

# Comparison between Conventional Bacterial and Algal-Bacterial Aerobic Granular Sludge Systems in the Treatment of Saline Wastewater

Philip Semaha, Zhongfang Lei, Ziwen Zhao, Sen Liu, Zhenya Zhang, Kazuya Shimizu

**Abstract**—The increasing generation of saline wastewater through various industrial activities is becoming a global concern for activated sludge (AS) based biological treatment which is widely applied in wastewater treatment plants (WWTPs). As for the AS process, an increase in wastewater salinity has negative impact on its overall performance. The advent of conventional aerobic granular sludge (AGS) or bacterial AGS biotechnology has gained much attention because of its superior performance. The development of algal-bacterial AGS could enhance better nutrients removal, potentially reduce aeration cost through symbiotic algae-bacterial activity, and thus, can also reduce overall treatment cost. Nonetheless, the potential of salt stress to decrease biomass growth, microbial activity and nutrient removal exist. Up to the present, little information is available on saline wastewater treatment by algal-bacterial AGS. To the authors' best knowledge, a comparison of the two AGS systems has not been done to evaluate nutrients removal capacity in the context of salinity increase. This study sought to figure out the impact of salinity on the algal-bacterial AGS system in comparison to bacterial AGS one, contributing to the application of AGS technology in the real world of saline wastewater treatment. In this study, the salt concentrations tested were 0 g/L, 1 g/L, 5 g/L, 10 g/L and 15 g/L of NaCl with 24-hr artificial illuminance of approximately  $97.2 \mu\text{mol m}^{-2}\text{s}^{-1}$ , and mature bacterial and algal-bacterial AGS were used for the operation of two identical sequencing batch reactors (SBRs) with a working volume of 0.9 L each, respectively. The results showed that salinity increase caused no apparent change in the color of bacterial AGS; while for algal-bacterial AGS, its color was progressively changed from green to dark green. A consequent increase in granule diameter and fluffiness was observed in the bacterial AGS reactor with the increase of salinity in comparison to a decrease in algal-bacterial AGS diameter. However, nitrite accumulation peaked from 1.0 mg/L and 0.4 mg/L at 1 g/L NaCl in the bacterial and algal-bacterial AGS systems, respectively to 9.8 mg/L in both systems when NaCl concentration varied from 5 g/L to 15 g/L. Almost no ammonia nitrogen was detected in the effluent except at 10 g/L NaCl concentration, where it averaged 4.2 mg/L and 2.4 mg/L, respectively, in the bacterial and algal-bacterial AGS systems. Nutrients removal in the algal-bacterial system was relatively higher than the bacterial AGS in terms of nitrogen and phosphorus removals. Nonetheless, the nutrient removal rate was almost 50% or lower. Results show that algal-bacterial AGS is more adaptable to salinity increase and could be more suitable for saline wastewater treatment. Optimization of operation conditions for algal-bacterial AGS system would be

important to ensure its stably high efficiency in practice.

**Keywords**—Algal-bacterial aerobic granular sludge, bacterial aerobic granular sludge, nutrients removal, saline wastewater, sequencing batch reactor.

## I. INTRODUCTION

GLOBALLY there is an increase in the growth of industries and this trend is projected to continue to provide processed goods for human consumption. Saline wastewater is a byproduct of many industrial activities, from the agro-processing, textile and tannery to petrochemical industries. The need to treat these large volumes of wastewater is apparent. Although the conventional AS process has been useful in this regard, it is less efficient due to plasmolysis effect of salt. In the AS process, high salt levels lead to a less diverse microbial population [1], affect particle size, biochemical properties and overall performance [2]. The induced increase in water density and buoyancy force also reduces the separation of floc/sludge [3], [4] during the sedimentation process, which is a major drawback.

In 1991, Mishima and Nakamura [5] reported AGS. It has thereafter attracted much attention as a novel technology for biological wastewater treatment compared to the conventional AS. Being considered as the self-aggregates coagulated under hydrodynamic shear forces [6], the aerobic granules are aggregates with functional strains that are embedded in the matrix of extracellular polymeric substances (EPS), inorganic compounds and various minerals [7]. Aerobic granules are characteristically regular, smooth and nearly round shaped, possessing excellent settleability, dense and strong microbial structure, high biomass retention and the ability to withstand high organic loading and tolerance to toxicity [8]. AGS has simultaneous nitrification denitrification (SND) capacity [9] and it has been used to explore the treatment of high strength wastewaters containing organics, nitrogen, phosphorus, toxic substances, xenobiotics and salts [2], [4], [7], [8], [10]-[13].

The need to conserve energy and resources is fundamental to all aspects of wastewater collection, treatment and reuse [14]. However, in the biological process, aeration is the most energy-intensive operation in wastewater treatment, amounting to 45-75% of plant energy costs [15]. Ahmad et al. [16] reported that algae growth occasionally occurs in WWTPs and as such in AGS systems. Algae are aquatic organisms containing photosynthetic pigments that enable them to synthesize structural materials and storage compounds from carbon

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dioxide and water. Their cellular activities significantly affect the oxygen resources of surface waters [17]. Through symbiotic microalgal–bacterial relationship, microalgae provide O<sub>2</sub> necessary for aerobic bacteria to biodegrade organic pollutants and consume the CO<sub>2</sub> released from bacterial respiration [18]. This algae-bacteria symbiosis has been the basis for natural water purification [19]. Zhang et al. [20] found that algae and bacteria could form granular consortia through biological processes in a photobioreactor with or without algae as the inoculum. This synergistic algal-bacterial relationship could potentially reduce energy and overall operational cost. Compared to general AGS, algal-bacterial AGS is more promising [16], as it exhibits great potential for reducing greenhouse gases emission and its granular biomass with high P and N contents could be useful in resource recovery and reutilization [21], when taking the growing global focus on sustainable and clean energy use into consideration.

References [6] and [22] investigated the effect of NaCl on conventional bacterial AGS, a novel biotechnology for wastewater treatment, especially on its nutrients performance. Reportedly, they observed a relative decrease in biomass and nutrient removal efficiency as salinity increased. According to [23], high salt concentration affects organic matter, nitrogen and phosphorus removal. This is expected, as salt stress can restrain cell division, enlargement and growth. Nutrient removal capacity, on the other hand, indicates the amount of nutrient removed by the available biomass over time. This could be more useful to enhance the practical use of aerobic granules in saline wastewater treatment. To the authors' best knowledge, little information is available on algal-bacterial AGS treatment of saline wastewater, let alone to say the comparison between the two granular (algal-bacterial and conventional bacterial AGS) systems. For this reason, the objective of this research was to compare the nutrients removal performance (focusing on removal capacity) and granular characteristics between the two systems in treating wastewater with varying salt concentrations (0, 1, 5, 10 and 15 g/l NaCl).

## II. MATERIALS AND METHODS

### A. Synthetic Wastewater Composition and Seed Sludge

The synthetic influent wastewater composition was: 400 mg COD (sodium acetate), 50 mg NH<sub>4</sub><sup>+</sup>-N/L (NH<sub>4</sub>Cl), 10 mg PO<sub>4</sub><sup>3-</sup>-P/L (KH<sub>2</sub>PO<sub>4</sub>), 10 mg Ca<sup>2+</sup>/L (CaCl<sub>2</sub>), 5 mgMg<sup>2+</sup>/L (MgSO<sub>4</sub>·7H<sub>2</sub>O), 5 mg Fe<sup>2+</sup>/L (FeSO<sub>4</sub>·7H<sub>2</sub>O), and 2.5 ml/L of trace elements solution. The composition of the trace elements solution was prepared according to [24]. A step-wise increase of NaCl was done every 10 days, except for days 0-10. The NaCl concentrations were: 0 g/L (days 0-5), 1 g/L (days 6-10), 5 g/L (days 11-20), 10 g/L (days 21-30) and 15 g/L (days 31-40). Mature conventional bacterial and algal-bacterial AGS collected from laboratory-scale SBRs were used in this research. The initial granule diameter was 1.5 mm and 5.8 mm for conventional and algal-bacterial AGS, respectively.

### B. Experimental Set-Up and Operation Conditions

Two identical cylindrical SBRs, bacterial AGS (Rc) and algal-bacterial AGS (Ra) with 1 liter volume each were used in

this study. The working volume was set at 0.90 L. The reactors were operated at the same time with pumps and all operations being controlled by a Programmable Logic Controller (PLC). The two reactors were operated at room temperature (25 ± 2 °C) for 40 days. The algal-bacterial AGS (Ra) reactor was operated under 24-hour light affixed by a rod to face the reactor. Each 6 h operation cycle consisted of 1 min feeding, 60 min no-aeration period, 296 min aeration period, 1 min settling, 1 min effluent discharge and 1 min idling. The solids retention time (SRT) was set at 30 days.

### C. Analytical Methods

Granule sampling was done 10 min before effluent discharge every 5 days, at the end of the 4<sup>th</sup> cycle. Effluent samples were collected, centrifuged at 3200 rpm and filtered through 0.45 μm filter membrane. Standard methods [25] were used to determine ammonia nitrogen (NH<sub>4</sub><sup>+</sup>-N), nitrite nitrogen (NO<sub>2</sub><sup>-</sup>-N), nitrate nitrogen (NO<sub>3</sub><sup>-</sup>-N), and orthophosphate (PO<sub>4</sub><sup>3-</sup>-P). Granule size was measured by a stereo microscope (STZ-40TBa, SHIMADZU, Japan) with a program Motic Images Plus 2.3S (version 2.3.0) and a mathematical set rule.

### D. Calculations

Removal capacity (RC) was computed as:

$$RC(\text{mg/g} - \text{MLVSS/day}) = \frac{VE \times C_{\text{inf.}} \times V_r \times n \times R}{X_{\text{vss}} \times V_r} \quad (1)$$

VE: exchange vol. ratio of wastewater per cycle (%); C<sub>inf.</sub>: influent concentration for designated pollutant (mg/L); n: no. of cycles per day; R: removal efficiency for designated pollutant (%); X<sub>vss</sub>: mixed liquor volatile suspended solid concentration (g/L); V<sub>r</sub>: total volume of liquid in the SBR reactor (L).

$$\text{Removal efficiency (\%)} = \frac{C_{\text{inf.}} - C_{\text{eff.}}}{C_{\text{inf.}}} \times 100 \quad (2)$$

where C<sub>inf.</sub> and C<sub>eff.</sub> (mg/L) are the influent and effluent concentrations for designated pollutants.

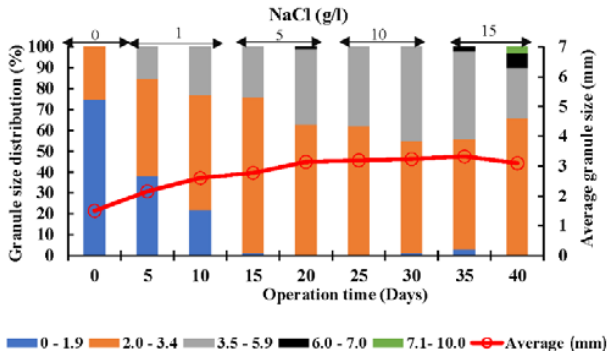
## III. RESULTS AND DISCUSSION

### A. Granule Characteristics

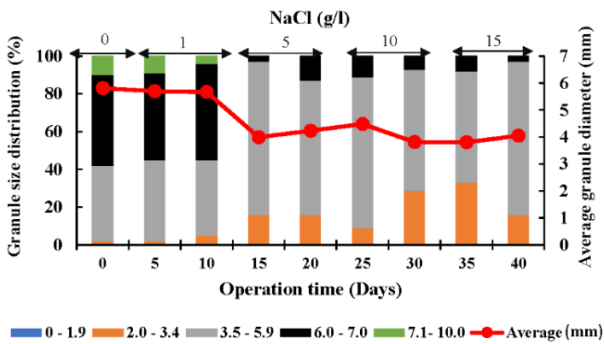
In Rc reactor, there was no apparent change in granule color. However, at 15 g/L NaCl concentration, the granules looked less compact compared to Ra granules which had compact cores. Ra granule color changed from green to dark green at higher NaCl concentration, and the flagella like structures on granule surface disappeared with salinity increase to produce granules with smooth rounded surfaces. As shown in Fig. 1, a consistent increase in granule size was observed in Rc where it was dominated initially by 75% and 25% of 0-1.9 and 2.0-3.4 mm granules under no salinity. This was changed with the increase of salinity, as granule size became larger with 62% and 66% of 2.0-3.4 mm sized granules on day 25 and day 40 under 10 g/L and 15 g/L NaCl salinity, respectively. However, the average granule size slightly decreased from 3.2 mm to 3.1 mm during days 25-40. Wang et al. [6] reported granule size

decrease from 5.2 mm to 4.2 mm when NaCl concentration varied from 10 g/L to 15 g/L. The granule surfaces were round and smooth as reported by [26], and the granule size increase was also observed by [22], which ranged between 3.35 mm and 2.63 mm under 15 g/L and 2.5 /L to 15 g/L NaCl conditions, respectively.

g/L and 15 g/L NaCl, respectively. At 15 g/L NaCl, the granules were lighter with fluffy edges although filamentous growth was not observed. Ra granules were much compact as compared to Rc. However, in both reactors, the biomass growth decreased at higher salt concentration with more broken granules in Rc than Ra.



(a)



(b)

Fig. 1 Granule size distribution and average diameter in conventional AGS (a) and algal-bacterial AGS (b)

Conversely in Ra, the granule size decreased throughout the operation from 48% of 6.0-7.0 mm and 40% of 3.5-5.9 mm granules under no salinity condition to 80% and 81% of 3.5-5.9 mm sized granules and 11% and 3% 6.0-7.0 mm granules at 5

TABLE I  
AVERAGE GRANULE SIZE DISTRIBUTION AND MIXED LIQUOR VOLATILE SUSPENDED SOLIDS (MLVSS) OVER THE OPERATION DURATION

Operation duration (day)	Average diameter (mm)		MLVSS (g/L)	
	Rc	Ra	Rc	Ra
0	1.5	5.8	3.1	2.6
5	2.2	5.7	1.3	4.4
10	2.6	5.7	1.2	2.7
15	2.8	4.0	0.7	2.4
20	3.1	4.2	1.2	1.0
25	3.2	4.5	0.6	1.9
30	3.2	3.8	0.4	1.2
35	3.3	3.8	0.5	1.2
40	3.1	4.0	0.4	0.9

*B. Nitrogen Removal*

Effluent total nitrogen (TN) decreased with salinity increase in both reactors (Fig. 2). Wang et al. [22] reported 98% TN removal at 15 g/L when gradually adapting mature aerobic granules to increased salinities from 2.5 g/L – 15 g/L although this was realized at higher dissolved oxygen range of 2.5 mg/L – 8.0 mg/L. It was however contrary to the downward trend from 72.43% - 55.92% at 5 g/L – 15 g/L NaCl reported by [6]. Nonetheless, the decreased biomass concentration at higher salinities was consistent with previous works [6], [22] in response to increasing salinity. Li et al. [27] also reported that higher TN removal was achieved in treating seawater-based wastewater with relatively higher salt proportions. It is interesting to note however that, although TN concentration in Ra was relatively lower than in Rc at all different salinities tested, in Rc the RC was higher and increased at higher salinity, mainly attributable to its biomass instability and decreased biomass concentration along with the operation.

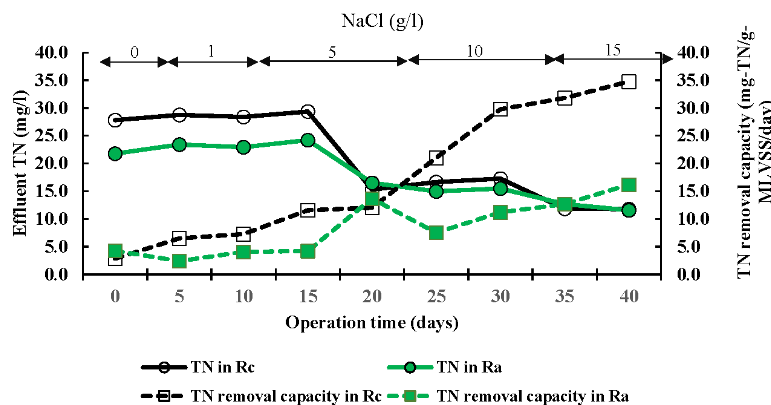


Fig. 2 Effluent TN concentration and TN RC

Effluent  $\text{NH}_4\text{-N}$  concentration was not affected by salinity  $\geq 5$  g/L NaCl. However, at 10 g/L NaCl concentration, some  $\text{NH}_4\text{-N}$  was detected in both reactors, 4.1 mg/L - 4.2 mg/L in Rc and 2.1 mg/L - 2.7 mg/L in Ra (Fig. 3). Both reactors recovered to achieve almost 100% removal on day 40 at 15 g/L NaCl, with effluent  $\text{NH}_4\text{-N}$  concentration of 0 mg/L and 0.3 mg/L in Rc and Ra, achieving  $\text{NH}_4\text{-N}$  removal rates of 91-100% and 94-99% by Rc and Ra, respectively. This could be due to acclimation of nitrifying bacteria to the increased salinity in both reactors. This observation is in agreement with previous works [6], [22]. However, the RC increased although it was relatively small. The previous researches [6], [28] also used mixed carbon sources (sodium acetate and glucose, 1:1). According to [28], carbon source influences microbial species in granules and consequently nutrients removal activities. Tay et al. [29] reported the dominance of filamentous bacteria in glucose-fed granules as compared to the compact microstructure of acetate-fed granules which formed dense and resistant granules compared to the former ones reported by [30]. This could mean that acetate-fed granules might be much adaptable to salinity stress. In this work, sodium acetate was the sole carbon source.

According to [31], high salinity inhibits ammonia oxidizing bacteria and decrease ammonium removal efficiency. Although this could be true, an increase in RC as observed in both

reactors could mean that after acclimation, the remaining microbial communities are able to efficiently remove  $\text{NH}_4\text{-N}$  to achieve nutrients removal notwithstanding the inhibitory effect of high salinity. The RC in Rc was 15.3 – 45.5 mg-N/mg-MLVSS/day, which was 4.5 – 20.9 mg-N/mg-MLVSS/day in Ra at 1 g/L and 15 g/L NaCl, respectively. The detected nitrite ( $\text{NO}_2\text{-N}$ ) concentrations in both reactors peaked to 10 mg/L at 5 g/L NaCl, and remained stable till the end of the experiment on day 40 at 15 g/L NaCl. A similar 9.7 mg/L of  $\text{NO}_2\text{-N}$  was reported by [22] at the same salinity in treating saline wastewater. High nitrite ( $\text{NO}_2\text{-N}$ ) concentration in reactors whilst treating saline wastewater is often due to salinity inhibition. Figueroa et al. [13] reported nitrite accumulation in SBR in treating diluted fishing canning wastewater (with salinity ranged 11- 15 g/L NaCl) using mature aerobic granules, which might be related to inhibitory effect caused by both ammonia accumulation and sodium chloride presence. A highest nitrate ( $\text{NO}_3\text{-N}$ ) concentration of 3.8 mg/L was detected at 10 g/L NaCl, which was however decreased with salinity increase at 15 g/L NaCl, to 1.8 mg/L and 1.4 mg/L in Rc and Ra, respectively. This  $\text{NO}_3\text{-N}$  concentration was lower than 12.0 mg/L  $\text{NO}_3\text{-N}$  reported by [22], under similar salinity conditions.

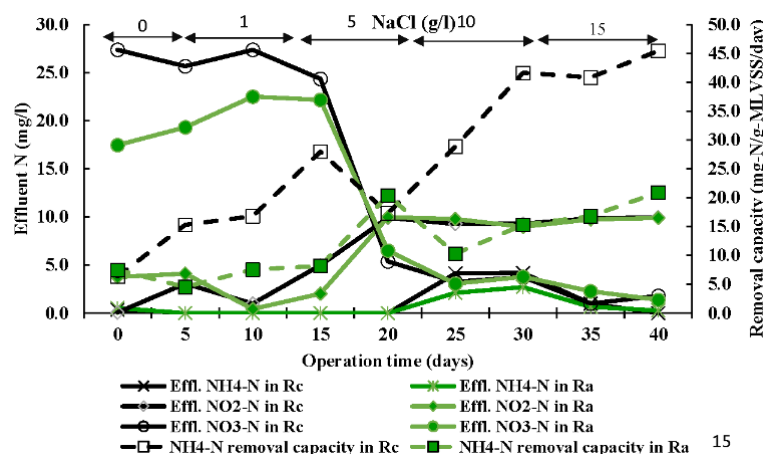


Fig. 3 Nitrogen profile and  $\text{NH}_4\text{-N}$  RC

### C. Phosphorus Removal

As illustrated in Fig. 4, there was no  $\text{PO}_4\text{-P}$  removal at the initial addition of 1 g/L NaCl on day 5 in Rc. This was however improved with the subsequent addition on day 10 with an effluent  $\text{PO}_4\text{-P}$  concentration of 1.9 mg/L, better than the initial effluent concentration of 3.6 mg/L at 0 g/L NaCl. Thereafter, the effluent  $\text{PO}_4\text{-P}$  increased to 4.2 mg/L from day 15 (5 g/L NaCl) to 5.3 mg/L on day 40 (15 g/L NaCl). Phosphorus removal was deteriorated on day 20 (5 g/L NaCl) with effluent 6.7 mg/L and 6.0 mg/L to 5.3 mg/L and 5.1 mg/L of  $\text{PO}_4\text{-P}$  in Rc and Ra, respectively. However, the removal capacities in both reactors decreased with the salinity increase at 5 g/L NaCl, 4.2 mg-P/g-MLVSS/day and 2.8 mg P/mg-MLSS/day by Rc

and Ra respectively to 0.7 mg-P/mg-MLSS/day at 15 g/L by both reactors on day 40. Effluent P concentrations from Ra were relatively stable and better as compared to Rc with salinity increase, except at 1 g/L NaCl on day 10, about 6.7 mg/L and 1.9 mg/L in Ra and Rc, respectively. Although phosphorus removal rate relatively increased from 5-15 g/L NaCl (day 20 - day 40) with effluent P concentration of 6.0-5.1 mg/L and 6.7-5.3 mg/L in Ra and Rc, respectively, the RC was almost the same by both Ra and Rc. Where there was an increase in Ra, Rc decreased but recovered to achieve higher RC throughout the operation. On day 15 and day 10, Ra and Rc achieved their highest removal rates of 58% and 81% at 5 and 1 g/L, respectively, whereas both reactors reached about 49% at 15

g/L NaCl on day 40, which are similar with the 50.21% reported by [6] who used mature AGS through gradual increase NaCl concentration to 15 g/L. However, [22] reported that phosphorus removal was deteriorated at 5 g/L NaCl without recovery when NaCl was gradually increased from 2.5 g/L to 15 g/L, during which the exact values were not mentioned. This observation may suggest that short-term exposure might be better for microbes to keep stable P removal under saline stress. A 10-day or 20-day interval for each salinity ratio adapted in

this research or [6] was relatively good to enhance P removal as compared to the 30-day interval reported by [22].

Pronk et al. [32] also reported that phosphate uptake rates were decreased when nitrite accumulated at increased salinity in evaluating the side effect of high salinity on aerobic granules. The phosphate uptake rate declined to approximately 40% of the maximum uptake rate when nitrite ( $\text{NO}_2\text{-N}$ ) accumulated up to 13 mg/L. In this work the highest nitrite accumulation in both reactors was 10 mg/L at 5 g/L NaCl.

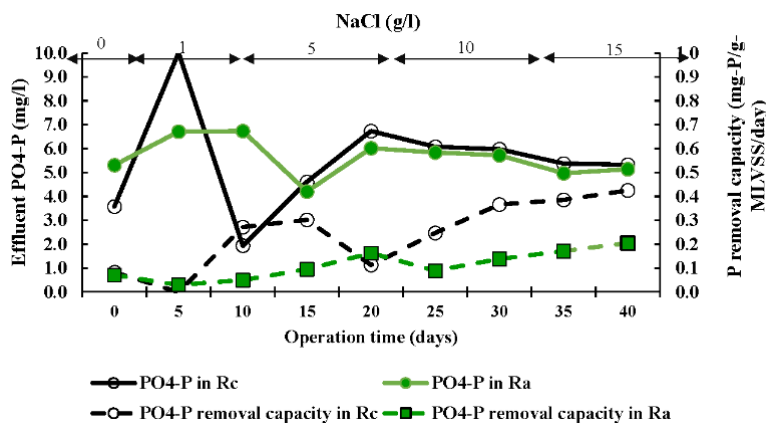


Fig. 4 Effluent orthophosphate removal and P RC

#### IV. CONCLUSIONS

Increase in influent salinity to 10 g/L NaCl caused an increase in the diameter of conventional AGS. However, in the algal-bacterial AGS system increase in salinity from 0-15 g/L NaCl resulted in decreased granule size with stable compact structure.

Both conventional and algal-bacterial AGS systems had high nitrogen oxidation with nitrification and denitrification efficiency range between 100-99% and 100-80%, respectively.

Conventional AGS exhibited better total N RC than algal-bacterial AGS with the increase in influent salinity. The former also had higher P RC with salinity increase than algal-bacterial AGS possibly due to its lower biomass concentration, while both systems achieved almost 50% removal at 15 g/L NaCl.

Seen from this work, algal-bacterial AGS reflected better granular stability and adaptation capability to the increased influent salinity. Therefore, future research is necessary on the optimization of operation condition for algal-bacterial AGS to enhance its high stably performance in practice. In addition, microbial community analysis is also important to understand the mechanisms involved in the nutrients removal by the two granular systems.

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