Development of granular sludge for textile wastewater treatment

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Article info
Article history:
Received 18 January 2010
Received in revised form
22 April 2010
Accepted 2 May 2010
Available online 25 May 2010

Keywords:
Granulation
Granule characterization
Textile wastewater
Sequencing batch reactor
Color removal

Abstract
Microbial granular sludge that is capable to treat textile wastewater in a single reactor under intermittent anaerobic and aerobic conditions was developed in this study. The granules were cultivated using mixed sewage and textile mill sludge in combination with anaerobic granules collected from an anaerobic sludge blanket reactor as seed. The granules were developed in a single sequential batch reactor (SBR) system under alternating anaerobic and aerobic condition fed with synthetic textile wastewater. The characteristics of the microbial granular sludge were monitored throughout the study period. During this period, the average size of the granules increased from 0.02 ± 0.01 mm to 2.3 ± 1.0 mm and the average settling velocity increased from 9.9 ± 0.7 m h⁻¹ to 80 ± 8 m h⁻¹. This resulted in an increased biomass concentration (from 2.9 ± 0.8 g L⁻¹ to 7.3 ± 0.9 g L⁻¹) and mean cell residence time (from 1.4 days to 8.3 days). The strength of the granules, expressed as the integrity coefficient also improved. The sequential batch reactor system demonstrated good removal of COD and ammonia of 94% and 95%, respectively, at the end of the study. However, only 62% of color removal was observed. The findings of this study show that granular sludge could be developed in a single reactor with an intermittent anaerobic–aerobic reaction phase and is capable in treating the textile wastewater.

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1. Introduction

The development of aerobic granules as a novel treatment technology for wastewater has been extensively reported using sequencing batch reactor (SBR) systems. The system has been used to treat different types of wastewater and pollutants such as dairy effluent (Arrojo et al., 2004), soybean-processing wastewater (Su and Yu, 2005), nitrogen and phosphorus-rich effluent (Cassidy and Belia, 2005), phenol effluent (Carucci et al., 2009) and also municipal wastewater (de Kreuk and van Loosdrecht, 2006). The use of anaerobic granules as seeding material for aerobic granules development has recently been reported by Linlin et al. (2005).

In recent years, the ability of biodegradation for textile dyeing wastewater and dyestuffs involving both anaerobic and aerobic processes has been widely reported in the literature (Ong et al., 2005; Isik and Sponza, 2008; Franciscon et al., 2009). Color removal and complete mineralization of the dyes have been achieved through the combination of both processes. For azo dyes, the cleavage of N≡N bond, which results in the removal of color, occurs during anaerobic stage along with the generation of aromatic amines, a toxic
compound which is detrimental to human health (van der Zee and Villaverde, 2005). Under aerobic condition, mineralization of the amines takes place to complete the treatment process. Several studies have been carried out using anaerobic granular sludge but this doesn’t lead to complete removal of the dyes. Study conducted using granular sludge grown in anaerobic/aerobic system for complete dye removal is apparently missing.

Since complete dye degradation requires both anaerobic and aerobic conditions, several studies have been focused on treatment systems which utilized two different reactors to fulfill both conditions (Ong et al., 2005; Moosvi and Madamwar, 2007; Isik and Sponza, 2008). The operation of such a system is rather complicated as the anaerobic microorganisms in the anaerobic tank need to be separated before the wastewater can be pumped to the aerobic tank. To simplify the system, a study was conducted to develop microbial granular sludge that can survive and function in both anaerobic and aerobic conditions and hence requires only one reactor. Potential strict anaerobic microorganisms can survive easily since oxygen only penetrates partially in the granules during aerated phase of the process. The study focused on the development of this type of granular sludge and the effectiveness of the system in treating synthetic textile dyeing wastewater.

2. Materials and methods

2.1. Wastewater composition

Synthetic wastewater with the following composition was used: NH4Cl 0.16 g L⁻¹, KH2PO4 0.23 g L⁻¹, K2HPO4 0.58 g L⁻¹, CaCl2·2H2O 0.07 g L⁻¹, MgSO4·7H2O 0.09 g L⁻¹, EDTA 0.02 g L⁻¹ and trace solution 1 mL L⁻¹. The carbon sources used in this experiment were glucose (0.5 g L⁻¹), ethanol (0.125 g L⁻¹) and sodium acetate (0.5 g L⁻¹). The trace elements used were based on the composition recommended by Smolders et al. (1995). The composition of the trace element was H3BO3 (0.15 g L⁻¹), FeCl3·4H2O (1.5 g L⁻¹), ZnCl2 (0.12 g L⁻¹), MnCl2·4H2O (0.12 g L⁻¹), CuCl2·2H2O (0.03 g L⁻¹), NaMoO4·2H2O (0.06 g L⁻¹), CoCl2·6H2O (0.15 g L⁻¹), and KI 0.03 g L⁻¹. Mixed dyes consisted of Sumifix Black EXA, Sumifix Navy Blue EXF and Synozol Red K-4B with total concentration of 50 mg L⁻¹ was used in this study. The mixture gave an initial COD of 1270 mg L⁻¹; 1020 ADMI (American Dye Manufacturing Index) and average ammonia concentration of 38 mg L⁻¹. The pH of the synthetic wastewater was adjusted to 7.0 ± 0.5 before feeding.

2.2. Reactor set-up

The schematic representation of the reactor set-up is given in Fig. 1. A column reactor was designed based on Wang et al. (2004) and Zheng et al. (2005) with several modifications. The column was designed for a working volume of 4 L with internal diameter of 8 cm and total height of 100 cm. The wastewater was fed into the reactor from the bottom of the column. Air was supplied into the reactor by a fine air bubble diffuser also from the bottom of the column. The decanting of the wastewater took place via an outlet port located at 40 cm height from the bottom of the reactor. The mean cell residence time (SRT) was set by the discharge of suspended solids with the effluent.

2.3. Analytical methods

The morphological and structural observations of the granules were carried out by using a stereo microscope equipped with digital image processing and analyzer (PAX-ITv6, ARC PAX-CAM). The microbial compositions within the granules were observed qualitatively with scanning electron microscope (FESEM-Zeiss Supra 35 VPFESEM). The granules were left to dry at room temperature prior to gold sputter coating (Bio Rad Polaron Division SEM Coating System) with coating current of 20 mM for 45 s. Other parameters such as mixed liquor suspended solid (MLSS), mixed liquor volatile suspended solid (MLVSS), COD, color and NH4 were analyzed according to the Standard Methods (APHA, 2005).

The granules developed in the SBR column were analyzed for their physical, chemical and biological characteristics. Physical characteristics include settling velocity, sludge volume index (SVI) and granular strength. The settling velocity was determined by averaging the time taken for an individual granule to settle at a certain height in a glass column filled with tap water. The SVI assessment was carried out according to the procedure described by Beun et al. (1999). Determination of the granular strength was based on Ghangrekar et al. (2005). Shear force on the granules was introduced through agitation using an orbital shaker at 200 rpm for 5 min. At certain amplitude of the shear force, parts of the granules that are not strongly attached within the granules were detached. The quantity of the ruptured granules was separated by allowing the fractions to settle for 1 min in a 150 ml measuring cylinder. The dry weight of the settled granules and the residual granules in the supernatant were measured. The ratio of the solid in the supernatant to the
total weight of the granular sludge used for granular strength measurement was expressed in percentage as an integrity coefficient (IC). This percentage indirectly represents the strength of the granules. Smaller IC value indicates stronger granule and vice versa.

The granules were analyzed chemically for their mineral content which includes Ca$^{2+}$, Mg$^{2+}$, Na$^{+}$, K$^+$ and Fe$^{3+}$. The mineral content was determined using Perkin Elmer Analyst 400 Flame Atomic Absorption Spectrophotometer (FLAA). The microbial activity of the microbial granular sludge was conducted by measuring the oxygen utilization rate (OUR), specific oxygen utilization rate (SOUR) and specific methanogenic activity (SMA). The OUR (mg O$_2$ L$^{-1}$ h$^{-1}$) measurement was performed according to the Standard Methods (APHA, 2005). The SMA measurements were conducted according to Erguder and Demirer (2008) with several modifications where a 500 mL BOD bottle seeded with FSG with final concentration of 2 g VSS L$^{-1}$ and basal medium (250 mL effective volume). The bottle was flushed with N$_2$ gas mixture for 5 min to obtain an anaerobic condition. The bottle was then sealed with rubber septum. Acetic acid (HAc) was fed into the serum bottle at a concentration of 3000 mg L$^{-1}$. The experiments were conducted in a temperature controlled condition of 30 ± 2 °C. The production of methane gas (CH$_4$) was determined daily for 7 days using liquid displacement methods containing concentrated KOH stock solution (20 g L$^{-1}$). After each gas measurement, the bottle was manually shaken. At the end of the SMA assay, the VSS content in the bottle was measured. The SMA was calculated as the maximum CH$_4$ produced per gram of VSS per hour (mL CH$_4$/g VSS h$^{-1}$) (Zitomer and Shrout, 1998).

2.4. Experimental procedures

A mixture containing an equal volume of sludge from a municipal sewage treatment plant and a textile mill wastewater treatment plant that gave a total volume of 2 L was used in this experiment. The sludge inoculums were sieved with a mesh of 1.0 mm to remove large debris and inert impurities. The sludge mixture was acclimated for two months with 2 L of synthetic wastewater containing dye degrading microbes. The dye degrading microbes used in this study was based on the study conducted by Nawahi (2009) and Ibrahim et al. (2009). Together with the sludge mixture, about 100 mL of anaerobic granules with size less than 1 mm diameter were used as seed for the granulation process. The anaerobic granules were collected from an anaerobic sludge blanket reactor system treating paper mill industrial effluent. The MLSS of the anaerobic granules were 3.3 g L$^{-1}$. The pH during the reaction process varied in the range of 6.0–7.8 and the temperature of the reactor system was set at 30 ± 2 °C. The reactor system was operated for a period of 66 days. Two liters of the wastewater remained in the reactor after the decanting stage yielding a volumetric exchange rate (VER) of 50%. 20 mL of sample from the influent and effluent (wastewater released after decanting stage) of the reactor system were collected for the measurement of COD, ammonia and color removal (Fig. 1).

3. Results and discussions

3.1. Biomass profile

The change in biomass concentration (i.e. MLSS) from the start-up until the end of the study is shown in Fig. 2. During the first few days of the experiment, almost half of the sludge was washed-out from the reactor causing a rapid decrease in the biomass concentration. The MLSS reduced from initial concentration of 5.5 g L$^{-1}$ to 2.9 g L$^{-1}$ mainly due to the short settling time used in the cycle (i.e. 5 min). During this initial stage, the anaerobic granules were also observed to disintegrate into smaller fragmented granules and small debris resulted from shear force caused by the aeration during the aerobic stage. These small fragments have poor settling ability and were washed out from the reactor. This caused an increase of the suspended solids concentration in the effluent as shown in Fig. 2. As the experiment continued and granules with adapted biomass were formed, the concentration of the biomass in the reactor increased and finally reached 7.3 ± 0.9 g MLSS L$^{-1}$ when the experiment was discontinued on the 66th day. The MLVSS followed the same trend as MLSS,

![Fig. 2 – Change in biomass concentration during the formation of granular sludge in the SBR. (●) MLSS, (□) MLVSS, (♦) Suspended solid in the effluent.](image-url)
ranging from 1.9 ± 0.5 g L⁻¹ to 5.6 ± 0.8 g L⁻¹. The SRT also increased from 1.4 days at the initial stage to 8.3 days on the 66th day, indicating the accumulation of the biomass in the reactor.

3.2. Bioactivities of the granules

A typical DO concentration profile for one complete cycle and the OUR profile during both of the aerobic reaction phases are shown in Fig. 3. Stage PI and PII show the first and second stage of anaerobic reaction phase, respectively. At these anaerobic stages, most of the dye degradation is expected to occur where amines, as the byproduct, were released (Sponza and Isik, 2005). Stage PII and PIV represent the first and second stage of aerobic reaction phase, respectively. Most of the substrates provided to the reactor system were anticipated to be consumed within a few minutes of the first aerobic reaction phase (PII), known as the feast period. During the feast period, the DO concentration in the reactor was low (about 4 mg L⁻¹). The high utilization of DO during the feast period was also indicated by the high OUR which was 281 mg L⁻¹ h⁻¹. The amines, which were produced during anaerobic reaction phase (PI), were mineralized under this aerobic condition (PII) as they cannot be further degraded under anaerobic phases (Sponza and Isik, 2005).

When all the carbon sources (substrate and amines) in the wastewater were utilized, endogenous respiration process took place, which is referred as the famine period. The transition from the feast to famine phase was clearly observed with the drastic increase of the dissolved oxygen and the extreme drop of the OUR within few minutes of the aerobic reaction phase (PII). The DO concentration immediately increased to around 7.0 mg L⁻¹ which was closed to the DO saturation level. The OUR also reduced to 14 mg L⁻¹ h⁻¹ indicating low utilization of DO.

Since there was no addition of substrate during the second aerobic reaction phase (PIV), the consumption of DO during this phase was also low. This is shown by high DO level reaching saturation value of 7.6 mg L⁻¹. A sharp increase in the OUR was observed at the beginning of this phase but at a lower value than the one observed in Stage PII. Apparently, the residual dyes which were not degraded in Stage PI and PII were transformed into smaller molecules (e.g. amines) during the second stage of the anaerobic phase (PIII). These smaller molecules were further mineralized in Stage PIV which resulted in a sharp increase in the OUR. As the concentration of these molecules were reduced, the OUR also became lower until it reached a minimum of 11 mg L⁻¹ h⁻¹ Table 1 shows the OUR value during both of the aerobic reaction phases.

The SOUR of the microbial granular sludge was determined before the termination of the experiment. The SOUR was 51.1 ± 6.8 mg DO g⁻¹ VSS h⁻¹. This value was slightly lower than those of the aerobic granules reported by Tay et al. (2001) which ranged from 55.9 to 69.4 mg DO g⁻¹ VSS h⁻¹ and higher than the coupled granules reported by Erguder and Demirer

![Fig. 3](image.png)

**Table 1** – The OUR levels during the aerobic reaction phase of one complete cycle.

<table>
<thead>
<tr>
<th>Aerobic reaction phase</th>
<th>OUR (mg L⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st stage (PII)</td>
<td>281 ± 39</td>
</tr>
<tr>
<td>2nd stage (PIV)</td>
<td>167 ± 51</td>
</tr>
<tr>
<td>Begin react</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>End react</td>
<td>11 ± 2</td>
</tr>
</tbody>
</table>
(2005) (6–47 mg DO g⁻¹ VSS h⁻¹). The specific methanogenic activity (SMA) of the microbial granular sludge is lower (10.3 mL CH₄ g⁻¹ VSS h⁻¹) than the one reported by Erguder and Demirer (2005) (14–42 mL CH₄ g⁻¹ VSS h⁻¹). However, despite the low SMA emission, it provides the evidence of the existence of methanogens within the microbial granular sludge. Obviously, the granular sludge offered the methanogens sufficient protection from the toxic oxygen concentration in the bulk liquid.

3.3. Morphology of granular sludge

A week after inoculating the reactor, visual and microscopic observations of granule formation were made. At this stage, the developed granules were composed mostly of loosely clumped sludge which could easily break up into pieces if placed under vigorous shaking. Within a week, the anaerobic seed granules had undergone morphological changes from spherical in shape and black in color with average diameter of 1 mm into smaller grey granules. It is likely that the sulfides in the anaerobic sludge were oxidized due to exposure to the aerobic condition. On day 30, two different types of granules were clearly observed in the reactor as shown in Fig. 4.

Fig. 4a shows mainly irregular shaped, yellow colored granules (Type A) that are solely developed from the activated sludge. In Fig. 4b, the anaerobic granules that have fragmented into smaller pieces have formed different sizes of granules (Type B) that contained pieces of anaerobic granules. The outer layer of the latter were yellow in color indicating the domination of aerobic or facultative microorganisms while the darker spots within the granules indicate the presence of anaerobic fragments originated from anaerobic granules. The formation of Type A granules could be elucidated by the mechanisms explained by Beun et al. (1999). The development was initiated from the mycelial pellets that were retained in the reactor due to high settling velocity. These mycelial pellets eventually become the support matrix for the bacteria growth. Bacteria that were able to attach to this matrix were retained and suppressed the filamentous growth and became the dominant species in the reactor.

The formation of Type B granules has been discussed by Linlin et al. (2005). These granules were formed through a series of physical and morphological changes. The anaerobic granules initially disintegrated into smaller size flocs and debris when exposed to aeration forces in the SBR column. Some of the granules and debris that were too small were washed-out with the effluent while the heavier ones were retained in the column and acted as nuclei for the formation of the aerobic granules. This type of granules that consisted of combination of aerobic and anaerobic portions within the granules could increase the possibility of degradation process that requires both aerobic and anaerobic conditions for complete degradation particularly for textile wastewater that contains azo-dyes.

Fig. 4c shows the sludge particles during the initial stage of the experiment with an average size of 0.02 ± 0.01 mm while Fig. 4d shows the granules at the final stage (day 66) of the experiment with the average particle diameter size of 2.3 ± 1.0 mm with maximum size reaching up to 4 mm.

Fig. 4 – Morphological development of granular sludge from anaerobic granular sludge and aerobic activated sludge. Pictures were taken using stereo microscope with magnification of 6.3 ×, Scale bar equals to 1 mm. (a) Granules developed from the activated sludge. (b) Granules developed from anaerobic granules patches. (c) Sludge particles during early stage of experiment. (d) Microbial granular sludge at the 66 days of the experiment.
The microstructure of the microbial granular sludge was examined using SEM and shown in Fig. 5. The SEM observation of the mature granules shows the domination of non-filamentous coccoid bacteria that are tightly linked and embedded to one another and form a rounded shape on the surface of the granule, covered with extrapolysaccarides (EPS) (Fig. 5a). The absence of filamentous bacteria in the developed granules may be due to the experimental conditions that did not favor the growth of filamentous bacteria at such high concentration of DO during aerobic phase (i.e. 7.0 ± 0.5 mg L⁻¹) and considerably high organic loading rate (2.4 kg COD m⁻³ d⁻¹) (Chudoba, 1985; Zheng et al., 2006). Fig. 5b shows the presence of cavities between the clumped bacteria. These cavities are anticipated to be responsible in allowing smooth mass transfer of substrates or metabolite products in and out of the granules.

3.4. Physical characteristics of granular sludge

3.4.1. Size
The shear force imposed in the development of granules in this experiment, in terms of superficial upflow air velocity which was 1.6 cm s⁻¹, resulted in the development of microbial granular sludge with average diameter of 2.3 ± 1.0 mm. According to Peng et al. (1999), the diameter of the developed aerobic granules is in the range of 0.3–0.5 mm which is much smaller as compared to anaerobic and anoxic granules that could develop up to 2 to 3 mm. The strong shearing force produced by mixing and aeration during the reaction phase could prevent the development of bigger granules which can be achieved in an anaerobic system (van Benthum et al., 1996; Kwok et al., 1998).

3.4.2. Settling velocity
The average settling velocity of the seed sludge and seed anaerobic granular sludge was 9.9 ± 0.7 m h⁻¹ and 42 ± 8 m h⁻¹ respectively. The average settling velocity of the anaerobic granular seed is in accordance with those reported by Schmidt and Ahring (1996) which is in the range of 18–100 m h⁻¹. The average settling velocity of the granular sludge developed in this study increased from 17.8 ± 2.6 m h⁻¹ to 80 ± 8 m h⁻¹ at the end of experiment. The settling velocity obtained in this study is almost three times greater than the settling velocity of the aerobic granules reported by Zheng et al. (2005) (i.e. 18–31 m h⁻¹).

The increase in settling velocity has given significant impact on the biomass concentration in the reactor. The relationship between settling velocity of the granules and the concentration of the MLSS is shown in Fig. 6. Despite the short settling time (5 min), the high settling velocity possessed by the developed microbial granular sludge enabled the granules to escape from being flushed out during the decanting phase. Such conditions have caused more microbial granular sludge to be retained in the system and resulted in the increase of biomass concentration in the reactor. In this experiment, the SRT value was 1.4 days during the start-up (partly low due to wash out of inoculated sludge) and rose up to 8.3 days at the end of experiment. As less biomass was washed-out during the decanting period, the increase in SRT is another manifestation of good settling characteristics resulting from the high settling velocity.

The SVI value has improved from 276.6 mL g⁻¹ at the initial stage of the experiment to 69 mL g⁻¹ at the end of the experiment indicating the good settling properties of the granular sludge.
granules which is favorable in wastewater treatment plant operation. The change of the SVI value and the SRT as a function of time are given in Fig. 7. The SVI value achieved in this experiment is in agreement with the result reported by McSwain et al. (2004) with SVI values of 115 ± 36 mL g⁻¹ (settling time 10 min) and 47 ± 6 mL g⁻¹ (settling time 2 min). The higher settling velocity and lower SVI value of the mature microbial granular sludge as compared to previous reports by other researchers indicate that the formation of granules seeded with anaerobic granules would develop better settling properties of the granules. It may also be due to the specific reaction condition of anaerobic/aerobic setting in the experiment that induced the well settling of the granule.

3.4.3. Granular strength

The granular strength of the granules was measured based on the integrity coefficient (IC) as described by Ghangrekar et al. (2005). The smaller the IC value, the higher the strength and ability of the granules to remain as high structural integrity granules during aeration phase that caused the shear force. Fig. 8 shows the IC profile of the developed granular sludge as a function of time. The IC reduces as the granules developed. With an initial value of 30 ± 0.3, the IC was reduced to about 9.4 ± 0.5 at the termination of the experiment. A sharp reduction of IC was observed after 40 days of the experimental run. According to Ghangrekar et al. (2005) granules with integrity coefficient of less than 20 were considered high strength granules. The reduction in IC value indicates the increase in the strength of the bond that holds the microorganisms together within the developed granules.

During the early stage of the granule development, the microbes within the granules were loosely bounded to each other. When the microbes were loosely linked together, the granules may contain more cavities which make the granules less dense, as shown by low settling velocity value. As more microbes were linked together, the granules increased in size. Under the applied selective pressures (i.e. short settling time, hydrodynamic shear force, feast-famine regime) within the reactor, microbes may produce more EPS (Qin et al., 2004). As reported by Adav et al. (2008), the EPS could contribute greatly to the strength and the stability of anaerobic granules. When more EPS are being produced by the microbial cells, they form a cross-linked network and further strengthen the structural integrity of the granules. The cavities within the granules may be filled with the EPS as it is a major component of the bio-granule matrix material in both anaerobic and aerobic granules. This caused the granules to become denser and stronger as shown by their high settling velocity and low IC value at the end of the experiment.

3.4.4. Mineral content

The concentration of minerals in granular sludge, newly developed and matured granular sludge were determined in mg/g of dry sludge and presented in Table 2. The concentration of Na⁺ and K⁺ are not much different in the sludge, newly developed and matured granules. However, there is an obvious increase in the concentration of Ca²⁺ and Mg²⁺ within the matured granules. The concentration of Fe²⁺ was slightly reduced in the matured and newly developed granules as compared to the sludge.

Basically, an unchanged concentration of Na⁺ and slightly decreasing K⁺ concentration in the sludge and matured granules may indicate that these monovalent cations may not be involved in the granulation process. It has been reported that high concentration of the Na⁺ and K⁺ may cause adverse

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Sludge (1 week)</th>
<th>Newly developed microbial granular sludge</th>
<th>Matured microbial granular sludge (10 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺</td>
<td>1.53 ± 0.02</td>
<td>2.0 ± 0.6</td>
<td>4.65 ± 0.04</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.13 ± 0.01</td>
<td>0.322 ± 0.003</td>
<td>1.75 ± 0.08</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.22 ± 0.07</td>
<td>0.25 ± 0.04</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>K⁺</td>
<td>1.31 ± 0.07</td>
<td>1.15 ± 0.03</td>
<td>0.93 ± 0.05</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>2.32 ± 0.02</td>
<td>1.90 ± 0.04</td>
<td>1.98 ± 0.08</td>
</tr>
</tbody>
</table>
effect on the granules formation. It could cause reduction in sludge concentration, settling velocity of the sludge, granular strength and treatment efficiency (Ghangrekar et al., 2005). The monovalent cation, Na\(^{+}\) has also been reported causing detrimental impact on the flocculation system (Sobeck and Higgins, 2002). Nevertheless, there are contradictory reports related to the effect of these monovalent cations in granulation development processes. Fernandez et al. (2008) reported that the concentration of granular biomass has improved significantly in the reactor system fed with high concentration of inorganic salt influent. A quick decrease of solids concentration in the effluent was observed after the addition of NaCl.

The development of the granular sludge in this study showed higher accumulation of Ca\(^{2+}\) and Mg\(^{2+}\) towards the end of the experiment. This may indicate the involvement of the inorganic elements in the granulation process. Based on the divalent cation bridging theory, the presence of Ca\(^{2+}\) and Mg\(^{2+}\) promotes equivalent floc properties (Sobeck and Higgins, 2002). According to Ren et al. (2008), granule-rich Ca\(^{2+}\) showed more rigid granular structure and higher strength as compared to granule without Ca\(^{2+}\) accumulation.

3.5. Removal performance

The performance of the reactor system from start-up until the end of granules development period based on the removal of COD, color and ammonia is given in Fig. 9. At the initial stage of the operation, the percentage removal for COD and ammonia was 71% and 67% respectively (Fig. 9a and b). The removal efficiency increased to 94% for COD and 95% for ammonia at the end of the experiment. The increase in the removal efficiency indicates the occurrence of high biological activity in the reactor system. During the first month, the removal efficiency for COD and ammonia fluctuated but the removal became stable for the remaining period. The removal efficiency for color was fluctuating almost throughout the study period (Fig. 9c). The percentage of color removal was about 25% during the start up and increased to 62% at the end of the experiment. The average of color removal was 55%. This low percentage of the color removal may be due to insufficient adaptation time. As dye substances are recalcitrant and difficult to be degraded, more time is required to accumulate sufficient organisms which degrade the dyes in the reactor. The inconsistent percentage for color removal may also be contributed by the unstable condition of the aromatic amines, the byproduct of dye degradation which easily oxidized and recolor when exposed to oxygen during the aerobic phase. The increase of color during autoxidation of aromatic amines was confirmed by several researchers (Cruz and Buitron, 2001; Libra et al., 2004; Sponza and Isik, 2005).

The inconsistency of color removal may be also influenced by the absorption of color into sludge biomass throughout the experiment. The absorption of color into the sludge biomass has been reported by other researchers (Otero et al., 2003; Wang et al., 2006; Sirianuntapiboon and Srisornsak, 2007).

Fig. 10 shows the percentage removal of COD, ammonia and color in a complete 340-min reaction phase of the SBR system recorded on the 66th days of experiment. The profile and the percentage removal for COD and ammonia were almost the same while the removal of color was much lower. After 340 min of intermittent anaerobic and aerobic modes, about 93%, 95% and 62% of COD, ammonia, and color respectively were removed.

During the first anaerobic phase (PI) (0–40 min), approximately 15% and 4% of COD and ammonia respectively, were removed. In the first aerobic phase (PII) (40–170 min), about 68% of the COD was removed while 80% of the ammonia was oxidized. Most of the organic compounds and nitrification of ammonia were achieved during this stage. The supply of oxygen at this stage enabled good oxidation of these compounds (Brauer and Henning, 1986). The nitrate produced...
will be removed through the denitrification process that will occur in the second stage of anaerobic phase. The second anaerobic phase (PIII) (170–210 min) showed only about 5% of COD and ammonia being removed while the remaining (about 4%) was removed in the second aerobic phase (PIV) (210–340 min). As for color, about 45% and 16% were removed during anaerobic and aerobic phases respectively. The result shows the ability of anaerobic microbes within the granule to degrade the dye. The high percentage for color removal indicated active cleavage of dye compound took place during both of the anaerobic phases (PI and PIII).

The degradation and decolorization of dye during anaerobic condition has been widely reported in the literatures (dos Santos et al., 2007). In anaerobic condition, the electrons from the electron donor are transferred to the N=N bond of the azo dye causing the cleavage of the bond forming aromatic amines. The amines are then degraded under aerobic condition reducing the COD value of the wastewater. In addition to the degradation mechanism, dye removal may also occur via adsorption onto the biomass (Aksu, 2001 and Crini, 2006). Amines, the colorless byproduct of anaerobic degradation of dye compound are unstable compound that could easily be oxidized during the presence of oxygen. These autoxidation of the amines may form different intensity colored compound (Cruz and Buitron, 2001; Libra et al., 2004; Sponza and Isik, 2005). This reaction may cause the reduction on the overall percentage of color removal during aerobic condition (PII and PIV). Based on the removal performance of the system, it has been proven that the developed microbial granular sludge is capable to perform the degradation process during anaerobic and aerobic phases. This indicates the presence of aerobic, facultative and anaerobic microorganisms in the microbial granular sludge. According to Li and Liu (2005), when the granules grew to a size larger than 0.5 mm, the diffusion of oxygen into the inner part of the granules became a limitation. This may give an indication of the presence of anaerobic microorganisms within centre part of the microbial granular sludge since the average size of microbial granular sludge developed in this study was 2.3 ± 1.0 mm. Aerobic microorganisms may be found at the outer layer of the granules which can easily access the oxygen molecule and mainly responsible for the COD removal. The facultative microorganisms may be found in any part of the microbial granular sludge due to its capability to live both under anaerobic and aerobic condition.

4. Conclusion

- Stable microbial granular sludge could be cultivated in a single SBR system with the application of intermittent anaerobic and aerobic reaction mode during the reaction phase.
- The matured granules showed the domination of non-filamentous bacteria that were tightly linked and embedded to one another and covered with EPS. The SVI value of the biomass has decreased from 276.6 mL g⁻¹ to 69 mL g⁻¹ at the end the 66 days, also indicating the excellent settling properties of the granules.
- The development of the granular sludge is positively correlated with the accumulation of divalent cationic Ca²⁺ and Mg²⁺ in the granules suggesting the role played by the cations in the granulation process.
- The results indicate the viability of the single reactor system for treating textile wastewater under intermittent anaerobic and aerobic phase strategy.
- The OUR/SOUR and SMA analyses indicate the presence of anaerobic and aerobic microorganisms activities in the granular sludge which is capable to perform degradation process both in anaerobic and aerobic conditions.

Acknowledgements

The authors wish to thank the Ministry of Science, Technology and Innovation (MOSTI), Ministry of High Education (MOHE) and Universiti Teknologi Malaysia for the financial supports of this research (Grants No.: 79137, 78211 and 75221).

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