Halophilic and nonhalophilic aerobic granular sludge formation in hypersaline synthetic oilfield produced water

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Abstract

This study investigated the ability of two different bacterial cultures to form aerobic granular sludge (AGS) in hypersaline synthetic oilfield produced water. The formation of AGS was explored in both halophilic and mixed cultures through measurement of extracellular polymeric substance (EPS) production and composition, accumulation of selected divalent cations, and AGS size and integrity. EPS production was higher in the halophilic culture than the mixed culture as salt content increased. In addition, alginate content in the halophilic culture was consistently higher as well. Both cultures exhibited the same behaver of cation accumulation, with concentrations of Ca^{+2} and Mg^{+2} in extracted EPS decreasing at higher salt content. Finally, image analysis showed that the average granule size of the mixed culture was higher for salt contents less than 4% (w/v). However, the granules were larger and more stable in the halophilic culture as salinity increased further. Overall, seeding a reactor with halophilic bacteria produced better AGS stability at salt concentrations greater than 4%.

Introduction

Oilfield produced water (PW) is a saline aqueous solution that is a byproduct of oil production and contains a range of organic and inorganic compounds. It is considered the highest volume waste generated from the oil and gas industry (Pendashteh et al. 2012). Since PW contains salt concentrations higher than 35 grams per liter, it can be classified as a hypersaline wastewater (Sharghi and Bonakdarpour 2013).

The composition of PW is highly variable, and depends substantially on the characteristics of the surrounding geological formation (Li 2013). Magnesium, calcium, sodium, potassium and chloride are all typically present in concentrations up to hundreds of grams per liter (Sharghi et al. 2013). Other abundant inorganic components can be present, including carbonate, bicarbonate, sulfate and some metallic elements, such as strontium and barium (Li 2013). Additionally, PW contains hydrocarbon compounds from solubilized crude oil and production chemicals such as n-alkanes, poly cyclic aromatic hydrocarbons (PAHs), phenolic compounds, and benzene derivatives. The wide range of constituents present and the high salt content make handling and treatment of PW a major challenge and limit the options for safe reuse or disposal.

Biological treatment of PW to remove organic constituents is a potentially cost-effective process that can increase options for reuse or further treatment. The primary limitation to biological treatment is the high salinity, which severely affects microbial metabolism and can cause cell lysis (Pendashteh et al. 2012). Additionally, the presence of salts can negatively influence the structure and settling properties of the sludge biomass (Ramos et al. 2015). High concentrations of monovalent cations, such as Na^+, may weaken the extracellular polymeric substance (EPS) matrix and reduce the biomass settling properties. Some studies have reported, however, that divalent cations can bind to negatively charged sites on extracellular biopolymers and act as bridges to increase the stability of the microbial matrix (Higgins and Novak 1997, Kara et al. 2008).

Aerobic granular sludge (AGS) has received significant attention in the last decade. In addition to other benefits, the formation of granules improves the ability of sequencing batch reactors (SBRs) to withstand shocks due to changes in wastewater content (Ramos et al. 2015).

Combining this technology with halophilic microorganisms that can thrive in hypersaline wastewater could lead to a better treatment process for produced water. The objective of this study is to investigate the potential for formation of aerobic granules under hypersaline conditions in SBR reactors using both a mixed culture of aerobic biomass and a halophilic bacteria culture.

Materials and Methods

Experimental set-up

Two 3.0 L bench scale SBR reactors were utilized in the current study. All reactor characteristics, including cycle time details, are shown in Table 1. The first reactor was inoculated with a mixed culture of activated sludge collected from the aeration tank of a municipal wastewater treatment plant in Lawrence, KS. The second reactor was inoculated with a pure culture of *Sporosarcina halophila* (ATCC 43097). *S. halophila* is a gram positive, obligate aerobe, mildly halophilic bacterium. It grows optimally in a range of 2–5% NaCl and at pH 7.8. It is slightly mesophilic, with an optimum temperature of 30°C, but can grow from 15-37˚C (Castillo-Carvajal et al. 2014, Claus et al. 1983).

Synthetic produced water preparation

A synthetic produced water was used for these experiments (Table 2). This PW was based on ion compositions reported in the existing literature, but modified to include essential nutrients for microbial growth (Pendashteh et al. 2012, Sharghi and Bonakdarpour 2013, Sharghi et al. 2013). Sodium acetate was the sole carbon source. The C/N/P ratio of the synthetic PW was adjusted to approximately 100/10/1 by adding NH_4Cl and KH_2PO_4.

Table 1: SBR characteristics and cycle time details

Parameter	Value
Diameter (cm)	6.5
Height (cm)	88.5
Volume (L)	3
Organic Daily Loading Rate (kg-COD)/m^3-d	2.6
VER (%)	50
Cycle time (h)	6
Anaerobic Feeding (min.)	30
Aeration (min.)	322
Settling (min.)	3
Decant (min.)	5
Settling Velocity (m/h)	9
Superficial Upflow Gas Velocity (cm/sec)	2.2

Analytical methods

Mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) concentrations were measured twice a week according to Standard Methods (APHA 2005). A stereomaster microscope (Fisher Scientific) was used to capture granule images twice a week during the experiment. The average granule diameter and aspect ratio were analyzed using Fiji image processing software. EPS extraction was carried out using the heat method described by Li and Yang (2007). Total carbohydrate and alginate contents were determined by a phenol-sulfuric acid assay according to Dubois et al. (1956) with D-glucose and sodium alginate used as standards, respectively. Protein content was determined by the Lowry assay with bovine serum albumin used as the standard (Lowry et al. 1951).

Table 2: Synthetic oilfield produced water composition

Compound	Concentration (mM)	Compound	Concentration (µM)
NaCl	0-1283	FeCl3. $6H_2O$	11.1
CaCl2 .2H2O	0.41	H_3BO_3	4.85
KCl	26.8	$CuSO_4.5H_2O$	0.24
MgCl2 .6H2O	0.25	KI	0.36
$NaHCO_3$	9.52	$MnCl_2.4H_2O$	1.21
NH_4Cl	16.1	$Na_2MoO_4.2H_2O$	0.50
KH_2PO_4	0.73	$ZnSO_4.7H_2O$	0.83
Mg SO_4	4.15	$CoCl_2.6H_2O$	1.26
NaCH3COO	39.0		

Results and discussions

Both reactors were fed synthetic PW with a total dissolved solids concentration of 7500 mg per liter until the average granule diameter became constant. Fine bio-aggregated particles started to appear after 14 days of cultivation time, reaching a steady average diameter on the 37th day. At that point, salt (as NaCl) was increased stepwise over time from 1% to 7.5% (w/v) or 10 to 75 g NaCl L^{-1}.

The concentration of MLVSS in SBR_1, which was inoculated with the mixed culture, was 927 mg/ L, compared to 275 mg/ L in the halophile inoculated reactor (SBR_2), at the beginning of the cultivation time. As the study period went on (and salinity increased), the biomass concentrations in SBR_2 eventually exceeded those in SBR_1, and remained consistently higher. Above 5% salinity, MLVSS concentrations in SBR_2 remained between 10,000 and 11,000 mg/l, with MLVSS/MLSS ratios around 0.82. In contrast, the biomass content in SBR_1 at these conditions declined to about 5700 mg/ L, with MLVSS/MLSS ratios around 0.80. Both reactors formed granules in the saline PW, but these granules had a different shape and external appearance, as discussed in more detail below.

Role of EPS content and composition on AGS formation

The AGS granulation process is affected by EPS production and composition (McSwain et al. 2005). In addition, the physicochemical properties of AGS, such as structure integrity, adsorption ability and dewaterability are all associated with EPS production (Li et al. 2017). In this study, it is hypothesized that the total extracted EPS consists of proteins (PN) and carbohydrates (PS) as the major components. The EPS content for both the halophilic and mixed cultures, normalized to total MLVSS, are depicted in Figure 1. The mixed culture exhibited higher EPS production at low salt content, which may be a response to salt stress. As salt concentration increased to 3.5%, the average EPS content increased in both cultures, although the increase was much larger in the mixed culture. Increased salinity can stimulate the biomass to produce more EPS to cope with the higher external osmotic pressure as observed in a previous study by Corsino et al. (2017). As the salt increased above 3.5 % (w/v), the EPS content declined significantly in the mixed culture, while it decreased only slightly in the halophilic culture and then remained constant through 7.5% salinity. Overall, as the salt content increased, EPS production was more stable in the halophilic reactor (SBR_2).

To find the effect of hypersaline PW on the distribution of protein and carbohydrate in the total EPS, PN/PS ratios were calculated for both reactors (Figure 2). The results indicated that the total EPS was predominantly composed of PN throughout the experiment. Maximum PN/PS ratios were achieved when the salt content was 4 and 5 % for the mixed and halophilic cultures, respectively. No consistent difference in PN/PS ratio was observed between the two cultures. Since alginate-like exopolysaccharides (ALE) are known to be involved in AGS structure and support granule flexibility against crushing (Lin et al. 2013, Meng and Liu 2017), EPS was also tested for ALE content at specific salt levels (4, 6, and 7.5) %. The results, shown in Figure 3, indicate that halophilic bacteria produce higher amounts of ALE at all three salt concentrations, with the difference increasing at higher salt contents.

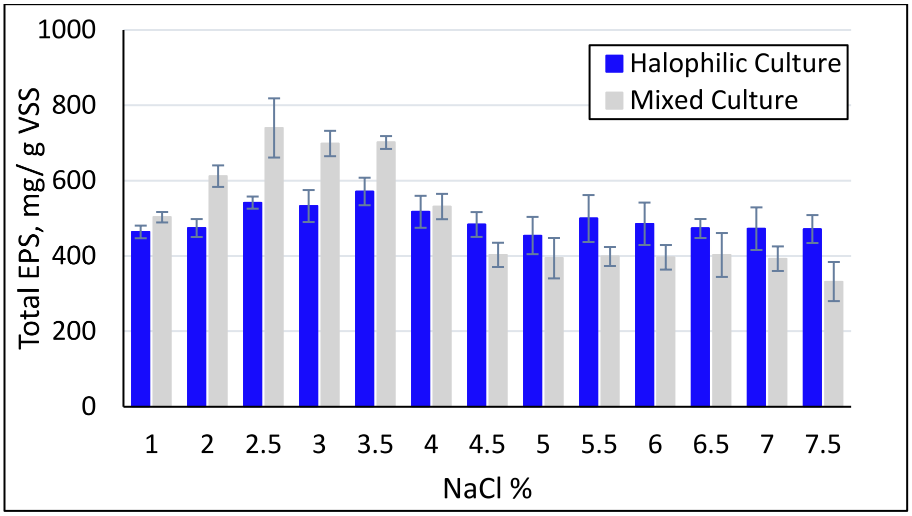

Figure 1: Total EPS production in halophilic and mixed cultures.

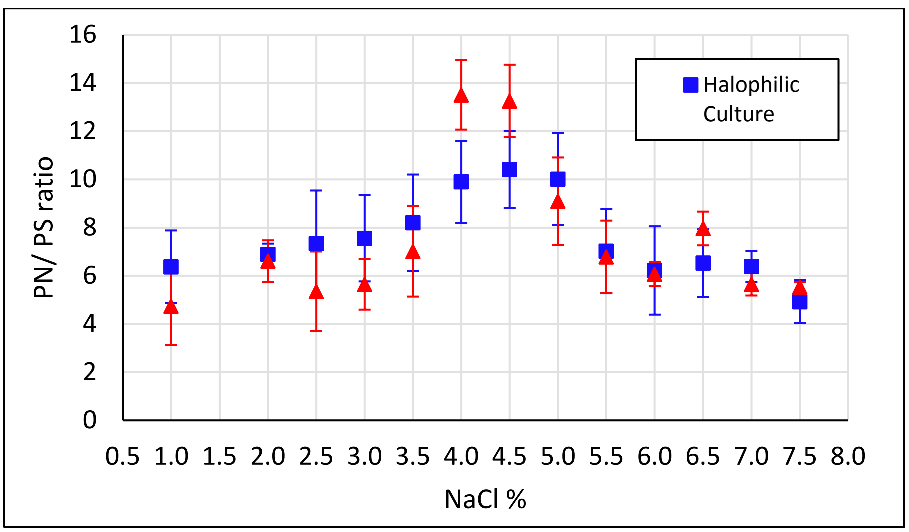

Figure 2: PN/PS ratio in halophilic and mixed cultures.

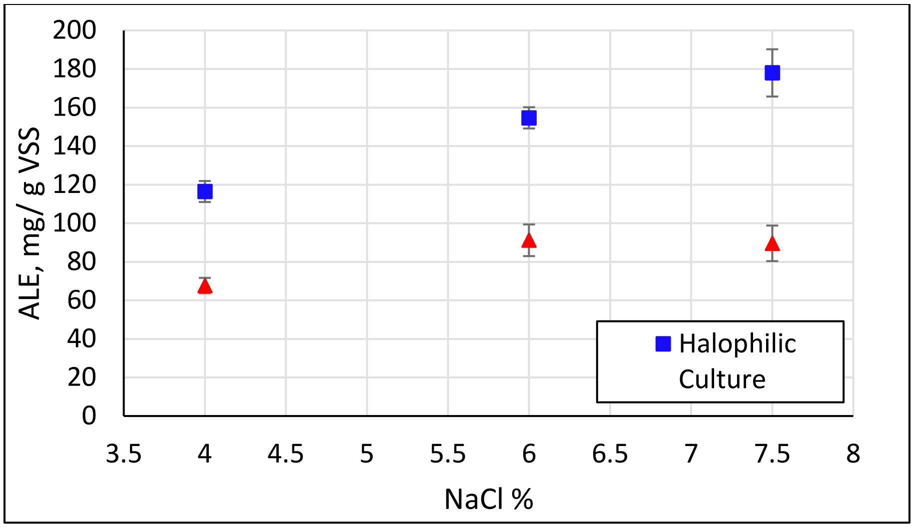

Figure 3: Alginate-like polysaccharides at different salt concentrations.

Role of divalent cations in AGS granulation

It has been reported that some divalent cations can play an important role in bioflocculation through bridging negatively charged sites on bio cells surfaces to biopolymers or by binding biopolymers with each other (Kara et al. 2008). In addition, the presence of divalent and trivalent cations can induce double layer compression which leads to improved granulation of AGS (Higgins and Novak 1997). To investigate the role of the major divalent cations in formation of AGS, Ca^{+2} and Mg^{+2} concentrations in extracted EPS were monitored. Figures 4 and 5 show the Ca^{+2} and Mg^{+2} concentrations in both halophilic and mixed cultures, respectively. It is clear that the concentrations of both cations decreased as the salt content increased in both cultures. This reduction is likely due to exchange of divalent cations for Na^{+} as the sodium concentration increased in the bulk solution. Na^{+}, which has a small ionic size and a large hydration layer, can cause a deteriorating effect on AGS structure (Kara et al. 2008). These decreased divalent cation concentrations could lead to reduced bridging within the EPS, eventually weakening the AGS structure (Higgins and Novak 1997, Li et al. 2017).

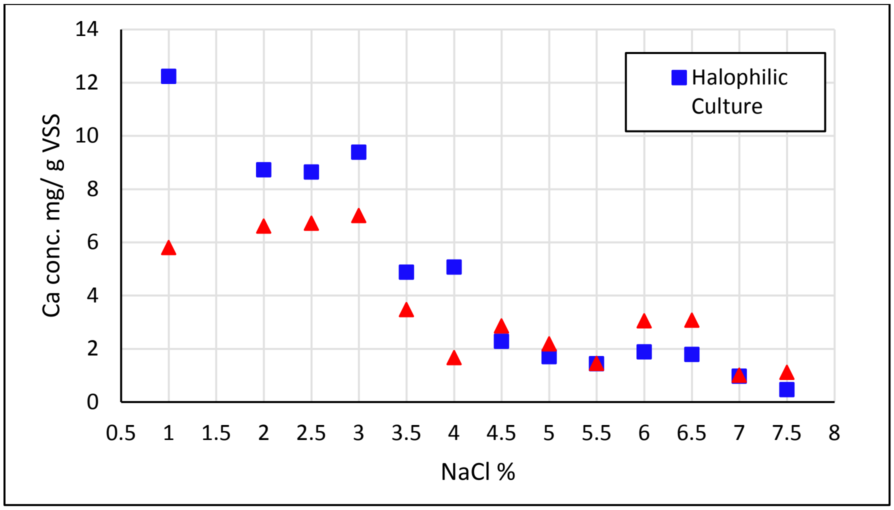

Figure 4: Calcium content in EPS extracted from halophilic and mixed cultures.

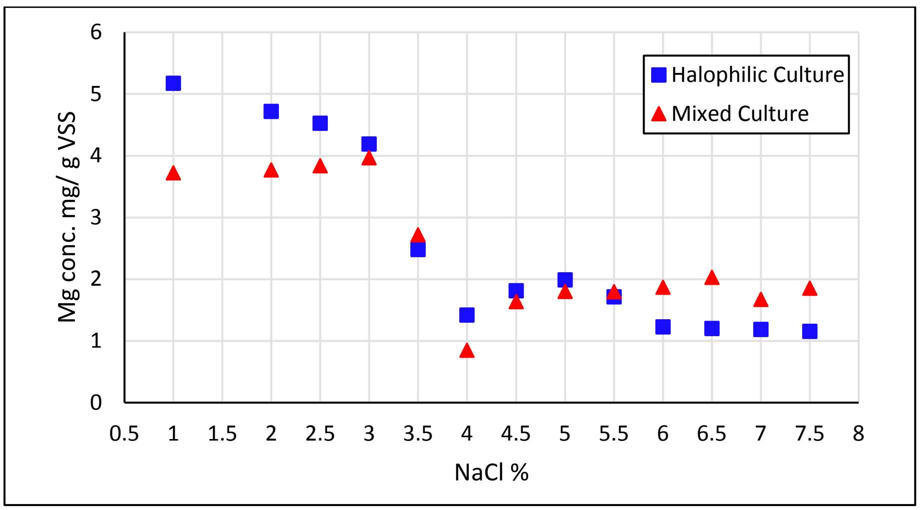

Figure 5: Magnesium content in EPS extracted from halophilic and mixed cultures.

Effect of salinity on the size and integrity of AGS

Image analysis showed that the average granule diameter in mixed culture was higher during the initial cultivation compared to the halophilic culture (Figure 6). As the salt content increased above 3.5%, the average granule diameter of the mixed culture dropped from 1.1 ± 0.23 to 0.57 ± 0.5 mm. This reduction in granule size indicates that the AGS of the mixed culture had started to deteriorate. In contrast, the average granule size in the halophilic culture increased until 4% salt concentration, and then decreased only slightly as salinity increased. The average size was 0.95 ± 0.05 mm. The matured AGS of the halophilic culture (Figure 7 (b)) had an irregular shape, light yellow appearance, and the surface of the granules was not as smooth as the mixed culture granules. The average aspect ratio was 0.67. In contrast, the matured AGS of the mixed culture (Figure 7 (e)) had a regular shape, a dark yellow appearance, and a solid and smooth surface. The aspect ratio for these granules averaged 0.8. It is clear that, while both reactors could establish granules at lower salinities, granule size and integrity in the mixed culture reactor was much more heavily affected by salt concentrations above 4%. On the other hand, AGS in the halophilic reactor showed better performance in this harsh environment. According to image analysis of AGS for both cultures, EPS decline was synchronous with the reduction in average granule size (Figure 6). In addition, by comparing Figure 6 to Figure 1, we conclude that the highest granule sizes corresponded with the highest EPS content in both reactors, which indicates that EPS plays a crucial role in AGS formation.

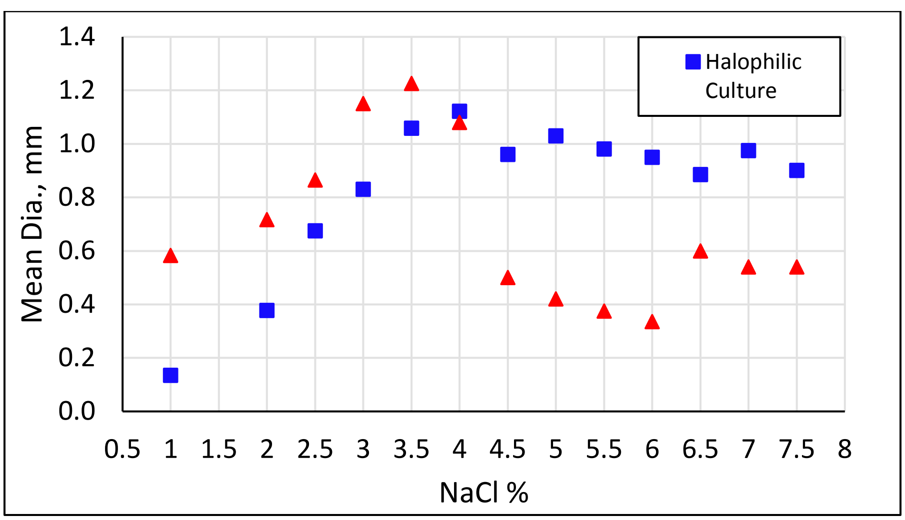

Figure 6: Average granules size for halophilic and mixed cultures.

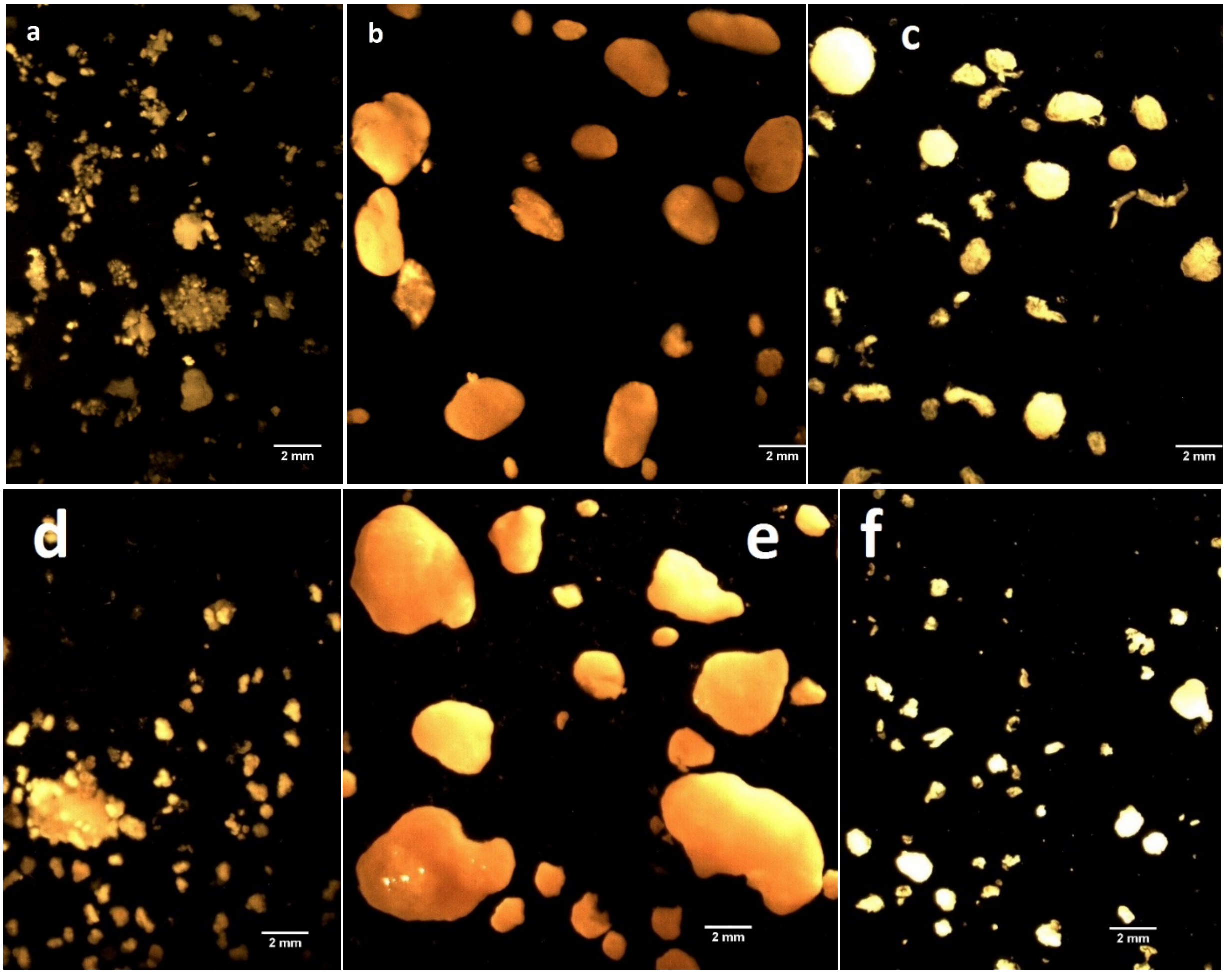

Figure 7: Granular sludge: (a) formation of halophilic AGS at 1 % NaCl (b) matured halophilic AGS at 3.5 % NaCl (c) halophilic AGS at 7.5 % NaCl (d) formation of mixed AGS at 1 % NaCl (d) matured mixed AGS at 3.5 % NaCl; (d) mixed AGS at 7.5 % NaCl.

Conclusions

Both the halophilic and mixed cultures have shown a good ability to produce granules and EPS in salt content less than 3.5% (w/v). However, halophilic microorganisms appear to have an advantage over mixed culture bacteria as the salinity increases, as shown in both granule characteristics and EPS formation. The average size of halophilic AGS was consistently higher when salt content increased above 4% (w/v). In addition, EPS content also remained higher in the halophilic reactor at high salinity. The concentration of divalent cations (Ca^{+2} and Mg^{+2}) in the EPS of both reactors decreased as the dissolved Na^{+} concentration in the reactor increased.

However, only the mixed culture reactor showed a decline in AGS integrity, so we cannot establish a direct link between this behavior and granule stability. The halophilic reactor did exhibit higher ALE production, which may be related to the better AGS integrity observed in this reactor at higher salinities.

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References

APHA (2005) Standard Methods for the Examination of Water and Wastewater American Public Health Association, Washington, DC.

Castillo-Carvajal, L.C., Sanz-Martín, J.L. and Barragán-Huerta, B.E. (2014) Biodegradation of organic pollutants in saline wastewater by halophilic microorganisms: a review. Environmental Science and Pollution Research 21(16), 9578–9588.

Claus, D., Fahmy, F., Rolf, H. and Tosunoglu, N. (1983) Sporosarcina halophila sp. nov., an obligate, slightly halophilic bacterium from salt marsh soils. Syst Appl Microbiol 4, 496-506.

Corsino, S.F., Capodici, M., Torregrossa, M. and Viviani, G. (2017) Physical properties and Extracellular Polymeric Substances pattern of aerobic granular sludge treating hypersaline wastewater Bioresource Technology 229 152–159.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry 28, 350-356.

Higgins, M. and Novak, J. (1997) The Effect of Cations on the Settling and Dewatering of Activated Sludges: Laboratory Results. Water Environment Research 69(2), 215-224.

Kara, F., Gurakan, G. and Sanin, F. (2008) Monovalent cations and their influence on activated sludge floc chemistry, structure, and physical characteristics. Biotechnology and Bioengineering 100(2), 231-239.

Li, H. (2013) Produced Water Quality Characterization and Prediction for Wattenberg Field, Colorado State University.

Li, X., Luo, J., Guo, G., Mackey, H.R., Hao, T. and Chen, G. (2017) Seawater-based wastewater accelerates development of aerobic granular sludge: A laboratory proof-of-concept. Water Research 115 210-219.

Li, X.Y. and Yang, S.F. (2007) Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. Water Research 41, 1022-1030.

Lin, Y.M., Sharma, P.K. and Loosdrecht, M.C.M.v. (2013) The chemical and mechanical differences between alginate-like exopolysaccharides isolated from aerobic flocculent sludge and aerobic granular sludge. Water Research 47, 57-65.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurements using Folin phenol reagent. J. Biol. Chem 193, 265-275.

McSwain, B.S., Irvine, R.L., Hausner, M. and Wilderer, P.A. (2005) Composition and Distribution of Extracellular Polymeric Substances in Aerobic Flocs and Granular Sludge. Applied and Environmental Microbiology 71(2), 1051–1057.

Meng, S. and Liu, Y. (2017) Transparent exopolymer particles (TEP)-associated membrane fouling at different Naþ concentrations. Water Research (111), 52-58.

Pendashteh, A.R., Abdullah, L.C., Fakhru'l-Razi, A., Madaeni, S.S., Abidin, Z.Z. and Biak, D.R.A. (2012) Evaluation of membrane bioreactor for hypersaline oily wastewater treatment. Process Safety and Environmental Protection 90, 45-55.

Ramos, C., Suárez-Ojeda, M.E. and Carrera, J. (2015) Long-term impact of salinity on the performance and microbial population of an aerobic granular reactor treating a high-strength aromatic wastewater. Bioresource Technology 198, 844–851.

Sharghi, E.A. and Bonakdarpour, B. (2013) The study of organic removal efficiency and halophilic bacterial mixed liquor characteristics in a membrane bioreactor treating hypersaline produced water at varying organic loading rates. Bioresource Technology 149 486–495.

Sharghi, E.A., Bonakdarpour, B., Roustazade, P., Amoozegarb, M.A. and Rabbanic, A.R. (2013) The biological treatment of high salinity synthetic oilfield produced water in a submerged membrane bioreactor using a halophilic bacterial consortium. Journal of Chemical Technology & Biotechnology 88(11), 2016-2026.