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Biological treatment of saline domestic wastewater by using a down-flow hanging sponge reactor

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ABSTRACT

In this study, the effect of salinity on the removal of organic matter and nitrogen concentrations in bioreactor was investigated using a hybrid bench scale down-flow hanging sponge (DHS) system for 145 days of operation. The reactor had three identical sections that were filled to 30% volume with Bio-Bact to serve as attached media. The DHS reactor was fed with domestic wastewater that was mixed with increasing concentration of sodium chloride from 0.5 to 3.0% stepwise. The influent and effluent concentrations of BOD_5, COD_{Cr}, NH_4^+-N, and TN were analyzed to evaluate the performance of the DHS reactor during the operational period. Results indicate that when salinity was increased from 0.5 to 3.0%, the removal efficiency gradually decreased from 80.3% to 61.5% for COD_{Cr}, 76.4%–65.0% for BOD_5, 64.1%–48.4% for NH_4^+-N, and 50%–36% for TN. Besides, the changes in biofilm characteristics with increasing salinity were observed during the operational period. The results indicate that salinity has a significant influence on the removal of organic matters and nitrogen transformation in the biofilm of the bioreactor. Even so, the DHS reactor revealed a good potential for treating saline wastewater.

1. Introduction

Highly saline wastewater is defined as wastewater that has a salt concentration of more than 1% (Kargì and Dinçer, 1999). Saline wastewater is commonly generated in many industries such as sea food processing, leather production, pharmaceutical processing, and chemical production (Sudarno et al., 2011; Wang et al., 2015). Domestic/kitchen wastewater may also contain high salt concentration depending on food types. Many coastal or island areas use seawater for toilet use to save freshwater (Cortes-Lorenzo et al., 2012). Apart from the high level of salt, these wastewater sources also contain other pollutants such as organic and inorganic compounds. Therefore, the removal of these compounds present in the wastewater prior to discharge into the receiver is necessary (Sharma et al., 2020). Several chemical and physical approaches have been effectively employed to treat the saline wastewater including, but not limited to, evaporation (Lefebvre et al., 2005), coagulation - flocculation (Ellouze et al., 2003), ion exchange (Tchobanoglus et al., 2003), and membrane techniques (Afonso and Borquez, 2002). However, the high salinity presents in the environment can cause cell plasmolysis in microorganisms and damage them (Vyrides and Stuckey, 2009; Bassin et al., 2011; Ab Hamid et al., 2020). Furthermore, it is difficult to remove organic compounds from wastewater that has a high saline concentration (Ludzack and Noran, 1965; Zhang et al., 2019). Thus, it is necessary to remove the nitrogen and organic compounds having high saline concentration using biological technique before discharging into water bodies.

Most of the microbial members present in wastewater treatment systems are non-halophilic and weakly tolerate high salinity (Linarić et al., 2013; Qiu and Ting, 2013). Several investigations have described the effect of salinity on the performance of microorganisms in

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bioreactors and suggested that it had unfavorable effects on treatment activities (Kargi and Dincer, 1996; Dincer and Kargi, 1999; Uygur, 2006; Zhang et al., 2020). Sun et al. (2009) reported that the salinity of seawater hindered the activity of nitrite-oxidizing bacteria (NOB). Results suggested that seawater may be the major reason for maintaining ammonia-oxidizing bacteria and hindering NOB under the operating conditions (Wang et al., 2019). Abu-ghararah and Sherrard (1993) elucidated that salinity had a minute effect on nitrogen removal when the salt concentration reached 0.4%. Other authors have also reported that salinity considerably affected nutrient removal (Uygur and Kargı, 2004). Dinçer and Kargi revealed that a salt content of >3% had harmful effects on the rate and efficiency of nitrification (Dinçer and Kargi, 2001). Another study showed that the nitrification process was strongly inhibited when salt concentrations increased in the range of 0–60 g L^{-1} (Sánchez et al., 2004). Previous studies have also demonstrated that chemical oxygen demand (COD_{Cr}) removal was negatively affected when salt concentrations were above 5% in wastewater (Kargi and Dincer, 1996; Kargi, 2002).

The down-flow hanging sponge (DHS) bioreactor is one of the typical models of the attached growth method for biological wastewater treatment. This model has several advantages in terms of removal rate, organic loading, and remaining high biomass when compared with other models (Tanikawa et al., 2019; Tran et al., 2020). Therefore, the application of DHS model is popular worldwide among environmental engineers (Machdar et al., 2000; Tandukar et al., 2007; Uemura et al., 2010; Mahmoud et al., 2011; Natori et al., 2012; Matsubayashi et al., 2016). However, the application of the DHS bioreactor to treat saline wastewater has not been researched in detail so far.

In a previous study, we operated a DHS bioreactor to treat domestic wastewater for 81 days (Nguyen et al., 2018). The results showed that this model performed stably during the operations and achieved maximum removal of COD_{Cr} (80%), biochemical oxygen demand (BOD_5) (83%), NH_4^+-N (65%), and TN (60%). The experimental data corresponded well with the Stover–Kincannon model with a high coefficient of determination (Nga et al., 2019). In this study, the DHS bioreactor was operated to investigate the effect of salinity (0.5–3%) on organic and nutrient removals, ammonium oxidation, and biomass characteristics. The correlations between BOD_5, COD_{Cr}, NH_4^+-N, and TN removal with salinity conditions were also evaluated.

2. Materials and methods

2.1. Experimental apparatus

A pilot-scale DHS bioreactor is presented in Fig. 1. The DHS bioreactor had a height of 1.5 m and a square edge of surface of 0.2 m. The reactor consisted of three identical segments connected vertically in series. Each segment was filled with sponge and polyurethane Bio-Bact spheres occupying 30% of the volume of each segment to act as attached media. Air intake vents were located between the segments for aeration, the air was diffused from the outside to the reactor through these vents naturally without the need for supply by aeration pumps. The effluent was collected from the bottom of the DHS reactor to a 10 L sedimentation tank, in the sampling time, this tank was brought out and keep idling for 30 min before taking samples. The sponge and polyurethane Bio-Bact spheres together had a surface area of 3300 $m^2.m^{-3}$ and a void ratio of 90%.

2.2. Wastewater source

The input wastewater was taken from the storage tank of a dormitory of an X university in Vietnam. Firstly, wastewater was pumped into a container and the suspended solids were allowed to settle. Then, the wastewater was sampled for analysis and the salinity was adjusted by adding NaCl to reach the design salt level before feeding to the reactor. The characteristic of the wastewater used in this experiment is presented in Table 1.

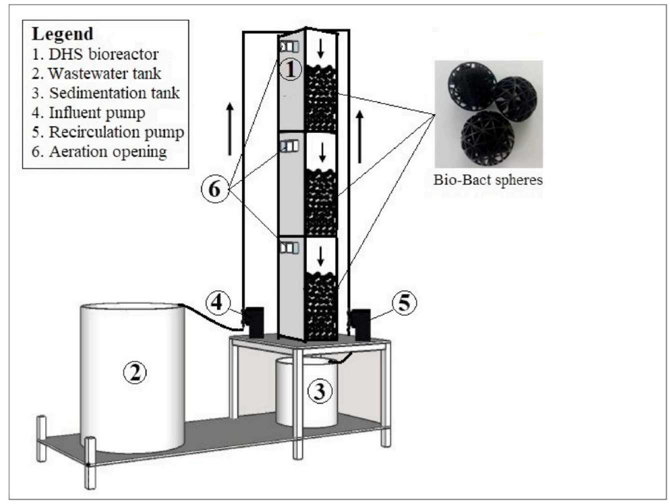

Fig. 1. Schematic diagram of the DHS bioreactor.

2.3. Operational conditions

The wastewater from the wastewater tank was pumped to the top of DHS bioreactor at the designed flow. From the top of the DHS reactor, the wastewater was distributed through a distribution system installed at the top of the reactor and flows through the three segments, and flows down to the sedimentation tank at the bottom (Fig. 1). Before performing this experiment, the reactor was operated for 81 days to evaluate the treatment of domestic wastewater at different organic loading rates. It was established that the DHS reactor performed stably at 1 kg COD_{Cr} $m^{-3}.day^{-1}$ (Nguyen et al., 2018; Nga et al., 2019). In this experiment, the effect of salinity on organic removal and ammonium oxidation in this reactor was ascertained in 145 days. Before starting the experiment, the seed sludge was taken from a domestic wastewater treatment plant with capacity of 30.000 $m^3.day^{-1}$, was then acclimated with salt at a low concentration of 0.2% in an aeration tank for 30 days. After that, the sludge was diluted to have a mixed liquor suspended solids (MLSS) content of 3000 $mg.L^{-1}$ and pumped to the reactor for the formation of biofilms on the sponge and spheres. During the experiment, salinity was maintained at different levels by increasing sodium chloride concentration from 0.5% to 3% at an increment of 0.5%. The duration of each phase was varied in the range of 20–30 days. Detailed experimental conditions for the six phases with various salinity concentrations of 0.5, 1, 1.5, 2, 2.5, and 3%, respectively, are indicated in Table 2. The out flow was circulated with a ratio of 1:1 for lengthening the hydraulic retention time and enhancing treatment efficiency.

Table 1
Characteristics of influent wastewater.

Parameter	Unit	Range
pH	–	7.2 ± 0.5
TSS	mg L^{-1}	20 ± 9
BOD_5	mg L^{-1}	156 ± 18
COD_{Cr}	mg L^{-1}	231 ± 19
DO	mg L^{-1}	0.4 ± 02
NH_4^+-N	mg L^{-1}	42 ± 19
$NO^-{}_3$-N	mg L^{-1}	0.16 ± 0.08
N Total	mg L^{-1}	44 ± 12
P Total	mg L^{-1}	6.5 ± 3
Alkalinity	$mgCaCO_3{\cdot}L^{-1}$	331 ± 1
Salinity	%	0.5–3.0 (depended on phases)

Table 2
Operating conditions.

Phase	Time course (day)	Organic loading rate (kg COD_{Cr}.m^{-3}. d^{-1})	Salinity (%)
Phase 1	0–20	1	0.5
Phase 2	21–50	1	1.0
Phase 3	51–75	1	1.5
Phase 4	76–101	1	2.0
Phase 5	102–125	1	2.5
Phase 6	126–145	1	3.0

2.4. Analytical methods

The American Public Health Association (APHA) standard, "Standard Methods for the Examination of Water and Wastewater" (APHA, 1998), was used for the determination of BOD_5, COD_{Cr}, nitrate nitrogen (NO_3^--N), nitrite nitrogen (NO_2^--N), ammonium nitrogen (NH_4^+-N), MLSS, and mixed liquor volatile suspended solids (MLVSS). To maintain biomass in the reactor, the sludge samples were taken only at the end of the experiment. The sponge media with sludge were carefully removed from the reactor. The biomass layer was separated from the sponge media by squeezing and washing the sponge with distilled water. Other parameters such as DO, pH, and salinity were directly tested by Water Quality Checker WQC-22A (DKK-TOA, Japan). All the analytical methods are described in a previous study (Nguyen et al., 2018).

3. Results and discussion

3.1. Removal of organic pollutants

Fig. 2 shows the change in COD_{Cr} profile during the experimental period. It showed that the COD_{Cr} concentration in the influent was stable from the start until the day of 102, however there was a variation after that due to student vacation. The average COD_{Cr} contents of the influent, effluent, and removal efficiency throughout the operation of DHS bioreactor were approximately 305.3 ± 29.4 mg.L^{-1}, 89.8 ± 12.6 mg. L^{-1}, and 70.3 ± 5%, respectively. During phase 1, the average of COD_{Cr} removal efficiency was 79%. At phase 2, it sharply dropped to 70.4% at day 51 of the experiment, and the average COD_{Cr} removal during this phase was 72.8%. In phases 3, 4, and 5, the removal efficiency gradually decreased from 72.8% to 65.6% after 125 days of operational time. Phase 6 demonstrated a significant decline in the treatment of COD_{Cr} and the efficiency dropped to 61% at the end of the experiment. These results indicate that when salinity increased from 0.5% to 3.0% the COD_{Cr} removal dropped from 79% to 61% after 145 days. As the other operational factors were fixed, these results demonstrate that microorganisms are inhibited by an increase in the concentration of salt in the influent. The sodium chloride concentration at 3.0% could cause a remarkable reduction in the COD_{Cr} removal efficiency in the DHS bioreactor. The microbial growth is reported to be affected by the salt concentration. Microbes are reported to have good metabolism and growth in an isotonic solution (for example NaCL 0.85 wt%) (He et al., 2017). The increase in salinity of the solution directly affects the osmotic pressure of the solution. The increase osmotic pressure (negative) will in turn take water out of the microbial cell, due to which the cell may die (Oren, 2020). The death of microbes certainly affects the COD removal efficiency (He et al., 2017). Uygur and Kargı (2004) also reported that the ability of microorganisms decomposing organic matter were affected when the salt concentration was above 10 gCl$^-$.L$^-$. Another study also demonstrated that the average COD_{Cr} removal efficiency decreased from 92% to 90% when the salinity increased from 0% to 2%, but it significantly dropped from 90% to 25% as the salinity increased from 2% to 8% in an aerobic granular sequencing batch reactor treating synthetic wastewater (Wang et al., 2015). However, based on the average COD_{Cr} removal efficiency, this system performance was better or worse than that of the results in previously studies (Ramos et al., 2007; Uemura et al., 2012; Wang et al., 2016) depending on concentration of salinity in the wastewater and the operating conditions. In contrast, Mahmoud and colleagues achieved the removal of COD (90%) and BOD5 (95%) in the treatment of domestic wastewater using DHS model (Mahmoud et al., 2011). Tawfik investigated the effect of hydraulic retention time (HRT) on performance of DHS reactor treating agricultural wastewater and found out that when HRT varied from 5.26 to 1.5 h, the COD removal rate decreased 89.3% to 72.2% (Fleifle et al., 2013). The differences result among studies could possibly be due to the effect of salinity on the performance of the bioreactor has the same trend but different intensity because of varying experimental conditions.

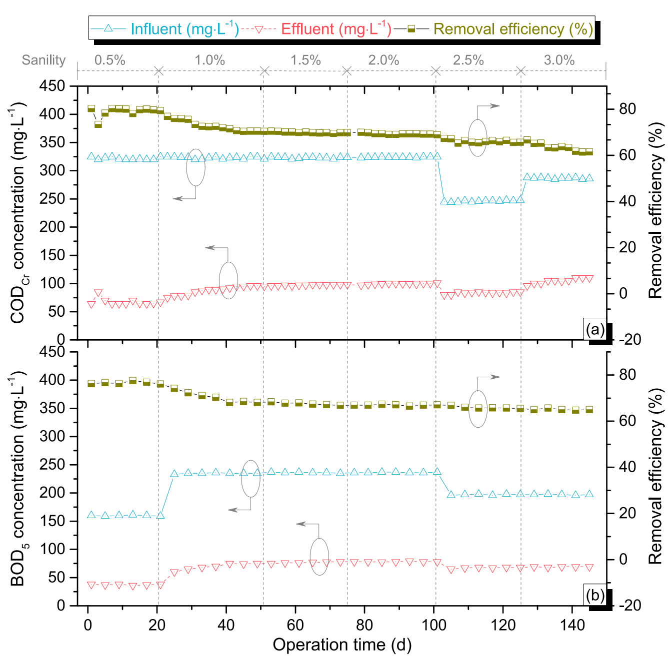

Fig. 2. Profile of organic matter: a) COD_{Cr} removal; b) BOD_5 removal.

The changes in BOD_5 in the influent and effluent with time are presented in Fig. 2b. The variations in BOD_5 and COD_{Cr} are similar. At a salinity of 0.5%, the average BOD_5 removal efficiency was 76.7%, and it decreased to 70.4% when salinity increased to 1%. It continuously dropped in through next phases of the experiment. In summary, the average BOD_5 removal efficiency reduced from 76.5% to 65.1% after six phases when the salinities increased from 0.5% to 3.0%. The decline in removal efficiency with an increase in salt concentration could be attributed to the inhibition of salinity on the metabolism of the microbial community (Wang et al., 2015, 2016; Zhao et al., 2016).

3.2. Oxidation of ammonium nitrogen

Fig. 3a shows the profile of NH_4^+-N throughout operational time. It was observed that during early stage of the start-up, the removal efficiency of NH_4^+-N was high (>64%) and stable, providing that the nitrifying bacteria in the DHS reactor were well adapted and developed stably. This phenomenon can be explained that the nitrifying bacteria community was available in the seed sludge obtained from a domestic wastewater treatment plant, which was acclimated with salt at a low concentration of 0.2% in an aeration tank for 30 days before performing this experiment can adapt and grow well in DHS reactor. The average removal efficiency of NH_4^+-N was 64.1% in the first phase. When the salinity increased from 0.5% to 1.5% after three phases, the removal efficiency significantly dropped to 53%. From phase 4 to 6 the NH_4^+-N oxidation was less drastic than that of the previous phases. This indicated that when the salt concentration in the influent wastewater increases from 0.5 to 1.5%, the effect on nitrifying bacteria is evident, however, when the salt concentration increases from 2.0% to 3.0%, the

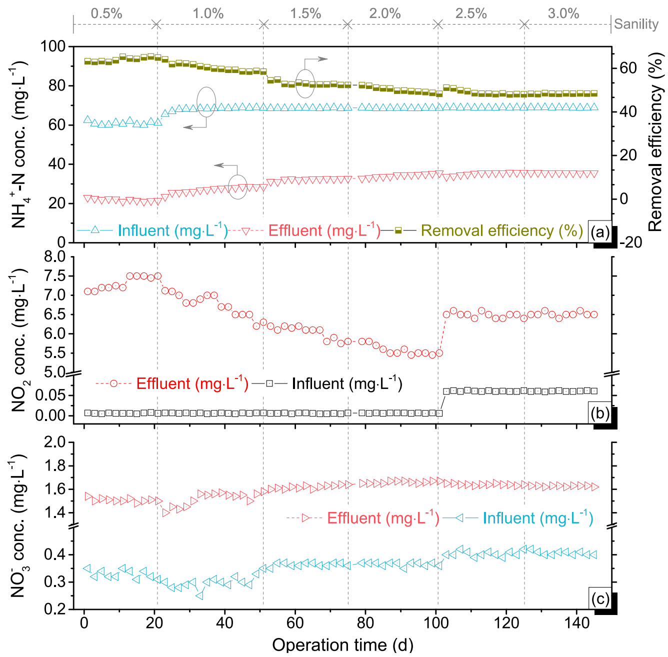

Fig. 3. Profiles of nitrogen formed during the experiment: a) Ammoniacal nitrogen; b) Nitrite nitrogen; c) Nitrate nitrogen.

nitrification efficiency did not change significantly, possibly because the nitrifying bacteria strains began to elevate to a higher adaptive level. Overall, the NH_4^+-N removal rate decreased throughout the operational time, reducing from 64.1% to 48.4% after 145 days with increase in salinity from 0.5% to 3.0%. This tendency was also elucidated in previous studies (Uemura et al., 2012; Wang et al., 2015, 2016; Zhao et al., 2016). Wang and colleagues reported that the removal of NH_4^+-N was strongly affected by salinity (Wang et al., 2015, 2016). When the salinities were increased stepwise from 3% to 8%, the average NH_4^+-N removal reduced from 88% to 21% in a granular sequencing batch reactor, and 92%–27% in an anoxic–aerobic sequencing batch biofilm reactor (Wang et al., 2015, 2016). Zhao et al. (2016) investigated the effect of salinity on the performance of a sequencing batch reactor and demonstrated that at 2.0% of salinity, the removal rate of NH_4^+-N was 95%, at 2.5% it reduced to 65%, and then it sharply reduced to 25% at 3.0% (Zhao et al., 2016). They explained that when salinity increases, the tolerance of the microorganisms was lower and oxygen availability was less, resulting in the inhibition of microbial activities.

Fig. 3b illustrates NO_2^--N concentrations during the experiment. It is observed that NO_2^--N was stable in the range of 7.1–7.5 mg.L^{-1} after the first day of the experiment. After that, the NO_2^-N concentration gradually decreased and reached 5.5 mg.L^{-1} after 101 days when the salinity was increased from 0.5% to 2%. When the salinity increased further to 2.5% and 3%, the NO_2^--N concentration was stable at approximately 6.5 mg.L^{-1} until the end of the experiment at day 145. This result demonstrates that nitrite oxidation occurred. The variation in NO_3^--N concentration during the experiment is shown in Fig. 3c. It shows that NO_3^--N was very low in the influent and in the effluent this anion was stable in the range of 1.4–1.7 mg.L^{-1}. This result indicates that the accumulation of nitrate did not happen during the experiment. Under aerobic conditions, nitrification would occur and convert ammoniacal nitrogen to nitrate nitrogen in two phases. The first phase occurs in the presence of the *Nitrosomonas spp* genus, in which NH_4^+ is oxidized into NO_2^- by following the equation: $NH_4^+ + 1.5\ O_2 => NO_2^- + 2H^+ + 2H_2O$. Subsequently, nitrite-oxidizing bacteria (*Nitrobacter spp*) oxidize NO_2^- to NO_3^- according to equation $NO_2^- + 0.5\ O_2 => NO_3^-$. The increase in NO_2^- and NO_3^- concentrations in the effluent when compared to the influent proves the occurrence of the nitrification process. Interestingly, NO_2^- concentration was much higher than that of nitrate. This can be explained by the fact that the *Nitrobacter spp* bacteria was inhibited by salinity in the wastewater, and thus they could not convert NO_2^- to NO_3^- causing the accumulation of nitrite nitrogen. Some previous studies have also found out that NO_2^- accumulation occurred strongly at high salt concentrations (Dinçer and Kargi, 2001; Chen et al., 2003). Contrary to these results, other researchers have reported that NO_2^--N and NO_3^--N were accumulated in the effluent of the anoxic–aerobic sequencing batch biofilm, when the salinity was 8% (Wang et al., 2016). Different experimental conditions can provide different results, and therefore, it is necessary to investigate the effect of salt concentration on the nitrification process with variable settings.

3.3. Total nitrogen removal

Fig. 4 presents the variations in the total nitrogen (TN) concentrations in the influent and effluent during the experimental period. The TN concentration was approximately 63 mg.L^{-1} during the start-up time, and it was stable in the influent at approximately 70 mg.L^{-1}. However, the TN in the effluent gradually increased with the increase in salinity. Consequently, the removal rate of TN was reduced from 50% at day 20–36% at day 145 with an increase in salt concentration from 0.5% to 3% after the six steps of increase. TN was not as high as that of NO_2^- and NO_3^-, and it has the same trend as that of removal rate of ammoniacal nitrogen. This shows that the performance of the DHS model was affected by the increase in salinity. A higher salt concentration in the influent resulted in a lower TN removal rate by the reactor. Previous studies have shown that nitrite-oxidizing bacteria have the ability to adapt to lower salinity than ammonia-oxidizing bacteria (Sinha and Annachhatre, 2007; Natori et al., 2012). However, previous studies have not explicitly discussed the effect of salinity on removing TN in wastewater. The TN that was removed from the system can be attributed to the assimilation of TN by microorganisms to produce new cells.

3.4. Biomass properties

At the steady states of each phase, representative Bio-Bact spheres of each segment were collected for morphological observations and the analysis of biomass. It was observed that biofilm distributions in the spheres and sponge were less uniform and appeared slightly darker when salinities increased, especially at the highest level. Biofilm samples were separated from the media to determine the MLSS concentration. The results show that when the salinities increased from 0.5% to 3%, biomass MLSS concentration in the sponge also decreased from 2741 ± 6.6 mg.L^{-1} to 2591 ± 4.6 mg.L^{-1}, and biomass MLVSS was 74–81% of the MLSS. This suggests that the microbial community was affected by salt concentration resulting in the decrease of removal efficiencies of organic matter and nitrogen. It was observed that the salinities could cause change in the microbial community in the sludge during the operational period (Luo et al., 2016; Zhao et al., 2016; De Vrieze et al.,

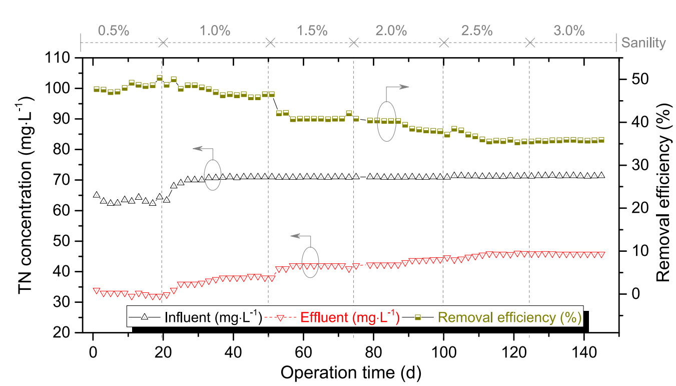

Fig. 4. Profile of total nitrogen during the experiment.

2017). Luo et al. demonstrated that in the membrane bioreactor, when salinity increased, the halophilic microbes became dominant and halophobic microbes were reduced (De Vrieze et al., 2017). Another study found out that salinity had a remarkable effect on the abundance of microorganisms in a sequencing batch reactor (Zhao et al., 2016). Several microbial species have salinity tolerance such as *Actinobacteria* and *Lutimaribacterlitoralis* (Wang et al., 2016; Zhao et al., 2016). Because of certain limitations in the experimental conditions, studies on the identification and diversity of the microbial community in the biofilm were not carried out. However, results on the changes in sludge morphology, MLSS, and MLVSS imply that microbial populations also underwent changes in the experimental conditions.

3.5. *Effect of salinity on removal efficiency*

To understand the effect of salt concentration on the performance of the reactor, the plots of removal efficiencies of different parameters were plotted against the NaCl concentration (Fig. 5). Interestingly, the relationships were all linear with high values of correlation coefficient (R^2). The R^2 values for the removal of COD_{Cr}, BOD_5, NH_4^+-N, and TN were 0.804, 0.791, 0.932 and 0.923, respectively (Fig. 5). Using these regression models, it is possible to precisely predict removal efficiencies with change in salinity of the influent. The negative value of the slope of all the regression trend lines demonstrates the inverse relationship between salinity and removal efficiency. In addition, the absolute value of the slope of these straight lines was in the descending order of NH_4^+-N, COD_{Cr}, TN, and BOD_5. This result indicated that the removal of NH_4^+-N was the most affected and BOD_5 was the least affected with an increase in salinity among these parameters. This section provides a deeper understanding of the performance of a bio-treatment system with reference to salinity. However, further research is required to determine, verify and predict the trend of reactor performance under the effect of specific factors.

4. Conclusions

In summary, the effect of salinity on removing organic matter and nitrogen present in domestic wastewater was investigated in a bench-scale down-flow hanging sponge bioreactor during a 145-day experiment. The findings of this study highlight that the treatment efficiency of the pollutants in the bioreactor was inversely proportional and they followed a linear relationship with the concentration of salt in the influent. When salinity was increased stepwise from 0.5% to 3.0%, the removal efficiencies of COD_{Cr}, BOD_5, NH_4^+-N, and TN gradually decreased. And, the salinity has a higher influence on the nitrogen removal in the bio-growth conditions of the bioreactor compared to organic matters removal. The result also showed that the removal of NH_4^+-N was the most affected and BOD_5 was the least affected by increasing salt concentration among water quality parameters investigated. The morphology observation and biomass concentration present in the attached media were also significantly changed during the operational period with increasing salt concentration. Even so, the DHS system revealed a good potential for treating saline wastewater. However, the results obtained from this study still have many aspects that need to be exploited and identified at pilot scale to move towards recommendations for practical application. Thus, further research with pilot scale to give specific parameters in order to accurately evaluate treatment efficiency, investment and operating costs as well as feasibility is essential.

Author contribution statement

Dinh T. Nga: Conceptualization, Writing - original draft. Nguyen T. Hiep: Data curation, Formal analysis. Arvind Kumar Mungray Writing - review & editing. La Duc Duong: Methodology, Writing - review & editing. Phuong Nguyen-Tri: Writing - review & editing. D. Duc Nguyen: Conceptualization, Supervision Methodology, Writing - review &

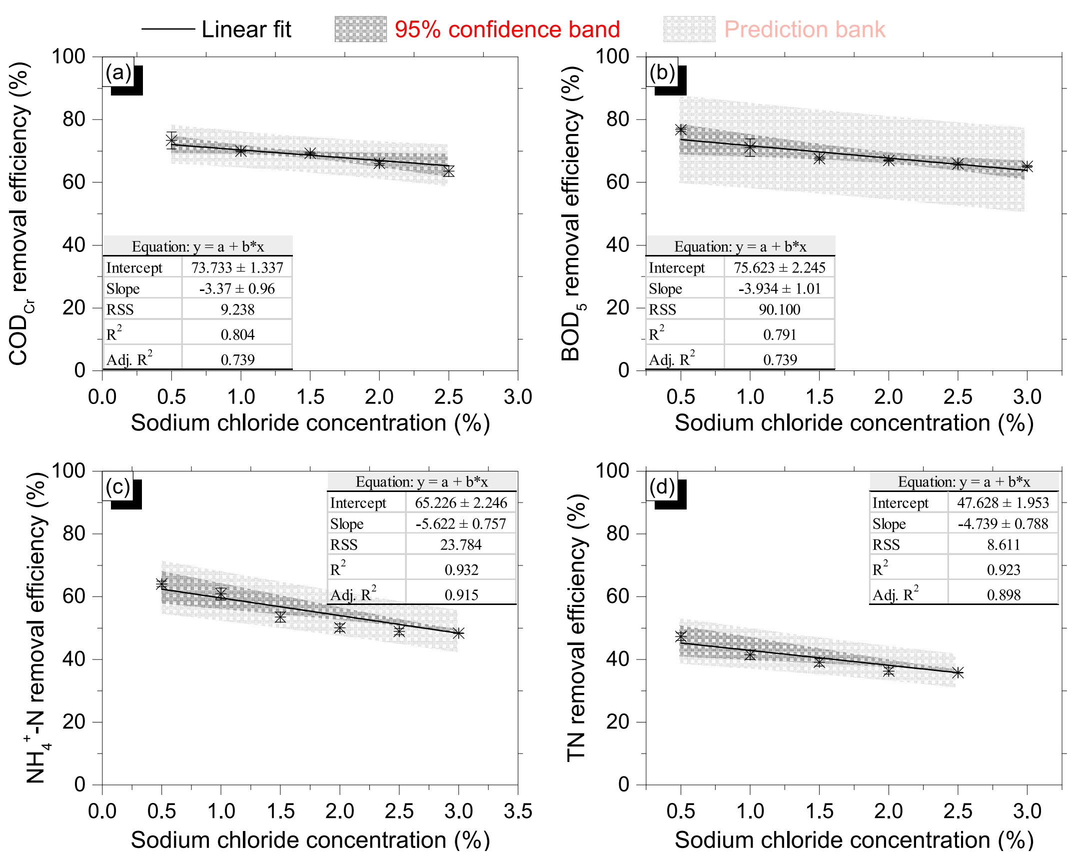

Fig. 5. The relationship between salinity and removal efficiencies of pollutants (RSS: Residual Sum of Squares).

editing. W. Jin Chung: Writing - review & editing. S. W. Chang: Funding acquisition, Writing - review & editing. Phan D. Tuan: Visualization, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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