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The Effectiveness of an Anoxic-Oxic-Anoxic-Oxic Sequencing Batch Reactor System (A2/O2-SBR) to Treat Electroplating Wastewater and the Bacterial Community within the System

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ABSTRACT

This study presents an examination of the effectiveness of an anoxic-oxic-anoxic-oxic sequencing batch reactor (A2/O2-SBR) for treating electroplating wastewater (EPWW). The A2/O2-SBR was monitored for 60 days and the bacterial composition in the treatment system was determined. The system consisted of four reactors, which had the following anoxic/oxic ratios: reactor-I, 0:9 h; reactor-II, 2:7 h; reactor-III, 4.5:4.5 h; and reactor-IV, 7:2 h. The combined cycle time of the reactors was 12 h, the hydraulic retention time (HRT) was five days, and the total volume of mixed liquor suspended solids (MLSS) was 2,000 mg/L. The results demonstrate the importance of an anoxic period for the treatment of heavy metals. Most of the ammonium nitrogen (NH_4^+-N), Total Kjeldahl nitrogen (TKN), and total nitrogen (TN) were removed during the oxic period. However, as the anoxic period increased, the amounts of TKN, nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), and TN declined. Reactor-IV showed a high removal efficiency for heavy metals (Zn^{2+}, 89.74%; Cd^{2+}, 81.37%), TKN (89.20%), and TN (84.25%), and also effectively treated NH_4^+-N (78.84%), biochemical oxygen demand (BOD_5; 93.5%), and chemical oxygen demand (COD; 84.9%). Reactor-IV showed an appropriate difference in the dissolved oxygen (DO) concentration between the anoxic period and oxic period (2.12-2.00 mg/L). The main bacterial phyla in the treatment system were Proteobacteria, Actinobacteria, and Firmicutes, while *Pseudomonas vancouverensis* and *Cryobacterium arcticum* were the most common species. The anoxic period and bacterial community have significantly demonstrated the ability to remove Zn^{2+} and Cd^{2+} for effective treatment of EPWW.

1. INTRODUCTION

Heavy metals are common in various industries, including galvanizing, electroplating, leather tanning, and film photography. Galvanization is an electroplating process in which zinc is used to coat steel and increase its corrosion resistance. EPWW contains heavy metals and nutrients that can contaminate the environment (Coelho et al., 2021; Wang et al., 2021). Therefore, treating EPWW is necessary (Marques et al., 2013). Biological processes can be used to eliminate heavy metals and are both cost-effective and environmentally friendly (James and Vijayanandan, 2022).

Sequencing batch reactor (SBR) systems enhance the activated sludge process for wastewater treatment. However, wastewater characteristics may influence pollution removal efficiency (Singh et al., 2022). SBR systems demonstrate exceptional stability

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and adaptability, making them efficient for biological wastewater treatment (Zhang et al., 2023). The reaction phase in SBR systems includes both anoxic and oxic periods, and enhances pollution removal (Liu et al., 2017; Gao et al., 2021). An abundance of oxygen (O_2) allows aerobic organisms to perform nitrification, using O_2 as an electron acceptor. Conversely, microorganisms utilize NO_3^--N as an electron acceptor under anoxic conditions, resulting in denitrification (Yan et al., 2019). Heavy metals, nitrogen compounds, phosphates, and COD are reduced in industrial wastewater under anoxic conditions (Feng et al., 2013; Jena et al., 2020; Lu et al., 2021). Preserving both anoxic and oxic processes helps to accelerate pre-denitrification in anoxic phase. This inhibits the development of NO_3^--N and NO_2^--N within the system (Gong et al., 2012; Lu et al., 2021). The anoxic/oxic system removes nitrogen through nitrification, denitrification, ammonification, and anaerobic ammonium oxidation (anammox) process.

The anoxic/oxic process can be used to treat EPWW, removing organic matter (OM) and nitrogen compounds (Yan et al., 2018; Gao et al., 2021). Feng et al. (2013) reported that an anoxic/aerobic-membrane bioreactor (A/O-MBR) can remove 80-95% of heavy metals like zinc (Zn^{2+}), copper (Cu^{2+}), lead (Pb^{2+}), and cadmium (Cd^{2+}). By using ion exchange, extracellular precipitation, and intracellular accumulation, denitrifying bacteria remove heavy metals better than nitrifying bacteria (Wang et al., 2016; Su et al., 2020). Moreover, microorganism multiplication in various environments demonstrates the need for DO. Microorganisms determine wastewater treatment system efficiency (Quan et al., 2012). The main phyla in anoxic/oxic habitats are Proteobacteria, Bacteroidetes, Firmicutes, Chloroflexi, Acidobacteria, and Verrucomicrobia (Xie et al., 2024). Yong et al. (2018) found that *Pseudomonadaceae* generate organic acids to chelate and precipitate heavy metals.

The anoxic-oxic-anoxic-oxic (A2/O2-SBR) process was demonstrated to effectively treat less heavy metal contaminated in EPWW (Yan et al., 2018; Gao et al., 2021). This study examines how different oxic and anoxic conditions change the bacterial communities in EPWW that contains heavy metals, as well as how they affect the removal of heavy metals, the breakdown of OM, and the growth of bacteria. Pilot-scale experiments are necessary to advance practical applications of the A2/O2-SBR process. Managing the wastewater treatment system requires monitoring DO levels during anoxic and oxic phases. The goal of this study was to evaluate A2/O2-SBR system, which constantly treats EPWW, and identify the best anoxic/oxic ratios. Additionally, studying the diversity of microbial populations during wastewater treatment will improve our understanding of these systems.

2. METHODOLOGY

2.1 Wastewater characteristics

EPWW from electro-galvanized steel production was obtained from the electroplating industry in southern Thailand. The production process is not continuous and the wastewater is occasionally released, resulting in a single collection during peak production to evaluate the efficiency of the A2/O2-SBR in reducing high pollution levels. Table 1 presents the characteristics of the EPWW.

Table 1. The EPWW characteristics

Parameter	Value
Cadmium (Cd^{2+})	20.57±0.21 mg/L
Zinc (Zn^{2+})	30.62±0.13 mg/L
Ferrous (Fe^{2+})	0.273±0.120 mg/L
Manganese (Mn^{2+})	0.120±0.110 mg/L
Nickel (Ni^{2+})	0.052±0.160 mg/L
Copper (Cu^{2+})	0.004±0.100 mg/L
Biochemical Oxygen Demand (BOD_5)	285.0±3.0 mg/L
Chemical Oxygen Demand (COD)	402.0±2.5 mg/L
Ammonium-nitrogen (NH_4^+-N)	3.31±0.04 mg/L
Total Kjeldahl Nitrogen (TKN)	14.50±0.13 mg/L
Nitrite-nitrogen (NO_2^--N)	0.34±0.02 mg/L
Nitrate-nitrogen (NO_3^--N)	0.43±0.01 mg/L
Total nitrogen (TN)	15.73±0.25 mg/L
pH	7.43±0.04

2.2 A2/O2 SBR operation

Activated sludge was obtained from domestic wastewater treatment plants in Bangkok, Thailand. The four reactors in this study were made from clear acrylic plastic cylinders, with each reactor having a diameter of 18 cm, a height of 40 cm, and a working volume of 7.5 L (Figure 1(a)). The mixer system used a 60-rpm gear motor and paddle agitator. Air was diffused to the reactor, and the mixing and air pump were timed.

Four reactors were used with a cycle time of 12 h, an HRT of 5 days, and a MLSS concentration of 2,000 mg/L. All reactors were run for 9 h during the reaction phase and operated for 60 days. During the reaction phase, reactor-I, reactor-II, reactor-III, and reactor-IV had different anoxic/oxic ratios (0:9 h, 2:7 h, 4.5:4.5 h, and 7:2 h, respectively) as depicted in Figure 1(b).

(a) SBR reactors in the experiment

(b) Operational coditions of the A2/O2-SBR at different anoxic/oxic ratios

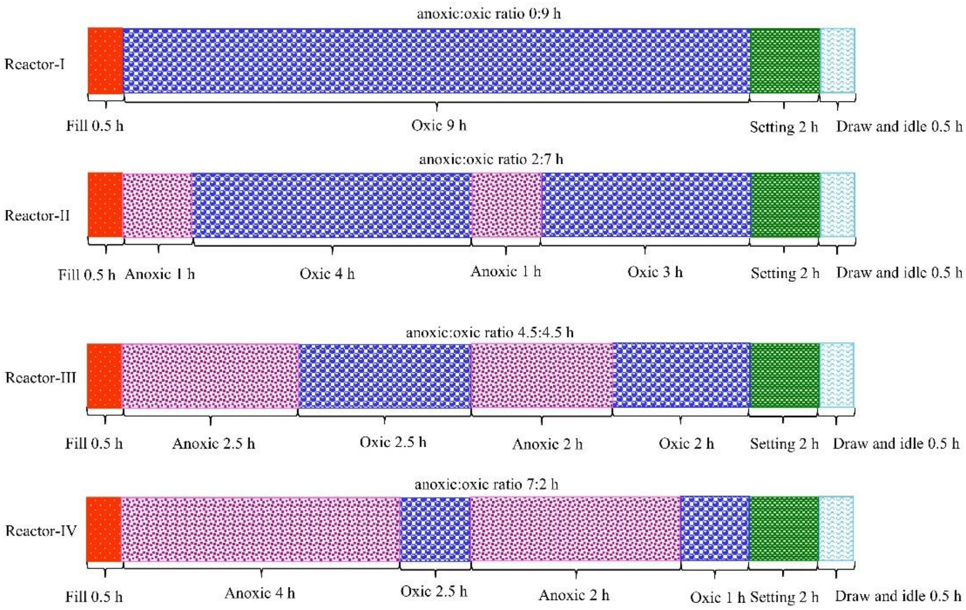


Figure 1. Experimental of A2/O2-SBR for treating EPWW (a) SBR reactors in the experiment, (b) Operational conditions of the A2/O2-SBR at different anoxic/oxic ratios

2.3 Analytical methods

The BOD_5, COD, TKN, NH_4^+-N, NO_2^--N, and NO_3^--N were measured following standard methods used for examining water and wastewater (Rice et al., 2012). The concentrations of heavy metals were determined using an atomic absorption spectrophotometer (AA). The sample sludge from reactor-I and the most effective reactor were used on

Day 0 (blank) and Day 60 to investigate bacterial diversity. The GF-1 kit from Vivantis technologies was used to extract bacterial DNA from the sludge after 20 min of centrifugation at 10,000 rpm. DNA was amplified, purified, and sequenced using 16S rRNA primers, following the Illumina 16S metagenomics protocol.

3. RESULTS AND DISCUSSION

3.1 Heavy metal removal

The results of this study are shown the AA machine was unable to detect Fe^{2+}, Mn^{2+}, Ni^{2+}, and Cu^{2+} in the effluent wastewater, indicating that low levels of heavy metals can be removed with the treatment system. The concentrations of Zn^{2+} and Cd^{2+} in all reactors decreased over time, as represented in Figure 2(a) and Figure 2(c), because activated sludge could be a process for removing heavy metals through active and passive mechanisms involving intracellular accumulation and cellular metabolism processes (Ayangbenro and Babalola, 2017). Several researchers have reported that the physicochemical properties of anionic functional groups in extracellular polymeric substances (EPS) and cell surfaces act as adsorbents for sludge. Zn^{2+} and Cd^{2+} accumulate within cells through the Zn-transported system (Zhang et al., 2022; Bi et al., 2014).

The metal ions that fill the active binding sites make the biosorption process more challenging due to their slow adsorption rate until they reach the saturation point, which ultimately results in an equilibrium state (Fathollahi et al., 2021; Kalita and Baruah, 2023). The type of microorganisms and their resistance to heavy metals influence their capacity to adsorb and precipitate heavy metals on the cell surface. Numerous studies indicate that denitrifying bacteria exhibit greater resistance to heavy metals than nitrifying organisms (Feng et al., 2013; Su et al., 2020). Therefore, the oxic and anoxic conditions of the treatment system influence the remediation of heavy metals. According to Sirianuntapiboon (2013), SBR incorporating an anoxic phase can eliminate cyanide and Zn^{2+}. The investigation revealed that *Nitrobacter* sp. was the most affected nitrifying bacteria, while heterotrophic or denitrifying bacteria showed no signs of damage. Moreover, Cd^{2+} has demonstrated higher toxicity to nitrifying bacteria in comparison to denitrifying microbes (Feng et al., 2013; Dai et al., 2019).

Figure 2(b) and Figure 2(d) demonstrate that all reactors removed up to 58% of Zn^{2+} and 49% of Cd^{2+} from the first period (Anoxic1) due to the presence of more vacant and unoccupied active biosorption sites on the biosorbent surfaces. However, the second anoxic period (Anoxic2) was the most effective treatment for Zn^{2+} and Cd^{2+}, surpassing the first and second oxic periods (Oxic1 and Oxic2). The results confirmed that the bio-sludge kept more Zn^{2+} and Cd^{2+} as the anoxic period increased, as shown in Figures 2(e) and Figure 2(f).

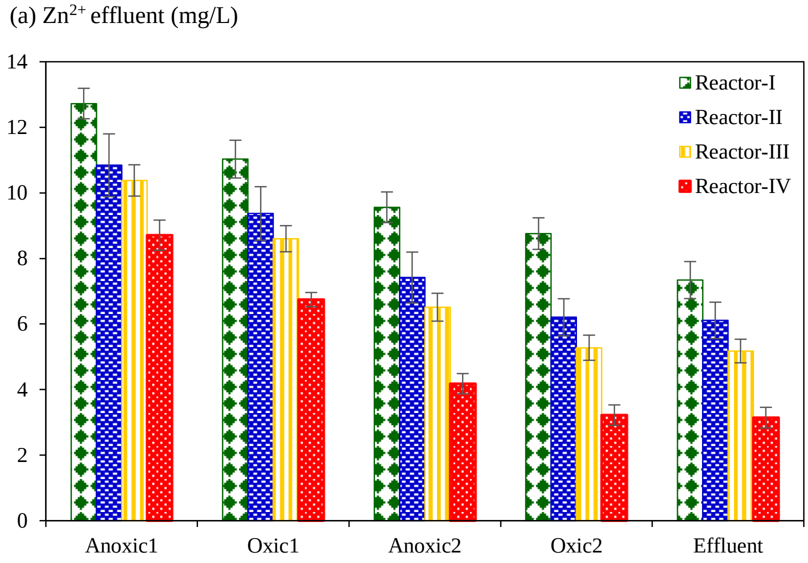
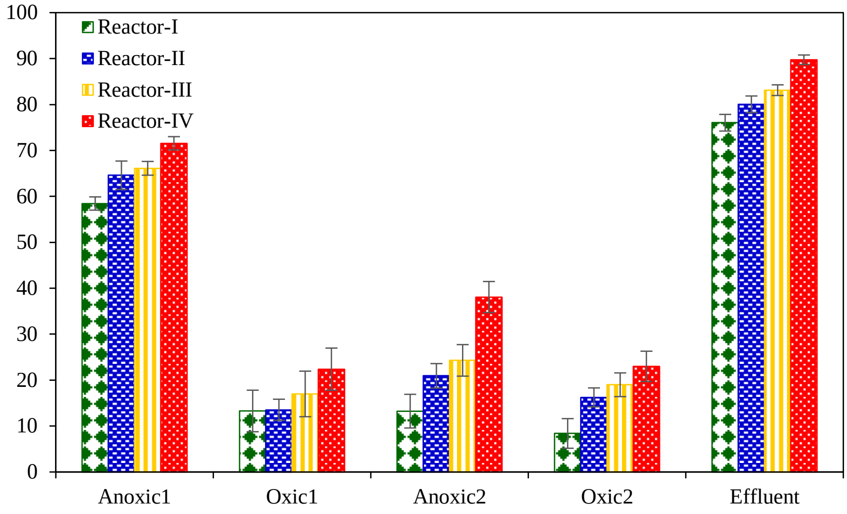
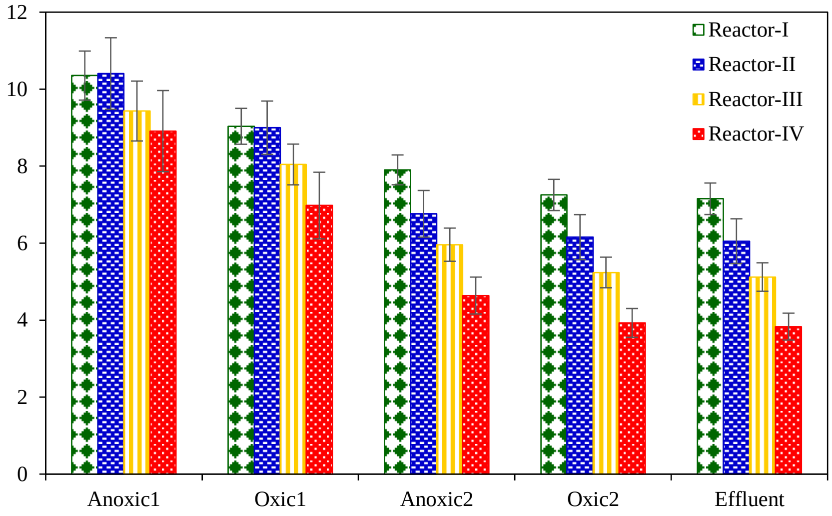

(a) Zn^{2+} effluent (mg/L)

Figure 2. Heavy metal concentrations, removal efficiency in wastewater and heavy metal concentrations in bio-sludge of A2/O2-SBR for EPWW treatment (a) Zn^{2+} concentrations, (b) Zn^{2+} removal efficiencies, (c) Cd^{2+} concentrations, and (d) Cd^{2+} removal efficiencies, (e) Zn^{2+} concentrations and (f) Cd^{2+} concentrations in bio-sludge

(b) Zn^{2+} removal efficiency (%)



(c) Cd^{2+} concentrations (mg/L)



(d) Cd^{2+} removal efficiencies (%)

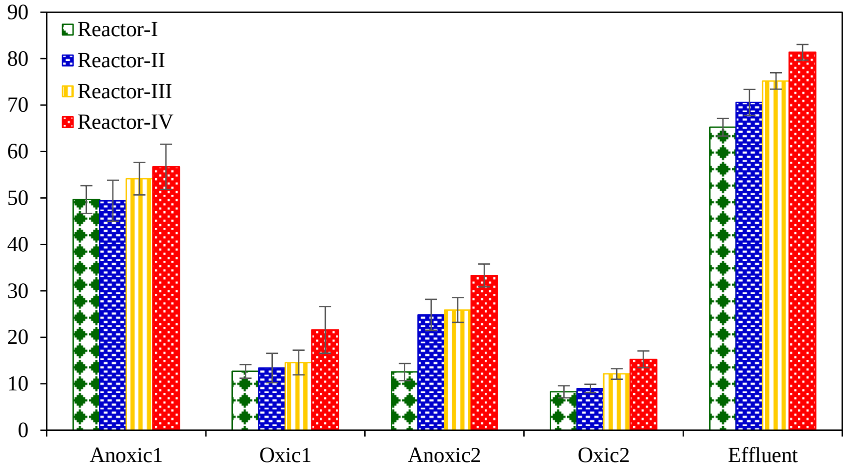
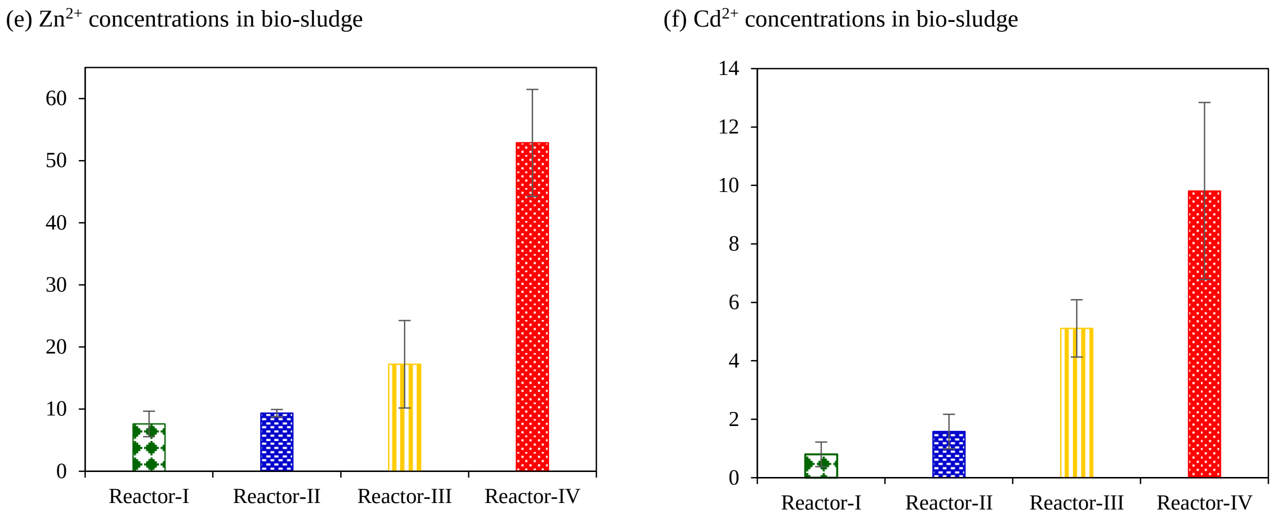


Figure 2. Heavy metal concentrations, removal efficiency in wastewater and heavy metal concentrations in bio-sludge of A2/O2-SBR for EPWW treatment (a) Zn^{2+} concentrations, (b) Zn^{2+} removal efficiencies, (c) Cd^{2+} concentrations, and (d) Cd^{2+} removal efficiencies, (e) Zn^{2+} concentrations and (f) Cd^{2+} concentrations in bio-sludge (cont.)



(e) Zn^{2+} concentrations in bio-sludge

(f) Cd^{2+} concentrations in bio-sludge

Figure 2. Heavy metal concentrations, removal efficiency in wastewater and heavy metal concentrations in bio-sludge of A2/O2-SBR for EPWW treatment (a) Zn^{2+} concentrations, (b) Zn^{2+} removal efficiencies, (c) Cd^{2+} concentrations, and (d) Cd^{2+} removal efficiencies, (e) Zn^{2+} concentrations and (f) Cd^{2+} concentrations in bio-sludge (cont.)

3.2 BOD_5 and COD removal

Figure 3(a) and Figure 3(c) show that most BOD_5 and COD values decreased after the first period of the reaction phase and slightly decreased in the later period because bacteria require OM for growth. OM is oxidized by heterotrophic bacteria to carbon dioxide, after which the nitrogen compounds can be decomposed by autotrophic bacteria, especially nitrifying bacteria, through the process of decomposing inorganic compounds. All of the above processes occurred under oxic conditions. During anoxic periods, low DO levels inhibit microbial activity, leading to reductions in OM removal (Yan et al., 2018). The oxic phase is more effective than the anoxic phases in reducing BOD_5 and COD because autotrophic bacteria do most of the work in oxic conditions (Gabarro et al., 2014; Gao et al., 2021).

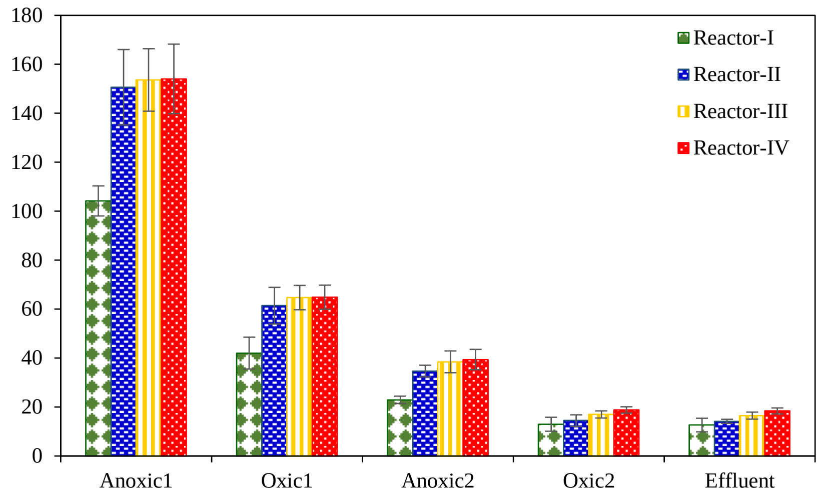
The COD degradation by the A2/O2-SBR occurred when aerobic microorganisms oxidized OM under aerobic conditions, while heterotrophic bacteria utilized organic carbon as an electron donor under anoxic conditions (Huang et al., 2020; Guo et al., 2023). Reducing the length of the oxic period in wastewater treatment process led to a lower BOD_5 and COD removal efficiency. However, the BOD_5 and COD removal efficiencies were greater than 90% and 84% (Figure 3(b) and Figure 3(d)). This result is consistent with that of Li et al. (2018), who reported a COD removal efficiency of 89.1% at a Cd^{2+} concentration of 40 mg/L. Zhang et al. (2022) reported that activated sludge could reduce COD by 73.64% when the Zn^{2+} concentration was 20 mg/L. In addition, Liu et al. (2017) reported that a two-step anoxic/oxic membrane bioreactor system effectively treated landfill leachate and removed 80.1% of COD.

3.3 Nitrogen compound removal

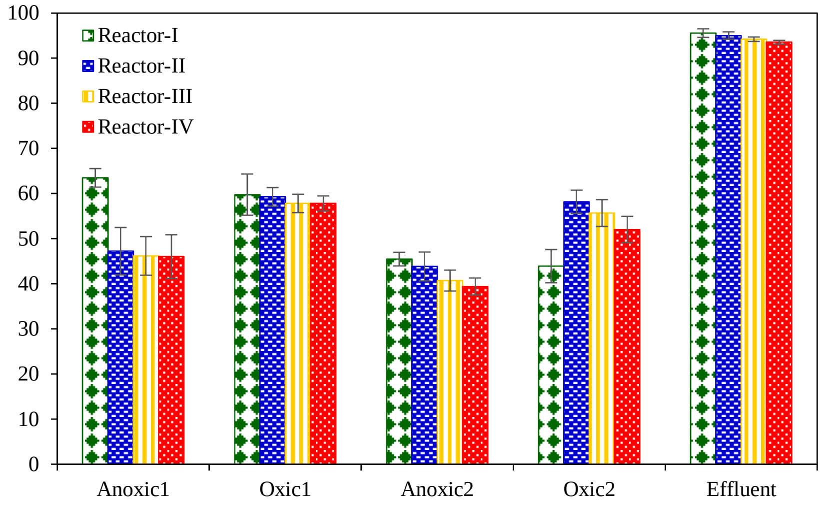
3.3.1 NH_4^+-N and TKN removal

Figure 4 (a) shows the concentrations of NH_4^+-N. Most NH_4^+-N is removed under oxic conditions but can also be removed under anoxic conditions through these processes. Under oxic conditions, in the nitrification process, ammonia-oxidizing bacteria (AOB) convert ammonia into NO_2^--N in the first step, and then, NO_2^--N is oxidized to NO_3^--N by nitrite-oxidizing bacteria (NOB) (Wang et al., 2016). Under anoxic conditions, in the ammonium oxidation process (anammox), anammox bacteria use NO_2^--N instead of O_2 to convert NH_4^+-N. and NO_2^--N into N_2 (Jaroszynski et al., 2011; Lu et al., 2021). In addition, Figure 4(b) shows that the NH_4^+-N removal efficiency of reactor-I steadily decreased over time due to the absence of OM. However, reactor-II, reactor-III, and reactor-IV had greater NH_4^+-N removal efficiencies in the oxic periods than in the anoxic periods because most NH_4^+-N was treated during the oxic periods. However, when anoxic period increased in length, the efficiency of NH_4^+-N removal decreased due to the decreased DO levels, leading to the inhibition of ammonification process (Tang et al., 2021; Lu et al., 2021). Additionally, Cd^{2+} and Zn^{2+} inhibited the ammonium oxidation enzyme activity of the nitrifying and AOB (Marques et al., 2013; Bi et al., 2014; Li et al., 2018).

(a) BOD_5 concentrations (mg/L)



(b) BOD_5 removal efficiencies (%)



(c) COD concentrations (mg/L)

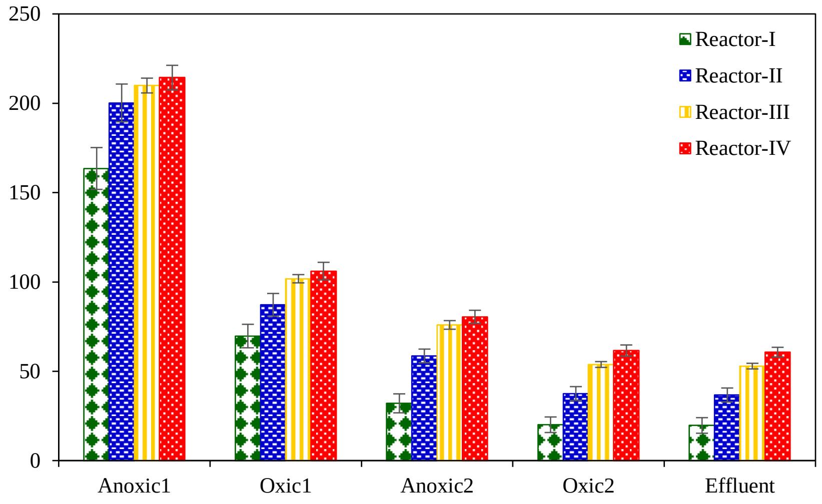


Figure 3. BOD_5 and COD concentrations and their removal efficiency of A2/O2-SBR for EPWW treatment (a) BOD_5 concentrations, (b) BOD_5 removal efficiencies, (c) COD concentrations, and (d) COD removal efficiencies

(d) COD removal efficiencies (%)

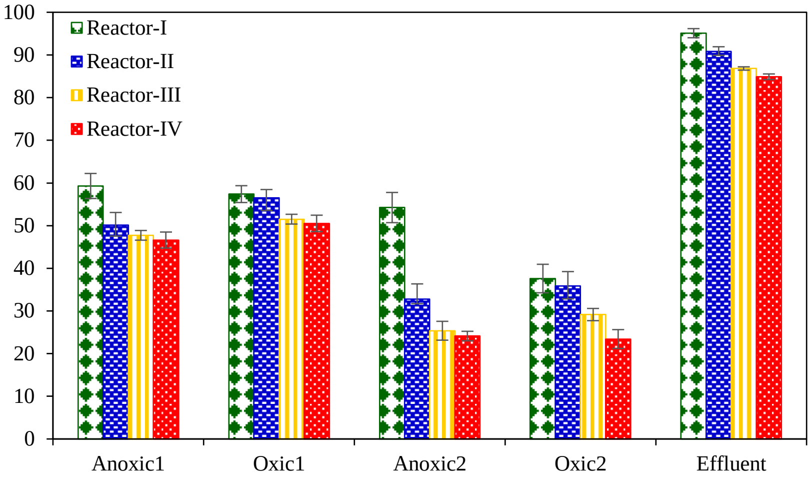


Figure 3. BOD_5 and COD concentrations and their removal efficiency of A2/O2-SBR for EPWW treatment (a) BOD_5 concentrations, (b) BOD_5 removal efficiencies, (c) COD concentrations, and (d) COD removal efficiencies (cont.)

However, during the anoxic periods of reactor-II, reactor-III, and reactor-IV, NH_4^+-N removal efficiencies were 39-42% due to the anammox process (Jaroszynski et al., 2011). This result was observed in the A2/O2-SBR treatment system when the anoxic period increased in length, resulting in a decreased NH_4^+-N removal efficiency. Similarly, Hu et al. (2011) reported that in an anoxic/aerobic SBR, the removal efficiency of NH_4^+-N decreased as the anoxic period increased.

TKN concentrations in this study are shown in Figure 4(c), which shows that TKN concentrations decreased after the first period. Figure 4(d) shows that TKN removal efficiency of reactor-I continuously decreased at 6 h and began to stabilize at 9 h, while adding anoxic periods in other reactors improved TKN removal efficiencies during the oxic period. However, increasing the anoxic ratios for these systems increased TKN removal efficiency. In addition, the results show that most TKN is eliminated by autotrophic nitrifying bacteria under oxic conditions (Guo et al., 2023). Under anoxic conditions, TKN is eliminated by denitrification process, and anammox process.

When comparing the removal efficiency of NH_4^+-N with that of TKN during every period, it was found that when the anoxic period increased in length, NH_4^+-N removal efficiency decreased. On the other hand, TKN removal efficiency in anoxic period was less than that in oxic period, but TKN removal efficiency in the effluent increased with an increasing anoxic ratio in A2/O2-SBR. The results showed that A2/O2-SBR, which increased anoxic ratio was promoted the removal of organic nitrogen.

(a) NH_4^+-N concentrations (mg/L)

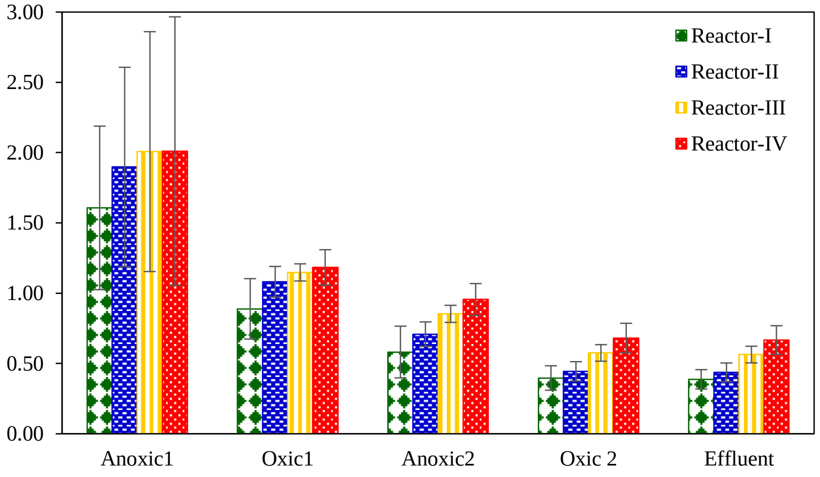
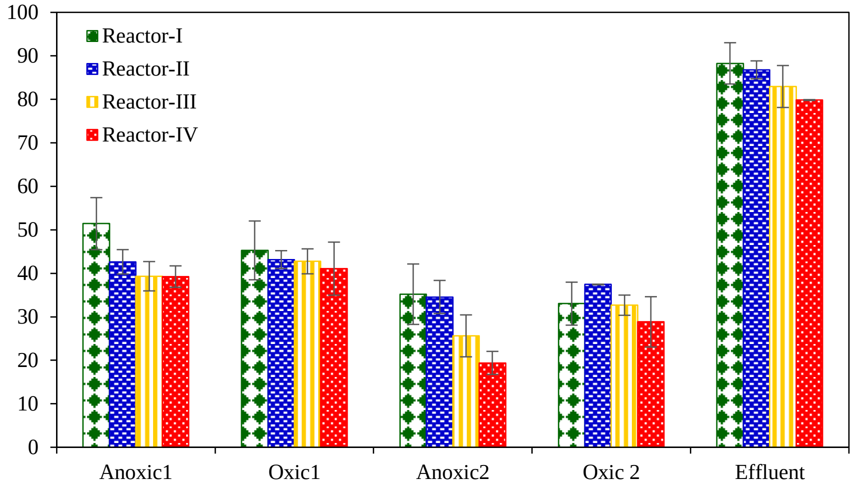
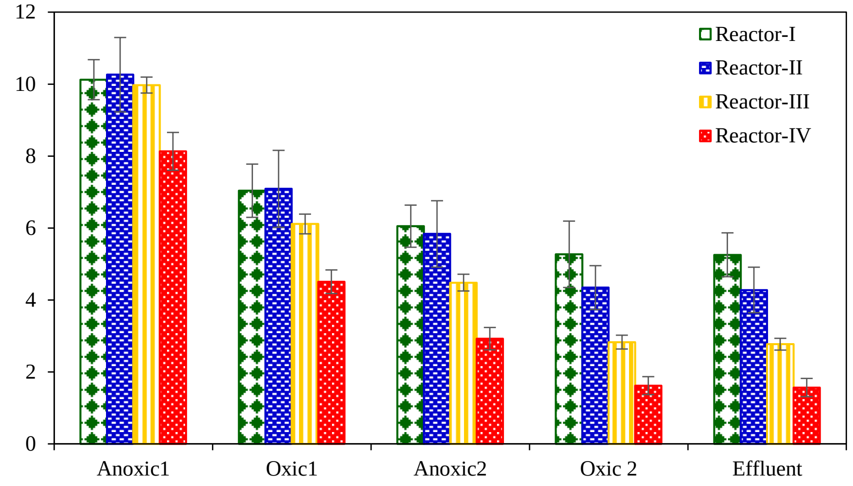


Figure 4. NH_4^+-N and TKN concentrations and their removal efficiency of A2/O2-SBR for EPWW; (a) NH_4^+-N concentrations, (b) NH_4^+-N removal efficiencies (c), TKN concentrations, and (d) TKN removal efficiencies

(b) NH_4^+-N removal efficiencies (%)



(c) TKN concentrations (mg/L)



(d) TKN removal efficiencies (%)

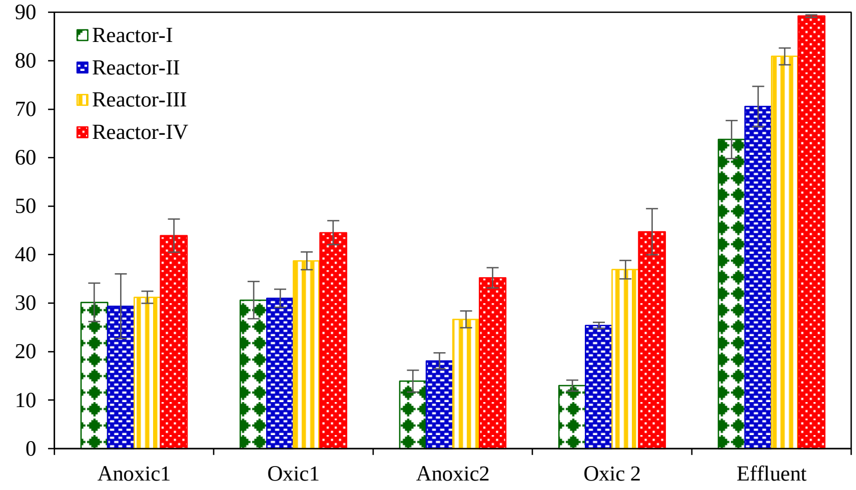
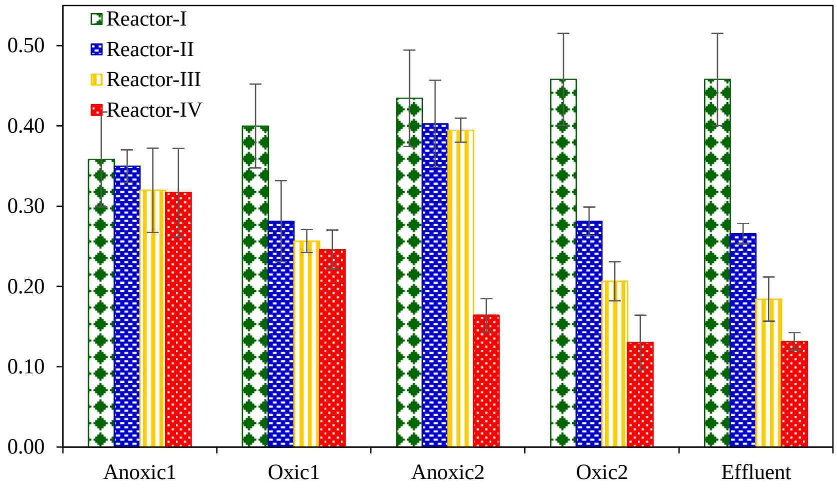


Figure 4. NH_4^+-N and TKN concentrations and their removal efficiency of A2/O2-SBR for EPWW; (a) NH_4^+-N concentrations, (b) NH_4^+-N removal efficiencies (c), TKN concentrations, and (d) TKN removal efficiencies

3.3.2 NO_2^--N and NO_3-N removal

NO_2^--N and NO_3^--N concentrations in EPWW were 0.34±0.20 and 0.43±0.10 mg/L. Figure 5(a) and Figure 5(b) show that NO_2^--N and NO_3-N concentrations increased in reactor-I but decreased in other reactors. Nitrification occurred in reactor-I, causing the accumulation of NO_2^--N and NO_3^--N in this reactor. However, reactor-II, reactor-III, and reactor-IV experienced increases in the anoxic period combined with denitrification, with NO_3^--N being converted into N_2O or N_2, which reduced the accumulation of NO_2^--N and NO_3^--N. This result is consistent with that of Wu et al. (2018), who reported that the secondary-stage A/O process of a pilot-scale two-stage anoxic/oxic process was effective in removing nitrogen by reducing NO_3^--N to N_2. However, Cd^{2+} toxicity has a detrimental effect on microorganism activity, leading to reductions in NO_2^--N and NO_3^--N removal (Su et al., 2019), which caused NO_2^--N and NO_3^--N to accumulate in this study.

(a) NO_2^--N concentrations (mg/L)



(b) NO_3^--N concentrations (mg/L)

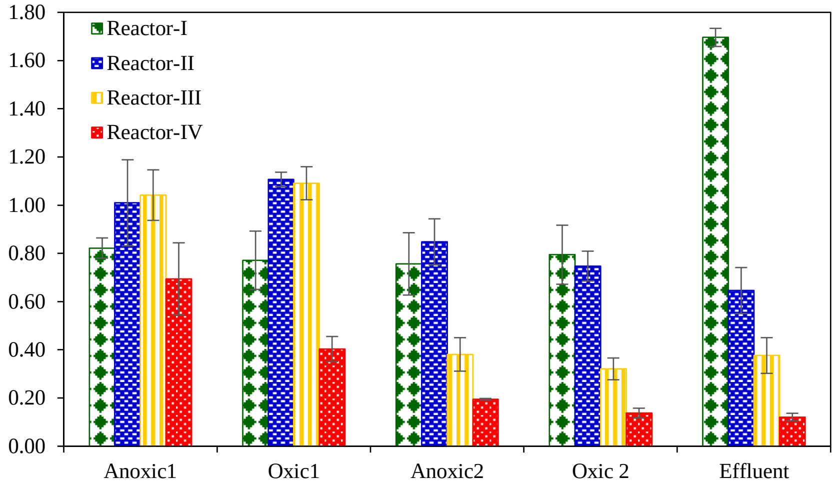
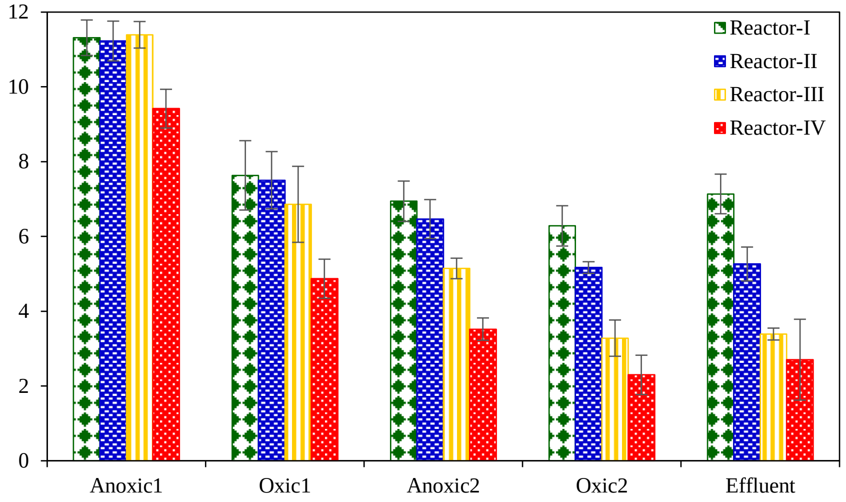


Figure 5. Concentrations of NO_2^--N and NO_3^--N in EPWW treated using the A2/O2-SBR (a) NO_2^--N concentrations and (b) NO_3^--N concentrations

3.3.3 TN removal

TN is the sum of NO_3^--N, NO_2^--N, and TKN. This study showed that TN concentrations in all reactors gradually decreased over time, as shown in Figure 6(a). TN removal efficiencies of the A2/O2-SBR reactors are shown in Figure 6(b). Reactor-I removed most of TN within the first 6 h, and only a small amount remained after treatment. However, in other reactors, the nitrification process converted most of NH_4^+-N and TKN to NO_3^--N under oxic conditions, leading to the removal of most TN under oxic conditions (Huang et al., 2020). Increasing the anoxic period in the system improved the efficiency of TN removal during every period of the reaction phase. The continuous operation of anoxic/oxic systems affects the treatment of nitrogen compounds (Zheng et al., 2021; Hu et al., 2011). Yan et al. (2018) reported that the first-stage anoxic/oxic cycle in a two-stage anoxic/oxic process transforms most TN into NO_3^--N through complete nitrification and incomplete denitrification, followed by the conversion of NO_2^--N to NO_3^--N. EPWW contains Zn^{2+} and Cd^{2+}, which are toxic to nitrifying bacteria and denitrifying bacteria (Su et al., 2019; Zhang et al., 2023); however, the findings show that reactor-IV had the highest TN removal efficiency at 84.25±6.10%. According to research by Hu et al. (2020), the efficiency of TN removal in the anoxic/oxic process was greater than 80%.

(a) TN concentrations (mg/L)



(b) TN removal efficiencies (%)

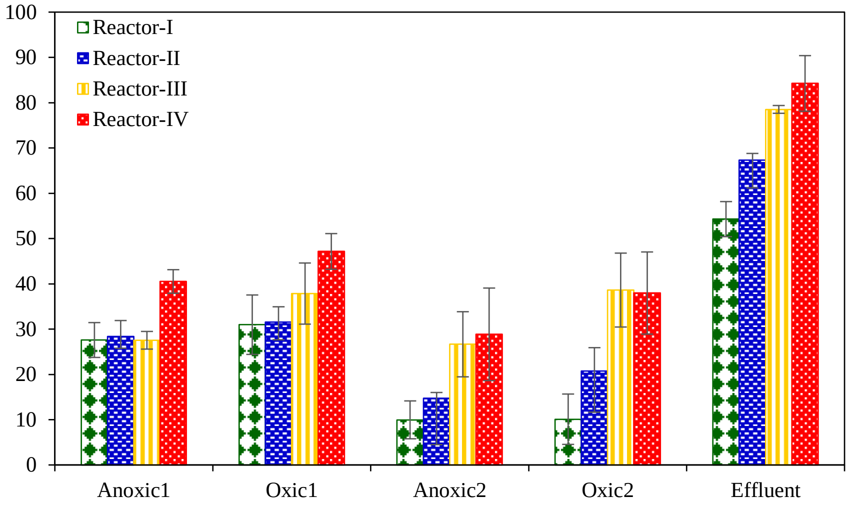


Figure 6. Concentrations and removal efficiency of TN in EPWW treated using the A2/O2-SBR (a) TN concentrations and (b) TN removal efficiencies

3.4 DO levels

Table 2 displays the DO levels in this study. The average DO concentration was 0.1-0.4 mg/L during the anoxic periods and 2.2-6.5 mg/L during oxic periods, which was adequate for denitrifying and nitrifying bacteria (Yan et al., 2019). Anammox bacteria can also grow at DO concentrations below 2.5 mg/L (Kimura et al., 2011). Raising the anoxic/oxic ratio during the reaction phase of these systems reduced the difference in DO levels between the two periods. Between anoxic and oxic periods, reactor-I had DO values of 6.5-6.6 mg/L, and reactor-II, reactor-III, and reactor-IV had DO values of 5.3-5.4, 5.3-5.5, and 2.1-2.2 mg/L, respectively. Increasing the anoxic period while decreasing the oxic period resulted in greater Zn^{2+}, Cd^{2+}, TKN, and TN elimination. Over 80% of BOD_5 and COD were removed.

Minor DO concentration differences between oxic and anoxic periods affect both obligate aerobic and facultative anaerobic bacteria. Yong et al. (2018) observed a close relationship between DO levels and OM elimination, as high DO levels during oxic and short anoxic periods impeded denitrification. Gao et al. (2021) found that excess DO (3-4 mg/L) from oxic unit to anoxic unit damaged denitrifying microorganisms. In a single reactor wastewater treatment system, regulating DO levels to suit different microbial taxa is crucial. A2/O2 reduced DO variations between oxic and anoxic phases, improving nitrogen and OM removal. Increasing the anoxic period in reactor-IV effectively removed Zn^{2+} (89.74%) and Cd^{2+} (81.37%), with BOD_5 and COD removal efficiencies greater than 93.55% and 84.9%, respectively.

3.5 Sludge properties

Table 3 shows A2/O2-SBR performance. As anoxic period increased, excess sludge and suspended particles decreased, but sludge retention time (SRT) increased due to lower biomass output (Jena et al., 2016). High SRT improves sludge flocculation (Singh et al., 2022). Sludge settled well with sludge volume index (SVI) values of 48-55. The food to microorganism ratios (F/M) in this investigation were 0.22-0.26 kg BOD/kg MLSS.

Table 2. DO values of the A2/O2-SBR for treatment of EPWW

Reaction phase steps	Reactor-I*	Reactor-II	Reactor-III	Reactor-IV
Anoxic1	6.5±0.1 mg/L	0.4±0.1 mg/L	0.2±0.1 mg/L	0.1±0.1 mg/L
Oxic1	6.6±0.1 mg/L	5.8±0.1 mg/L	5.7±0.1 mg/L	2.3±0.2 mg/L
Anoxic2	6.5±0.1 mg/L	0.4±0.1 mg/L	0.3±0.1 mg/L	0.1±0.1 mg/L
Oxic2	6.6±0.1 mg/L	5.7±0.1 mg/L	5.6±0.1 mg/L	2.2±0.1 mg/L

Remark * Reactor-I controlled the oxic conditions throughout the study.

Table 3. Sludge properties in the A2/O2 SBR for the treatment of EPWW

Parameter	Reactor-I	Reactor-II	Reactor-II	Reactor-IV
Excess sludge (mg/d)	529±217	364±123	206±50	147±34
SVI (mL/g)	54±9	55±6	48±5	50±4
SRT (days)	5±2	7±2	12±4	15±4
SS (mg/L)	57.29±5.70	51.14±5.00	50.43±5.00	49.07±4.40
F/M (kg_{BOD}/Kg_{MLSS})	0.22±0.10	0.24±0.10	0.25±0.10	0.26±0.10
pH	7.02±0.20	7.17±0.10	7.29±0.10	7.33±0.20

3.6 Microbial community

Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, and Verrucomicrobia were the most common phyla in the samples (Figure 7(a)). Reactor-IV also contained Deinococcus-Thermus and Planctomycetes. In reactor-IV, under anoxic conditions, Proteobacteria constituted 60.8%, while under oxic conditions, they comprised 58.5%. Actinobacteria were more common in oxygenated (17.4%) than anoxic (6.1%) conditions, which suggests that they prefer environments with less O_2 for the metal-removal process. Firmicutes exhibited a higher prevalence in anoxic conditions, with a representation of 12.4% compared to 8.3% in oxic environments. Gram-negative bacteria possessing lipopolysaccharide surfaces exhibit resistance to Cd (Huang et al., 2017; Su et al., 2020). These results indicate a high removal efficiency of heavy metals under anoxic conditions (Figure 2). Furthermore, Firmicutes and Thermomonas are involved in the digestion of ammonia nitrogen (Chen et al., 2019; Jin et al., 2022).

Figure 7(b) depicts the microbial species present in reactor-I and reactor-IV. In reactor-I, *Thermomonas heamalytica* and *Bacillus funiculus* were the most common bacteria. In reactor-IV, *Pseudomonas vancouverensis*, *Clostridium pasterurianum*, and *Sulfuriflexus mobiliz* were the most common bacteria. Hussain et al. (2022) showed that many bacteria, such as those in the genera *Bacillus*, *Staphylococcus*, and *Pseudomonas*, can facilitate Zn^{2+} fixation. Reactor-IV had a high abundance of nitrifying and denitrifying bacteria, especially *P. vancouverensis*, which likely helped with nitrogen and metal removal. *Pseudomonas* species can also remove Cd^{2+} (Igbinosa et al., 2012; Abbas et al., 2014). *T. heamalytica* facilitates nitrogen removal, while *B. funiculus* removes heavy metals, demonstrating the critical role of bacteria in reactor-I. In reactor-IV, *Pseudomonas* reached a relative abundance of 20% and a metal removal efficiency of over 30% when DO levels were low.

DO concentrations and time period affect bacterial proliferation (Liu et al., 2022), which in turn affects the removal efficiency of A2/O2-SBR. Bacterial taxonomy is linked to the removal of heavy metals (Fathollahi et al., 2021). Microorganisms are crucial for wastewater, nitrogen, and heavy metal treatment. Improved Proteobacteria and Actinobacteria populations may help remove metals from the wastewater, having different roles in oxygenated and anoxic environments.

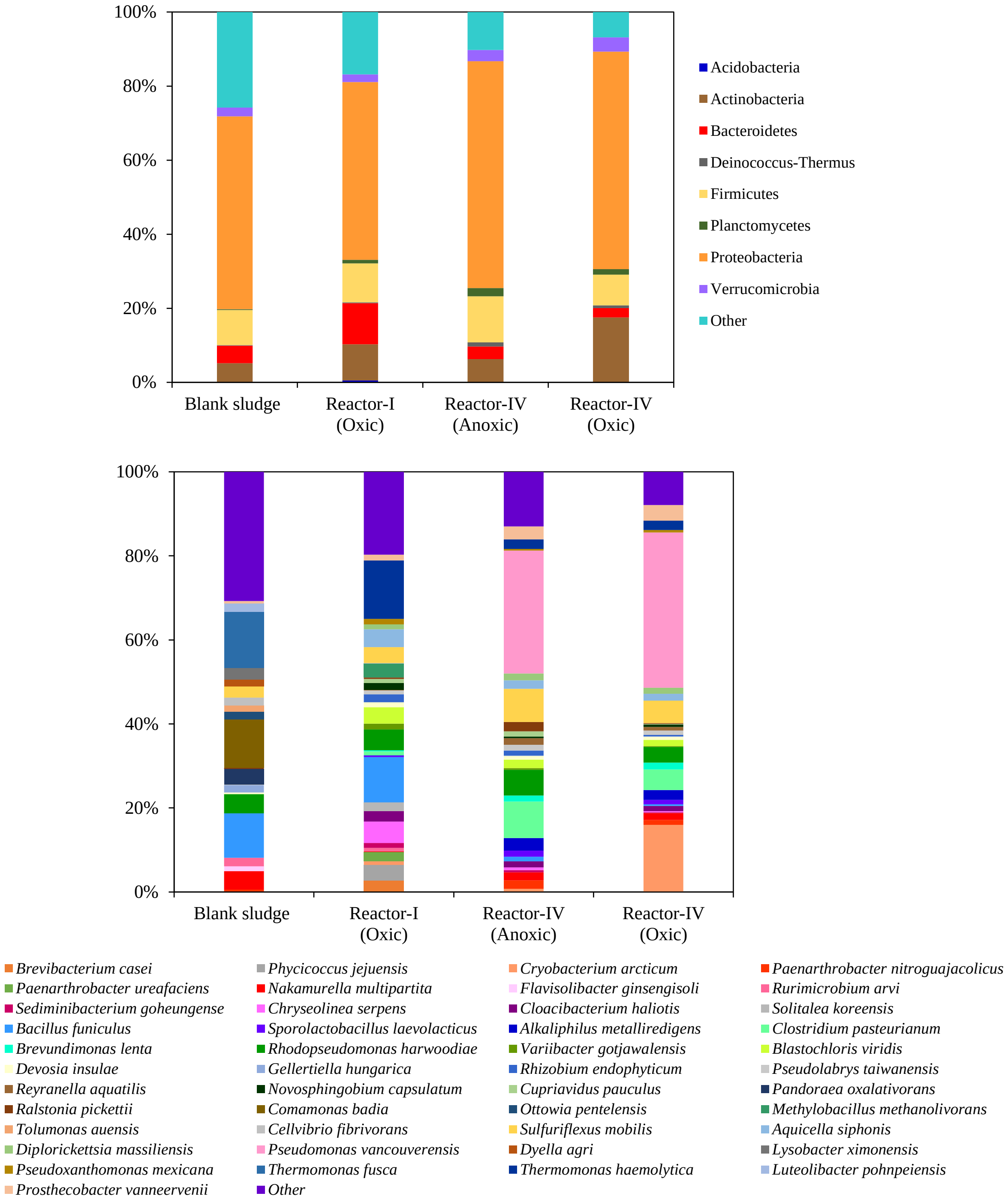


Figure 7. Relative abundances of bacterial in the blank sludge, reactor-I, and reactor-IV (with relative abundances greater than 1%) (a) phyla and (b) species

4. CONCLUSION

This study demonstrates that A2/O2-SBR system cleans electroplating effluent. Heavy metals (Zn^{2+} 89.74%, Cd^{2+} 81.37%), TKN (89.20%), and TN (84.25%) were removed at the best rates in reactor-IV, likely because it had a longer anoxic phase. Keeping the concentration of DO between 2.1 and 2.2 mg/L was beneficial for bacteria like *P. vancouverensis*, *R. harwoodiae*, and *S. mobilis*, which removed nitrogrn and heavy metals, improving the wastewater treatment. The A2/O2-SBR is a reliable and adaptable wastewater treatment technology. This study provides valuable insights for metal contamination management, including the importance of specific bacterial species for the elimination of heavy metals and nitrogen compounds and how the bacterial community can be modified to improve reactor performance. Future pilot studies should focus on the development and implementation of the A2/O2-SBR system in industrial wastewater treatment.

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