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Seawater-based wastewater accelerates development of aerobic granular sludge: A laboratory proof-of-concept

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ABSTRACT

This study aimed to develop an aerobic granular sludge process for the efficient treatment of highly saline wastewater and understand the granulation process in a seawater-based multi-ion matrix. Five identical sequencing batch airlift reactors (SBARs) are used to treat synthetic saline sewage with different proportions of real seawater (0%–100%). The results confirm that aerobic granular sludge can be successfully developed with various proportions of seawater up to 100% and show that seawater not only significantly accelerates granulation but also generates stronger granular structures than does freshwater. The increased presence of gel-forming alginate-like exopolysaccharides in the granules explains why a greater proportion of seawater leads to higher density and improves the cohesive strength of the granules. SEM-EDX analysis further revealed substantial presence of both Ca^{2+} and Mg^{2+} phosphate in the granule core as well as in the outer layers providing extra bridging forces in addition to alginate-like exopolysaccharides for accelerating the granule formation and maintaining the structure. It is hoped that this work could explore another approach for saline sewage treatment and bring some clues for the mystery of granulation mechanism.

1. Introduction

An estimated 50 percent of the world's population lives within 100 km of a coastline (World Bank, 2009), and this figure is expected to grow rapidly in the next half century due to migration, given the geographical and economic advantages of coastal areas compared to inland regions. Associated with this growth is an increase in the production of saline wastewater. One such cause is the growth of the aquaculture industry, which has increased by a factor of five between 1990 and 2013 (FAO, 2014). While providing both food and income to coastal areas (Naylor and Burke, 2005), it also generates saline wastewater typically composed of dissolved solids (30 g/L), nitrogen (60 mg/L) and phosphorus (5 mg/L) (Díaz et al., 2012). A second contributor is seawater intrusion into coastal freshwater aquifers, caused by both the over extraction of groundwater resources and rising sea levels. For example, Moptma-Miner (1994) estimates that 58% of Spain's coastal hydrogeological units have been impacted by seawater intrusion due to over extraction, while Weert et al. (2009) estimate that 204 million people were impacted globally by saline water intrusion into groundwater in 2000. Meanwhile, for coastal areas with constant head boundaries, seawater could intrude up to several kilometers inland due to rising sea levels (Werner and Simmons, 2009). As groundwater accounts for a third of all freshwater consumption and is the sole water source for many cities, brackish groundwater wells must be frequently desalinized (Bear et al., 1999). Wastewater can become salinized through the disposal of desalination brine in sewers or the infiltration of saline groundwater into sewer networks.

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Conventional biofilm processes are typically used for the treatment of wastewater from aquaculture and other sources (Eding et al., 2006) but are constrained in urban environments by their large footprint (Dickenson, 1997), unstable performance (Vallet et al., 2009), and reactor blockages due to precipitation on biomass support materials (Sánchez et al., 2012). This shortcoming in turn limits the quality of treatment, causing ammonium, nitrite and suspended solids to accumulate (Ebeling and Timmons, 2012). An alternative technology to allow the compact, stable and efficient biological treatment of seawater-laden wastewater is therefore necessary.

Aerobic granular sludge (AGS) is a promising alternative technology due to its compact layered structure, which allows increased biomass retention and concentration, as well as its greater resistance to adverse conditions compared with conventional activated sludge (Tay et al., 2005). Applications of AGS in the treatment of dairy (Schwarzenbeck et al., 2004), abattoir (Cassidy and Belia, 2005) and livestock wastewater (Othman et al., 2013) have been studied intensively. However, Jeison et al. (2008) found one potential obstacle in that the particle size and physical strength of granules decreased significantly when salinity was increased from 1 to 10 g Cl^-/L. Pronk et al. (2014) made similar findings and observed granule disintegration after increasing salinity to 20 g Cl^-/L, similar to that of seawater-based aquaculture wastewaters. Conversely, Li and Wang (2008) report that increasing salinity up to 50 g Cl^-/L results in much larger, more compact and active granules. Corsino et al. (2016) also achieved robust results using aerobic granular sludge to treat fish processing wastewater at salinity levels up to 50 g Cl^-/L. The wide variations between findings from previous studies leads us to reconsider the effect of salinity on aerobic granular sludge processes. Moreover, almost all studies to date have focused on simple saline matrices, usually containing only salt (NaCl) (Li and Wang, 2008; Bassin et al., 2011; Taheri et al., 2012). Seawater is significantly more complex, containing seven ions at concentrations greater than 100 mg/L. The impact of multi-ion matrices on the granulation process requires further investigation, especially that of abundant metal divalent cations (e.g. Ca^{2+}, Mg^{2+}) in seawater. Multi-ion matrices in seawater can have a significant impact on extracellular polymer structure, which is critical to the granulation process (Lin et al., 2013b).

This study therefore explores the effects of high salinity on the aggregation of AGS for the treatment of seawater-based wastewaters. Five identical sequencing batch airlift reactors (SBARs) fed with various proportions of real seawater (0, 20, 50, 80 and 100%) were used to develop AGS. A series of batch experiments were further conducted to study the possible causes of the granulations observed with different proportions of seawater. Our findings could expand the potential applications of aerobic granules to the treatment of seawater-based wastewaters.

2. Materials and methods

2.1. Reactor design and operation

Five identical SBARs (R1, R2, R3, R4 and R5) with a working volume of 1.1 L each (100 cm in height and 5 cm in diameter) were inoculated with 4 g/L of activated sludge taken from a local sewage treatment plant in Hong Kong. A 2 L/min airflow rate was applied to each reactor to produce a superficial upflow air velocity of 1.2 cm/s (Tay et al., 2004). The ambient temperature in the laboratory was 23 ± 1 °C. Each SBAR was operated for 140 days with a 2.4 h operating cycle comprising 6 min feeding, 120 min aeration, 5 min settling, 5 min decanting and 8 min idling. The volumetric exchange ratio of each reactor was set at 50%, corresponding to a hydraulic retention time (HRT) of 4.8 h. The performance of the reactors was recorded every two days. The physical and chemical properties of the sludge were also measured every week.

2.2. Synthetic saline sewage

Synthetic saline wastewater was prepared by diluting the synthetic stock solution (Table A.1 in Supplementary Information (SI)) with a mixture of seawater and freshwater. Seawater was collected from the Hong Kong seawater-toilet flushing network, which undergoes straining and electrochlorination. Salinity at the point of collection was 3.2% with no residual free chlorine. Different proportions of seawater were mixed with the synthetic stock wastewater, as shown in Table A.2 of the SI, to achieve final seawater proportions of 0% (R1), 20% (R2), 50% (R3), 80% (R4) and 100% (R5) to the nearest percentage point respectively. The influent was comprised of acetate, glucose and yeast with a chemical oxygen demand (COD) concentration of 400 mg/L. Ammonium chloride was also added to increase the influent ammonium concentration to 100 mg-N/L. The ratio of bicarbonate ($NaHCO_3$) to ammonium-nitrogen (N) in the influent was fixed at 4 to maintain a pH of 7.2.

2.3. Batch experiment

The batch experiment was conducted in two smaller 500 ml reactors to investigate the dynamics of rapid granulation using different proportions of seawater. Each batch reactor was inoculated with 2 g/L of activated sludge taken from the same local sewage treatment plant. The batch reactors followed the same operation schedule as the SBARs and were run for 30 days. Both batch reactors were fed with the same synthetic wastewater as described in Section 2.2 but mixed with 0 (R_A) and 100% (R_B) seawater respectively. Samples were collected daily to measure the diameter of the sludge and the mass concentration of metal ions in the sludge. The physical and chemical properties of the sludge were measured daily.

2.4. Analytical methods

COD was calculated based on the Standard Methods (APHA, 2005). Total nitrogen (TN) was measured with a total nitrogen measurement module equipped on a total organic carbon analyzer (TOC-VCPH, Shimadzu). A flow injection analyzer (QuikChem 8500, Lachat Instruments) was applied to examine the concentration of ammonium nitrogen, while nitrates and nitrites were determined with an ion chromatograph (HIC-20A super, Shimadzu). The physical properties of the sludge were measured, namely mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), Sludge Volume Index (SVI), particle size distribution, morphology, cohesion and specific area. MLSS, MLVSS and SVI in SBARs were measured every two days according to the Standard Methods (APHA, 2005) with the exception of SVI, which was also measured for a duration of 5 min. Wet density was determined using a pycnometer with water (3 replicates). The particle size distribution of the sludge was determined with a laser diffraction particle size analyzer (LSI3 320, Beckman Coulter). The electrostatic charge of the biomass surface was expressed as its zeta potential and determined with a Zetasizer 300 (Malvern) following the method developed by Su et al. (2014). The sludge samples were fixated in a saline mixed solution of 2% paraformaldehyde, 2% glutaraldehyde, and 1× phosphate-buffered overnight at 4 °C and subsequently lyophilized. The sludge microstructure and morphology were then examined with a scanning electron microscope (SEM) (JSM 6300F, JEOL). Several granules were sectioned with a scalpel prior to fixation. A cross-sectional analysis was conducted in the central area of the granules using energy

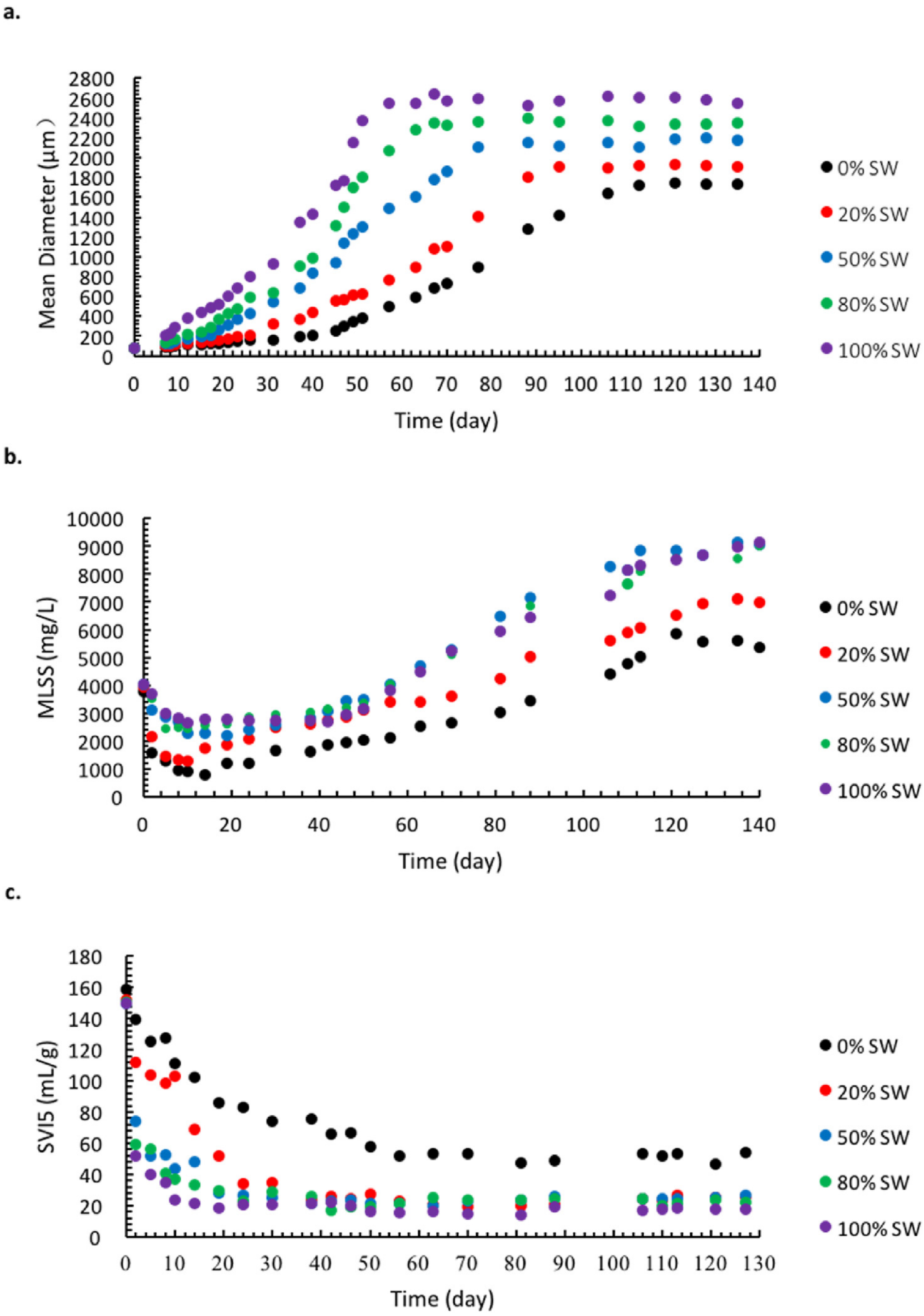

Fig. 1. Profile of sludge indices for: (a) MLSS, (b) mean diameter of granules, and (c) SVI_5.

dispersive X-ray (EDX) connected to the SEM. A cohesion test was carried out following Wan et al. (2011) with some modifications described in Mackey et al. (2016). The test involved low and high mixing intensities of $G = 250\ s^{-1}$ and 13,230 s^{-1}. After conducting microwave digestion on the sludge (Sandroni and Smith, 2002), the concentration of metal ions was analyzed with an inductively coupled plasma-optical emission spectrometer (725 ES, VARIAN). Saturation indices for potential precipitates in the bioreactor were determined using the Visual MINTEQ software. The temperature and pH were fixed at 23 °C and 7.2 respectively. Input cation and anion concentrations in the seawater were obtained from previous research using the same seawater source (Dai et al., 2014). Extracellular polymeric substances (EPS) were first extracted from the sludge using a formaldehyde-NaOH method (Liu and Fang, 2002). The polysaccharide (PS) content in EPS was then quantified using a phenol-sulfuric acid method with glucose as the standard (Dubois et al., 1956). The PN content in EPS was also determined using a modified Lowry colorimetric method (DC Protein Assay, BioRad) with bovine serum albumin as the standard (Frølund et al., 1995). The alginate-like exopolysaccharides (ALE) in AGS and its block composition were measured using the method used by Lin et al. (2010) at the end of the experiment.

3. Results

3.1. The formation of aerobic granules

Fig. 1a shows the changes in the mean diameter of the aerobic

Table 1
Synthetic wastewater-based AGS granulation time reduced by different methods.

Strategies	Time for granulation (days)	References
Static magnetic field	25	(Wang et al., 2012)
50% crashed granules mixed	20	(Pijuan et al., 2011)
Ca^{2+} augmentation	17	(Liu et al., 2010)
Mg^{2+} augmentation	16	(Liu et al., 2010)

granules in each SBAR. The time taken for granulation (i.e. mean diameter ≥ 200 μm and $SVI_5/SVI_{30} \approx 1$) in R5 (100% seawater) was 7 days (SVI_5/SVI_{30} provided in Fig. A.7 of SI). This was a month shorter than that of R1 (0% seawater) and less than half the time taken for most rapid granulation enhancement methods reported in the literature (Table 1). After 140 days of cultivation, the average diameter of aerobic granules had stabilized in all reactors. The final mean granule size showed a strong positive correlation with the proportion of seawater such that the final mean diameter in R1 (0% seawater) was 1700 μm, compared to 2500 μm in R5 (100% seawater) (Fig. 1a).

Changes in MLSS and SVI_5 for each SBAR were recorded throughout the operation, as shown in Fig. 1b–c. During the first 10 days, poorly-settling sludge in each reactor was washed out to varying degrees under the short settling time applied (5 min) (Fig. 1b). Washout was greatest in the highest salinity reactors, most likely due to greater buoyancy forces. Thereafter, the MLSS concentrations of all reactors increased (Fig. 1b) after granulation (Fig. 1c) and biomass growth. Comparing the final MLSS concentrations and SVI_5 in the five SBARs, a greater proportion of seawater increased the settling of aerobic granules (e.g. 25 ± 5 mL/g of SVI_5 in R5 vs. 50 ± 5 mL/g of SVI_5 in R1) (Fig. 1c) as well as the total biomass concentrations.

3.2. Characterization of the rapid formed granules

3.2.1. Physical strength

The results of the cohesion test showed no apparent flocculation of sludge in any of the five SBARs with a mixing intensity (G value) of 250 s^{-1} as shown in Fig. 2, indicating that the sludge was granular rather than flocculent in structure (Wan et al., 2011). When a higher G-value (13,230 s^{-1}) was applied, a breakup of granules was clearly observed in all reactors (Fig. 2) although the degree of breakup, in both absolute and proportional terms, was lower in the more saline reactors. The mean diameter of the sludge decreased by 50% in R1 but only by 20% in R5 after mixing at the highest intensity, suggesting that the presence of seawater considerably increases the physical strength of AGS.

3.2.2. Inorganic precipitation

The MLVSS/MLSS ratios decreased by more than 50% as the

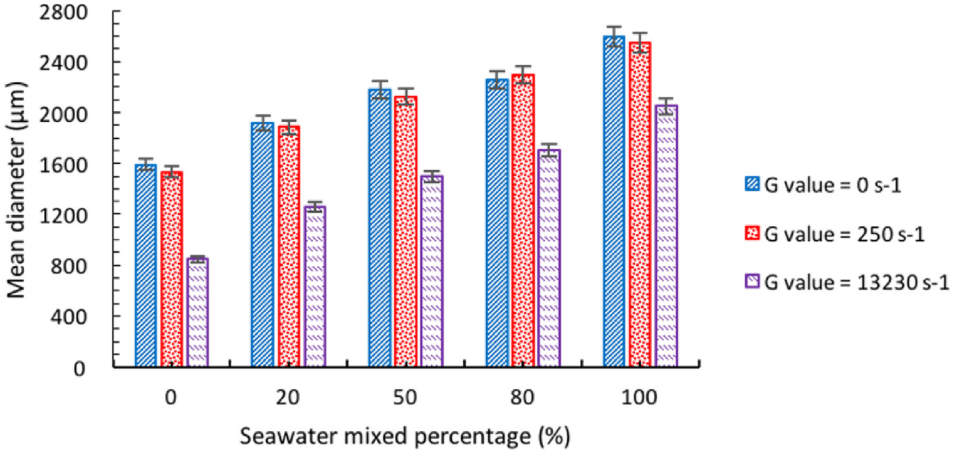

Fig. 2. Changes in the mean particle size of the granular sludge cultivated with different proportions of seawater in influent during the cohesion tests.

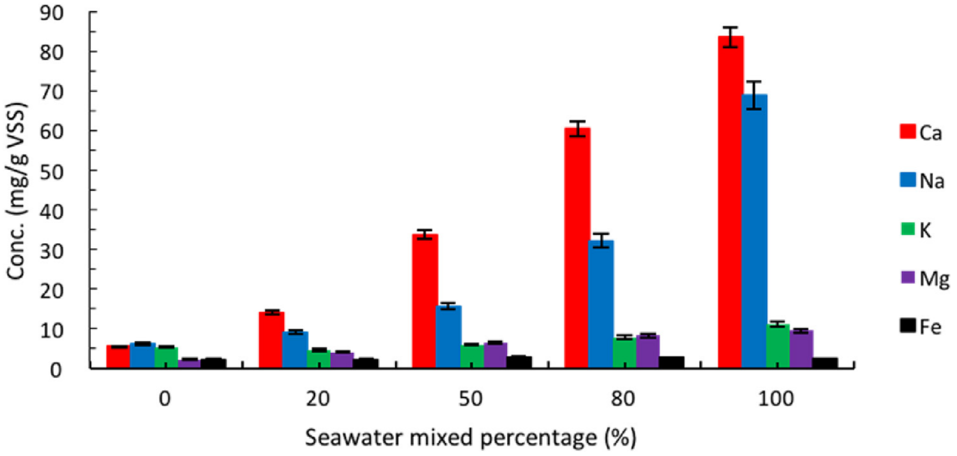

Fig. 3. The concentrations of metal ions in aerobic granules cultivated with different proportions of seawater.

proportion of seawater fractions was raised from 0 to 100% (Fig. A.2 in SI). With an increase in the proportion of seawater, greater proportions of calcium, sodium, and to a lesser degree magnesium and potassium were found incorporated in the granules, making up 9% of MLSS in the pure seawater system (Fig. 3). Inorganic precipitates were observed by SEM (Fig. A. 3. in SI) in the core area of granule. The EDX analysis (Table A.4 in SI) indicated the presence of both calcium and magnesium phosphate precipitates while calculation of the saturation indices in the influent (Table A.6. in SI) implied preformed precipitates could be Hydroxyapatite (HAP), Whitlockite ($Ca_3(PO_4)_2$) and $Ca_4H(PO_4)_3 \cdot 3H_2O$.

3.2.3. *Variation of extracellular polymeric substances (EPS)*

The production and composition of extracellular polymeric substances (EPS)are known to affect the granulation process of AGS (McSwain et al., 2005). The physicochemical properties of the microbial aggregates including structure, surface charge, flocculation, settling and dewatering properties, and adsorption ability are all associated with EPS production (Sheng et al., 2010). The production and composition of EPS in granules in all SBARs were measured after 30, 70, 110 and 140 days respectively, as shown in Fig. 4. Total EPS production decreased with an increase in the proportion of seawater (Fig. 4), while the PN content of EPS in all reactors remained relatively stable.

3.2.4. *Effects of alginate-like exopolysaccharides*

Apart from bulk PS and PN measurements, alginate-like exopolysaccharides (ALE), which constitute a key structural component of PS, were extracted from the EPS. Alginates are known to form rigid, non-deformable gels due to their highly specific interactions with divalent cations (eg. Mg^{2+}, Ca^{2+}) across a broad temperature and pH range (Lin et al., 2008). In terms of chemical structure, alginate macromolecules are composed of mannuronic acid (M) and guluronic acid (G) residues. GG blocks are generally associated with gel-forming capacity while MM and MG blocks provide the chains with flexibility (Melvik and Dornish, 2004). The concentration and block composition of ALE in the granules were determined at the end of the experiment. Fig. 5 shows that the ALE content and the proportion of GG blocks in ALE increased with greater proportions of seawater. The ALE content and proportion of GG blocks in the ALE of granules were six times and two times higher in R5 than in R1 respectively, implying a greater gel-forming capacity in R5.

3.3. *Granulation batch tests*

In order to further investigate how seawater enhances AGS, the granule formation process from flocs was repeated in batch experiments and the results are summarized in Fig. 6. The granulation process in R_B (100% seawater) was significantly faster than in R_A (0% seawater), reaching a diameter of 200 μm in six days, whereas the

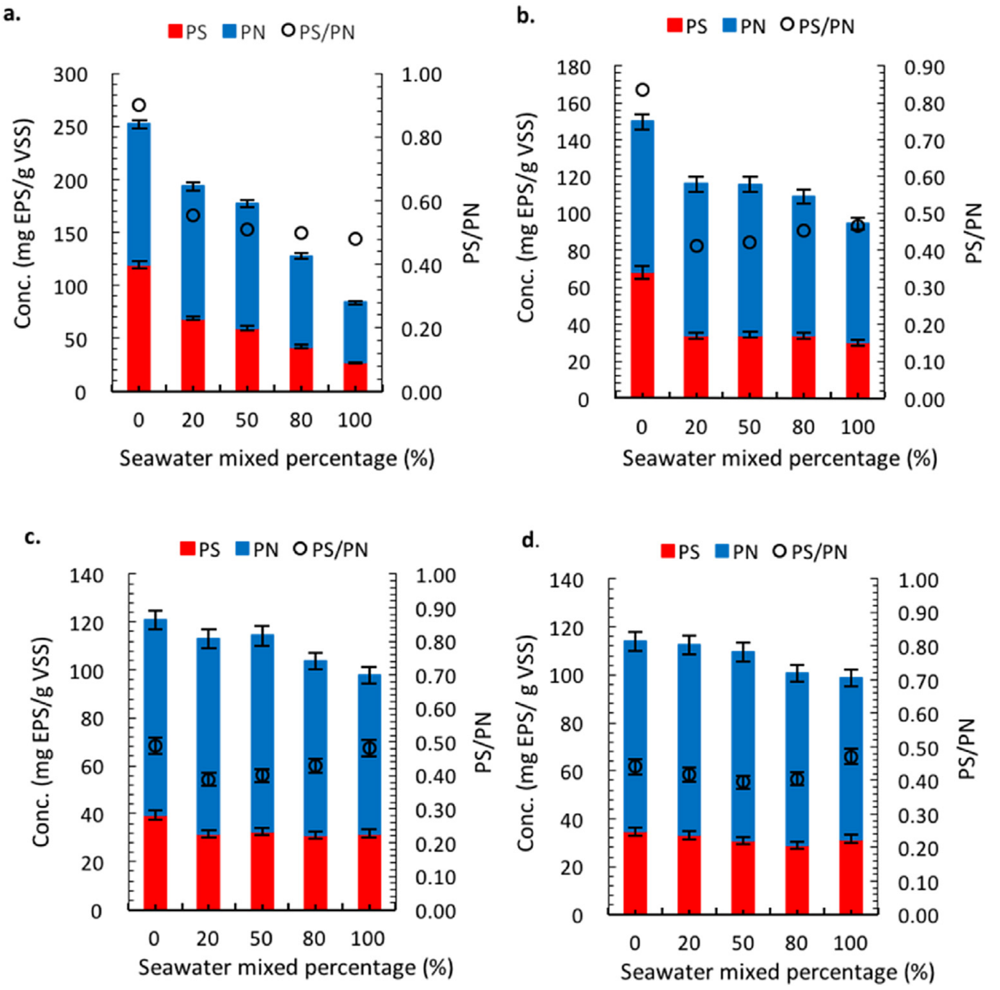

Fig. 4. Main EPS production of the granular sludge cultivated with different proportions of seawater after (a) 30 days, (b) 70 days, (c) 110 days and (d) 140 days.

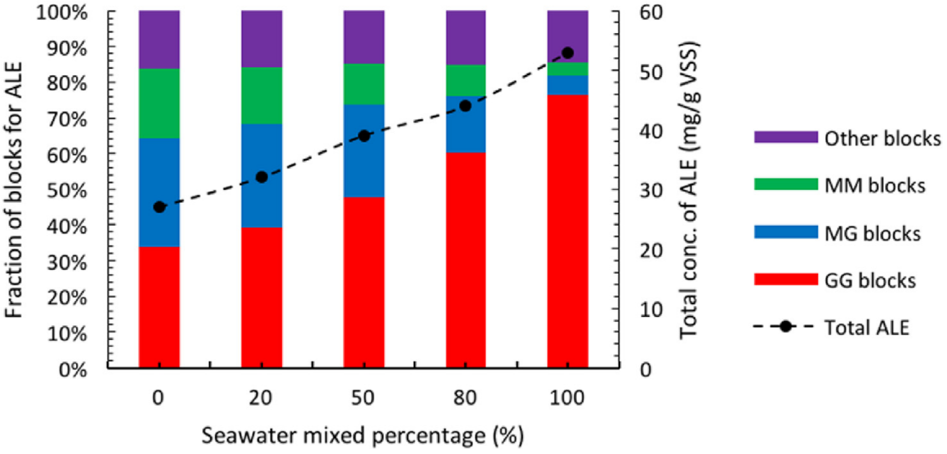

Fig. 5. The total concentration of alginate-like EPS (proportion of blocks obtained by partial hydrolysis) isolated from aerobic granules in reactors with different proportions of seawater.

sludge in R_A only reached 130 μm at the end of the entire 30-day test duration (Fig. 6). As indicated as Phase II in Fig. 6, a notable increase in sludge density occurred in R_B after six days. While R_A showed a gradual increase in density, the magnitude of this increase was much smaller than that of R_B. The density increase in R_B was accompanied by significant increases in Ca^{2+} and phosphate (Fig. A.5 in SI). The zeta potential of R_A increased slightly from −26.0 to −20.9 mV over the 30-day duration. In R_B, however, it increased significantly from −23.9 to +2.7 within the first two days, increasing gradually thereafter and finally ending at +6.5 mV.

3.4. Performance of the SBARs

The ammonia oxidizing efficiency was suppressed at the initial stage in reactors with higher salinity level. However, all reactors were able to remove 90% NH_4^+-N after 140 days' operation (Fig. 7a). A longer time was required to reach such performance at high salinity levels (≥50% seawater). The efficiency of TN removal in the reactors containing seawater (R2-R5) was initially limited but gradually increased with the adaption of biomass (Fig. 7b). After 140 days of acclimation, the efficiency of TN removal via simultaneous aerobic nitrification-denitrification was 50% in R5, two and a half times higher than that of R1. In terms of COD removal, all five SBARs could achieve over 85% COD removal efficiency throughout the operation period (Fig. 7c). The maximum oxidation rates of ammonia in R5 was 6.4 ± 0.2 mg N/g-VSS·h, 34% lower than that of R1 (8.6 ± 0.4 mg N/g-VSS·h). The maximum nitrite oxidation rate was 4.1 ± 0.2 mg N/g-VSS•h in R1 but was reduced almost to zero in R2 to R5 (Table 2). The results were also confirmed by nitrite accumulation in R2 to R5 throughout the entire experiment (Fig. A.6 in SI). The improved nitrogen removal is therefore attributed to both shortcutting, allowing a more efficient use of carbon, and a greater anoxic core due to increased granule diameters with larger proportions of seawater.

4. Discussion

4.1. Effect of salinity on granulation

This study finds that an increase in seawater proportion is associated with enhanced aerobic granulation. Due to the higher density of seawater (1025 g/L), more than 70% of the sludge was washed out initially (Fig. 1.b) in the most saline reactor (R5), including observed granule fragments and early-stage granules.

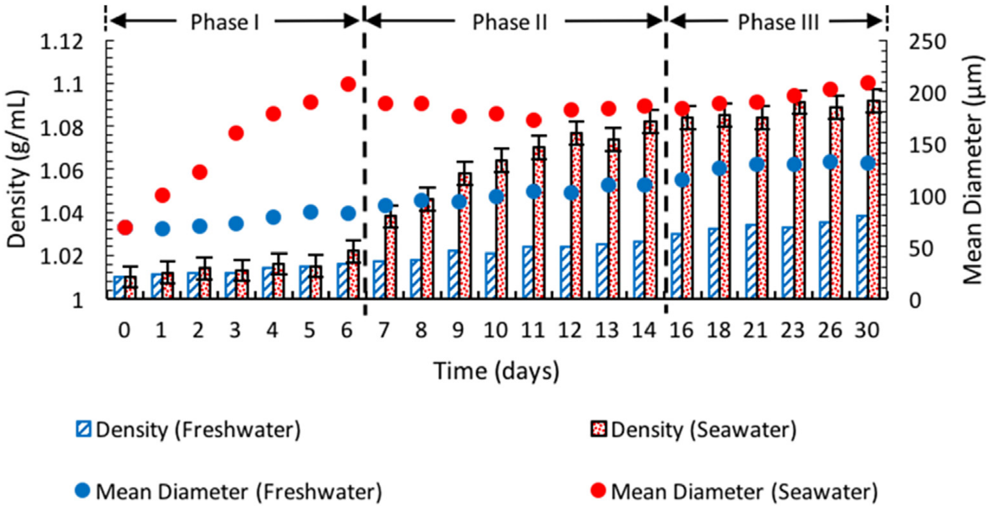

Fig. 6. Average diameter and density of granules in batch tests.

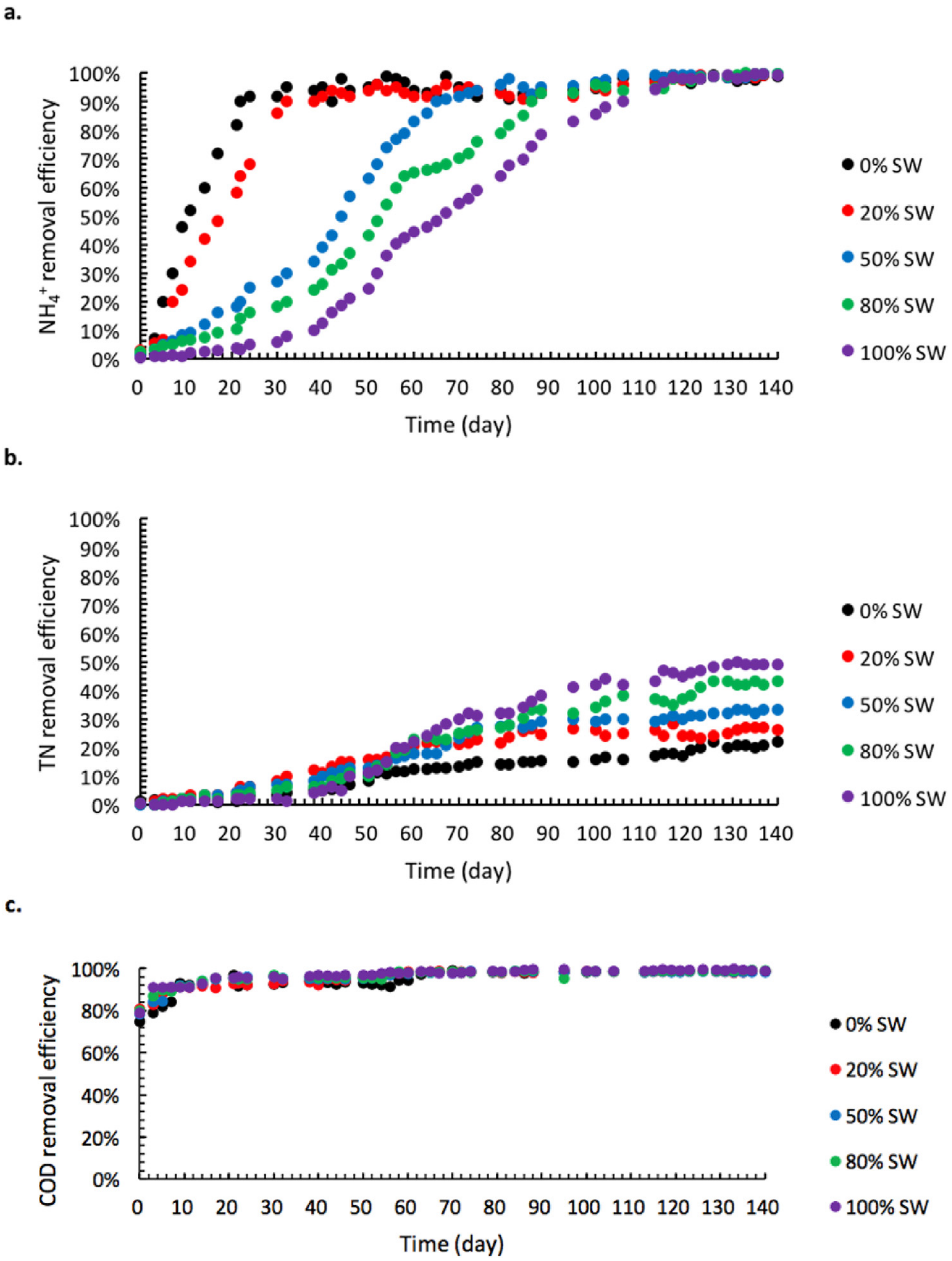

Fig. 7. The performance of N removal in each reactor during the entire operation (a) ammonium removal, (b) TN removal and (c) COD removal.

The higher buoyancy forces lead to a much greater selection pressure for dense well-settling aggregates. Additionally, the substantial presence of metal ions in seawater can neutralize the negative charges around bacteria, thereby facilitating the initial aggregation process (López-Maldonado, et al., 2014). This was supported by zeta-potential measurements from the batch tests comparing freshwater and seawater systems (Fig. A.4 in SI).

A number of previous studies also indicate that increasing salinity, particularly sodium, results in an increased production of proteins (Kara et al., 2008; Cui et al., 2015). This may be due to a protective mechanism that resists salinity as well as cation-π interactions of monovalent salts with extracellular proteins. Such behavior was not observed in this study, which found a slight overall decrease in both polysaccharides and proteins. As EPS

Table 2
Maximum oxidation rates (mg N/g VSS·h) of ammonium and nitrite.

	Seawater Mixed (%)				
	0	20	50	80	100
Max. NH$_4^+$ oxidation rate	8.6 ± 0.4	8.4 ± 0.3	6.6 ± 0.2	6.6 ± 0.3	6.4 ± 0.2
Max. NO$_2^-$ oxidation rate	4.1 ± 0.2	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01

compounds depend on both the microbial community and their selective environment, the use of a brackish seed sludge in this study may have favored bacteria producing EPS compounds less influenced by salinity (notably Na^+) than other reported studies (Wingender et al., 1999). The role of specific ions is discussed in the following section.

4.2. Roles of monovalent and divalent cations on EPS and aggregation

According to the cohesion tests, increasing the proportion of seawater enhanced the physical strength of AGS while also improving its rate of granulation and granule diameter. This is in direct contrast with a number of studies that suggest that high Na^+ concentrations may reduce gel-like EPS and bonding strength (Bruus et al., 1992; Sobeck and Higgins, 2002; Kara et al., 2008; Seviour et al., 2008) by replacing divalent cation bridges of alginate-like polysaccharides (de Kerchove and Elimelech, 2006). On the other hand, both Mg^{2+} and Ca^{2+} have been demonstrated to significantly improve both the granulation process and the strength of AGS through cationic bridging (Bruus et al., 1992; Sobeck and Higgins, 2002; Lin et al., 2013a,b). The exact role of monovalent and divalent ions in sludge flocculation and granulation remains contested.

Three major theories exist for the involvement and effects of monovalent and divalent salts in sludge aggregation. These are the Derjaugin, Landau, Verwey, and Overbeek (DLVO) theory, which describes the competition between attractive van der Waals forces and repulsive electrostatic double layer forces; divalent cation bridging (DCB) theory, in which divalent cations are critical to the structural bridging of PS; and alginate theory, a subset of DCB in which Ca^{2+} is the primary cation bridging alginate-like PS. According to DLVO theory, higher ionic concentrations, regardless of cation charge, would reduce the double layer and improve aggregation, supporting the behavior observed in this study.

Nevertheless, DLVO has been associated with batch studies, whereas DCB theory has been shown to be dominant in long-term studies such as this (Sobeck and Higgins, 2002). On the other hand, in DCB theory, the presence of monovalent salts has a negative impact on aggregation by ion exchange and competitive ionic binding with potential divalent cationic bridging sites (Sobeck and Higgins, 2002; Cui et al., 2015). This causes a release of divalent cations into the bulk liquid (Cui et al., 2015), a decrease in gellated EPS (although total EPS may increase) and a subsequent decrease in particle size and settling ability (Sobeck and Higgins, 2002; Kara et al., 2008; Cui et al., 2015). This has been noted when the monovalent-to-divalent cation ratio exceeds 2 on an equivalent basis (Higgins and Novak, 1997), significantly lower than the ratio in seawater. DCB theory is therefore unlikely to be useful. On the other hand, ALE content increased significantly as the seawater proportion increased. While the corresponding ratio of Ca^{2+} to other ions remained stable, the overall concentration of Ca^{2+} both in the bulk liquid and in the sludge did increase with increased seawater, indicating that alginate type bonding may also be an active component of the enhanced granulation in the presence of seawater. This is further supported by the presence of Ca^{2+} not only in the precipitate core but throughout the EPS, as detected by SEM-EDX on the biofilm matrix of granule cross-sections (Table A.5 in SI).

4.3. Precipitates inside the granules

Precipitate formation in the cores of granules was a universal phenomenon with increasing metal ions concentration. Overall, the VSS/TSS ratio decreased by a factor of 2.3 such that around half of the weight of the granules was associated with extracellular inorganics with 100% seawater. EDX analysis in this study revealed that both calcium and magnesium phosphates formed in the core area of granule while saturation indices of influent were only saturated for calcium phosphates (e.g. Hydroxyapatite, Whitlockite). The formation of precipitates in granules can occur in two ways: (1) precipitation prior to granulation, thereby acting as nuclei for subsequent cell attachment and granule growth (Wan et al., 2014); or (2) precipitation following granulation due to increasing granule size, producing denitrification in the core and causing an increase in local pH, inducing precipitation (Juang et al., 2010). The presence of both calcium and magnesium phosphates implied precipitates formation in both ways in this study. Calcium phosphates mainly formed prior to granulation due to oversaturation while magnesium phosphates probably formed after the molding of the ALE gel layer. Lin et al. (2012) claimed that the higher affinity for Ca^{2+} but lower affinity for Mg^{2+} of ALE might lead to spatial-temporal gradient of Mg^{2+} concentration which makes the inner zone of granules saturated for struvite-like precipitates. Similar mineral precipitates were also reported in prior research (Ren et al., 2008; Lin et al., 2013a). These precipitates demonstrably increased sludge density, thereby benefitting the settling velocity (Winkler et al., 2013) and enhancing granule strength to maintain long-term operation stability (Lee et al., 2010; Lin et al., 2013a). Despite significantly lower concentrations than equivalent concentrations of Mg^{2+} in seawater, Ca^{2+} was the dominant cation incorporated into the sludge matrix. As the VSS/TSS ratio decreased rapidly over the first ten days and then remained steady in the reactors containing seawater, precipitation appears to have expedited granulation in this study. This is likely to have been a key factor in the more rapid granulation observed in reactors with larger seawater proportions by providing nuclei for biofilm attachment and granule development.

4.4. Polysaccharides (PS) and proteins (PN) in extracellular polymeric substances (EPS)

PS are reported to contribute to the granular structure by forming a backbone (Adav et al., 2008). However, PN can also improve the integrity of AGS by enhancing surface hydrophobicity and reducing negative charge on the surface (McSwain et al., 2005). Only a minor decrease in PN was observed with salinity in this study. Conversely, a decrease in PS through aerobic granulation and with salinity was observed in this as well as other studies (McSwain et al., 2005; Schmidt and Ahring, 1994; Laspidou and Rittmann, 2002). Such changes can be attributed to variations in the composition of the extracellular PS produced, microbial species present, and types of limiting substrate (Mancuso Nichols et al., 2004). Despite an overall reduction in PS, a clear increase in alginate-like compounds was observed and is discussed further.

Alginate-like exopolysaccharides as a trigger for granulation?

Alginate is a common polysaccharide produced in aerobic microbial cultures including AGS and has been suggested as a dominant structural EPS compound for aerobic granulation (Lin et al., 2013b). The alginate-promoting granulation process is attributed to the ability of alginate to form a gel with Ca^{2+} and to wrap with protein (Lin et al., 2010). The greater GG block composition of ALE allows the formation of a particular chemical structure containing diamond-shaped holes whose dimensions are ideal for crosslinking with divalent cations, especially Ca^{2+}, which forms alginate gels (Christensen et al., 1990).

Such interactions may have occurred around the core precipitates following the first layer of microbial attachment to trigger granulation. ALE potentially entraps protein after the pre-granules form (precipitates wrapped with gel) (Lin et al., 2010), maintaining the granule's high PN/PS ratio.

4.5. Proposed dynamic mechanism of rapid granulation

Based on the results from the granulation batch test, the rapid granulation in R_B can be understood as consisting of three phases (Fig. 6). In the first, the mean diameter of the sludge rapidly increased to about 200 μm after six days of operation but its density remained relatively constant around 1.01 g/ml. Similar density values were reported in early formation granules on phosphate rich brackish wastewater by Mackey et al. (2016) and denser than phosphate removing granules treating non-saline wastewater by Bassin et al. (2012). During the first phase, the adsorption of sodium, and to a much lesser degree Ca^{2+} and Mg^{2+}, onto the surface of the sludge was observed (Fig. A.5 in SI). This adsorption is most likely responsible for the significant neutralization of the zeta potential observed, which facilitated aggregation. In the second phase, the mean diameter of the sludge decreased by only 10% but its density increased from 1.01 to 1.08 g/mL. This phenomenon can be ascribed to the spontaneous precipitation of mineral phosphates or the accumulation of these ions in the sludge matrix due to the increase in ALE content (Fig.A.5 in SI). Winkler et al. (2013) indicated such densities would require around 5% precipitates content. The third phase is a stable growth period for the aerobic granules during which mean diameter and density gradually increased. The magnesium content in the sludge increased during this phase compared to the second phase.

In summary, the rapid granulation through seawater mixing can be summarized as follows: Firstly, a salinity-induced decrease in electrostatic charge on the surface of cells allows sludge flocculation. Secondly, mineral ions accumulate in aggregates as ALE cross-linkages and inorganic precipitates. The formation of ALE cross-linkages subsequently establishes the network structure of the EPS. This structure then supports precipitation at the core, which constitutes the starting point of aerobic granulation. Lastly, the structure grows stably on the initially-formed granule cores.

4.6. Nitrogen removal in saline condition

The presence of significant TN removal under conditions of complete nitrite accumulation imply that shortcut nitrogen removal behaviour was occurring in the reactors treating seawater-laden wastewater. Partial denitrification is induced by the inhibition of nitrite oxidizing bacteria (NOB) growth by salinity and washout under granulation. Comparably, ammonia oxidizing bacteria (AOB) are less affected (Bassin et al., 2011; Pronk et al., 2014). Nevertheless, restrained NH_4^+-N removal efficiency was still obvious at the initial period when larger proportions of seawater were involved. The decreasing nitrite accumulation from Day 100 in 0% seawater reactor indicated eventual enrichment of NOB in the system. The initially suppressed performance was mainly attributed to washing out of seeding flocs in all reactors and low specific oxygen utilization rate at high salt concentrations (Wang et al., 2015). Gradually improving TN removal performance through the study in all reactors revealed that denitrifiers had the ability to acclimate to rising salinity (Glass and Silverstein, 1999, Park et al., 2001). Gradually increasing MLVSS concentrations throughout the study may have also contributed to improving TN removal but could not explain higher TN removal observed in seawater dominated reactors. One reason may be the reduction in oxygen transfer efficiency under high salinity promoting more organisms in the granule core to operate with anoxic metabolism. Park et al. (2001) claimed that direct acclimation was more efficient than stepwise acclimation when converting freshwater denitrification systems to marine systems. This may also explain the better TN removal performance in reactors treating saline water after acclimation than freshwater.

5. Conclusion

This study has successfully developed AGS using various proportions of seawater mixed with synthetic municipal sewage. The addition of seawater significantly accelerated the granulation process in comparison with freshwater and resulted in a stronger granular structure due to the abundance of metal ions and the increased production of gel-forming ALE. The process of rapid granulation with seawater is summarized as follows: 1) electrostatic repulsion on cell surfaces is neutralized, enhancing the aggregation of biomass; 2) metal ions form ALE cross-linkages and precipitates to initiate the aerobic granulation process; 3) the inorganic core of the granules provides a medium for cell adhesion and aggregation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.03.002.

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