

## Development of Microbial Granule Using Low Strength Textile Wastewater Under Influence of Static Magnetic Field

Ahmad Hanis Omar<sup>a</sup>, Khalida Muda<sup>\*b</sup>,  
Zaiton Abdul Majid<sup>c</sup>, Augustine Chioma Affam<sup>d</sup>,  
Nasiman Sapari<sup>e</sup>, Emmanuel Olisa<sup>f</sup>,  
Norelyza Hussein<sup>g</sup>, Normala Hashim<sup>h</sup>,  
Nur Syamimi Zaidi<sup>i</sup>

<sup>a,b,g,h,i</sup> Faculty of Civil Engineering, Universiti  
Teknologi Malaysia,

81310 UTM Johor Bahru, Johor, Malaysia

<sup>c,g,h,i</sup> Centre for Sustainable and Water Security  
(IPASA) UTM,

81310 Johor Bahru, Johor, Malaysia

<sup>e</sup>Faculty of Science, Universiti  
Teknologi Malaysia,

81310 UTM Johor Bahru, Johor, Malaysia

<sup>d</sup>Civil Engineering Department, School of Engineering  
and Technology,

University College of Technology Sarawak,  
96000 Sibu, Sarawak, Malaysia

<sup>e,f</sup>Civil Engineering Department, Universiti  
Teknologi Petronas,  
Bandar Seri Iskandar,

31750 Tronoh, Perak, Malaysia

\*Corresponding Author Email: [khalida@utm.my](mailto:khalida@utm.my)

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### Abstract

Most studies reported on the development of aerobic granules using high strength wastewater. Low strength wastewater however, is reported as type of wastewater which normally not able to support fast development of granulation. Although with such limitation, this study is aimed at forming the granules using low strength textile wastewater but with facilitation of static magnetic field. This study is conducted in a single sequencing batch reactor of two-stage anaerobic-aerobic reaction phase under the exposure of magnetic field. Granules were cultivated using combination of textile sludge and municipal wastewater treatment plant. From this study, the granules increased to about  $0.15 \pm 0.10$  cm of average size. This had thus increased the settling velocity of the granules. Granule strength that is expressed as integrity coefficient was observed to decrease from  $26.3 \pm 0.5$  to  $12.5 \pm 0.5$  owing to an increase in the strength of the granules. The removal performances of chemical oxygen demand (COD), total organic carbon (TOC) and color were observed quite high viz. 93.5%, 92.9% and 44.4%, respectively. Generally, the study indicated that granular sludge is potential to be developed even using low strength wastewater with facilitation of static magnetic field.

**Key Words:** Low strength wastewater; Textile wastewater; Granulation; Static magnetic field.

## 1 Introduction

The textile industry is one of dominant industry that contribute to generation of effluent wastewater due to high consumption of water for various wet processing operations. The effluent wastewater mainly contains chemical compound such as dyes, alkalis, acids, soaps of metals and surfactants dispersing agents (1). Dye such as aromatic and heterocyclic dyes become hardly to degrade due to its complicated and stable structure which also exist in textile wastewater and other complex matrix (2). The release of textile industry effluent in the environment without proper treatment may

cause serious environmental pollution. According to Waghmode et al. (3), aromatic amines compound may present in wastewater that contains azo dye due to reduction of azo bond ( $-N=N-$ ) under anaerobic condition which in several cases are toxic, mutagenic and carcinogenic (4). Amongst a variety of physicochemical and biological treatment, the two-stage biological anaerobic-aerobic system is appropriate for treating azo dye-containing wastewater (5). It is considered environmentally friendly as this treatment process can lead to complete mineralization of textile effluent at low cost.

In form of biological treatment, there have been various types of microbes that have been used to decolorize dyes. Microbial strains such as *Bacillus* sp. and *Shewanella putrefaciens* (6, 7) have been reported as the successful azo dye decolorization. Some of these microbes can perform decolorization anaerobically while most of it can normally perform aerobically. Anaerobic azo dye decolorization usually leads to the formation of aromatic metabolites that can resist further anaerobic degradation. However, under aerobic condition complete mineralization of the amines can occur (8). Due to such limitation, several studies have employed two-stage reactors to execute both anaerobic-aerobic phases (9, 10). This execution is complex because the anaerobic microbes need to be removed from the wastewater before the wastewater can be transferred into aerobic reactor. Hence, as to enhance this operation, sequencing batch reactor (SBR) was used for these microbes granulation development, which it is operated under aerobic and anaerobic conditions simultaneously.

As a matter to enhance the development of microbes granulation, various internal factors such as hydrodynamic shear force, hydraulic residence time, substrate composition i.e. feed, organic loading rate (OLR) and settling time have been examined to enhance secretion of extracellular polymeric substances (EPS). These substances can help in enhancing the adhesion ability among the microbes thus, helps in fastening the development of granulation process (11). As for external factors, the usage of magnetic field to improve the performance of biological wastewater treatment gains wide attention lately. The intense magnetic field (more than 1 T) may hinder physical process of organisms (9) whereas Ji et al. (12) observed that slightly weak magnetic field maybe useful for bacterial growth. Studies employing static magnetic field in wastewater

treatment have proven that magnetic field could enhance microbial growth, accelerate biodegradation of organic materials (13-16) and increase nitrification rate (12). Its positive effect on biomass and glutathione production has also been reported (17). Wang et al. (18) also reported the improvement of granules development under the influence of magnetic field. Initially, development of granules was about 41 days in the absence of magnetic field but as 48 mT of magnetic field intensity was applied, the granules development period was shortened to 25 days. Still until today, studies that reported on the application of magnetic field specifically on the granulation are quite minor.

Common studies also highlighted that high strength wastewater that can be indicated by high OLR could efficiently develop the granules. Very lack of studies was conveyed in terms of using low OLR to develop the granules. Studies by Tay et al. (19) demonstrated the difficulty of granules cultivation with lower OLR. The study showed that OLR with lower rate than 2 kg COD/m<sup>3</sup> illustrated slow development of aerobic granules and took longer time to attain stable condition. Hence, the purpose of this study is to present a potential way to cultivate the granules with feasibility to develop using low strength wastewater. In order to occupy with the possible limitation of low OLR, static magnetic field was then used as the external factor of enhancement.

## 2 Methodology/Materials

### 2.1 Configuration of reactor

Magnetic bio-granules reactor system was designed and fabricated to operate at working volume of 3 L. Internal diameter of the reactor was 0.08 m with the total height of 1.5 m. The influent was fed from bottom of the reactor while decanting of the effluent was done at 0.4 m from bottom of the reactor's column. Air supply into the reactor was done using bubble diffusers. The operating configuration involving filling, anaerobic-aerobic, settling and decanting sequence phase were shown in Table 1. The bulk permanent magnets were arranged around the reactors and the exhibited magnetic field intensity was about 13.9–0.2 mT. The reactor system was operated at hydraulic retention time (HRT) of 8 hrs for a period of 24

weeks. It was operated until the matured bio-granules were formed in the reactor.

Table 1 Operating conditions of the magnetic bio-granules reactor system

| Operating Parameters | Time (min) |
|----------------------|------------|
| Fill                 | 5          |
| Idle                 | 10         |
| Anaerobic            | 55         |
| Aerobic              | 165        |
| Idle                 | 10         |
| Anaerobic            | 55         |
| Aerobic              | 165        |
| Settling             | 5          |
| Decanting            | 5          |
| Idle                 | 5          |

## 2.2 Compositions of wastewater

The wastewater used in this study was a synthetic textile type of wastewater. The composition of this synthetic wastewater were  $\text{KH}_2\text{PO}_4$  (0.23 g/L),  $\text{NH}_4\text{Cl}$  (0.16 g/L),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.09 g/L), EDTA (0.02 g/L), trace solution 1 mL/L,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (0.07 g/L) and  $\text{K}_2\text{HPO}_4$  0.58 (g/L). Sodium acetate (0.5 g/L), ethanol (0.125 g/L) and glucose (0.5 g/L) were used as carbon sources. Meanwhile, the trace elements used in this study were based on research by Smolder et al. (1995), namely  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  (0.12 g/L),  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  (0.03 g/L),  $\text{FeCl}_3 \cdot 4\text{H}_2\text{O}$  (1.5 g/L),  $\text{H}_3\text{BO}_3$  (0.15 g/L),  $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$  (0.06 g/L),  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (0.15 g/L), KI 0.03 g/L and  $\text{ZnCl}_2$  (0.12 g/L). Reactive Black 5, Disperse Orange 1 and Reactive Blue 4 were the dyes used. The wastewater consisted COD of  $500 \pm 100$  mg/L, TOC of  $205 \pm 20$  and color  $1000 \pm 100$  American Dye Manufacturing Index (ADMI). The pH was fixed at 7.0–0.5 before treatment was commenced.

## 2.3 Analytical methods

Size of the granule was observed by a stereomicroscope with camera (HUVITZ, HSZ-ILST6, Korea). Physical and biological characteristics of the microbial granules were determined. The physical characteristics include the settling velocity and granular strength. The settling velocity was obtained according to method described by Beun et al.(20). The granular strength was determined according

to Ghangrekar et al.(21) which was measured as integrity coefficient (IC). Determination of the sludge size was done based on sieving method (22). Parameter of chemical oxygen demand (COD), color, total organic carbon (TOC) and biomass concentration were measured based on Standard Methods (23). Other studies conducted include FTIR, UV/V is and FESEM analysis.

## 2.4 Experimental procedure

A 1.5 L synthetic wastewater and 1.5 L sludge from a textile wastewater treatment plant which giving a total of 3000 mL for the operational volume were mixed. The mixture gave a total sludge concentration of 4.8 g/L and organic loading rate (OLR) of 0.83 kgCOD/m<sup>3</sup>.d. Acclimatization of sludge mixture was performed before the reactor was operated for 24 weeks with hydraulic retention time (HRT) of 8 hrs. Temperature of the reactor was set at  $26 \pm 3^{\circ}\text{C}$ .

## 3 Results and Discussions

### 3.1 Physical characterization of microbial granules

#### 3.1.1 Microbial granule size

Shear force exerted on the granules due to the superficial up flow air velocity was 0.015 m/s. This led to microbial granule development, which the growth is about  $0.15 \pm 0.10$  cm of average particle diameter size (maximum size up until 0.3 cm). These microbial granules were form after undergone a numbers of physical and morphological changes. Figure 1 shows the microbial granules growth at the early stage (week 1) until the matured microbial granules form at the final stage (week 25). After 12 weeks of operation (Figure 1d), small granules with average diameter  $0.5 \pm 1.0$  mm were observed. Afterwards, the number and average diameter of the granules increased slowly (Figure 1e) until the matured granules form at the final stage. The formation of these granules is very slowing due to used of low strength wastewater and took longer time to reach stable condition. These result showed similarity with studies reported

by Tay et al. (19) that demonstrated lower OLR caused difficulties to cultivate and form the granules.

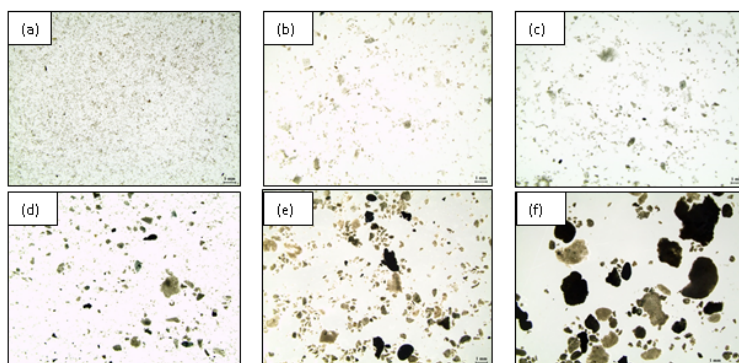


Figure 1 Microscopic image (a) Week 1, (b) Week 4, (c) Week 8, (d) Week 12, (e) Week 20 and (f) Week 25 of microbial granules development (Images were taken at magnification 6.7x, scale bar = 1 mm)

### 3.1.2 Biomass profiles and settling velocity of the granules

Figure 2 shows the profile of biomass concentration which corresponding to the settling velocity of granules. At the beginning of the experiment, disintegration of the anaerobic granules was observed. This was due to the aeration shear force that led to the breakdown of fragments of the granules. As a result of these fragmentations, the settling velocity of the granules was low and consequently was removed from the reactor. This was observed as the biomass concentration (i.e. MLSS) was decreased at the early stage of the experiments. Later after more than 40 days, an increase in the MLSS was observed and this can be explained probably due to the granules have already been adapted well to the biological culture. Towards the end of the experiment, the total biomass concentration was about  $6.7 \pm 0.9$  g/L.

Nevertheless, the observed settling velocity, which is between 25 to 70 m/hr, is actually aligned with the literatures that reported on the settling velocity of anaerobic granules. According to Schmidt and Ahning (24), the settling velocity of normal anaerobic granules was 18 to 100 m/hr while Zheng et al. (25) reported the range of

between 18 to 31 m/hr. This particular condition amongst others helped the granules to remain in the reactor and not being washed out as well as increased the concentration of the biomass.

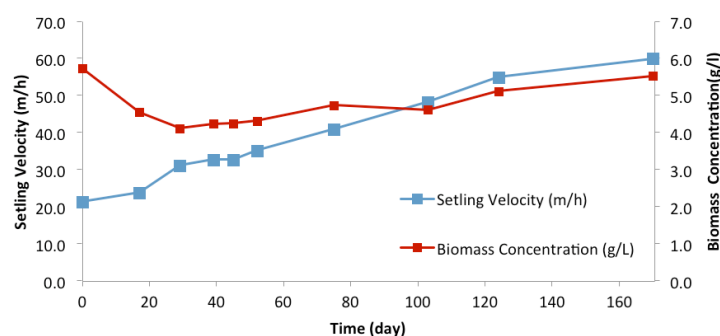


Figure 2 Profile of biomass concentration and settling velocity during the experiment

### 3.1.3 Granular strength

Integrity coefficient (IC) index was used to measure strength of the developed granules (21). High IC index indicates lower granular strength and vice versa. Figure 3 shows the change of IC index throughout the operational time.

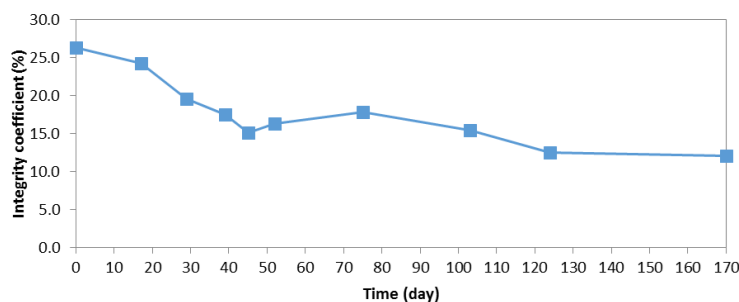


Figure 3 Profile of integrity coefficient (IC) during the reactor operation

It was observed that as the development of the granules continued, there was a reduction in the IC value. The initial IC index of  $26.3 \pm 0.5\%$  was reduced to  $12.5 \pm 0.5\%$  towards the end of the

experiment. According to Ghangrekar et al. (21), value of IC index below than 20% shows that the developed granules is in high strength. Reduction in IC index shows that there is an increase in bonding strength of the microbial cells forming the granules. At the beginning of the experiment, the microbial cells were very loose and slightly bound together. As time progressed, the microbial cells were bounded. This was likely to occur because of the extra-cellular polymeric substances (EPS) which was secreted by the microbes under the condition of shorter settling time, aeration shear force and anaerobic-aerobic regime (26).

#### 3.1.4 Analysis of SEM

SEM was used to observed microstructure of the granules (Figure 4). The image showed non-filamentous bacteria mostly dominated within that developed granule. These bacteria were also well linked as a result of an embedded round shape found on the granule's surface and covered most likely with EPS. The bacterial growth conditions may not have favored filamentous bacteria, hence cause their absence in the granules. This is probably due to high concentrations of dissolved oxygen and organic loading in the reactor (25). As can be seen in Figure 4, when the magnetic field was applied, bacteria clumped together and cavities were formed. These cavities are known to be responsible for the ease in movement of substrates or the metabolites into and outside of the granules. The energy dispersive x-ray microanalysis further characterizes the elemental composition of the microbial granule.



Figure 4 SEM microstructure of the granule. Cavities that present between bacteria are clumped inside the granules which been exposed with magnetic field (a) sludge after granule development; scale bar = 50 m (b) raw sludge; scale bar = 80 m

## 3.2 Removal performances of the reactor

### 3.2.1 COD and TOC removal efficiency

The efficiency of the system during the development of the granules in terms of COD and TOC removal is given in Figure 5 and 6. After acclimatization of the sludge with the wastewater, the reactor was operated for several weeks. The COD and TOC removal efficiency were 93.5% and 92.9%, respectively. The obtained high removal efficiency indicates an increase in the biological activity throughout the operational time of the reactor system. According to Li and Liu (27), oxygen in the inner part of the granules was limited which illustrated the existence of anaerobic microorganisms at the core of microbial granules. Meanwhile, aerobic microorganisms assist in COD and TOC removal where these microorganisms may present at the surface layer of the microbial granules.

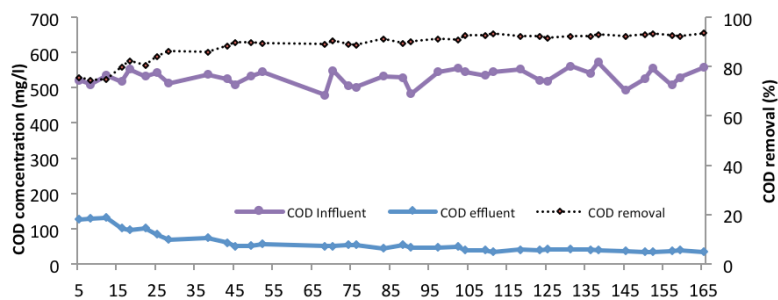


Figure 5 Removal of COD throughout the reactor's operation

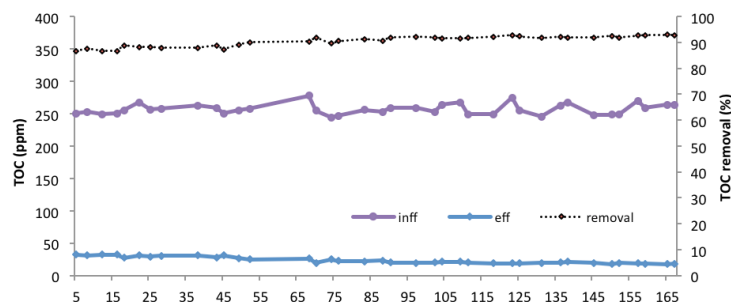


Figure 6 Removal of TOC throughout the reactor's operation

### 3.2.2 Color removal efficiency

Inconsistency in color removal can be affected by the absorption of color into activated sludge during biological treatment (28). Color removal may also be affected by reduction in duration of the anaerobic phase, which leads to the insufficient time for degradation of the N=N- bond which is the cause of color in textile wastewater. Dyes are non-biodegradable and may require longer degradation time in a bioreactor or even in the environmental matrix. Color removal efficiency may be inconsistent as a result of, degradation byproduct of dye that may oxidize and re-bond when oxygen is available especially when aeration is commenced in the bioreactor as well as unstable condition of the aromatic amines. Increase in color during autoxidation of aromatic amines has been reported severally (10). In our study, after stabilization of the reactor, the efficiency of the color removal increased from 22.6 to 38.9% when anaerobic phase

time was increased from 55 to 165 min (Figure 7). Effluent color concentration was 417 ADMI at the end of the experiment.

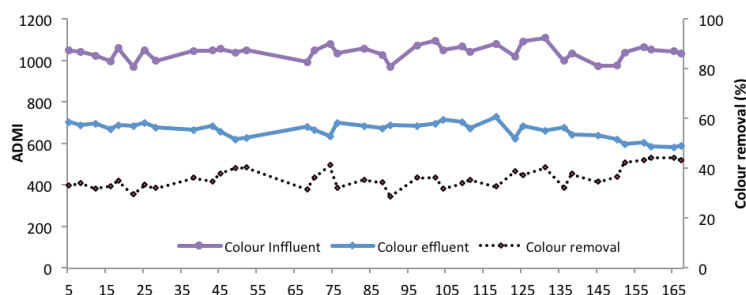


Figure 7 Color removal during the reactor operation

### 3.3 Dissolved oxygen (DO) and pH profile

Monitoring of pH and DO parameters for the purpose to control the biological nutrient removal has received much attention. However, in this study it was monitored to detect pre-upset conditions and potential problems during the process (29). The DO level and pH value depict the oxidation and biological reactions of both batch and continuous processes (30). The pH declines during nitrification reaction, while aggravates during denitrification process. The end of nitrification and denitrification reactions are depicted via two important breakpoints (31). During the aeration phase, the minimum is known as the ammonia valley which depicts the completion of nitrification process. Meanwhile, during anoxic phase, the maximum nitrate apex is a sign of denitrification reaction completion. The pH and DO monitoring is significant in determine the optimization of aerobic and anaerobic phase as aeration activity can be stopped after the completion of nitrification process and anaerobic phase can be avoided when denitrification is completed (29). The pH and DO profiles of a complete cycle of the two stage anaerobic-aerobic reaction phases of the reactor is shown in Figure 8 and it was used to monitor and ensure the system was working adequately.

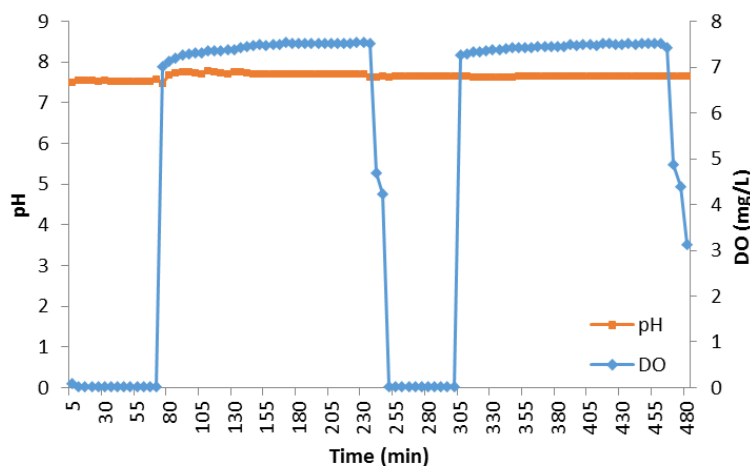


Figure 8 One complete cycle profile for DO and pH during the two stage reaction phases of the reactor (a) II and IV: aerobic and, (b) I and III: anaerobic phase

### 3.4 UV-Vis spectra analysis

The UV-Vis spectrum of the textile wastewater before and after treatment was analyzed. Modification of the absorbance peaks indicates the decolorization and degradation of the treated textile wastewater as shown in Figure 9. Before treatment, UVvisible spectra of RB5 had two main absorption bands, in UV region (310 nm) that has two adjacent rings and the visible region (595 nm) which has a long conjugated system that linked by two azo groups (32). In the RB4, it was categorized based on the absorption peaks at wavelength 595 (that indicates of chromophore group), wavelength 296 (that depicts anthraquinone function) and wavelength 256 (indicates dichlorotriazine group or the other transitions of the chromophore) (33). The RB4 dye solution is decolorized through the cleavage of chromophore in dye structures. The spectrum of untreated wastewater in this study showed peaks at 225 nm and 595 nm in UV region and in visible region respectively. The absorbance peak at 225 indicates the presence of naphthalene group, while wavelength peak at 595 nm is due to chromophore group (34). The findings are in line with the study conducted by Ong et al. (34)

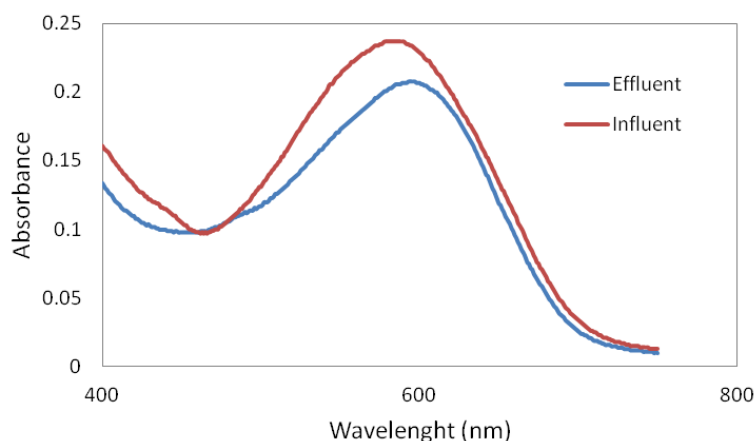


Figure 9 UV/Vis spectrum of the untreated and treated wastewater

### 3.5 Fourier-transform infrared (FTIR) analysis

FTIR analysis of untreated and treated textile wastewater by two-stage sequencing batch reactor is shown in Figure 10. The FTIR spectrum of untreated wastewater shows a distinct peak that corresponds to toxic functional groups present within the dye molecules. The peak of value  $3333.83\text{ cm}^{-1}$  corresponds to O-H stretching and aromatic N-H stretching as in amines. The peak at  $1642.26\text{ cm}^{-1}$  represents azo ( $\text{-N=N-}$ ) bond stretching, and peak at  $678.57\text{ cm}^{-1}$  corresponds to  $\text{=C-H}$  bending as in alkene. The FTIR spectrum of treated wastewater shows some modifications in comparison to the untreated wastewater. The bands at  $3333.83\text{ cm}^{-1}$  moved to  $3333.14\text{ cm}^{-1}$ ,  $1642.26\text{ cm}^{-1}$  band was modified to  $1642.40\text{ cm}^{-1}$ ,  $678.57\text{ cm}^{-1}$  band was slightly shifted to  $668.07\text{ cm}^{-1}$ . FTIR spectrum of treated and untreated wastewater showed a difference in absorbance and band position and this indicates a probable degradation of the wastewater.

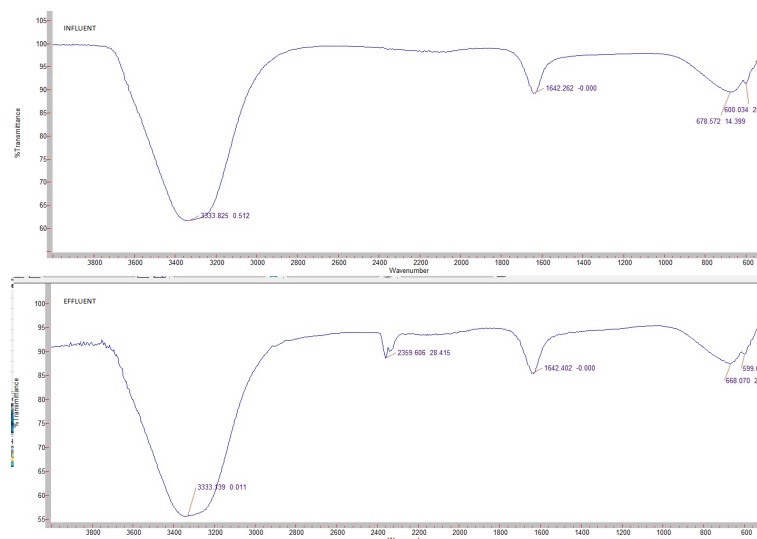


Figure 10 FTIR spectrum of untreated and treated textile wastewater

This study shows that operation of a two-stage anaerobic-aerobic reaction phase sequencing batch reactor is able to develop microbial granules under presence of magnetic field. In another study, it was reported that the application of magnetic field enhanced the aggregation of sludge, increase the iron accumulation, reduce granulation time and stimulated more EPS (18). In another experiment, magnetic field of 78 mT increased about 25% of wastewater treatment performance (16). Magnetic field induction of up to 20 mT affects positively on bacterial growth in activated sludge as well as on wastewater biodegradation (12).

## 4 Conclusion

The operation of a two-stage anaerobic-aerobic reaction phase sequencing batch reactor was found to be able to develop microbial granules with low strength textile wastewater under the influence of 13.9 0.2 mT static magnetic field. Although these microbial granules need a long time to grow up and develop, but this microbial granules still successful in degrading the textile wastewater. Improvement in the granular strength as a result of increase in the

biomass concentration led to an increase in the COD, TOC and color removal efficiencies indicating that the process was effective. Modification of characteristic organic bonds of the textile wastewater in the FTIR study indicates that biodegradation occurred. The results show that application of anaerobic-aerobic reaction phase SBR strategy under the influence of magnetic field is feasible. At this time, it can be concluded that static magnetic field has a positive effect towards the operation of a biological bioreactor.

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