Long term Evaluation of the Effect of Salinity on Organic Removal and Ammonium Oxidation in a down-flow Hanging Sponge Reactor

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ABSTRACT: The effect of salinity on organic removal and ammonium oxidation in a down-flow hanging sponge reactor was investigated by conducting a long-term continuous experiment over a period of 800 days. The DHS reactor, constructed by connecting three identical units, was fed with artificial wastewater containing 500 mg-N/L of ammonium nitrogen and 1400 mg- COD/L of phenol. Salinity of the influent was controlled by the addition of 8.0 to 25 g-Cl/L of NaCl. The DHS reactor was operated at a hydraulic retention time of 12 h in a temperature controlled room at 25°C. No significant inhibition of organic removal and ammonium oxidation was observed at salinities of up to 20 g-Cl/L, at which levels ammonium oxidation and COD removal both exceeded 90%, respectively. However, at a salinity of 25 g-Cl/L, organic removal and ammonium oxidation were both severely inhibited. In addition, the ratio of effluent nitrite nitrogen to influent ammonium nitrogen increased from 3.4% at salinities of 8.0 g-Cl/L to 33% at salinities of 20 g-Cl/L.

Key words: Ammonium oxidation, Phenol, Salinity, Nitritation, DHS

INTRODUCTION

Down-flow hanging sponge (DHS) reactors employ polyurethane sponge media to retain biomass. Wastewater is trickled from the top of the reactor and purified by microorganisms within and on the surface of the sponge media as influent flows vertically downward through the reactor. Since the sponge media of DHS reactors are exposed to the atmosphere, oxygen dissolves continually into the wastewater. Consequently, there is no need for external aeration. Furthermore, since a large amount of activated sludge accumulates on the inside and outside of the sponge media, an ecosystem with an extremely long food chain develops and the production of excess sludge is restricted (Uemura and Harada, 2010).

The DHS reactor was originally developed as a post-treatment process for the up-flow anaerobic sludge blanket (UASB) systems used to treat sewage, especially for developing countries. Recently, our laboratory has attempted to apply the DHS process to industrial wastewater treatment. For example, a recent assessment of DHS reactor performance for treating artificial wastewater containing high concentrations of phenol and ammonium nitrogen (NH_4^+-N) confirmed that the reactor was effective for both COD removal and ammonium oxidation (Uemura *et al.*, 2010). Interestingly, of the oxidized nitrogen species present in the effluent of this previous study, nitrite nitrogen (NO_2^--N) was the most abundant.

Conversion of NH_4^+-N to NO_2^--N, not to nitrate nitrogen (NO_3^--N), is referred to as nitritation (Sinha and Annachhatre, 2007). In the event that a stable nitritation process could be established, the development of a cost-effective nitrogen removal wastewater treatment system could be realized through the implementation of either an anaerobic ammonium oxidation (ANAMMOX) process, or a short-cut denitrification process further downstream (Sinha and Annachhatre, 2008). To date, factors such as free ammonium concentration, temperature, dissolved oxygen (DO), and salinity, have been reported to

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promote nitration (Sinha and Annachhatre, 2008; Chen *et al.*, 2003). In a previous study (Uemura *et al.*, 2010), the salinity of the seawater used to dilute influent wastewater was considered to have induced nitration. However, in that study, the marked variation in the salinity of the sea water during the experimental period made it difficult to clarify the relationship between influent salinity and nitration.

On this background, this study sought to evaluate the effect of salinity on both ammonium oxidation and the removal of organics using a DHS reactor in a long-term, continuous experiment lasting approximately 800 days. In addition, particular emphasis was placed on clarifying the relationship between salinity and nitration.

MATERIALS & METHODS

The experimental setup used in this study is the same as that described previously (Uemura *et al.*, 2010). The DHS reactor was constructed by connecting three identical DHS units in series. Each DHS unit consisted of 60 triangular polyurethane sponges (Marusuzu, MSC-E16, Japan) attached to a plastic plate (2000 mm high x 70 mm wide). The void volume of the sponge medium was 98.4%. The approximate distance between sponges was <5 mm and the total sponge volume in each DHS unit was 240 cm³. The theoretical HRT of each unit, which was calculated based on the sponge volume, was set at 4 h (total HRT = 12 h).

The artificial wastewater was pumped into the top of the first DHS unit, with the effluent of the first unit then being pumped to the 2nd and 3rd units in order. Prior to this study, the DHS reactor was operated for more than 400 days to treat artificial coke-plant wastewater diluted with real sea water (Uemura *et al.*, 2010). The DHS reactor was placed in a temperature controlled room set at 25°C. The NaHCO$_3$ solution was added to the top of the second and third units to supplement inorganic carbon (IC) and maintain them at 350 mg-C/L (2900 mg-CaCO$_3$/L) based on the findings of a previous study (Uemura *et al.*, 2010).

Artificial wastewater containing mineral and buffer solutions, as well as 600 mg/L phenol (1400 mg-COD/L), 500 mg-N/L NH$_4$Cl and 3600 mg/L NaHCO$_3$ was prepared according to the methods used in a previous study (Uemura *et al.*, 2010). Furthermore, mineral solutions were added to the wastewater, which were made as prescribed for the 5-day BOD test described in "*StandardStandard Methods for the Examination of Water and Wastewater*"(APHA, 1995). The salinity of the influent was adjusted with NaCl to be in the range of 8.0 to 25 g-Cl⁻/L. The period during which the salinity was adjusted to 8.0 g-Cl⁻/L was referred to as Phase 1 (day 0-24), which was followed by Phase 2 (10

g-Cl⁻/L, day 25-39), Phase 3 (15 g-Cl⁻/L, day 40-320), Phase 4 (20 g-Cl⁻/L, day 321-395), and Phase 5 (25 g-Cl⁻/L, day 396-656). In Phase 6 (day 657-792), the salinity was decreased to 10.9 g-Cl⁻/L to investigate the recovery of DHS reactor performance.

Routine monitoring of NH$_4^+$-N, NO$_2^-$-N, NO$_3^-$-N, and COD in the wastewater and the effluent of each unit was conducted. The COD was analyzed using a HACH water quality analyzer (HACH DR-890). All other analytical procedures were performed according to American Standard Methods for the Examination of Water and Wastewater (APHA, 1995). To determine the volatile suspended solid (VSS) of the attached sludge, sludge samples were harvested from the first, 12th, 24th, 36th, 48th, and 60th sponges of each DHS unit to give a total of 18 sponges collected from all the units.

RESULTS & DISCUSSION

Fig. 1 shows the COD concentrations in the influent and effluents of each unit over time. Unfortunately, COD data were not available for Phases 1 and 2. The average COD loading rate of the whole reactor during Phases 3 to 6 was 2.77 ± 0.28 (standard deviation) kg/m³/d, and the residual COD in the effluent of the third unit (final effluent) was both very stable and low in Phase 3 (15 g-Cl⁻/L). However, as salinity increased to 20 and 25 g-Cl⁻/L (Phases 4 and 5), the residual COD tended to increase, particularly in the beginning of Phase 5. One hundred days after the start of Phase 5, the residual COD became relatively stable, but it was still affected by high salinity. After decreasing salinity to 10.9 g-Cl⁻/L in Phase 6, COD removal gradually recovered to more than 90% in 61 days.
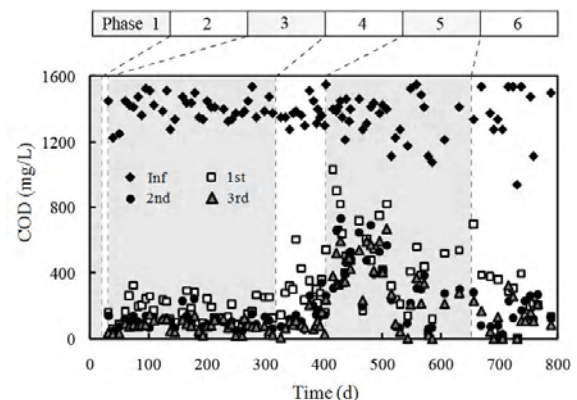

Fig. 1. Variation of COD in the influent and effluents of each unit

The average COD removal in the steady states of Phase 3 (day 40-312) was 87.3 ± 4.8% by the first unit, 92.7 ± 3.0% by the first and second units, and 94.5 ±

2.4% by all units (data not shown). The COD in the influent was thus primarily removed by the first unit, which corroborated the findings of a previous study (Uemura *et al.*, 2010). During the steady states of Phase 4 (day 326-393), when the salinity was adjusted to 20 g-Cl⁻/L, average COD removals were $77.5 \pm 8.5\%$ by the first unit, $90.4 \pm 3.8\%$ by the first and second units, and $93.6 \pm 3.1\%$ by all units. Though COD removal by the first unit was 10% less than that observed in Phases 3 to 4, total COD removal by all units remained above 90%. On the other hand, the average COD removals in the steady states of Phase 5 (day 606-656) were $55.5 \pm 7.2\%$ by the first unit, $78.2 \pm 0.8\%$ by the first and second units, $81.8 \pm 1.4\%$ by all units, indicating that COD removal by each unit decreased during Phase 5.

Ramos *et al.* (2007), for example, conducted on the treatment of wastewater containing phenol under high salinity conditions using a down-flow-up-flow biofilm reactor, which demonstrated to remove more than 95% of influent phenol (2800 mg-COD/L) at a COD loading rate of 1.0 kg/m³/d and salinity conditions of 30 g-Cl⁻/L. In this study, the COD removal was 82 % by all units at a COD loading rate of 2.8 kg/m³/d even under high salinity conditions of 25 g-Cl⁻/L. Such high removal efficiencies obtained using the DHS reactor adequately demonstrated the high potential of this rector for COD removal under high salinity conditions. After termination of the continuous experiment, the average sludge concentrations retained by each DHS unit were determined based on sponge volume being 29.6 g-VSS/L, 18.9 g-VSS/L and 18.0 g-VSS/L in the first, second and third units, respectively. It is thus likely that the large amounts of sludge retained in the sponge media of the DHS reactors contributes to the superior COD removal efficiency under relatively high salinity conditions.

Fig. 2 shows NH_4^+-N, NO_2^--N, and NO_3^--N concentrations in the influent and effluent of the third unit for Phases 1 through 6. While NH_4^+-N was oxidized with high efficiency (97%) during Phases 1 to 3, NO_2^--N began to accumulate and NO_3^--N decreased in the final effluent in Phase 3. However, later in the same phase, the production of NO_2^--N was observed to stabilize. In Phase 4, the oxidation of NH_4^+-N was slightly inhibited, but a tendency toward NO_2^--N accumulation continued. In the beginning of Phase 5, the NH_4^+-N in the final effluent increased drastically and production of NO_2^--N and NO_3^--N temporarily decreased. Over the 160 days from the beginning of Phase 5, effluent NH_4^+-N tended to decrease gradually and a slight recovery in NO_2^--N and NO_3^--N production was observed. However, 160 days after the beginning of Phase 5, the oxidation of NH_4^+-N started to deteriorate once more. At present, the reason for this

sudden deterioration of ammonium oxidation is not clear. After the salinity decreased to 10.9 g-Cl⁻/L (Phase 6), the recovery of ammonium oxidation proceeded drastically, with almost complete oxidation of NH_4^+-N to NO_3^--N established in the latter stage of Phase 6. In addition to restoring COD removal, the recovery of ammonium oxidation was also achieved by decreasing salinity to 10.9 g-Cl⁻/L.
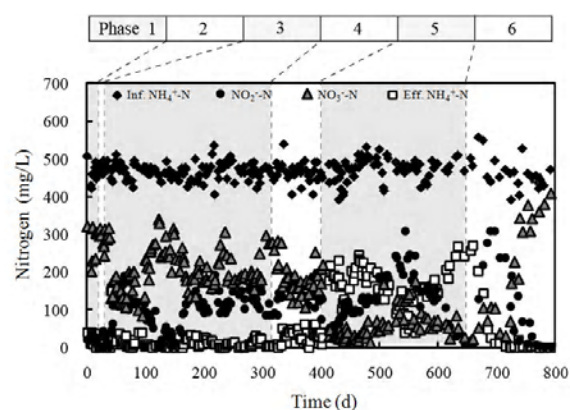

Fig. 2. Variation of NH_4^+-N in the influent and NH_4^+-N, NO_2^--N, and NO_3^--N in the effluents of each unit

The average concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N in the effluents of each unit during relatively stable periods, i.e., day 1 to 23 for Phase 1, day 24 to 38 for Phase 2, day 296 to 320 for Phase 3, day 337 to 384 for Phase 4, and day 631 to 656 for Phase 5, are shown in Figs 3 to 5. While similar tendencies in water quality were observed in Phases 1 and 2, and Phases 3 and 4, the pattern observed in Phase 5 differed from that observed in the other phases.

Although some NH_4^+-N was observed to accumulate in the effluents of the first and second units during Phases 1 through 4, the NH_4^+-N levels decreased to approximately 28 mg-N/L in the effluent of the third unit, which gave 93% of overall ammonium oxidation. However, in Phase 5, the residual NH_4^+-N in the final effluent increased to 251 mg-N/L. As a result, ammonium oxidation decreased to 46% (Fig. 3). Thus, though the DHS reactor exhibited a high capacity for ammonium oxidation at salinities of up to 20 g-Cl⁻/L, the ammonium oxidation was significantly inhibited at 25 g-Cl⁻/L.

Phenol is known to be inhibitory to nitrification. However, in this study, the continuous aerobic treatment of wastewaters with high levels of phenol and ammonium was efficiently undertaken in the DHS reactor. As was observed in our previous study (Uemura *et al.,* 2010), almost all of phenol was primarily removed in the 1st unit, while oxidation of NH_4^+-N occurred mainly in the two downstream units. This
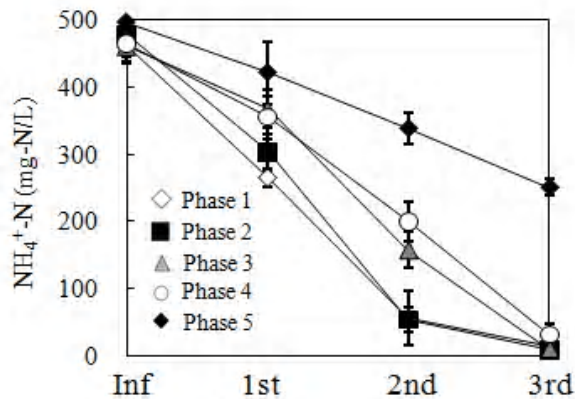
Fig. 3. Average NH$_4^+$-N concentrations in the influent and effluents of each unit

suggests that phenol inhibition for nitrifiers can be avoided in the 2nd and 3rd units.

In Phases 1 and 2, NO$_2^-$-N increased from 50-81 mg-N/L in the first unit to 105-137 mg-N/L in the second unit, before decreasing to approximately 15 mg-N/L in the third unit. On the other hand, during Phases 3 and 4, NO$_2^-$-N decreased markedly in the first unit, before increasing to 137-150 mg-N/L in the second unit and then remaining constant in the third unit. In Phase 5, NO$_2^-$-N production was negligible in all of the units, implying that the activity of ammonium oxidizing bacteria (AOB) was almost totally inhibited (Fig. 4).
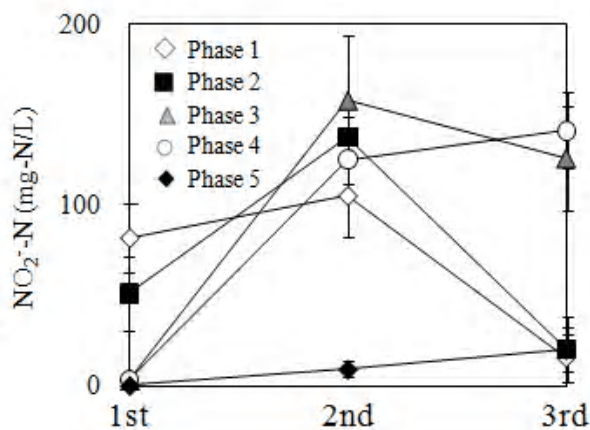

Fig. 4. Average NO$_2^-$-N concentrations in the effluents of each unit

Concentrations of NO$_3^-$-N were negligible in the effluent of the first unit throughout the entire experimental period, suggesting that nitrite oxidizing bacteria (NOB) were poorly presented in the unit. However, an approximately linear production of NO$_3^-$-N was observed from the first to third unit in Phases 1

and 2. The concentration of NO$_3^-$-N in the effluent of the second unit decreased from approx. 150 mg-N/L in Phases 1 and 2, to approximately 29 mg-N/L in Phases 3 and 4 before increasing again to approximately 180 mg-N/L in the third unit in Phases 3 and 4. No significant production of NO$_3^-$-N was observed in any of the units during Phase 5 (Fig. 5). The threshold salinity inhibiting the activity of AOB and NOB is considered to be between 10 and 15 g-Cl$^-$/L, with marked inhibition occurring above 20-25 g-Cl$^-$/L. Furthermore, it is suggested that this inhibitory effect of salinity is more apparent in NOB than it is in AOB. Similarly, Chen *et al.*, (2003) reported that inhibition of nitrite oxidation started at salinities of approximately 10 g-Cl$^-$/L in a batch reactor.
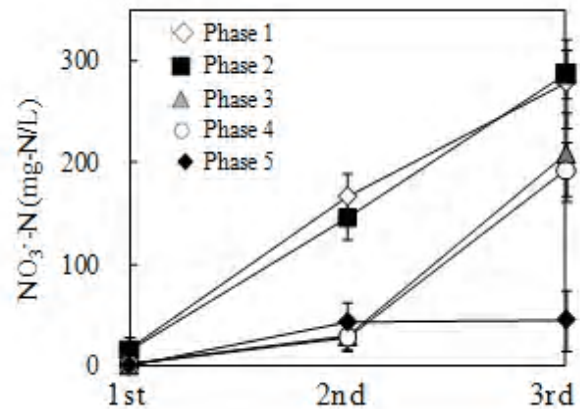

Fig. 5. Average NO$_3^-$-N concentrations in the effluents of each unit

For convenience, the sum of NH$_4^+$-N, NO$_2^-$-N, and NO$_3^-$-N, is referred to as inorganic nitrogen (IN) in this study. Over the course of this long-term continuous experiment, a slight reduction has been observed in IN through all the units (data not shown). This decrease can be basically attributed to denitrification. The surface of the DHS biofilm is kept aerobically with high DO level but a distinctive DO gradient occurs along the sponge-inward depth, resulting in anaerobic conditions at deep inside portions of the sponge (Araki *et al.*, 1999). These results demonstrate the possibility that denitrification occurred deep inside the sponge under DO-limited conditions. The residual organics (phenol and its intermediates) as well as organics derived from sludge autolysis may function as electron donors in the denitrification. The simultaneous removal of phenol and ammonium was also reported by Yamagishi *et al*. (2001) in an activated sludge process.

Fig. 6 shows the fraction of NO$_2^-$-N in the effluent of the third unit to the NH$_4^+$-N in the influent, i.e. the fraction of nitritation, at each salinity examined. As shown in the figure, nitritation was promoted by

salinities of up to 20 g-Cl$^-$/L, particularly from 10 to 15 g-Cl$^-$/L. This may have occurred because the inhibitory effect of salinity on AOB and NOB appears at approximately 10-15 g-Cl$^-$/L, and because the inhibitory effect is more apparent in NOB than it is in AOB. As reported here, similar increases in the rate of nitritation in response to increased salinities have been observed previously (Chen *et al.*, 2003; Cui *et al.*, 2006; Yu *et al.*, 2004; Inttasungkha *et al.*, 1999). However, other studies have reported that no relationship exists between nitritation and salinity (Campos *et al.*, 2002; Abe *et al.*, 2007). Interestingly, almost all of the studies reporting a positive correlation between increased salinity and nitritation were conducted using a sequencing batch reactor (SBR), while those reporting no such relationship employed continuously fed reactors such as activated sludge units and air-lift type bioreactors (Campos *et al.*, 2002; Abe *et al.*, 2007). Because the SBR employs a one-cycle operation consisting of wastewater supply, aeration, sludge sedimentation, and the discharge of treated water, conditions such as pH, DO, and substrate concentrations, especially NH_4^+-N, can vary markedly over short time periods.

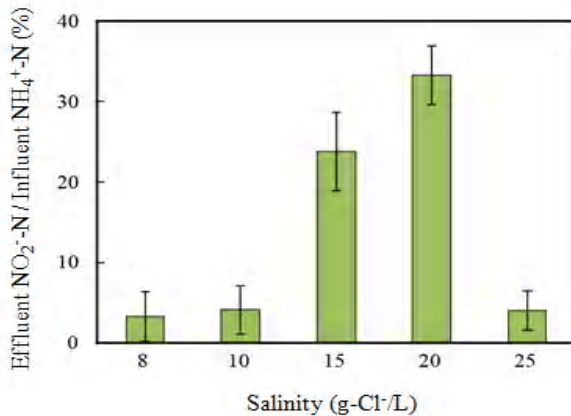**Fig. 6. Ratio of NO_2^--N to NH_4^+-N at different salinities**

Generally, parameters such as the free ammonia nitrogen (FA), temperature, and DO are considered to be keys to stimulating nitritation (Chung *et al.*, 2005; Hanaki *et al.*, 1990). Because the DHS reactor employs a plug-flow mode, variation in the parameters that affect nitritation is expected between the upper and lower portions of the reactor; for example, see the FA concentrations in the influent and effluents of each unit that were calculated using the equation of Anthonisen *et al.* (1976) in Table 1. The FA calculations in the effluents of each unit showed a tendency to increase from Phase 1 to 5, possibly due to inhibition of AOB by the increased salinity. IC addition to the tops of the second and third units was undertaken to increase the alkalinity which was neutralized as the wastewater flowed through the reactor. Therefore, due to the gradual decrease in NO_2^--N and NO_3^--N concentrations during each experimental phase, the pH in the wastewater gradually increased due to an excessive supply of IC. Anthonisen *et al.* (1976) reported that FA inhibited AOB and NOB at concentrations of 10-150 mg-N/L and 0.1-1.0 mg-N/L, respectively, implying that NOB were more sensitive to FA than AOB. Therefore, the likely effect of salinity on the nitritation observed in this study could be characterized as follows: AOB were inhibited by the increase in salinity and the concomitant increase in FA, which in turn inhibited the NOB and resulted in the accumulation of NO_2^--N.

Another possible reason for the observed increase in nitritation is that, compared to the AOB, the NOB are more sensitive to salt. Furthermore, the decrease in the rate at which oxygen dissolves into wastewater as salinity increases lowers the DO of the media and promotes nitritation (Hill, 2009). Future research considering the relationships between these parameters in DHS reactor systems need to be investigated further to clarify the mechanism of nitritation in response to increased salinities.

Table 1. FA in the effluent from each unit during each experimental phase (in mg/L)

Unit	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
Influent			21.4 (8.60)		
1st	17.4 (2.76)	21.7 (4.28)	30.4 (9.98)	32.1 (6.85)	37.5 (8.02)
2nd	1.80 (1.43)	3.60 (1.37)	13.7 (6.25)	16.6 (5.85)	71.0 (15.0)
3rd	3.02 (2.94)	3.05 (2.97)	2.95 (4.43)	6.71 (4.44)	118 (11.6)

Figures in parentheses represent standard deviations

CONCLUSION

The following conclusions can be drawn from this study:

1) The DHS reactor showed excellent COD removal up to salinities of 20 g-Cl⁻/L, with COD removals of 94.1% being achieved by all of the units at an average COD loading rate of 2.7 kg-COD /m³/d during Phases 3-4. COD removal was primarily achieved by the first unit. Although COD removal decreased slightly at a salinity of 25 g-Cl⁻/L, the DHS reactor still achieved 81.8% COD removal.

2) The oxidation of ammonium nitrogen occurred primarily in the two downstream units. An average ammonium oxidation of 93.9% was achieved during Phases 1 through 5, but it was significantly inhibited at a salinity of 25 g-Cl⁻/L.

3) The proportion of effluent NO_2^--N to influent NH_4^+-N increased from 3.4% at 8.0 g-Cl⁻/L to 33% at 20 g-Cl⁻/L, meaning that nitritation was promoted by increased salinity.

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