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Biological textile dye removal mechanism of direct blue 15 (DB15) by anoxic/oxic-SBR system

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ABSTRACT

The anoxic/oxic-sequencing batch reactor system was operated with synthetic textile wastewater containing direct blue 15 (DB15) at various anoxic:oxic ratios of 8:2, 2:8 and 0:10 to observe system removal efficiency and performance. The results showed that the color removal efficiency increased through addition of anoxic period in the reaction step of operation program. Moreover, the color removal efficiency increased with increasing anoxic period. The highest color removal efficiency (87.69% ± 0.23%) was detected at anoxic:oxic ratio of 8:2, whereas it was lowest (66.62% ± 0.14%) at anoxic:oxic ratio of 0:10. However, the anoxic condition did not negatively affect the chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) removal efficiencies. The COD and BOD_5 removal efficiencies were about 95%–96%. For the gel chromatography study, DB15 was first degraded by denitrifying bacteria under anoxic conditions, then the metabolites as aromatic amines were further adsorbed and degraded by heterotrophic and nitrifying bacteria under oxic conditions.

Keywords: Sequencing batch reactor system; Oxic; Anoxic; Direct blue 15; Azo dye; Gel filtration chromatography

1. Introduction

Azo dyes, consisting of an aromatic compound with one or more –N=N– groups in their chemical structure, are widely used in textile industries at over 60%–70% [1–5]. For the dying process, more than 15%–50% of dyes and their intermediates were contaminated into the environment, presenting serious environmental problems due to the poor biodegradable chemical structure of the azo group.

In the past, physiochemical methods were widely used for the removal of dyes from textile wastewater [6–8]. However, limitations of those methods include high cost, complicated operational process and chemical sludge wastes production, which is hard to dispose of and toxic to the environment [9]. In recent years, many researchers focused on the biological treatment process because it is more cost-effective and environmentally friendly. Several reports demonstrated that decolorizing azo dyes by special microorganisms start by cleavage of azo bond with azoreductase enzymes under anoxic conditions generating aromatic amines. Moreover, azoreductase enzymes were produced from special anaerobic microbes and the metabolite of azo reduction by the above microbial process as aromatic amines showed carcinogenic effects to humans and aquatic life [10–12]. However, the aromatic amines could be easily biodegraded under aerobic conditions [13,14]. Then, oxic and anoxic conditions should be applied during operation to reach complete removal of azo textile dyes without affecting the environment and aquatic life. From the above information, it could be suggested that the sequencing batch reactor (SBR) system operated under anoxic/oxic conditions is a suitable biological wastewater treatment system to treat textile wastewater containing azo dyes [15,16]. In this study, synthetic textile wastewater (STW) containing 40 mg/L direct blue 15 (DB15) (STW + DB15) was used to observe SBR system

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efficiency and performance under various anoxic:oxic ratios of 8:2, 2:8 and 0:10. Moreover, the DB15 removal mechanism and type of main microbe affecting azo dyes were investigated.

2. Materials and methods

2.1. Sequencing batch reactor reactor

The SBR reactor (10 L total volume each) was made from acrylic plastic (5 mm thick) as shown in Fig. 1. The reactor was 18 cm in diameter and 40 cm in height with a working volume of 7.5 L. A low speed gear motor, model P 630A-387, 100 V, 50/60 Hz, 1.7/1.3 A (Japan Servo Co. Ltd., Japan) was used to drive the paddle-shape impeller. The impeller speed was adjusted to 60 rpm. One set of air pumps, model EK-8000, 6.0 W (President Co. Ltd., Thailand), was used to supply air for both reactors. The air pump equipment was enough to supply air to both reactors. The dissolved oxygen of each reactor was not less than 2.0 mg/L.

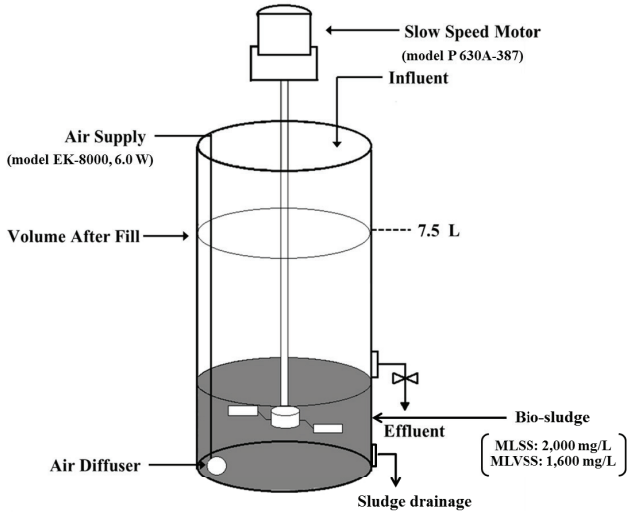

Fig. 1. Flow diagram of SBR system.

2.2. Bio-sludge preparation

Bio-sludge was collected from the Bangkok municipal central wastewater treatment plant (Siphaya plan), Thailand. The characteristics of the bio-sludge were 10,000 mg/L concentration, 12–15 d sludge age (solids retention time [SRT]) and mixed liquor volatile suspended solids (MLVSS) was 80% of mixed liquor suspended solids (MLSS). The bio-sludge was acclimatized in STW without DB15 for 2 weeks before use in the experiments.

2.3. Direct blue 15

Direct dye: DB15 (molecular formula: $C_{34}H_{24}N_6Na_4O_{16}S_4$ and molecular weight: 992.804037 g/mol) [17] was selected for use in this study. The chemical characteristics of DB15 are described in Table 1.

2.4. Synthetic textile wastewater

The STW containing 40 mg/L DB15 (STW + DB15) used in this study contained biochemical oxygen demand (BOD_5) and DB15 at 800 and 40 mg/L, respectively, as shown in Table 1.

2.5. Operation of SBR system

Three sets of SBR reactors were used in this experiment. The total and working volume of each reactor was 10.0 and 7.5 L, respectively. Optimum operation parameters of SBR system were as follows: *hydraulic retention time* of 5 d and MLSS of 2,000 mg/L. The system was operated for two cycles a day (12 h/cycle). Each cycle included: fill up for 0.5 h, reaction step for 10 h and settle and idle for 1.5 h (stop aeration) as mentioned in Table 2. In the reaction step, the anoxic and oxic conditions were applied at anoxic:oxic ratios of 8:2, 2:8 and 0:10 h as shown in Table 3. Then, the SBR system was operated under the aforementioned conditions, and was called anoxic/oxic-SBR system.

2.6. Gel filtration chromatography

A gel filtration chromatography technique was used to determine the molecular weight pattern of DB15 and its

Table 1
Chemical composition and properties of synthetic wastewater

Chemical composition		Chemical properties		Chemical structure of the direct blue 15
Composition	Concentration (mg/L)	Properties	Concentration	
Glucose	834	COD, mg/L	2,000 ± 40	
Urea	107	BOD_5, mg/L	800 ± 15	
KH_2PO_4	44	TKN, mg/L	50 ± 1.48	
$NaHCO_3$	688	NH_4^+–N, mg/L	4.21 ± 0.24	
$FeCl_3$	7.25	Organic-N_2, mg/L	45.99 ± 1.48	
$MgSO_4{\cdot}7H_2O$	38	NO_2^-–N, mg/L	0.90 ± 0.02	
$CaCl_2$	14	NO_3^-–N, mg/L	1.39 ± 0.02	
Direct blue15	40	TN, mg/L	52.57 ± 1.50	
		pH	8.2	

metabolites, which were treated by the SBR system. 1.5 mL of STW + DB15 solutions was added into the top of a Sephadex G-50 (1.2 × 50 cm^2) column, previously equilibrated with 0.05 M phosphate buffer (pH 7.0) and eluted with the same buffer at a flow rate of 0.5 mL/min. The effluents were collected at 5 mL. The color intensity of each fraction was measured at 607 nm as mentioned above.

2.7. Analytical methods

The color removal efficiency was assayed by measuring the decrease in color intensity as the absorbance at 607 nm. All experiments were performed in triplicate. The influents and effluents of the SBR system were centrifuged at 3,000 rpm for 10 min to separate the bio-sludge mass and then, the supernatants were used to determine the absorbance using UV–Vis spectrophotometer (GENESYS 10S, USA) at 607 nm. The color removal yield was calculated by the equation below:

$$\%\ \text{color removal efficiency} = \frac{\text{Influent absorbance} - \text{Effluent absorbance}}{\text{Influent absorbance}} \times 100$$

Table 2
Operation parameters of anoxic/oxic-SBR system[a] with synthetic wastewater

Parameter	Anoxic:oxic ratio (h)		
	8:2	2:8	0:10
MLSS (mg/L)	2,000	2,000	2,000
HRT	3	3	3
Dye concentration (mg/L)	40	40	40
Flow rate (mL/d)	2,500	2,500	2,500
Hydraulic loading	0.33	0.33	0.33
F/M ratio	0.13	0.13	0.13
Organic loading (kg BOD_5/m^3·d)	0.27	0.27	0.27
Colorant loading (g DB15/m^3·d)	0.1	0.1	0.10
Cycle (h)	12	12	12
- Fill (h)	0.5	0.5	0.5
- React (h)	10	10	10
- Anoxic (h)	8	2	0
- Oxic (h)	2	8	10
- Settle (h)	1	1	1
- Idle (h)	0.5	0.5	0.5

[a]The anoxic/oxic-SBR system was different from the conventional-SBR system as it applied the anoxic period in the reaction step (Table 2).

Total Kjeldahl nitrogen (TKN) and chemical oxygen demand (COD) were measured using closed reflux titrimetric method. BOD_5 was measured using azide modification of iodometric method. Ammonia nitrogen (NH_4^+–N) was measured using nesslerization method. Nitrite nitrogen (NO_2^-–N: colorimetric method), nitrate nitrogen (NO_3^-–N: cadmium reduction method), total solids, MLSS, MLVSS and sludge volume index (SVI) were measured according to standard methods for the examination of water and wastewater [18]. The pH was measured using a digital pH meter (WTW, series: inolab 720, Germany).

2.8. Statistical analysis method

Each experiment was repeated at least three times. All data were subjected to two-way analysis of variance using SAS Windows Version 6.12 [19]. Statistical significance was tested using the least significant difference at $p < 0.05$ and the results were shown as the mean ± standard deviation.

3. Results

3.1. Performance and efficiency of anoxic/oxic-SBR system with STW–DB15 at various anoxic:oxic ratios

The anoxic/oxic-SBR system was operated with STW + DB15 (Table 1) at various anoxic:oxic ratios of 8:2, 2:8 and 0:10. The effect of anoxic:oxic ratios on the removal efficiency and bio-sludge performance are described as follows.

3.1.1. pH and dissolve oxygen

Systems pH and dissolve oxygen (DO) were measured during operation (12 h/cycle) of anoxic/oxic-SBR system as shown in Fig. 2. Influent pH was in the alkaline range in all experiments tested as shown in Table 3. The system showed the same pH profile results during the system fill up step. The system pH slightly decreased during the fill up step as shown in Fig. 2. Moreover, the system pH decreased during anoxic period of reaction step, but increased to more than 8.00 during oxic period of the reaction step as shown in Fig. 2.

For the DO profile, the system produced interesting results as shown in Table 3 and Fig. 2. The system DO dropped rapidly during anoxic period of the reaction step. The system at anoxic:oxic ratio 8:2 decreased to 0.3 ± 0.1 mg/L during anoxic period, however, the DO rapidly increased up to 3.65 ± 1.0 mg/L as shown in Table 3 and Fig. 2. On the other hand, the DO of conventional-SBR was quite stable at 6.3 ± 0.2 mg/L during reaction step (anoxic:oxic ratio of 0:10) as shown in Table 3 and Fig. 2.

Table 3
pH, DO and color removal efficiencies and effluent properties of anoxic/oxic-SBR system operated with STW + DB15 at various anoxic:oxic ratios of 8:2, 2:8 and 0:10

Anoxic:oxic ratio	Color (mg/L)				pH		DO (mg/L)	
	Influent	Effluent		% Removal	Anoxic period	Oxic period	Anoxic period	Oxic period
		Anoxic period	Oxic period					
8:2	40.07 ± 0.12	5.04 ± 0.09	5.02 ± 0.09	87.69 ± 0.23	7.60 ± 0.22	8.50 ± 0.30	0.30 ± 0.10	3.65 ± 1.0
2:8	40.07 ± 0.12	19.16 ± 0.08	12.97 ± 0.08	67.81 ± 0.20	7.78 ± 0.30	8.20 ± 0.40	1.60 ± 0.20	6.25 ± 0.20
0:10	40.07 ± 0.12	13.44 ± 0.07		66.62 ± 0.14	8.35 ± 0.24		6.30 ± 0.20	

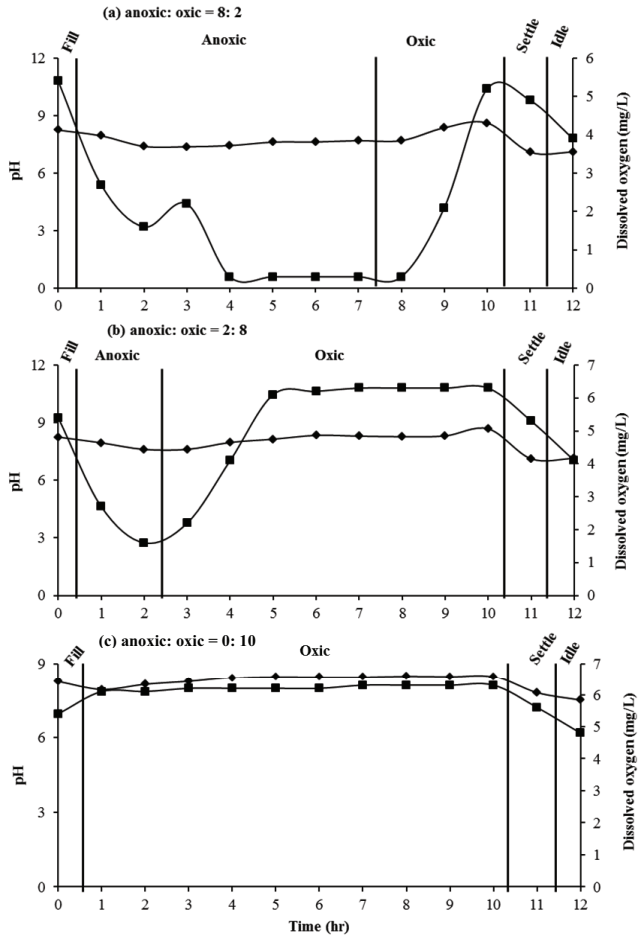

Fig. 2. pH (♦) and DO (■) profiles of anoxic/oxic-SBR system operated at various anoxic:oxic ratios of (a) 8:2, (b) 2:8 and (c) 0:10.

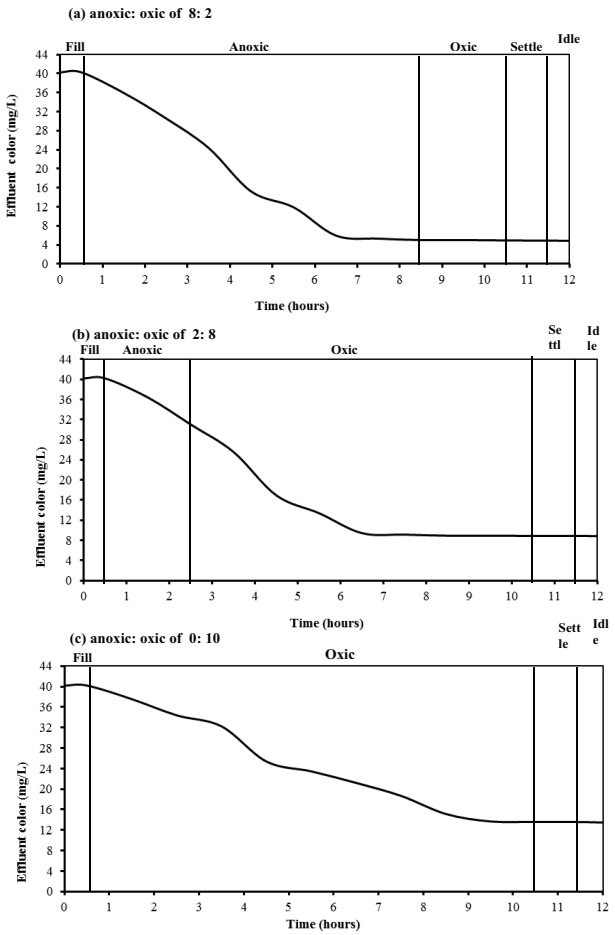

Fig. 3. Effluent color profile of anoxic/oxic-SBR system operated at various anoxic:oxic ratios of (a) 8:2, (b) 2:8 and (c) 0:10.

3.1.2. DB15

DB15 removal efficiencies of anoxic/oxic-SBR system with STW + DB15 at various anoxic:oxic ratios were observed as shown in Fig. 3 and Table 3. The results indicated that the addition of anoxic period in the reaction step could increase the color removal efficiency as shown in Table 3 and Fig. 3. The wastewater color of the system rapidly decreased during anoxic period of reaction step. Moreover, the color removal efficiency increased with increasing anoxic period. The highest color removal yield was 87.69% ± 0.23% while, the color removal efficiency of the conventional-SBR system (anoxic:oxic ratio of 0:10) was only 66.62% ± 0.14% as shown in Table 3. In addition, the color removal ability of the system rapidly increased and became stable after 9–10 h operation as shown in Fig. 3.

3.1.3. BOD_5 and COD

Effluents characteristics of anoxic/oxic-SBR system operated at various anoxic:oxic ratios of 8:2, 2:8 and 0:10 are shown in Table 4 and Fig. 4. The system was not significantly different in terms of COD and BOD_5 removal efficiencies as shown in Table 4 and Fig. 4. The COD and BOD_5 removal efficiencies of the system at anoxic:oxic ratios of 8:2, 2:8 and 0:10 were all about 96% as shown in Table 4.

3.1.4. Nitrogen compounds

The effluent and influent nitrogen compounds of the system as TKN, NH_4^+–N, NO_2^-–N, NO_3^-–N, organic nitrogen (org-N) and total nitrogen (TN) are shown in Table 5 and Fig. 5. The effluent nitrogen compound profiles of the system operated at various anoxic:oxic ratios produced interesting results as shown in Fig. 5. NH_4^+–N of STW + DB15 rapidly decreased in the conventional-SBR system (anoxic:oxic of 0:10), while NH_4^+–N of STW + DB15 in the anoxic/oxic-SBR system slightly decreased during anoxic period as shown in Fig. 5. For the determination of effluent NO_3^-–N, it decreased after addition of anoxic period in the reaction step. The effluent NO_3^-–N in the anoxic/oxic-SBR system at anoxic:oxic ratio 8:2 was lowest (2.07 ± 0.11 mg/L), while it was highest (12.97 ± 0.28 mg/L) at anoxic:oxic ratio 0:10 as shown in Table 5. Moreover, the systems showed the same patterns of effluents NO_2^-–N and org-N in all experiments. The highest TN and org-N removal efficiencies of 82.56% and 92.20%, respectively, were detected at anoxic:oxic ratio 8:2 (food/microbe [F/M] of 01.3) as shown in Table 5.

3.1.5. Bio-sludge performance

The addition of the anoxic period in the reaction step could increase bio-sludge age (SRT) of SBR system as

Table 4
COD and BOD_5 removal efficiencies and effluents properties of anoxic/oxic-SBR system operated with STW + DB15 at various anoxic:oxic ratios of 8:2, 2:8 and 0:10

Anoxic:oxic ratio	Organic loading (kg $BOD_5/m^3 \cdot d$)	COD		BOD_5		SS	Bio-sludge qualities			
		Effluent (mg/L)	% Removal	Effluent (mg/L)	% Removal	Effluent (mg/L)	SRT (d)	SVI (mL/g)	MLSS (mg/L)	F/M
8:2	0.27	81 ± 6	96.1 ± 0.3	32 ± 2	96.0 ± 0.3	13 ± 1	11 ± 1	55 ± 5	2,116 ± 71	0.13
2:8	0.27	80 ± 5	96.2 ± 0.3	32 ± 2	96.1 ± 0.3	19 ± 1	9 ± 1	61 ± 8	2,240 ± 38	0.13
0:10	0.27	85 ± 3	95.8 ± 0.2	34 ± 2	95.8 ± 0.2	26 ± 2	6 ± 2	67 ± 4	2,364 ± 48	0.13

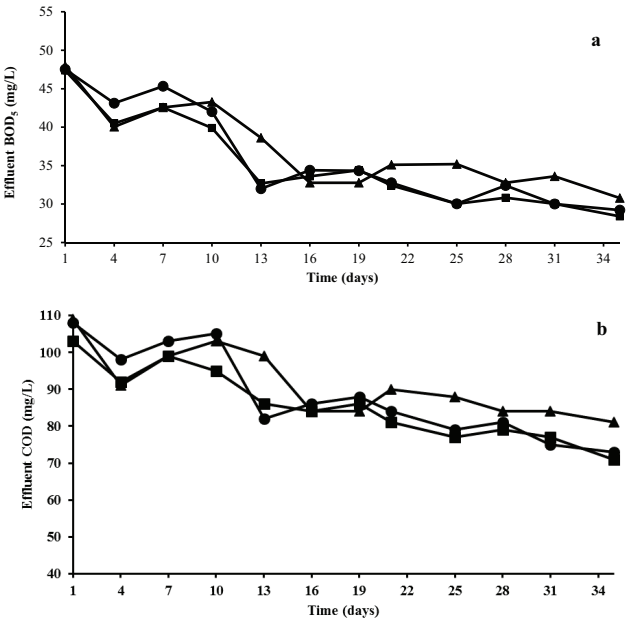

Fig. 4. Effluents BOD_5 (a) and COD (b) profiles of anoxic/oxic-SBR at various anoxic:oxic ratio of (●) 8:2, (■) 2:8 and (▲) 0:10.

shown in Table 4. The systems SRTs were 11 ± 1, 9 ± 1 and 6 ± 2 d at anoxic:oxic ratios of 8:2, 2:8 and 0:10, respectively (Table 4). Moreover, the effluent suspended solids (SS) decreased with increasing anoxic period in the reaction step. The effluent SS of the system at anoxic:oxic 8:2 was lowest (13 ± 1 mg/L), while it was highest (26 ± 2 mg/L) at anoxic:oxic 0:10. However, the systems SVI were almost identical in all experimental conditions. It was more than 70 mL/g, which was classified as a good value.

3.2. Gel filtration chromatography

The molecular weight distribution by gel filtration chromatography was examined for influent and effluent of the system with STW + DB15 at various anoxic:oxic ratios of 8:2, 2:8 and 0:10 as shown in Fig. 6. The effluent of the anoxic/oxic-SBR system gave similar molecular weight distribution patterns as shown in Fig. 6, but the higher molecular weight group rapidly decreased under anoxic period of the reaction step. Moreover, the increase of anoxic period of the reaction step, the number of larger molecular weight groups was increased as shown in Fig. 6.

The adsorbed DB15 and its metabolites were washed from bio-sludge using 0.1% Triton X100. Then, the eluted solutions were determined for molecular weight distribution by gel filtration chromatography as mentioned in section 2.6. The chromatogram patterns of adsorbed DB15 and their metabolites showed the interesting results that it consisted of smaller molecular weight groups as shown in Fig. 7.

4. Discussions

The application of anoxic period in the reaction step of SBR system operation produced interesting results where by the removal efficiencies and bio-sludge performance improved by the addition of anoxic period in the reaction step. However, the anoxic/oxic-SBR system performance was different from the conventional-SBR system. The system pH at anoxic:oxic 8:2 and 2:8 was lower than that of 0:10 due to the type of microbes present [20–22]. The anoxic condition induced the anoxic and microaerophilic bacteria groups resulting in volatile fatty acid and CO_2 production, then the system pH dropped [23–25]. Moreover, the color intensity of the wastewater rapidly decreased during anoxic period. It could suggest that DB15 removal microbes were mainly anoxic bacteria or microaerophilic bacteria groups. It was strongly confirmed that the bio-sludge age of the system with longer anoxic period was longer than that with shorter anoxic or non-anoxic period resulted by the increasing of number of denitrifying bacteria and decreasing of heterotrophic bacteria. And, the specific growth rate of denitrifying bacteria was lower than that of heterotrophic bacteria [25,29]. From previous works, the azo group of the textile dyes can be easily biodegraded under anaerobic conditions [2,4,11–13]. The bacterial degradation of azo dyes involves cleavage of azo bond under anoxic conditions, leading to formation of aromatic amines. Further degradation of aromatic amines generally requires oxic conditions [11,26]. To confirm the above suggestions, the effluents of the experiments and the eluted DB15 and their metabolites from the bio-sludge were determined for molecular weight distribution by gel filtration technique. It was confirmed that the larger molecular weight was easily and rapidly degraded under anoxic conditions as show in Fig. 6. Then, the amount of smaller molecular weight DB15 part was reduced under oxic conditions. Moreover, the smaller molecular weight DB15 part was adsorbed onto the bio-sludge, while the larger molecular weight DB15 part could not be detected on the bio-sludge as shown in Figs. 6 and 7. In addition, the system operated under anoxic/oxic conditions showed

Table 5
Nitrogen compounds removal efficiencies and effluents properties of anoxic/oxic-SBR system operated with STW + DB15 at various anoxic:oxic ratios of 8:2, 2:8 and 0:10

Anoxic: oxic ratio	Organic loading (kg BOD5/m^3 d)	Org-N (mg/L)		NH_4^+–N (mg/L)		NO_2^-–N (mg/L)		NO_3^-–N (mg/L)		Removal efficiencies (%)	
		Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	TKN	TN
8:2	0.27	45.99 ± 1.48	4.21 ± 0.24	4.30 ± 0.02	1.89 ± 0.09	0.90 ± 0.02	0.50 ± 0.01	1.39 ± 0.02	2.07 ± 0.11	88.23 ± 0.41	83.48 ± 0.63
2:8	0.27	45.99 ± 1.48	4.49 ± 0.15	4.30 ± 0.02	1.53 ± 0.15	0.90 ± 0.02	0.50 ± 0.02	1.39 ± 0.02	4.09 ± 0.19	88.60 ± 0.42	80.37 ± 0.80
0:10	0.27	45.99 ± 1.48	4.33 ± 0.23	4.30 ± 0.02	1.37 ± 0.10	0.90 ± 0.02	0.45 ± 0.03	1.39 ± 0.02	12.97 ± 0.28	89.05 ± 0.39	63.97 ± 1.35

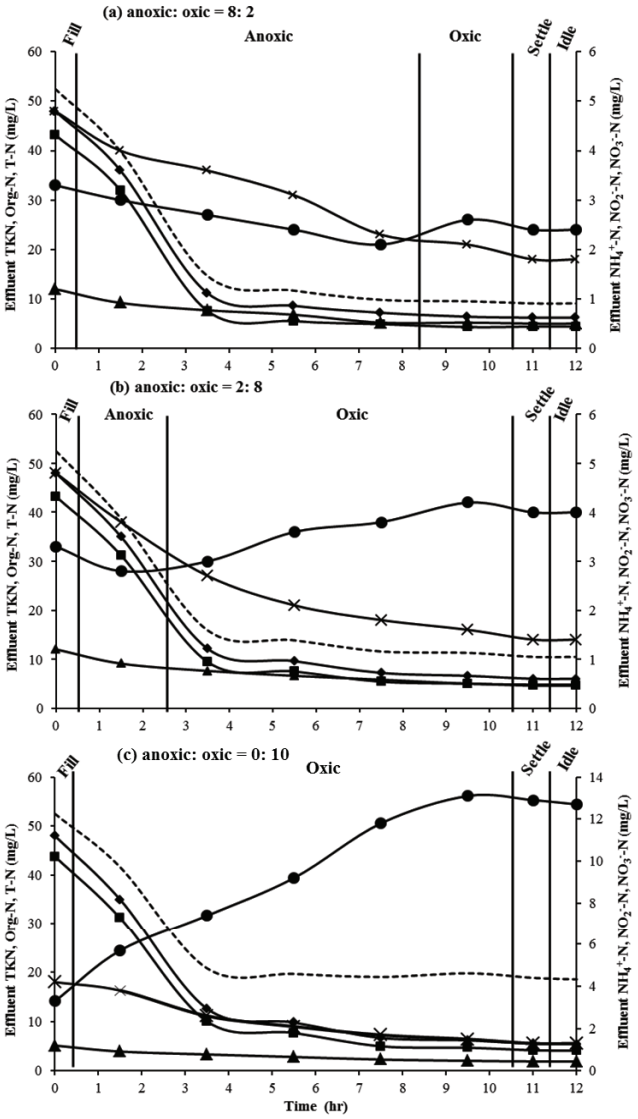

Fig. 5. Effluents TKN (♦), TN (---), organic nitrogen (■), NH_4^+–N (×), NO_2^-–N (▲) and NO_3^-–N (●) profiles of anoxic/oxic-SBR system operated at various anoxic:oxic ratios of (a) 8:2, (b) 2:8 and (c) 0:10.

higher nitrogen removal efficiency. Effluent TN rapidly decreased at the anoxic period. Also, the NO_3^-–N of STW + DB15 increased from 1.39 ± 0.02 to 12.97 ± 0.28 mg/L during operation at anoxic:oxic 0:10. From the above results, the main azo degrading bacteria under anoxic condition were denitrifying bacteria, which is the first finding in this paper [27–32]. Because, it was different from the previous report that azo group was easily degraded under anaerobic condition [12,23,26,27]. Moreover, the metabolites from biodegradation of azo group of acid dye under anoxic conditions (possibly aromatic amines) were easily biodegraded under oxic conditions as shown in Fig. 6 [26,33–35]. Moreover, the chromatogram pattern of the eluted DB15 solutions from the bio-sludge produced interesting results consisting of smaller molecular weight part. It could be concluded that the azo dye as DB15 was first degraded by denitrifying bacteria under

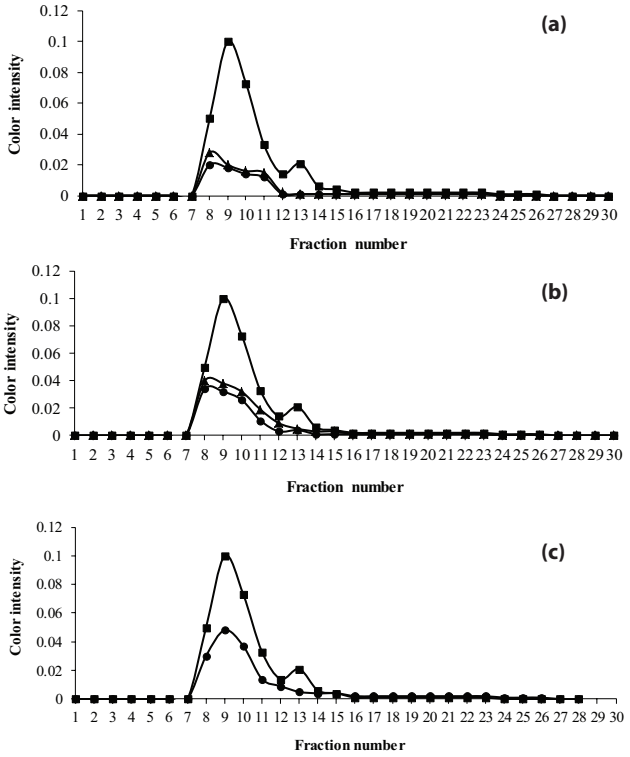

Fig. 6. Chromatograms of DB15 in STW + DB15 treated by anoxic/oxic-SBR system at anoxic:oxic ratios of (a) 8:2 h, (b) 2:8 h and (c) 0:10 h were obtained using gel filtration on a Sephadex G-50 column. Symbol: (■) influent, (▲) effluent of anoxic period and (●) effluent of oxic period.

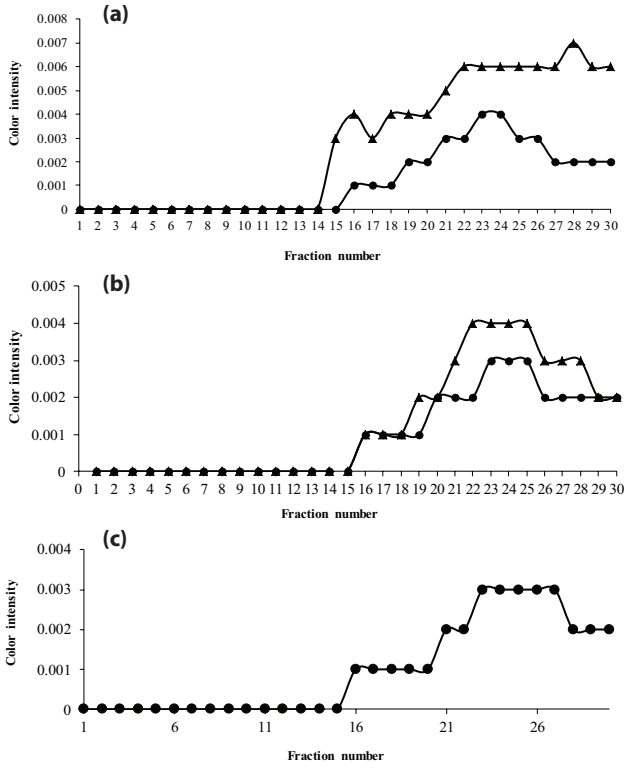

Fig. 7. Chromatograms of DB15 were eluted from bio-sludge of anoxic/oxic-SBR system operated with STW + DB15 at anoxic:oxic ratios of (a) 8:2 h, (b) 2:8 h and (c) 0:10 h. Symbol: (▲) effluent of anoxic period and (●) effluent of oxic period.

anoxic conditions resulting in azo group degradation to aromatic amines [4,13,31–33,36]. Next, they were adsorbed and degraded by heterotrophic bacteria under oxic conditions [1,13,31]. From above result and suggestion, it could suggest that the azo dye degradation mechanism involved as follows: first, the azo group of direct dye was degraded to be aromatic amine (metabolize) by denitrifying bacteria under anoxic condition. Later, according to result on the chromatogram patterns of eluted DB15 (Fig. 7), the metabolize as aromatic amine was adsorbed and consequence degraded by heterotrophic and nitrifying bacteria under oxic condition.

5. Conclusion

The effects of various anoxic:oxic ratios of 8:2, 2:8 and 0:10 on the efficiency of anoxic/oxic-SBR system operated SBR for color and organic removal was done with STW + DB15. The conclusions from this study are as follows: the main color removal mechanism occurred in the anoxic period. The highest color removal efficiency (87.69% ± 0.23%) was detected at anoxic:oxic 8:2, while it was lowest (66.62% ± 0.14%) at anoxic:oxic 0:10. The other advantage was that the addition of anoxic period in the reaction step did not affect the COD and BOD_5 removal efficiencies. In conclusion, the color removal ability consisted of two possible mechanisms as follows: first, the azo group of DB15 was degraded by denitrifying bacteria under anoxic conditions. Then, the metabolites as aromatic amines were adsorbed and degraded by heterotrophic and nitrifying bacteria under oxic conditions.

Acknowledgments

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Symbols

BOD_5	—	Biochemical oxygen demand
COD	—	Chemical oxygen demand
DB15	—	Direct blue 15
DO	—	Dissolved oxygen
HRT	—	Hydraulic retention time
MLSS	—	Mixed liquor suspended solids
NH_4^+–N	—	Ammonium nitrogen
NO_2–N	—	Nitrite nitrogen
NO_3–N	—	Nitrate nitrogen
Org-N	—	Organic nitrogen
SBR	—	Sequencing batch reactor
STW	—	Synthetic textile wastewater
TKN	—	Total Kjeldahl nitrogen
TN	—	Total nitrogen

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