

Long-term stability and decolourization of azo, anthraquinone and triphenylmethane dyes of aerobic granules

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Abstract

The long-term stability of aerobic granules is critical for decolourization of different dyes in textile wastewater. Here, we investigated dye decolourization and the stability of acetate-cultivated granules after exposure to dyes. Results show that granules can maintain excellent structure stability with the presence of azo and triphenylmethane dyes during a 200-day operation period, achieving biomass concentrations as high as 8-12 g/L and 90% and 100% decolourization efficiency, respectively. Aerobic granules, however, partially disintegrated after exposure to anthraquinone, resulting in dye decolourization efficiency ranging from 50-80% and a biomass concentration as low as around 0.5 g/L due to biomass wash-out. The study indicates that long-term granule stability is much dependent on the dye classes. The enrichment of specific species in granules for dye decolourization has not been affected by the granule structure. The specific dye decolourization rate and dye to microorganism ratio for anthraquinone were 5-6.5 and 13.5-16.4 times, respectively, higher than those for azo and triphenylmethane dyes, but the total reactor performance for anthraquinone decolourization is much poorer than azo and triphenylmethane dyes due to low biomass retention in the reactor. The results suggest the importance of stability of aerobic granules for biomass retention to achieve better treatment performance of dye-containing wastewater. For the first time, the long-term stability and decolourization performance of aerobic granules for treating anthraquinone and triphenylmethane dyes are reported here and compared with azo dye, which can be used to guide the treatment of real textile wastewater containing azo, anthraquinone and triphenylmethane dyes by aerobic granules.

Keywords: Aerobic granules, azo, anthraquinone, triphenylmethane, decolourization, long-term stability

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1. INTRODUCTION

Synthetic reactive dyes from the textile industry have caused a serious concern to water ecosystems due to their recalcitrant and toxic natures. It has been reported that more than 280,000 t of textile dyes are annually discharged in textile industrial effluents worldwide (Maas and Chaudhari, 2005). Based on the chromophoric group's chemical structure, dyes are classified as azo, anthraquinone, triphenylmethane, heterocyclic and polymeric dyes (Hadibarata et al., 2012), among which azo, anthraquinone and triphenylmethane account for most textile dyestuffs produced.

To alleviate environmental pollution and severe health risk of dyes, physical, chemical and biological methods have been developed to treat dye-containing wastewater. Compared with physical and chemical technologies, biological approach is more environmentally friendly and cost-effective, and it is thus believed promising to decolorize and degrade dyes in wastewater standalone (Popli and Patel, 2015) or to be integrated with advanced oxidation for complete mineralization (Rodrigues et al., 2014). Different strains have been isolated to decolorize or degrade dyes in different conditions such as anaerobic, microaerobic, aerobic or alternating anaerobic and aerobic conditions. Consortia have been proved to be more efficient in treating dye-containing wastewater (Waghmode et al., 2011; Mishra and Maiti, 2018). In practice, however, acclimatized sludge with mixed culture is most promising to treat dye-containing wastewater because it could be operated for a long period without much concerns about the wash-out of specific strains from treatment systems and continuous frequent inoculation. Aerobic granular sludge as a kind of biomass retention technology has attracted strong attention for dye-containing wastewater treatment since 2010. The compact structure and large size of granules allow them to withstand toxic compound shock and unfavourable environmental conditions. Meanwhile, more diverse microbial population could be enriched for more efficient pollutant removal.

To use aerobic granular sludge to decolourise or to degrade dyes, some researchers developed granules from suspended sludge with azo dye containing wastewater. It has been demonstrated widely that aerobic granules can be formed easily with azo dyes at low concentrations such as 50 mg/L mixed with other readily biodegradable carbon sources such as glucose, starch, acetate or ethanol. For example, Muda et al. (2010) reported the development of stable, mature and well-settled AG within a 65-day operation with a mixture of external carbon sources such as glucose, ethanol and acetate with a COD concentration of 1270 mg/L in the presences of a mixture of three azo dyes with a total 50 mg/L concentration under intermittent anaerobic and aerobic conditions. Mata et al. (2015) reported better granulation results with the addition of 20 mg/L azo dye AR14 into synthetic wastewater with starch as carbon source (COD concentration of 1000 mg/L) in non-tubular SBR in their 62-day experiment.

Ibrahim et al. (2010) and Kee et al. (2015) developed granules from a mixture of bacterial species isolated from textile sludge or soil contaminated with textile wastewater and sterilised activated sludge fed with real dyeing wastewater. To some extent, the formation of granules with the presence of low concentration of azo dyes is not surprising because aerobic granules have been developed with synthetic wastewater containing acetate or glucose at the presence of other types of recalcitrant toxic compounds (Maszenan et al., 2011; Zhu et al., 2011). Furthermore, aerobic granules could even be developed with toxic compounds such as phenol (Jiang et al., 2002) and tert-butyl alcohol (Zhuang et al., 2005) as a sole carbon source, let alone granulation with readily biodegradable carbon sources mixed with low concentrations of other types of recalcitrant and toxic compounds such as dyes. Although granulation was studied with the presence of azo dye, there still lack evidence on granulation with the presence of anthraquinone and triphenylmethane, another two main classes of dyes widely used in textile industry, and on how efficiently they could be decolorized or degraded by granules.

With more commercial applications of aerobic granules for municipal wastewater treatment (Pronk et al., 2015), a more efficient and rapid way to start up a new reactor for dye containing wastewater treatment is to inoculate excessive granular sludge from municipal wastewater treatment plants. The stability of aerobic granules after exposure to different operating conditions with dyes' presence becomes more critical for stable dye decolourization and minimization. The instability of aerobic granules for dye treatment has been observed in some studies. Sadri Moghaddam and Alavi Moghaddam (2016) reported that after successful granulation with synthetic wastewater containing 50 mg/L azo dye acid red 18 in SBR, the increase in the azo dye concentration from 50 to 100 mg/L on day 60 immediately caused the disintegration of aerobic granules into smaller-sized filamentous flocs and SVI ~~rose~~increase to 340 ml/g, indicating an unfavourable effect of dye concentration on the aerobic granules' structure stability and characteristics. The disintegration of aerobic granules in this study might be caused by the sudden shock of azo dye from 50 to 100 mg/L. Muda et al. (2011) operated one reactor for around 300 days with varying HRT for wastewater with the presence of a total of 50 mg/L concentration of mixed azo dyes. They reported that the decrease in granule size with a percentage smaller than 300 µm increased from less than 15% at the beginning to more than 50% at the end of reactor operation, which they attributed to the extension of HRT over the time. The decrease in granule size over time showed the variability and instability of granule characteristics over the long-term operation period, but it is unclear if this was caused by operating conditions only or by the presence of dyes. Franca et al. (2015) operated two SBRs in parallel with one added with 20 mg/L azo dye and the other as the control without dye and observed that from 77 days the granules/flocs ratio in the dye-fed SBR2 slightly increased, even after increasing dye concentration from 20 to 60 mg/L whilst total granule disintegration was observed in the dye-free control SBR1. Although the disintegrations of granules in SBR1 was unexplainable, we could see that the presence of azo dye from 20 to 60mg/L did not negatively affect granule stability compared with the control. Most aerobic granule

studies for dye treatment were operated for short periods such as 2-3 months. It is thus hard to know if granules can maintain their structural stability for a long time with dyes' presence.

So far, almost all studies on dye decolourization and degradation by using aerobic granules focused on azo dye only. In practice, however, real textile wastewater likely contains azo, anthraquinone and triphenylmethane dyes. To apply aerobic granules for real textile wastewater treatment, we need to answer another two essential and important research questions apart from granulation. The first question is if the granules can withstand toxic and recalcitrant dye compounds for long periods after being exposed to dyes, ~~by maintain~~to maintaining intact granule structure and stable reactor performance. The second question is if the effects of different classes of dyes on granule stability are the same. Although azo dye is dominant in textile wastewater, the presence of other classes of dyes such as anthraquinone and triphenylmethane is unavoidable sometimes. Regarding the long-term stability of granules with the presence of dyes especially with different chemical structures, it would be worth determining how each class of dye affects granule structure first. Furthermore, if granules cultivated by benign wastewater such as municipal wastewater could be inoculated to start up reactors for textile wastewater treatment, it would be much more efficient from the application point of view.

This study therefore aims to acclimatize acetate-cultivated granules for dye decolourisation, to evaluate the long-term stability of aerobic granules after being exposed to individual azo, anthraquinone and triphenylmethane dyes, and to compare the decolourization efficiency of three different dyes by aerobic granules in the similar conditions. The ultimate goal of this study is to explore the application potential and operation practice of aerobic granules for the treatment of real textile wastewater containing multiple classes of dyes.

2. MATERIALS AND METHODS

2.1 Medium

To cultivate aerobic granules, synthetic wastewater with the following composition was used: COD (sodium acetate) 1000 mg L^{-1} , NH_4Cl 115 mg L^{-1} , K_2HPO_4 45 mg L^{-1} , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 30 mg L^{-1} , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 25 mg L^{-1} , $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 20 mg L^{-1} , and trace elements same as those used by Chen et al. (2015). After aerobic granular sludge reached steady state, reactive azo dye, i.e., Acid Red 14 (Chromotrope FB, Sigma-Aldrich, 50% dye content), triphenylmethane dye, i.e., Brilliant Green (Sigma-Aldrich, 90% dye content), and anthraquinone dye, i.e., Reactive Blue 19 (Remazol Brilliant Blue R, Sigma-Aldrich) was added, respectively, to the above-mentioned synthetic wastewater to simulate wastewater containing different classes of dye. The dye concentration was increased step-

wise from 5 to 85 mg L^{-1} during the long-term operation period to investigate dye decolourisation and granule stability.

2.2 Reactor operation for the cultivation of aerobic granules

Three bubble columns were used to cultivate aerobic granules with an internal reactor diameter of 6.5 cm and a working volume of 2.5 L. Fine air bubbles for aeration were supplied through an air sparger at the reactor bottom to all reactors. The reactors were operated sequentially at ambient temperature with a cycle time of 4 h, which included 5 min of influent filling, 228 min of aeration, 2 min of settling and 5 min of effluent discharging from the middle port of the reactor with a volumetric exchange ratio of 50%. This operation resulted in 8-hr hydraulic retention time (HRT). A programmable logic controller was installed to achieve the automatic cyclic operation of the reactors.

2.3 Reactor operation for dye decolourisation using microbial granules

After aerobic granules reached steady state in the three bubble columns with a biomass concentration of 3.4 g L^{-1} , the wastewater was shifted to synthetic wastewater containing dye. Meanwhile, the cycle time was extended to 6 hours with the same feeding, settling and discharging times, but the reaction time in the cycles was divided into an anaerobic phase with nitrogen rate of 0.5 L min^{-1} from 5 to 180 minutes and an aerobic phase from 180 to 353 minutes with aeration rate of 3.0 L min^{-1} for dye decolourisation. HRT was extended to 12 hours accordingly due to the extension of cycle time. Reactors fed with wastewater containing Acid Red 14, Brilliant Green or Reactive Blue 19 in this study were denoted as R1, R2 and R3, respectively.

2.4 Analytical methods

Chemical oxygen demand (COD), mixed liquor suspended solids (MLSS), sludge volume index (SVI₃₀) and mixed liquor volatile suspended solids (MLVSS), and total organic carbon (TOC) were analysed in accordance to standard methods (Eaton et al., 2005). Sludge volume index (SVI₅) was measured in a similar manner as SVI₃₀ by modifying the settling time from 30 to 5 min. For reactors with aerobic granules, MLSS in the reactors was calculated from biomass density and biomass bed volume measured according to Beun et al. (1999).

The wavelength of maximum absorbance for Acid Red 14 (520 nm), for Brilliant Green (623 nm) and for Reactive Blue 19 (595 nm) was determined experimentally by using a spectrophotometer.

Samples taken from reactors were centrifuged at 8000 rpm for 10 min to obtain the supernatant.

The absorbance of the supernatant at individual maximum wavelength of dye absorbance was used

to quantify Acid Red 14, Brilliant Green and Reactive Blue 19, respectively. The decolourization (%) of dye was calculated as the formula: $\text{decolourization (\%)} = [(A_i - A_e)/A_i] \times 100$, where A_i is the initial absorbance of the dye in the influent and A_e is the absorbance of the dye in the effluent.

3. RESULTS AND DISCUSSION

3.1 Decolourisation of azo, anthraquinone and triphenylmethane dyes during long-term operation by inoculating acetate-cultivated granules

Figure 1 shows the decolourization of Acid Red 14 (AR14) in R1, Brilliant Green (BG) in R2, and Reactive Blue 19 (RB19) in R3, respectively. Although azo concentration of 20-50 mg/L has been widely reported in the studies with aerobic granules (Muda et al., 2011; Kolekar et al., 2012; Lourenço et al., 2015), step-wise increase in dye concentration was adopted in this study because of the observation of granule disintegration in R3 with the presence of anthraquinone dye in the first start-up trial. The step-wise increase in dye concentration in all three reactors does not only allow sufficient time for sludge acclimatized to enrich relevant species in acetate-cultivated granules for dye decolorization, but also ensure the same operation strategy used in three reactors fed with three different classes of dyes for a fair comparison.

In the first ten days, 10-20mg/L of dye concentrations in the influent resulted in high residual dye concentrations in the effluent, especially in R1 and R3. Meanwhile, the partial disintegration of aerobic granules in R3 was observed. In this case, dye addition was stopped and reactors were continued to operate with synthetic wastewater. On day 25, sludge in R3 was discarded, and aerobic granular sludge in reactors R1 and R2 was discharged and mixed to re-inoculate three reactors evenly with a biomass concentration of 2.3 g/L in each reactor for re-startup of the three reactors aiming for dye decolorization. The dye addition started from 5 mg/L in the second trial. It can be seen from Figure 1 that during the long-term operation period with a step-wise increase in dye concentration to 85 mg/L, decolourization of AR14 was maintained at around 90%. BG has the best performance with almost 100% decolourization rate during long-term operation period with the dye concentration increased to 85 mg/L. According to the trends in Figure 1, it is expected that R1 and R2 can deal with higher dye concentrations with satisfactory decolourisation performance. R3 with RB19, however, experienced poorer decolourization compared with R1 and R3 with dye decolourisation rate ranging from 50 to 80%. Regarding granule stability, partial granule disintegration was observed when RB 19 was increased from 5 to 10 mg/L and the sludge became a mixture of suspended sludge and small granules. Due to the relatively lower decolourization rate,

the dye concentration in R3 was just increased to 40 mg/L after 110 days and maintained at this value until the end of the experiment.

The results in this study suggest that acetate-cultivated granules can be acclimatized for the decolourization of the three most representative textile dyes, i.e., azo, anthraquinone and triphenylmethane dyes. A similar study was reported to successfully acclimatize acetate-cultivated granules for degradation of phenol (Tay et al., 2005a) or chlorophenol (Carucci et al., 2009) and for partial nitrification (Wang et al., 2016). Specific species could be enriched in granules for the degradation of a specific type of pollutants. The conversion of acetate-cultivated granules for dye decolourization has a practical implication because this means in the future it is feasible to take aerobic granular sludge treating municipal wastewater to start up new reactors for industrial wastewater treatment with toxic or recalcitrant compounds to shorten reactor start-up time.

Since anaerobic/aerobic operation mode was adopted in all three SBR reactors, the dye decolourisation efficiency during anaerobic and aerobic phases was analysed as well and the results were shown in Figure 2. It can be seen that the vast majority of decolourization occurred during the anaerobic phase for AR 14 and BG while circa 10 to 25% of decolourization of RB 19 was carried out in the aerobic phase and around 50% in anaerobic phase after 50 days. From days 25 to 50, both anaerobic and aerobic phases contributed to dye decolourization because anaerobic phase alone was not sufficient to completely decolour dyes in all three reactors at the initial acclimatization period. For example, on day 30, it was found that the total BG removal rate was above 85% with the aerobic phase contributing more than the anaerobic phase. From day 30 to 40, dye ~~removed colour~~ in the aerobic phase quickly reduced to almost zero ~~while-with~~ almost 100% of BG ~~was removed colour removed~~ in the anaerobic phase. Przysaś et al. (2012) also reported either anaerobic or aerobic condition for triphenylmethane BG removal. They isolated one bacterial strain s45 (*Burkholderia cepacia*) for 50 mg/L BG removal and found this bacterium achieved 100% GB removal efficiency in either aerobic condition or static (anaerobic) condition although GB removal rate was higher in aerobic than anaerobic condition. In addition, microorganisms which can remove triphenylmethane dye have been isolated in anaerobic (Wu et al., 2013; Khan et al., 2015) or aerobic (Sani and Banerjee, 1999; Ogugbue and Sawidis, 2011) condition. That triphenylmethane dye GB can be removed in either anaerobic or aerobic phase effectively by aerobic granules in this study indicates that specific species enriched in aerobic granules could be anaerobic or aerobic or facultative regarding dye decolorization. A similar phenomenon was observed in R1 and R3 too. Although anaerobic azo dye decolourization was extensively reported (Pandey et al., 2007), a few

microorganisms that can decolorize azo dye in aerobic condition were still found (Stolz, 2001; Kodam et al., 2005). Similar to azo dye, bacterial species that can remove anthraquinone dyes in aerobic (Gurav et al., 2011) or anaerobic (Holkar et al., 2014) condition were isolated as well. For example, the decolourization rate of anthraquinone Acid Blue 25 (100 $\mu\text{mol/L}$) by *Bacillus cereus* DC11 is more than 55% under anaerobic conditions but below 5% under aerobic conditions (Deng et al., 2008) whilst *G. geotrichum* achieved 45% of anthraquinone dye Vat Red 10 in aerobic condition (Gurav et al., 2011).

In general, however, dye decolourization efficiency in anaerobic condition especially for azo dye is much higher than in aerobic condition (Mishra and Maiti, 2018). Decolourization of dyes in either anaerobic or aerobic conditions by different species allows aerobic granular sludge reactor with alternating anaerobic and aerobic conditions to enrich specific species favourable to either anaerobic or aerobic condition. This implies that anaerobic and aerobic phases do not need to be precisely controlled but still can maintain good dye decolourization efficiency. Furthermore, even with a mixture of three main classes of textile dyes such as azo, anthraquinone and triphenylmethane dyes, the same alternating anaerobic and aerobic conditions or the same lengths of anaerobic and aerobic phases could be used for good dye removal performance. For dye degradation, an aerobic phase is essential because it is believed to allow mineralization of metabolic intermediates such as aromatic amines from decolourization of dyes to detoxify dyes to a maximum extent (Pandey et al., 2007).

Inoculated granules to three reactors were from aerobic conditions. After the cycle operation was changed from only aerobic phase to alternating 3-h anaerobic and 3-h aerobic phases, granules in R1 and R2 during the 200-day operation period always showed excellent stability, indicating the robustness and resilience of aerobic granules to varying conditions. This is favourable to practical application as granules cultivated from different conditions could be inoculated to start up new reactors.

3.2 Effects of dyes on physical characteristics of microbial granules

Figure 3 showed the physical morphologies of granules from the three reactors on operation day 200. It can be seen that the sludges in R1 fed with RA14 and R2 with BG were mixtures of small/young and large/mature granules. No obvious suspended sludge was observed. Granules in these two reactors maintained remarkably intact and compact structures. Because of dye adsorption, granules displayed corresponding dye colour. However, granules in R3 is a mixture of

small granules and suspended sludge, which was observed particularly when RB19 concentration increased from 5 to 10 mg/L. Since only 2-minute settling time was used in the reactors, the disintegration of inoculated granules in R3 led to the significant wash-out of sludge.

Meanwhile, the growth of fast-settling suspended sludge was observed which was mixed with small granules as shown in Figure 3. This indicates that the effects of dyes on granule structure stability depend on dye type. Azo and triphenylmethane dyes did not show any negative impact on the structure of acetate-cultivated granule, but anthraquinone dye was very unfavourable to granule stability in this study. Starting up reactors twice validated the adverse effects of anthraquinone dye RB19 on granule stability. In addition, the step-wise increase in azo and triphenylmethane dye concentration in this study is conducive to granule stability because it was reported that the increase in azo dye from 50 directly to 100mg/l led to granule disintegration (Sadri Moghaddam and Alavi Moghaddam, 2016). The granule size reduction was observed in the long-term operation of aerobic granular sludge reactors by Muda et al. (2011) for azo dye decolourization when adjusting HRT. When multiple factors such as HRT, cycle time and OLR were changed simultaneously in the study by Muda et al. (2011), it is not easy to determine if the decrease in aerobic granule size was due to the changes to operating conditions or from the dye used in the study. In this study, we kept all other operating conditions constant. It is thus confident to say that granules can maintain structure stability after exposure to azo and triphenylmethane dyes in a step-wise increased manner. The presence of azo and triphenylmethane dyes do not affect biomass accumulation, which reached until 8-12 g/L (Figure 4) over time just as it accumulated in reactors fed with wastewater but no dyes (Liu et al., 2011). High biomass retention makes the granular sludge more robust and resilient to decolorize dyes.

Figure 4 shows that SVI_5 in all three reactors ranged from 15 to 60 mL/g, a typical volume index range of granular sludge. Nevertheless, from the sludge morphology shown in Figure 3, it can be known that low SVI_5 does not necessarily represent a granule dominant sludge. This was in agreement with the report by Liu et al. (2011), in which a sludge volume percentage of less than 200 μm was proposed to indicate if sludge is dominated by granules. Furthermore, a low and similar SVI_5 did not retain biomass in R3 as in R1 and R2 because biomass retention is also dependent on settling time applied to reactors. In this study, only 2-minute settling time was adopted, which resulted in the wash-out of suspended sludge and small granules with low settling velocities (Toh et al., 2003). Thus, maintaining a granule structure is crucial to retain sufficient biomass in reactors, which is beneficial to sludge to withstand high toxic compound load by reducing the toxic compound load to per unit of microorganisms. It can be found from Figure 4, that on day 200, dye to microorganism ratio (D/M), i.e., the toxic compound load to per unit of microorganisms per day, was 13.5-16.4

times higher in R3 with RB 19 than R1 and R2 although dye concentration in influent fed to R3 was only around half of that to R1 and R2. The higher D/M ratio might be one of the reasons that R3 was not able to re-develop granule dominant sludge during long-term operation period even the favourable operational conditions to granulation (Qin et al., 2004; Liu et al., 2007) was adopted. From the second start-up of reactors with 5 mg/L dye concentration on day 25 to day 200, D/M in R1 and R2 increased 3.3 and 4.0 times, respectively, to deal with more dyes while it rose by 53 times in R3. The significant increase in D/M implies the importance of biomass retention in granular sludge reactors. Higher MLVSS is preferred because it can reduce D/M, and thus allow a higher influent dye concentration for a higher dye colorization efficiency. However, biomass concentration information was missing in most related studies, leading to less possibility to compare results and little general conclusion about the capability of granules for dye decolourization. Muda et al. (2011) reported MLVSS data although they attributed higher color removal percentage in phase IV to both factors i.e., OLR and MLVSS. But when we used their reported data to calculate, it is found that the increase in the colour removal percentage from 67% in phase I to 83% in phase IV with the same ratio of anaerobic to aerobic phase in all 4 phases was accompanied with D/M reduced from 0.00313 to 0.00096/day. This indicates that D/M could be used to explain or predict dye color removal efficiency particularly when multiple operational parameters such as cycle time, OLR and biomass concentration were changed at the same time. However, it needs to be pointed out that besides D/M, lengths of anaerobic and aerobic phases (Muda et al., 2011) and the availability of sufficient external carbon sources (Ong et al., 2005) could also affect dye color removal efficiency.

3.3 Decolourisation of dyes in sequencing batch cycles with/without acetate addition

Given the fact that 100% of dye decolourization efficiency was always achieved in R2 fed with BG, cycle studies on days 199 and 200 with/without acetate were focused on R1 with AR14 and R3 with RB19. It can be seen from Figure 5 that at the same dye concentration, the presence of acetate increased AR 14 removal rate in the anaerobic phase significantly. As shown in Figure 6, AR14 removal rate with the acetate was 23.1 mg/L·h while it reduced by 46%, i.e., 12.6 mg/L·h with the dye only (Figure 6). Considering biomass concentrations in two individual cycles were slightly different, the specific dye removal rates based on biomass were calculated. It was found that without readily biodegradable carbon sources such as acetate, the specific dye removal rate was reduced by 38% from 1.7 to 1.1 mg/g MLVSS·L·h). These data suggest that co-substrate can increase the removal rate of AR14. The enhancement of azo dye decolourization efficiency by co-substrate has been well established (Zhang et al., 2019), but co-substrate can also increase the azo dye decolourization rate. It is possible that acetate enhanced the reductive cleavage of azo dye apart from maintaining bacterial growth and activity.

In R3 fed with RB19, however, the effects of acetate in wastewater on dye removal rate was not as obvious as that on AR14. The dye removal rate without acetate seems even higher than that with acetate. But the biomass concentrations in two cycles different. Therefore, the dye removal rate is not able to show the activity of biomass for dye decolorization. It can be seen from Figure 6 that the specific dye removal rate was still lower when no acetate was added into wastewater. It reduced by 15 % from 8.4 to 7.1 mg/g MLVSS·L·h, showing the benefit of external carbon source to RB19 decolourization as well. External carbon and nitrogen sources are usually added for anthraquinone dye degradation. Some believed that external carbon sources were to promote bacterial growth and activity and to maintain the diversity of the microbial community (Li et al., 2019) while others showed that anthraquinone dye removal was significantly enhanced by the addition of carbon sources through the co-metabolic process (Xu et al., 2006; Giwa et al., 2012). This difference might be primarily from the difference of species used or enriched for anthraquinone degradation with different metabolic pathways. The common carbon sources used in the laboratory studies are glucose, sucrose, D-maltose, L-rhamnose (Kurade et al., 2016). The specific anthraquinone removal rate is only 15% higher with the presence of acetate, suggesting that acetate might be more used for bacterial growth or activity maintenance than co-metabolism with dye. Higher specific azo and anthraquinone dye removal rates with the presence of acetate indicate the necessity of other carbon sources for dye decolorization.

In the literature, dye removal rate mg/L·h was widely used for the comparison of dye removal performance by different biomass or bacteria (Franca et al., 2020). In this study, the removal rate of anthraquinone dye RB19 was around 2.6 -3.4 mg/L·h while 0.29-2.5 mg/L·h decolourization rates in the literature by using fungal isolates (Yemendzhiev et al., 2009; Attéké et al., 2013; Mounguengui et al., 2014) or consortium of *Proteus mirabilis* and *Proteus vulgaris* (Parmar and Shukla, 2015) were reported. Chaudhari et al. (2017) reported 0.67-6.32 mg/L·h of anthraquinone dye RB4 at static conditions by using aerobic granules. Thus, anthraquinone dye removal rate by using aerobic granules in Chaudhari's and this study appears higher than suspended biomass. However, the dye removal rate is affected dramatically by biomass concentration. Thus, the specific dye removal rate is a fairer parameter for the comparison of biomass activity for dye removal while the dye removal rate is better for the comparison of reactor performance. There was, however, widely lacking biomass concentration data in the literature. For example, It is hard to know if higher dye decolorization rate such as 6.32 mg/L·h in Chaudhari's study than 3.4 mg/L·h in our study was due to higher biomass concentration in their batch tests because in our study only 0.3-0.5 mg/L biomass

was retained in R3 due to granule disintegration during the long-term operation period. In addition, it was found that Chaudhari et al. (2017) used granules with a size of 7-9mm for the dye test, significantly larger than an average size of aerobic granules such as 1-2mm that could be achieved and stabilised in most studies on aerobic granules. Meanwhile, only batch tests for 16 cycles with each cycle of 48 hours were carried out without a description of granule morphology, leading to little information on effects of the anthraquinone dye on granule stability during long-term operation. In this study, however, the disintegration and instability of granules were observed after being exposed to anthraquinone dye RB19 even under the condition even with a step-wise increase in dye concentration aiming to minimize toxic anthraquinone day shock on granules.

The removal rate of AR14 was almost 4 times of that of RB19 while the specific anthraquinone decolourization rate such as 7-8 mg/g MLVSS ·L·h was 5-6.5 folds of that of azo dye, suggesting a much higher specific biomass activity in R3 for RB19 decolourization and the promising potential of sludge acclimatized for anthraquinone dye removal. However, the instability of granule structure caused great concern for the application of aerobic granules to anthraquinone dyes because high dye removal rate was not able to be achieved at low biomass concentration due to granule disintegration and over-growth of suspended sludge. Furthermore, the reactor with low biomass concentration is fragile when facing toxic compound shock and fluctuation of operation conditions.

One of the most significant advantages of granular sludge to treat dye containing wastewater is the high biomass retention in reactors. In addition, large granule size with mass transfer resistance inside can make granules more resilient to unfavourable conditions especially with toxic compounds (Tay et al., 2005b). When granule stability was negatively affected by anthraquinone dye such as in R3, the dye removal rate was significantly lower. Therefore, the bottleneck to use aerobic granule for anthraquinone dye or a mixture of dyes containing anthraquinone is how to improve granule stability and thus increase biomass retention in reactors for highly efficient dye colour removal. The stability of granules should be future research focus for anthraquinone dye removal.

4. CONCLUSIONS

This study investigated decolourization of azo, anthraquinone and triphenylmethane dyes by inoculating acetate-cultivated aerobic granules. Based on the research work done in this study, the main conclusions are drawn as: i) Change of cycle operation of aerobic granular sludge reactors from pure aerobic phase to alternating anaerobic and aerobic phases in one cycle do not affect granule stability and long-term operation. In addition, aerobic granular sludge cultivated with acetate can be acclimatized to removing three types of dyes, i.e. azo, anthraquinone and triphenylmethane dyes. These allow new reactor start-up for dye decolourization by inoculating mature granules from

different processes feasible, providing flexible and simple reactor start-up strategies for textile wastewater treatment; ii) Both azo and triphenylmethane dyes do not have any negative impact on granule structure stability and biomass retention during the long-term operation period. 100% and 90% removal efficiency could be achieved for triphenylmethane dye GB and azo dye AR14, respectively. However, the addition of anthraquinone dye RB19 to influent resulted in the disintegration of granules, over-growth of suspended sludge, poor biomass retention and low biomass concentration in the reactor, leading to high dye-to-microorganism ratio and low dye decolourization efficiency and rate. Granules are very promising to treat azo and triphenylmethane dyes for long-term stability but not anthraquinone.

For the first time, this work studied long-term stability of granules when treating dye-containing wastewater with azo, anthraquinone and triphenylmethane dyes, and compared decolourization performance of three classes of dyes in the same reactor configuration and operating conditions. The study identified different effects of dyes with different chemical structures on long-term granule stability. The findings on specific dye decolourization rate and dye-to-microorganism ratio of granular sludge for three different classes of dyes provide a foundation for further investigation to improve granule stability when treating textile wastewater with multiple classes of dyes.

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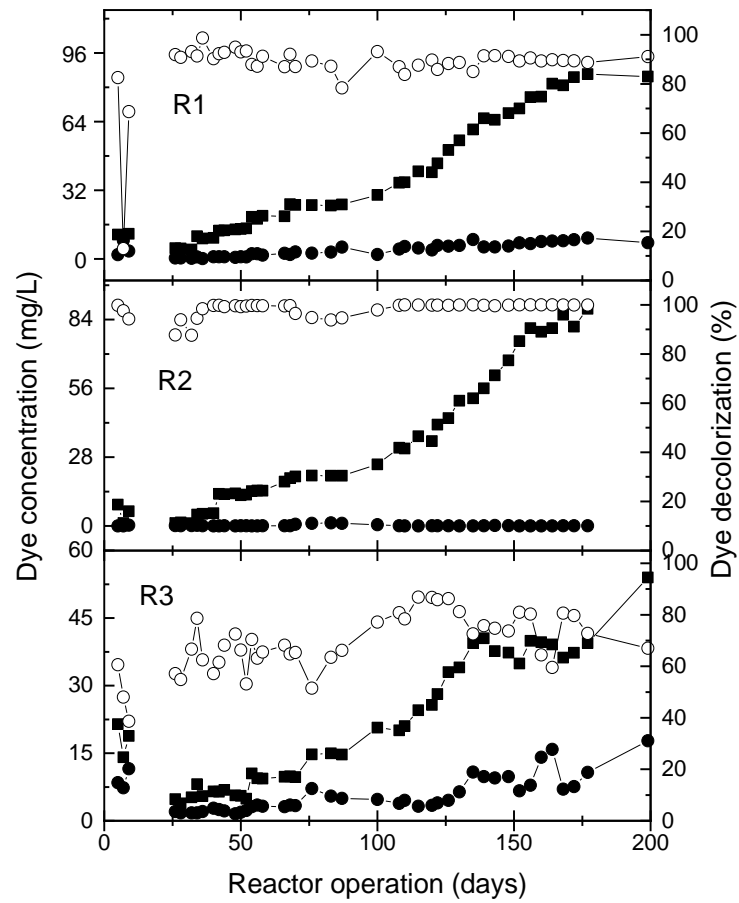


Figure 1 Dye decolourisation by microbial granules over the operation time with a step-wise increased influent dye concentration in wastewater, R1: AR14; R2: BG; R3: RB19; symbols ■, ●, and ○ represent influent dye concentration, effluent dye concentration and dye decolourisation percentage, respectively.

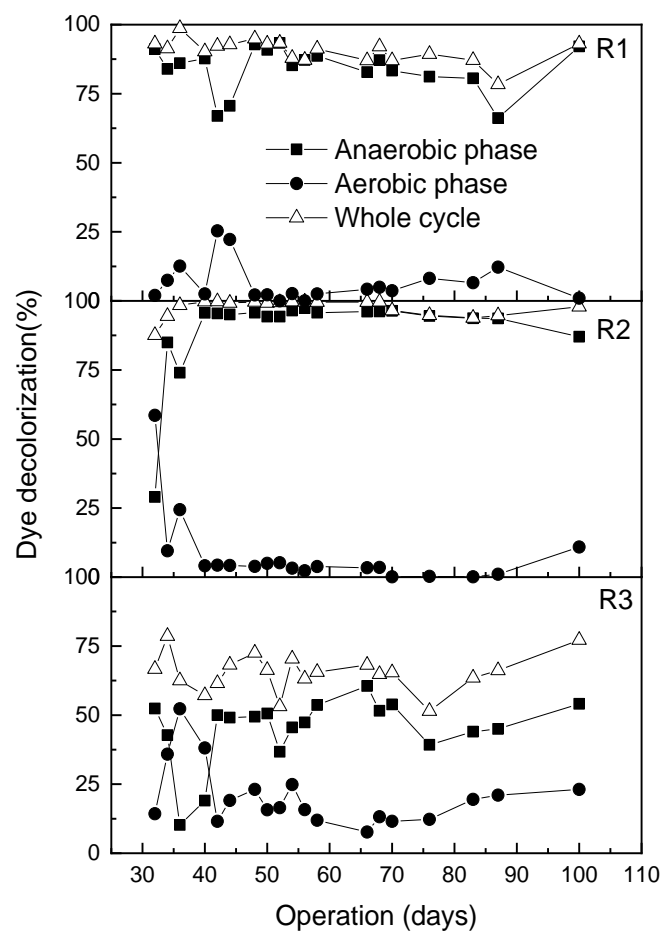


Figure 2 Dye decolourization during the anaerobic phase, the aerobic phase and the whole cycle in the three reactors with AR14 in R1, BG in R2, and RB19 in R3.

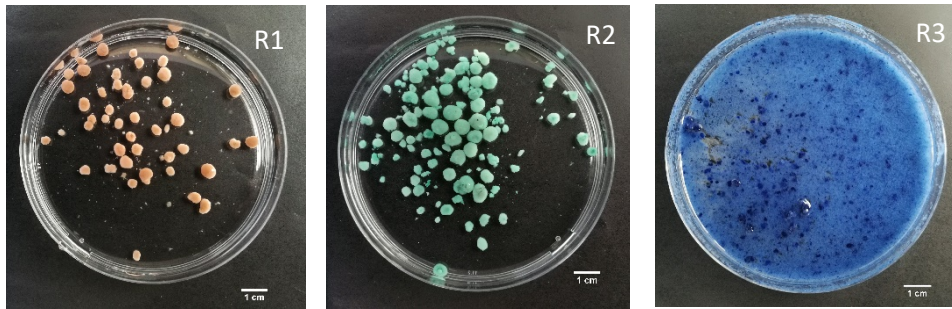


Figure 3 Morphology of microbial granules from three reactors on day 177 for dye decolourisation with AR14 in R1, BG in R2, RB19 in R3.

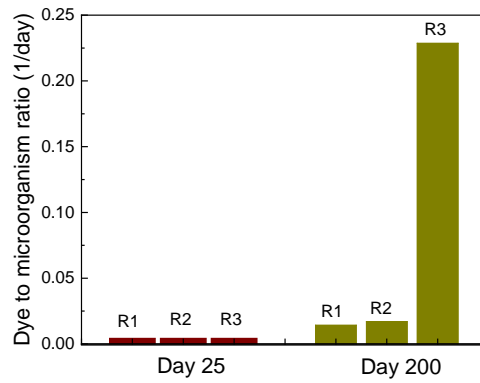
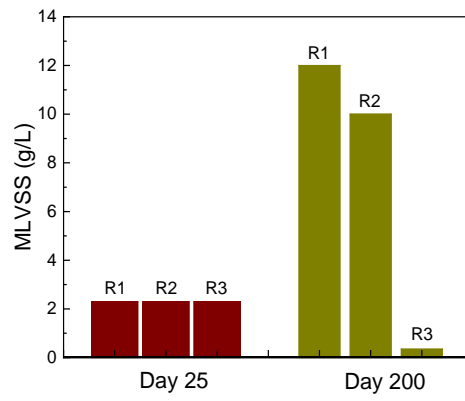
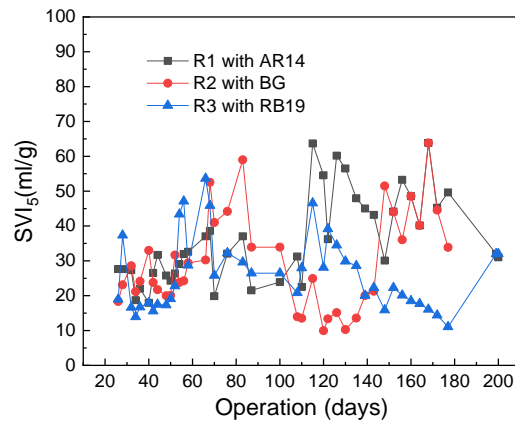


Figure 4 SVI₅ in three reactors with azo, anthraquinone and triphenylmethane dyes during long-term operation period, and MLVSS and dye to microorganism ratio on days 25 and 200

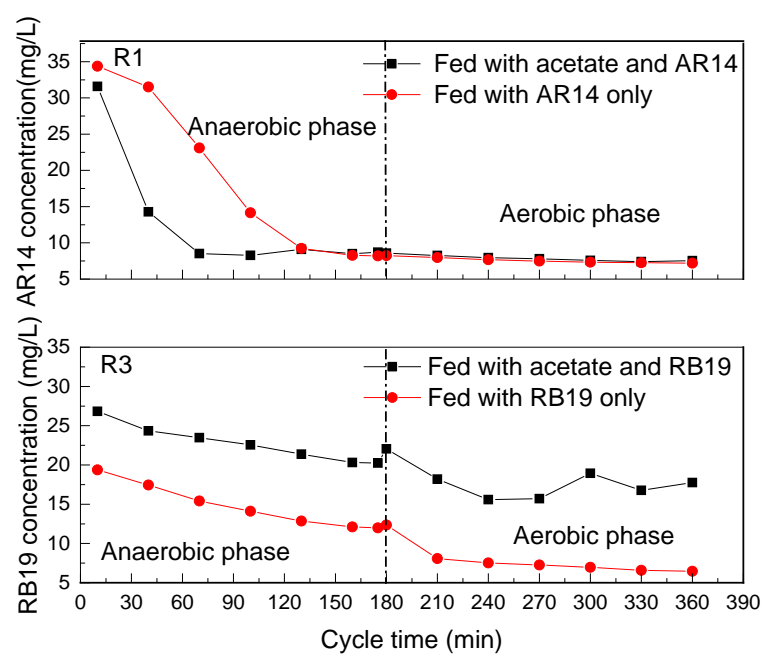
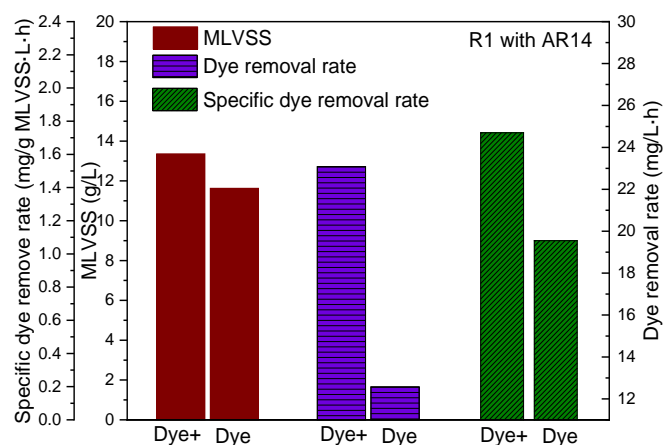
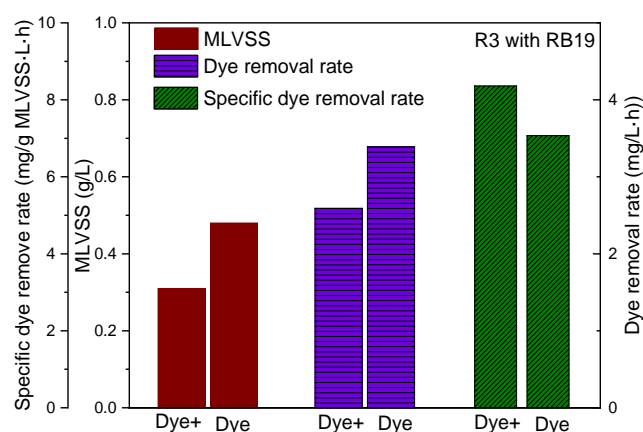


Figure 5 Dye decolourization in a typical cycle of reactors R1 and R3 fed with the mixed carbons of acetate and dye, and the single carbon source of dye only, respectively, on operation day of 199.



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609 Figure 6 Removal rates and specific removal rates of azo dye AR14 in the cycle of R1, and
 610 anthraquinone dye RB19 in the cycle of R3, respectively, with/without acetate addition; 'Dye+'
 611 represents dye with acetate addition in the influent, 'Dye' represents dye without acetate addition
 612 in the influent. (note: The calculation of dye removal rates and specific removal rates are based on
 613 initial dye concentration and stable dye concentration within the removal period of the cycles)

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