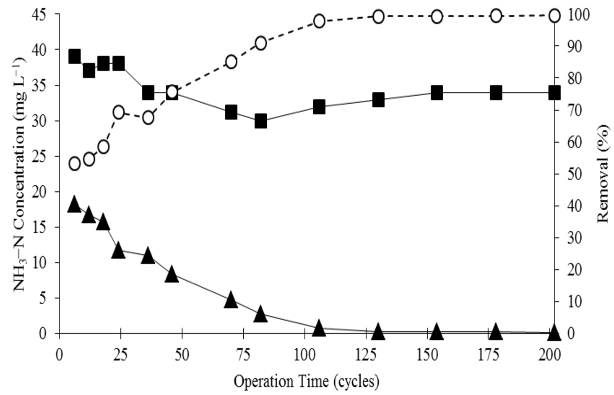

Figure 6 Profile of NH_3–N removal performance in the SBR at 50 ± 1 °C within 202 cycles (82 days): Influent (■), Effluent (▲) and Removal percentage (○)

3.3.2 Ammonia and Total Phosphorus Removal

At the beginning of the bioreactor start-up, the removal efficiency of ammoniacal nitrogen was 53.2 % and then improved when granules started to form (see Figure 6). Subsequently, the effluent ammoniacal nitrogen concentration decreased gradually from 18.2 mg L^{-1} to 11.7 mg L^{-1}. The ammoniacal nitrogen removal efficiency kept increasing slowly and became stable afterwards. In the formation process of aerobic granular sludge, the effluent ammoniacal nitrogen concentration demonstrated a good effluent quality, and remained below 10 mg L^{-1} which proved an excellent removal efficiency of ammoniacal nitrogen by AGS. After 202 cycles (on 82 days) of operation, the highest removal efficiency for ammoniacal nitrogen reached about 99.6 %. Nitrification process in the bioreactor system can be enhanced by the nitrifying bacteria community within the aerobic granules which became predominant after the biodegradation of organics. Based on the findings of Belmonte *et al*. [48], granules formation process could encourage the proliferation of nitrifying bacteria and this would improve the nitrification process in the bioreactor system which could contribute to ammoniacal nitrogen removal above 90 %, and this was the exact case in our study. The sufficient amount of oxygen supplied in the bioreactor system also allowed for good oxidation of ammoniacal nitrogen during the aerobic reaction phase to establish an effective nitrification process.

Figure 7 shows phosphorus removal. As can be seen in Figure 7, phosphorus removal rate was unsteady during the first few cycles of SBR operation. However, the removal rate of phosphorus increased gradually after 36 cycles (33 days) of granulation at 50 ± 1 °C, and achieved about 81.7 % at the end of the experiment. In this study, the results of removal efficiency of the phosphorus were similar to the results obtained by de Kreuk *et al*. [49]. Since the SBR system operation consisted of feeding, aeration, settling and discharge phases, microbial growth in the bioreactor was unstable especially during the early stage of the granulation process. Some studies reported that the alternation of non-aerated/aerated phase in the bioreactor could favour the AGS formation and stability [49, 50]. The non-aerated feeding phase could have promoted the stability of granule system by selecting the slow-growing microorganisms, for example, PAOs [13]. Thus, it is believed that AGS formation could hypothetically benefit through the cultivation of PAOs. While during the aerated phase, all the typical biological nutrient removal reactions could occur simultaneously [49]. This is possible because of oxygen gradient inside the granule, resulting in an aerobic outer shell and a core that is anoxic/ anaerobic. PAOs grow in the outer shell due to the high DO concentration from the bulk liquid and utilise the residual oxygen in the solution for phosphorus removal [27, 50, 51].

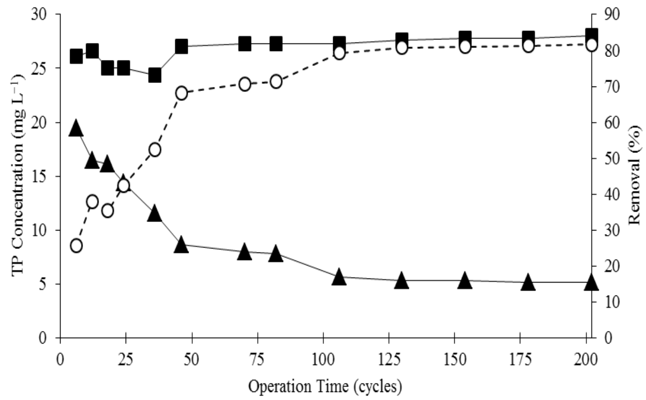

Figure 7 Profile of TP removal performance in the SBR at 50 ± 1 °C within 202 cycles (82 days): Influent (■), Effluent (▲) and Removal percentage (○)

3.4 Challenges and Strategies for Cultivation Aerobic Granules with Real Domestic Sewage at High Temperature

Real domestic sewage commonly has a much lower COD value by comparison to industrial sewage. Hence, it was expected that low-strength sewage used in this study would affect the granulation process or long start-up of the aerobic granular system from activated sludge [52, 53]. Thus, in this experiment short cycle time of 3 hours was applied to increase the organic loading rate (OLR) so that build-up of granular biomass could be sped up or otherwise, the system might be hampered if the sewage was too diluted or cycle time was too long. In addition, the non-aerated feeding that was implemented during the influent feeding phase also played an assistance role of the system to encourage the growth of slow-growing microorganisms such as nitrifiers, PAO and GAO so that optimum capacity of the required microorganisms was enabled to achieve in the system [13,18].

Aerobic granular sludge cultivation was a competitive process between loose bioflocs and compact aggregates under certain fastidious pressure, which was affected by many operation conditions such as hydraulic retention time (HRT), organic loading rate, cycle time, and settling time [25]. Inoculation reactor with aerobic granules as seeds has been proven to assist and accelerate the start-up process of the aerobic granular system, and even overcome the sensitivity of granule formation process under operation conditions during the start-up period [36, 54]. Even though this study demonstrated the capability of activated sludge to start-up aerobic granular sludge system with real domestic sewage at high temperature, it is advisable to use aerobic granules as seeds for quick start-up of the bioreactor to treat low-strength domestic wastewater. Moreover, previous literature by Liu *et al*. [25] also described that it was much easier for domestic wastewater to be acclimated to both nitrification and COD removal by using acetate-fed granules as seeds.

In addition, inoculum sludge used in this study was taken from the wastewater treatment plant (WWTP) located in the Madinah city, Saudi Arabia with daytime temperatures averaging about 40 – 50 °C , and nights about 28 °C, it was an advantage that the inoculum sludge used was well-adapted to high temperatures. The presence of thermophilic microorganisms in the inoculum sludge that were well-adapted to the high temperature condition had a significant advantage in shortening the period of acclimatization phase during the aerobic granular sludge growth. As a result, this study succeeded to develop aerobic granular sludge with the start-up of the bioreactor system directly at high temperature condition (50 ± 1 °C). Without the presence of thermophilic microorganisms in the inoculum sludge that are well-adapted to the high temperature condition, it will require a longer period of acclimatization phase during the aerobic granular sludge growth. This is because the bioreactor system has to be started-up at a lower temperature before being gradually increased to a higher temperature. Some of the previous work by de Kreuk *et al*. [26] and Ebrahimi *et al*. [30] had also demonstrated this approach in more detail.

Although the inoculum sludge used as seeds in this study had gone through a little more change of acclimatization phase due to the change of conditions (e.g., the mode of SBR system and high temperature), the inoculum sludge was still capable to adapt to high temperature in treating real domestic wastewater. This was important for the practical application of aerobic granular sludge development at high temperature.

4.0 CONCLUSION

The major goal of this study was to evaluate the possibility of aerobic granular sludge (AGS) formation using the SBR system in treating real domestic wastewater at high temperature (50 ± 1 °C). Our results indicate that the formation process was achieved, and the characteristics of aerobic granules based SBR process can be appropriate on the granules formation, stability and settling properties of the biomass in the bioreactor as well as nutrient removal during the treatment of low-strength domestic wastewater at high temperature. It is believed that the main possible reason for the good performance achieved by the AGS was due to the inoculum sludge used to seed the bioreactor was well-adapted to high temperature. Hence, the results from this lab-scale study may support the potential application of the AGS technology in the future especially in hot climate zones for example in Saudi Arabia. Three major conclusions were drawn from this study.

Firstly, stable mature granules were cultivated in the bioreactor with diameters between 2.0 and 5.0 mm. Secondly, The biomass concentration was 5.8 g L^{-1} for MLSS and 4.1 g L^{-1} for MLVSS while the SVI_5 was 73.4 mL g^{-1} indicating good biomass accumulation in the bioreactor and acceptable settling layouts of aerobic granular sludge. Lastly, COD and ammoniacal nitrogen removals were achieved at a maximum of 84.4 % and 99.6 %, respectively while the removal efficiency for phosphorus was up to 81.7 %.

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