Journal of Hazardous Materials 172 (2009) 172–179

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Performance and dye-degrading bacteria isolation of a hybrid membrane process

Sheng-Jie You [a,*], Jun-Yu Teng [b,1]

[a] Department of Bioenvironmental Engineering and R&D Center for Membrane Technology, Chung Yuan Christian University, No. 200, Rd. Chung-Pei, Chungli 320, Taiwan
[b] Department of Civil Engineering, Chung Yuan Christian University, Chungli 320, Taiwan

ARTICLE INFO

Article history:
Received 4 February 2009
Received in revised form 25 June 2009
Accepted 26 June 2009
Available online 4 July 2009

Keywords:
Azo dye
Membrane bioreactor
Reactive Black 5
Lactococcus lactis
Wastewater reuse

ABSTRACT

Textile dyeing wastewater contains harmful compounds, which are toxic to both marine organisms and human beings if it discharged into an aquatic environmental without suitable treatment. In this study, the wastewater containing the azo dye, Reactive Black 5 (RB5), was partially treated in an anaerobic sequencing batch reactor which was further treated either in an aerobic membrane bioreactors (AOMBR) or in combined aerobic membrane bioreactor/reverse osmosis (AOMBR/RO) process. The results showed that in the anaerobic sequencing batch reactor the RB5 dye was degraded to form aromatic amine intermediate metabolites, which were further mineralized in the AOMBR. It was also observed that although all effluents from the AOMBR and AOMBR/RO processes met the Taiwan EPA's effluent criteria, irrespective of which membranes were used in the aerobic tank, the effluent from the AOMBR/RO process met the criteria for reuse for toilet flushing, landscaping, irrigation, and cooling water purposes, where as the AOMBR effluent only met the criteria for cooling water due to incomplete color removal. Five anaerobic high dye-degrading bacteria were isolated, which were identified to be the same species of *Lactococcus lactis* by 16S rRNA sequencing. The *L. lactis* showed complete degradation of RB5 and further studies showed that it can also able to degrade Reactive Red 120 and Reactive Yellow 84 efficiently within 6 h.

1. Introduction

Among dyestuffs, reactive, acid, and direct azo dyes, are more commonly used in the textile industry [1]. Reactive azo dyes, containing one to four azo bonds (N=N), are usually used to dye cellulose fibers, wool and nylon, which generally get adsorbed by forming covalent bonds with the fibers [2]. Reactive dyes are very soluble in water, therefore, are poorly adsorbed. It has been reported that approximately 10–50% of reactive dye lost during the dyeing and polishing processes [1,3]. The release of colored wastewaters represents not only a serious environmental problem, but also causes a public health concern [4]. Several wastewater treatment processes, such as chemical oxidation/coagulation, advanced oxidation, photocatalysis, adsorption and biodegradation, can be used to treat the dye containing effluent [5–7]. Among these processes, the conventional aerobic activated sludge biodegradation process (ASP), is used worldwide and considered to be the cheapest treatment process. However, it has been observed that the aerobic biodegradation is not really economical, due to low removal efficiency for reactive and other anionic soluble dyes. Also, due to low biodegradability under aerobic condition requires a large reactor volume [2,8]. On the contrary, anaerobic activated sludge can degrade dye more rapidly but produce toxic aromatic amines intermediate. These amines are generally not further degraded and will accumulate under anaerobic conditions. Mineralization of aromatic amines is more common by aerobic bacteria and therefore, a two-stage biological process, anaerobic followed by aerobic reactors, is suggested to be used for treatment of wastewater containing reactive dye [6,9].

In the two-stage biological process, first the reactive dye is partially degraded by the anaerobic activated sludge process which result in reduction of the color due to a reductive cleavage of azo bond and production of colorless aromatic amines. However, since these aromatic amines are more toxic than the dye itself, they are further degraded aerobically in the second stage [10]. The literature survey on biodegradation of the dye wastewater showed that many different type of anaerobic reactor configurations like sequential batch reactor (SBR) [11], fluidized beds [12], upflow anaerobic sludge blanket reactor (UASB) [13], rotating biological contactor (RBC) [14], and fixed/packed bed reactor [15] have been used for treatment of synthetic dyestuffs and high decolorization and degradation efficiencies were obtained. It was also concluded that the use of two-stage anaerobic–aerobic conditions could allow complete mineralisation of these xenobiotic azo-compounds.

The SBRs are sequenced into fill–react–settle–decant phases, and offer promising possibilities for estimation of parameters, as

* Corresponding author. Tel.: +886 3 2654911; fax: +886 3 2654911.
E-mail addresses: sjyou@cycu.edu.tw (S.-J. You), nickprometheus@yahoo.com.tw (J.-Y. Teng).
[1] Tel.: +886 3 2654911; fax: +886 3 2654911.

0304-3894/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2009.06.149

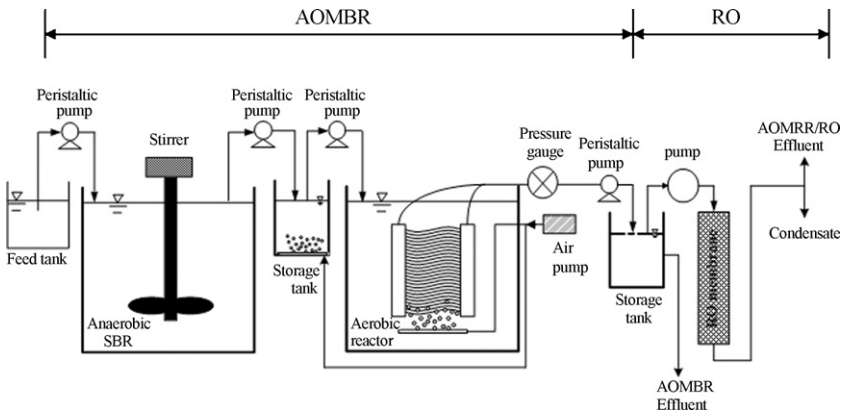

Fig. 1. Schematic diagram of the AOMBR/RO processes.

they are by nature, dynamic in behavior, and allow repeatable behavior to establish initial conditions, and evaluate parameters [16]. The SBR technology has been used for nitrogen and phosphorus removal and other wastewater treatment due to its good operational flexibility, simple running and compact layout [17].

After this two-stage biological treatment, the wastewater usually meets the effluent criteria, but not the reuse criteria. Thus, extra-treatment processes, such as coagulation, advanced oxidization and membrane processes, are always added to meet wastewater reused criteria [18–20]. The additional membrane process, either microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) or reverse osmosis (RO), is needed to reclaim dye containing wastewater. Recently, many full-scale commercial membrane bioreactors (MBRs) plants have begun to operate for domestic wastewater treatment and are replacing the conventional (activated sludge + settling) treatment process. MBRs incorporate MF or UF-membranes which are immersed into the conventional activated sludge process. Such MBR combined with RO processes are now started operating worldwide for domestic and industrial wastewater treatment and reclamation. In the combined MBR/RO process, the MBR unit removes biodegradable organic matter, while the RO unit removes non-biodegradable organic or inorganic matter. The MBR/RO process showing a growing interest and rapid development because of their great potentiality in terms of water disinfection, possibility to reuse water and also offers other benefits of short hydraulic retention time (HRT). In addition, since the MBR process the membrane replaces the sedimentation tank of the traditional ASP. Thus, no sludge is wasted and all the suspended solids, including microorganisms, are retained in the reactor. Therefore the sludge retention time (SRT) can be increased further in order to increase the biomass concentration and the high loading rate capability [21]. It is also possible that some specific bacteria, including those which can degrade difficult degrading textile dyeing wastewater, are enriched in the MBR process to enhance the biodegradation efficiency [22–24].

Textiles contributed 16.4 billion US dollars to Taiwan's economic output in 2004, but large quantities of toxic, low biodegradable, heavily colored wastewater were produced, which have caused an adverse effect on the natural water environment in Taiwan. In the present study the pilot scale sequenced anaerobic (ANSBR) and aerobic MBR (AOMBR) systems were used for biodegradation wastewater containing Reactive black 5 dye.

In the present study, we have developed a two-stage biological process i.e., an anaerobic sequencing batch reactor (ANSBR) followed by an aerobic membrane bioreactor (AOMBR) to meet the standard Taiwan EPA effluent discharge criteria. The biodegradation and color removal performance of this combined system was studied using simulated textile effluents containing a reactive black 5 (RB5) azo dye. The effluents of the AOMBR were further treated by RO unit to achieve Taiwan standard wastewater reused criteria. In addition, the high anaerobic color degrading bacteria were also isolated and screened in this study.

2. Materials and methods

2.1. Process configuration

Schematic diagrams of the AOMBR and AOMBR/RO processes are illustrated in Fig. 1. The AOMBR process combines a 36 l anaerobic SBR and a 18 l aerobic MBR. The ANSBR is sequenced into fill 0.5 h–react 21.5 h–settle 0.5 h–decant 1.5 h phases, which are decided based on pretest study. No activated sludge was discarded as waste during the operation period. The HRT, MLSS, ORP, and pH of the anaerobic SBR were 48 h, 2700 mg/l, −300 mv, and 6.8–7.2, respectively. The effluent of ANSBR were stored in an aerobic storage tank and further flowed into aerobic MBR. Four different commercial membranes, namely "M", "N", "K" and "C" (produced b y the R&D Center for Membrane Technology of Chung Yuan Christian University, Taiwan) were installed in the aerobic MBR unit simultaneously, which were showed in Table 1.

The effluent from the AOMBR process was drained out by using a peristaltic pump, which is operated in cyclic mode of 10 min filtration followed 5 min relaxation. The HRT, MLSS, ORP, DO and pH in the aerobic MBR unit were 24 h, 2100 mg/l, higher than 250 mv, higher than 3.0 mg/l and 6.8–7.2, respectively. The specific aeration intensity was maintained at 20 $m^3\ m^{-2}\ h^{-1}$. Finally, in order to meet the different targets for wastewater reuse criteria of Taiwan EPA, we added a commercial RO unit (TFC material, Dow Co. Ltd.) to the back of the AOMBR process, thus forming the AOMBR/RO process, as shown in Fig. 1. The RO unit consists of a semi-permeable dissymmetrical membrane composed of selective skin layers which are supported by porous sub-layers. The RO was operated at 15 kg/cm pressure, 50–150 l/h flow and water recovery ratio of 50%.

The experiments were performed from June 5, 2005 to June 15, 2007, in which the first 80 days was utilized for culturing period. The data in Section 3 are averaged from 25 August, 2005 to June 15, 2007. Both the AOMBR and AOMBR/RO processes operated at 20 °C

Table 1
The membranes used in this study.

Membrane	Type[a]	Material	Pore size (μm)	Area (m^2/set)
M	H	Polyethylene	0.4	0.200
N	F	Non-woven	30	0.020
K	F	Chlorinated polyethylene	0.4	0.125
C	F	Polytetrafluoroethylene	0.22	0.020

[a] H: hollow fiber, F: flat sheet.

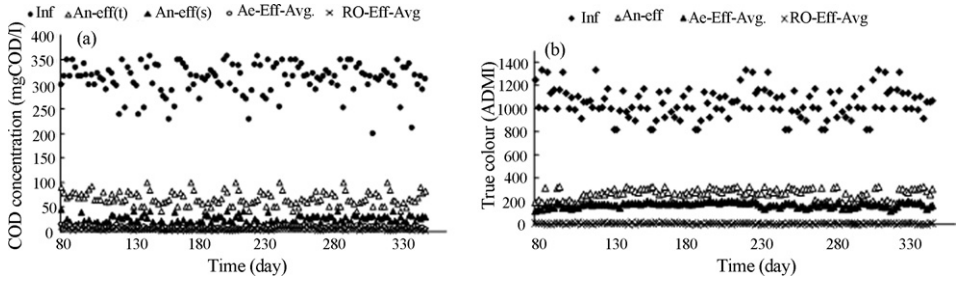

Fig. 2. (a) COD and (b) true color removal performance for each unit in the AOMBR/RO process.

laboratory. The seed sludge was taken from the Kuagnin industrial park wastewater treatment plant, which treats industrial wastewater effluent consisting of 38.2% of highly colored textile dyeing wastewater.

2.2. Wastewater and analytical methods

The substrate used for the reactor cultivation contained (per liter): milk powder, 177.1 mg; sucrose, 17.7 mg; acetate, 32.6 mg; $(NH_4)_2SO_4$, 12.5 mg; urea, 39.1 mg; $FeCl_3$, 0.13 mg, KH_2PO_4, 17.7 mg, and Reactive Black 5, 6.25 mg. Given these constituents, the concentrations of COD, BOD, SS, and ADMI (American Dye Manufacturers Institute, the unit of true color) were 300 mg COD l^{-1}, 200 mg BOD l^{-1}, 200 mg SS l^{-1}, and 1000 ADMI, respectively, simulating concentrations in influent wastewater from the Kuagnin industrial park wastewater treatment plant. The 6.25 mg l^{-1} Reactive Black 5 was corresponding to 4.5 mg l^{-1} COD, revealed that the contribution of dye to COD was very low. All the processes were monitored 2–3 times per week. All the samples taken from the reactors were first analyzed for suspended solid (SS), turbidity (NTU) and total coliform (CFU). Then the samples were filtered rapidly using Whatman GF/A filter paper and the filtrate were analyzed to find their true color, COD, BOD, total hardness, total alkalinity, chloride, total dissolved solids, according to the Standard Methods [25]. The UV–vis spectra of the effluents of each unit were also performed by spectrophotometer.

2.3. Anaerobic color degradation and bacteria isolation

Sludge taken from the ANSBR unit was cultured and isolated in a TGC agar medium (thioglycollate medium, Difco) at 37 °C for 48 h in an anaerobic oven. Sixty separate colonies on the medium were transferred to an individual TGC broth medium for culturing at 37 °C for 48 h in an anaerobic oven. The isolates were further tested after 36 h to determine the degradation rate of the anaerobic color in the TGC broth containing 50 mg/l of RB5. The DNA of the five high anaerobic color degrading bacteria was further extracted, sequenced and compared with the NCBI database. One isolate was tested further to determine the anaerobic color degradation rate for TGC broth that contained 50 mg/l of either Reactive Red 120 (RR120) or Reactive Yellow 84 (RY84).

3. Results and discussion

3.1. Overall process performance

Fig. 2(a) and Table 2 show the average COD, BOD, true color (ADMI), SS concentration and corresponding removal efficiency of influent and the effluent from the ANSBR, aerobic MBR and RO process observed during 1 year of operation. It can be observed that the average COD of the ANSBR, aerobic MBR, and RO effluents were 67.7 ± 13.9, 7.9 ± 2.3, and 3.1 ± 1.9 mg l^{-1}, respectively, which corresponding to the total COD removal efficiencies of 78.2, 97.5, and 99.0%, respectively. Table 2 also shows that the average BOD of the ANSBR, aerobic MBR, and RO effluents were 46.0 ± 8.4, 2.9 ± 1.0 and 1.1 ± 0.7 mg BOD l^{-1}, respectively, which corresponding to the removal efficiencies of 78.5, 98.6, and 99.5%, respectively. It was observed that nearly 74–78% of total COD, BOD, true color and SS removal efficiency were observed in an ANSBR alone, where as remaining 17–20% and 2–3% of total were removed in subsequent aerobic MBR and RO process, respectively. The high COD and true color removal efficiency in ANSBR as compared to aerobic MBR proves its excellent ability for treatment of reactive dyes. The measured soluble COD and SS of the anaerobic effluent were 23.8 mg COD l^{-1}, and 42.3 mg SS l^{-1}, respectively, implying that nearly 69.6% of the anaerobic effluent COD was contributed by particulate matter, mostly due to unsettled biomass. Thus, the actual COD removal efficiency of the ANSBR was 92.3%. Where as the total and soluble COD removal efficiency of the aerobic MBR unit increased only 19.3 and 5.2%, respectively.

In addition, Fig. 2(b) and Table 2 show the average true color of the effluent after the AOMBR/RO process. The average ADMI of the

Table 2
Effluents and removal efficiencies from each unit in the AOMBR/RO process.

	COD[a]	$BOD_{5,20°C}$	True color	SS
Effluent (mg/l or ADMI)				
Taiwan effluent criteria	<100	<30	<550	<30
Influent	310.6 ± 31.7	213.6 ± 22.8	1039.1 ± 153.4	149.4 ± 34.8
Anaerobic SBR effluent	67.7 ± 13.9	46 ± 8.4	264.2 ± 49.9	42.3 ± 12.6
Aerobic MF effluent[c]	7.9 ± 2.3	2.9 ± 1.0	169.0 ± 22.4	ND[b]
RO effluent[c]	3.1 ± 1.9	1.1 ± 0.7	8.0 ± 3.9	ND
Removal efficiency (%)				
Anaerobic SBR effluent	78.2	78.5	74.6	71.7
Aerobic MF effluent[c]	97.5	98.6	83.7	>99.99
RO effluent[c]	99.0	99.5	99.2	>99.99

[a] The COD value here was total COD but not soluble COD.
[b] Not detected.
[c] Averaged from the effluents of four different MF membranes.

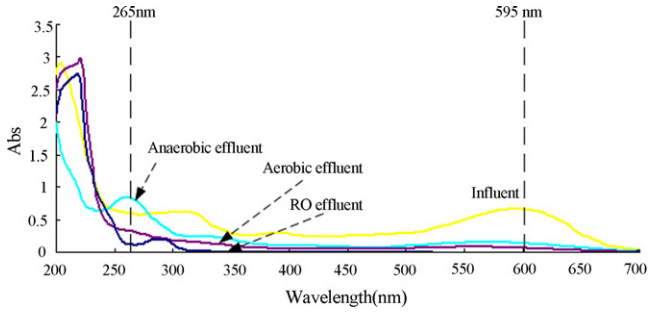

Fig. 3. Total removal performance of each membrane in the AOMBR/RO process.

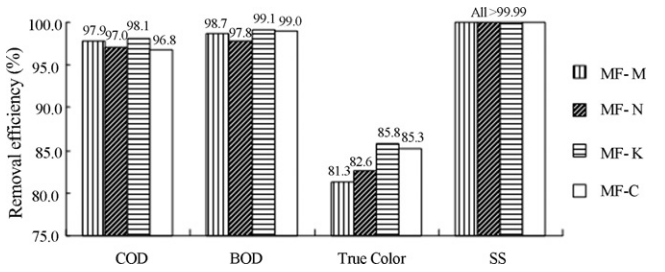

Fig. 4. Overall removal performance of membrane in the AOMBR process.

anaerobic SBR, aerobic MBR, and RO effluents were 264.2 ± 49.9, 169.0 ± 22.4 and 8.0 ± 3.9 ADMI, respectively, which corresponding to the removal efficiencies of 74.6, 83.7, and 99.2%, respectively. Finally, Table 2 also shows that the average SS of the effluent from the anaerobic SBR was 42.3 ± 12.6, while no SS was detected in the effluent from the aerobic MBR or RO units. This revealed that the SS removal efficiencies of the anaerobic SBR, aerobic MBR and RO units were 71.7, >99.99, and >99.99%, respectively.

Although removal efficiency of aerobic MBR was not high, the aerobic MBR must be used because two major mechanisms were takes place in the aerobic MBR unit: the mineralization of aromatic amines into non-toxic metabolites [11] and the retaining of particulate matter in the reactor. The degradation of aromatic amines into other non-toxic metabolites has already been discussed in several references [10,26]. Delée et al. [27] stated that 62–95% and 60% of the color and COD removal can be achieved by anaerobic treatment, while an additional 30% of the COD, which are mostly contributed by aromatic amines, can be removed by the subsequent aerobic treatment. In this study, the color removal efficiency for ANSBR was 74.6%, which was closed to that observed in Delée's study [27]. However, 92.3% of the COD degradation occurred during the ANSBR, while only 5.2% of the COD degradation occurred during the subsequent aerobic MBR. This value was much higher than that observed in Delée et al.'s study [27]. This might be due to the presence of untreated dye and other organic compounds in the effluent.

Fig. 3 shows the UV–vis absorbance spectrum of the influent, ANSBR effluent, aerobic MBR effluent and RO effluent. The influent shows the maximum absorbance at 595 nm (A_{595}) which corresponds to chromophoric azo group of RB5 dye which is responsible for color. It was also observed that the absorbance at 595 nm of the influent, ANSBR effluent, aerobic MBR effluent and RO effluent were 0.673, 0.158, 0.084, and 0.007, respectively. Thus individual color removal efficiencies of the ANSBR, aerobic MBR and RO units were 76.5, 11.0, and 11.5%, respectively. This indicates that the ANSBR removed most of the RB5. In addition, the maximum absorbance was shifted from 595 nm to 265 nm (A_{265}) after anaerobic treatment, which indicates the formation of a predominant intermediate metabolite under anaerobic degradation. Mohanty et al. [26] also observed such similar phenomenon. In their study, the maximum absorbance was shifted from 597 nm to 267 nm after anaerobic treatment of Reactive Black 5 containing wastewater. They also analyzed the anaerobic sample by HPLC and found that the peak at 267 nm in UV–vis spectra was corresponds to amine compound. The A_{265} of the ANSBR effluent was 0.850 and it was further reduced to 0.321 in the aerobic MBR effluent and to 0.129 in the RO effluent. This shows that only 62.2% of intermediate metabolite degradation occurred in the aerobic reactor and there were still some of toxic intermediate metabolite remains in the effluent of aerobic MBR. The HRT of aerobic MBR unit was set for 24 h. Thus the aerobic COD degradation rate was estimated to 0.66 mg COD/l h, which was much lower than the anaerobic COD degrading rate of 5.96 mg COD/l h. Isik and Sponza [28] have studied the aromatic amine degradation in a UASB/CSTR sequential system treating Congo Red dye. In their continuous operations in the anaerobic/aerobic stage, they observed nearly 88% of COD, 99% of color and 91% of total aromatic amine (TAA) removal at a HRT of 3.60 days and at a CR dye concentration of 4000 mg/l. Isik and Sponza [29] have studied anaerobic UASB/aerobic treatment of a simulated textile wastewater. In this study they have reported the effect of HRT (0.96–15 days) of aerobic CSTR reactor on COD and amine removal. For maximum COD removal, the optimum HRT was found to be 2.2 days. At optimum HRT the better utilization of COD at high level and aromatic amines are readily degraded/mineralized by the aerobic microorganisms. Therefore although in present study the aerobic degrading rate was quite low, it will be possible to achieve complete degradation of these intermediate metabolite by increasing the aerobic HRT to optimum level.

3.2. Performance and reuse potential of the different membranes

3.2.1. Performance of different membranes in the AOMBR and AOMBR/RO processes

The performances of the four different membranes installed in the aerobic MBR were compared in Fig. 4. It was observed that the overall removal efficiencies of COD, BOD, true color (ADMI) and SS were 96.8–98.1%, 97.8–99.1%, 81.3–85.8%, and almost 100%, respectively. This indicates that although the pore size, material and frame type of the membranes were quite different, the removal efficiencies were similar. The formation of cake layer on the membrane surface begins immediately after the start of filtration. Thus pore size of this cake layer becomes much smaller than the pore size of the membrane [21,30]. Thus, although the pore size of the membranes was varied from 0.22 μm to 30 μm, the removal performances were quite similar, possibly due to the smaller pore size of the formed cake layer. Many researchers have studied the performance of coarse size membrane sometimes referred as dynamic membrane where the deposition of porous sludge layer over the membrane acts as filter which performs similar to the fine size membrane depending on the porosity of sludge layer formed and also on size and concentration of sludge [31]. Liu et al. [32] have studied the performance of dynamic membrane bioreactor for municipal sewage treatment using silk membrane (0.1 mm) showed that nearly 78% COD removal and 91.3% turbidity removal could be achieved and the perfect separation ability of membrane can be obtained within 30 min at low MLSS (3 g/l) and within 5 h at high MLSS (7.5 g/l). Meng et al. [31], have compared the performance nonwoven (3 and 5 μm) with hollow fiber (0.4 μm) membrane for treatment of synthetic domestic wastewater showed that there was very little difference in the effluent water quality between the nonwoven bioreactor and the membrane bioreactor. Nearly 97–98% of COD and TOC removal also about 95–96% BOD removal were achieved in both the reactors therefore the pore size of nonwovens had little effect on the organic carbon reduction, probably due to the formation of a dynamic layer on the nonwoven membrane surface.

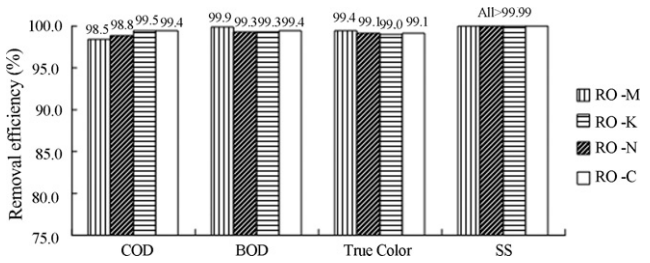

Fig. 5. Overall removal performance of membranes in the AOMBR/RO process.

The removal performance of RO effluent corresponding to each AOMBR membrane effluent are shown in Fig. 5. The overall removal efficiencies for COD, BOD, true color (ADMI), and SS were 98.5–99.5%, 99.3–99.9%, 99.0–99.4%, and higher than 99.99%, respectively.

3.2.2. Comparison against Taiwan EPA effluent criteria

The comparison of the effluents produced by the AOMBR and AOMBR/RO processes with the Taiwan effluent criteria is shown in Table 3. It was observed that both processes satisfy the Taiwan EPA criteria for effluent. It was also observed that there was no significant difference for different membranes, although the pore size of membrane-N was much larger (30 μm) than that of the other three membranes (0.22–0.4 μm). Furthermore, the removal efficiency of the RO unit for COD, BOD, and SS did not show a significant increase; because of this the concentrations of these constituents in the AOMBR effluents were quite low. The RO units could significantly enhance the removal efficiency of true color. The average ADMI of the AOMBR effluent was between 147.3 and 197.4 ADMI and it was decreased to 0.2–1.5 ADMI after treatment by the RO unit, giving an average ADMI removal efficiency of RO unit as 99.75%. Thus, in order to meet just Taiwan EPA effluent criteria it does not require the addition of the RO.

3.2.3. Comparison with the Taiwan EPA wastewater reuse criteria

Table 4 shows a comparison of the AOMBR and AOMBR/RO effluents with the Taiwan EPA's wastewater reuse criteria for toilet flushing, landscaping and irrigation, and cooling water. It was observed that the effluent produced by the AOMBR processes does not meet the color and *E. coli* standard reuse criteria for toilet flushing, landscaping or irrigation purposes but it mates reuse criteria for cooling water. In addition, no *E. coli* was detected in membrane MF-C in the aerobic MBR units, while 2–21 CFU/100 ml were detected in the other three membranes. For the treated wastewater to be reused for toilet flushing, landscaping and irrigation, the combined available chlorine should be higher than 0.4 mg/l according to Taiwan EPA criteria. The goal of 0.4 mg/l for combined available chlorine can be achieved by adding suitable quantity of chlorine and ammonia compounds. Therefore, the effluent samples were further treated with 3 mg/l of NaOCl, as showed in Table 4. It was observed that the addition of chlorine not only increased the combined chlorine concentration >0.4 mg/l (data not shown here), but also lowered the number of *E. coli* to the non-detectable (ND) level. However, even after the suitable addition of chlorine, the effluent from the AOMBR still did not meet the color standards necessary for toilet flushing, landscaping and irrigation purposes. In addition, it was observed that the effluent from the AOMBR/RO processes satisfies all the Taiwan EPA wastewater reuse criteria. According to the above results, we can suggest appropriate treated wastewater reuse targets for both the AOMBR and AOMBR/RO processes. Wastewater treated by the former process can only be reused as cooling water, while wastewater treated by the AOMBR/RO process can be reused for toilet flushing, landscaping, irrigation and cooling water.

Table 3
Comparison of AOMBR and AOMBR/RO effluents to the Taiwan EPA effluent criteria.

	COD (mg/l)	$BOD_{5,20\,^{\circ}C}$ (mg/l)	True color (ADMI)	SS (mg/l)
Taiwan EPA criteria	100	30	550	30
AOMBR				
MF-M	6.6 ± 2.6	2.7 ± 0.7	194.7 ± 15.9	ND
MF-N	9.2 ± 4.9	4.8 ± 0.4	181.0 ± 4.8	ND
MF-K	5.9 ± 1.2	2.0 ± 0.7	147.3 ± 4.8	ND
MF-C	9.8 ± 1.2	2.1 ± 0.1	153.1 ± 6.3	ND
AOMBR/RO				
RO-M	4.8 ± 4.4	6.4 ± 3.2	0.2 ± 0.1	ND
RO-N	3.7 ± 2.4	9.4 ± 4.7	1.5 ± 0.4	ND
RO-K	1.7 ± 1.0	10.4 ± 4.8	1.5 ± 0.7	ND
RO-C	2.0 ± 1.9	8.9 ± 3.8	1.3 ± 0.2	ND

3.3. Isolation of the bacteria responsible for high degradation of Reactive Black 5 (RB5)

In this study we also isolated higher decolorization performance bacteria by using a TGC medium. After screening, the dyeing-degradation batch experiments results showed that only five high color degrading isolates, AN-13, AN-20, AN-23, AN-27 and AN-51, were responsible for more than 99% of the RB5 degradation within 6 h of experimental time. In addition, 20 medium color degrading and 25 low color degrading isolates identified which can degrade more than 99% and 70% of RB5 within 36 h, respectively, while 10 isolates showed no RB5 degradation performance. Fig. 6 shows the dye degradation profile and cell growth curves of the five high RB5 degrading bacteria. It was observed that the average dye-degrading rate during the first 2.5 h was higher (14.55 mg dye/l h) than the rate in the following 3 h (3.21 mg dye/l h). On average, more than 70% of the dye was degraded during the first 2.5 h and over 95% during the first 4.5 h. On the other hand, no cell growth was observed in the first 2 h. The average cell growth rate was 0.508 OD_{600}/h from 2 to 3.5 h and 0.042 OD_{600}/h in the following 2 h. The average specific dye degradation rate for the first 2 h was estimated to be 553.35 mg dye/OD_{600} l h, while the rate for the following 3.5 h was 6.68 mg dye/OD_{600} l h. This significant difference may be due to no cell growth was observed in the first 2 h. This suggests that the efficient RB5 degrading bacteria could cleave the azo bond with a very short lag phase. In this present study it was observed for high RB5 dye-degrading bacteria isolate had very short lag phase of about 2 h. In literature most of the studies have not reported the cell growth profile for such a short initial period and few studies [33–34] have reported that decolorization occurs during logarithmic growth phase and COD/BOD reduction during the maximum stationary growth phase. It was also observed that the average pH of the medium was decreased from 7.0 to 4.25 during 5.5 h experimental period; this indicates that these five isolates may produces some acidic intermediate compounds during dye/medium degradation.

The five high dye-degrading bacteria were further sequenced using the 16S rRNA gene and the results were further compared with the NCBI database. All the high dye-degrading bacteria belonged to *Lactococcus lactis*, as shown in Table 5. Four sub-species of *L. lactis*, i.e., *L. lactis subsp. Cremoris*, *L. lactis subsp. lactis*, *L. lactis subsp. Hordniae*, and *L. lactis subsp. diacetylactis*, were reported [35]. In this study, AN-13, AN-20, AN-23 and AN-51 were identified to be sub-species of *L. lactis subsp. lactis*. The *L. lactis subsp. lactis* produces lactic acid by fermentation of lactose which leads to the decrease in pH. It was also reported that the cell growth of *L. lactis* will not occur at pH below 4.4 [36]. This similar phenomenon was also observed in the dye-degrading bacteria isolation study, where none of these five high dye-degrading bacteria grew after 3.5 h when the pH decreased to 4.25 in average (as showed in Fig. 6). A comparison of the 16S rRNA sequences of these five isolates

Table 4
Comparison of AOMBR and AOMBR/RO effluents to the Taiwan EPA wastewater reuse criteria.

	1[a] *E. coli* (CFU/100 ml)	2 $BOD_{5,20\,°C}$ (mg/l)	3 Color	4 Odor	5 Turbidity (NTU)	6 Total hardness (mg/l as $CaCO_3$)	7 Total alkalinity (mg/l as $CaCO_3$)	8 Chloride (mg/l)	9 TDS[b] (mg/l)	10 SS[b] (mg/l)
Criteria	ND	<10	NU[c]	NU[c]	<5	<450	<350	<500	<1000	<10
AOMBR										
MF-M	21/ND[d]	2.7	Colored	NU	0.39	101.6	25.7	6.1	415.5	ND
MF-N	2/ND[d]	4.8	Colored	NU	0.47	79.1	23.3	6.1	415.5	ND
MF-K	5/ND[d]	2.0	Colored	NU	0.16	55.6	39.6	5.8	342.6	ND
MF-C	ND/ND[d]	2.1	Colored	NU	0.18	58.0	37.6	9.2	318.3	ND
AOMBR/RO										
RO-M	ND	0.2	NU	NU	0.24	6.4	5.3	1.1	43.0	ND
RO-N	ND	1.5	NU	NU	0.13	6.5	5.7	ND	25.0	ND
RO-K	ND	1.5	NU	NU	0.10	3.2	7.3	ND	24.0	ND
RO-C	ND	1.3	NU	NU	0.08	4.8	6.5	2.2	24.7	ND

[a] Treated water reused for toilet flushing: criteria 1, 2, 3 and 4.
Treated water reused for landscaping and irrigation: criteria 1, 2, 3, 4 and 5.
Treated water used for cooling water: criteria 2, 6, 7, 8, 9 and 10.
[b] TDS: total dissolved solids; SS: suspended solids.
[c] NU: not uncomfortable when it was used.
[d] *E. coli* number after the addition of 3 mg/l of NaOCl.

showed 96–98% of similarities of between them, therefore they can be considered to be the same species. To the best of our knowledge there has been only one study focused on dye degradation by *L. lactis* [37] where it was found that the dye reduction of *L. lactis* was stimulated by sugar rather than by organic acids. In addition, Seesuriyachan et al. [38] found that other lacto bacteria, i.e. *Lactobacillus casei*, had azo dye-degrading ability. However, in other studies, *Bacillus subtilis*, *Pseudomonas* spp., *Geobacillus strarothermophilus*, *Rhodobacter sphearoides* and *Enterococcus faecalis* showed high azo dye reduction ability, which was not the case in our study [39].

In this study the five high RB5 dye-degrading bacteria were isolated. In order to see their ability to degrade other reactive azo dyes, further studies were carried out to test the degradation ability of the AN-13 isolates for other azo dyes, i.e., Reactive Red 120 (RR120) and Reactive Yellow 84 (RY84). Fig. 7 shows the degradation profile of RB5, RR120 and RY84 dye. It was observed that not only RB5, but also RR120 and RY84 could be degraded completely within 6 h, which was similar to the dye-degradation experiments in Fig. 6. This indicats that *L. lactis* had the ability to degrade different azo dyes with a high degradation rate.

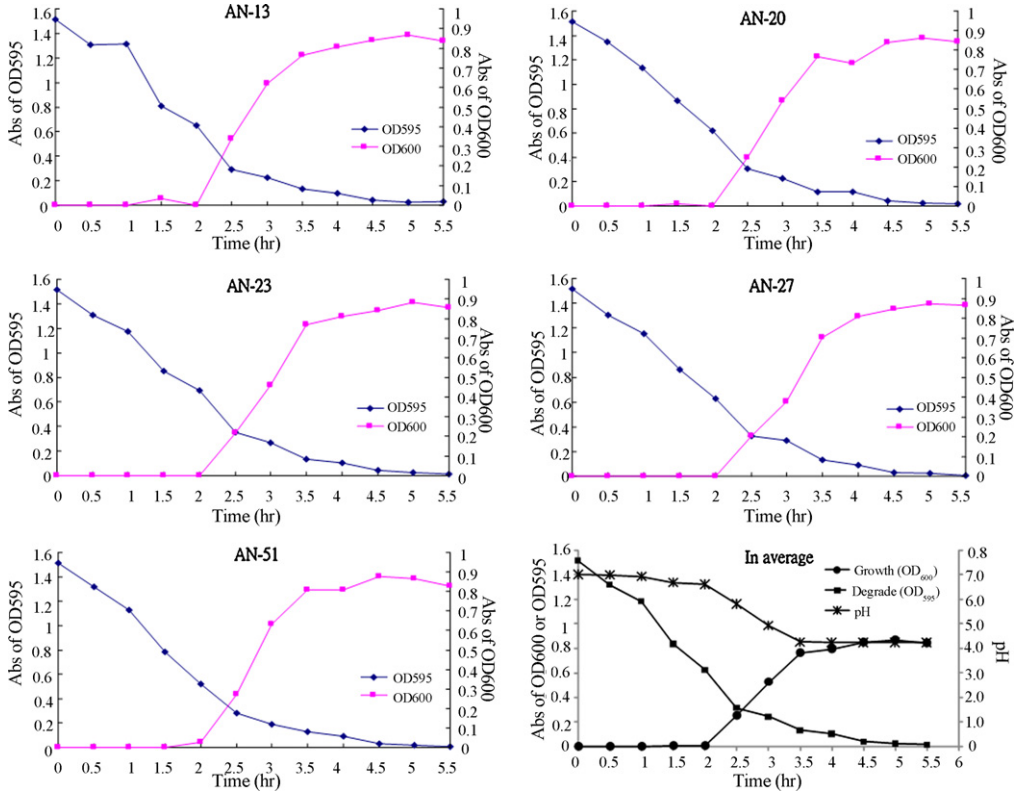

Fig. 6. Illustration of color removal performance by the five high degradation performance isolates.

Table 5
Phylogenetic relationship between the five high dye-degrading isolates.

Isolates no.	Phylogenetic relationship			
	Species	Source in NCBI	% of similarity	Match bps.
AN-13	*Lactococcus lactis subsp. lactis*	DQ171718.1	97%	1149/1181
AN-20	*Lactococcus lactis subsp. lactis*	AB285124.1	81%	334/409
AN-23	*Lactococcus lactis subsp. lactis*	AB285124.1	97%	1154/1184
AN-27	*Lactococcus lactis*	AY675242.1	98%	1179/1201
AN-51	*Lactococcus lactis subsp. lactis*	DQ171718.1	97%	1156/1190

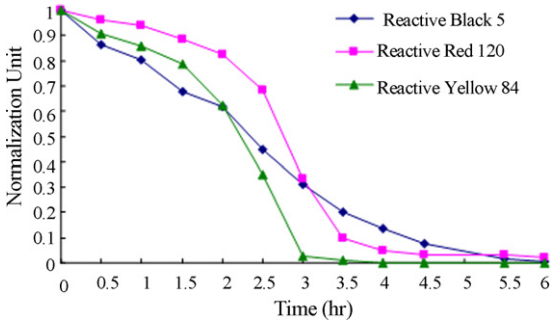

Fig. 7. Illustration of color removal performance of three different azo dyes by the AN-13 isolate.

4. Conclusion

In this study, the performance of the ANSBR, AOMBR and AOMBR/RO processes for RB5 degradation were observed. The effluents produced from these processes were compared with the effluent and treated wastewater reuse criteria of the Taiwan EPA. The following conclusions are made based on this experimental study.

1. The COD, BOD, true color (ADMI) and SS concentrations of the AOMBR effluents were 7.9, 2.9, 169 and 0 mg/l, while that of the AOMBR/RO effluent were 3.1, 1.1, 8.0 and 0 mg/l, respectively, therefore both the effluent fulfills the Taiwan EPA effluent criteria.
2. The AOMBR/RO process could treat wastewater up to level which satisfies all the Taiwan EPA wastewater reuse criteria for toilet flushing, landscaping, irrigation, and cooling purpose.
3. Five high dye-degrading bacteria were isolated, which can degrade almost 50 mg/l of Reactive Black 5 within 5.5 h, were isolated.
4. This study further isolated 20 medium dye-degrading bacteria and 25 low dye-degrading bacteria which could degrade more than 99 and 70% of Reactive Black 5 within 36 h, respectively, while 10 isolates showed no measurable Reactive Black 5 degradation performance.
5. The 16S rRNA of the above five high dye-degrading bacteria were further sequenced and found to be the same species, *L. lactis*. The rate of Reactive Black 5 degradation by *L. lactis* could be as high as 14.55 mg/l h for the first 2.5 h without a significant lag phase time.
6. The *L. lactis* can degrade not only Reactive Black 5 but also Reactive Yellow 84 and Reactive Red 120 with high efficiency.

Acknowledgements

This study was supported by a grant received from the National Science Council (No. NSC 96-2621-Z-033-002-MY3) and the Center-of-Excellence Program on Membrane Technology/Ministry of Education, Taiwan, Republic of China.

References

[1] K. Vijayaraghavan, Y.S. Yun, Utilization of fermentation waste (*Corynebacterium glutamicum*) for biosorption of Reactive Black 5 from aqueous solution, J. Hazard. Mater. 141 (2007) 45–52.
[2] U.K. Khare, P. Bose, P.S. Vankar, Impact of ozonation on subsequent treatment of azo dye solution, J. Chem. Technol. Biotechnol. 82 (2007) 1012–1022.
[3] C. O'Neill, F.R. Hawkes, D.L. Hawkes, N.D. Lourenço, H.M. Pinheiro, W. Delée, Colour in textile effluents-sources, measurement, discharge consents and simulation: a review, J. Chem. Technol. Biotechnol. 74 (1999) 1009–1018.
[4] A.B. dos Santos, F.J. Cervantes, J.B. van-Lier, Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology, Bioresour. Technol. 98 (2007) 2369–2385.
[5] S.H. Lin, F. Peng, Continuous treatment of textile wastewater by combined coagulation, electrochemical oxidation and activated sludge, Water Res. 30 (1996) 587–592.
[6] T.H. Kim, C.H. Park, E.B. Shin, S.Y. Kim, Decolorization of disperse and reactive dye solutions using ferric chloride, Desalination 161 (2004) 49–58.
[7] F.I. Hai, K. Yamamoto, K. Fukushi, Hybrid treatment systems for dye wastewater, Crit. Rev. Environ. Sci. Technol. 37 (2007) 315–377.
[8] P.C. Vandevivere, R. Bianchi, W. Verstraete, Treatment and reuse of wastewater from the textile wet-processing industry: review of emerging technologies, J. Chem. Technol. Biotechnol. 72 (1998) 289–302.
[9] D.T. Sponza, M. Isik, Reactor performances and fate of aromatic amines through decolorization of Direct Black 38 dye under anaerobic/aerobic sequentials, Process Biochem. 40 (2005) 35–44.
[10] J.A. Libra, M. Borchert, L. Vigelahn, T. Storm, Two stage biological treatment of a diazo reactive textile dye and the fate of the dye metabolites, Chemosphere 56 (2004) 167–180.
[11] C.B. Shawa, C.M. Carliellb, A.D. Wheatleya, Anaerobic/aerobic treatment of coloured textile effluents using sequencing batch reactors, Water Res. 36 (2002) 1993–2001.
[12] M. Haroun, A. Idris, Treatment of textile wastewater with an anaerobic fluidized bed reactor, Desalination 237 (2009) 357–366.
[13] M. Isik, D.T. Sponza, Monitoring of toxicity and intermediates of C.I. Direct Black 38 azo dye through decolorization in an anaerobic/aerobic sequential reactor system, J. Hazard. Mater. 114 (2004) 29–39.
[14] Y. Zaoyan, S. Ke, S. Guangliang, D. Jinshan, M. Huanian, Anaerobic–aerobic treatment of a dye wastewater by combination of RBC with activated sludge, Water Sci. Technol. 26 (1992) 2093–2096.
[15] I.K. Kapdan, M. Tekol, F. Sengul, Decolorization of simulated textile wastewater in an anaerobic–aerobic sequential treatment system, Process Biochem. 38 (2003) 1031–1037.
[16] D.J. Batstone, M.J. Torrijos, C. Ruiz, J.E. Schmidt, Use of an anaerobic sequencing batch reactor for parameter estimation in modeling of anaerobic digestion, Water Sci. Technol. 50 (2004) 295–303.
[17] N.D. Lourenc, J.M. Novais, H.M. Pinheiro, Effect of some operational parameters on textile dye biodegradation in a sequential batch reactor, J. Biotechnol. 89 (2001) 163–174.
[18] S. Ledakowicz, M. Solecka, R. Zylla, Biodegradation, decolourisation and detoxification of textile wastewater enhanced by advanced oxidation processes, J. Biotechnol. 89 (2001) 175–184.
[19] G.B. Tabrizi, M. Mehrvar, Integration of advanced oxidation technologies and biological processes: recent developments, trends, and advances, J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng. 39 (2004) 3029–3081.
[20] A.M. EI-Eein, J. Libra, U. Wiesmann, Cost analysis for the degradation of highly concentrated textile dye wastewater with chemical oxidation H_2O_2/UV and biological treatment, J. Chem. Technol. Biotechnol. 81 (2006) 1239–1245.
[21] T. Stephenson, S. Judo, B. Jefferson, K. Brindle, Membrane Bioreactors for Wastewater Treatment, IWA Publishing, London, 2000.
[22] C. Meehan, A.J. Bjourson, G. McMullan, *Paenibacillus azoreducens* sp. nov., a synthetic azo dye decolorizing bacterium from industrial wastewater, Int. J. Syst. Evol. Microbiol. 51 (2001) 1681–1685.
[23] H. Zhang, A. Kallimanis, A.I. Koukkou, C. Drainas, Isolation and characterization of novel bacteria degrading polycyclic aromatic hydrocarbons from polluted Greek soils, Appl. Microbiol. Biotechnol. 65 (2004) 24–31.
[24] J. Maier, A. Kandelbauer, A. Erlacher, A. Cavaco-Paulo, G.M. Gubitz, A new alkali-thermostable azoreductase from *Bacillus* sp. strain SF, Appl. Environ. Microbiol. 70 (2004) 837–844.
[25] APHA, Standard Methods for the Examination of Water and Wastewater, 17th ed., American Public Health Association, Washington, D.C., 1989.
[26] S. Mohanty, N. Dafale, N.N. Rao, Microbial decolorization of reactive black-5 in a two-stage anaerobic–aerobic reactor using acclimatized activated textile sludge, Biodegradation 17 (2006) 403–413.
[27] W. Delée, C. O'Neill, F.R. Hawkes, H.M. Pinheiro, Anaerobic treatment of textile effluents: a review, J. Chem. Technol. Biotechnol. 73 (1998) 323–335.
[28] M. Isik, D.T. Sponza, Aromatic amine degradation in a UASB/CSTR sequential system treating Congo Red dye, J. Environ. Sci. Health Part A-Toxic/Hazard. Subst. Environ. Eng. 38 (2003) 2301–2315.
[29] M. Isik, D.T. Sponza, Anaerobic/aerobic treatment of a simulated textile wastewater, Sep. Purif. Technol. 60 (2008) 64–72.

[30] L.D. Nghiem, S. Hawkes, Effects of membrane fouling on the nanofiltration of pharmaceutically active compounds (PhACs): mechanisms and role of membrane pore size, Sep. Purif. Technol. 57 (2007) 176–184.
[31] F. Meng, S.R. Chae, A. Drews, M. Kraume, H.S. Shin, F. Yang, Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material—review, Water Res. 43 (2009) 1489–1512.
[32] H. Liu, C. Yang, W. Pu, J. Zhang, Formation mechanism and structure of dynamic membrane in the dynamic membrane bioreactor, Chem. Eng. J. 148 (2009) 290–295.
[33] S. Sandhya, S. Padmavathy, K. Swaminathan, Y.V. Subrahmanyam, S.N. Kaul, Microaerophilic–aerobic sequential batch reactor for treatment of azo dyes containing simulated wastewater, Process Biochem. 40 (2005) 885–890.
[34] J.S. Knapp, P.S. Newby, The microbiological decolorization of an industrial effluent containing a diazo linked chromophore, Water Res. 29 (1995) 1807–1809.
[35] G.J. Holt, R.N. Krieg, H.P. Sneath, T.J. Staley, T.S. Williams, Bergey's Manual of Determinative Bacteriology, 9th ed., Williams and Wilkins, Maryland, 1994.
[36] W.S. Kim, J.N. Ren, W. Dunn, Differentiation of *Lactococcus lactis* subspecies lactis and subspecies cremoris strains by their adaptive response to stresses, FEMS Microbiol. Lett. 171 (1999) 57–65.
[37] S.A. Learoyd, R.G. Kroll, C.F. Thurston, An investigation of dye reduction by food-borne bacteria, J. Appl. Bacteriol. 72 (1992) 479–485.
[38] P. Seesuriyachan, S. Takenaka, A. Kuntiya, S. Klayraung, S. Murakmi, K. Aoki, Metabolism of azo dyes by *Lactobacillus casei* TISTR 1500 and effects of various factors on decolorization, Water Res. 41 (2007) 985–992.
[39] J.S. Chang, C. Chou, Y.C. Lin, P.J. Lin, J.Y. Ho, T.L. Hu, Kinetic characteristics of bacterial azo-dye decolorization by *Pseudomonas luteola*, Water Res. 35 (2001) 2841–2850.