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Performance evaluation of the bench-scale hybrid alternating aerobic/anoxic activated sludge-membrane bioreactor system for the wastewater treatment and reuse

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ABSTRACT

In this study, the effect of total dissolved solids (TDS) on the simultaneous removal of TDS and biological nitrogen was investigated in a bench-scale hybrid alternating aerobic/anoxic activated sludge-membrane bioreactor (AAA-MBR). The aerobic granular sludge was cultivated in an aerobic sequencing batch reactor with municipal wastewater (organic loading rate: 2.1–4.3 kg COD/m^3 day) and used as a biosorbent for TDS removal in the pretreatment reactor. The AAA-MBR system, consisting of two AAA zones and a reaction zone where the membrane modules are submerged, was operated for 30 d under four TDS conditions (average TDS concentrations of 0, 2,003, 3,003, and 4,032 mg/L for Phase I, II, III, and IV, respectively). The influent wastewater was synthesized to simulate the reverse osmosis concentrate (ROC) obtained from a full-scale municipal wastewater reclamation plant that recycles secondary sewage effluents. The reduction efficiency of the chemical oxygen demand (COD) (>98.8% removal) and rate of nitrification (>93.9%) and denitrification (>86.1%) were not significantly influenced by the TDS concentration (<2,003 mg/L) of the influent. Thus, the AAA-MBR process could be an effective ROC recycling alternative to the conventional methods to reduce TDS content, COD, and biological nitrogen at a low cost with less consumption of chemical adsorbents or coagulants.

Keywords: Aerobic/anoxic; Aerobic granular sludge; Biosorption; Biological nitrogen removal; Membrane bioreactor

1. Introduction

Water reclamation of secondary treated sewage effluent via microfiltration (MF) or ultrafiltration (UF) prior to reverse osmosis (RO) membrane filtration has become increasingly popular. Demands for these and other technologies have grown as water supply and management issues have been exacerbated with population growth and water quality degradation [1]. The RO membranes push saline water to the less saline side with an applied hydraulic pressure (ΔP), which is greater than the osmotic pressure ($\Delta\pi$), across a membrane [2,3]. The RO processes are efficient in removing organic and inorganic contaminants in drinking water or for water reuse but produce concentrated hypersaline waters in a RO concentrate (ROC) stream [4,5]. The ROC contains large amounts of dissolved salts, nutrients (*e.g.*, nitrogen and phosphorus), and recalcitrant micropo-

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llutants that are resistant to degradation under biological wastewater treatment conditions (*e.g.*, pharmaceuticals, pesticides, and endocrine disrupting compounds). The production of the ROC limits the application of RO technology in water reclamation practices [6–8]. The direct disposal of ROC into surface water is economical, but may pose a serious threat to aquatic ecosystems [9]. ROC management is important for reducing the environmental risk associated with these filtration technologies. In addition, there are legal and regulatory concerns regarding the long-term effects of organic- and inorganic-bound nutrients, which can induce eutrophication in the receiving water body and deteriorate water quality [10].

ROC recycling offers a sustainable alternative to traditional ROC management methods (*e.g.*, disposal or treatment). A major challenge facing ROC recycling in municipal wastewater treatment plants involves its high concentrations of nutrients and low ratio of biochemical oxygen demand (BOD_5) to chemical oxygen demand (COD). Zero liquid discharge (ZLD) or near-ZLD (>95% water recovery) may provide a potential solution to problems associated with nutrient removal in ROC by reducing the discharge volume [11]. However, large capital investments and pretreatment to remove mineral scale precursors limit the achievable recovery of the water. Conventional treatment processes (*e.g.*, adsorption/biosorption using activated carbon or demineralization via chemical softening and electro-coagulation) remain ineffective because of their low removal rates and high operation costs due to chemical addition and regeneration [12,13]. Advanced oxidation processes including electro-/O_3/H_2O_2/UV oxidation and chemical treatment can provide high removal rates of ammonium nitrogen (NH_4^+-N), but reduction of the total nitrogen (TN) is often low due to the partial transformation of NH_4^+-N to nitrate nitrogen (NO_3^--N) [14,15].

The alternating aerobic/anoxic (AAA) activated sludge system is a continuous flow process which meets the specific requirements for nitrification and denitrification by implementing a simple sequence of "air-on/off" phases [16]. For example, NH_4^+ can be oxidized to NO_3^- by autotrophs under aerobic conditions (nitrification), while NO_3^- can be reduced to N_2 by heterotrophs under anoxic conditions (denitrification). The AAA process enhances the nitrogen removal efficiency under alkaline deficient conditions since the alkalinity consumed during nitrification can be partially recovered during the subsequent denitrification process [17,18]. In addition, the AAA system significantly reduces energy consumption compared to conventional methods, as the aeration process accounts for more than 60% of the total energy consumption in a treatment plant [19]. Recently, membrane bioreactors (MBRs) has been combined with conventional activated sludge systems (*e.g.*, a hybrid anaerobic-anoxic-aerobic MBR process (A^2O-MBR) [20, 21]) to remove refractory contaminants and ammonia in industrial wastewater due to its high efficiency and cost-effectiveness [22]. To the best of our knowledge, there are no reports regarding biological nitrogen removal at high total dissolved solids (TDS) loading rates in a hybrid AAA-MBR system.

The main characteristic of the AAA process is that the alkalinity previously consumed in the aerobic nitrification phase can be partially recovered during the subsequent anoxic denitrification phase, thus maintaining a relatively stable system and achieving nitrogen removal [8].

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This study aims (1) to examine the biosorption of the TDS using aerobic granular sludge in a pretreatment basin. The use of aerobic granular sludge as a novel biosorbent may be effective in the removal of TDS in ROCs in the context of a wastewater reclamation plant [23]. (2) To identify bacterial abundance and distribution during the denitrification process in the hybrid AAA-MBR system, and (3) to verify the long-term performance of a bench scale hybrid AAA-MBR for biological TDS, COD, and nitrogen removal.

2. Materials and methods

2.1. Experimental set-up and operating conditions

The bench-scale experiments were performed in an AAA-MBR system followed by pretreatment using aerobic granular sludge for TDS removal (Fig. 1). The aerobic granular sludge was cultivated in an aerobic sequencing batch reactor (SBR), which received municipal wastewater inputs with an organic loading rate of 2.1–4.3 kg COD/m^3 d. The SBR had a working volume of 9.1 L, a height of 1,100 mm, and an inner diameter of 120 mm. The pretreatment tank was operated in biosorption mode, consisting of four discrete cycles (4 h aerated FILL and REACT, 1 h SETTLE, 0.5 h DRAW, and 0.5 h IDLE) under continuous stirring.

The whole experiment was carried out as four different phases varying the average TDS concentrations. The synthetic ROC was fed into the top of the pretreatment reactor, and the flow rate was controlled by a variable-speed peristaltic pump to maintain constant hydraulic retention time (HRT) of 24 h. The HRTs were calculated based on the volumes of the reactors. Throughout the experiments, the mixed liquor from the pretreatment tank was continuously pumped to the AAA-MBR system with an effluent recycle ratio of 2 (160 L/d). The AAA-MBR was divided into two AAA zones and a reaction zone where the membrane modules were submerged. The flat-sheet membrane modules were composed of polyethylene terephthalate with a nominal pore size of 0.25 μm and effective membrane surface area of 0.15 m^2 (YMK Co., Ltd, South Korea). Membranes were chemically cleaned using a 3 wt% sodium hypochlorite solution for 24 h to reduce membrane fouling before the next experimental phase. The ceramic air diffuser, which produces fine air bubbles, was installed at the bottom of the AAA-MBR system to provide dissolved oxygen (DO) and prevent membrane fouling. The level of DO was maintained at 1.5–3.0 and 0.1–0.15 mg/L for the aerobic and anoxic conditions of the AAA zones, respectively, and 5.0–7.0 mg/L for the reaction zone. The two AAA zones and a reaction zone had constant HRTs of 4.6, 4.7, and 1.9 h, respectively. The

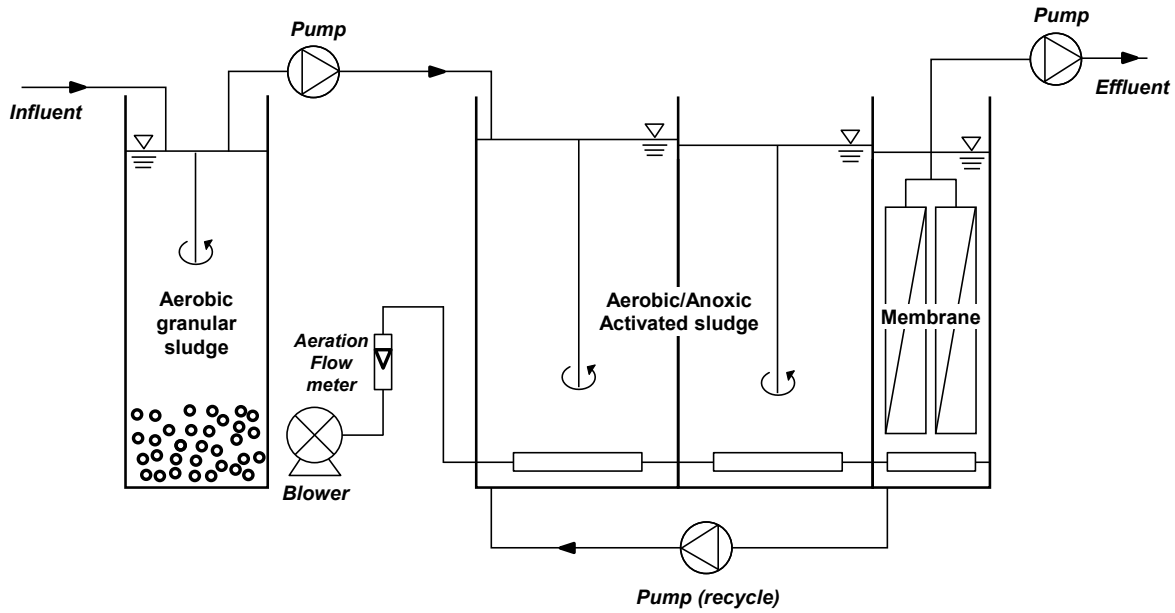


Fig. 1. Schematic diagram of the experimental setup for alternating aerobic/anoxic activated sludge-membrane bioreactor system.

temperature of the AAA-MBR was controlled at 23–25°C using a temperature control system. At the end of MBR, the excess sludge was wasted directly from reactor to control the solid (or sludge) retention time (SRT) at 30 d. The effluent samples were taken from the pretreatment reactor and MBR. Analysis of the samples were conducted for physicochemical (TDS, COD, NH_4^+-N, and NO_3^--N), microbiological (operational taxonomic units (OTUs) and Chaol and Shannon diversity indices), and operational parameters (mixed liquor suspended solids (MLSS)) as per standard methods (discussed below).

2.2. Influent characteristics

Four wastewaters were synthesized to simulate the ROC from a full-scale wastewater reclamation plant featuring sewage secondary effluent reclamation. The synthetic ROC was prepared by dissolving NH_4Cl, $CaCl_2$, $MgSO_4$, $NaNO_3$, KH_2PO_4, $MgSO_4$, $NaHCO_3$, and KCl in deionized (DI) water (18.2 MΩ·cm), which was obtained from a Barnstead nanopure DI water system (USA). Methanol (3,200 mg/L) was used as the external carbon source for denitrification. All chemicals used were of analytical-reagent grade. The influent characteristics at four experimental phases are summarized in Table 1. The simulated wastewater samples were stored in the refrigerator (4°C) and warmed to 25°C before use. The average TDS concentrations in the ROC of the four phases were 0, 2003, 3003, and 4032 mg/L, while the COD concentrations were 3220, 3213, 3228, and 3,238 mg/L, respectively.

2.3. Analytical methods

The effective specific surface area of the aerobic granular sludge was measured using the Brunauer-Emmett-Teller (BET) method. The aerobic granular sludge before and after TDS biosorption was observed by scanning (SEM; JSM-6500F, JEOL Ltd., Japan) and transmission electron microscopies (TEM; JEM-1010, JEOL Ltd., Japan) at 80 kV. Chemical analyses were performed according to the Standard Methods for the examination of water and wastewater [24]. The TDS was determined by drying at 105°C on a glass-fiber filter (Whatman GF/C) using the APHA method 2540 C. MLSS was measured by drying at 105°C on a pre-weighed evaporating dish following the analytical procedure outlined in the Standard Methods. COD was measured on a Hach (DBR 200, USA) COD test kit using the potassium dichromate method (APHA method 5220B). NH_4^+-N and NO_3^--N were determined using a UV spectrophotometer (Hach DR-4000, USA), direct Nesslerization (APHA method 4500 NH_3 C), and the ultraviolet spectrophotometric screening method (APHA method 4500 NO_3^- B), respectively. The pH and DO of the ROCs were monitored using a pH meter (Mettler-Toledo, Switzerland) and DO probe (Mettler-Toledo, Switzerland). A two sample *t*-test was performed with a 95%confidence level ($\alpha = 0.05$) used to evaluate the significance of differences between nitrogen removal by the AAA-MBR system at different initial TDS concentrations.

2.4. DNA extraction, amplification, and 16S rRNA pyrosequencing

At the end of each experimental phase, the activated sludge samples were collected from the effluent and purified for the extraction of total microbial community DNA. A 20 mL sample was centrifuged at 5,000 rpm for 10 min, and the pellets were subsequently re-suspended in 250 µL Tris-EDTA buffer solution (10 mM TRIS, 1 mM EDTA, pH 7.6) followed by rinsing with TRIS/EDTA [25]. Bacterial genomic DNA for each sample was extracted in triplicate and purified using a GeneAllExgene™ Soil SV Kit (GeneAll Biotechnology, Seoul, Korea) according to the proto-

Table1
Characteristics of the synthetic wastewater used in the experiments

Phase	pH	TDS[a] (mg/L)	COD[b] (mg/L)	NH_4^+-N (mg/L)	NO_3^--N (mg/L)
I	7.1 ± 0.5	0	3,220 ± 290	5.15 ± 1.4	74.2 ± 3.8
II	7.4 ± 0.3	2,003 ± 59.0	3,213 ± 237	5.19 ± 1.1	75.2 ± 3.9
III	7.2 ± 0.6	3,003 ± 243	3,228 ± 482	5.12 ± 1.0	74.5 ± 3.3
IV	7.5 ± 0.2	4,032 ± 278	3,238 ± 272	5.06 ± 0.9	74.6 ± 3.6

[a] TDS: total dissolved solids
[b] COD: chemical oxygen demand

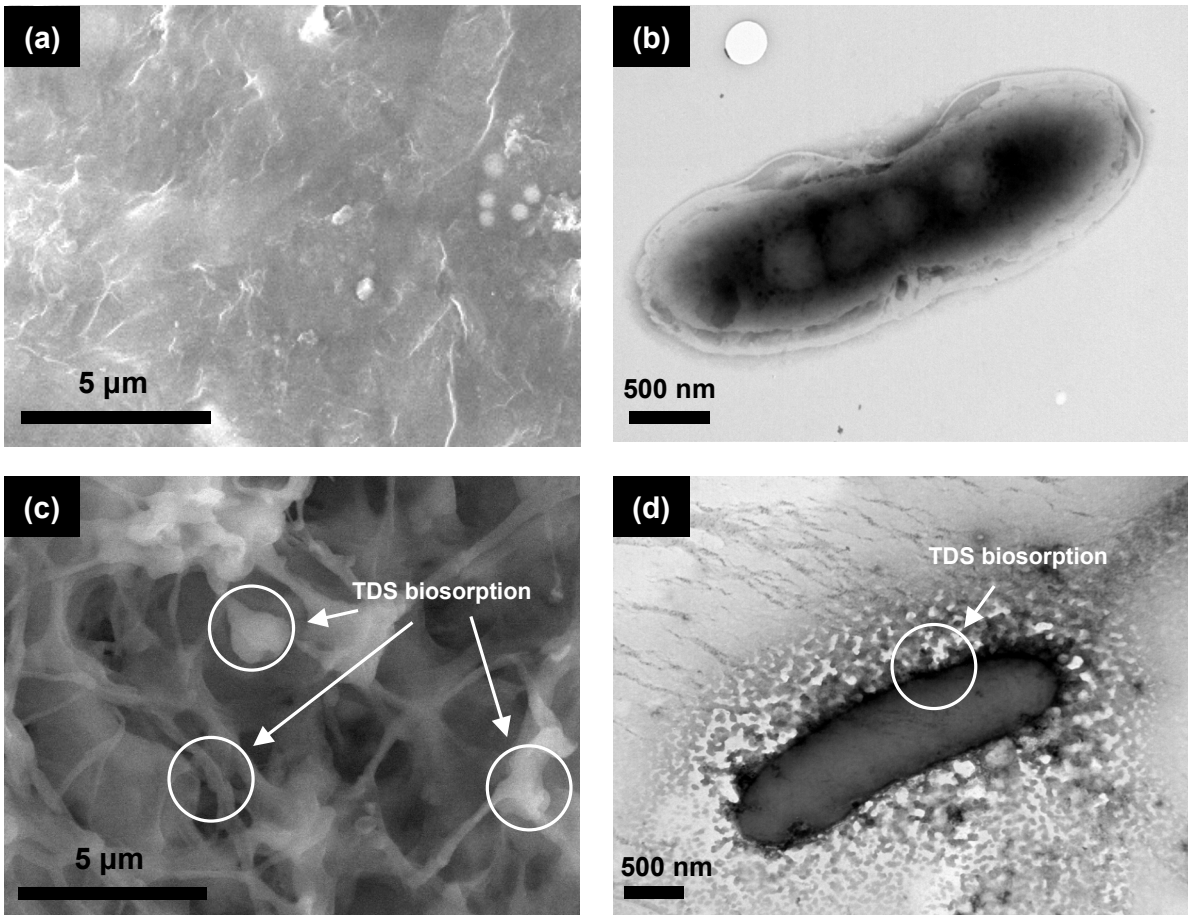


Fig. 2. SEM of the aerobic granular sludge (a) before and (c) after TDS biosorption. TEM micrograph of the aerobic granular sludge (a) before and (c) after TDS biosorption. Experimental conditions: $[TDS]_0$ = 2,000 mg/L; $[Aerobic\ granular\ sludge]_0$ = 1.0 g/L; pH 7.

col of the manufacturer [26]. The extracted DNA samples were frozen in a −20°C freezer overnight or until processing (up to 1 week). The amplification of the bacterial 16S rRNA gene including three variable regions (V1–V3) was performed using barcoded fusion primers, as previously described [27–30]. The polymerase chain reaction (PCR) for DNA amplification was performed after purification using a QIA quick PCR Purification Kit (QIAGEN, Limburg, Netherlands) [31]. Metagenomic pyrosequencing was performed on a 454 GS Junior system (Roche, Basel, Switzerland) according to the manufacturer's instructions. The obtained sequences were compared with those in the EzTaxon extended database using the BLASTN algorithm [32]. The OTUs (neighbor clustering distance 0.03) as well as Chaol and Shannon diversity indices were estimated using CL community™ software (www.chunlab.com) [28].

3. Results and discussion

3.1. Characterization of the TDS-adsorbed aerobic granular sludge

The surface morphology of the aerobic granular sludge incubated for 24 h in the SBR under aerobic condition was characterized by SEM and TEM. Fig. 2 shows that before TDS biosorption, the bare aerobic granular sludge exhibited an uninterrupted smooth surface surrounded by heterotrophic

bacterial biofilms. After TDS biosorption, the aerobic granular sludge sample was extracted from the pretreatment tank of the AAA-MBR system to analyze the deposit morphology on the surface. A dense network of sharp-edged bacterial biofilms was observed on the surface of the TDS-sorbed aerobic granular sludge at a TDS concentration of 2,000 mg/L (Fig. 2c). This is indicative of tightly bound extracellular polymeric substances (EPS) whose compositions and morphology are significantly dependent on the synthetic conditions of the sludge (*e.g.*, SRT and concentration) [33,34].

3.2. Biological nitrogen removal in the hybrid AAA-MBR

A bench-scale AAA-MBR system was used to investigate the effect of TDS content on biological nitrogen removal from synthetic ROC. Aerobic granular sludge was selected to pretreat the influent TDS, as in our previous studies [23, 35]. The BET surface area of the aerobic granular sludge was determined from the nitrogen adsorption-desorption isotherms. The aerobic granular sludge had a specific surface area of 828.76 m^2/g and maximum TDS biosorption capacity of 1,698 mg/g at a concentration of 0.25 g/L of aerobic granular sludge. The hydraulic retention times of the AAA activated sludge system and MBR were adjusted to 9.3 and 1.9 h, respectively, and a fixed ratio of 2 was used for internal recycling. TDS, COD, NH_4^+-N, and NO_3^--N concentrations along the reactor sections (*i.e.*, pretreatment, AAA, and MBR) were analyzed to obtain better insight into the removal of TDS, COD, and nitrogen. Fig. 3 shows the operation results for COD, NH_4^+-N, and NO_3^--N removal over 30 days of AAA-MBR operation including pretreatment using the aerobic granular sludge (Phase I). Overall, stable removal performance with respect to organic matter and nitrogen was observed in the AAA-MBR process.

The COD in the synthetic ROC ranged from 3,020 to 3,510 mg/L (COD_{inf}) with an average value of 3,220 mg/L. In the pretreatment tank, the influent COD was reduced by more than 75.5%. This indicates that most of the COD was removed by biosorption in the presence of aerobic granular sludge in an aerobic environment. The remaining COD was reduced from 649 ± 229 mg/L (COD_{pre}) to 24 ± 15 mg/L (COD_{eff}), which results in an overall >98.8% removal efficiency. This high COD removal efficiency indicates that large macromolecules were effectively biodegraded into smaller molecular substrates in the AAA-MBR process [36].The initial NH_4^+-N concentration was maintained in the influent at 5.2 ± 1.4 mg/L (NH_4^+-N_{inf}). As shown in Fig. 3, nitrification occurred in the pretreatment tank with an outlet NH_4^+-N concentration of 0.3 ± 0.2 mg/L (NH_4^+-N_{pre}). This indicated that 93.9% of NH_4^+-N had been oxidized during pretreatment. Xia et al. [37] reported that nitrification can be accelerated in the presence of suspended solids and exhibited a non-linear increase with increasing suspended solids content. This is primarily due to a large surface area of solids that can facilitate nitrification by increasing the chances of interaction between nitrifying bacteria and nitrogen. When no suspended solids are present in freshwater systems, NO_2^--N is not been completely transformed to NO_3^--N until NO_2^--N significantly accumulated in the cultivation system. This indicates that the density of nitrifying bacteria in the aerobic granular sludge was sufficient to efficiently nitrify the influent NH_4^+-N. The average final effluent concentration of NO_3^--N was 9.5 mg/L (NO_3^--N_{eff}) with an average total removal efficiency of 87.3%. This demonstrates that most of the nitrogen was removed in the AAA-MBR reactor.

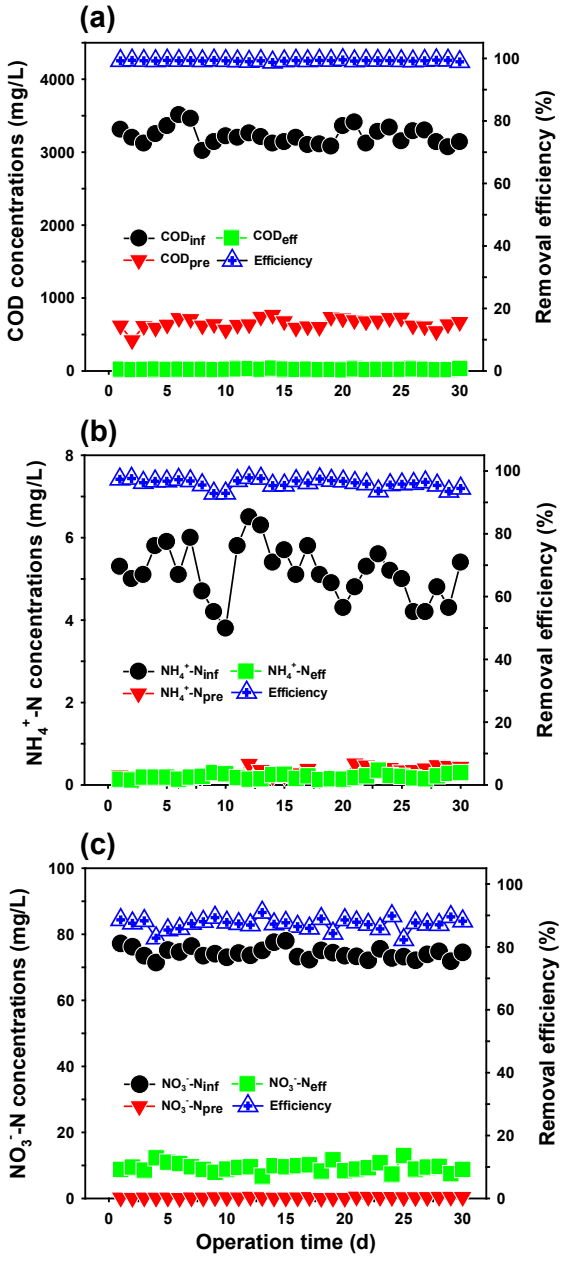


Fig. 3. Variation of (a) TDS, (b) COD, (c) NH_4^+-N, and (d) NO_3^--N concentrations in the hybrid alternating aerobic/anoxic-membrane bioreactor system over 30 d of operation (Phase I). Experimental conditions: $[TDS]_0$ = 0–32 mg/L; $[COD]_0$ = 3,020–3,510 mg/L; $[NH_4^+$-$N]_0$ = 3.8–6.5 mg/L; $[NO_3^-$-$N]_0$ = 71.4–78.0; [Aerobic granular sludge]$_0$ = 0.25g/L.

3.3. Effect of TDS on biological nitrogen removal in the hybrid AAA-MBR

The initial TDS concentration was varied to evaluate the response and tolerance of the AAA-MBR process to higher

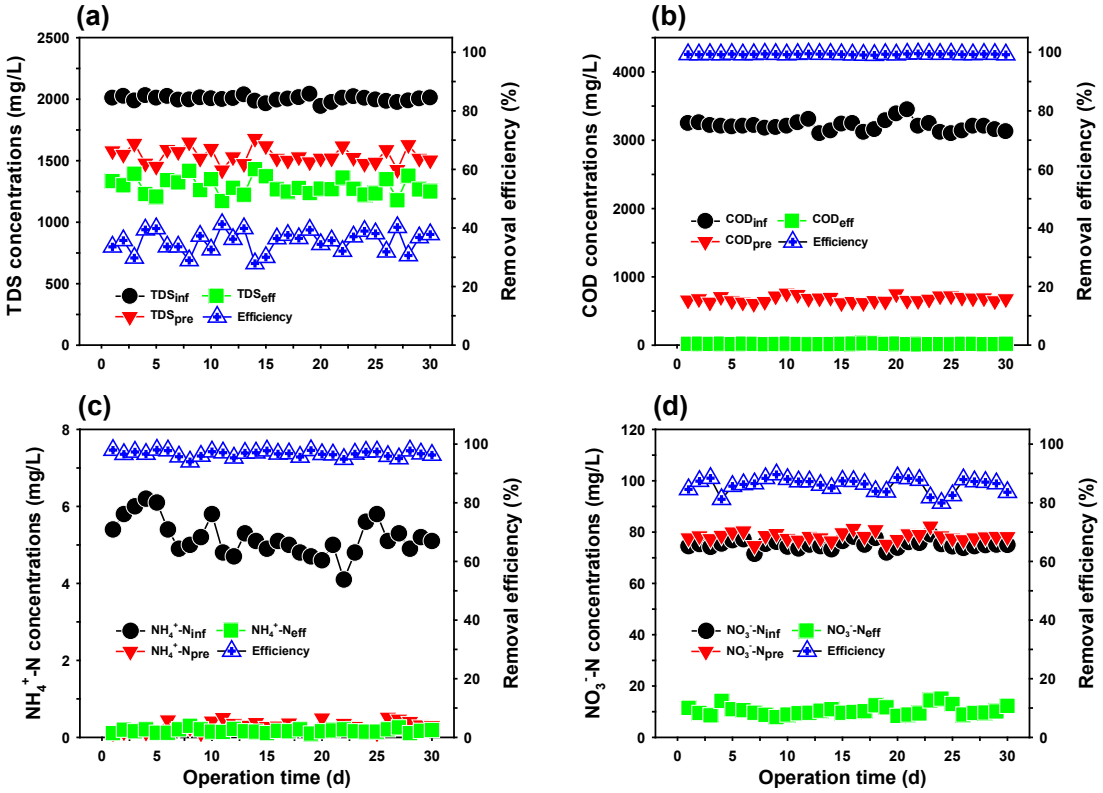


Fig. 4. Variation of (a) TDS, (b) COD, (c) NH_4^+-N, and (d) NO_3^--N concentrations in the hybrid alternating aerobic/anoxic-membrane bioreactor system over 30 days of operation (Phase II). Experimental conditions: $[TDS]_0$ = 1,944–2,043 mg/L; $[COD]_0$ = 3,100–3,450 mg/L; $[NH_4^+\text{-}N]_0$ = 4.1–6.2 mg/L; $[NO_3^-\text{-}N]_0$ = 71.5–79.1; $[\text{Aerobic granular sludge}]_0$ = 0.25 g/L.

influent TDS concentrations. The TDS concentration was varied between 1,944 and 4,310 mg/L, while the other conditions were held constant and the system was operated for 30 days. The COD concentrations ranged from 2,990 to 3,710 mg/L in most of the trials. Fig. 4 shows the variation in the TDS, COD, NH_4^+-N, and NO_3^--N concentrations along the hybrid AAA-MBR reactors at an initial TDS concentration of 2,003 ± 40 mg/L (Phase II). From Fig. 4, 23.0% of the TDS was biosorbed by the aerobic granular sludge in the pretreatment tank, and the remaining 12.4% was removed in the following reactors.

The COD removal efficiency was 79.0% (from 3,213 ± 237 to 676 ± 86.2 mg/L) in the pretreatment tank with aerobic granular sludge. In the following AAA-MBR reactors, 20.4% more COD was removed (from 676 ± 86.2 to 21 ± 8.7 mg/L), which indicates that the initial TDS concentration did not significantly affect the COD or its removal efficiency in the final effluent (Fig. 5). The NH_4^+-N concentration in the influent ranged between 4.1 and 6.2 mg/L in Fig. 4c. A similar degree of nitrification to Phase I (94.4%) was achieved in the pretreatment tank ($NH_4^+\text{-}N_{pre}$ = 0.29 ± 0.23 mg/L). The average NO_3^--N concentration in the final effluent was 10.5 mg/L, with a86.1% average removal efficiency. This result was not significantly different from the trial in the absence of TDS (P> 0.05). This suggests that the hybrid AAA-MBR process can be effectively operated at higher TDS concentrations.

Figs. 6 and 7 show the variation in TDS, COD, NH_4^+-N, and NO_3^--N concentrations in the pretreatment tank and AAA-MBR as well as the total removal efficiencies at higher

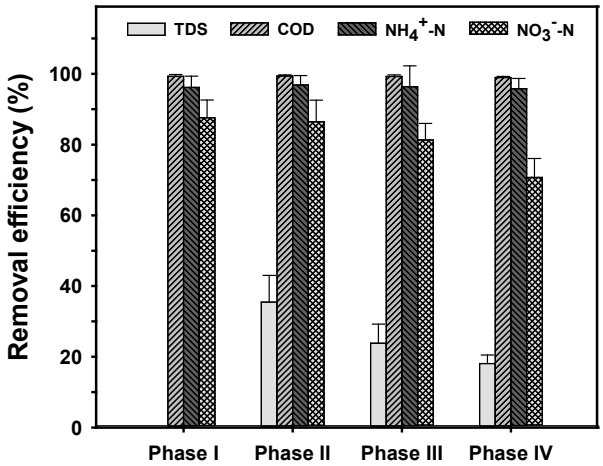


Fig. 5. Removal performance of TDS, COD, NH_4^+-N, and NO_3^--N in the hybrid alternating aerobic/anoxic-membrane bioreactor system under different initial TDS concentrations. Experimental conditions: $[TDS]_0$ = 0–4,310 mg/L; $[COD]_0$ = 2,990–3,710 mg/L; $[NH_4^+\text{-}N]_0$ = 3.8–6.5 mg/L; $[NO_3\text{-}N]_0$ = 71.2–79.1; $[\text{Aerobic granular sludge}]_0$ = 0.25g/L; Operation period = 30 d.

initial TDS concentrations (>2,760 mg/L, Phase III and IV). The system receives influent with a COD between 2,990 and 3,710 mg/L, similar to that in Phase I and II. Increasing the initial TDS concentration from 2,003 to 4,032 mg/L caused

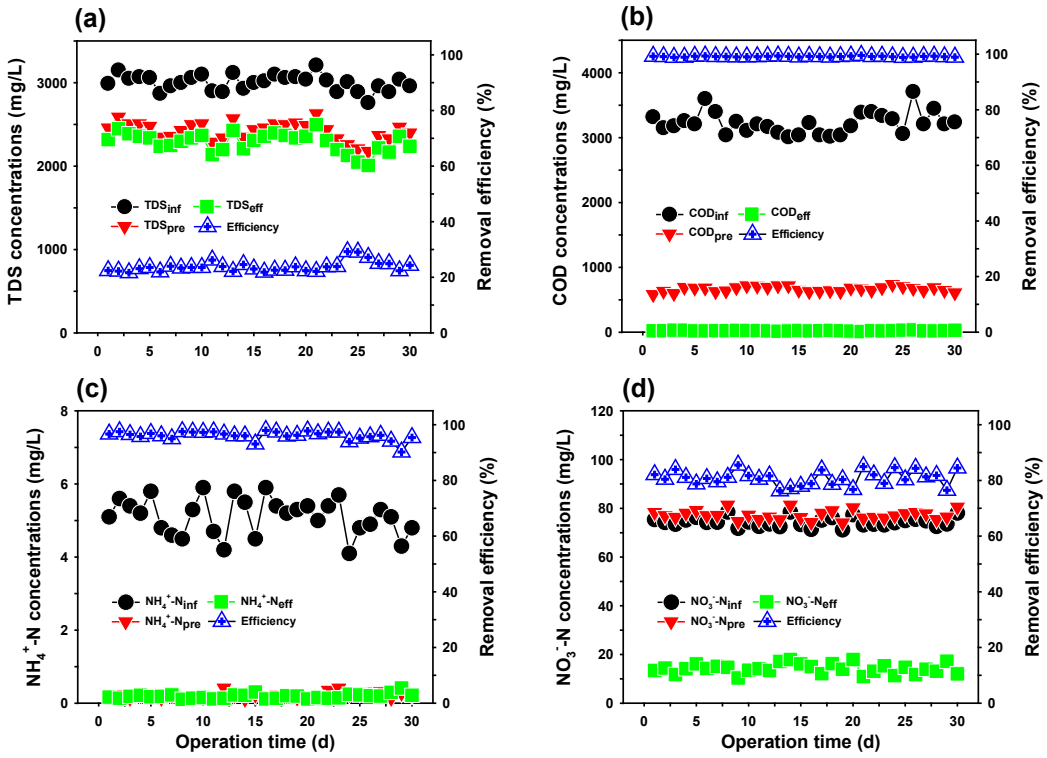


Fig. 6. Variation of (a) TDS, (b) COD, (c) NH_4^+-N, and (d) NO_3^--N concentrations in the hybrid alternating aerobic/anoxic-membrane bioreactor system over 30 d of operation (Phase III). Experimental conditions: $[TDS]_0$ = 2,700–3,210 mg/L; $[COD]_0$ = 3,010–3,710 mg/L; $[NH_4^+\text{-}N]_0$ = 4.1–5.9 mg/L; $[NO_3^-\text{-}N]_0$ = 71.2–78.5; $[\text{Aerobic granular sludge}]_0$ = 0.25 g/L.

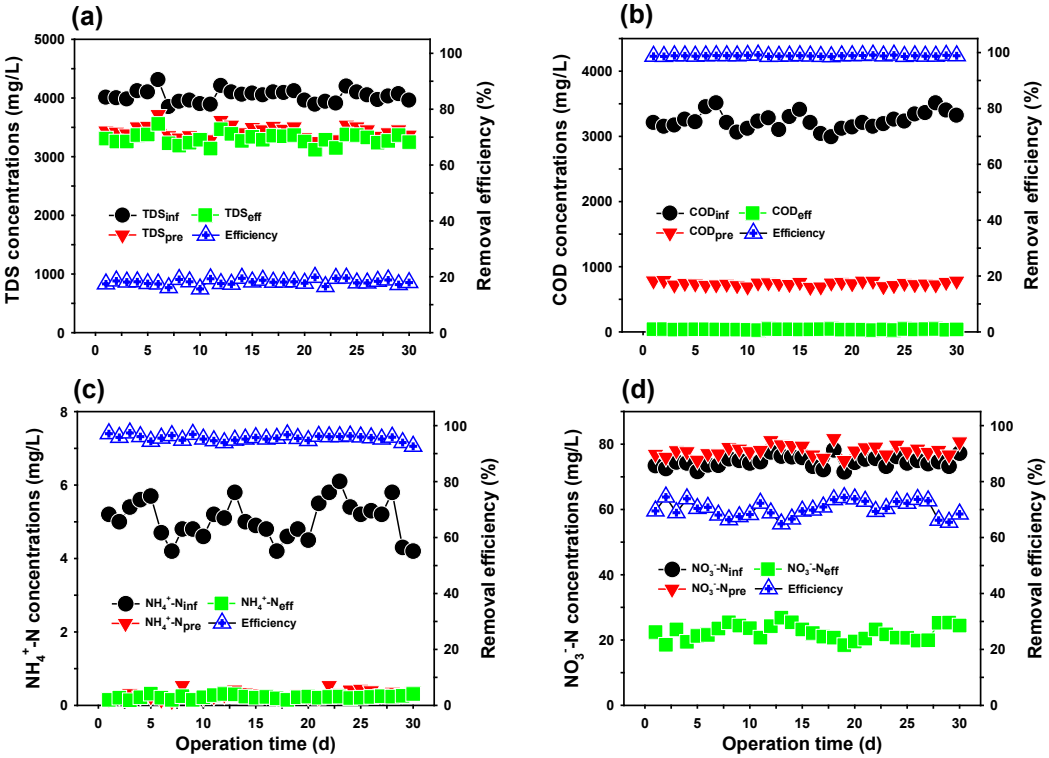


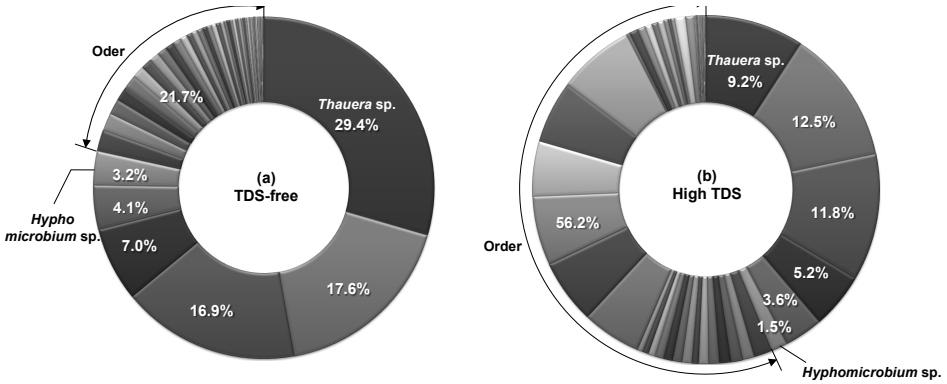
Fig. 7. Variation of (a) TDS, (b) COD, (c) NH_4^+-N, and (d) NO_3^--N concentrations in the hybrid alternating aerobic/anoxic-membrane bioreactor system over 30 d of operation (Phase IV). Experimental conditions: $[TDS]_0$ = 3,850–4,310 mg/L; $[COD]_0$ = 2,990–3,510 mg/L; $[NH_4^+\text{-}N]_0$ = 4.2–6.1 mg/L; $[NO_3^-\text{-}N]_0$ = 71.5–78.2; $[\text{Aerobic granular sludge}]_0$ = 0.25 g/L.

a slight decrease in the TDS removal efficiency (23.0, 19.2, and 14.8% in the pretreatment tank and 12.4, 4.6, and 3.2% in AAA-MBR for Phase II, III, and IV, respectively) when the amount of aerobic granular sludge was fixed (Fig. 5). The pretreatment reactor removed most of the influent COD (approximately 79.5% removal) by aerobic granular sludge. The total COD levels in Phase III and IV were reduced from 2,990–3,710 mg/L to 14–45 mg/L for a 98.8–99.2% removal efficiency. A similar range of COD removal efficiency was also found in Phase I and II. This confirms that the COD removal was not strongly influenced by the initial TDS concentration.

Significant nitrification was observed at higher TDS concentrations, as shown in Figs. 6 and 7. In the pretreatment reactor, the influent NH_4^+-N concentrations of 4.1–5.9 and 4.2–6.1 mg/L (NH_4^+-N_{inf}) for Phase III and IV decreased to 0.11–0.42 and 0.11–0.54 mg/L (NH_4^+-N_{pre}), respectively. This indicates that approximately 95.6 and 94.0% of the initial NH_4^+-N was oxidized to NO_3^--N, demonstrating that a similar degree of nitrification occurred in the pretreatment reactor of the hybrid AAA-MBR system compared to those in Phase I and II (93.9 and 94.4%). It was found that 80.9 and 70.2% of the NO_3^--N was biologically removed in Phase III and IV, respectively, lower than the removal rates in Phase I and II (87.3 and 86.1%, respectively). TheNO_3^--N removal efficiencies in Phase III and IV were significantly different from those in the absence of TDS (Phase I) ($P < 0.05$, Fig. 5).

3.4. Bacterial abundance and distribution

Denitrification occurs in bacterial cells where nitrogen oxides and oxyanions act as terminal e^- acceptors instead of O_2 for energy production under oxygen-depleted conditions, and nitrate is subsequently reduced to produce gaseous nitrogen [38,39]. The nitrification (SNR) and denitrification rates (SDNR) of the AAA-MBR system were obtained for the four experimental phases. The SDNR decreased with increasing initial TDS (SDNR = 0.035, 0.034, 0.032, and 0.028 g NO_3^--N/g VSS per day for Phase I, II, III, and IV, respectively), while 0.0025 g NH_4^+-N/g VSS per day for the SNR was obtained with low influent NH_4^+-N loadings (3.8–6.5 mg/L) for all experimental phases. The 16S rRNA gene pyrosequencing technique was used to examine the dynamic composition of microbial communities during denitrification (Fig. 8). A total of 55 bacterial communities were identified in the absence of influent TDS in which the *Thauera* sp., *Rhodobacteraceae*, *Rikenellaceae*, *Sphingobacteriales*, *Rhodocyclaceae*, and *Hyphomicrobiumnitrativorans NL23* sp. were the six most abundant microbial communities with and without the influent TDS. *Thauera* sp. and *Hyphomicrobium* spp. are well-known denitrifying bacteria commonly found in sewage treatment plants [40–42]. Recently, the *Hyphomicrobiumnitrativorans NL23* sp. was identified as a major player in methanol-fed denitrification systems [43–45]. The *Rhodobacteraceae* and *Rhodocyclaceae* communities are characterized as heterotrophic denitrifying populations in activated sludge [46,47]. When the influent was TDS-free, *Thauera* sp. (29.4%) were the most dominant bacterial taxa followed by *Rhodobacteraceae* (17.6%), *Rhodocyclaceae* (4.1%), and *Hyphomicrobiumnitrativorans NL23* sp. (3.2%). This indicates that denitrifying bacteria were dominant, comprising 54.3% of the total bacterial community. However, the proportions of *Thauera* sp. and *Hyphomicrobiumnitrativorans NL23* sp. decreased to 9.2 and 1.5%, respectively, with



Key functional groups (Order > Family > Genus)	Abundance (%)	
	(a) Without TDS	(b) With TDS
Rhodocyclales > Rhodocyclaceae > Thauera	29.4	9.2
Rhodobacterales > Rhodobacteraceae	17.6	12.5
Bacteroidales > Rikenellaceae	16.9	11.8
Sphingobacteriales	7.0	5.2
Rhodocyclales > Rhodocyclaceae	4.1	3.6
Rhizobiales > Hyphomicrobiaceae > Hyphomicrobium nitrativorans NL23	3.2	1.5

Fig. 8. Identification of the major OTUs and relative abundance (%) of the bacterial species detected during denitrification (a) in the absence and (b) presence of influent TDS in the hybrid AAA-MBR system. The samples were analyzed by 16S rRNA-based pyrosequencing. Experimental conditions: $[TDS]_0$ = 0 and 4,032 mg/L; $[COD]_0$= 2,990–3,510 mg/L; $[NH_4^+\text{-}N]_0$ = 3.8–6.5 mg/L; $[NO_3^-\text{-}N]_0$ = 71.4–78.2; [Aerobic granular sludge]$_0$ = 0.25 g/L.

higher TDS content (avg. 4,032 mg/L), which reduced the SDNR to 0.028 g NO_3^--N/g VSS per day.

4. Conclusions

The results presented herein suggest that the hybrid AAA-MBR system with pretreatment using aerobic granular sludge is efficient for the simultaneous removal of TDS and nitrogen from synthetic ROC produced from a municipal wastewater reclamation plant. An increase in the average TDS concentration in the influent from 0 to 2,003 mg/L had negligible effects on TDS and nitrogen removals. However, very high initial TDS concentrations (>2,760 mg/L) inhibited TDS removal (from 35.4 to 18.1%) and denitrification efficiency (from 87.3 to 70.2%) due to the reduction in *Thauera* sp. and *Hyphomicrobiumnitrativorans NL23* spp. communities. This efficient hybrid AAA-MBR process has potential for use as a ROC recycling alternative to significantly reduce the high concentrations of TDS, COD, and nitrogen at a low operational cost and reduced consumption of chemical adsorbents and coagulants. Further research is required to better understand the performance of the membrane module and membrane fouling in the hybrid AAA-MBR.

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