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CULTIVATION AND CHARACTERISTICS OF AEROBIC GRANULAR SLUDGE FOR SIMULTANEOUS ORGANICS AND NUTRIENTS REMOVAL PERFORMANCES AT HIGH TEMPERATURE

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Abstract: With inoculum sludge from a conventional activated sludge wastewater treatment plant, a sequencing batch reactor fed with synthetic wastewater was operated at 50 ± 1 °C to study the formation of aerobic granular sludge (AGS) for simultaneous organics and nutrients removal with a complete cycle time of 3 h. The AGS were successfully cultivated with excellent settling ability and demonstrated exceptional performance in the organics and nutrients removal with influent loading rate and COD/N ratio of 1.6 kg COD m^{-3} d^{-1} and 8.3, respectively. Stable, regular, dense and fast settling granule (average diameter, 2.0 mm and sludge volume index, 44.73 mL g^{-1}) were developed in a single reactor. In addition, 85% COD removal efficiency was observed in the system at the maturation stage of the granulation, while its ammonia nitrogen and total phosphorus removal efficiencies were up to 88% and 70%, respectively. The study demonstrated the capabilities of AGS formation in a single, high and slender column type-bioreactor at high temperature which is suitable to be applied for hot climate and low humidity condition (e.g. Saudi Arabia).

Keywords: *Aerobic granular sludge; sequencing batch reactor; high temperature*

1.0 Introduction

Domestic wastewater treatment in urban areas is one of the crucial elements to be count in the development of a country in order to sustain individual's health and welfare. Untreated wastewater can cause spreading of disease in the form of several types of endemic and epidemic illnesses (Al-Rehaili, 1997). There are various kinds of wastewater treatment applications nowadays ranging from modest, low priced and less efficient processes to very advanced, highly efficient and pricey operations. The selection among these processes should acknowledge local area circumstances such as climate and weather, social attributes, economy, availability of enforceable standards, availability of land and power, demanded operation skills and its applicable, monitoring actions, effluent discharge options as well as effluent reuse applications and conditions (Al-Rehaili, 1997). Several types of the widely used technologies at present for wastewater treatment including activated sludge process (ASP), sequencing batch reactor (SBR), up-flow anaerobic sludge blanket reactors associated with facultative aerobic lagoon (UASB–FAL) and constructed wetlands (CWs) (Kalbar *et al*., 2012).

Aerobic granular sludge (AGS) has been widely studied in these recent years. AGS is made up of a dense cluster of symbiotic organisms, with good biological activity performance and excellent mass transfer efficiency. Aerobic granular sludge-based reactors represent an appealing option over conventional activated sludge systems due to their small footprint and low excess sludge production (de Bruin *et al*., 2004). The sludge developed in such systems acquires high biomass concentration, better settling properties, high chemical oxygen demand (COD) removal efficiency, and good phosphorus removal capacity (de Kreuk *et al*., 2005a). In addition, simultaneous nitrification–denitrification can occur simultaneously in granules due to the bulk oxygen concentration and granule size (Beun *et al*. 2001; Mosquera-Corral *et al*. 2005). Aerobic granular sludge has mainly been cultivated using sequencing batch reactor (SBR) systems, some using airlift or bubble column reactors. Several studies within lab scale were broadly demonstrated to identify the most crucial aspects influencing the development of aerobic granular sludge such as organic loading rate, settling time, hydrodynamic shear force and substrate composition (Adav *et al*., 2008). However, the formation of aerobic granular sludge is a challenging ecological process, in which many components need to be further inspected.

In the aerobic granular sludge technology, temperature was a crucial factor which contributed to the efficiency and rates of bioconversion in the system. At high temperature, the effluent quality of the treated wastewater might be affected likewise the organics and nutrients removal performances. In most studies on aerobic granular sludge, SBRs (AGS-SBR) have been operated at ambient temperature, e.g., 20–25 °C (Morgenroth *et al*., 1997; de Kreuk and van Loosdrecht, 2004; Whang and Park, 2006) or lower (de Kreuk *et al*., 2005b). Because detailed information of the high temperature effects on aerobic granulation is limited, it becomes main purpose of the present study to investigate the granulation process, stability, density and performances of aerobic granules at high temperature as high as 50 °C.

In this article, aerobic granulation was cultivated in SBR. The morphology of granular sludge, their settling properties and treatment efficiencies were also discussed. The aim of this study is to enrich the knowledge of cultivation procedure, and to encourage the application of aerobic granular sludge in wastewater treatment.

2.0 Materials and Methods

2.1 Experimental Procedure and Rector Operation

Experiments were carried out in a double-walled cylindrical column bioreactor (internal diameter of 6.5 cm and total height of 100 cm) with a working volume of 3 L. During the start-up period, 1.5 mL of activated sludge from a municipal sewage treatment plant was added into the bioreactor system as inoculums in the bioreactor. The bioreactor was operated in SBR mode at a cycle of 3 h: 60 min of feeding, 110 min of aeration, 5 min of settling and 5 min of effluent withdrawal. A set of two peristaltic pumps were used to feed and to discharge the wastewater in the reactor system. During the filling phase, wastewater was introduced through ports located at the bottom of the bioreactor. While fine air bubbles for aeration were supplied by means of air bubble diffusers were placed at the bottom. The effluent was withdrawn through the outlet ports positioned at medium height in the column bioreactor which had a volumetric exchange ratio (VER) of 50 %. The sludge retention time was set by the discharge of total suspended solids (TSS) with the effluent. The bioreactor was operated at the temperature of 50 ± 1 °C, using water bath sleeves and a thermostat.

Measurements of the parameters such as mixed liquor suspended solid (MLSS), mixed liquor volatile suspended solid (MLVSS), chemical oxygen demand (COD), ammonia nitrogen (NH_3-N) and total phosphorus (TP) were carried out according to Standard Methods for the Examination of Water and Wastewater (APHA, 2012). The sludge volume index (SVI) method was performed according to the procedure done by de Kreuk *et al*. (2005b). A stereo microscope equipped with digital image analyser (PAX-ITv6, ARC PAX-CAM) was used to examine the morphological and structural observations of aerobic granules at regular intervals.

2.2 Synthetic Wastewater Characteristics and Seed Sludge

The reactors were fed with the same medium as the one used by de Kreuk *et al*. (2005a). It was prepared as two stock solutions that were mixed with distilled water prior to feeding. Solution A contained sodium acetate (65.1 mM), $MgSO_4 \cdot 7H_2O$ (3.7 mM) and KCl (4.8 mM). Solution B contained NH_4Cl (35.2 mM), K_2HPO_4 (4.4 mM), KH_2PO_4 (2.2 mM), and 10 mL L^{-1} trace element solution. The trace element stock solution contained: EDTA 342.2 mmol L^{-1}, $ZnSO_4 \cdot 7H_2O$ 15.3 mmol L^{-1}, $CaCl_2 \cdot 2H_2O$ 111.3 mmol L^{-1}, $MnCl_2 \cdot 4H_2O$ 51.1 mmol L^{-1}, $FeSO_4 \cdot 7H_2O$ 35.9 mmol L^{-1}, $Na_2Mo_7O_{24} \cdot 2H_2O$ 2.7 mmol L^{-1}, $CuSO_4 \cdot 5H_2O$ 12.6 mmol L^{-1}, and $CoCl_2 \cdot 6H_2O$ 13.5 mmol L^{-1}. Per cycle, 150 mL of both solutions were added to the reactor together with 1200 mL of tap water.

Activated sludge was used as inoculums, which were taken from Al Madinah Sewage Treatment Plant in Saudi Arabia. The amount of inoculum was about 1.5 L, with a mixed liquor suspended solid (MLSS) concentration 13.7 g L^{-1} and a mixed liquor volatile suspended solid (MLVSS) concentration 9 g L^{-1}. The seed sludge appeared fluffy, irregular and loose structure with dark brown color.

3.0 Results and Discussion

3.1 Aerobic Granular Sludge Formation and Morphology Observation

During the early stage, seed sludge appeared as a fluffy, irregular, loose-structure morphology and rich with filamentous organisms under microscopic examination as shown in Figure 1. The sludge color progressively turned from dark brown to light yellowish brown throughout the experimental period due to the washout of the microorganisms and low concentrations of biomass because of poor settling properties of the young sludge. During the initial stage of granulation, the loose flocs were also easily broke up into small pieces if placed under vigorous shaking.

After few days, the flocs-like sludge started to disappear and changed to small granules with average diameter 0.9 mm. Under high shear force, the flocs became denser causing the aggregation of the biomass to secrete more exopolysaccharides (EPS) (Dulekgurgen *et al*., 2008). Interactions between inter-particle bridging process among EPS, microbial cells and ion contributes to the evolution of seed sludge from flocs to granules (Sheng *et al*., 2010). EPS can promote the cell hydrophobicity and change the surface charges on the microorganisms (Zhu *et al*., 2012), which can increase microbial cell adhesion and granulation.

In the following weeks, the small granules became more regular in shape and progressively increased in size, while more flocculent sludge washed out from the bioreactor, resulting in the accumulation of the aerobic granules with high settling ability. Finally, mature granules formed after 30 days of inoculation with average diameter of 2.0 mm. The compact mature granules were smooth with a solid surface and contribute to a stable operation of the bioreactor.

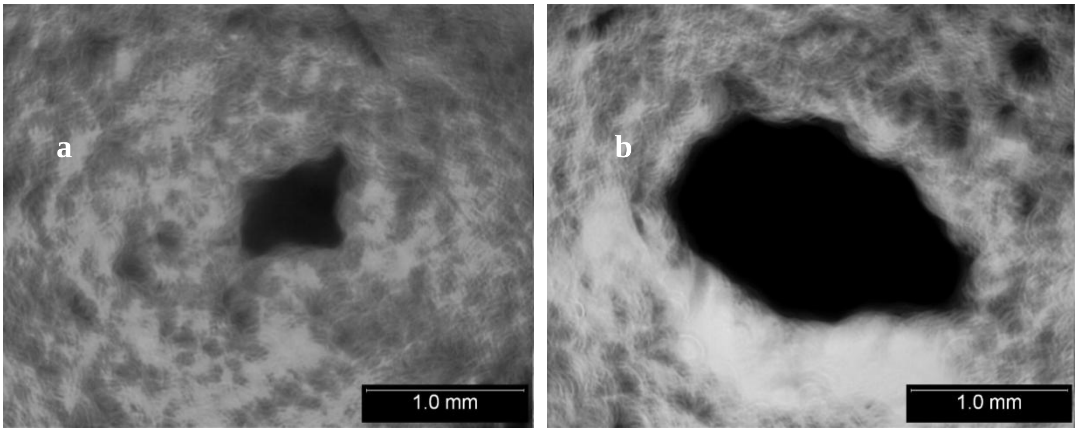

Figure 1: Images of granules at 50 °C after startup at (a) day-15 and (b) day-30

3.2 Biomass Profile and Settling Properties Of Granules

Figure 2 displays the MLSS, MLVSS and SVI progression in the SBR system from the first day until day-30 of the experiment. During the initial stage of the experiment, most of the sludge was washed-out from the bioreactor leading a significant decline in the biomass concentration and increased the effluent solids concentration. Figure 2 also reveals a rapid decrease of MLSS from 13.7 g L^{-1} to 3.3 g L^{-1} in the first 6 days, probably due to the short settling time applied in the cycle system. During the days between day-7 and day-14, both MLSS and MLVSS concentration kept rising but with occasional decrease perhaps due to some microorganisms of the seed sludge in the bioreactor were adapting themselves to the synthetic wastewater. The concentration of the biomass was improving as the small granules started to appear in the bioreactor on the day-15. Subsequently, both MLSS and MLVSS concentrations increased uniformly and achieved steady state at about 12.5 g L^{-1} and 10.5 g L^{-1}, respectively during day-30. The same trend was also spotted for the MLVSS content which ranging from initial concentration of 9 g L^{-1} to 10.5 g L^{-1} at the end of the experiment. The MLVSS to MLSS ratio is about 0.84 and a stable condition of biomass concentration indicates a good accumulation of biomass in the bioreactor.

Due to the increase of biomass content, the developed aerobic granules fed with synthetic wastewater at 50 °C possess a good settling ability in terms of SVI. The SVI value has improved from 119.12 mL g^{-1} at the beginning of the experiment to 44.73 mL g^{-1} upon the completion of the study. Initially, it was observed the SVI value rose until day 9 of the experiment probably because of biomass washout in the effluent, thus lowering the MLSS concentration in the reactor. Afterwards, the SVI decline slowly with the accumulation of denser and quick settling properties of biomass towards the end of the study. Mature aerobic granules possessed lower SVI value compared to the conventional activated sludge indicated the great settling attributes of the granules. Additionally, the fast settling properties of the granules encouraging well separation of biomass from the treated effluent, hence leaves a clear supernatant in the reactor system before being discharged.

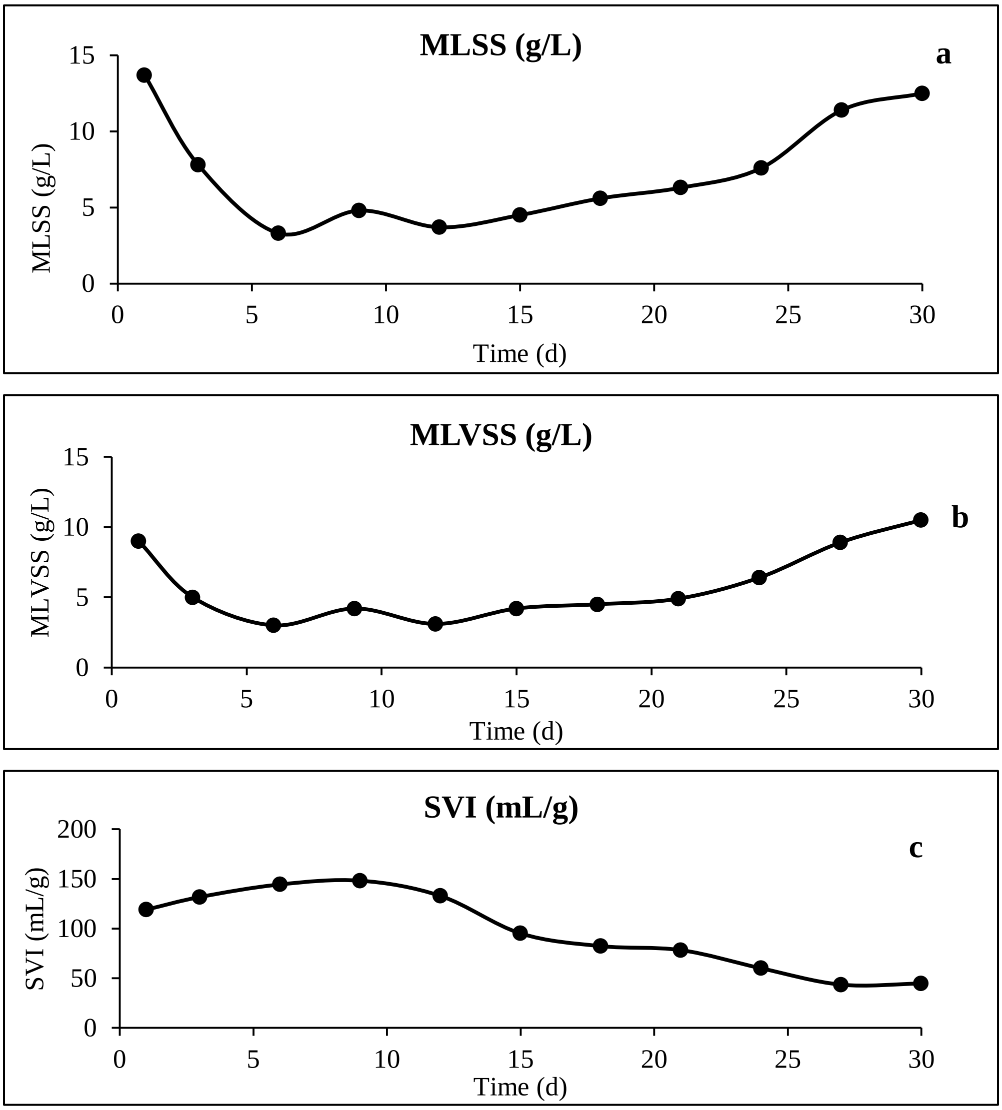

Figure 2: Variation of biomass concentrations and sludge volume index in SBR within 30 days for (a) MLSS concentration (b) MLVSS concentration and (c) SVI

3.3 Organics and Nutrients Removal Efficiencies of Granules

Figure 3 illustrates the removal rate of organics and nutrients in the SBR system with respect to COD, ammonia nitrogen and total phosphorus from beginning until the end of granules development period. At the beginning of the bioreactor operation with the concentration of influent COD was 400 mg L^{-1}, the removal rate of COD dropped from 63% to 41%, probably due to the adapting process of the sludge with synthetic wastewater. In the first 18 days, the removal rate of COD kept increasing but with sporadic decrease. Thereafter, the COD removal efficiency improved uniformly and became stable for the remaining period. When the granular sludge started to evolve from flocculent sludge in the bioreactor system, it enhanced the degradation ability for COD removal efficiency up to 85%, which is comparable to previous work done by Abdullah *et al*. (2011) in treating palm oil mill effluent (POME) and Rosman *et al*. (2013) in treating rubber wastewater using aerobic granular sludge. This result indicates the high biological activity occurred during microbial aerobic degradation process of synthetic wastewater.

The ammonia nitrogen removal efficiency was 72% at the beginning of the bioreactor operation with the concentration of influent ammonia nitrogen was 50 mg L^{-1} and then dropped significantly to 61%. Subsequently, the removal rate of ammonia nitrogen improved when granules started to form. Figure 3 reveals the removal efficiencies for ammonia nitrogen kept increasing but with occasional decrease. Afterwards, the removal efficiencies for ammonia nitrogen increased gradually and achieved steady state at about 88% during day-30. The ammonia nitrogen concentration in the effluent shows a significant better quality and maintained below 10 mg L^{-1} upon the formation of aerobic granular sludge which indicates effective ammonia nitrogen removal efficiency. Nitrifying bacteria population within the aerobic granules became predominant after the biodegradation of organics which help in nitrification process. Belmonte *et al*. (2009) stated that nitrification process could be improved by promoting the development of granules that enhance the retention of large amounts of nitrifying bacteria in the bioreactor system leading higher removal efficiency for ammonia. The sufficient oxygen level supplied in the bioreactor system enabled a good oxidation for ammonia nitrogen and more than 80% of ammonia nitrogen being removed during the aerobic reaction phase which shows a stable and excellent nitrification process happened in the system.

Figure 3 display the removal efficiency for total phosphorus was 50% at the beginning of the experiment with the concentration of influent total phosphorus was 20 mg L^{-1}. Eventually, the removal rate increased moderately and achieved around 70% during day-30. Due to the SBR operation system consists of sequencing phase of feeding, aeration, settling and discharge of effluent, microorganism growth in bioreactors are subject to occasional variations. The presence of aerobic starvation stage encourages the bioactivity of accumulating organisms for the uptake and release of phosphate. However, by comparing the results with previous work done by de Kreuk *et al*. (2005a), the removal rate of total phosphorus in this study is lower. This is possibly due to the increase of nitrite concentrations that might hinder the bioactivity of phosphorus accumulating organisms, contributing to the growth retardation, which requires further investigation.

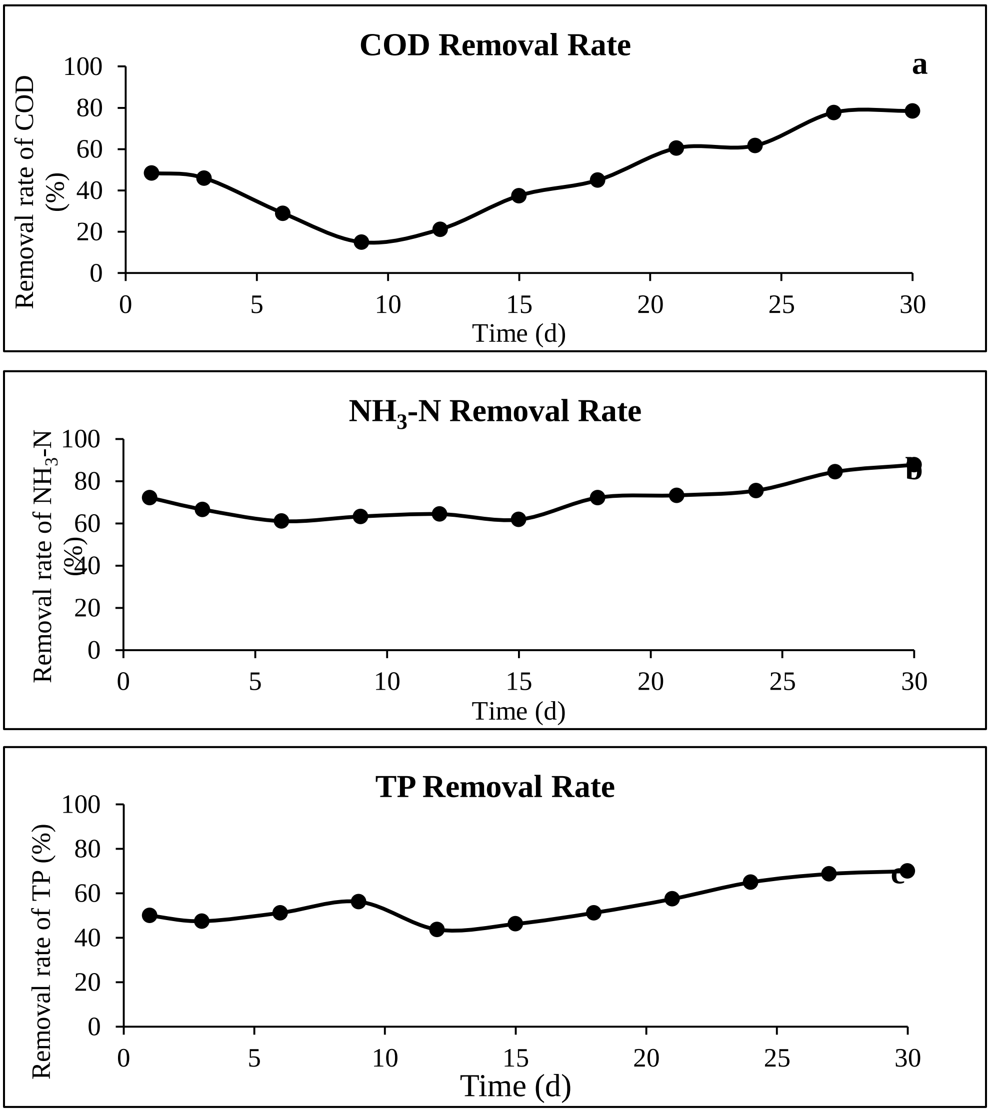

Figure 3: Profile of removal performances in the SBR system within 30 days for (a) COD, (b) ammonia nitrogen and (c) TP.

4.0 Conclusions

Development of stable and compact aerobic granules in SBR system was successful with an excellent settling ability and an average diameter of 2.0 mm fed with synthetic wastewater. A good COD removal rate of 85% was achieved after 30 days of operation with influent loading rate and COD/N ratio of 1.6 kg COD m^{-3} d^{-1} and 8.3, respectively. In addition, 88% ammonia nitrogen removal rate and 70% total phosphorus removal rate were also observed in the single bioreactor system. Therefore, the study presented herein suggested feasibility of the developed aerobic granular sludge for the treatment of domestic wastewater at high temperature of 50°C which is suitable to be applied for hot climate and low humidity condition for instance in Saudi Arabia.

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