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Development of bio-granules using selected mixed culture of decolorizing bacteria for the treatment of textile wastewater

Thuan Chien Kee[a], Hui Han Bay[a], Chi Kim Lim[a], Khalida Muda[b], Zaharah Ibrahim[a,*]

[a]*Faculty of Biosciences and Medical Engineering, Department of Biosciences and Health Sciences, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia*
Tel. +6075534122; email: zaharah@fbb.utm.my
[b]*Faculty of Civil Engineering, Department of Environmental Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia*

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ABSTRACT

In this study, four dye-degrading bacteria, *Bacillus pumilus* strain ZK1, *Bacillus cereus* strain ZK2, *Brevibacillus panacihumi* strain ZB1, and *Lysinibacillus fusiformis* strain ZB2 were used for the development of granule. Sterile sludge was used as seeding agent in a sequencing batch reactor under intermittent facultative anaerobic–aerobic system followed by subsequent textile wastewater treatment. Physical and morphological characteristics of the granules were determined after 112 d of development time. The average size of the mature granules reached 3.3 ± 1 mm, with integrity coefficient of 25 ± 2, settling velocity of 56 ± 5 m h^{-1}, and sludge volume index of 35 ± 5.5 mL g^{-1}. Mixed liquor suspended solids and mixed liquor volatile suspended solids were 12.9 ± 0.8 and 11 ± 0.6 g L^{-1}, respectively. The developed granules showed 61% of decolorization and 46% of COD removal at HRT of 24 h. The population distribution of the bacteria consortium in mature granules developed into subsequent ratio of 1:4:9:11 from initial inoculum ratio of 1:1:1:1 of *B. pumilus* strain ZK1, *B. cereus* strain ZK2, *B. panacihumi* strain ZB1, and *L. fusiformis* strain ZB2, respectively. The results obtained indicated that this mixed culture of bacteria has good potential application for the treatment of textile wastewater.

Keywords: Granulation; Textile wastewater; Sequencing batch reactor; Decolorization; COD; Bacterial population distribution

1. Introduction

Textile industry is a major source of wastewater as it uses large amounts of water in its preparation and dyeing processes. Azo dyes are synthetic dyes and the main constituent of textile wastewater with over 700,000 metric tonnes manufactured worldwide every year [1]. Azo dyes are being extensively used in textile industries due to its stability and variation in colors. However, when textile is being processed, low efficiency in dyeing may cause huge volume of dyestuff to flow into the effluent, ending up in the environment [2]. The amount of applied azo dyes lost in textile effluents has been reported to be in the range of 10–50% depending on dye types and fabrics [1]. The presence of dye effluent will influence the

*Corresponding author.

penetration of sunlight into water bodies which in turn will adversely affect the aquatic life. In addition, these dye stuffs released from the dyeing industries are recalcitrant, harmful, and potentially toxic to all life forms [3]. Over the years, the biological treatment of azo dyes has become increasingly important due to its eco-friendly and economical features compared to the physical and/or chemical treatment processes. These have lead to the need for the application of biological treatment process, especially via the facultative anaerobic–aerobic treatment system that was developed in previous studies [4].

The bacterial culture utilized in biological treatment is either in suspension or immobilized onto support materials. Comparatively, immobilized cells or biofilms were reported to be more efficient than that of suspended cells [5]. Granulation, one of the current immobilization mechanisms, can retain higher density biomasses in reactor than other immobilization techniques, and therefore can provide more efficient color and COD removal in textile wastewater treatment processes [6]. Moreover, the effectiveness of granulation treatment was further enhanced by the use of acclimatized mixed cultures in terms of biodegradation rates and mineralization [7]. Due to the ability of bacteria to distribute and organize themselves in the particular micro-environment through cell–cell signaling, the resulting high complexity bacteria community is beneficial to granular stability and biodegradation tasks [8,9]. Therefore, a combination of granulation using acclimatized mix culture [7] and facultative anaerobic–aerobic system [10] used in sequencing batch reactor (SBR) would provide both reducing and oxidizing conditions that are capable of handling varying effluents common in textile mills.

Current development of granules for wastewater treatment in local textile industry generally involved the use of synthetic wastewater containing single or several combinations of dyes. Cultivation of granules using real textile wastewater, on the other hand, was less reported. Therefore, the aim of this study was to explore the characteristics of aerobic granules formed using real textile wastewater as sole substrate in a sequential facultative anaerobic/aerobic SBR. A novel decolorizing bacterial consortium was used to develop acclimatized granules with enhanced decolorizing ability to treat high fluctuation of color and COD. Effects of the HRT to treat color and COD were investigated in detail. The change in the population of the microbes was also monitored during the development of granules. This study will further help to understand the process and performance of the SBR system for the treatment of high-strength industrial textile effluent.

2. Methods

2.1. Characterizations of textile wastewater and textile sludge

The textile wastewater and textile sludge used in this study were collected from a local textile treatment plant. The wastewater and the sludge were autoclaved at 12˚C, 15 kPa for 15 min. The wastewater was characterized according to parameters; COD, pH, and ADMI while the sludge were physically characterized using parameters; sludge volume index (SVI), settling velocity (SV), integrity coefficient (IC), mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS).

2.2. Preparation of bacterial inoculum

The microorganisms used in this study consist of *Bacillus pumilus* strain ZK1, *Bacillus cereus* strain ZK2, *Brevibacillus panacihumi* strain ZB1, and *Lysinibacillus fusiformis* strain ZB2. The bacteria were isolated from local textile effluent and were acclimatized in sterilized textile wastewater. Each bacterium was grown overnight in textile wastewater enriched with nutrient broth. The cultures were agitated in a rotary shaker at 37˚C, until the exponential growth phase was reached, before added into the reactor at ratio of 1:1:1:1.

2.3. Reactor set-up

Fig. 1 illustrates the water-jacketed glass column reactor (height: 90 cm; internal diameter: 6 cm) that was used for the development of granules as well as for the wastewater treatment. The working volume of the reactor was 1 L. For granules development, the HRT was set at 6 h. The reactor was operated in a sequencing stage of feeding, reacting, settling, and decanting. The reaction cycle consisted of 170 min of facultative anaerobic stage and 170 min of aerobic stage, followed by another identical four cycles with total cycle length of 360 min as seen in Table 1. The 500 mL seed sludge obtained from local textile treatment plant was sterilized and added into the column, followed by 500 mL of sterilized textile wastewater with organic loading rate of ≈2 kg COD m^{-3} d^{-1}. The system was equipped with automatic peristaltic and air pumps. In each cycle, the bacteria in ratio 1:1:1:1 as well as the sterilized textile wastewater were added

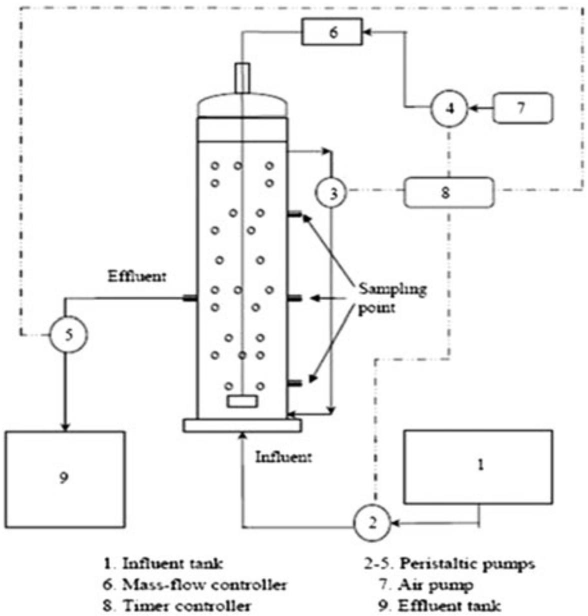

Fig. 1. Schematic diagram of lab-scale SBR system.

Table 1
Operating parameters of the applied treatment system

Parameter	SBR treatment system
Influent color (ADMI)	1,400–1,800
Influent COD (mg L^{-1})	800–1,000
Effective volume (L)	1.0
Feeding (min)	5
Reaction	
Facultative anaerobic (min)	170
Aerobic (min)	170
Settling (min)	5
Decant (min)	5
Idle (min)	5
Cycle (h)	6
Temperature (℃)	ambient

into the reactor during filling stage. During decanting stage, 500 mL of the wastewater was washed out from the reactor, achieving volumetric exchange rate of 50%. Aeration system was used throughout the facultative anaerobic phase to provide sufficient mixing in the system. Air was introduced using a diffuser located at the bottom of the reactor to provide aeration to the system during aerobic stage with flow rate of 2.88 L min^{-1}, which gives superficial air velocity of 1.70 cm s^{-1}. Following that, optimization of HRT during treatment of textile wastewater was carried out using the developed granules. After the treatment, the color, pH, and COD of the textile wastewater were analyzed according to the Standard Methods [11] using a HACH DR 6000 spectrophotometer.

2.4. Analytical analysis of developed granules

The developed granules were analyzed for its MLSS and MLVSS according to the Standard Methods [11]. This is followed by determination of IC [12], SV [13], and SVI [14]. Granular size was observed via Leica stereomicroscope. The morphology of the granules was examined using field emission scanning electron microscope (FESEM), (Zeiss 350VP). For the bacterial population distribution study, 1 mL of random granules was mixed with 2 mL of saline solution. The bacterial suspension was serially diluted before being pour-plated on nutrient agar and incubated at 37℃ for 24 h. Triplicate plates were done for each dilution. The colonies formed were counted and only colony counts between 30 and 300 were selected as colony forming units to avoid inaccurate counting due to overcrowding of colonies [15]. Plates with sterilized textile wastewater serve as negative control; whereas, plates with acclimatized inoculum culture will be the positive control.

3. Results and discussions

3.1. Characterizations of textile wastewater and seed sludge

Characterization on real textile wastewater as shown in Table 2 revealed that the wastewater has high color intensity at the range of 1,400–1,800 ADMI. In addition to that, the COD at the range of 800–1,000 mg L^{-1} was recorded. The pH of the wastewater was near basic (7.0–8.5). The physical characterizations of seed sludge were recorded in Table 3. Based on the observation, the seed had mean diameter of 0.02 mm as shown in Fig. 2(a).

3.2. Bio-granulation

The physical and morphological characteristics of the granules formed were examined at different stages during their development. Fig. 2 shows the microscopic images of the developed granules. Within

Table 2
Characteristics of real textile wastewater

Parameter	Range
pH	7.0–8.5
COD (mg L^{-1})	800–1,000
Color (ADMI)	1,400–1,800

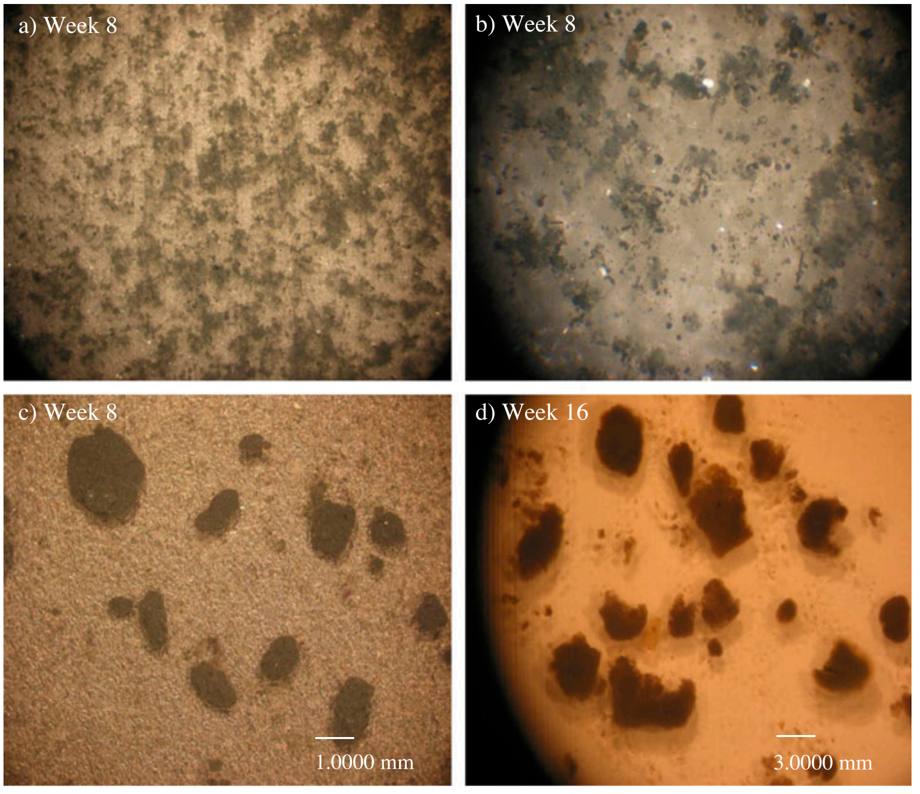

Fig. 2. Images of seed sludge at (a) initial stage (b) after two weeks (c) after eight weeks and (d) after sixteen weeks of granules development under stereo microscopic observation (6.3× magnification).

16 weeks of development, the granules increased in size to an average of 3.3 ± 0.9 mm (Fig. 2(d), Table 4).

During granulation, increase in biomass resulted in denser granules hence increased the granular strength. The increases of granular size in turn caused the rise in settling speed. Excellent settling property of the granules, which enhances the solid separation process, resulted in higher amount of biomass retained in the reactor. In this study, the average SV achieved was 56.1 ± 5.0 m h^{-1} (Fig. 3(a)) and this value was much higher compared to other reported studies [16–19].

The SVI value was at an average of 35.1 ± 5.5 mL g^{-1} indicating good settling properties. According to Lotito et al. [20], sludge sample with SVI lower than 80 cm^3 g^{-1} gives better settle-ability while SVI value higher than 120 cm^3 g^{-1} may impose settling problem. Hence, the developed granules with SVI value of 35.1 ± 5.5 mL g^{-1} (Fig. 3(b)) indicated good settling properties. The MLSS and MLVSS of the developed granules were in the range as reported by Beun et al. [14].

With the increase in the settling property of the granules, the biomass concentration in the reactor was increased to 11 mg L^{-1} after 16th week of development. The strength of the granules is expressed in terms of IC. The IC of the granules was reduced from 70 ± 5 in the first week to 25 ± 2 in the 16th week (Fig. 3(c)). The physical characteristics of developed granules after 16th week of development time are tabulated in Table 4.

Table 3
Physical characteristics of the seed sludge

Characteristics	Seed sludge
SVI (mL g^{-1})	150.4 ± 13.4
Average diameter (mm)	0.02 ± 0.01
Average SV (m h^{-1})	2.6 ± 0.6
Granular strength (IC)	70 ± 5
MLSS (g L^{-1})	9.1 ± 0.6
MLVSS (g L^{-1})	5.8 ± 0.4

3.2.1. FESEM analysis

The FESEM analysis showed the uneven and rough surface morphology of seed sludge (Fig. 4(a)). This characteristic showed that the seed sludge is suitable for initial housing of granules formation. Fig. 4(b) showed the predominant rod-shaped bacterial cells that adhered together to form an organized clumping struc-

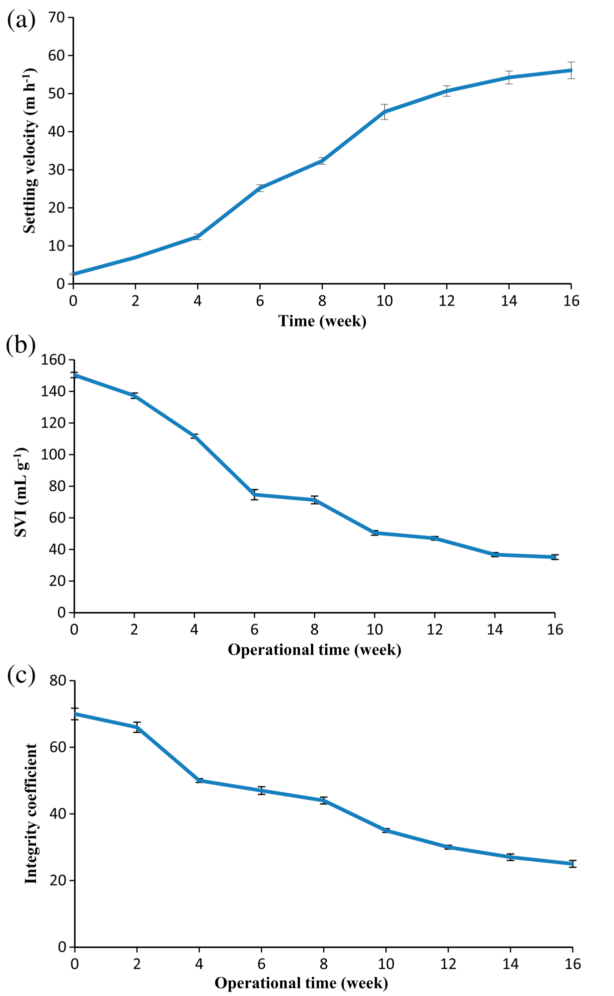

Fig. 3. (a) SV (b) SVI and (c) IC profile of the developed granules.

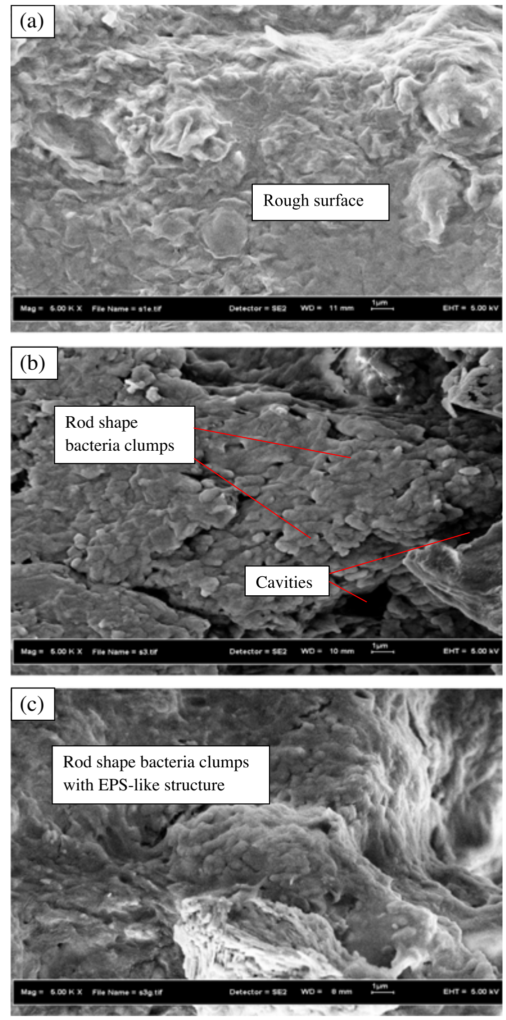

Fig. 4. FESEM observations on (a) surface of initial sludge (b) cross section surface of granules after sixteen weeks and (c) surface of granules after sixteen weeks (5000× magnification).

ture on the cross-section surface of developed granules. The presence of extracellular polymeric substances can be clearly seen in Fig. 4(c), which relate the biological adhesion and microbial aggregation on the outer surfaces of the developed granules [21]. Both Fig. 4(b) and (c) showed numerous cavities in between the bacterial biomasses. According to Tay et al. [22] and Toh et al. [23], the cavities might be responsible for translocation of metabolite substances in and out the granules.

3.2.2. Bacterial population distribution of the developed granules

Study on the bacterial community structure revealed that after 16 weeks of bio-granulation, population distribution of ZK1, ZK2, ZB1, and ZB2 in the mature granules was developed from initial inoculum ratio of 1:1:1:1 into final ratio of 1:4:9:11 with ZB2 being the dominant species (Fig. 5), showing successful incorporation with varied dominancy. The population of ZB1 and ZB2 had increased from 25 to 36% and from 25 to 43%, respectively. In contrast, the population of ZK1 and ZK2 was reduced from 25 to 4% and from 25 to 17%, respectively. The change in bacterial community structure may due to certain interaction between members within selected consortium, which comprised of either active or passive bacterial competition. It might also possibly due to active bacterial competition such as secretion of antimicrobial molecules that scattered away competitors without actually killing the competitors [24]. This explanation is also supported by other studies showing that *Pseudomonas aeruginosa* produces rhamnolipid and fatty acid *cis*-2-decenoic acid against biofilms of *Bordetella bronchiseptica* [25] and a number of other

Table 4
Physical characteristic of developed granules

Characteristics	16th week granular sludge
SVI (mL g^{-1})	35.1 ± 5.5
Average diameter (mm)	3.3 ± 0.9
Average SV (m h^{-1})	56.1 ± 5.0
Granular strength (IC)	25 ± 2
MLSS (g L^{-1})	12.9 ± 0.8
MLVSS (g L^{-1})	11 ± 0.6

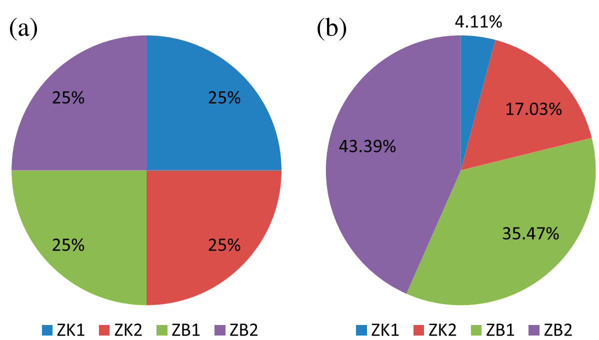

Fig. 5. (a) Initial inoculums ratio and (b) inoculum ratio on 16th week's granules.

species [26]. There may also be mechanism that functions directly by restrict/remove nutrient from one organism and supply it to another. Oehmen et al. [27] reported that carbon and phosphorus accumulation by polyphosphate-accumulating organisms in certain wastewater treatment assists in their dominance over other species. Whereas, rapid growth rate (passive competition), a drive to exploit nutrient uptake by one organism at the expense of another, can also be the possible reason for the population shift.

3.2.3. Treatment of real textile wastewater

In this study, the developed granules were used in treating real textile wastewater with special focus on color (ADMI) and COD removal. Studies on the HRT showed that at 6 h HRT, the color removal was 18%. Increase in HRT to 24 h resulted in increase of color removal to 57% as shown in Fig. 6. Based on the observation, longer HRT would increase the interaction phase between granules and wastewater, thus enhancing the removal of color since longer facultative anaerobic condition was more favorable in color removal. On the other hand, the COD removal demonstrated the same trend as color removal. The 6 h HRT resulted in 14% reduction of COD while increase of HRT to 24 h showed increase in COD reduction by 39% as illustrated in Fig. 6. However, when the HRT was prolonged to 48 h, decrease in COD as well color removal was gradually observed at 44 and 20%, respectively.

The decrease of color removal after 48 h HRT could be due to auto-oxidation reactions of aromatic amines [28]. In addition to that, coupled reactions that took place in the same SBR system causing high accumulation of aromatic amine during long facultative anaerobic phase may cause toxic effect that inhibited the viability of aerobic bacteria in the bio-granules during the aerobic phase [29]. Hence, HRT more than 24 h would eventually reduce the efficiency of bio-granules in treating textile wastewater and the period of facultative anaerobic phase ought to be sufficient for improved color and COD removal efficiency [30]. The HRT 24 h emerged to become the optimum retention time for the treatment in the pre-screening study; therefore, further experiments were done on manipulation of the time allocated for facultative anaerobic and aerobic within 24 h time range.

As reported by Sponza and Işik [31], increase of HRT offered sufficient time for conversion of complex initial real textile wastewater substances into inter-metabolites in intermittent facultative anaerobic and aerobic systems. Furthermore, in order to improve textile wastewater treatment efficiency in terms of azo bond cleavage (facultative anaerobic phase) and mineralization of aromatic amines (aerobic phase), determination of better time distribution among facultative anaerobic and aerobic phase is necessary. Therefore, further experiment was done by designing the time ratio of facultative anaerobic and aerobic phase into 12:12, 15:9, 18:6 and 21:3 h and labeled as sample 1, sample 2 sample 3, and sample 4, respectively.

Based on observation as illustrated in Fig. 7, the removal performance for color and COD with sample 1, 2, 3, and 4 showing color: COD ratio as follows: 57:39, 53:34, 61:46, and 56:42 respectively. Again, the

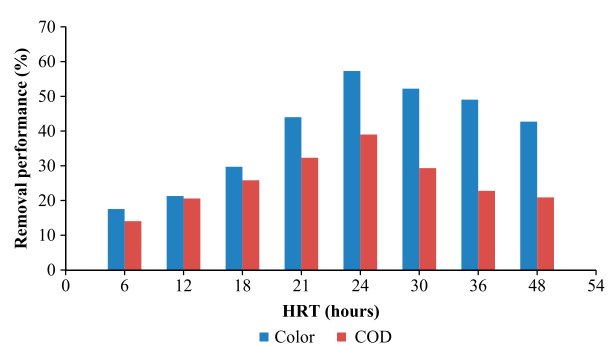

Fig. 6. ADMI and COD removal profile of raw textile wastewater at different HRT.

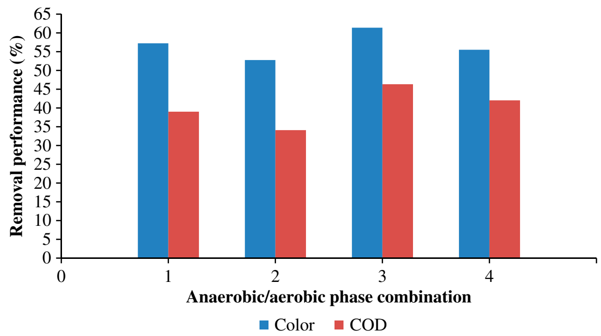

Fig. 7. ADMI and COD removal profile of raw textile wastewater on different combination of anaerobic/aerobic phase: 1: 12 h anaerobic, 12 h aerobic; 2: 15 h anaerobic, 9 h aerobic; 3: 18 h anaerobic, 6 h aerobic; 4: 21 h anaerobic, 3 h aerobic.

prolonged facultative anaerobic phase might increase the color removal efficiency due to longer interaction time for bacteria and in turn lead to the increase of subsequent degradable intermediates for improvement of COD removal [32]. Similarly, Sun et al. [33] also found that increase of HRT leading to the high COD removal. However, it is important to note that prolong HRT should be avoided because excessive accumulation of aromatic amines would result in increase of toxicity level, which might trigger fatal of granular bacteria. Subsequently, this phenomenon would reduce the final color and COD removal [28]. In this study, the best distribution of time between facultative anaerobic and aerobic treatment was found at ratio of 18:6. This ratio resulted in 61 and 46% of color and COD removal, respectively.

4. Conclusion

In this study, granules consisting of selected novel decolorizing bacteria, used to treat real textile wastewater, were successfully developed under the sequential facultative anaerobic-aerobic cycle. The granules showed good characteristics with SV of $56 \pm 5\ \mathrm{m\,h^{-1}}$, IC of 25 ± 2, diameter of 3.3 ± 1 mm, and SVI of 35 ± 5.5. It is noteworthy that the granules developed were capable of degrading raw textile wastewater, with HRT 24 h resulting in the removal of color (61% with initial values ranging from 500 to 2,000 ADMI) and COD (46% with initial values ranging from 400 to $1{,}500\ \mathrm{mgL^{-1}}$). Population distribution of ZK1, ZK2, ZB1, and ZB2 in the mature granules that was developed from initial inoculum ratio of 1:1:1:1 into final ratio of 1:4:9:11 with ZB2 being the dominant species. Results obtained from this study demonstrated that the granules were successfully developed using the novel combination of bacteria and were capable of treating real textile wastewater.

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