Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.21573

95 (2017) 208–219
November

Effect of anoxic conditions on the efficiency and bacterial diversity of bio-sludge in a sequencing batch reactor (SBR) system with wastewater containing Cr^{3+} and Ni^{2+}

Suntud Sirianuntapiboon[a,*], Methinee Rarunroeng[a], Kanidta Chairattanawan[b]

[a]*Department of Environmental Technology, School of Energy Environment and Materials, King Mongkut's University of Technology Thonburi, Bangmod, Thungkru, Bangkok 10140, Thailand, Tel. +66 1-662-4708656; Fax: +66 1-662-4279062, +66 1-662-4708660; email: suntud.sir@kmutt.ac.th (S. Sirianuntapiboon), meny_aum1@hotmail.com (M. Rarunroeng)*

[b]*Department of Applied Science, Office of General Education, Sripatum University, Phahonyothin Road, Chatuchak, Bangkok 10900, Thailand, Tel./Fax: +66 1-662-579-1111 Ext. 2217; email: kanidta.ch@spu.ac.th*

Received 17 May 2017; Accepted 18 October 2017

ABSTRACT

The efficiency and bacterial diversity of bio-sludge in oxic-sequencing batch reactor (SBR) and anoxic/oxic-SBR systems with synthetic industrial estate wastewater without heavy metals (SIWW) and SIWW containing 3.0 mg/L of Cr^{3+} or Ni^{2+} (SIWW + Cr^{3+} or SIWW + Ni^{2+}) at a mixed liquor suspended solids of 2,000 mg/L and a hydraulic retention time of 1.5 d were investigated. The highest Ni^{2+} and Cr^{3+} removal efficiencies of 93.1% ± 0.9% and 95.4% ± 0.2%, respectively, were detected in anoxic/oxic-SBR systems with SIWW + Cr^{3+} and SIWW + Ni^{2+}, respectively. The average Cr^{3+} and Ni^{2+} adsorption abilities of bio-sludge from the anoxic/oxic-SBR system were 24.0 ± 5.0 mg Ni^{2+}/g bio-sludge and 20.0 ± 4.0 mg Cr^{3+}/g bio-sludge, respectively. In addition, 3.0 mg/L of Cr^{3+} or Ni^{2+} had a strong repressive effect on the growth and activity of heterotrophic carbonaceous biochemical oxygen demand removal bacteria. However, the addition of an anoxic period in the reaction step increased heavy metal removal efficiency as a result of denitrifying bacteria. The other advantage of the anoxic/oxic-SBR system was that it showed high nitrogenous compound removal yields. To observe bacterial diversity in the anoxic/oxic-SBR system during operation, rDNA analysis technique was applied. It was found that the addition of 3.0 mg/L of Cr^{3+} or Ni^{2+} did not show significant negative effects on nitrifying and denitrifying bacteria, but some species disappeared from the system, particularly nitrifying bacteria, under anoxic conditions.

Keywords: Adsorption; Anoxic; Cr^{3+}; Ni^{2+}; Sequencing batch reactor; Oxic

1. Introduction

The advantages of the establishment of an industrial estate park are its suitability for resource utilization management, transportation systems, and waste and emission treatment and management [1,2]. Moreover, suitable areas for future exploitation, resources, and facilities required for operation, such as water, electricity, and waste treatment systems, can also be prepared [2,3]. As mentioned above, an industrial estate park should ideally consist of a single type of industry, such as food processing, textiles, or electronics. Unfortunately, some industrial estate parks often consist of various types of industries, for example (in Thailand), a food processing factory together with an electroplating factory [2,3]. In that case, the waste generated, especially wastewater, may contain not only organic matter but also inorganic matter such as heavy metals (HMs), resulting in difficult treatment and management [1,2]. Theoretically, a chemical treatment

* Corresponding author.

process is suitable for inorganic wastewater and a biological treatment process is suitable for organic wastewater [2,4–8]. Moreover, a typical biological wastewater treatment system such as a conventional activated sludge system [2] cannot be used for treatment of wastewater containing both organic and inorganic matter such as HMs due to the toxicity of HMs on the growth and activity of bio-sludge [2,9,10].

To date, many researchers have reported that HMs such as lead, cadmium, copper, and zinc could be absorbed by special microorganisms, for example, yeast, bacteria, fungi, or bio-sludge of an activated sludge systems [9–20]. However, the HM adsorption capacity depends upon the types of microbes and HMs under analysis [9,10,21–26]. According to the above information and that acquired from previous works, the application of a sequencing batch reactor (SBR) system to treat wastewater containing both organic matter and HMs is one way to easily modify the oxic and anoxic operating conditions in the reaction step. Doing so to the operating program stimulates specific microbes to treat each pollutant as biochemical oxygen demand (BOD_5), nitrogenous compounds, or HMs [9,10,26,27]. Moreover, previous works have reported that the nitrogen removal mechanism is related to HM removal ability [27,28], and that the main microbial group for the removal of HMs is nitrogenous removal bacteria, which function as nitrifying and denitrifying bacteria [27,28].

In this study, oxic-SBR and anoxic/oxic-SBR systems were treated with synthetic industrial estate wastewater without heavy metals (SIWW), SIWW + Cr^{3+}, and SIWW + Ni^{2+} to investigate the effect of anoxic conditions on HM removal efficiency. In addition, the bacterial diversity of bio-sludge in the anoxic/oxic-SBR system during operation was also investigated.

2. Materials and methods

2.1. Types of wastewater

Three types of synthetic industrial estate wastewater (SIWW) were prepared according to the composition of raw wastewater from Ladkrabang Industrial Estate, Bangkok, Thailand, which consists of wastewater from various types of industries, such as food processing, electroplating, and automobile industries [27]. The types of SIWW used in this study were as follows: SIWW without HMs (SIWW), SIWW containing 3.0 mg/L Ni^{2+} (SIWW + Ni^{2+}), and SIWW containing 3.0 mg/L Cr^{3+} (SIWW + Cr^{3+}). Chemical compositions of SIWWs are shown in Table 1.

2.2. Acclimatization of bio-sludge

Bio-sludge was collected from a bio-sludge storage tank at the Bangkok Municipal Sewage Treatment Plant (moderated activated sludge system type), Bangkok, Thailand (Sripaya Sewage Treatment Plant). This bio-sludge was acclimatized with SIWW at a hydraulic retention time (HRT) of 1.5 d for 10 d, which resulted to dominate the suitable microbes for SIWWs before using as an inoculum in the oxic-SBR and anoxic/oxic-SBR systems.

2.3. Sequencing batch reactor

Six 10 L SBR reactors made from 5-mm thick acrylic plastic were used in this study, as shown in Fig. 1. Each reactor had an 18 cm diameter and a 40 cm height, with a working volume of 7.5 L and a total volume of 10 L. Complete mixing in the reactor was adjusted by controlling the speed of the turbine-shaped impeller to 60 rpm. 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 for driving the impeller. One set of air pumps (model EK-8000, 6.0 W; President Co., Ltd., Thailand) were used for supplying air for two sets of reactors. (The system had about 2 or 3 mg/L of oxygen, as evidenced by the dissolved oxygen in the system.)

2.4. Operation of oxic-SBR and anoxic/oxic-SBR systems

SBR reactors were operated at 1 cycle/d under an HRT of 1.5 d. Exactly 1.4 L of 10 g/L acclimatized bio-sludge (10,000 mg/L solid content) was inoculated in each reactor, and SIWW, SIWW + Ni^{2+}, or SIWW + Cr^{3+} were added (final volume of 7.5 L) within 1 h. The operating program was 24 h, as mentioned above, including 1 h for the filling step, 19 h for the reaction step, and 3 h for the settling step. After the bio-sludge was fully settled, the supernatant was drawn out within 0.5 h and the system was kept under anoxic conditions (idle) for 0.5 h. After that, the reactor was filled with fresh wastewater to a volume of 7.5 L and the operations were repeated. Excess bio-sludge was removed during the draw and idle periods to control the mixed liquor suspended solids (MLSS) of the system (Table 2). For the reaction step, two operating conditions were applied: the reaction step, which consisted of an anoxic/oxic/anoxic/oxic sequence of 5/5/5/4 h (this system was called an anoxic/oxic-SBR system) [27]; and the reaction step, which was fully aerated for 19 h (this system was called an oxic-SBR system), as shown in Table 2 [27]. Samples (both effluent and bio-sludge) were taken for chemical analysis and analysis of bio-sludge performance during operation, as shown in Table 2.

2.5. Chemical analysis

BOD_5, chemical oxygen demand (COD), organic-N, NH_4^+–N, NO_3^-–N, NO_2^-–N, Cr^{3+}, Ni^{2+}, suspended solids (SS), total dissolved solids (TDS), and the pH of the influent and effluent of both SBR systems were determined according to standard methods for the examination of water and wastewater [29]. MLSS was determined by weighing the total SS of the mixed liquor (dry basis) from the SBR reactor. Mixed liquor volatile suspended solids (MLVSS) was the organic matter of MLSS. Moreover, MLVSS was the weight loss of MLSS after being burned at 550°C in a muffle furnace. Total nitrogen (TN) was the summation of organic-N, NH_4^+–N, NO_3^-–N, and NO_2^-–N. The bio-sludge volume index (SVI) was determined as the volume (mL) per gram of bio-sludge. Bio-sludge age was determined by the ratio of total biomass (as MLVSS) of the system to the amount of excess bio-sludge wasted per day.

2.6. Collecting and pretreatment of bio-sludge samples

Bio-sludge samples were collected from reactors of both oxic- and anoxic/oxic-SBR systems at 0 and 30 d of operation. Bio-sludge samples were centrifuged at 6,000 × *g* for 20 min. The cell pellets were collected and stored at −20°C until used for DNA extraction.

Table 1
Chemical compositions and properties of various types of synthetic industrial wastewaters (SIWWs)

Chemical properties			
Parameters	SIWW[a]	SIWW + Ni^{2+}[b]	SIWW + Cr^{3+}[c]
COD (mg/L)	480 ± 7	480 ± 6	480 ± 5
BOD_5 (mg/L)	230 ± 3	230 ± 3	230 ± 3
Organic-N (mg/L)	8.2 ± 0.3	8.1 ± 0.4	8.1 ± 0.4
NH_4^+–N (mg/L)	8.4 ± 0.2	8.5 ± 0.2	8.5 ± 0.2
NO_2^-–N (mg/L)	2.4 ± 0.2	2.2 ± 0.2	2.2 ± 0.2
NO_3^-–N (mg/L)	5.2 ± 0.3	5.3 ± 0.3	5.3 ± 0.3
TN (mg/L)	24.2 ± 0.3	24.1 ± 0.3	24.1 ± 0.3
Ni^{2+} (mg/L)	0.00 ± 0.00	3.04 ± 0.06	0.00 ± 0.00
Cr^{3+} (mg/L)	0.00 ± 0.00	0.00 ± 0.00	3.02 ± 0.04
SS (mg/L)	11 ± 4	21 ± 3	21 ± 3
Organic/SS	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Inorganic/SS	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
TDS (mg/L)	341 ± 20	354 ± 4	383 ± 43
Organic/TDS	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Inorganic/TDS	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Chemical composition (mg/L)			
Glucose	282	282	282
$Cr(NO_3)_3.9H_2O$	–	–	18.0
$NiCl_2$	–	6.6	–
Urea	21.4	21.4	21.4
KH_2PO_4	8.72	8.72	8.72
$FeSO_4.7H_2O$	4.978	4.978	4.978

[a]SIWW: synthetic industrial estate wastewater without heavy metals.
[b]SIWW + Ni^{2+}: SIWW containing 6.6 mg/L $NiCl_2$.
[c]SIWW + Cr^{3+}: SIWW containing 18.0 mg/L $Cr(NO_3)_3.9H_2O$.

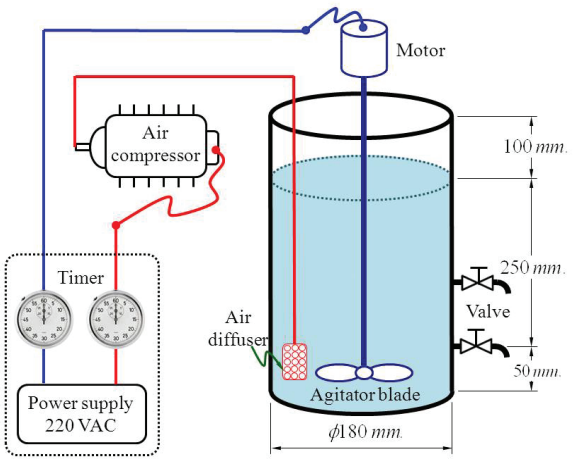


Fig. 1. Schematic diagram of the SBR system.

2.7. DNA extraction and purification

Total DNA was extracted from 5 g (wet weight) of cell pellet samples using a modified sodium dodecyl sulfate-based DNA extraction method [30]. Genomic DNA was purified further by using a Gel/PCR DNA Fragments Extraction Kit (Geneaid, Taiwan). Purified DNA was determined by agarose gel electrophoresis (0.8% agarose) and UV visualization, after ethidium bromide (EB) staining.

2.8. PCR amplification of 16S rRNA gene

Purified DNA was amplified by polymerase chain reaction using nucleotide sequences of universal primers [338F, 5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG ACT CCT ACG GGA GGCA-3′; 518R, 5′-ATTACCGCGGCTGCTGG-3′] [31]. The thermocycle program included an initial denaturing at 95°C for 10 min, followed by 30 cycles of denaturing at 95°C for 30 s, annealing at 60°C for 1 min, 72°C for 30 s, and a final extension step at 72°C for 7 min. The PCR reaction was performed in a total volume of 50 μL, containing 50 ng of the DNA template, 10 mM of each primer, 0.2 mM dNTPs, 3 mM $MgCl_2$, and 1U *Taq* polymerase (Qiagen, Germany). The amplified products were visualized on 1% agarose gel stained with EB.

Table 2
The operating of the SBR system used for treating SIWWs

Parameter	SIWW	SIWW + Ni^{2+}	SIWW + Cr^{3+}
HRT (d)	1.5	1.5	1.5
MLSS (mg/L)	2,000	2,000	2,000
Flow rate (mL/d)	5	5	5
F/M ratio	0.075	0.075	0.075
BOD_5 loading (g/d)	1.15	1.15	1.15
Volumetric BOD_5 loading (kg BOD_5/m^3 d)	0.15	0.15	0.15
Volumetric Ni^{2+}loading (g Ni^{2+}/m^3 d)	0.00	2.02	0.00
Volumetric Cr^{3+}loading (g Cr^{3+}/m^3 d)	0.00	0.00	2.01

Note: Each operation cycle of SBR system was 24 h. Each cycle consisted of four steps as fill up step, reaction step, settling step, and draw and idle step, consecutively.

The total period of reaction step about 19 h. On the reaction step operation was controlled to be oxic and anoxic consecutively as below:

One cycle of operation (24 h) Step of operation (h)	On the reaction step of SBR operation Anoxic:oxic ratio (h)	
	10:9 (Anoxic/oxic-SBR system) The system was operated as anoxic[a]/oxic/anoxic/oxic condition consequence in the reaction step	0:19 (Oxic-SBR system) The system was operated as oxic condition[a] in the reaction step
1: Fill	1	1
2: React	5/5/5/4 Anoxic[b]/oxic/anoxic/oxic	19 Oxic[b]
3: Settling[a]	3	3
4: Draw and idle	1	1

[a]The samples were taken for determined the chemical properties at settling step only.
[b]The samples were taken after each operation step to analyze after centrifugation at 6,000 × *g* as follows: (1) The supernatants of the samples were for chemical properties analysis. (2) The bio-sludge of the samples for heavy metals was analyzed.

2.9. Analyses of PCR products by DGGE

Denaturing gradient gel electrophoresis (DGGE) was performed with a DGGE-2000 system (CBS Scientific Company, Del Mar, CA). PCR products were loaded onto 8% (w/v) polyacrylamide gels in a 1× TAE buffer made with a denaturing gradient ranging from 50% to 70% (where 100% denaturant contains 7 M urea and 40% formamide). Electrophoresis was performed at 60°C for 14 h at 80 V. After electrophoresis, the gels were stained for 15 min with EB, visualized with a UV transilluminator and captured using BioVision CN 1000/26M (Vilber Lourmat, France). The target DGGE bands were excised, suspended overnight in 20 μL of Milli-Q water, and stored at 4°C. The DNA fragments recovered from the gels were used as templates for re-amplification, using a primer set without a GC clamp. The amplified PCR products were purified and sequenced by First BASE (Malaysia). The sequences obtained were compared with sequences in the BLAST/NCBI database (http://www.ncbi.nlm.nih.gov/BLAST) to determine their approximate phylogenetic affiliations [32].

2.10. Statistical analysis

Each experiment was repeated three times. All data were subjected to two-way analysis of variance using SAS for Windows version 6.12 [33]. Statistical significance was tested using the least significant difference at a level of $p < 0.05$. The results shown are mean ± standard deviation.

3. Results

The bacterial distribution and diversity of bio-sludge and bio-sludge efficiency, as well as the performance of oxic-SBR and anoxic/oxic-SBR systems, were investigated with SIWW, SIWW + Ni^{2+}, and SIWW + Cr^{3+} at MLSS of 2,000 mg/L and HRT of 1.5 d for 30 d.

3.1. BOD_5 and COD

The anoxic/oxic-SBR system showed higher BOD_5 and COD removal efficiencies than the oxic-SBR system with the same operating conditions, as shown in Table 3. Anoxic/oxic-SBR system removal efficiencies were 5% higher

Table 3
COD and BOD_5 removal efficiencies and effluent properties of oxic-SBR and anoxic/oxic-SBR systems with SIWWs

Type of wastewater	Type of SBR system	Oxic: anoxic	BOD_5		COD		Effluent qualities			
			Effluent (mg/L)	Removal (%)	Effluent (mg/L)	Removal (%)	SS (mg/L)	Organic/SS	TDS (mg/L)	Organic/TDS
SIWW[a]	Oxic-SBR	19:0	35 ± 2	85 ± 1	75 ± 15	85 ± 1	13 ± 2	0.7 ± 0.1	160 ± 5	0.4 ± 0.1
SIWW + Ni^{2+}[b]		19:0	69 ± 7	70 ± 3	120 ± 14	75 ± 3	23 ± 3	0.6 ± 0.1	150 ± 3	0.6 ± 0.1
SIWW + Cr^{3+}[c]		19:0	65 ± 3	72 ± 2	120 ± 5	75 ± 2	24 ± 3	0.6 ± 0.1	148 ± 2	0.5 ± 0.1
SIWW[a]	Anoxic/ oxic-SBR	9:10	26 ± 2	89 ± 1	48 ± 2	90 ± 1	15 ± 2	0.7 ± 0.0	130 ± 7	0.6 ± 0.1
SIWW + Ni^{2+}[b]		9:10	40 ± 3	83 ± 1	82 ± 4	83 ± 1	25 ± 3	0.5 ± 0.1	150 ± 4	0.6 ± 0.1
SIWW + Cr^{3+}[c]		9:10	46 ± 3	80 ± 1	82 ± 3	83 ± 1	24 ± 4	0.5 ± 0.1	125 ± 10	0.6 ± 0.1

[a]SIWW: synthetic industrial estate wastewater without heavy metals.
[b]SIWW + Ni^{2+}: SIWW containing 6.6 mg/L $NiCl_2$.
[c]SIWW + Cr^{3+}: SIWW containing 18.0 mg/L $Cr(NO_3)_3.9H_2O$.

compared with the oxic-SBR system. Moreover, the addition of HM (Ni^{2+} or Cr^{3+}) in the SIWW (SIWW + Ni^{2+} and SIWW + Cr^{3+}) had a more positive effect on BOD_5 and COD removal efficiencies in the oxic-SBR system than in the anoxic/oxic-SBR system. BOD_5 and COD removal efficiencies of the system with SIWW + Ni^{2+} and SIWW + Cr^{3+} were 10% higher than with SIWW for the same operating conditions (Table 3).

3.2. Nitrogenous compounds

The anoxic/oxic-SBR and oxic-SBR systems with SIWWs showed interesting results for TN removal efficiencies, as shown in Table 4. TN removal efficiency of oxic-SBR systems was decreased concomitantly by adding HM (Ni^{2+} or Cr^{3+}). Interestingly, TN removal efficiencies of the oxic-SBR system with SIWW + Cr^{3+} or SIWW + Ni^{2+} were 20% and 26% lower than with SIWW. On the other hand, Ni^{2+} or Cr^{3+} did not have strong negative effects on the nitrogenous compound removal efficiency of the anoxic/oxic-SBR system. The TN removal efficiency of the anoxic/oxic-SBR system was two times higher than that of the oxic-SBR system in all experiments (Table 4). Moreover, in the oxic-SBR system, effluent NO_3^-–N was about 80% higher than influent NO_3^-–N in all experiments. Also, the effluents NH_4^+–N and NO_3^-–N of the anoxic/oxic-SBR system were lower compared with the oxic-SBR system in all experiments, as shown in Table 4. HM did not show any negative effect on nitrogenous compound removal efficiencies.

3.3. HM ($Cr^{3+}$and Ni^{2+})

The anoxic/oxic-SBR system showed higher HM removal efficiencies than the oxic-SBR system. The Cr^{3+} and Ni^{2+} removal efficiencies of the anoxic/oxic-SBR system were 95.4% ± 0.2% and 93.1% ± 0.9%, respectively (Table 5). Moreover, the bio-sludge of the anoxic/oxic-SBR system showed higher HM adsorption yields than that of the oxic-SBR system. The bio-sludge of the oxic-SBR system showed maximum Cr^{3+} and Ni^{2+} adsorption yields of 17.2 mg Cr^{3+}/g of bio-sludge and 14.5 mg Ni^{2+}/g of bio-sludge, respectively. In contrast, the bio-sludge of the anoxic/oxic-SBR system showed maximum Cr^{3+} and Ni^{2+} adsorption yields of 30.2 mg Cr^{3+}/g of bio-sludge and 25.7 mg Ni^{2+}/g of bio-sludge, respectively.

3.4. SS and TDS

The amount of effluent SS in SIWWs increased by adding HM (Cr^{3+} and Ni^{2+}), as shown in Table 3. In addition, anoxic conditions in the reaction step did not have any negative effect on the effluent SS. The effluent SS of the anoxic/oxic-SBR and oxic-SBR systems was almost the same for the same operating conditions (Table 3). Unfortunately, the organic content of the effluent SS in the systems with SIWW + Ni^{2+} and SIWW + Cr^{3+} was lower than with SIWW. Moreover, the organic content of the effluent SS of the anoxic/oxic-SBR system was lower than that of the oxic-SBR system. For TDS observation, neither system showed any significant effect on effluent TDS, which were in the range of 125–160 mg/L. However, the organic content of effluent TDS in the anoxic/oxic-SBR system was higher than that of the oxic-SBR system (Table 3).

3.5. Bio-sludge performances

Cr^{3+} and Ni^{2+} in the SIWW + HM system increased the sludge retention time (SRT). The SRTs of the oxic-SBR system with SIWW, SIWW + Ni^{2+}, and SIWW+Cr^{3+} were 6 ± 1, 9 ± 2, and 9 ± 1 d, respectively. In contrast, they were 9 ± 1, 14 ± 1, and 14 ± 1 d for the anoxic/oxic-SBR system, as shown in Table 5. Moreover, the SVIs of the oxic-SBR system with SIWW, SIWW + Ni^{2+}, and SIWW + Cr^{3+} were 95 ± 2, 130 ± 6, and 150 ± 9 mL/g, respectively. The MLVSS/MLSS of the systems with SIWW was higher compared with SIWW + HM (SIWW + Ni^{2+} or SIWW + Cr^{3+}) in all experiments (Table 5).

3.6. Microbial distribution

An analysis of bacterial distribution in the bio-sludge of the anoxic/oxic-SBR system with SIWW, SIWW + Ni^{2+}, and SIWW + Cr^{3+} was also carried out. It was found that the number and type of bacteria in the bio-sludge of the anoxic/oxic-SBR system with all types of wastewater were reduced after cultivation for 30 d. Interestingly, some species of bacteria disappeared in the process, as shown in Tables 6–8.

Table 4
Nitrogenous compounds removal efficiencies and effluent properties of oxic-SBR and anoxic/oxic-SBR systems with SIWWs

Type of wastewater	Type of SBR system	Oxic: anoxic	Organic-N (mg/L)		NH_4^+–N (mg/L)		NO_2^-–N (mg/L)		NO_3^-–N (mg/L)		TN	
			Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Effluent (mg/L)	Removal (%)
SIWW[a]	Oxic-SBR	19:0	8.2 ± 0.3	3.3 ± 0.1	8.4 ± 0.2	4.4 ± 0.5	2.4 ± 0.2	1.4 ± 0.1	5.2 ± 0.2	9.3 ± 0.6	18.3 ± 0.5	24.1 ± 3.0
SIWW + Ni^{2+}[b]		19:0	8.2 ± 0.3	3.8 ± 0.4	8.4 ± 0.2	5.5 ± 0.5	2.4 ± 0.2	1.4 ± 0.2	5.2 ± 0.2	8.8 ± 0.7	19.4 ± 0.8	19.7 ± 3.5
SIWW + Cr^{3+}[c]		19:0	8.2 ± 0.3	3.7 ± 0.1	8.4 ± 0.2	6.2 ± 0.5	2.4 ± 0.2	1.5 ± 0.1	5.2 ± 0.2	9.1 ± 0.4	20.2 ± 0.8	18.2 ± 3.3
SIWW[a]	Anoxic/oxic-SBR	9:10	8.2 ± 0.3	4.1 ± 0.3	8.4 ± 0.2	3.6 ± 0.2	2.4 ± 0.2	1.4 ± 0.1	5.2 ± 0.2	4.3 ± 0.1	13.4 ± 0.3	44.0 ± 2.0
SIWW + Ni^{2+}[b]		9:10	8.2 ± 0.3	4.5 ± 0.2	8.4 ± 0.2	3.8 ± 0.1	2.4 ± 0.2	1.2 ± 0.2	5.2 ± 0.2	5.1 ± 0.2	14.4 ± 0.2	40.0 ± 1.0
SIWW + Cr^{3+}[c]		9:10	8.2 ± 0.3	4.8 ± 0.4	8.4 ± 0.2	3.9 ± 0.1	2.4 ± 0.2	1.3 ± 0.2	5.2 ± 0.2	5.1 ± 0.2	15.1 ± 0.4	38.0 ± 2.0

[a]SIWW: synthetic industrial estate wastewater without heavy metals.
[b]SIWW + Ni^{2+}: SIWW containing 6.6 mg/L $NiCl_2$.
[c]SIWW + Cr^{3+}: SIWW containing 18.0 mg/L $Cr(NO_3)_3.9H_2O$.

Table 5
Heavy metals and bio-sludge properties of oxic-SBR and anoxic/oxic-SBR systems with SIWWs

Type of wastewater	Type of SBR system	Oxic: anoxic	Nickel (Ni^{2+})				Chromium (Cr^{3+})				Bio-sludge properties			
			Effluent (mg/L)	Removal (%)	Bio-sludge (mg/g bio-sludge)		Effluent (mg/L)	Removal (%)	Bio-sludge (mg/g bio-sludge)		F/M	MLVSS/MLSS	Bio-sludge age (d)	SVI (mL/g)
					Average ($\bar{x} \pm SD$)	Maximum			Average ($\bar{x} \pm SD$)	Maximum				
SIWW[a]	Oxic-SBR	19:0	–	–	–	–	–	–	–	–	0.075	0.8 ± 0.1	6 ± 1	95 ± 2
SIWW + Ni^{2+}[b]		19:0	0.35 ± 0.05	88.5 ± 1.5	11.7 ± 6.0	14.5	–	–	–	–	0.075	0.7 ± 0.1	9 ± 2	130 ± 6
SIWW + Cr^{3+}[c]		19:0	–	–	–	–	0.30 ± 0.05	90.0 ± 1.0	11.5 ± 3.0	17.2	0.075	0.7 ± 0.1	9 ± 1	150 ± 9
SIWW[a]	Anoxic/oxic-SBR	9:10	–	–	–	–	–	–	–	–	0.075	0.8 ± 0.0	9 ± 1	80 ± 4
SIWW + Ni^{2+}[b]		9:10	0.20 ± 0.03	93.4 ± 1.0	20.0 ± 4.0	25.7	–	–	–	–	0.075	0.7 ± 0.0	14 ± 1	95 ± 5
SIWW + Cr^{3+}[c]		9:10	–	–	–	–	0.15 ± 0.00	95.0 ± 1.0	24.0 ± 5.0	30.2	0.075	0.7 ± 0.0	14 ± 1	95 ± 3

[a]SIWW: synthetic industrial estate wastewater without heavy metals.
[b]SIWW + Ni^{2+}: SIWW containing 6.6 mg/L $NiCl_2$.
[c]SIWW + Cr^{3+}: SIWW containing 18.0 mg/L $Cr(NO_3)_3.9H_2O$.

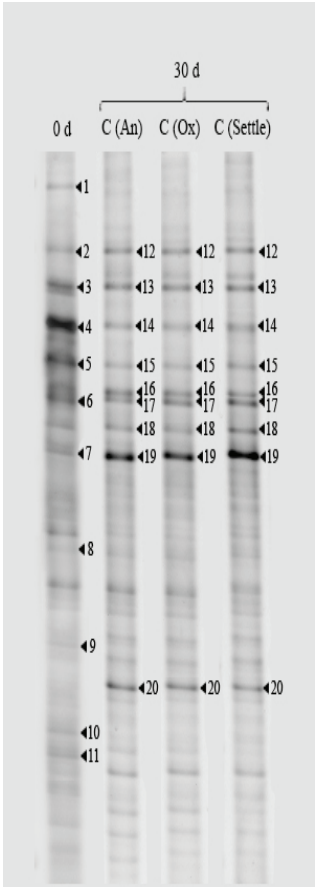
However, the sequence of excited bands of mixed liquor in the anoxic/oxic-SBR system with SIWW did not show any significant difference during the anoxic, oxic, and settling periods, as shown in Table 6. Nitrifying bacteria were the types of bacterial species that disappeared the most (Table 6). Populations of *Pseudomonas* spp., *Cytophaga* spp., and *Flavobacterium* spp. were also reduced. However, adding an anoxic period in the reaction step of the system (anoxic/oxic-SBR system) with SIWW + Ni^{2+} and SIWW + Cr^{3+} did not have a significant effect on bacterial distribution. The types of bacteria were almost the same with the three types of wastewater tested, as shown in Tables 6–8.

4. Discussion

In previous studies [3,9,10,26,27], the use of oxic and anoxic/oxic-SBR systems to treat wastewater containing both organic matter and HMs was carried out to observe the highest HM removal efficiency and bacterial distribution of bio-sludge. However, the operating conditions and types of microbes also need to be considered for optimal HM removal efficiency [3,20,27]. Unfortunately, HMs had a repressive effect on the growth and activity of microbes, with each type of HM showing different levels of repression [3,6,34–37]. It was confirmed that the BOD_5 and COD removal efficiencies of the oxic-SBR system with SIWW + Ni^{2+} and SIWW + Cr^{3+} were lower than that with SIWW, as a result of the strong repressive effect of HMs such as Ni^{2+} and Cr^{3+} on the growth and activity of heterotrophic carbonaceous BOD_5 removal bacteria [3,27]. In addition, the effluent SS with SIWW + HM was higher than that with SIWW, which was due to the dead heterotrophic bacterial cell residue [3,20,27]. It was strongly confirmed that the organic content of the TDS with SIWW + HM was higher than that with SIWW, which resulted from the organic matter which was released from dead heterotrophic carbonaceous BOD_5 removal bacteria [2]. Moreover, the organic content in the effluent SS with SIWW + HM was lower than that with SIWW, which was caused by the HM (Ni^{2+} or Cr^{3+}) content of the effluent SS. However, the addition of an anoxic period in the reaction step of the operating process (anoxic/oxic-SBR system) increased the removal of organic matter, such as BOD_5, COD, and nitrogenous compounds together with HMs, as shown in Tables 4–6. It was confirmed that 3.0 mg/L of HM as Ni^{2+} or Cr^{3+} could repress the growth and activity of heterotrophic carbonaceous BOD_5 removal bacteria, while it did not show significant repressive effect on nitrogenous compound removal bacteria (nitrifying and denitrifying bacteria). Both nitrifying and denitrifying bacteria showed higher HM adsorption ability than heterotrophic carbonaceous BOD_5 removal bacteria. It was also confirmed that the decrease of heterotrophic carbonaceous BOD_5 removal bacteria resulted in an increase in the bio-sludge age [2,3,8], because the specific growth rate of the heterotrophic carbonaceous BOD_5 removal bacteria was higher than that of the nitrogenous compound removal bacteria [2,3,27,38]. Moreover, the addition of an anoxic period in the reaction step of the operating program (anoxic/oxic-SBR system) increased the number of denitrifying bacteria, which increased TN removal efficiency [2,3,27]. Importantly, denitrifying bacteria were the main bacterial group for the HM adsorption mechanism [3,10,27]. From the above information, it could be concluded that the greatest advantage of the anoxic/oxic-SBR system was that it simultaneously stimulated and increased the number of specific bacterial strains that could remove nitrogenous compounds and HM [2,3,10,14,18,25,27,39,40]. This could suggest that the specific bacteria strains were nitrifying and denitrifying bacteria; however, denitrifying bacteria showed the highest HM adsorption ability [2,3,8]. Unfortunately, HMs produced more negative effects on the growth and activity of heterotrophic carbonaceous BOD_5 removal bacteria than on those of nitrogenous BOD_5 removal bacteria (nitrifying and denitrifying bacteria, as mentioned above) [2,3,9,10,27]. BOD_5 and COD removal efficiencies were reduced by the addition of HMs; but the anoxic/oxic-SBR system could be used to increase carbonaceous BOD_5 removal efficiency, as a result of the detoxification of HMs by nitrogenous BOD_5 removal microbes [2,27]. It was confirmed that the adsorbed-HMs in the bio-sludge of the anoxic/oxic-SBR system was higher than that of bio-sludge of the oxic-SBR system. As has been noted in previous studies, HM removal efficiency depended on the type of HM. It was confirmed in this study that both SBR systems showed higher Cr^{3+} removal efficiency than Ni^{2+} removal efficiency. Since Cr^{3+} (molecular weight 51.9962, atomic number 24) has a smaller molecular size than Ni^{2+} (molecular weight 58.6934, atomic number 28) [41], Cr^{3+} could more easily be adsorbed onto the bio-sludge than Ni^{2+}. Interestingly, this was confirmed by the fact that the Cr^{3+} adsorption ability of bio-sludge with SIWW + Cr^{3+} was 23.18 ± 08.64 mg/g bio-sludge, while Ni^{2+} adsorption ability of bio-sludge with SIWW + Ni^{2+} was only 14.49 ± 8.94 mg/g bio-sludge, as shown in Table 5. Moreover, the MLVSS/MLSS with SIWW + HM was lower than with SIWW, as a result of the HMs as Ni^{2+} and Cr^{3+} that were adsorbed onto the bio-sludge, as shown in Table 5. The above results suggest that the anoxic/oxic-SBR system was the most suitable to treat organic wastewater containing HMs.

To confirm the above results and suggestions, a bacterial distribution determination by the rDNA analysis technique was carried out. It was found that the types of microbes in the bio-sludge of the anoxic/oxic-SBR system with SIWWs were reduced after 30 d cultivation. Also, the types of bacteria with SIWW + HM were less than that with SIWW, as shown in Tables 6–8. Moreover, the BOD_5 and COD removal efficiencies were reduced by adding Cr^{3+} or Ni^{2+} in the wastewater, as mentioned above. As noted, HM as Cr^{3+} or Ni^{2+} provided a strongly repressive effect on the heterotrophic carbonaceous BOD_5 removal bacteria [13,20,27,35]. Moreover, the addition of 3.0 mg/L of Cr^{3+} or Ni^{2+} did not show significant negative effects on the type and number of nitrogen removal bacterial groups (both nitrifying and denitrifying bacteria), as shown in Tables 6 and 7. Unfortunately, some species disappeared from the system, particularly nitrifying bacteria such as *Pseudomonas* spp., *Cytophaga* spp., *Flavobacterium* spp., etc., under anoxic conditions. This could suggest that the anoxic/oxic-SBR system was most suitable for treatment of organic wastewater containing HM as Cr^{3+} or Ni^{2+}. Moreover, it confirmed that nitrifying and denitrifying bacteria were the main bacterial groups with HM adsorption ability and that denitrifying bacteria had higher HM adsorption ability than nitrifying bacteria.

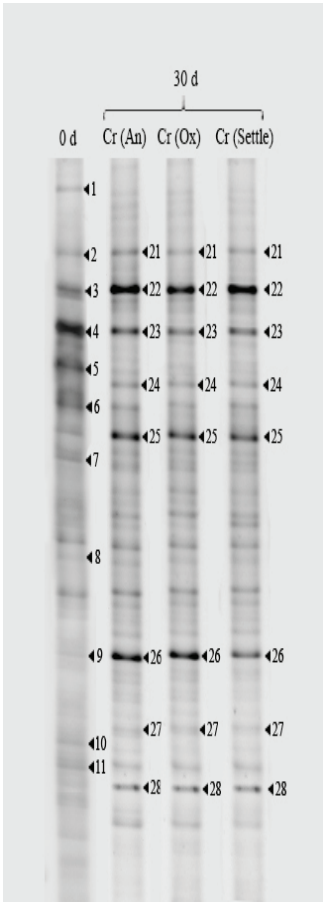
Table 6
The analysis of the sequenced excised bands of 30 d cultivated mixed liquor of anoxic/oxic-SBR system with SIWW on the DGGE by NCBI blast



Bands no.	Strain	Eubacterial classification	Type of gram bacteria	Microbial communities	Operation time (d)			
					0	30 (An[a])	30 (Ox[b])	30 (Settle[c])
1	*Pseudomonadales pseudomonas* sp. 32-rlima2	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
2	*Pseudomonadales pseudomonas* sp. 32-rlima2	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
3,12,13,14, 15,16,17,19	Uncultured *bacterium*	Division TM7	Positive	Anaerobic digestion	+	+	+	+
4	*Pseudomonadales* clone PPW-294	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
5	*Pseudomonadales* clone PPW-294	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
6	*Cytophagales flectobacillus* sp. LR16	Bacteroidetes	Negative	Anaerobic bacteria	+	–	–	–
7	*Flavobacteriales flavobacterium, Gillisia illustrilutea* strain IC157	Bacteroidetes	Negative	Anaerobic bacteria	+	–	–	–
8	*Rhodocyclales thauera* sp. JPB-3.02	β-Proteobacteria	Negative	Ammonia oxidizing bacteria	+	–	–	–
9,20	*Actinomycetales, Micropruina glycogenica* strain Lg2	Actinobacteria	Positive	Phosphorus removed activity	+	+	+	+
10	*Rhizobiales rhizobium* sp. MBJ2	α-Proteobacteria	Negative	Nitrogen-fixing bacteria	+	–	–	–
11	*Cellulomonas, Cellulosimicrobium* sp. I37.1	Actinobacteria	Positive	Degrade cellulose	+	–	–	–
18	Uncultured *bacterium* clone FS45	Unclassified bacteria	Positive	Facultative bacteria or anaerobic bacteria	–	+	+	+

Remark: +, Visible band; –, no visible band.
[a]Anoxic step; [b]Oxic step; [c]Settle step.

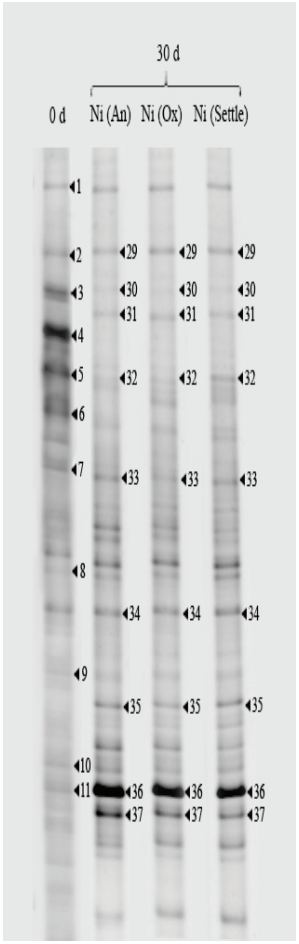
Table 7
The analysis of the sequenced excised bands of 30 d cultivated mixed liquor of anoxic/oxic-SBR system with SIWW + Cr^{2+} on DGGE by NCBI blast



Bands no.	Strain	Eubacterial classification	Type of gram bacteria	Microbial communities	Operation time: d (condition in the reaction step)			
					0 d	30 (An[a])	30 (Ox[b])	30 (Settle[c])
1	*Pseudomonadales pseudomonas* sp. 32-rlima2	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
2	*Pseudomonadales pseudomonas* sp. 32-rlima2	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
3,21,22, 23,24	Uncultured *bacterium*	Division TM7	Positive	Anaerobic digestion	+	+	+	+
4	*Pseudomonadales* clone PPW-294	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
5	*Pseudomonadales* clone PPW-294	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
6	*Cytophagales flectobacillus* sp. LR16	Bacteroidetes	Negative	Anaerobic bacteria	+	–	–	–
7	*Flavobacteriales flavobacterium, Gillisia illustrilutea* strain IC157	Bacteroidetes	Negative	Anaerobic bacteria	+	–	–	–
8	*Rhodocyclales thauera* sp. JPB-3.02	β-Proteobacteria	Negative	Ammonia oxidizing bacteria	+	–	–	–
9,26	*Actinomycetales, Micropruina glycogenica* strain Lg2	Actinobacteria	Positive	Phosphorus removed activity	+	+	+	+
10	*Rhizobiales rhizobium* sp. MBJ2	α-Proteobacteria	Negative	Nitrogen-fixing bacteria	+	–	–	–
11	*Cellulomonas, Cellulosimicrobium* sp. I37.1	Actinobacteria	Positive	Degrade cellulose	+	–	–	–
25	Uncultured *bacterium* clone FS45	Unclassified bacteria	Positive	Facultative bacteria or anaerobic bacteria	–	+	+	+
27	*Actinomycetales micropruina* clone F5K2Q4C04H50GM	Actinobacteria	Positive	Phosphorus removed activity	–	+	+	+
28	*Actinomycetale smicropruina* clone F5K2Q4C04H50GM	Actinobacteria	Positive	Phosphorus removed activity	–	+	+	+

Remark: +, Visible band; –, no visible band.
[a]Anoxic step; [b]Oxic step; [c]Settle step.

Table 8
The analysis of the sequenced excised bands of 30 d cultivated mixed liquor of anoxic/oxic-SBR system with SIWW + Ni^{2+} on DGGE by NCBI blast



Bands no.	Strain	Eubacterial classification	Gram bacteria	Microbial communities	Operation time: d (condition in the reaction step)			
					0 d	30 d (An[a])	30 d (Ox[b])	30 d (Settle[c])
1	*Pseudomonadales pseudomonas* sp. 32-rlima2	γ-Proteobacteria	Negative	Nitrifying bacteria	+	+	+	+
2	*Pseudomonadales pseudomonas* sp. 32-rlima2	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
3,29,30, 31,32,33	Uncultured *bacterium*	Division TM7	Positive	Anaerobic digestion	+	+	+	+
4	*Pseudomonadales* clone PPW-294	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
5	*Pseudomonadales* clone PPW-294	γ-Proteobacteria	Negative	Nitrifying bacteria	+	–	–	–
6	*Cytophagales flectobacillus* sp. LR16	Bacteroidetes	Negative	Anaerobic bacteria	+	–	–	–
7	*Flavobacteriales flavobacterium, Gillisia illustrilutea* strain IC157	Bacteroidetes	Negative	Anaerobic bacteria	+	–	–	–
8	*Rhodocyclales thauera* sp. JPB-3.02	β-Proteobacteria	Negative	Ammonia oxidizing bacteria	+	–	–	–
9	*Actinomycetales, Micropruina glycogenica* strain Lg2	Actinobacteria	Positive	Phosphorus removed activity	+	–	–	–
10	*Rhizobiales rhizobium* sp. MBJ2	α-Proteobacteria	Negative	Nitrogen-fixing bacteria	+	–	–	–
11	*Cellulomonas, Cellulosimicrobium* sp. I37.1	Actinobacteria	Positive	Degrade cellulose	+	–	–	–
34	*Actinomycetales actinocorallia* sp. 9_55	Actinobacteria	Positive	Phosphorus removed activity	–	+	+	+
35	*Actinomycetales, Propionicimonas, Tessaracoccus* sp. NSG39	Actinobacteria	Positive	Propionate producing bacteria	–	+	+	+
36	*Actinomycetales, Propionicimonas, Tessaracoccus* sp. NSG39	Actinobacteria	Positive	Propionate producing bacteria	–	+	+	+
37	*Actinomycetales micropruina* clone F5K2Q4C04H50GM	Actinobacteria	Positive	Phosphorus removed activity	–	+	+	+

Remark: +, Visible band; –, no visible band.
[a]Anoxic step; [b]Oxic step; [c]Settle step.

5. Conclusions

Anoxic/oxic-SBR and oxic-SBR systems at an HRT of 1.5 d and MLSS of 2,000 mg/L were applied for the treatment of various types of SIWWs to investigate the highest HM removal efficiency. It was found that Cr^{3+} and Ni^{2+} could repress the growth and activity of heterotrophic carbonaceous BOD_5 removal bacteria, even though they did not have a significant repressive effect on nitrogenous compound removal bacteria (nitrifying and denitrifying bacteria). The growth and activity of both nitrifying and denitrifying bacteria could not be repressed, even at a concentration of Ni^{2+} or Cr^{3+} of up to 3.0 mg/L. The highest Cr^{3+} and Ni^{2+} removal efficiencies of 95.4% ± 0.2% and 93.1% ± 0.9%, respectively, were achieved in the anoxic/oxic-SBR system. Moreover, both SBR systems showed higher Cr^{3+} removal efficiency than Ni^{2+} removal efficiency. The maximum Cr^{3+} and Ni^{2+} adsorption yields detected were 30.2 mg Cr^{3+}/g of bio-sludge and 25.7 mg Ni^{2+}/g of bio-sludge, respectively.

This suggested that the addition of HMs (Ni^{2+} and Cr^{3+}) could increase the number of nitrogenous compound removal bacteria (nitrifying and denitrifying bacteria) and decrease the number of heterotrophic carbonaceous BOD_5 removal bacteria. Moreover, the anoxic/oxic-SBR system stimulated the growth and activity of denitrifying bacteria, resulting in an increase in bio-sludge age and TN removal yield. MLVSS/MLSS of bio-sludge with SIWW + HM was lower than that with SIWW, which resulted when HMs such as Ni^{2+} and Cr^{3+} were adsorbed onto the bio-sludge. Lastly, the anoxic/oxic-SBR system was more suitable for treating organic wastewater containing HMs. To confirm the above information, a bacterial diversity determination by rDNA analysis technique was applied. The results showed that heterotrophic carbonaceous BOD_5 removal bacteria were reduced in bio-sludge cultivated with SIWW + HM. Unfortunately, some species disappeared from the system, particularly nitrifying bacteria such as *Pseudomonas* spp., *Cytophaga* spp., *Flavobacterium* spp., etc., under anoxic conditions.

Acknowledgments

The authors wish to express deep gratitude to King Mongkut's University of Technology Thonburi for providing research materials and equipment for this project.

Symbols

BOD_5	—	Biochemical oxygen demand
COD	—	Chemical oxygen demand
Cr^{3+}	—	Chromium ion
F/M	—	Food (BOD_5 loading)/microbes (total bio-sludge)
HMs	—	Heavy metals
HRT	—	Hydraulic retention time
MLSS	—	Mixed liquor suspended solids
MLVSS	—	Mixed liquor volatile suspended solids
NH_4^+–N	—	Ammonium nitrogen
Ni^{2+}	—	Nickel ion
NO_2^-–N	—	Nitrite nitrogen
NO_3^-–N	—	Nitrate nitrogen
Organic-N	—	Organic nitrogen
SBR	—	Sequencing batch reactor
SS	—	Suspended solids
SIWW	—	Synthetic industrial estate wastewater without heavy metals
SIWWs	—	Various types of synthetic industrial wastewater
SIWW + HM	—	Synthetic industrial estate wastewater containing heavy metals
SIWW + Cr^{3+}	—	Synthetic industrial estate wastewater containing chromium ions
SIWW + Ni^{2+}	—	Synthetic industrial estate wastewater containing nickel ions
SVI	—	Sludge volume index
TDS	—	Total dissolved solids
TN	—	Total nitrogen

References

[1] Department of Industrial Works (Thailand), Standards of Industrial Effluents Quality due to Ministry of Industry Regulations, Department of Industrial Works, Ministry of Industry, Bangkok, Thailand, 1992.
[2] G. Tchobanoglous, F.L. Burton, H.D. Stensel, Wastewater Engineering: Treatment Disposal and Reuse, 4th ed., McGraw-Hill, New York, 2005.
[3] S. Sirianuntapiboon, M. Boonchupleing, Effect of bio-sludge concentration on the efficiency of sequencing batch reactor (SBR) system to treat wastewater containing Pb^{2+} and Ni^{2+}, J. Hazard. Mater., 166 (2009) 356–364.
[4] R.R. Bansode, J.N. Losso, W.E. Marshall, R.M. Rao, R.J. Portier, Adsorption of metal ions by pecan shell-based granular activated carbons, Bioresour. Technol., 89 (2003) 115–119.
[5] C.E. Janson, R.E. Kenson, L.H. Tucker, Treatment of heavy metal in wastewater, Environ. Prog., 1 (1982) 212–216.
[6] N.F. Fahim, B.N. Barsoum, A.E. Eid, M.S. Khalil, Removal of chromium(III) from tannery wastewater using activated carbon from sugar industrial waste, J. Hazard. Mater., 136 (2006) 303–309.
[7] M.L. Arora, E.F. Barth, M.B. Umphres, Technology evaluation of sequencing batch reactors, J. Water Pollut. Control Fed., 57 (1985) 867–875.
[8] F. Kargi, A. Uygur, Nutrient removal performance of a sequencing batch reactor as a function of the sludge age, Enzyme Microb. Technol., 31 (2002) 842–847.
[9] S. Sirianuntapiboon, T. Hongsrisuwan, Removal of Zn^{2+} and Cu^{2+} by a sequencing batch reactor (SBR) system, Bioresour. Technol., 98 (2007) 808–818.
[10] S. Sirianuntapiboon, O. Ungkaprasatcha, Removal of Pb^{2+} and Ni^{2+} by bio-sludge in sequencing batch reactor (SBR) and granular activated carbon-SBR (GAC-SBR) systems, Bioresour. Technol., 98 (2007) 2749–2757.
[11] S. Al-Asheh, Z. Duvnjak, Adsorption of copper and chromium by *Aspergillus carbonarius*, Biotechnol. Progr., 11 (1995) 638–642.
[12] B. Arican, C.F. Gokcay, U. Yetis, Mechanistics of nickel sorption by activated sludge, Process Biochem., 37 (2002) 1307–1315.
[13] B.Y. Chen, V.P. Utgikar, S.M. Harmon, H.H. Tabak, D.F. Bishop, R. Govind, Studies on biosorption of zinc(II) and copper(II) on *Desulfovibrio desulfuricans*, Int. Biodeterior. Biodegrad., 46 (2000) 11–18.
[14] Y. Chen, G. Gu, Short-term batch studies on biological removal of chromium from synthetic wastewater using activated sludge biomass, Bioresour. Technol., 96 (2005) 1722–1729.
[15] K. David, B. Volesky, Advances in the biosorption of heavy metals, Trends Biotechnol., 16 (1998) 291–300.
[16] B. Mattuschka, G. Straube, Biosorption of metals by a waste biomass, J. Chem. Technol. Biotechnol., 58 (1993) 57–63.
[17] X. Wang, L. Chen, S. Xia, J. Zhao, J.M. Chovelon, N.J. Renault, Biosorption of Cu(II) and Pb(II) from aqueous solutions by dried activated sludge, Miner. Eng., 19 (2006) 968–971.

[18] B. Arican, U. Yetis, Nickel sorption by acclimatized activated sludge culture, Water Res., 37 (2003) 3508–3516.
[19] A. Kapoor, T. Viraraghavan, Heavy metal biosorption sites in *Aspergillus niger*, Bioresour. Technol., 61 (1997) 221–227.
[20] R.C. Chakravarty, P. Banerjee, Mechanism of cadmium binding on the cell wall of an acidophilic bacterium, Bioresour. Technol., 108 (2012) 176–183.
[21] M. Amini, H. Younesi, N. Bahramifar, Biosorption of nickel(II) from aqueous solution by *Aspergillus niger*. Response surface methodology and isotherm study, Chemosphere, 75 (2009) 1483–1491.
[22] M.I. Ansari, A. Malik, Biosorption of nickel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial wastewater, Bioresour. Technol., 98 (2007) 3149–3153.
[23] M.H. Cheng, J.W. Patterson, R.A. Minear, Heavy metals uptake by activated sludge, J. Water Pollut. Control Fed., 47 (1975) 362–376.
[24] O. Gulnaz, S. Saygideger, E. Kusvuran, Study of Cu(II) biosorption by dried activated sludge: effect of physico-chemical environment and kinetics study, J. Hazard. Mater., 120 (2005) 193–200.
[25] S.A. Ong, P.E. Lim, C.E. Seng, M. Hirata, T. Hano, Effects of Cu(II) and Cd(II) on the performance of sequencing batch reactor treatment system, Process Biochem., 40 (2005) 453–460.
[26] S. Sirianuntapiboon, P. Chaiyasing, Removal of organic matters and heavy metals from wastewater by granular activated carbon-sequencing batch reactor system, Asian J. Energy Environ., 1 (2000) 125–142.
[27] M. Rarunroeng, S. Sirianuntapiboon, Effect of anoxic:oxic ratio on the efficiency and performance of sequencing batch reactor (SBR) system for treatment of industrial estate wastewater containing Cr^{3+} and Ni^{2+}, Desal. Wat. Treat., 57 (2016) 21752–21769.
[28] S. Sirianuntapiboon, A. Chaochon, Effect of Cr^{+3} on the efficiency and performance of the sequencing batch reactor (SBR) system for treatment of tannery industrial wastewater, Desal. Wat. Treat., 57 (2016) 5579–5591.
[29] American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 19th ed., APHA, Washington, D.C., USA, 1995.
[30] J. Zhou, M.A. Bruns, J.M. Tiedje, DNA recovery from soils of diverse composition, Appl. Environ. Microbiol., 62 (1996) 316–322.
[31] G. Muyzer, E.C. de Waal, A.G. Uitterlinden, Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, Appl. Environ. Microbiol., 59 (1993) 695–700.
[32] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, Basic local alignment search tool, J. Mol. Biol., 215 (1990) 403–410.
[33] SAS Institute, The SAS System for Windows, Release 6.12, SAS Institute, Cary, NC, 1996.
[34] U. Açikel, T. Alp, A study on the inhibition kinetics of bioaccumulation of Cu(II) and Ni(II) ions using *Rhizopus delemar*, J. Hazard. Mater., 168 (2009) 1449–1458.
[35] A. Öztürk, Removal of nickel from aqueous solution by the bacterium *Bacillus thuringiensis*, J. Hazard. Mater., 147 (2007) 518–523.
[36] M. Fereidouni, A. Daneshi, H. Younesi, Biosorption equilibria of binary Cd(II) and Ni(II) systems onto *Saccharomyces cerevisiae* and *Ralstonia eutropha* cells: application of response surface methodology, J. Hazard. Mater., 168 (2009) 1437–1448.
[37] A.I. Ferraz, T. Tavares, J.A. Teixeira, Cr(III) removal and recovery from *Saccharomyces cerevisiae*, Chem. Eng. J., 105 (2004) 11–20.
[38] M.H. Gerardi, Nitrification and Denitrification in the Activated Sludge Process, John Wiley & Sons, New York, 2002.
[39] P. Gikas, P. Romanos, Effect of tri-valent (Cr(III)) and hexa-valent (Cr(VI)) chromium on the growth of activated sludge, J. Hazard. Mater., 133 (2006) 212–217.
[40] Y.W. Lee, S.K. Ong, C. Sato, Effects of heavy metals on nitrifying bacteria, Water Sci. Technol., 36 (1997) 69–74.
[41] E.R. Scerri, The Periodic Table: Its Story and Its Significance, Oxford University Press, Oxford, UK, 2007.