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Biodegradation mechanism of direct blue 15 (DB15) and effect of NaCl on the removal efficiency in anoxic/oxic SBR system with synthetic textile wastewater containing DB15 (STWW + DB15)

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ABSTRACT

The application of an anoxic/oxic sequencing batch reactor (SBR) system for treatment of synthetic textile wastewater containing Direct Blue 15 (STWW + DB15) was carried out to observe the DB15 biodegradation mechanism and its intermediates. The effect of various NaCl concentrations on the system removal efficiency was also investigated. The results showed that NaCl had a more repressive effect on the growth and activity of heterotrophic carbonaceous compound removal bacteria than on nitrogenous compound removal bacteria (both nitrifying and denitrifying bacteria). The highest biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), DB15, total nitrogen (TN) and total Kjeldahl nitrogen (TKN) removal efficiencies of 96.29% ± 0.74%, 95.81% ± 0.30%, 90.61% ± 0.82%, 87.9% ± 0.24% and 85.03% ± 0.20%, respectively, were detected for STWW + DB15 at hydraulic retention time of 3 d and mixed liquor suspended solids of 2,000 mg/L. The lowest BOD_5, COD, DB15, TN and TKN removal efficiencies of 30.01% ± 3.48%, 33.26% ± 0.92%, 26.93% ± 1.42%, 51.49% ± 0.31% and 49.77% ± 0.87%, respectively, were detected for STWW + DB15 containing 14% NaCl (w/v). Moreover, NaCl had a stronger repressive effect on nitrifying bacteria than on denitrifying bacteria. Observation of the biodegradation mechanism of DB15 in the anoxic/oxic SBR system revealed that the azo bond of DB15 was first degraded to 1-amino-8-naphthol-3,6-disulfonic acid and 2-ethoxy-4-methylaniline at the anoxic period of the reaction step, and then 1-amino-8-naphthol-3,6-disulfonic acid was further degraded to 2-naphthol at the oxic period of the reaction step.

Keywords: Direct Blue 15; Sequencing batch reactor system; Anoxic; Oxic; Nitrification; Denitrification

1. Introduction

The textile industry is one of the main export industries of Thailand, and azo dye is one of the most common textile dyes used in the dyeing process; more than 60%–70% of textile dye consumption is azo-type dye [1,2]. Previous studies have found that about 2%–10% of textile dyes are lost during the manufacturing process and end up as contaminants in the wastewater [3–5]. Moreover, a high NaCl concentration of 40–100 g/L is normally used in the dyeing process to ensure maximum fixation of dye particles onto the cellulosic fiber [6,7]. Thus, the textile wastewater contains not only organic matter and dye material but also a high concentration of NaCl [8–10]. High salt concentrations of up to 15%–20% have been detected in textile wastewaters from dye-stuff industries [11]. Moreover, it is well known that NaCl is an inhibitor

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of the growth and activity of bio-sludge in the biological wastewater treatment process [8,9,12].

Numerous studies have demonstrated that the decolorization of azo dyes by microorganisms is started by cleavage of the azo bond by the azo-reductase enzyme under anaerobic conditions, resulting in the formation of aromatic amines (1-amino-8-naphthol-3,6-disulfonic acid and 2-ethoxy-4-methylaniline) as metabolites. These aromatic amines are toxic to humans and animals and cannot be degraded under anaerobic conditions; however, they can easily be degraded under aerobic conditions [13–16]. Then, it could inform that usual biological azo dye degradation occurred under anaerobic/oxic conditions. A new finding in our previous work [17,18] was that azo dyes could be degraded under anoxic/oxic conditions. The main azo dye removal mechanism was found to occur during the anoxic period of the biological treatment process by denitrifying bacteria [17,18]. Theoretically, anoxic/oxic conditions could successfully be applied in an SBR system. To confirm the above suggestion [17,18], the Direct Blue 15 (DB15) decolorization mechanism was observed in an anoxic/oxic sequencing batch reactor (SBR) system with synthetic textile wastewater containing Direct Blue 15 (STWW + DB15). In addition, the effect of various NaCl concentrations on the efficiency and performance of the anoxic/oxic SBR system was determined.

2. Materials and methods

2.1. Chemicals

DB15 (tetrasodium 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxynaphthalene-2,7-disulfonate]; $C_{34}H_{24}N_6Na_4O_{16}S_4$; molecular weight (MW) 992.8 g/mol; CAS No. 2429-74-5) was selected as the azo dye for use in this study. The chemical characteristics of DB15 are shown in Table 1. The chemical agents for preparing nutrient agar media and STWW were purchased from QReC (New Zealand) and HiMedia (India). Diethyl ether and methylene chloride (chromatography grade; Merck, Germany) were used for sample extraction and gas chromatography–mass spectrometry (GC–MS) determination.

2.2. Synthetic textile wastewater

STWW used in this study was prepared according to the raw textile wastewater used in previous reports [17,18]. STWW contained 40 mg/L DB15 (STWW + DB15). The biochemical oxygen demand (BOD_5) and total Kjeldahl nitrogen (TKN) of STWW were 800 and 50 mg/L, respectively, as shown in Table 1. Moreover, STWW + DB15 was supplemented with various concentrations of NaCl (STWW + DB15 + nNaCl) of 2%, 6%, 10% and 14% (STWW + DB15 + 2NaCl, STWW + DB15 + 6NaCl, STWW + DB15 + 10NaCl and STWW + DB15 + 14NaCl, respectively).

2.3. Bio-sludge preparation

Bio-sludge was collected from the excess bio-sludge storage tank at the central sewage treatment plant in Bangkok, Thailand (Sripaya sewage treatment plant). The excess bio-sludge concentration was about 10,000 mg/L. The bio-sludge was acclimatized in STWW without DB15 for 2 weeks before using as inoculum in the experiments.

2.4. Sequencing batch reactor systems

Five reactors were made from acrylic plastic (5 mm thick), as shown in Fig. 1. The total and working volumes were 10.0 and 7.5 L, respectively. A low-speed gear motor, 100 V, 50/60 Hz, 1.7/1.3 A (model P 630A-387; Japan Servo Co., Japan), was used to drive the paddle-shaped impeller. The speed of the impeller was adjusted to 60 rpm. Air pumps, 25 W, output 45 L/min, pressure >0.015 MPa (model ACO-208; HAILEA Group, China), were used for supplying air (one set of air pumps for each reactor). Excess bio-sludge was drawn during the draw and idle period to control the stability of mixed liquor suspended solids (MLSS) of the system.

2.5. Operation of the anoxic/oxic SBR system

The anoxic/oxic SBR system was operated at 2 cycles/d (12 h/cycle) at a hydraulic retention time (HRT) of 3 d and with MLSS of 2,000 mg/L. Each cycle included: 0.5 h fill up, 10 h reaction step and 1.5 h settle and idle (to stop aeration). During the reaction period (10 h), the system was sequenced

Table 1
Chemical composition and properties of synthetic textile wastewater containing DB15 (STWW + DB15)

Chemical composition		Chemical properties	
Composition	Concentration (mg/L)	Properties	Concentration
Glucose	1,875	COD, mg/L	2,000
Urea	107	BOD_5, mg/L	1,000
KH_2PO_4	44	TKN, mg/L	50
$NaHCO_3$	688	Direct blue 15 (DB15), mg/L	40
$FeCl_3$	7.25	pH	8.2
$MgSO_4{\cdot}7H_2O$	38		
$CaCl_2$	14		
Direct blue 15 (DB15)	40		

TKN, total Kjeldahl nitrogen (summation of org-N and NH_4^+-N).

into anoxic and oxic periods of 8:2. The operating parameters of the anoxic/oxic SBR system are described in Table 2. The system pH was maintained in a range of 7.5–8.0. The experiments were carried out for a period of 40 d at room temperature (25°C–30°C). Effluents were collected after the anoxic and oxic periods of the reaction and settle steps.

2.6. Chemical analysis

DB15 removal efficiency was determined by measuring the decrease in color intensity (optical density or absorbance) at different time intervals. All experiments were performed in triplicate. Samples collected after anoxic and oxic periods of the reaction and settle steps were centrifuged at 3,000 rpm for 10 min to remove the bio-sludge mass; the supernatants were then used to determine the optical density (absorbance) with a UV–Vis spectrophotometer (GENESYS™ 10S; Thermo Fisher Scientific, USA) at λ_{max} of 607 nm. The DB15 removal efficiency was calculated by the color removal efficiency equation below:

$$\% \text{ DB15 removal efficiency} = \frac{\text{Influent absorbance} - \text{Effluent absorbance} \times 100}{\text{Influent absorbance}}$$

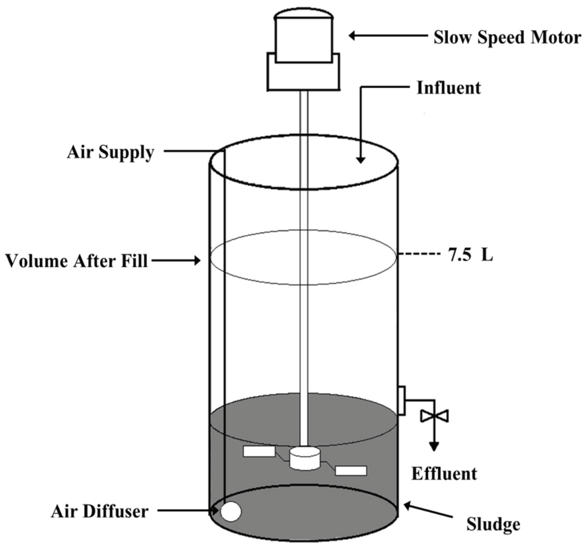


Fig. 1. Flow diagram of SBR systems.

The following parameters were determined based on standard methods for the examination of water and wastewater [19]: TKN, chemical oxygen demand (COD) using closed reflux titrimetric method, BOD_5 using azide modification of iodometric method, ammonia nitrogen (NH_4^+–N) using Nesslerization method, nitrite nitrogen (NO_2^-–N) using colorimetric method, nitrate nitrogen (NO_3^-–N) using cadmium reduction method, total solids, MLSS and sludge volume index (SVI). Org-N was the result of the amount of TKN minus the amount of NH_4^+–N.

2.7. GC–MS analysis

The effluents of the anoxic/oxic SBR system collected at anoxic and oxic periods of the reaction step were centrifuged at 5,000 rpm for 20 min. Supernatants were extracted using an equal volume of diethyl ether. The upper layer of the solution (diethyl ether layer) was collected and dried over anhydrous Na_2SO_4 and evaporated to dryness in a rotary vacuum evaporator (Buchi, Switzerland). The vacuum-evaporated samples were dissolved in methylene chloride for gas chromatography–mass spectroscopy (GC–MS) analysis of metabolites using an Agilent/HP 6890 gas chromatograph

Table 2
Operation parameters of anoxic/oxic SBR system with STWW + DB15 containing various NaCl concentrations of 0%, 2%, 6%, 10% and 14% (w/v): STWW + DB15 + NaCl

Parameter	NaCl concentrations (% w/v)				
	STWW + DB15	STWW + DB15 + 2NaCl	STWW + DB15 + 6NaCl	STWW + DB15 + 10NaCl	STWW + DB15 + 14NaCl
MLSS (mg/L)	2,000	2,000	2,000	2,000	2,000
HRT	3	3	3	3	3
Flow rate (mL/d)	2,500	2,500	2,500	2,500	2,500
Hydraulic loading	0.33	0.33	0.33	0.33	0.33
F/M ratio	0.13	0.13	0.13	0.13	0.13
Organic loading (kg BOD_5/m^3.d)	0.33	0.33	0.33	0.33	0.33
DB15 loading (kg DB15/m^3.d)	0.01	0.01	0.01	0.01	0.01
1 Cycle (h)	12	12	12	12	12
Fill (h)	0.5	0.5	0.5	0.5	0.5
React (h)	10	10	10	10	10
Anoxic (h)	8	8	8	8	8
Oxic (h)	2	2	2	2	2
Settle (h)	1	1	1	1	1
Idle (h)	0.5	0.5	0.5	0.5	0.5

with a 5973 mass selective detector (Agilent Technologies, USA). A DB-1ms column (30 m long, 0.25 mm i.d., nonpolar) was employed for GC–MS analysis. Helium was used as a carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 280°C. The oven condition was kept constant at 80°C for 2 min, then increased up to 200°C at a rate of 10°C/min; after that it was raised up to 280°C at a rate of 20°C/min. The metabolites were identified on the basis of mass spectra, and using the NIST library for identification of the intermediates.

3. Results

3.1. Effect of various NaCl concentrations on the performance and efficiency of the anoxic/oxic SBR system with STWW + DB15 + nNaCl

In order to determine the effect of various NaCl concentrations on the efficiency and performance of the anoxic/oxic SBR system with STWW + DB15, STWW + DB15 + 2NaCl, STWW + DB15 + 6NaCl, STWW + DB15 + 10NaCl and STWW + DB15 + 14NaCl, the removal efficiencies and bio-sludge performance resulting from the effects of various NaCl concentrations were investigated as follows.

3.1.1. DB15 removal

The effects of various NaCl concentrations on DB15 removal efficiency are shown in Fig. 2 and Table 3. The anoxic/oxic SBR system with STWW + DB15 showed higher color removal efficiency than that with STWW + DB15 + nNaCl. Moreover, DB15 removal efficiency decreased with an increase in NaCl concentration. The highest DB15 removal efficiency of 90.61% ± 0.82% was detected for STWW + DB15, while it was lowest (26.93% ± 1.42%) for STWW + DB15 + 14NaCl, as shown in Table 3. Lower concentrations of NaCl of 2%–6% showed slightly repressive effects on DB15 removal efficiency (about 86%–89%), while a higher NaCl concentration of up to 10% had a strongly repressive effect on DB15 removal efficiency (Table 3). DB15 removal occurred exclusively at the anoxic period of the reaction step. Unfortunately, the removal efficiency was slightly decreased at the oxic period of the reaction step (Fig. 2).

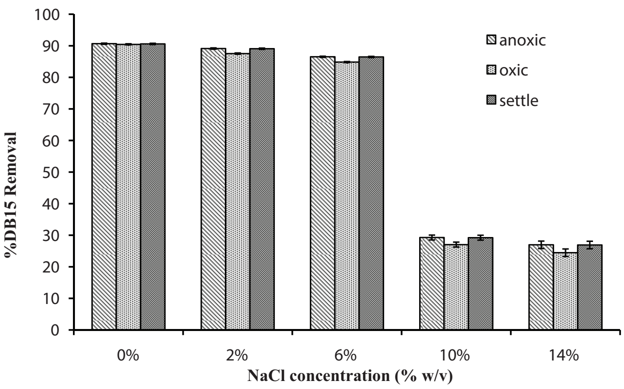


Fig. 2. Effect of various NaCl concentrations on color (DB15) removal efficiencies of anoxic/oxic SBR system with STWW + DB15 + nNaCl.

Table 3
Effect of NaCl concentrations on DB15, BOD_5 and COD removal efficiencies and effluent properties of anoxic/oxic SBR system operated with STWW + DB15 containing various NaCl concentrations of 0%, 2%, 6%, 10% and 14%

NaCl concentration (% w/v)	DB15 loading (kg DB15/m^3.d)	Organic loading (kg BOD_5/m^3 d)	F/M ratio	DB15 concentration (mg/L) Influent	Effluent Anoxic	Oxic	Settle	%DB15 removal	BOD_5 concentration (mg/L) Influent	Effluent Anoxic	Oxic	Settle	%BOD removal	COD concentration (mg/L) Influent	Effluent Anoxic	Oxic	Settle	%COD removal
0	0.01	0.33	0.13	40.05 ± 0.36	3.73 ± 0.09	3.82 ± 0.09	3.76 ± 0.07	90.61 ± 0.82	1,023 ± 36.47	46 ± 4.21	40 ± 3.42	38 ± 4.10	96.29 ± 0.74	2,005 ± 33.28	92 ± 10.24	86 ± 11.14	84 ± 11.32	95.81 ± 0.30
2	0.01	0.33	0.13	40.05 ± 0.36	4.35 ± 0.36	4.99 ± 0.32	4.37 ± 0.08	89.08 ± 0.80	1,023 ± 36.47	110 ± 6.52	91 ± 5.31	90 ± 5.78	91.20 ± 0.94	2,005 ± 33.28	289 ± 14.73	238 ± 6.31	232 ± 1.52	88.4 ± 0.57
6	0.01	0.33	0.13	40.05 ± 0.36	5.39 ± 0.09	6.07 ± 0.37	5.42 ± 0.09	86.48 ± 0.92	1,023 ± 36.47	225 ± 9.23	200 ± 1.12	204 ± 6.24	80.06 ± 1.29	2,005 ± 33.28	584 ± 6.55	521 ± 2.91	519 ± 1.40	74.03 ± 0.12
10	0.01	0.33	0.13	40.05 ± 0.36	28.32 ± 0.10	29.21 ± 0.32	28.34 ± 0.19	29.25 ± 1.02	1,023 ± 36.47	646 ± 11.08	624 ± 8.29	637 ± 8.74	37.73 ± 2.42	2,005 ± 33.28	1,204 ± 10.49	1,120 ± 10.08	1,119 ± 9.83	43.99 ± 1.33
14	0.01	0.33	0.13	40.05 ± 0.36	29.25 ± 0.42	30.24 ± 0.50	29.27 ± 0.42	26.93 ± 1.42	1,023 ± 36.47	731 ± 6.25	703 ± 6.75	716 ± 7.65	30.01 ± 3.48	2,005 ± 33.28	1,417 ± 15.17	1,336 ± 9.06	1,333 ± 9.83	33.26 ± 0.92

3.1.2. BOD_5 and COD

Table 3 shows that NaCl had a repressive effect on the BOD_5 and COD removal efficiencies. Moreover, the removal efficiencies were decreased with an increase in NaCl concentration, as shown in Table 3. The highest BOD_5 and COD removal efficiencies of 96.29% ± 0.74% and 95.81% ± 0.30%, respectively, were detected for STWW + DB15, while the lowest BOD_5 and COD removal efficiencies of 30.01% ± 3.48% and 33.26% ± 0.92%, respectively, were found for STWW + DB15 + 14NaCl. COD and BOD_5 removal abilities were strongly repressed by a NaCl concentration of up to 10 mg/L, as shown in Table 3. COD and BOD_5 of STWW + DB15 were almost completely removed in anoxic conditions. However, their removal abilities could be increased at the oxic period of the reaction step, as shown in Fig. 3.

3.1.3. Nitrogenous compounds

The results of the nitrogen compound removal profiles are shown in Table 4 and Fig. 4. The TN and TKN removal efficiencies were the highest for the system with STWW + DB15: 87.9% ± 0.24% and 85.03% ± 0.20%, respectively, as shown in Table 4. But the removal efficiencies were repressed by adding NaCl. Moreover, the removal efficiencies were decreased with an increase of NaCl concentration; an NaCl concentration of up to 10 mg/L had a strongly repressive effect on their removal efficiencies. The lowest TN and TKN removal efficiencies of 51.49% ± 0.31% and 49.77% ± 0.87%, respectively, were detected at an NaCl concentration of 14% (STWW + DB15 + 14NaCl), as shown in Table 4. For observation

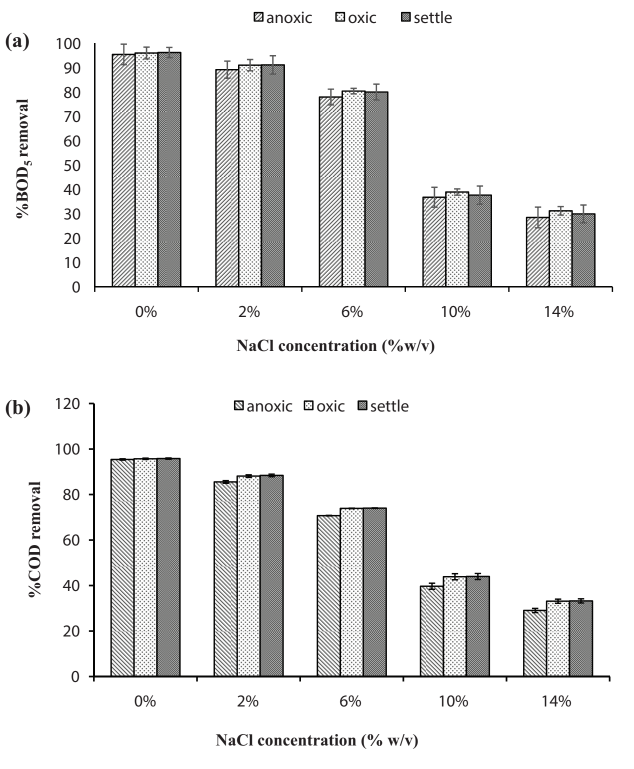


Fig. 3. Effect of various NaCl concentrations on BOD_5 (a) and COD (b) removal efficiencies of anoxic/oxic SBR system with STWW + DB15 + nNaCl.

Table 4
Effect of NaCl concentrations on nitrogen compounds removal efficiency and effluent nitrogenous compounds of anoxic/oxic SBR system operated with STWW + DB15 containing various NaCl concentrations of 0%, 2%, 6%, 10% and 14%

NaCl concentration (% w/v)	TKN (mg/L)			Org-N (mg/L)		NH_4^+-N (mg/L)		NO_2^--N (mg/L)		NO_3^--N (mg/L)		Total-N (mg/L)		
	Influent	Effluent	% removal	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	% Removal
0	50.61 ± 0.50	6.11 ± 0.12	87.93 ± 0.24	45.51 ± 0.23	4.17 ± 0.15	5.10 ± 0.75	1.94 ± 0.02	0.9 ± 0.02	0.47 ± 0.01	1.40 ± 0.01	1.04 ± 0.01	52.90 ± 0.58	7.92 ± 0.14	85.03 ± 0.20
2	50.61 ± 0.50	7.30 ± 0.14	85.58 ± 0.32	45.51 ± 0.23	5.34 ± 0.01	5.10 ± 0.75	1.96 ± 0.04	0.9 ± 0.02	0.51 ± 0.01	1.40 ± 0.01	1.09 ± 0.02	52.90 ± 0.58	8.91 ± 0.05	83.16 ± 0.18
6	50.61 ± 0.50	9.09 ± 0.11	82.04 ± 0.36	45.51 ± 0.23	6.85 ± 0.24	5.10 ± 0.75	2.24 ± 0.02	0.9 ± 0.02	0.52 ± 0.01	1.40 ± 0.01	1.12 ± 0.02	52.90 ± 0.58	10.72 ± 0.22	79.74 ± 0.33
10	50.61 ± 0.50	22.84 ± 0.12	54.87 ± 0.28	45.51 ± 0.23	19.82 ± 0.55	5.10 ± 0.75	3.02 ± 0.01	0.9 ± 0.02	0.72 ± 0.01	1.40 ± 0.01	1.24 ± 0.01	52.90 ± 0.58	24.79 ± 0.60	53.16 ± 0.61
14	50.61 ± 0.50	24.55 ± 0.10	51.49 ± 0.31	45.51 ± 0.23	21.23 ± 0.50	5.10 ± 0.75	3.32 ± 0.01	0.9 ± 0.02	0.77 ± 0.02	1.40 ± 0.01	1.26 ± 0.01	52.90 ± 0.58	26.58 ± 0.49	49.77 ± 0.87

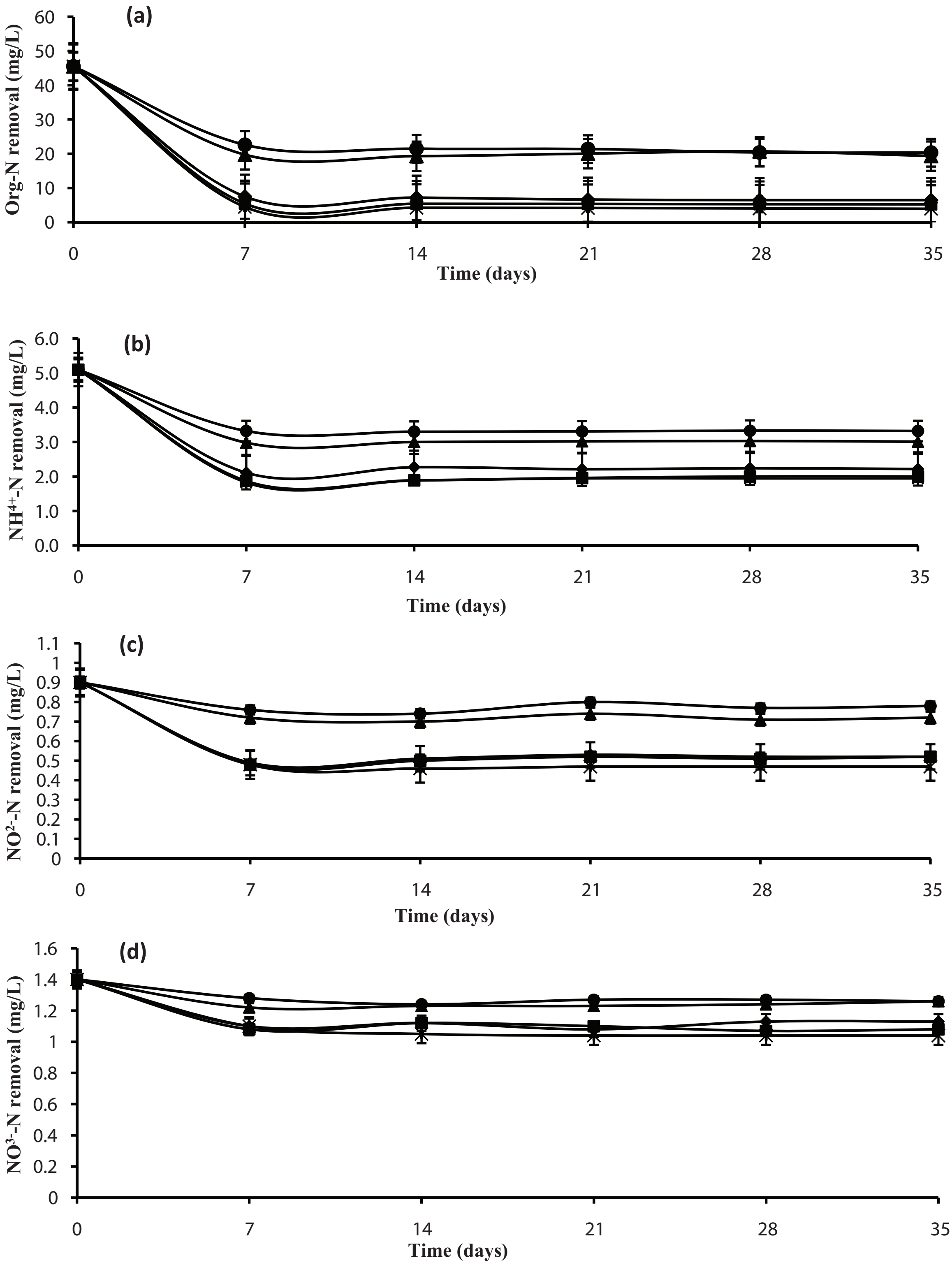


Fig. 4. Effect of various NaCl concentrations of 0% (×), 2% (■), 6% (♦), 10% (▲) and 14% (●) on (a) Org-N (b) NH_4^+-N, (c) NO_2^--N and (d) NO_3^--N, removal profile of anoxic/oxic SBR system with STWW + DB15 + nNaCl.

of effluent nitrogenous compounds, it was found that Org-N, NH_4^+-N, NO_2^--N and NO_3^--N of STWW + DB15 were rapidly removed during 7 d of operation, as shown in Fig. 4, but their removal yields were repressed by NaCl. Moreover, their removal yields were decreased with an increase of NaCl concentration, as shown in Fig. 4. NO_2^--N concentration in the effluent dropped from 0.90 ± 0.02 mg/L to 0.47 ± 0.01, 0.51 ± 0.01, 0.52 ± 0.01, 0.72 ± 0.01 and 0.07 ± 0.02 mg/L at 0%, 2%, 6%, 10% and 14% (w/v) NaCl, respectively, as shown in Fig. 4(c). The NO_3^--N removal trends are shown in Table 4 and Fig. 4(c). Similarly, effluent NO_3^--N was slightly increased with an increase of NaCl concentration; effluent NO_3^--N of 1.04 ± 0.01, 1.09 ± 0.02, 1.12 ± 0.02, 1.24 ± 0.01 and 1.26 ± 0.01 mg/L was detected at NaCl concentrations of 0%, 2%, 6%, 10% and 14% (w/v), respectively.

3.1.4. Bio-sludge performance

Bio-sludge properties are shown in Table 5. The anoxic/oxic SBR system with STWW + DB15 and STWW + DB15 + nNaCl showed interesting results for bio-sludge properties; a good SVI and bio-sludge age of 57 ± 3 mL/g and 17 ± 1 d, respectively, were detected with STWW + DB15. But the performance declined with the addition of NaCl. Moreover, SVI and solids retention time (SRT) of the system were increased with an increase of NaCl concentration. SVI of lower than 100 mL/g was detected at NaCl of up to 6% (w/v), and of up to 140 ± 4 mL/g at an NaCl concentration of 14% (w/v). The highest SRT of 21 ± 1 d was detected at an NaCl concentration of 14% (w/v). The system showed low effluent suspended solids (SS) of 6–7 mg/L in all experiments tested, as shown in Table 5.

3.2. GC–MS analysis

GC–MS analysis was carried out to determine the metabolites of DB15 during the biodegradation process in the anoxic/oxic SBR system. Samples from anoxic and oxic periods of the reaction step after 35 d operation of the system with STWW + DB15 were collected to determine the metabolites of the biodegradation process. The extracted samples were injected into the GC–MS system. The mass spectrum of the anoxic period sample was identified as 1-amino-8-naphthol-3,6-disulfonic acid, shown at a retention time of 19.10 min and MW of 319.31 m/z, and 2-ethoxy-4-methylaniline, shown at a retention time of 12.431 min and MW of 151.20 m/z. The mass spectrum of the oxic period sample was identified as 2-ethoxy-4-methylaniline, shown at a retention time of 12.431 min and MW of 151.20 m/z and 2-naphthol, shown at a retention time of 11.668 min and MW of 144.17 m/z (Table 6).

4. Discussion

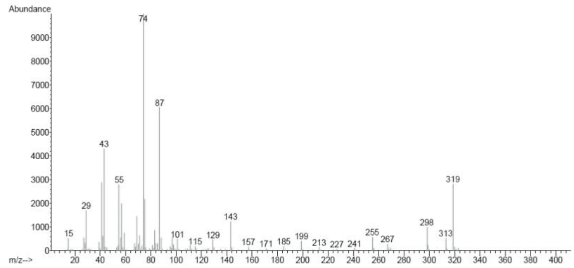
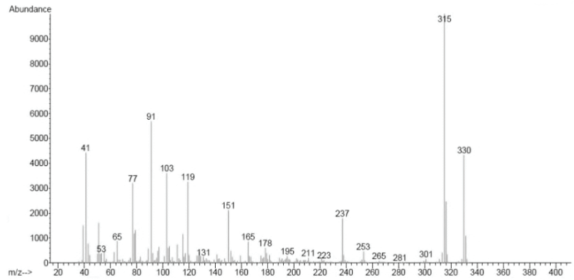
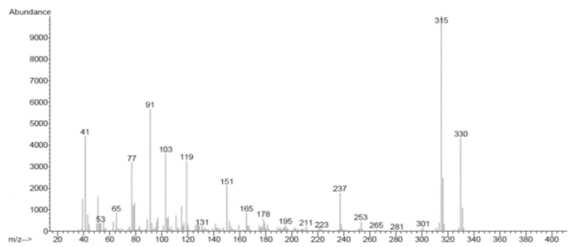
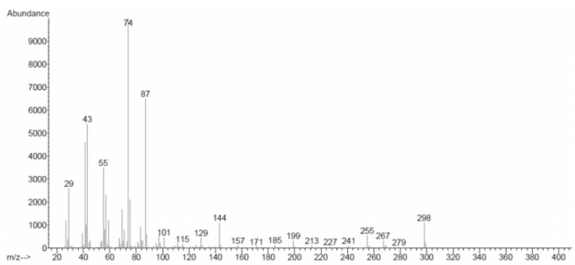
According to previous studies [2,17,18,20], an anoxic/oxic SBR system is most suitable for the treatment of textile industrial wastewater, resulting in high removal efficiencies, especially direct dyes of the azo group. An anoxic condition can increase textile dye removal efficiency without negative effects on organic removal efficiency as BOD_5 or COD [17,21,22], as confirmed by the results shown in Table 3 and Figs. 2 and 3. This might represent an advantage of an anoxic/oxic SBR system in treating textile wastewater, that is, low energy consumption compared with an oxic SBR system [7]. The system with STWW + DB15 showed the highest DB15 removal efficiency of 90.61% ± 0.82%. However, the removal efficiency was repressed by a high NaCl concentration of up to 10% (w/v). The DB15 removal efficiency dropped to 26.93% ± 1.42% with STWW + DB15 + 14NaCl. The other advantage of the anoxic/oxic SBR system is that DB15 removal occurred only at the anoxic period of the reaction step. The above information could suggest that nitrogenous compound removal bacteria showed higher color removal efficiency than heterotrophic carbonaceous compound bacteria, especially denitrifying bacteria [2,17,23]. The removal efficiency was slightly decreased at the oxic period of the reaction step. This is because the color removal ability consisted of adsorption and biodegradation mechanisms, and the adsorbed DB15 was desorbed from the bio-sludge, especially by denitrifying bacteria, under oxic conditions [20,22,24]. Moreover, the application of an anoxic period in the reaction step of the SBR operation (anoxic/oxic SBR system) could increase the nitrogenous compound removal efficiency, resulting in the promotion of nitrogenous compound removal bacteria, especially denitrifying bacteria, as shown in Table 4 and Fig. 4 [17,25].

However, the addition of a high concentration of NaCl of above 10% w/v in the textile wastewater could repress the anoxic/oxic SBR system efficiency, resulting in plasmolysis of the microbial cells of bio-sludge [8,9,26]. It was confirmed that the effluent SS of the system increased with an increase of NaCl concentration, as shown in Table 5. However, the system showed another interesting result: NaCl had a strongly repressive effect on the growth and activity of heterotrophic carbonaceous compound removal bacteria, resulting in a decrease of COD and BOD_5 removal efficiencies with an increase of NaCl concentration of up to 10% (w/v). However, NaCl only showed a slight effect on the growth and activity of nitrogenous compound removal bacteria, especially nitrifying bacteria [26–29]. The results shown in Table 4 and Fig. 4 indicate that effluent TKN increased with an increase of NaCl concentration, while effluent NO_3^--N of the system with STWW + DB15 and STWW + DB15 + nNaCl did not show any significant difference. The above results suggest that a high NaCl concentration had a more repressive effect on nitrifying bacteria than on denitrifying bacteria, resulting in a marked increase in effluent TKN, while there was no significant difference in the effect on effluent NO_3^--N for

Table 5
Effect of NaCl concentrations on bio-sludge properties and effluent SS of anoxic/oxic SBR system operated with STWW + DB15 + containing various NaCl concentrations of 0%, 2%, 6%, 10% and 14%

NaCl concentration (% w/v)	F/M ratio	MLSS (mg/L)	SVI (mg/L)	Bio-sludge age (d)	SS (mg/L)
0	0.13	2,118 ± 38	57 ± 3	17 ± 1	5.24 ± 1.1
2	0.13	2,116 ± 41	62 ± 2	17 ± 2	5.97 ± 1.6
6	0.13	2,114 ± 28	94 ± 2	18 ± 1	6.38 ± 1.2
10	0.13	2,100 ± 22	118 ± 4	20 ± 1	6.95 ± 1.4
14	0.13	2,094 ± 32	140 ± 3	21 ± 1	7.00 ± 1.0

Table 6
GC–MS data of metabolites obtained after biodegradation of DB15 in anoxic/oxic SBR system with STWW + DB15

Condition	Metabolites	Retention time (min)	MW. (m/z)[a]	Chromatogram
Anoxic	1-Amino-8-naphthol-3,6-disulfonic acid	19.10	319.31	
	2-Ethoxy-4-methylaniline	12.431	151.20	
Oxic	2-Ethoxy-4-methylaniline	12.431	151.20	
	2-Naphthol	11.668	144.17	

[a]m/z: mass/charge.

all NaCl concentrations tested [12,17,27,29–31]. This was in accordance with a report by Cortés-Lorenzo et al. [12], who found that NaCl of up to 24.1 g/L had a strongly repressive effect on the growth and activity of nitrifying bacteria. In contrast, 3.7 g/L NaCl did not have any significant effect on the nitrification process. Bassin et al. [32] also reported that the ammonia oxidation rate was not affected by an initial NaCl concentration of less than 10 g/L. Chen et al. [29] reported that an NaCl concentration of up to 18 g/L could promote saline-resistant species (*Nitrosococcus mobilis*) and repress non-saline-resistant species (*Nitrosomonas europaea* and *Nitrosomonas eutropha*).

The above results strongly confirm that an anoxic/oxic SBR system could be used for treatment of textile wastewater containing NaCl of up to 6% (w/v), with high removal efficiencies. And NaCl concentration of lower than 6% (w/v) might not affect bio-sludge activity; moreover, it could stimulate the growth of moderate halotolerant bacteria as the dominant species [12,29,32]. Another advantage of an anoxic/oxic SBR system might be that the system could be applied for treatment of textile wastewater containing a low NaCl concentration of up to 6% (w/v), with high removal efficiencies.

Observation of the biodegradation mechanism of DB15 in anoxic/oxic SBR system revealed interesting results. During the anoxic period of the reaction step, the azo group of DB15 was degraded to 1-amino-8-naphthol-3,6-disulfonic acid and 2-ethoxy-4-methylaniline, as shown in Table 6. Then, 1-amino-8-naphthol-3,6-disulfonic acid was further degraded to 2-naphthol (Table 6). This concurred with a report by Kalyani et al. [33], who found that the biodegraded product of azo dyes was 2-naphthol. Therefore, it could be concluded that the bio-sludge of the anoxic/oxic SBR system did not only break the azo bond of DB15 but also degraded 1-amino-8-naphthol-3,6-disulfonic acid to 2-naphthol. According to the results and information above, the proposed pathway for biodegradation of DB15, as depicted in Fig. 5, is that DB15 was adsorbed into the microbial cells of the bio-sludge [19,22,24]. Then the adsorbed DB15 was biodegraded under anoxic and oxic conditions in the anoxic/oxic SBR system [1,34]. In the first step of the degradation of DB15, azo-reductase cleaved the azo bond (N=N) of DB15 into 2-ethoxy-4-methylaniline and 1-amino-8-naphthol-3,6-disulfonic acid, which were the intermediates for the preparation of azo dyes [35]. Furthermore, amine oxidation and desulfonation of 1-amino-8-naphthol-3,6-disulfonic acid gave the final identified product in the form of a low MW compound, 2-naphthol. Previous works [1,34,35] reported that azo dye was degraded under anaerobic/oxic conditions, as follows. First, under anaerobic conditions, azo dye (N=N bond) was degraded by anaerobes into aromatic amines and was accumulated [15,34–36]. Under anaerobic conditions, the system showed higher decolorization yield than under oxic conditions, as a result of oxygen inhibiting azo bond reductase enzyme activity [15,34–37]. Second, those aromatic amines (intermediates of the anaerobic degradation period) were further mineralized to be less toxic under oxic conditions [15,37–42].

Many aerobes have the ability to mineralize various aromatic amines [42–46]. Bacterial degradation of aromatic amines under oxic conditions produces ammonia as an end product. Under aerobic conditions, dioxygenase could cleave the aromatic ring of aromatic amines and release ammonia ions as the end product [47].

The above results suggest that the azo bond of DB15 was first anoxic-degraded and produced aromatic amines as intermediates under anoxic conditions, a finding which differs from previous studies [15,37–42]. This report might be the first to find that azo dye was adsorbed into the bio-sludge, and then it was anoxic-degraded into 2-ethoxy-4-methylaniline and 1-amino-8-naphthol-3,6-disulfonic acid. Finally, the 1-amino-8-naphthol-3,6-disulfonic acid was further oxic-degraded into 2-naphthol.

Finally, the present findings demonstrate that an anoxic/oxic SBR system is eminently suitable for the treatment of textile wastewater containing azo dye, resulting in an increase in the number of nitrogenous compound removal bacteria, especially denitrifying bacteria. Another advantage of the system is that denitrifying bacteria showed higher saline (NaCl) resistance than nitrifying bacteria and heterotrophic carbonaceous compound removal bacteria. The system could remove nitrogenous compounds together with azo dye with a high removal efficiency. However, the salt concentration is an important consideration, because the system could operate with high removal efficiency only at an NaCl concentration of no higher than 6% (w/v).

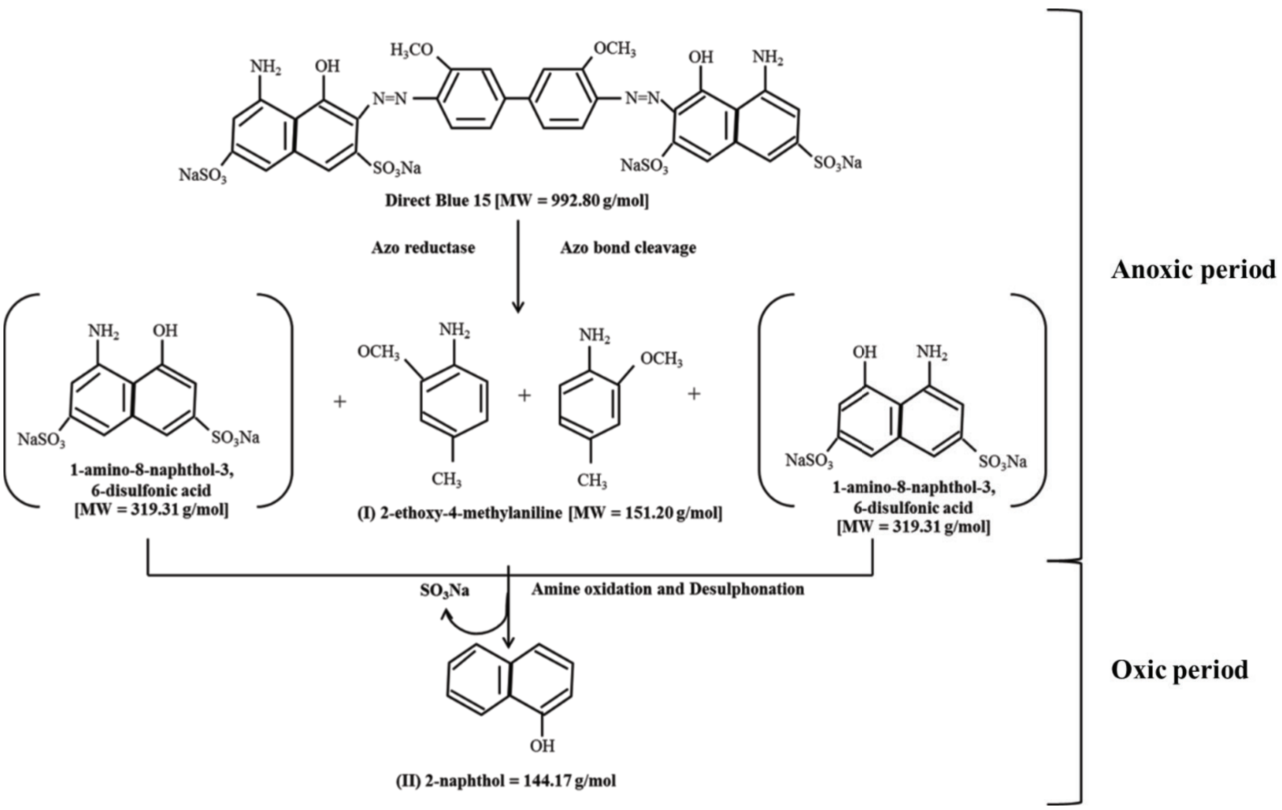


Fig. 5. Proposed pathway for biodegradation of DB15 in anoxic/oxic SBR system.

5. Conclusions

The anoxic/oxic SBR system is most suitable for application in the treatment of textile wastewater containing azo dye, as shown by the high removal efficiency. The system showed the highest BOD_5, COD, DB15, TN and TKN removal efficiencies of 96.29% ± 0.74%, 95.81% ± 0.30%, 90.61% ± 0.82%, 87.9% ± 0.24% and 85.03% ± 0.20%, respectively, for STWW + DB15 at HRT of 3 d and MLSS of 2,000 mg/L. However, the system efficiency was repressed by the addition of NaCl. Moreover, the removal efficiency was decreased with an increase of NaCl concentration. The lowest BOD_5, COD, DB15, TN and TKN removal efficiencies of 30.01% ± 3.48%, 33.26% ± 0.92%, 26.93% ± 1.42%, 51.49% ± 0.31% and 49.77% ± 0.87%, respectively, were detected for STWW + DB15 + 14NaCl. Nitrogenous compound removal bacteria, especially denitrifying bacteria, were the main microorganisms in the DB15 removal mechanism, which consisted of adsorption and biodegradation. During biodegradation, DB15 was degraded to 1-amino-8-naphthol-3,6-disulfonic acid and 2-ethoxy-4-methylaniline at the anoxic period of the reaction step; then 1-amino-8-naphthol-3,6-disulfonic acid was further degraded to 2-naphthol at the oxic period of the reaction step.

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Symbols

BOD_5	—	Biochemical oxygen demand
DB15	—	Direct Blue 15
COD	—	Chemical oxygen demand
GC–MS	—	Gas chromatography–mass spectroscopy
HRT	—	Hydraulic retention time
MLSS	—	Mixed liquor suspended solids
m/z	—	Mass to charge ratio
NH_4^+–N	—	Ammonium nitrogen
NO_3^-–N	—	Nitrate
NO_2^-–N	—	Nitrite
Org-N	—	Organic nitrogen
SBR	—	Sequencing batch reactor
SRT	—	Solids retention time
SS	—	Suspended solids
SVI	—	Sludge volume index
STWW	—	Synthetic textile wastewater
TKN	—	Total Kjeldahl nitrogen
TN	—	Total nitrogen
STWW + DB15	—	Synthetic textile wastewater containing Direct Blue 15
STWW + DB15 + nNaCl	—	STWW + DB15 containing various concentrations of NaCl

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