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Sequential Anaerobic-Aerobic Phase Strategy Using Microbial Granular Sludge for Textile Wastewater Treatment

Khalida Muda, Azmi Aris, Mohd Razman Salim and Zaharah Ibrahim

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1. Introduction

The textile industry involves a long chain of complex activities, from processing raw materials up to finishing the fabrics. These industries have created job opportunities to millions of people and have become one of the major incomes to many countries in the world. Unfortunately, the industry is also one of the major contributors to water pollution. The textile wastewater contains not only the colorant, which is one of the main pollutants, but also other chemicals that are added throughout the textile processing. The dye compounds present in textile wastewater are able to impose a major impact to a receiving water body even in small quantities.

Due to the non-biodegradable nature of textile wastewater, a conventional aerobic biological process is incapable of treating the wastewater. For a complete degradation of textile wastewater, a combination of anaerobic and aerobic reaction phases is necessary.

This chapter briefly reviews the characteristics of textile wastewater and available technologies. This is followed by an in-depth discussion on biogranulation technology and the application of a hybrid biogranular system in treating the textile wastewater.

2. The textile industry

The fabrics, either in the form of natural or chemical fibres, have reached millions of tonnes of production and have provided huge advantages to world economic values (Aizenshtein, 2004). In social terms it has provided benefits to more than 2.2 million workers through 114,000 textile-related companies. In 2001, the European textile and clothing industries contributed to about 3.4% of the EU manufacturing industrial revenue and granted 6.9% of

the work opportunities to the citizens (IPPC, 2003). According to recent statistics, the global textile market is worth more than US\$400 billions (Directory of Textile Manufacturers and Suppliers - <http://www.teonline.com/industry-overview.html>). It is predicted that the global textile production will grow up to 50% by 2014 as compared to the fabrication in 2005.

Globally, Malaysia is also known for its high quality textile and apparels. Since the early 1970s, when the country started to embark on being an export-oriented country, the growth of Malaysian's textile and apparel industry has increased tremendously and now provides an export value of 3.5 billion USD. This has listed the textile industry as the ninth largest contributor to total earnings of the manufactured exports in 2007. The industry has provided more than 67,000 work opportunities through 637 licensed textile production companies with investments of 2.6 billion USD (MIDA, 2007).

In Malaysia and many other developing countries, most of the textile mills are of small and medium scale. For these mills, the full installation of a wastewater treatment plant is quite difficult due to economic reasons. Hence, the mills have been discharging significant quantities of pollutants into the streams with fiber manufacturing and dyeing sectors being the predominant ones (Haroun and Azni, 2009).

2.1. Characteristics of textile wastewater

The textile industry consumes the largest portion of the colorant available in the world market. Due to the high customer demand, more than 100,000 commercial dyes exist in the market causing more than 700,000 tonnes of dyes to be produced annually (McMullan et al., 2001; Pearce et al., 2003). The result is a very high production of colored wastewater. The characteristics of textile wastewater (either quantitatively or qualitatively) vary greatly depending on the type of raw materials, chemicals, techniques or specific process operations at the mill, the equipment used and the production design of the textile processes (Bisschops and Spanjers, 2003; Dos Santos et al., 2006).

The textile industry consumes huge amounts of water in its wet processes. The average wastewater generation from a dyeing facility is estimated between 3800 and 7600 million m³ per day. Desizing, scouring, bleaching, mercerizing and dyeing are the common wet textile process operations. Among these, the mercerizing and dyeing processes consume the biggest specific volumes of water with a water usage of 230-310 L/kg and 8-300 L/kg of textile processed, respectively (Dos Santos et al. 2007).

Due to inefficiency of the textile processing activities, only 10% of the chemicals in the pre-treatment and dyes in dyeing operations remain on the fabric. In other words, about 90% of chemical substances will be discharged as textile effluent (IPPC, 2003). Others have reported that between 50 and 95% of the dyes are fixed on the fiber while the remainder is discarded in the subsequent textile-washing operations (EPA, 1997; Trovaslet et al., 2007). The amount of dye lost into the wastewater depends upon the type of dyestuff used, as well as the methods and application routes of the textile processing operation. Additionally, it depends on the intended color intensity that is required for each particular design (Willmott et al., 1998).

Textile wastewater is characterized with high chemical and biochemical oxygen demand, suspended solids, high values of conductivity and turbidity and intense color. This is caused by the presence of dye residues or intermediates and auxiliary chemicals added in the many stages in textile processing (Mohan et al., 2007a; Miranda et al., 2009). Textile processes with natural fibers generate higher pollution load as compared to synthetic fibers mainly due to the use of pesticides for preservation of the natural fibers (Correia et al., 1994).

Textile dyeing wastewater is also characterized by high salt content, which also imposes potential environmental problems. Typical cotton batch dyeing operations use quantities of salt that range from 20 to 80% of the weight of goods dyed, with common concentrations between 2,000 mg/L and 3,000 mg/L. Sodium chloride and sodium sulfate constitute the majority of the total salts used. Magnesium chloride and potassium chloride are used as raw materials in lower concentrations (EPA, 1997).

Common characteristics of textile wastewater from cotton textile wet processing for different processing categories are shown in Table 1. The highest concentration of organic pollutants (in terms of COD) is generated from bleaching while the highest concentration of total solids comes from the desizing process. The highest concentration of color, ranging from 1450-4750 ADMI, is generated from the dyeing process (Bisschops and Spanjers, 2003; Dos Santos et al., 2007). Metals such as copper, cadmium, chromium, nickel and zinc are also found in textile effluents, as they are the functional groups that form the integral part of the dye molecule (IPPC, 2003).

2.2. Treatment technology

At present, treatment of textile wastewater mainly involves physical and/or chemical processes. These include coagulation and flocculation (Harrelkas et al., 2009), precipitation (Solmaz et al., 2007), adsorption (Sayed and Ashtoukhy, 2009), membrane filtration and nanofiltration (Miranda et al., 2009), ion exchange (Wu et al., 2008), ultrasonic mineralization (Maezawa et al., 2007) and electrolysis (De Jonge et al., 1996). While these methods are often costly, they remove the pollutants by transferring them from one phase to another. Some of them generate highly concentrated sludge, hence creating disposal problems (Pearce et al., 2003) that may lead to soil contaminations. Excessive use of chemicals in dye treatment creates secondary pollution problems to the environment.

Process	COD (g/L)	BOD (g/L)	TS (g/L)	TDS (g/L)	pH	Color (ADMI)
Desizing	4.6-5.9	1.7-5.2	16.0-32.0	-	-	-
Scouring	8	0.1-2.9	7.6-17.4	-	10--13	694
Bleaching	6.7-13.5	0.1-1.7	2.3-14.4	4.8-19.5	8.5-9.6	153
Mercerising	1.6	0.05-0.10	0.6-1.9	4.3-4.6	5.5-9.5	-
Dyeing	1.1-4.6	0.01-1.80	0.5-14.1	0.05	5-10	1450-4750
Bleaching and Dyeing*	0.2-5.5	2.0-3.0	0.1-5.0	-	2-10	280-2000

*Characterization of textile wastewater in Malaysia (Ahmed et al., 2005; Ibrahim et al., 2009)

Table 1. Characteristics of textile wastewater (Bisschops and Spanjers, 2003; Dos Santos et al., 2006)

Treatment using ozonation, Fenton's reagent, electrochemical destruction and photocatalysis are some of the emerging techniques reported to have potential use for decolorization (Faouzi et al., 2006; Ay et al., 2009). However, such technologies usually involve complicated procedures and are economically unattainable (Chang and Lin, 2000).

Among the available techniques, the one that can offer effective pollutant removal at a lower cost is the desirable alternative. Of these, biological treatment is the obvious choice due to the relatively low operating cost.

While a conventional aerobic biological process is incapable of treating textile wastewater, studies have shown that the integration of anaerobic and aerobic processes are able to provide complete mineralization of colored substances (Knackmuss, 1996; Melgoza et al., 2004; van der Zee and Villaverde, 2005). It can be done by using either two separate anaerobic and aerobic reactors (Khelifi et al., 2008) or using integrated anaerobic/aerobic treatment in a single reactor (Frijters et al., 2006; Cinar et al., 2008). The wastewater is initially treated under an anaerobic condition followed by an aerobic condition. Under the anaerobic condition, the N=N bond of the azo dyes are cleaved, leading to the production of amines, the colorless byproducts. This is followed by complete mineralization under the aerobic condition. Different forms of biomass (i.e. suspension, film and granules) have been used in different types of reactor in the studies.

2.3. The water quality issues

The textile industry, in particular the wet industry, has been considered as one of the major water environment polluters. This is mainly due to the enormous amount of water and the complexity of the chemicals used in the manufacturing processes that end up in the wastewater. The poorly treated wastewater is still highly colored comprising of significant amounts of nonbiodegradable chemicals that are hazardous to the environment. Under anaerobic condition, some of the organics i.e. the azo dyes are transformed into more toxic chemicals (i.e. amines) that worsen the condition. The color will make a river inhabitable to a majority of aquatic plants and animals.

While there are many technologies available in treating the wastewater, a majority of them are relatively expensive to be applied by the small and mid-size industries. Furthermore, many of the physico-chemical technologies only transform the pollutants from one form or one phase to another and therefore do not provide any ultimate solution to the problem.

A conventional aerobic bioprocess fails to treat the wastewater due to the non-biodegradable nature of the wastewater. However, recent research and advancement in biological processes show that there is a huge potential of these new findings in providing low cost yet efficient technology to solve the textile wastewater problem.

3. Biogranulation treatment technology

Microbial granules form a self-immobilization community that is formed with or without support material. They are defined as discrete macroscopic aggregates containing dense

microbial consortia packed with different bacterial species. Each biogranule consists of millions of microorganisms per gram of biomass (Weber et al., 2007), formed via biological, physical and chemical forces. According to Calleja (1984), microbial granulation is a multicellular association in a physiological state that is causing the mixture of cells into a fairly stable and contiguous structure.

The main advantages of biogranules systems are mainly due to the biogranules good settling property and the fact that biogranules are formed without the need of any biomass carrier. The relatively large size and high-density biogranules give them a rapid settling rate, which enhances the separation of the treated effluent from the biomass and results in high solid retention time (SRT) (Ahn and Richard, 2003; Liu and Tay, 2004). Due to a better settling rate, the system also shows low suspended solid content discharged in the effluent (Wirtz and Dague, 1996).

Within the biogranules, the microorganisms are closely lumped together, hence generating syntrophic associations between the cells. This relationship occurs due to optimum distances between the cells at appropriate substrate levels and such condition enables high and stable performance of metabolism activities (Batstone et al., 2004).

The granulation system is first recognized in an up-flow anaerobic sludge blanket (UASB) system characterized by anaerobic biogranules. Much research has been carried out using innovative upflow sludge bed (USB) type reactors (Bachman et al., 1985; Lettinga et al., 1997). The applications of anaerobic granulation systems have been successfully demonstrated particularly in removing biodegradable organic matter from industrial wastewaters (Lettinga et al., 1980; Schmidt and Ahring, 1996). Later the attention has also been diverted to the development and applications of aerobic biogranules. The reason has been several drawbacks that have been observed in the anaerobic biogranules system, including long start-up periods, relatively high temperature requirements and ineffectiveness in dealing with nutrient and low organic strength wastewater (Liu and Tay, 2004).

Aerobic granulation systems have been used for organics, nitrogen, phosphorus and toxic substances removal, especially high strength wastewater (Yi et al., 2008; Kishida et al., 2009). In most cases, the system is in the form of a sequencing batch reactor (SBR) (Beun et al., 1999; Kim et al., 2008). The reaction phase of the system has been carried out either in anaerobic, aerobic or anoxic conditions, with or without mixing, depending on the purpose of the treatment.

3.1. Development of biogranules

Bacteria normally do not aggregate naturally to each other due to repulsive electrostatic forces via the presence of negatively charged protein compounds of the cell wall (Voet and Voet, 2004). However, under selective environmental conditions, microorganisms are capable to attach to one another and thus form aggregates.

Development of biogranules involves integration of physical, chemical and biological processes occurring in multiple stages (Calleja, 1984; Liu and Tay, 2002; Linlin et al., 2005;

Weber et al., 2007). The first stage of a biogranulation process is initiated by several forces, which include diffusion of mass transfer, hydrodynamic and gravitational forces, thermodynamic effects, as well as the tendency of cells to move towards one another. These forces result in cell-to-cell or cell-to-solid surface interactions. The second stage involves several physical forces (e.g. Van der Waals forces, surface tension, hydrophobicity, opposite charge attractions, thermodynamic of surface free energy, bridges by filamentous bacteria), and chemical and biochemical forces (e.g. cell surface dehydration, cell membrane fusions and signals among microbial communities). At this stage, the multicell connections are stabilized. The third stage is the maturing stage, which involves the production of substances that facilitate more cell-to-cell interactions; at this stage, highly organized microbial structures are formed. Several mechanisms of metabolite production will also change, such as higher production of extracellular polymer, growth of cellular cluster, metabolite change and environmental-induced genetic effects. The final stage involves shaping of the three dimensional granules by hydrodynamic shear forces.

Beun et al. (1999) have also described the path of aerobic granules formation in a reactor as illustrated in Figure 1. Immediately after inoculation, bacteria and fungi will be dominating the reactor system. At this early stage, mycelial pellets manage to retain in the reactor due to their good settling ability. Bacteria, which do not hold this characteristic, are discarded with the effluent. Due to the shear force imposed by air bubbles during the aeration phase, the filaments will be detached from the surface of pellets. The pellets then grow bigger until they reach a diameter of up to 5-6 mm. When the sizes of the pellets have grown even larger, self-defragmentation will take place due to the limitation of oxygen transfer in the inner parts of the grown pellets. The fragmented mycelial pellet will act as a matrix for bacteria to grow and form new colonies. The bacterial colonies grow larger and will form granules. As the granules are formed, the whole system will be governed by bacterial growth.

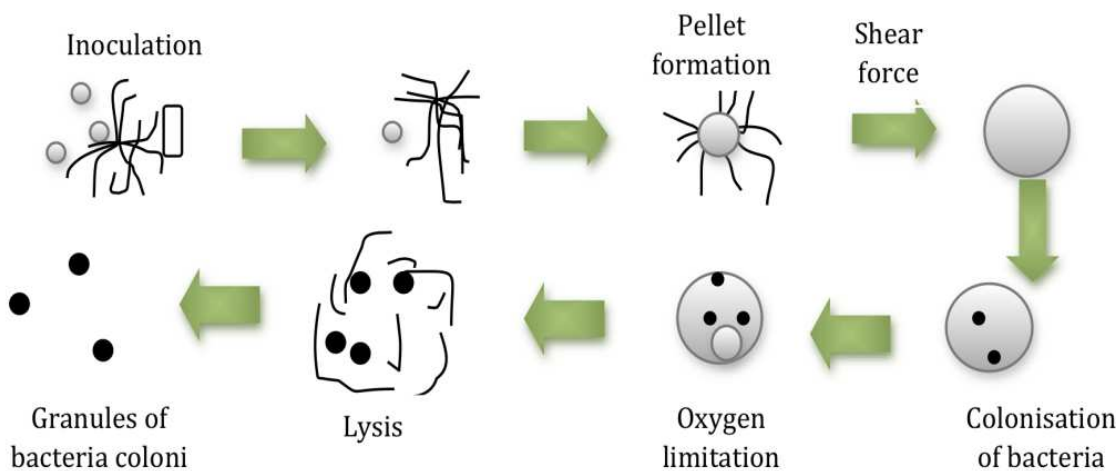


Figure 1. Schematic diagram of aerobic granulation developed without any carrier material (Beun et al., 1999)

Weber et al. (2007) have illustrated the involvement of several eukaryotic organisms in three consecutive phases. Microscopic analysis has revealed that eukaryotic organisms play a key

role in aerobic granule formation. Stalked ciliates of the subclass *Peritrichia* and occasionally, the fungi, are found to be involved in the biogranulation process development.

Development of biogranules seeded with anaerobic granular sludge in an SBR system has been demonstrated by Linlin et al. (2005). At the initial stage, the anaerobic granular seeds disintegrate into smaller flocs and debris due to the hydrodynamic shear force created by the air bubbles during the aerobic phase. Lighter and small sized flocs or debris will be washed out in the effluent during the decanting stage. The remaining heavier anaerobic granules remain and act as precursors that initiate the growth of new aerobic granules. The optimal combination of the shear force and the growth of the microorganisms within the aggregates govern the stable structure of the biogranules (Chen et al., 2008). The morphology of these aerobic granules is slightly different as compared to the aerobic granules as described by Beun et al. (1999).

3.2. Characteristics of biogranules

Biogranules are known for their outstanding features of excellent stability and high removal efficiency making biogranulation an innovative modern technology for wastewater treatment. The size of the biogranules is an important aspect that may influence the stability and performance of the reactor system. Biogranules with bigger sizes can easily be defragmented under high shear force resulting in high biomass washout. Meanwhile, if the size is too small, the biogranules cannot develop good settling properties, resulting in higher suspended substances in the effluent. Bigger biogranules with loose structure will be developed in an SBR system supplied with low superficial air velocity. Smaller biogranules but with high strength structures are observed being formed in systems aerated at higher superficial air velocity (Chen et al., 2007). Granular sizes range from 0.3 mm to 8.8 mm in diameter possessing different granular characteristics (Dangcong et al., 1999; Zheng et al., 2005).

The hydrodynamic shear force imposed through the aeration rate of the reactor system will control the development of biogranules (Chisti, 1999). The size of biogranules is the net result of the balance between the growth and the hydrodynamic shear force imposed by superficial air velocity (Yang et al., 2004). For the optimal performance and economic purposes, the operational diameter range for effective aerobic SBR granular sludge should be in the range of 1.0-3.0 mm (Toh et al., 2003).

The usual structure of an aerobic granule is normally spherical in shape with smooth surface areas, which can be influenced by the concentration and type of substrate used in the media compositions (Zhu and Wilderer, 2003; Adav and Lee, 2008). Based on electron microscope (SEM) observations, glucose-fed granules appear with fluffy outer surface due to the predominance growth of filamentous bacteria. On the other hand, the acetate-fed granules show a more compact microstructure with smooth surface. The non-filamentous and rodlike bacteria were observed dominating the acetate-fed granules that are tightly linked together (Tay et al., 2001).

Settleability of a biogranular sludge shows the capacity of the biogranules to settle within a specified period of time. Such properties will allow fast and clear separation between sludge

biomass and effluent. The settling velocity of aerobic granules is in the range of 30 to 70 m/h depending on the size and structure of the biogranules, which is comparable to the anaerobic granules. Settling velocity of activated sludge flocs is in the range of 8 to 10 m/h that is three times lower than to those of aerobic granules. Good settleability of sludge biomass is desirable in wastewater treatment plants to facilitate high percentage of sludge retention in a reactor system. Superior characteristics of settleability assist to maintain the stable performance, high removal efficiency and can handle high hydraulic loading of wastewater (Tay et al., 2001). Good settling property of biogranules is also shown by a low value of the SVI. The SVI of biogranules is lower than 100 mL/g (Peng et al., 1999 and Qin et al., 2004), much lower compared to the SVI of flocs (above 150 mL/g). The observed density of microbial aggregates is the consequence of balance interaction between cells (Liu and Tay, 2004). The density of the aerobic granule is reported to be in the range of 32 to 110 g VSS/L (Beun et al., 2002; Arrojo et al., 2006) and the specific gravity is in the range of 1.004 to 1.065 (Etterer and Wilderer, 2001 and Yang et al., 2004).

When biogranules grow bigger, the compactness of the granules decreases. This can be detected via a less solid and loose architectural assembly (Toh et al., 2003). Biogranules with high physical strength can withstand high abrasion and shear force. The physical strength of the biogranules is expressed as an integrity coefficient. This coefficient is an indirect quantitative measurement of the ability of the biogranules to withstand the hydrodynamic shear force (Ghangrekar et al., 2005). A good granular strength is indicated by an integrity coefficient of less than 20.

Biogranules are also characterized by high cell hydrophobicity and high EPS content. The former aspect is postulated to be the main triggering force in the initial stage of the biogranulation process and is a measure of the cell-to-cell interaction (Liu et al., 2003). The latter characteristic is postulated to be responsible for the aggregation between cells (Liu et al., 2004).

The presence of the EPS will enhance the polymeric interaction, which is one of the attractive forces that can promote the adhesion of bacterial cells. The networking between cell and EPS will assist the formation of biogranules (Zhang et al., 2007).

4. A hybrid biogranular system for textile wastewater treatment

The application of hybrid biogranular system in treating textile wastewater is reported in this section. In this study, the development of biogranules during the treatment of textile wastewater is investigated. The changes on the physical characteristics of the biogranules as well as the system performance in the removal of organic compound and color intensity of the textile wastewater are further discussed.

4.1. The system

The schematic representation of the reactor design is given in Figure 2. The design of the reactor is based on Wang et al. (2004) and Zheng et al. (2005) with several modifications. The column of the reactor has a working volume of 4 L with internal diameter of 8 cm and a total

height of 100 cm. The reactor is designed with a water-jacketed column for the purpose of temperature control. This can be achieved by allowing the circulation of hot water from a water heating circulation system to the water jacketed column of the system. The temperature of the heating system was set at 30°C. Air was supplied into the reactor by a fine air bubble diffuser located at the bottom of the reactor column. The reactor system was equipped with dissolved oxygen and pH sensors for the continuous monitoring throughout the experiment. The wastewater was fed into the reactor from the bottom of the reactor. The decanting of the wastewater took place via an outlet sampling port located at 40 cm above the bottom of the reactor. The reactor system has been designed with volumetric exchange rate (VER) of 50%. This means that only particles with settling velocity larger than 4.8 m/h remained in the column. Particles having smaller settling velocity will be washed out in the effluent. All operations of peristaltic pumps, circulation of influent, air diffuser and decanting process were controlled by means of a timer.

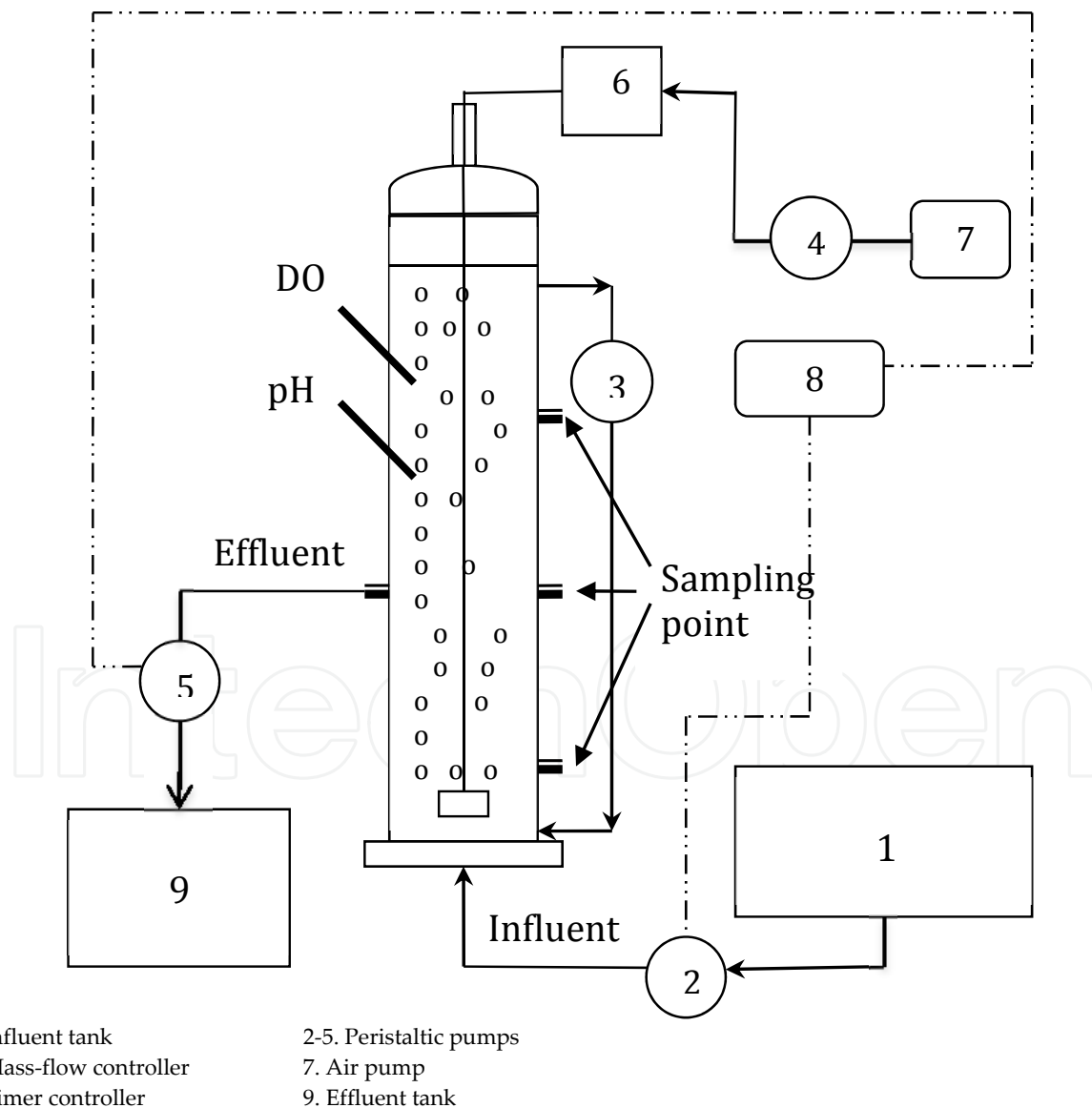


Figure 2. Schematic layout of the hybrid biogranular system

4.2. The operation and analysis

During the start-up period, 2 L of mixed sludge and 2 L of synthetic textile wastewater were added into the reactor system giving the working volume of 4 L with 5.5 g/L of sludge concentration after inoculation. The system was supplied with external carbon sources consisting of glucose, sodium acetate and ethanol with substrate loading rate of 2.4 kg COD/m³·d. The operation of the system started with 5 min filling of wastewater entering from the bottom of the reactor. The operation then continued with the react phase followed by 5 min settling, 5 min decanting and 5 min of idle time. The react time varies depending on the hydraulic retention time set for the system. Figure 3 shows the steps involved in one complete cycle of the hybrid biogranular system. During the biogranules development, the HRT of the reactor was set for 6 hours for one complete cycle. This will give a react time of 340 minutes. The react phase is divided into equal anaerobic and aerobic react periods. Table 2 shows the successive phase for one complete cycle of the reactor system.

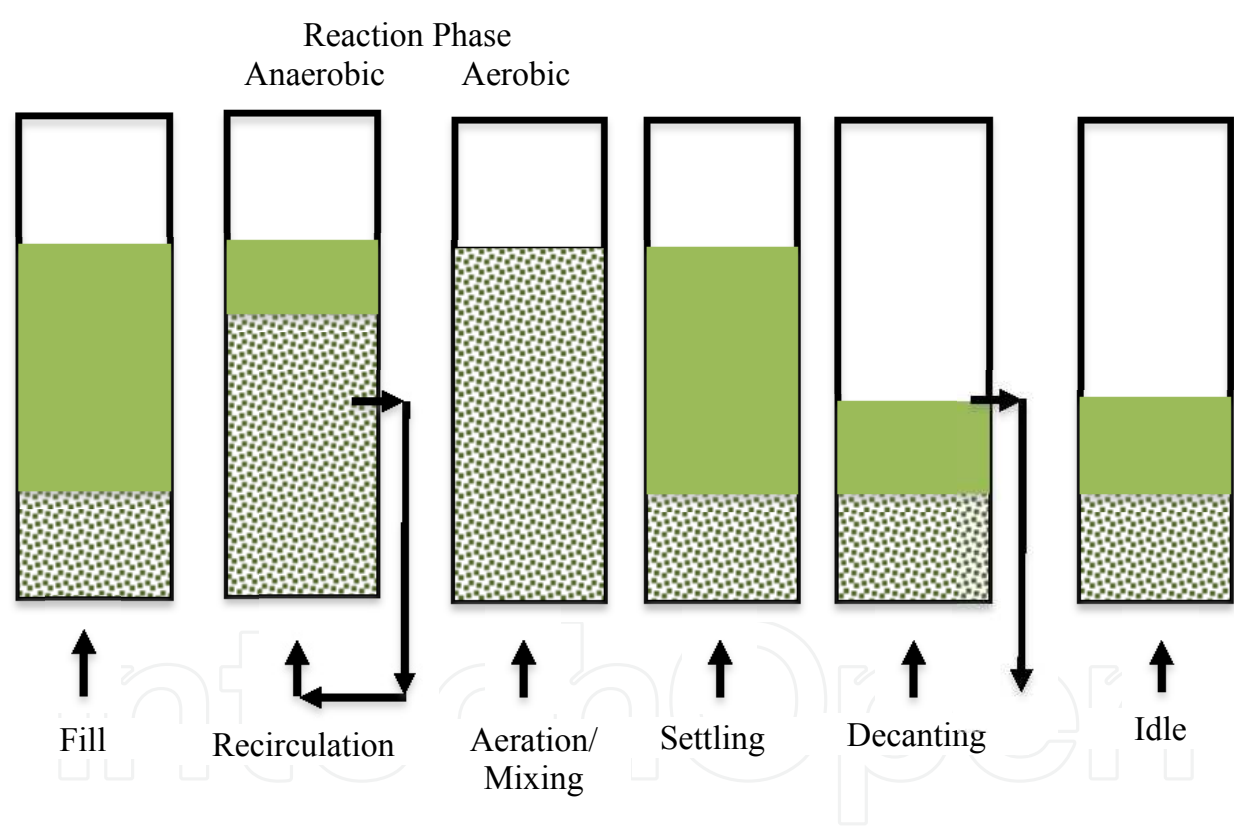


Figure 3. One complete cycle of the Hybrid Biogranular System

The operation of the reactor system was designed with intermittent anaerobic and aerobic react phases. The reaction phase started with an anaerobic phase followed by an aerobic phase. The reaction phase was repeated twice. During the anaerobic react phase, the wastewater was allowed to circulate from the upper level of the reactor and returned back through a valve located at the bottom of the system. The circulation process was carried out using a peristaltic pump at a rate of 18 L/h. The circulation system was stopped at the end of the anaerobic phase. The circulation process is required to achieve a homogeneous

distribution of substrate as well as a uniform distribution of the granular biomass and restricts the concentration gradient. The DO concentrations remained low during the anaerobic condition (0.2 mg/L) and reached saturation during the aerobic phase. The superficial air velocity during the aerobic phase was 1.6 cm/s. The system was operated without pH control causing variation in the range of 6.0 to 7.8 during the react phase.

Successive Phases	One complete cycle (6 hours)		
Filling	5 min		
React	1 st phase	Anaerobic	Aerobic
		40	130
	2 nd phase	40	130
Settling	5 min		
Decant	5 min		
Idle	5 min		
Total cycle length	360 min		

Table 2. One complete cycle of the hybrid biogranule system

In order to observe the changes on the characteristics of the biogranules due to the variations of HRT during textile wastewater treatment, the development of biogranules with sizes in the range of 0.3-2.5 mm was inoculated into the bioreactor at a ratio of 1:4 of the working volume of the reactor system. 1 L of acclimated mixed sludge was also added into the reactor system. The MLSS and MLVSS concentrations during the start-up of the experiment were 23.2 g/L and 18.4 g/L respectively. The operation steps of one complete cycle of the reactor system are shown in Table 3.

Sequence phase	Phase period	Air supply	Recirculation
Fill	15 min	Off	Off
Reaction			
Anaerobic	Varies*	Off	On
Aerobic	Varies*	On	Off
Settle	5 min	Off	Off
Decant	5 min	Off	Off
Idle	5 min	Off	Off

Table 3. Operation steps during single cycle operation

The morphological and structural observations of the granules were carried out using a stereo microscope equipped with digital image management and analyzer (PAX-ITv6, ARC PAX-CAM). The microbial compositions of the biogranules were observed qualitatively with a scanning electronic microscope (FESEM-Zeiss Supra 35 VPFESEM). The biogranules were left dried at room temperature prior to gold sputter coating (Bio Rad Polaron Division SEM Coating System) with coating current of 20 mA for 45 s. The microbial activity of the biogranules was

determined by measuring the oxygen utilization rate (OUR) following Standard Methods (APHA, 2005). The physical characteristics of the biogranules including settling velocity, sludge volume index, granular strength were measured throughout the experiment.

An initial value of 15 mL influent sample was taken from the influent tank before a new cycle operation started, while another 15 mL of the effluent sample was taken from the effluent tank after the effluent was released during the decanting phase as the final values. Samples were centrifuged for 5 min at 4000 rpm at 4°C in order to pellet down all of the suspended particles from the samples. The supernatant was used to measure the removal performance of the COD, color and ammonia removal. All of the measurements for COD, color and ammonia were performed according to Standard Method (APHA, 2005).

10 mL of sample was taken from the top portion of the reactor about 10 minutes after the filling stage ended and 10 mL of sample was taken from the effluent after the decanting stage for the measurement of the suspended particles in the influent and effluent. Another 10 mL of sample volume was taken during the aeration phase for the analysis of MLSS and MLVSS, which were measured according to Standard Methods (APHA, 2005).

The bed height of the biomass in the reactor was measured twice a week in order to estimate the SVI. The bed height was determined immediately after the settling time ended and before the wastewater was drained out during the decanting time. The SVI value can be calculated by measuring the bed volume of the sludge biomass in the reactor divided with the dry weight of the biomass in the reactor. The bed volume is the bed height of the sludge biomass that settled in the reactor 5 minutes after the aeration phase stopped. The bed volume is obtained by multiplying the bed height with the surface area of the bed column. The measurement of the SVI and the sludge retention time were calculated according to Beun et al. (1999). The settling velocity was determined by recording the average time taken for an individual granule to settle at a certain height in a glass column filled with tap water (Linlin et al., 2005).

Determination of the biogranules' strength was based on Ghangrekar et al. (1996). Shear force on the biogranules was introduced through agitation using an orbital shaker at 200 rpm for 5 minutes. At a certain degree of the shear force, parts of the biogranules that are not strongly attached within the biogranules will detach. The ruptured biogranules were separated by allowing the fractions to settle for 1 minute in a 150 ml measuring cylinder. The dry weight of the settled biogranules and the residual biogranules in the supernatant were measured. The ratio of the solids in the supernatant to the total weight of the biogranular sludge used for biogranular strength measurement was expressed as the integrity coefficient (IC) in percent. This percentage indirectly represents the strength of the biogranules. The higher the IC values the lesser the strength of the biogranules and vice versa.

4.3. Morphology and cellular characterization of biogranules

Development of biogranules was obtained within 66 days of operation period with 6 hours HRT. Morphology of the biogranules was investigated via visual and microscopic

observations. At the initial development stage, the biomass was composed more of loosely clumped sludge, which can easily break up into pieces under vigorous shaking. Within a week, the anaerobic seed granules underwent morphological changes from spherical in shape and black in color with average diameter of 1 mm into smaller grey granules due to exposure to the shear force during the aerobic react phase. On day 30, two different types of granules were clearly observed in the reactor as shown in Figure 4.

Figure 4a shows mainly irregular-shaped with yellow colored biogranules (Type A) that are solely developed from the activated sludge. In Figure 4b, the anaerobic granules that have fragmented into smaller pieces have formed different sizes of biogranules (Type B) containing 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 the anaerobic granules. The formation of Type A biogranules can 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 bacterial growth. Bacteria that were able to attach to this matrix were retained and suppressed the growth of filamentous microorganisms and became the dominant species in the reactor.

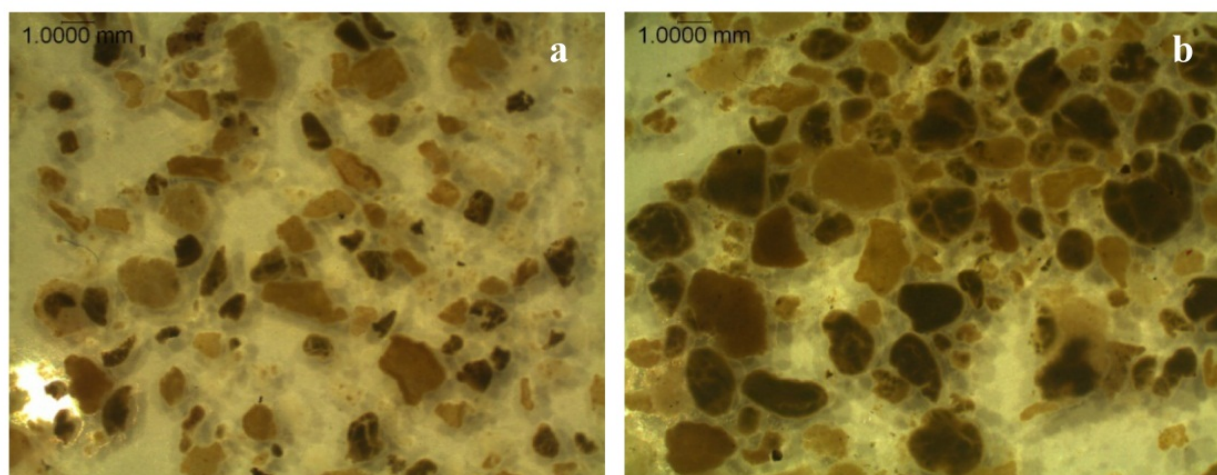


Figure 4. The morphological development of biogranules (scale bar at steady-state equals to 1mm). Pictures were taken using a stereo microscope with magnification of 6.3X. (a) Biogranules developed from the activated sludge. (b) Biogranules developed from anaerobic granules patches.

The formation of Type B granules has been discussed by Linlin et al. (2005). These biogranules 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 reactor 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 new granules. Having these combinations of aerobic and anaerobic portions within the biogranules will increase the possibility of complete degradation through the anaerobic/aerobic degradation process. Figure 5 shows the obvious

morphological differences between sludge particles with average sludge particles of 0.02 ± 0.01 mm (Figure 5a) during the initial stage of the experiment and matured biogranules (Figure 5b) at the final stage with average diameter of 2.3 ± 1.0 mm.

The microstructure of the biogranules was examined using SEM (Figure 6). The SEM observation of the mature biogranules shows the domination of non-filamentous coccoid bacteria. The bacteria are tightly linked and embedded to one another and form a rounded shape on the surface of the biogranule and covered with extracellular polysaccharides substances (EPS) (Figure 6a). Figure 6b shows the presence of cavities between the clumped bacteria. These cavities are anticipated to be responsible to allow a smooth mass transfer of substrates or metabolite products into and out of the granules (Tay et al., 2003 and Toh et al., 2003).

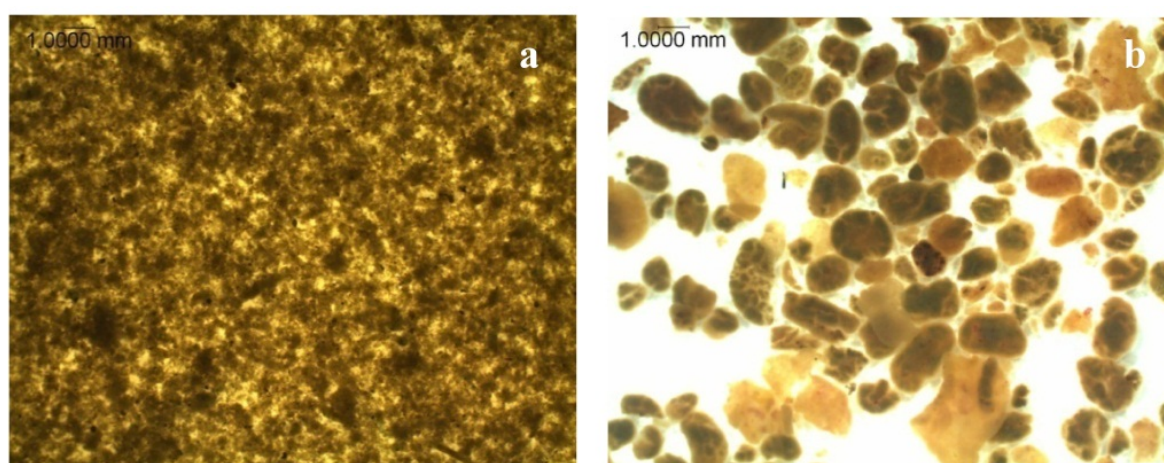


Figure 5. Pictures of sludge particles during the initial stage of the experiment (a) and matured biogranules at the 66 days of the experiment (b). Pictures were taken using a stereo microscope with magnification of 6.3X (scale bar equals to 1 mm)

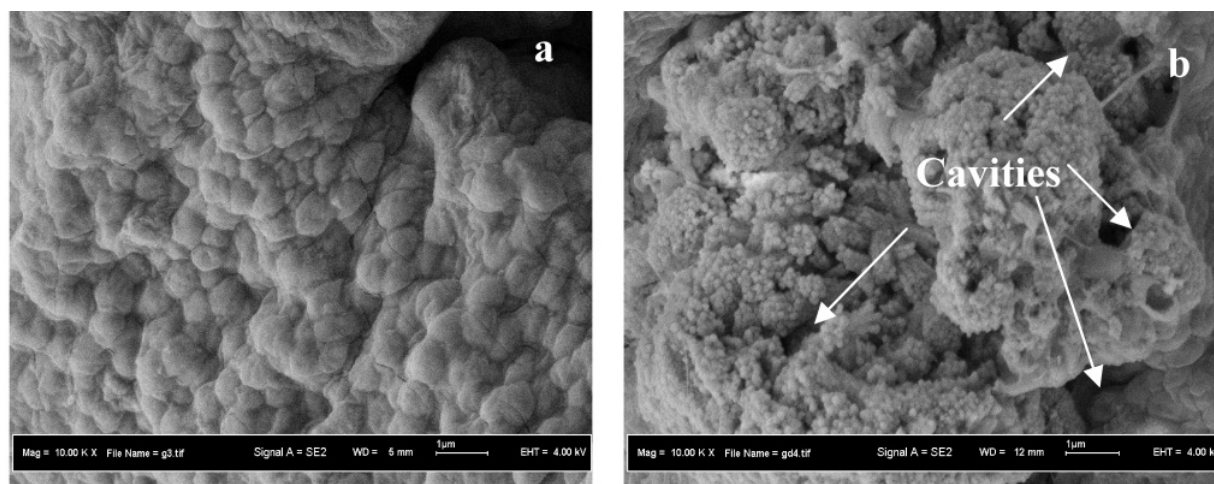


Figure 6. FESEM microstructure observations on mature biogranules under the magnification of 10,000K. (a) Coccoid bacteria tightly linked to one another. (b) Cavities that appear between bacteria clumped inside the biogranules

4.4. Physical characteristics of biogranules

The shear force imposed in the development of granules in this experiment, in terms of superficial upflow air velocity (i.e. 1.6 cm/s), resulted in the development of biogranules with an average diameter of 2.25 mm. The strong shearing force produced by aeration during the aerobic phase prevents the development of bigger aerobic granules. However, reduction in famine period may also lead to the formation of bigger aerobic granular sizes (Liu and Tay, 2006).

The average settling velocity of the sludge and anaerobic granular sludge used as the seeding were 9.9 ± 0.7 m/h and 42 ± 8 m/h respectively. The settling velocity of the biogranules increased from 17.8 ± 2.6 m/h to 83.6 ± 2.6 m/h at the end of experiment. The average settling velocity of the mature biogranules reached almost 80 ± 7.6 m/h, which was nearly three times greater than the settling velocity of the aerobic granules reported by Zheng et al. (2005).

The increase in settling velocity has given significant impact on the biomass concentration in the reactor. The relationship between the concentration of the MLSS and settling velocity of the granules is shown in Figure 7. Despite the short settling time (5 min), the high settling velocity possessed by the developed biogranules enabled the biogranules to escape from being flushed out during the decanting phase. Such conditions have caused more biogranules to retain in the reactor and resulted in the increase of biomass concentration.

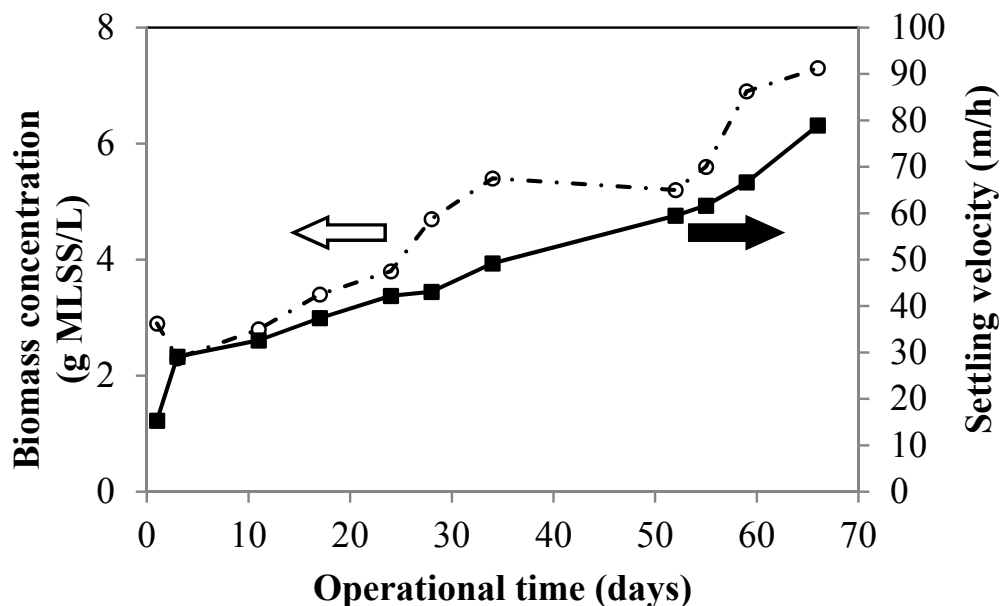


Figure 7. The relationship between the biomass concentrations retained in the reactor with the settling velocity of the biogranules (□) Settling velocity; (●) Biomass concentration

The SVI value has also improved from 277 mL/g at the initial stage to 69 mL/g at the mature development of biogranules. This indicates good settling properties of the biogranules, which is favorable in wastewater treatment plant operation. Figure 8 demonstrates the SVI profile along with the settling velocity. As the SVI value improved, the granular settling properties increased from 50 m/h to about 80 m/h. The SVI of biogranules seems to vary depending on the settling time of the reactor system. McSwain et al. (2004) reported the SVI of biogranules improved from 115 ± 36 ml/g to 47 ± 6 ml/g when the settling time decreased from 2 to 10 min. Biogranules developed with anaerobic seeding, showed higher settling velocity and improved SVI.

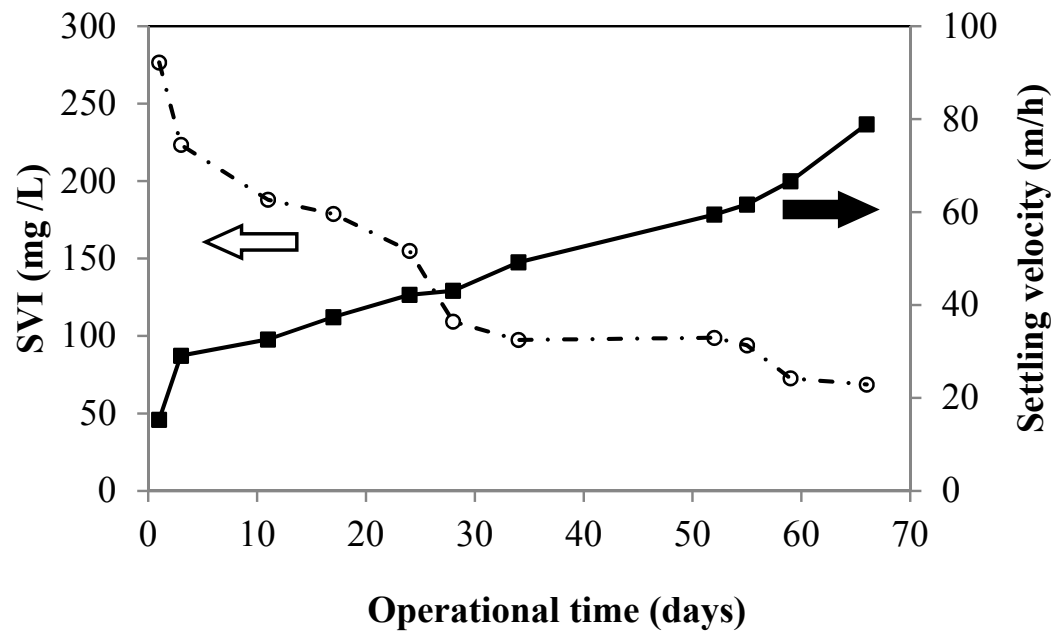


Figure 8. The relationship between the SVI values and settling velocity of the biogranules (▣) SVI, (○) Settling velocity

The granular strength of the biogranules was measured based on the integrity coefficient (IC) defined earlier. The smaller the value of IC, the higher the strength and ability of the biogranules to clump together and being prevented to break due to shear force of the aeration. Figure 9 shows the profile of IC of the developed biogranules as a function of time. The IC reduced as the biogranules developed. The initial value of IC was 30. Then the IC was reduced to about 9 as it reached a mature stage. According to Ghangrekar et al. (2005), biogranules 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 biogranules.

During the initial development, the microbes within the biogranules were loosely bounded to each other. At this stage, the biogranules may consist of more cavities causing the biogranules become less dense, as manifested by low settling velocity. As more microbes are linked together, the biogranules increase in size. Under certain selective pressures (i.e. short

settling time, hydrodynamic shear force, starvation of the microbial cell), microbes may produce more extrapolsaccarides (EPS) (Lin et al., 2003; Qin et al., 2004). As reported by Zhang et al. (2007) and Adav and Lee, (2008), the EPS contribute greatly to the strength and the stability of aerobic granules. When microbial cells produce more EPS, they form a cross-linked network and further strengthen the structural integrity of the granules. The cavities within the biogranules will be filled with EPS as it is a major component of the biogranules matrix material. This caused the biogranules to become denser and stronger as shown by their high settling velocity and lower IC value. The physical characteristics of the seed sludge and the matured biogranules are summarized in Table 4. The developed biogranules possess desirable biomass characteristics in the biological wastewater treatment system.

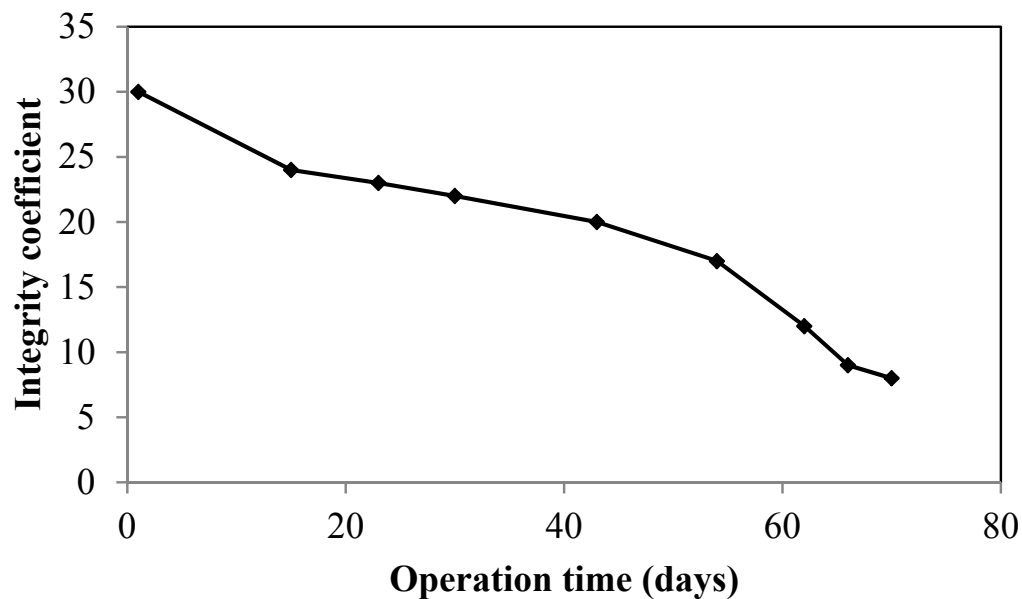


Figure 9. The profile of integrity coefficient representing the granular strength of the biogranules

Characteristics	Seed Sludge	Biogranules
SVI (mL/g)	277	69
Average diameter (mm)	0.02 ± 0.01	2.3 ± 1.0
Average settling velocity (m/h)	9.9 ± 0.7	80 ± 8
IC	92 ± 6	9.4 ± 0.5
MLSS (g/L)	2.9 ± 0.8	7.3 ± 0.9
MLVSS (g/L)	1.9 ± 0.5	5.6 ± 0.8

Table 4. Characteristics of seed sludge and biogranules

The profile of the biomass concentration (i.e. MLSS) after seeding with the anaerobic granules is shown in Figure 10. During the first few days, almost half of the sludge was washed out from the reactor causing a rapid decrease in the biomass concentration. The MLSS reduced from initial concentrations of 5.5 g/L to 2.9 g/L 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 biogranules and debris resulted from shear force caused by aeration. These small fragments have poor settling ability and were washed out from the reactor causing an increase of suspended solids concentration in the effluent.

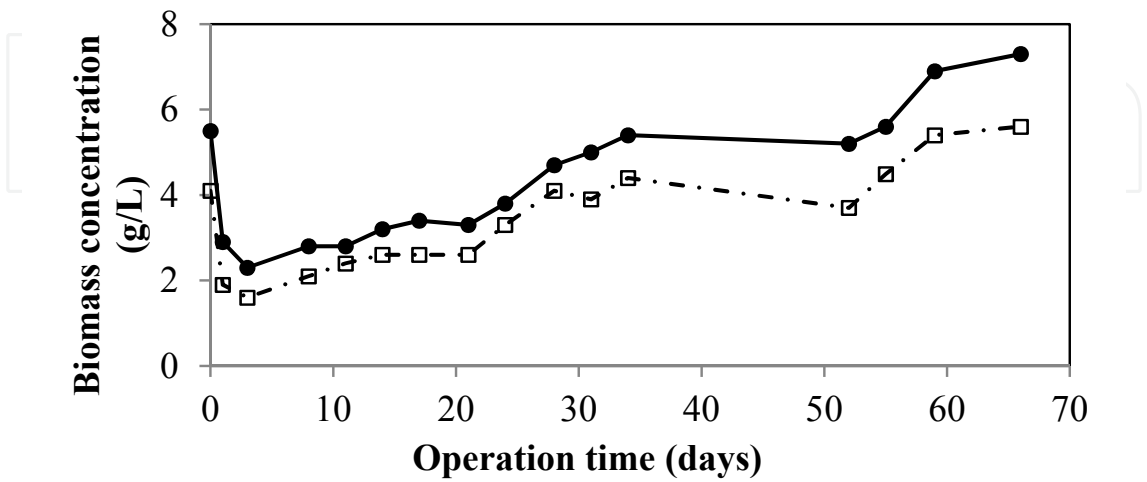


Figure 10. The profile of biomass concentration in the SBR. (●) MLSS, (□) MLVSS

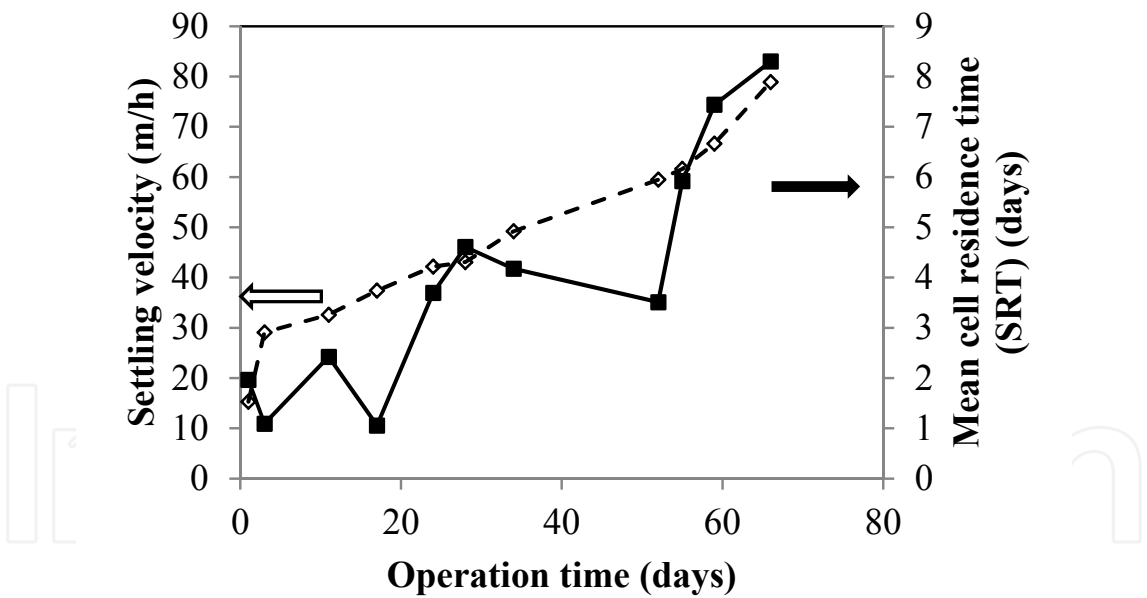


Figure 11. The settling velocity profile in relation to mean cell residence time (SRT). (■) SVI, (◇) SRT

As the experiment continued, the concentration of the biomass increased and reached 7.3 g MLSS/L on the 66th day. The profile of MLVSS follows the same trend of MLSS, ranging from 1.9 g/L to 5.6 g/L. The mean cell residence time (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. 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. Nonetheless, the benefit of high SRT will depend on the goal of the treatment process (Tchobanoglous et al., 2004). The SRT is affected by the settling velocity. The profiles of the settling velocity and the SRT as functions of time are given in Figure 11.

4.5. System profile

Changes in the HRT of the reactor system caused variation of the anaerobic and aerobic react times. It also may affect the loading rate imposed to the system if the substrate concentration is maintained. These conditions will affect the microbial activity within the biogranules and may influence the performance of the reactor system. The details of the experimental conditions of the reactor system are shown in Table 5.

The microbial activity was measured based on the OUR of a complete one cycle operation. The OUR was measured several times before each of the stages ended and showed that most of the external substrate was consumed more or less within the first 30 minutes of each aerobic reaction phase. Figures 12 and 13 show the profiles of the OUR throughout the experiment from Stage I to Stage VI.

Stage	Days covered	Phase (hours)				HRT (hrs)	OLR (kg COD/ m ³ ·day)
		1 st		2 nd			
		Anaerobic	Aerobic	Anaerobic	Aerobic		
I	49	1.42	1.42	1.42	1.42	6	2.5
II	43	2.92	2.92	2.92	2.92	12	1.3
III	51	5.92	5.92	5.92	5.92	24	0.6
IV	43	5.92	5.92	5.92	5.92	24	0.8
V	46	8.92	2.92	8.92	2.92	24	0.8
VI	46	2.92	8.92	2.92	8.92	24	0.8

$OLR = \frac{X}{V_{total}} \times \frac{V_{add}}{T}$, where X = COD concentration of the influent (mg/L); V_{add} = Volume of influent added in each cycle operation (mL); V_{total} = Total working volume of the experiment (mL); T = Hydraulic retention time (hour).

Table 5. Details of experimental conditions of the reactor system

The OUR profile (Figure 12) shows that the initial measurement of the OUR was reduced as the HRT increased (Stage I to Stage III). This is due to the reduction in the OLR as the HRT increased. Less oxygen is required as the organic load concentration is reduced. After a sharp increase of OUR at the beginning of each cycle in all stages, the OUR measurement was consistently low until the end of the cycle. The low value of the OUR indicates that most of the external substrates have been consumed. It also means that the microorganisms in the reactor system are under starvation phase. At this phase, no further degradation was observed even though the HRT was extended. During the starvation phase, endogenous respiration will take place, except at the beginning of the second phase of aerobic reaction where there was a short increase in the OUR. This increase is caused by the mineralization of

amines, the byproduct of dye degradation during the second anaerobic reaction phase. As the duration of anaerobic reaction phase increased, the short pulse increased as shown in Figure 13 (a and b) of Stage IV and V, respectively. Stage IV and Stage V were operated with the same HRT and organic loading but were different in the anaerobic and aerobic reaction phase ratio.

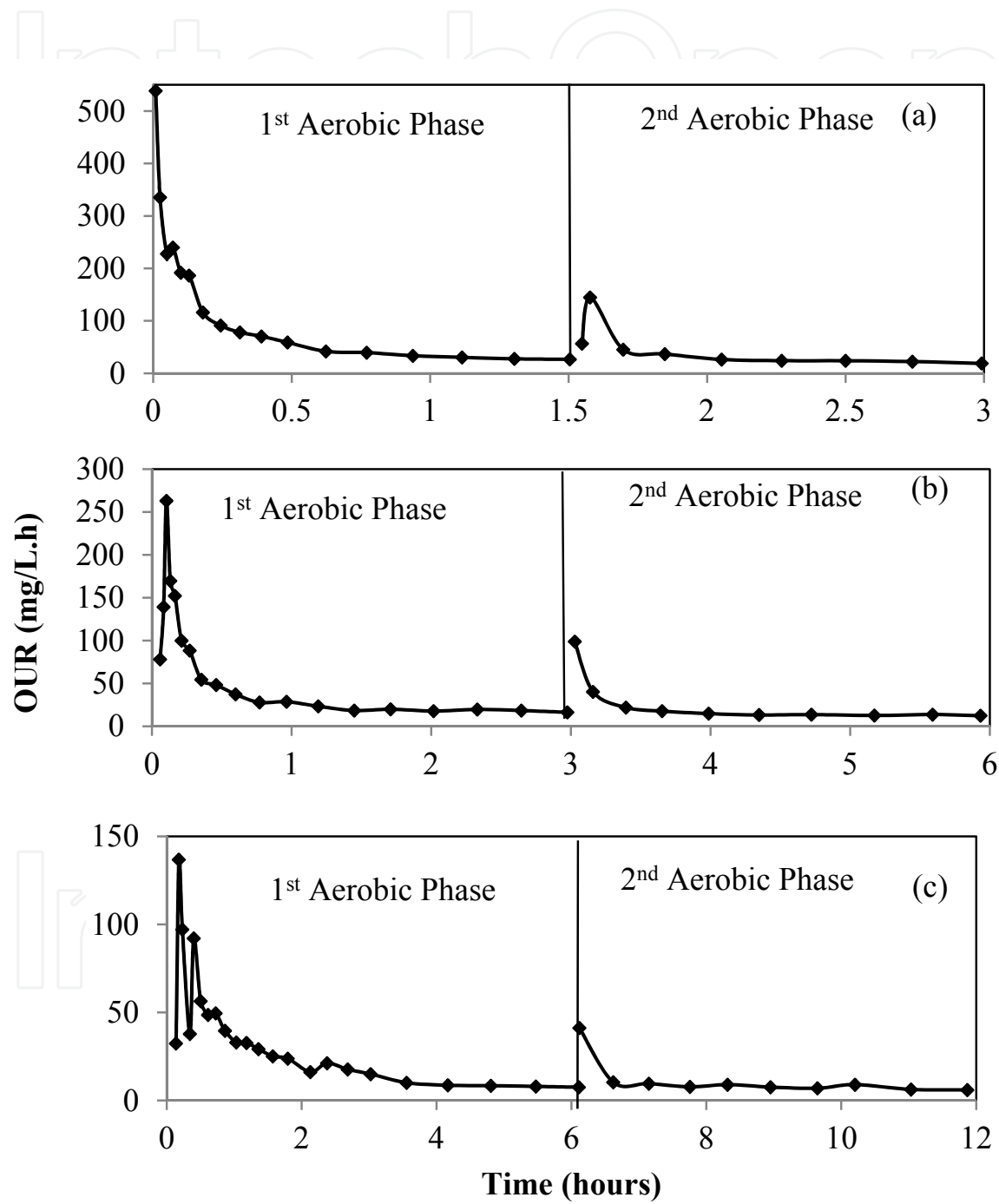


Figure 12. OUR profile of (a) Stage I (Aerobic phase 2.84 hours), (b) Stage II (Aerobic phase 5.84 hours) and (c) Stage III (Aerobic phase 11.84 hours)

The changes in the HRT will also affect the biomass accumulated within the reactor system. The HRT was increased from 6 hours in Stage I to 24 hours in Stage III, without the addition of any substrate. This resulted in the reduction of OLR supplemented into the reactor system from 2.5 to 0.6 kg COD/m³ day. The HRT for Stage III to VI was kept constant i.e. 24 hours, but the duration of anaerobic and aerobic react phases was varied. From Stage III onwards, the OLR was increased to 0.8 kg COD/m³ day by increasing the concentration of the carbon sources in the synthetic textile dyeing wastewater.

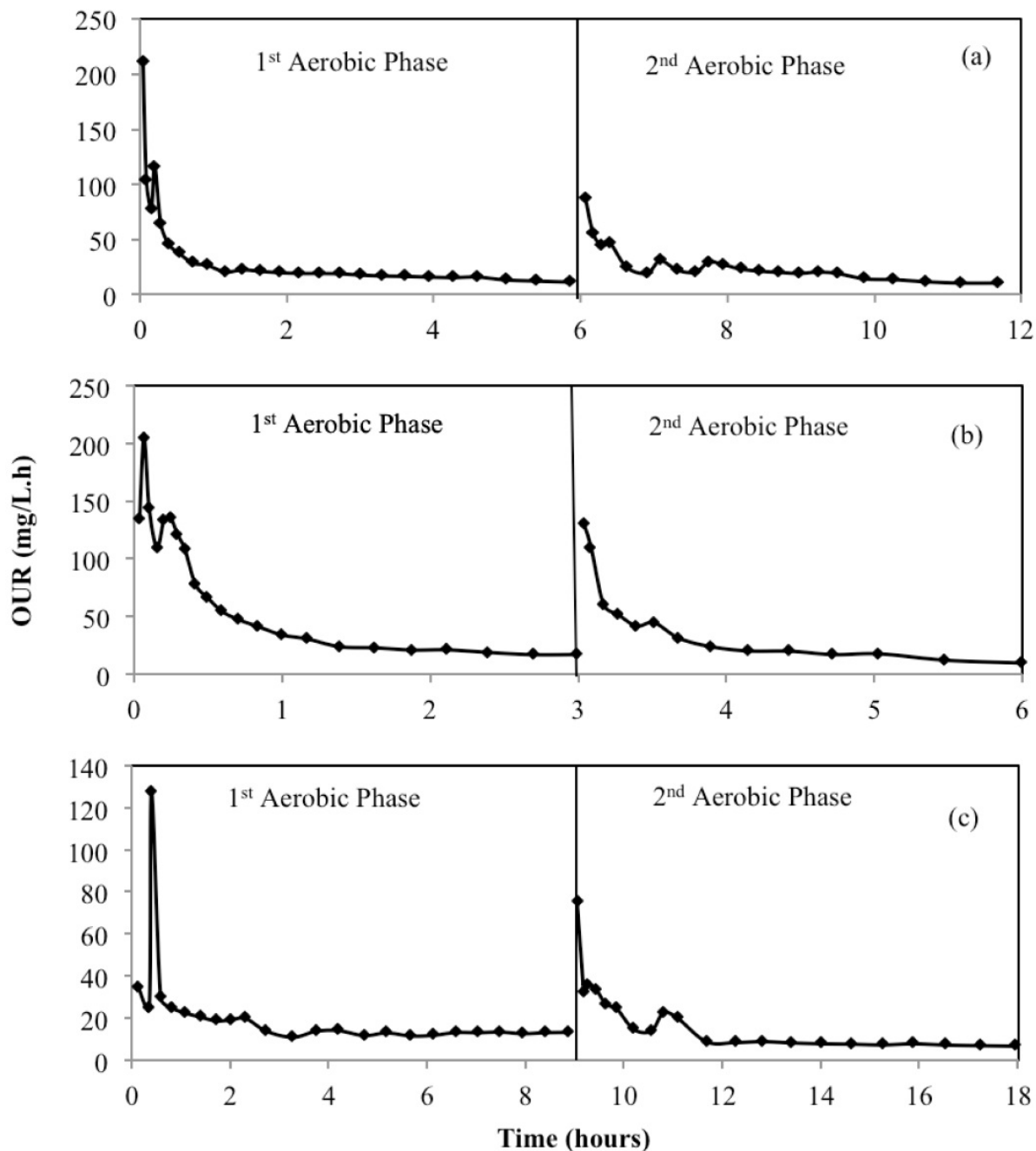


Figure 13. OUR profile of (a) Stage IV (Aerobic phase 11.84 hours), (b) Stage V (Aerobic phase 5.84 hours), (c) Stage VI (Aerobic phase 17.84 hours)

Table 6 shows the oxidation-reduction potential (ORP) values measured during the second phase of the anaerobic and aerobic reactions during the experiments. The ORP profile of all the stage corresponded very well with the dissolved oxygen. As the anaerobic react phase increased, more of negative values of the ORP were recorded. During the aerobic phase the ORP varies between +98 to +177 mV.

The biomass profile at steady state with stepwise increment of HRT (Stage I to III) and variation of react phases (Stage IV to VI) are shown in Table 8. As shown in Table 7, it is apparent that the biomass concentration (MLSS) in the reactor decreased and the VSS in the effluent were also reduced with the increase in the HRT (Stage I to III). The reduction of the biomass concentration in the reactor may be due to the lower value of OLR applied in the reactor system as the HRT increased.

Stage	Anaerobic React Phase	Aerobic React Phase
I	-124 ± 27	125 ± 19
II	-219 ± 33	129 ± 24
III	-358 ± 29	174 ± 34
IV	-355 ± 51	151 ± 17
V	-407 ± 21	112 ± 21
VI	-225 ± 28	177 ± 15

Table 6. Oxidation Reduction Potential

React Phase	Stage					
	I	II	III	IV	V	VI
Anaerobic (hours)	2.8	5.8	11.8	11.8	17.8	5.8
Aerobic (hours)	2.8	5.8	11.8	11.8	5.8	17.8
MLSS (g/L)	35.3 ± 1.6	28.7 ± 0.6	25.2 ± 1.8	30.5 ± 3.4	31.6 ± 3.7	23.3 ± 0.8
MLVSS (g/L)	31.9 ± 1.8	24.5 ± 2.2	18.5 ± 2.2	26.0 ± 3.4	22.4 ± 2.0	20.2 ± 0.8
VSS/SS	0.90	0.85	0.73	0.85	0.71	0.87
Effluent (VSS g/L)	0.34 ± 0.16	0.31 ± 0.11	0.26 ± 0.19	0.34 ± 0.11	0.33 ± 0.10	0.55 ± 0.22
SRT (day)	27.6 ± 13.4	42.4 ± 10.2	78.9 ± 23.9	70.1 ± 23.9	72.5 ± 23.3	41.6 ± 18.4

Table 7. Biomass concentrations at different stages of the experiment

When the OLR was increased to $0.8 \text{ kg COD/m}^3\cdot\text{day}$, there was an improvement in the biomass concentration where the biomass concentration have increased to $30.5 \pm 3.4 \text{ g/L}$ and $31.6 \pm 3.7 \text{ g/L}$ in Stage IV and Stage V as compared to $25.2 \pm 1.8 \text{ g/L}$ of biomass concentration in Stage III which run at the same HRT (24 hours) but with OLR $0.6 \text{ kg COD/m}^3\cdot\text{day}$. The increase in OLR has caused an increment in the biomass concentration in the reactor. A

slight increase in the biomass concentration was also observed along with the longer period of the anaerobic phase (Stage V), i.e. 18 hours.

The ratio of the volatile biomass (MLVSS) to total biomass (MLSS) reduced from Stage I to Stage III mainly due to decrease in the OLR as the HRT increased from 6 to 24 hours, whereas the MLVSS/MLSS ratio of the Stage III and Stage IV with 12 hours aerobic reaction phase was observed higher with the ratio of 0.73 and 0.85, respectively. The increment may be due to the increase of the OLR from 0.6 to 0.8 kg COD/m³ day (Stage III to Stage IV). Increase in the OLR means more carbon sources were supplied to the microorganisms in the reactor. When more food is available, more growth will take place and this is indicated by the increase in the MLVSS/MLSS ratio.

However, when the anaerobic period of the HRT is extended, the MLVSS/MLSS ratio decreased (0.71). Decrease in MLVSS/MLSS ratio may indicate an increase of inorganic accumulation within the granulation biomass. When the duration of aeration phase was increased up to 18 hours, the biomass started to reduce again (Stage VI) and increase of VSS in the effluent was once again observed. This may give an indication that too long of aerobic reaction phase is not suitable for granular biomass system. Prolong of aeration time may result in instability of the reactor performance. The profile of biomass concentration of the reactor system is given in Figure 14.

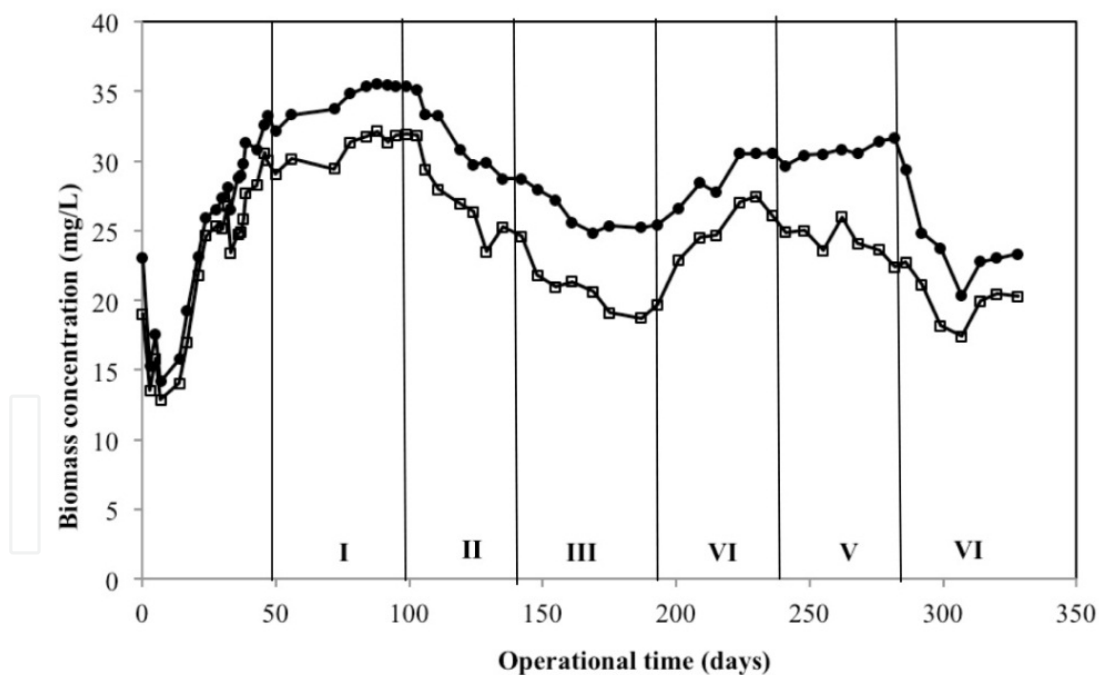


Figure 14. Profile of biomass concentration at different stages of the experiment. (●) MLSS, (□) MLVSS. Stage I: anaerobic (2.8 h): aerobic (2.8 h); Stage II: anaerobic (5.8 h): aerobic (5.8 h); Stage III and Stage IV: anaerobic (11.8 h): aerobic (11.8 h); Stage V: anaerobic (17.8 h): aerobic (5.8 h); Stage V: anaerobic (5.8 h): aerobic (17.8 h)

The SRT of the reactor system increased from 27.6 ± 13.4 to 78.9 ± 30.8 d when the length of the HRT increased from 6 to 24 hours (Stage I to Stage III). With HRT of 24 hours, increase of

anaerobic reaction phase up to 18 hours (Stage IV to Stage V) has slightly increased the SRT from 70.1 ± 23.9 to 72.5 ± 23.3 d. The SRT value changes in each stage of the experiment. According to Wijffels and Tramper (1995), the favorable sludge age for high removal efficiency for COD and nitrification process is more than 4 days. Based on the SRT obtained, this biogranular system is capable of the simultaneous degradation of nitrification process and COD removal. Since the treatment goal is to remove recalcitrant dyeing compound, the SRT value of all stages evaluated in this experiment was in the acceptable range from degradation of xenobiotic compounds (Grady et al. 1999).

React Phase	Stage					
	I	II	III	IV	V	VI
Anaerobic (hours)	2.8	5.8	11.8	11.8	17.8	5.8
Aerobic (hours)	2.8	5.8	11.8	11.8	5.8	17.8
SVI (mL/g)	13.1 ± 0.4	18.8 ± 1.5	21.4 ± 1.6	16.8 ± 1.3	15.5 ± 1.3	24.8 ± 0.9
SV (m/h)	41.3 ± 3.1	35.1 ± 0.8	24.5 ± 1.1	28.4 ± 1.3	33.4 ± 2.5	21.3 ± 0.5

Table 8. Physical properties of the biogranules at different stages of the react phase

The SVI value of the biogranules was used to evaluate the biogranules settling ability. It is anticipated that bigger biogranules will have higher settling velocity and hence, reduce the SVI value, indicating good settling ability. The SVI value improved when the anaerobic react phase was prolonged in Stage V indicating such reaction pattern will help to develop granules with better settling profile. According to Panswad *et al.* (2001a), inert biomass increased as the anoxic/anaerobic condition was prolonged. It is possible that the accumulation of inert particles within the biogranules increased and resulted in improved SVI properties. Table 8 showed the physical properties of the biogranules at different stages of the react phase.

Figure 15 shows the profile of SVI of the reactor system. The SVI value in Stage V was reduced from 16.8 ± 1.3 mL/g (in Stage IV) to 15.5 ± 1.3 mL/g. This is expected to be due to the accumulation of more inert solids within the biogranules as shown with low levels of MLVSS/MLSS ratio in Stage V (0.71). Despite changes in HRT that caused decrease in the size of biogranules, the SVI values of the whole experiments were good except for Stage VI. During Stage VI, the prolonged of the aerobic phase (i.e. 17.8 hours), which was operated at high superficial air velocity (2.5 cm/s), cause the biogranules to rupture. At this stage, the size of biogranules becomes smaller causing the settleability of the particles to reduce and was demonstrated with increase in SVI value.

Hydraulic retention time is an important parameter that control the contact time between the biomass and the wastewater in a reactor system. The HRT of a system must be long enough for the degradation process to take place. However, in the application of biogranules in the treatment system, the HRT should not be too long as it may cause the disintegration of the granules. According to Tay et al. (2002) and Wang et al. (2005), a short

HRT is favorable for rapid granulation process, while too long HRTs may lead to granulation system failure due to high biomass lost (Pan et al., 2004). An optimum HRT of biogranulation systems will be able to stabilize the reactor performance with good biomass retention and high removal performance. According to Pan et al. (2004), the optimum HRT for aerobic granulation systems ranging from 2 to 12 hours where stable aerobic granules with good settleability and microbial activities. However, the optimum HRT for treating different types of wastewater may vary depending on the type of wastewater and the targeted degradation compound.

4.6. Removal of color

Color removal was observed to increase from $66.7 \pm 1.6 \%$ to $76.5 \pm 0.8 \%$ as the HRT increased from Stage I to Stage III. Increase in the HRT allows longer contact time between the biogranules and the wastewater resulting in better color removal. Furthermore, when the OLR was increased from $0.6 \text{ kg COD/m}^3\cdot\text{day}$ (Stage III) to $0.8 \text{ kg COD/m}^3\cdot\text{day}$ (Stage IV), a significant improvement in color removal from $76.5 \pm 0.8 \%$ to $83.1 \pm 1.4 \%$ was observed. This may be due to the increase in the microbial population. Ong et al. (2005) reported that the percentage of color removal efficiency increased by 16% in anaerobic and 50% in aerobic SBR reactor systems when the OLR rate was increased from 2.66 to 5.32 g COD/L·day. An increase of color removal efficiency from 82% to 90% was also observed by Talarposhiti et al. (2001) when the COD loading was increased in a two-phase anaerobic packed bed reactor from 0.25 to 1 kg COD/m³·day.

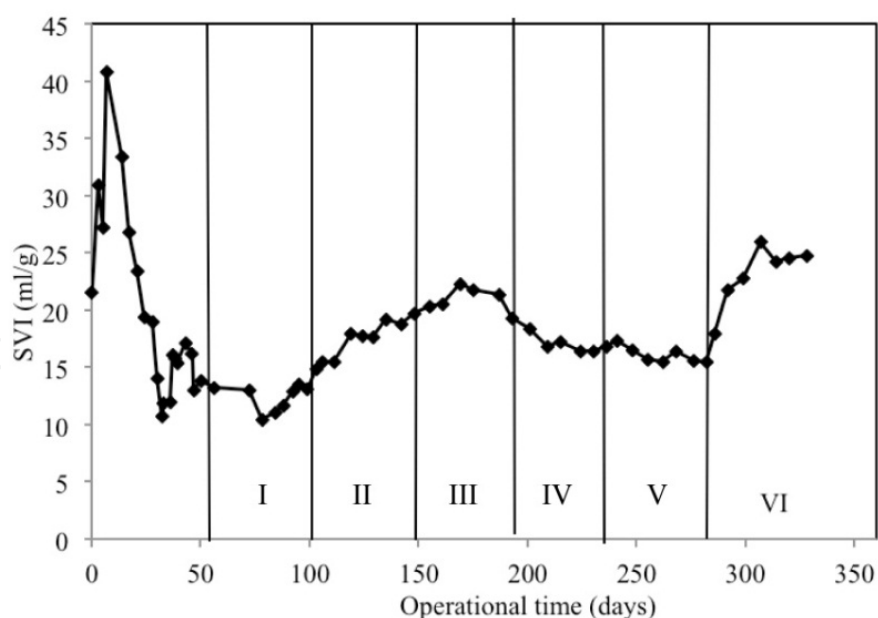


Figure 15. Sludge volume index profile of biogranules. Stage I: anaerobic (2.8 h): aerobic (2.8 h); Stage II: anaerobic (5.8 h): aerobic (5.8 h); Stage III and Stage IV: anaerobic (11.8 h): aerobic (11.8 h); Stage V: anaerobic (17.8 h): aerobic (5.8 h); Stage V: anaerobic (5.8 h): aerobic (17.8 h)

Since more color removal took place in the anaerobic condition (Banat et al., 1996; Dos Santos et al., 2007), the percentage of color removal was once again increased from Stage IV

($83.1 \pm 1.4\%$) to Stage V ($86.5 \pm 0.5\%$) when the anaerobic reaction phase was extended from 12 to 18 hours of the 24 hours reaction cycle. Improved decolorization process that occurs during the anaerobic stage enhances the overall wastewater biodegradation since more readily biodegradable substances can be degraded in the following aerobic treatment (Stolz, 2001). Figure 16 shows the profile of the color removal performance.

With respect to the mechanisms that are involved in color degradation, the addition of electron-donating substrate can considerably improve the decolorization reductive rate (Bras et al., 2001, Dos Santos et al., 2005). In anaerobic and aerobic sequential wastewater treatment system, the anaerobic stage was the main step for color degradation while the aerobic phase acted as the polishing step and enhancement in COD removal. Higher initial COD concentration did not improve color removal but caused deterioration in COD removal in the anaerobic-aerobic SBR system (Kapdan and Oztekin, 2006).

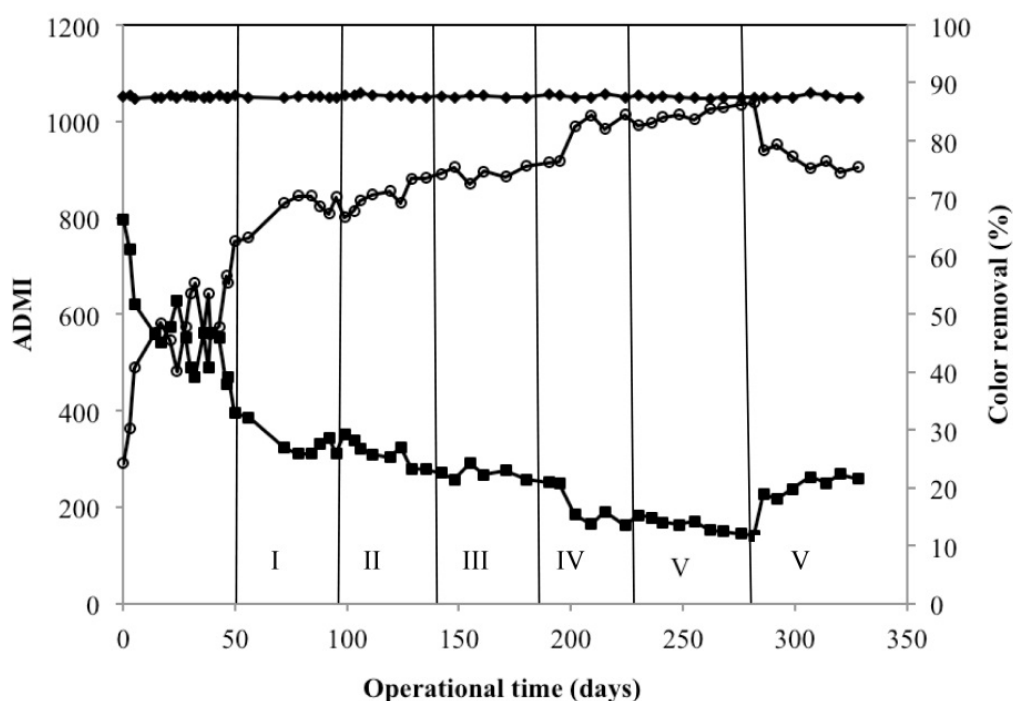


Figure 16. Profile of color removal performance of the reactor system at different stages of the experiment. (■) Influent color, (□) Effluent color, (○) Color removal. (100 ADM \approx 1 Pt-Co). Stage I: anaerobic (2.8 h): aerobic (2.8 h); Stage II: anaerobic (5.8 h): aerobic (5.8 h); Stage III and Stage IV: anaerobic (11.8 h): aerobic (11.8 h); Stage V: anaerobic (17.8 h): aerobic (5.8 h); Stage V: anaerobic (5.8 h): aerobic (17.8 h)

Psukphun and Vinitnantharat (2003) reported that the duration of the anaerobic phase should be long enough to obtain better COD and color removal. An increase in the HRT will provide enough time of the COD and inter-metabolites of simulated textile wastewater in anaerobic or/and anaerobic/aerobic systems (Isik and Sponza, 2008). Biodegradation of the azo bonds may require a certain contact time in order to achieve high removal efficiency. Depending only on the filling stage to provide anaerobic condition for the cleavage of azo bond compounds may not be adequate for textile wastewater treatment. However, the time

required for the cleavage of the azo bond may be affected by the complexity of the dye molecule structures. The suitable contact time of anaerobic and aerobic reaction phases may provide high removal performance for the cleavage of the N=N bond (anaerobic condition) and mineralization of aromatic amines (aerobic phase). Furthermore, the reduction of COD is more effective during the aerobic stage as compared to the anaerobic reaction condition (Smith et al., 2007). It shows that having longer anaerobic (18 hours) and shorter aerobic (6 hours) react phase resulted in the highest removal for color and a slight improvement in the efficiency of COD removal.

4.7. Removal of chemical oxygen demand

The time history of the COD concentration in the influent and effluent and the removal rate for all six stages is given in Figure 17. The biogranular system showed consistent COD degradation performance with $84.2 \pm 0.9\%$ removals after about 50 days of start-up period (acclimatization phase). The overall performance was almost consistent despite the fact that the duration of the experimental process was increased from 6 hours to 24 hours. This phenomenon may be due to the decreasing biomass concentration and reduction in the OLR as mentioned earlier. When the OLR was increased from $0.6 \text{ kg COD/m}^3\cdot\text{day}$ to $0.8 \text{ kg COD/m}^3\cdot\text{day}$ on the 194th day of the experiment (Stage III to Stage IV), the COD removal efficiency increased from about $84.4 \pm 0.4\%$ at the end of Stage III (day 193) to $90.7 \pm 0.2\%$ at the end of Stage IV (day 236).

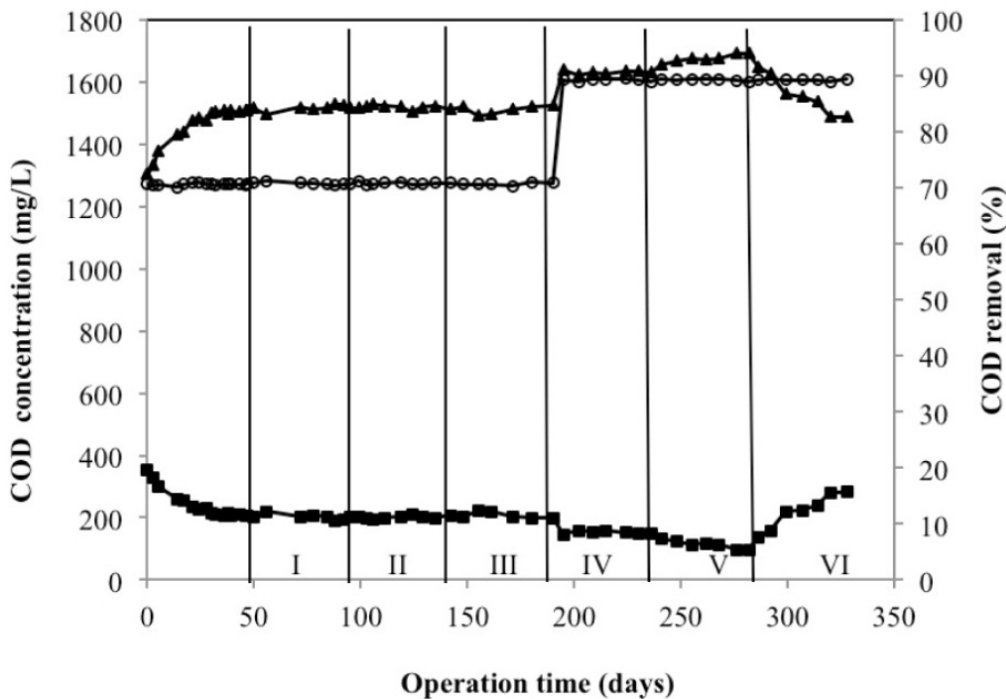


Figure 17. Profile of COD removal performance of the reactor system at different stages of the experiment. (○) Influent COD; (■) Effluent COD, (▲) COD removal. Stage I: anaerobic (2.8 h): aerobic (2.8 h); Stage II: anaerobic (5.8 h): aerobic (5.8 h); Stage III and Stage IV: anaerobic (11.8 h): aerobic (11.8 h); Stage V: anaerobic (17.8 h): aerobic (5.8 h); Stage V: anaerobic (5.8 h): aerobic (17.8 h)

At the final stage (Stage VI) of the experiment, a surge drop of COD removal efficiency was observed. As the aeration time was increased from 6 to 18 hours, the COD removal was reduced from $94.1 \pm 0.6\%$ to $82.6 \pm 0.8\%$. The drop in the COD removal efficiency was due to the increase in biomass loss into the effluent. The MLSS in Stage VI was 23.3 ± 0.8 g/L as compared to 31.6 ± 3.7 g/L observed in the previous stages. The effect of HRT on the COD and color removal performance by the biogranules at different stages is given in Table 10.

An increase in the percentage of COD removal efficiency was also observed when the period of anaerobic phase was increased from 12 hours to 18 hours. As noted in Table 9 the removal increased from 90.7 % in Stage IV to 94.1% in Stage V. Psukphun and Vinitnantharat (2003) claimed that the increase in the non-aeration phase in the SBR system will cause an alteration in the population of anaerobic microorganisms in the system. The state is expected to produce good COD and color removal for textile wastewater. However, according to Kapdan and Oztekin (2006), when the duration of anaerobic phase is too long, the contribution of aerobic react phase can be decreased. This is possibly due to the toxic effect of aromatic amines produced during dye degradation.

Reaction Phase	Stage					
	I	II	III	IV	V	VI
Anaerobic (hours)	2.8	5.8	11.8	11.8	17.8	5.8
Aerobic (hours)	2.8	5.8	11.8	11.8	5.8	17.8
COD (%)	84.2 ± 0.9	84.6 ± 1.1	84.4 ± 0.4	90.7 ± 0.2	94.1 ± 0.6	82.6 ± 0.8
Color (%)	66.7 ± 1.6	74.3 ± 0.4	76.5 ± 0.8	83.1 ± 1.4	86.5 ± 0.5	75.4 ± 0.3

Table 9. Profile of COD and color removal percentage at different stages of experiment

Owing to the condition in the reactor where different react phases occur in the same column, too long anaerobic reaction periods will cause high accumulation of aromatic amine in the same compartment. High concentrations of aromatic amines may inhibit the activity of aerobic microorganisms during the aerobic phase. In this study, even though the anaerobic react phase was extended up to 18 hours, there was no reduction in COD removal. This shows that there was no inhibition on the activity of aerobic microorganisms by the long accumulation of the byproducts produced from anaerobic degradation of the dye compound. The reason can be that the concentration of dye used was not sufficiently high to produce any toxicity effect on the microorganisms within the biogranules. Furthermore, the biogranules may not be affected by the dyestuff degradation byproducts due to the structural form of the biogranules. The biogranules structure, which consisted of EPS acts as a shield for microorganisms within the granules against any shock loading or toxic compound.

5. Conclusions

Stable biogranules can be cultivated in the SBR system with the application of intermittent anaerobic and aerobic reaction modes during the react phase. The matured biogranules showed the domination of non-filamentous bacteria that were tightly linked and embedded to one another and covered with EPS. The use of seed sludge in the development process affects the morphology of the developed granules. Matured biogranules had an average diameter of 2.3 ± 1.0 mm and a settling velocity of 80 ± 8 m/h and a low IC value of 9.4 ± 0.5 . This indicates successful development of excellent settling properties of biogranules. The cultivation of biogranules seeded with anaerobic granules resulted in better granules formation. The OUR/SOUR and SMA analysis proved the presence of anaerobic and aerobic microorganisms activities in the biogranules. They are capable of performing degradation both in anaerobic and aerobic conditions. The size and the SVI of the biogranules were very much affected by the variations of the HRT. An increase in the aeration react time resulted in the disintegration of the biogranules. Too long aerobic reaction times exposed the biogranules under prolonged starvation condition causing instability of the granular structure that lead to disruption of the biogranules.

The percentage of COD removal was not likely affected by the increase in the HRT, but mainly caused by the decrease in the granular biomass and OLR. However, the COD removal was improved with the increase in the anaerobic reaction phase. The percentage of color removal has improved with the increase of the HRT. An HRT with a prolonged anaerobic react time and reduced aerobic reaction time is considered as the best condition for the removal of color and the organic compound, resulting in maximum color and COD removal.

Author details

Khalida Muda, Azmi Aris, Mohd Razman Salim

*Department of Environmental Engineering,
Faculty of Civil Engineering,
Universiti Teknologi Malaysia, Skudai, Johor,
Malaysia*

Zaharah Ibrahim

*Department of Biological Sciences, Faculty of Biosciences and Bioengineering,
Universiti Teknologi Malaysia, Skudai, Johor, Malaysia*

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