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Rapid biodecolourization of eriochrome black T wastewater by bioaugmented aerobic granules cultivated through a specific method

Wang Hailei[a,b,*], Li Ping[a], Liu Guosheng[a], Li Xin[a], Yao Jianming[b]

[a] College of Life Sciences, Henan Normal University, Xinxiang 453007, China
[b] Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Sciences, Hefei 230031, China

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ABSTRACT

In this paper, an interesting phenomenon is revealed. Mn^{2+} has a significant influence on the morphologies of hypha and mycelium of *Phanerochaete* sp. HSD, and under the higher Mn^{2+} concentration, strain HSD can form very small micro-mycelium pellets (MMPs). Based on this phenomenon, a specific method to cultivate aerobic granules is presented. By seeding MMPs to bioreactor, bioaugmented aerobic granules (BAGs) were cultivated successfully, and granulation rate reached $53 \pm 2\%$ on day 15. MMPs result in the formation of aerobic granules containing MMPs as nuclei and also induce the formation of self-immobilized biogranules which do not have the MMP at their core. Furthermore, the feasibility of using BAGs to degrade eriochrome black T (EBT) wastewater is investigated too. The results prove that the treatment of EBT wastewater using BAGs is a feasible and promising way. BAGs not only can tolerate higher EBT loads, but also have better decolourization efficiency compared with conventional sludge and aerobic granules. Furthermore, the introduction of MMPs leads to the appearance of manganese peroxidase (MnP) in reactor, and MnP plays an important role in the treatment process of EBT wastewater.

1. Introduction

Azo dyes are the major group of dyestuffs and have been extensively used in textile, printing, and leather industries. They have been identified as the most problematic compounds in the effluents of many industries, especially some large molecules of them such as eriochrome black T (EBT), are resistant to fading from exposure to light, water and chemicals due to their complex chemical structure; hence they persist in nature [1].

There are many physico-chemical treatment methods for the removal of azo dyes from industrial wastewater such as Fenton reaction, ozonation degradation and adsorption [2–4]. However, these methods are non-selective, usually costly and not easily adapted [5]. The use of bacteria to treat dye effluents may result in the generation of dead-end aromatic amines which are generally more toxic than the parent compounds, and thus has poor adaptability and limited application to a wide range of dye wastewaters. In recent years, there has been an increasing interest in white rot fungi [6,7]. *Trametes versicolor*, for example, has been reported to be suitable for decolourizing some dyes because it can produce extracellular ligninolytic enzymes such as lignin peroxidase, laccase and manganese peroxidase (MnP), which can catalyze the oxidative degradation of the pollutants [8–10].

Aerobic sludge granulation is a new technology in biological wastewater treatment [11]. Compared with conventional activated sludge, aerobic granules feature a number of advantages such as denser and stronger microbial structure, better settling ability, more effective sludge–effluent separation, greater biomass retention and higher capability to withstand shock loads [12,13]. Aerobic granulation technology appears to have the potential to respond to the challenges of pollutant removal from wastewater and has been employed in treating many high-strength wastewaters containing organic compounds, nitrogen and phosphorus, phenol and so on [14–16]. Up to now, the mechanisms behind the formation of aerobic granules have not been fully understood yet. There are many factors that have been shown to have important influences on sludge granulation such as the loading rate, hydrodynamic shear force and hydraulic retention time [17,18].

In the previous work, a strain of higher MnP producer, *Phanerochaete* sp. HSD was screened in our lab and the fungus was capable of degrading azo dyes using its MnP [19]. In this study, an interesting phenomenon was revealed: Mn^{2+} affected the morphologies of hypha and mycelium pellet of strain HSD markedly and under higher Mn^{2+} concentrations, strain HSD could form very small micro-mycelium pellets (MMPs). Based on this phenomenon, a novel method to cultivate aerobic granules was presented. By

* Corresponding author at: College of Life Sciences, Henan Normal University, Jianshe Road 46#, Xinxiang 453007, China. Tel.: +86 3733326340; fax: +86 3733326916.
E-mail address: whl@henannu.edu.cn (W. Hailei).

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seeding the MMPs of strain HSD to sequencing batch reactor (SBR), bioaugmented aerobic granules (BAGs) were cultivated successfully and MnP also appeared in the reactor. Furthermore, the feasibility of using BAGs to degrade EBT wastewater was investigated too. To date, there is little information available in literature about cultivating aerobic granules using this method; moreover, few researchers have focused on treating EBT wastewater by BAGs. Thus, this study may be helpful to the application of aerobic granules in dyes wastewater treatment.

2. Materials and methods

2.1. Chemicals, microorganism and sludge

All chemicals used are of spectral or analytical grade. *Phanerochaete* sp. HSD was isolated from a tree full of rot from the pine hurst of Taihang Mountain in Henan Province, China. Both domestic sludge and conventional aerobic granules (CAGs) are obtained from Henan Key Laboratory for Environmental Pollution Control, and CAGs are cultivated by synthetic wastewater in which glucose is used as the carbon source.

2.2. Media and culture conditions

Strain HSD was incubated on potato dextrose agar (PDA) plate and sub-cultured for 3 days at 37° C, and then the spores were harvested using a camel hair brush. Spore suspensions were prepared and spore concentration was adjusted to 10^7 spores/ml and used as the inoculum for further studies. Two milliliter spore suspensions were seeded to nine 250 ml flasks each containing 100 ml basic medium, and then the flasks were cultured in a shaking incubator (150 rpm; 35 ± 2 °C) for 3–5 days. Basic medium had the following compositions: 10.0 g/l glucose, 2.0 g/l diammonium tartrate, 2.0 g/l KH_2PO_4, 0.8 g/l $MgSO_4{\cdot}7H_2O$, 0.1 g/l $CaCl_2$, 0.5 g/l Tween 80, 0.7% trace element solution including: 0.5 g/l glycine, 1.0 g/l NaCl, 0.1 g/l $FeSO_4{\cdot}7H_2O$, 0.1 g/l $CoSO_4$, 0.1 g/l $ZnSO_4{\cdot}7H_2O$, 10 mg/l $CuSO_4{\cdot}5H_2O$, 10 mg/l $AlK(SO_4)_2{\cdot}12H_2O$, 10 mg/l H_2BO_3, 10 mg/l $Na_2MoO_2{\cdot}2H_2O$. The pH of medium was adjusted to 4.5 by acetic acid–sodium acetate buffer solution. Furthermore, through the addition of $MnSO_4$, Mn^{2+} concentrations were adjusted to different levels (0.33 mmol/l, 0.67 mmol/l, 1.00 mmol/l, 1.33 mmol/l, 1.67 mmol/l, 2.00 mmol/l, 2.33 mmol/l, 2.67 mmol/l, 3.00 mmol/l) in order to investigate the effects of different Mn^{2+} concentrations on the morphologies of hypha and mycelium of the fungus.

2.3. The preparation of MMPs

MMPs were prepared using a medium in which Mn^{2+} concentration was 2.00 mmol/l and the other components were the same as the basic medium. Strain HSD was seeded to the medium under sterile condition and culture was carried out at 35 ± 2 °C for 5 days on a rotary shaker at 180 rpm. The cultures were centrifuged at 5000 rpm and the sediment, MMPs, were harvested. Under the microscope it was found that besides the hyphae, MMP consisted of quantities of chlamydospores of strain HSD.

2.4. The operation of SBR

Tests were carried out in a SBR system. The system, under the control of a microcomputer timer switch, was constituted by six 4 l cylindrical SBRs (55 cm in height and 10 cm in diameter), and each of them was equipped with thermometer, pH meter, dissolved oxygen (DO) analyzer, heater, aerator and stirrer. Initial sludge domestication process was as follows: at 30 °C, three reactors inoculated with 2 l domestic sludge (MLSS 14 g/l) and 0.78 g MMPs (dry weight), were called the control SBR (R1); the other reactors, conventional SBR (R2), were inoculated with 2 l domestic sludge, but no MMP. During start-up phase (day 1–3), reactors were fed with 1 l basic medium (exchange ratio, 25%). After day 3, the influent volume varied from 1 to 2 l (exchange ratio, 50%) and basic medium was changed to synthetic wastewater. Synthetic wastewater had the following compositions: 10.0 g/l glucose, 2.0 g/l NH_4Cl, 2.0 g/l KH_2PO_4, 0.8 g/l $MgSO_4{\cdot}7H_2O$, 0.1 g/l $CaCl_2$, 0.05 g/l EBT; furthermore, 0.075 mg/l of $MnSO_4$, instead of trace element solution, was added to synthetic wastewater. Operation process of SBR was as follows: feeding phase (10 min), aerobic phase (4.5 h), settling phase (30 min), discharge and idle phase (50 min). After a week, the glucose in synthetic wastewater was decreased gradually. On day 11, it was reduced to 1.0 g/l, and then the level was maintained. The concentration of EBT was increased according to the need of tests. Furthermore, the stored CAGs were activated using basic medium in a separate SBR (R3).

2.5. Decolourization of EBT wastewater

The capabilities of BAGs, conventional sludge and CAGs to decolourize EBT wastewater were compared in this work. Synthetic wastewater with different EBT concentrations (50–400 mg/l) was added to reactors. After the treatment, EBT concentration was measured at wavelength corresponding to the maximum absorbance (523 nm) by means of UV–vis spectrophotometer (Jenway 6305 UV–vis spectrophotometer, England). The adsorption and decolourization were determined according to the method of Li and Jia [20]. Furthermore, for higher concentration (200–400 mg/l) of EBT wastewater, the decolourization cycle of SBR was prolonged to 12 or 24 h according to the need of tests.

Moreover, a separate batch adsorption experiment with BAGs was conducted in order to confirm the function of adsorption in the decolourization process of EBT wastewater. In this experiment, different concentrations (100–800 mg/l) of EBT wastewaters were decolourized in the SBR, respectively and the adsorption rate was measured.

2.6. Analytical methods

Total suspended solids (TSS), chemical oxygen demand (COD), volatile suspended solids (VSS), mixed liquor suspended solids (MLSS) and sludge volume index (SVI) were analyzed by standard methods [21]. Integrality coefficient was measured using the method of Ghangrekar et al. [22], and the lower integrality coefficient, the greater is the strength of granules. Formation process of aerobic granules was viewed with bio-microscope (Nikon HFX-IIA). The surface and interior structure of granule were observed by scanning electron microscope (SEM, AMRAY-1000B, Japan) and transmission electron microscope (TEM, JEM-100CXII, Japan), respectively. Granulation rate (GR) of sludge was measured according to the method described by Wang et al. [11]. Particle size was determined by an image analysis system (Image-Pro Plus, V4.0, Media Cybernetics). MnP was assayed spectrophotometrically by the method of Kuwahara et al. [23]. One activity unit was defined as the amount of enzyme that oxidized 1 μmol of dimethoxyphenol per minute.

3. Results and discussion

3.1. The effects of Mn^{2+} concentrations on the biomass and morphologies of hypha and mycelium pellet of strain HSD

In previous reports, it was found that higher Mn^{2+} concentration could improve MnP yield of strain HSD [19], but the effects of different Mn^{2+} concentrations on the biomass and morphologies of hypha and mycelium of this strain were still unrevealed. Fig. 1(a) shows the curve of biomass has a decreasing trend with the increase of Mn^{2+} concentration, and it indicates that the higher Mn^{2+} concentration is harmful to the growth of this fungus. However, the decrease of biomass is not significant when Mn^{2+} concentration is lower than 1.67 mmol/l. The more interesting is that the size of mycelium pellets becomes smaller with the enhancement of Mn^{2+} concentration. The diameters of 99% mycelium pellets under the Mn^{2+} concentration of 2.00 mmol/l are 0.1–0.3 mm and these small pellets, look like silver sands, are called micro-mycelium pellets. Changes of the size of mycelium pellets varied with Mn^{2+} concentration are shown in Fig. 1(b). Moreover, Mn^{2+} concentration has a significant influence on the hyphal morphology of strain HSD. When Mn^{2+} concentration in the medium is higher than 1.67 mmol/l, the hyphae are anamorphic and intumescent under the microscope, and some dilatants appear at the hyphal tips or in the midst of hyphae; when Mn^{2+} concentration in the medium reaches 2.0 mmol/l, the pellet of strain HSD becomes incompact and it consists of quantities of chlamydospores. The micrographs of the normal hyphae, anamorphic hyphae and chlamydospores of strain HSD were shown in Fig. 2.

3.2. The domestication of aerobic granules

During start-up phase, microbes in both R1 and R2 propagated rapidly since the reactors were fed with basic medium. The colour of sludge changed from black to yellow gradually and the settling performance became better. The curves of MLSS (Fig. 3(a)) had a sharply descending trend during this phase. The reason is that much dispersed sludge that has bad settling performance is washed out with the effluent. After day 4, the biomass in reactors increased with the propagation of microorganisms, and the curves of MLSS started to ascend until they reached the peak value.

In R1, EBT could not be detected in the effluent on day 5, and in the meantime, a large number of aerobic granules appeared. On

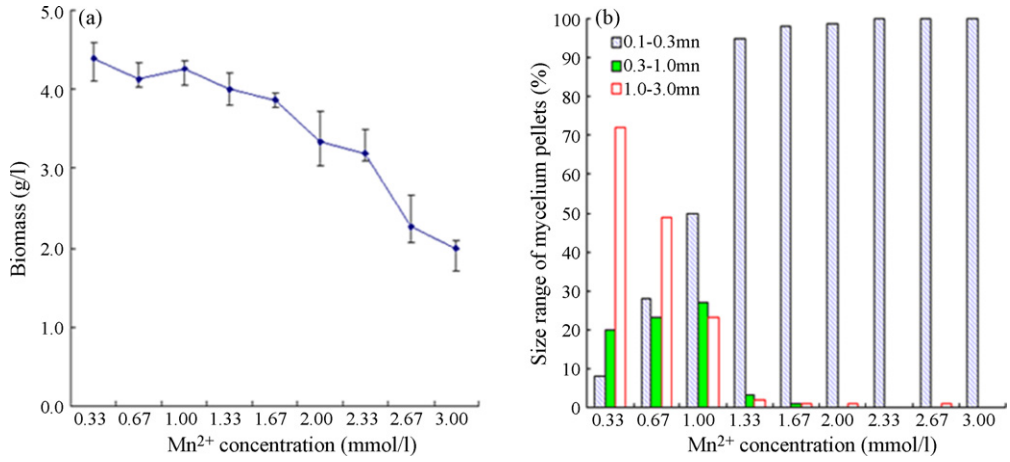

Fig. 1. The effects of different Mn^{2+} concentrations on the biomass and sizes of mycelium pellets of *Phanerochaete* sp. HSD. (a) the effects of different Mn^{2+} concentrations on the biomass;(b) size ranges of mycelium pellets varied with different Mn^{2+} concentrations.

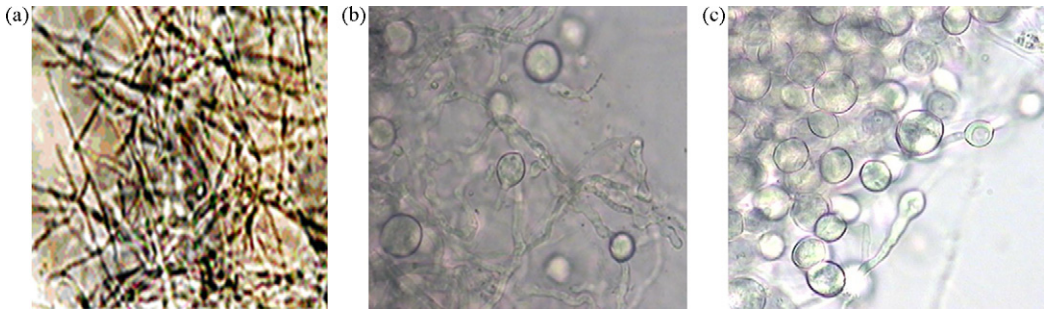

Fig. 2. The micrographs of the normal hyphae, anamorphic hyphae and chlamydospores of strain HSD. (a) The normal hyphae; (b) the anamorphic hyphae; (c) chlamydospores. The magnification is 400×.

day 7, the GR of sludge reached 37 ± 1%, and to day 15, it was up to 53 ± 2% (Fig. 3(b)). These aerobic granules cultivated by bioaugmentation were called bioaugmented aerobic granules in order to distinguish them with aerobic granules cultivated by other methods. Moreover, many protozoa could be found in the sludge under microscope.

On day 7, MLSS in R2 was 4.4 g/l, lower than that in R1. It is because that besides domestic sludge, MMPs are also seeded to R1. However, R2 is only inoculated with domestic sludge. Thus, MLSS of R1 is higher than that of R2 from the start. Certainly, we cannot exclude the possibility that the residual EBT restrains the growth of some microbes in R2. For 50 mg/l EBT wastewater, the decolourization rate of R2 was 92% after 6-h treatment; but in R1, EBT was degraded completely. Moreover, many protozoa also appeared in R2 after day 7, but, no aerobic granule appeared. Up to day 50, the sludge was still incompact, had no granulation trend.

Compared R2 with R1, the only difference was that R1 was inoculated with 4 g MMPs. Thus, the seeding of MMPs was the key reason for why aerobic granulation happened in R1. No aerobic granule appeared in R2, indicating that it was very difficult to cultivate aerobic granules using the poisonous EBT wastewater. So, seeding MMPs to the reactor was an effective and promising method to accelerate the formation of aerobic granules. However, how do the MMPs induce sludge granulation? The probable reason is that MMPs, these very small pellets, present primary matrixes in the process of sludge granulation. The matrixes can act as nuclei,

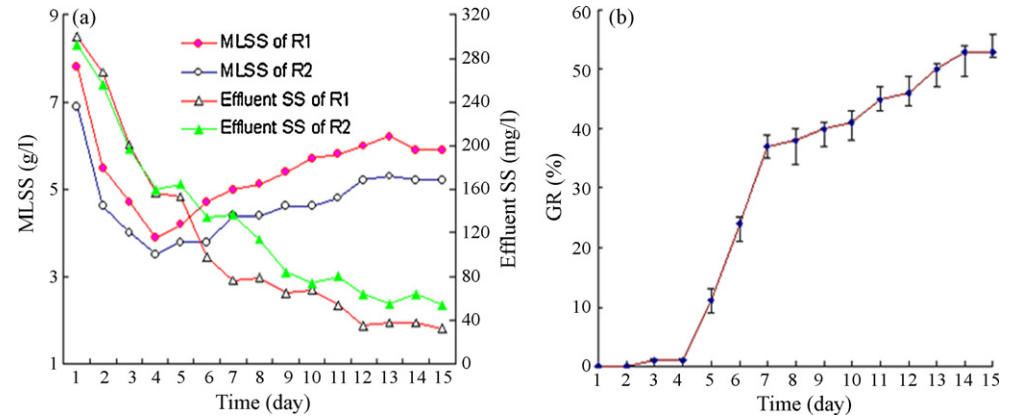

Fig. 3. Variations of MLSS, effluent SS and GR of sludge in reactors. (a) MLSS and effluent SS in R1 and R2 varied with time; (b) the GR of sludge in R1 varied with time.

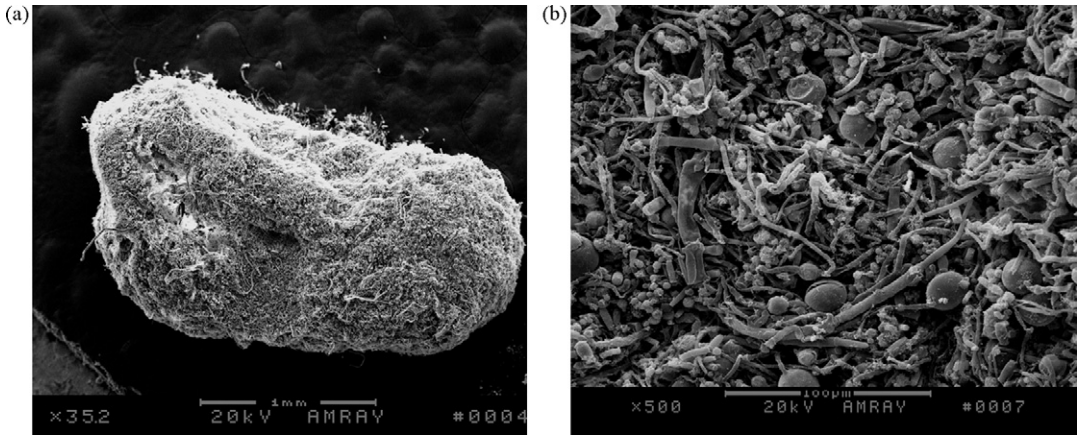

Fig. 4. Microscopy images of BAG. (a) SEM image of overview of granule; (b) SEM image of microbes on the surface of granule.

on which various microbes attracted by the adsorption, enlacement of hypha and other attractive forces can propagate and lead to the formation of aerobic granules at last. The primary matrixes are necessary for the initiation of granule development. This point has been proved by many reports [24,18]. For example, Liu and Tay pointed out the appearance of primary matrixes, which might be highly organized microbial structure such as flocs or fragments from biofilm, was a very crucial step to sludge granulation. Therefore, some researchers revealed that aerobic granules could form quickly using aerobic or anaerobic granules as the starting seeds [25,26]. In this work, the interior structure of aerobic granule was observed using TEM and it appeared that some granules contained a core made up of hyphae. MMP is also composed of the hyphae of strain HSD, however, whether the core is the MMP we seeded or not? This is another interesting question worth exploring. A test was carried out to answer this question. Three hundred MMPs (about 0.3 mm in diameter) were selected and each of them was inserted in a 0.4–0.5 mm brass wire with the diameter of 50 μm. These marked MMPs were seeded to a 0.5 l SBR and BAGs were domesticated in this reactor. 15 days later, 200 mature granules were picked out randomly and cut into slices, and then these slices were observed under microscope. The result showed that 16 aerobic granules contained brass wire, indicating that 8% of granules were developed on the basis of MMPs we seeded. Thus, the introduction of MMPs not only can result in the formation of aerobic granules containing MMPs as nuclei, but also can induce the formation of self-immobilized biogranules which do not have the MMP at their core. This result accorded with the conclusion of Ganesan and Saravanan [27]. They found that the introduction of activated carbon particles into aeration tank resulted in the formation of granules containing activated carbon particles as core nuclei and also induced the formation of granules which did not have any carrier particle at their core. However, our design had more advantages over their method, because the addition of MMPs not only promoted sludge granulation, but also introduced a superior microorganism which possessed higher ability to degrade the main ingredient of wastewater, EBT in this work, to the bioreactor.

But, why the addition of MMPs can trigger the formation of 92% of granules that do not use MMPs as nuclei? The possible reasons are: (i) MMP is incompact pellet and it consists of quantities of chlamydospores. The detached chlamydospores from pellets can form new pellets in the reactor, and the newly formed pellet also can present the nucleus for granule formation, although it does not contain a brass wire; (ii) it is found that strain HSD is a better microbial flocculant since it contains a large number of acidic polysaccharides, and now it is clear that these extracellular polymeric substances (EPS) can enhance the sludge granulation [28].

3.3. The morphology of BAG and its characteristics

BAGs were cultivated successfully in R1. Granules were cycloid or oval with compacter appearances, and the sizes of them varied between 1.0 and 4.7 mm with the average diameter 2.1 mm. The morphology and outer structure of the granules were observed using SEM. Fig. 4(a) indicated that BAGs had a compact but uneven surface. Rod-shaped and orbicular microorganisms including yeasts and bacteria existed on the surface of them, but filamentous microorganisms were dominant (Fig. 4(b)). The comparison of sludge characteristics in R1 and R2 was listed in Table 1. From it we could find that BAGs had many advantages over conventional sludge such as it had better settling performance, compacter microbial structure and lower water content.

Integrality coefficient is an index indicative of strength of granule against abrasion and shear, which granule often undergoes during reactor operation. As shown in Table 1, integrality coefficient of BAG was 0.0895, indicating that granule had better strength and stability. Furthermore, VSS/TSS of BAG in R1 was 89.1%, 21.3% higher than that of conventional sludge in R2, and also higher than the VSS/TSS ratios of biofilm and CAG in some previous reports [29–31]. As we know, for two kinds of sludge with the same TSS concentration, the one with higher VSS/TSS contains more biomass. Thus, BAG contains more biomass compared with equivalent TSS concentration conventional sludge and CAG.

3.4. Biodecolourization of EBT wastewater

Strain HSD is a strain of white rot fungus which has the better decolourization ability to azo dyes. However, to the higher concentration of EBT wastewater such as 300 mg/l, strain HSD can not decolourize it efficiently even after 20-h treatment [19]. Furthermore, it is reported that EBT is resistant to the removal by bacterial consortium (TJ-1), and to 200 mg/l of EBT, the decolourization rate is 42.2% after 12-h treatment [32]. Thus, to the naphthol azo dye—EBT, it is very difficult to be biodecolourized completely [33]. In this test, the feasibility of using BAGs to degrade EBT wastewater was studied. As shown in Fig. 5, BAGs could decolourize 100–400 mg/l EBT rapidly, and over 98.4% decolourization was obtained after 12-h treatment; especially for 100–300 mg/l EBT, the decolourization rates were up to 95.6%, 96.5% and 97.3%, respectively at hour 6. This proved that BAGs possessed better capability to decolourize EBT wastewater in contrast with the white rot fungus and bacterial consortium previously mentioned. Furthermore, Fig. 6(a) showed that BAGs could tolerate higher EBT loads and they were capable of decolourizing 400 mg/l EBT efficiently. Com-

Table 1
Comparison of sludge characteristics in R1 and R2.

Parameters	SVI (mL/g)	VSS/TSS (%)	Density (g/cm^3)	Integrality coefficient	Water content (%)
BAG	72 ± 2[a]	89.1 ± 0.2[b]	1.0046 ± 0.2[c]	0.0895	98.30 ± 0.02[d]
Conventional sludge	115 ± 4[a]	67.8 ± 0.2[b]	1.0017 ± 0.1[c]		99.76 ± 0.03[d]

The values are shown as average ± standard deviations.
[a] $n = 3$.
[b] $n = 3$.
[c] $n = 5$.
[d] $n = 5$.

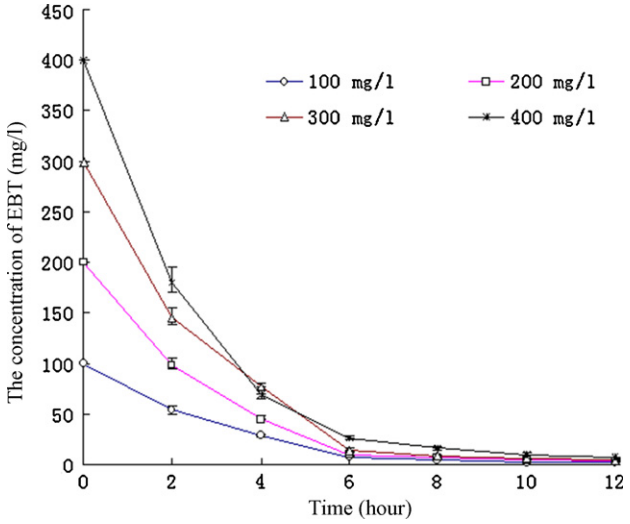

Fig. 5. Time courses of residual concentration of EBT during the decolourization process of BAGs.

pared with BAG, the ability of CAG was weaker. For 200 mg/l EBT, decolourization rate was 78.2%, and about 30% of decolourization was caused by the adsorption of granules. Conventional sludge in R2 could only treat EBT wastewater with the concentration of 50 mg/l. When the concentration surpassed 100 mg/l, effluent SS increased from 54 mg/l to 150 mg/l after 24 h and the significantly deteriorated performance of reactor was observed.

Fig. 6(b) is the COD removals of BAGs, conventional sludge and CAGs to 400 mg/l EBT wastewater. R1 possessed the higher COD removal ability, and COD removal rate reached 94.0 ± 0.7% at hour 24. In R2, COD removal rate was only 15.8 ± 0.4%, proving that conventional sludge was inefficient to treat high concentration of EBT wastewater. Furthermore, this figure revealed that R3 could not treat 400 mg/l EBT wastewater, either. COD removal rate was 19.4 ± 0.6% at hour 21.

Aerobic granules were believed to play a promising role in adsorption of toxic chemicals and dyes due to their higher surface area and porosity [28,34]. Zheng et al. [35] reported that the removals of cationic dye and rhodamine B by aerobic granules were governed by the Langmuir adsorption isotherm and maximum adsorption density was three times greater than that of sludge flocs. However, in this work, when a treatment cycle was completed, for 100–300 mg/l EBT wastewater, BAGs in the reactor were white; for 400 mg/l EBT wastewater, the colour of the effluent was gray but BAGs were still yellowish-white. The adsorption was almost negligible by analysis. The separate batch adsorption experiment with BAGs showed that the adsorption rate of granules was very low (<0.5%) when EBT concentration was lower than 400 mg/l. But, when the EBT concentration was higher than 500 mg/l, adsorption started to play an important role in the decolourization process. Thus, for 100–400 mg/l of EBT wastewater, adsorption is not the main reason for decolourization and most of the EBT is biodegraded by BAGs.

The previous facts demonstrate that BAGs not only can tolerate higher EBT loads compared with conventional sludge, but also possess better ability to biodegrade EBT wastewater. All these are the results of seeding MMPs to SBR. The appearance of MMPs in reactor

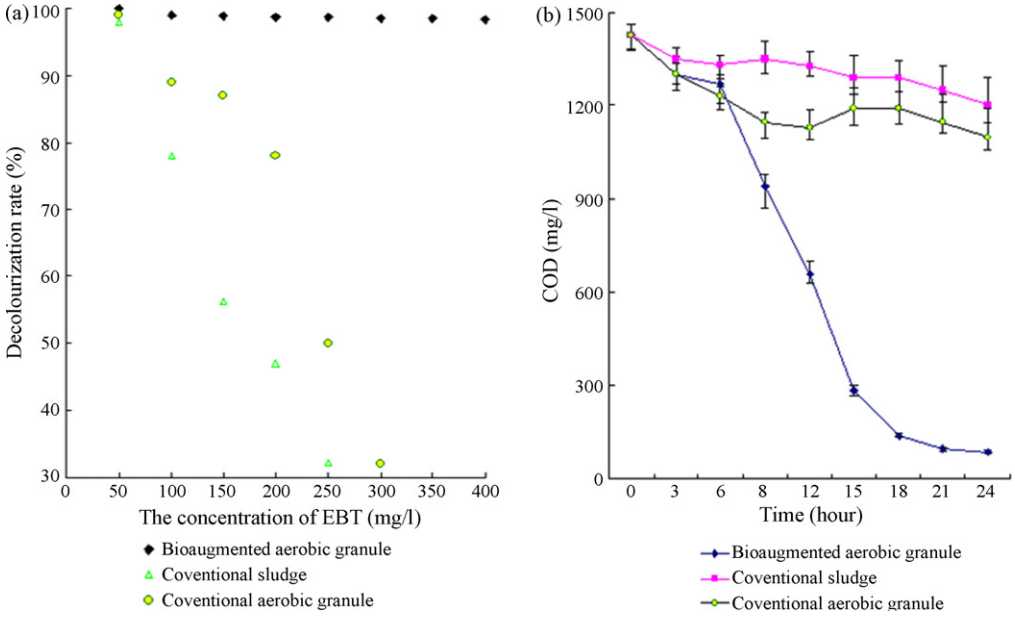

Fig. 6. Comparisons of decolourization rates and COD removals of BAGs, conventional sludge and CAGs to EBT wastewater. (a) Decolourization rates of BAGs, conventional sludge and CAGs to EBT wastewater with different concentrations; (b) COD removals of BAGs, conventional sludge and CAGs to 400 mg/l of EBT wastewater.

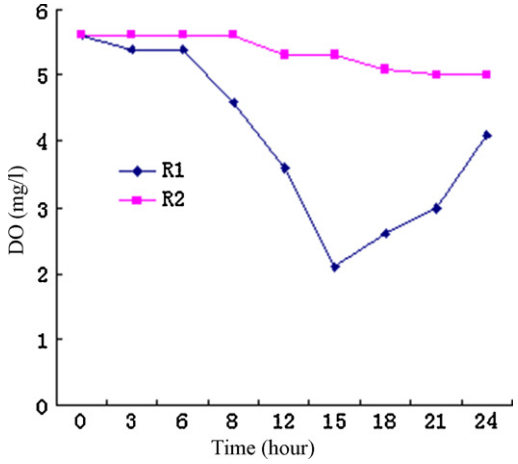

Fig. 7. The changes of DO concentration in R1 and R2 in the decolourization process of 400 mg/l of EBT wastewater.

(i) presents primary matrixes and quickens sludge granulation, so that the sludge has stronger microbial structure to withstand the impact of high-strength EBT wastewater; (ii) improves the biodegradation capability of reactor to EBT. In R1, MnP is detected although enzyme activities, 270–450 U/l, are not high; however, this enzyme can hardly be detected in R2. Strain HSD is a higher MnP producer, and the difference between R1 and R2 is that MMPs are seeded to the former, thus we can affirm that MnP in R1 is secreted by strain HSD. Up to now, it is clear that MnP can oxidize a variety of phenolic and non-phenolic substances including lignin and toxic pollutants [36]. In our previous work, we also proved that MnP played an important role in the reactions of cleavage of azo bond (–N=N–) or reduction of aromatic amines generated in the degradation process of EBT [19]. So, in R1, MnP should contribute to the decolourization of EBT wastewater. However, it is impossible that EBT is biodegraded by only MnP, many other enzymes secreted by the microbes in granules should be involved in this degradation process too, because many microorganisms can decolourize azo dyes efficiently such as *Pseudomonas luteola* [37], which has already been separated from the granules in our lab.

Moreover, the DO changes of in R1 and R2 revealed that BAGs could withstand the poison of EBT. In the decolourization process of 400 mg/l of EBT wastewater, DO concentration in R1 decreased to 2.1 mg/l at hour 15, and then the curve had an ascending trend (Fig. 7). As we know, the decrease of DO concentration means that the oxygen dissolved in wastewater is used by microbes in the reactor due to their respiration, and the more reduction of DO, the better microbial activity. In R2, the reduction of DO was very little in the whole process, indicating that microbial activity in sludge was bad. It is because EBT is toxic to microbes and it can suppress the growth of most of the microbes in conventional sludge. This can also explain why the COD removal rate in R2 is so low. Glucose concentration is 1.0 g/l in 400 mg/l EBT wastewater, and it means that more than 50% COD in wastewater is from glucose. However, COD removal of R2 is only $15.8 \pm 0.4\%$, suggesting that the microbes in sludge are so poisoned that cannot assimilate glucose. Why does not this phenomenon occur in R1? The reasons are (i) BAG possesses dense and complex space structure, and in this structure, there exist a large number of gaps; the microbes in the interior of BAG such as some strict aerobes and facultative aerobes can consume the oxygen through these gaps and avoid the direct contact with EBT molecular; (ii) the addition of strain HSD results in the appearance of MnP in R1, and MnP can cleave the azo bond (–N=N–) and reduce the aromatic amines generated in the degradation process of EBT, leading to the reduction of toxic substances.

In 2006, Ivanov et al. [38] and Wang et al. [11] reported that bioaugmentation could enhance the formation of microbial granules in aerobic wastewater treatment although different microbes were used in their tests. Subsequently, it was considered to be an effective method to cultivate aerobic granules. In this work, we proved that seeding MMPs to SBR was a specific method to cultivate aerobic granules. Compared with the previous methods such as seeding biogranules or activated carbon particles to the reactor [24–26], MMPs not only presented the primary matrixes to accelerate sludge granulation, but also improved the treatment capability of reactor to EBT wastewater by bioaugmentation. More importantly, MnP appeared in the reactor with the addition of MMPs, and since MnP could degrade EBT and lead to the decolourization of wastewater and reduction of toxic substances, it played an important role in the treatment process of EBT wastewater. To date, there is little information available in literature about cultivating aerobic granules using MMPs; moreover, few researchers have focused on treating EBT wastewater by BAGs. Thus, this study may be helpful to the culture and application of aerobic granules in wastewater treatment.

4. Conclusions

In view of the results obtained, it can be concluded that:

(1) Mn^{2+} has a significant influence on the morphologies of hypha and mycelium of strain HSD and under higher Mn^{2+} concentrations, strain HSD can form small MMPs.
(2) Seeding MMPs to SBR is a novel method to cultivate aerobic granules. MMPs can accelerate sludge granulation. In the bioreactor without MMPs, it is very difficult to cultivate aerobic granules using EBT wastewater. Furthermore, MMPs result in the formation of aerobic granules containing MMPs as nuclei and also induce the formation of self-immobilized biogranules which do not have the MMP at their core.
(3) Decolourization of EBT wastewater using BAGs is a feasible and promising way. BAGs not only can tolerate higher EBT loads, but also have better decolourization efficiency compared with conventional sludge and CAGs. Moreover, MnP appears in bioreactor after the introduction of MMPs and it plays an important role in the treatment process of EBT wastewater.

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