Electronic Supporting Information

Granulation of autochthonous planktonic bacterial community of seawater for saline wastewater treatment

Total number of pages: 14

Number of Tables: 5

Number of Figures: 8

Table S1. Summary of studies on biological nutrient removal of aerobic granular sludge under saline conditions

Salinity (g/l NaCl)	Inoculum	Reactor volume & cycle time	Wastewater	Granulation time	N and P removal	Reference
11 – 15	Activated sludge	1.5 L SBR; 3 h cycle	Fish canning effluent; COD:410 - 480 mg/l; NH_4^+-N: 45 – 60 mg/l.	Granules formed after 75 days	TN removal: 20 – 45%; Nitrite accumulated; P removal not reported.	16
2 – 10	Activated sludge	5 L SBR; 24 h cycle	Synthetic wastewater; COD: 500 – 3000 mg/l; Glucose as C source.	Granules seen within 20 days	N and P removals not reported.	17
11, 22, 33	Aerobic granules	2.7 L SBR; 3 h cycle.	Synthetic wastewater; Acetate as carbon source.	-	Complete ammonia removal; Nitrite accumulated; P removal inhibited.	18
15, 30, 50	Aerobic granules	Continuous flow reactor	Synthetic wastewater; acetate and propionate as C sources.	-	Ammonia removal: 90%; Nitrite accumulated; P removal not reported.	19
5.5 – 6.0	Activated sludge	60 L pilot-scale SBR	Municipal wastewater; NH_4^+-N: 34 mg/l.	Irregularly shaped granules	Ammonia removal: 98%; TN removal: 86%; Nitrite accumulated; P removal: 50%.	20
10 – 80	Aerobic granules	5.7 L SBR; 6 h cycle;	Synthetic wastewater; glucose as carbon source.	-	Ammonia removal: 21%; Nitrite accumulated; P removal not reported.	21
30, 38, 50, 75	Aerobic granules	3.5 L SBR	Fish canning wastewater diluted with tap water to adjust salinity	Granules became irregular at higher salinities.	Complete nitrification and denitrification; P removal not reported.	14
0 – 30	Activated sludge	1.1 L SBR; 2.4 h cycle.	Synthetic wastewater; glucose and acetate as carbon source; NH_4^+-N: 100 mg/l.	Initial granules formed in 7 days; Rapid granulation at high salinities.	Ammonia removal: 90%; Nitrite accumulated; P removal not reported.	24
2.5 – 15	Activated sludge	2.57 L SBR; 3 h cycle.	Synthetic wastewater; Acetate as carbon source; NH_4^+-N: 3.54 mM.	Mature granules formed after 70 days	Nitrification and denitrification by gradual adaption; P removal not reported.	23
17, 25, 34	No inoculum	3 L SBR; 6 h cycle.	Synthetic wastewater; acetate as carbon source; NH_4^+-N: 30 mg/l.	Granulation in 7 days	Complete ammonia and total nitrogen removal; P removal: >76%.	This study

Table S2. Composition of seawater-based wastewater used for cultivating granular sludge from seawater microbes. *Nutrients added to seawater.

Chemical	*Value**
Sodium acetate*	517 mg/l
Ammonia-N*	30 mg/l
Phosphate-P*	11 mg/l
Calcium	359 mg/l
Magnesium	1189 mg/l
Sulphur	11.5 mg/l
Sodium	7455 mg/l
Potassium	353 mg/l
Strontium	6 mg/l
Trace elements mix*[31]	1 ml/l
Parameter	*Value*
pH	8.1 ± 0.1
Salinity	34 g/l
Turbidity	4.5 ± 0.23 NTU
Colony forming units (CFU)	4×10^3 CFU/ml

Table S3. Summary of biological treatment of seawater-based wastewater at 34 ppt. Values represent mean and SD of measurements obtained from 60 to 100 days of SBR1 operation.

Parameter	Influent	Effluent
Chemical oxygen demand (mg l^{-1})	450±26	BDL
Ammonium-N (mg l^{-1})	31±0.9	BDL
Nitrite-N (mg l^{-1})	BDL	1.3±0.4
Nitrate-N (mg l^{-1})	BDL	2.5±0.6
Phosphorus-P (mg l^{-1})	11±0.5	2.5±0.5
Turbidity (NTU)	4.53±2.3	1.9±0.7
Microbial load (CFUs ml^{-1})	4.0×10^3	$3\text{-}5 \times 10^1$

BDL: Below detection limit

Table S4. Concentration of metal ions in the influent, effluent and granular sludge.

	Mg	Ca	Na	K	Sr	Fe
Influent* (mg L^{-1})	1189 ±21	359 ±31	7455 ±34	353 ±14	6 ±0.5	2 ±0.4
Effluent* (mg L^{-1})	857 ±46	256 ±54	5420±349	277 ±188	3.9 ±0.15	BDL
Biomass# (mg (g dry wt.)$^{-1}$)	153 ±8	153 ±4	113 ± 5	110 ±4	113 ±7	65 ±6

*Values represent average ±SD of minimum of 10 cycles; BDL- <2µg L^{-1}, #average ±SD on day 98.

Table S5. Functional bacteria detected in the granular sludge formed seawater microbes

Microorganisms	Prominent metabolic function
Acinetobacter	Phosphorus, PAO metabolism
Alcanivorax dieselolei	Nitrogen, denitrifier with high salt tolerance (15%)
Aquamicrobium aerolatum	Facultative aerobe
Azospira	Nitrogen, nitrogen fixer
Bacillus cereus	Sulphur, sulphate reducer
Bifidobacterium adolescentis	Nitrogen, ammonium oxidiser
Candidatus Accumulibacter	Phosphorus, PAO metabolism
Clostridium	Stickland reaction
Collinsella aerofacium	Obligate anaerobe
Erythrobacter nanhaisediminis	Nitrogen, aerobic denitrifier
Labzenria aggregata	Nitrogen, phototropic NO_2 reducer
Marinobacter bryozoorum	Nitrogen, denitrifier
Marinobacter hydrocarbonoclasticus	Nitrogen, denitrifier
Methylobacterium adhaesium	Nitrogen, nitrate as energy source
Neptuniibacter caesarienesis	Nitrogen, Strict aerobe with dissimilatory nitrate reduction
Nitratireductor pacificus	Nitrogen, denitrification (NO_3 to NO_2 only)
Nitroreductor	Nitrogen, NO_x reducer
Nitrosomonas marina	Nitrogen, ammonium oxidiser
Nitrospira	Nitrogen, ammonium oxidation via nitrite pathway
Nonlabens tegetincola	Phosphorus, hydrolyse phosphate, P- metabolism
Oceanicola granulosus	Nitrogen, denitrifier with PHA storage
Paracoccus aminovorans	Nitrogen, ammonium oxidizer
Phaeobacter gallaeciensis	Biotic/ abiotic surface colonizers, Fe- siderophores producers
Planctomycete MS30D1	Nitrogen, anammox
Planctomycetes	Nitrogen, Annamox
Polymorphum gilvum	Hydrocarbon degrading saline bacteria
Pseudomonas balearica	Nitrogen, denitrification
Pseudomonas citronellosis	Nitrogen, denitrifier
Pseudoxanthomonas mexicana	Nitrogen, denitrification (from NO_2 to N_2 only)
Rhodocyclales related organisms	Phosphorus, PAO metabolism
Roseibium denhamense	Nitrogen, denitrifier Phototropic bacteriochlorphyll producer with phosphatase activity
Roseibium hamelinense	Nitrogen/ phosphorus, denitrifier with phosphatase activity
Spirochaeta halophile	Nitrogen, ammonium utiliser with high salt tolerance (12%)
Stappia meyerae	Carbon, CO oxidation/ CO_2 fixation, denitrifier
Stenotrophomanas acidaminiphila	Nitrogen, NO_x reducer

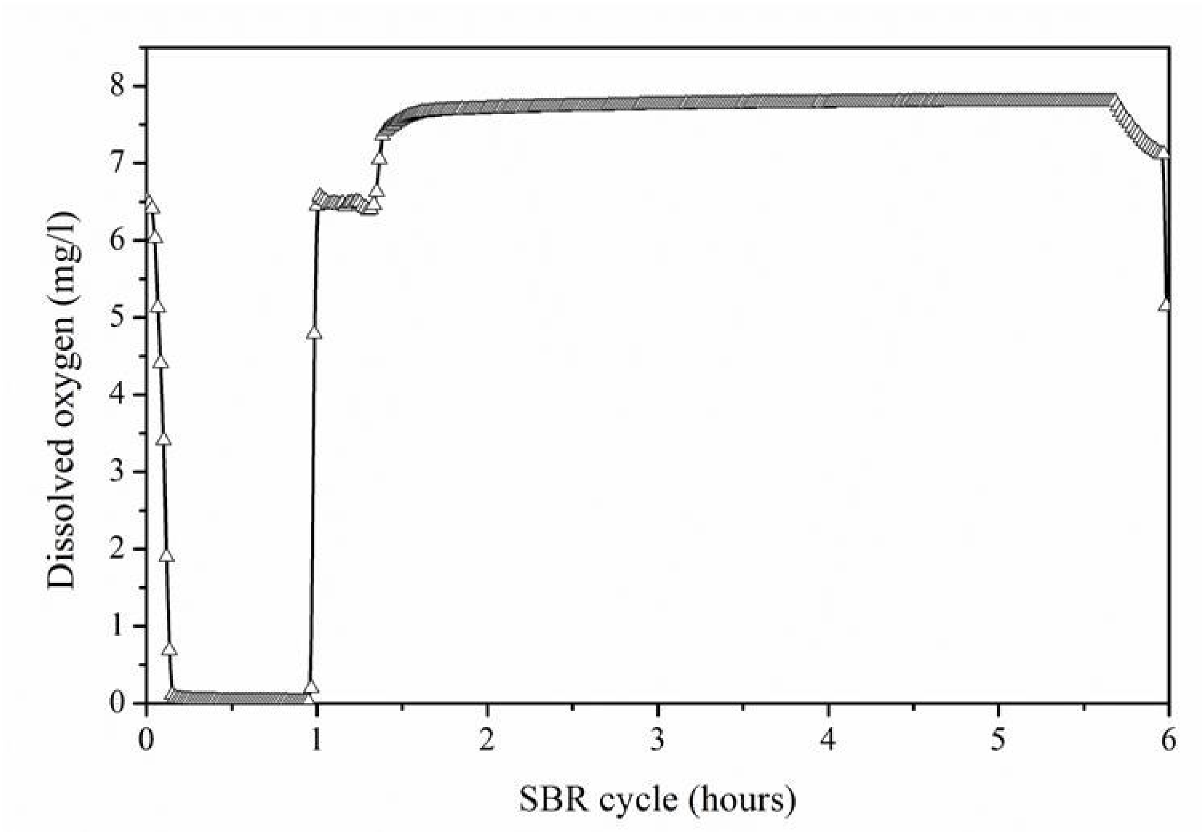

Figure S1. Dissolved oxygen profile of a single SBR cycle indicating anaerobic and aerobic phases.

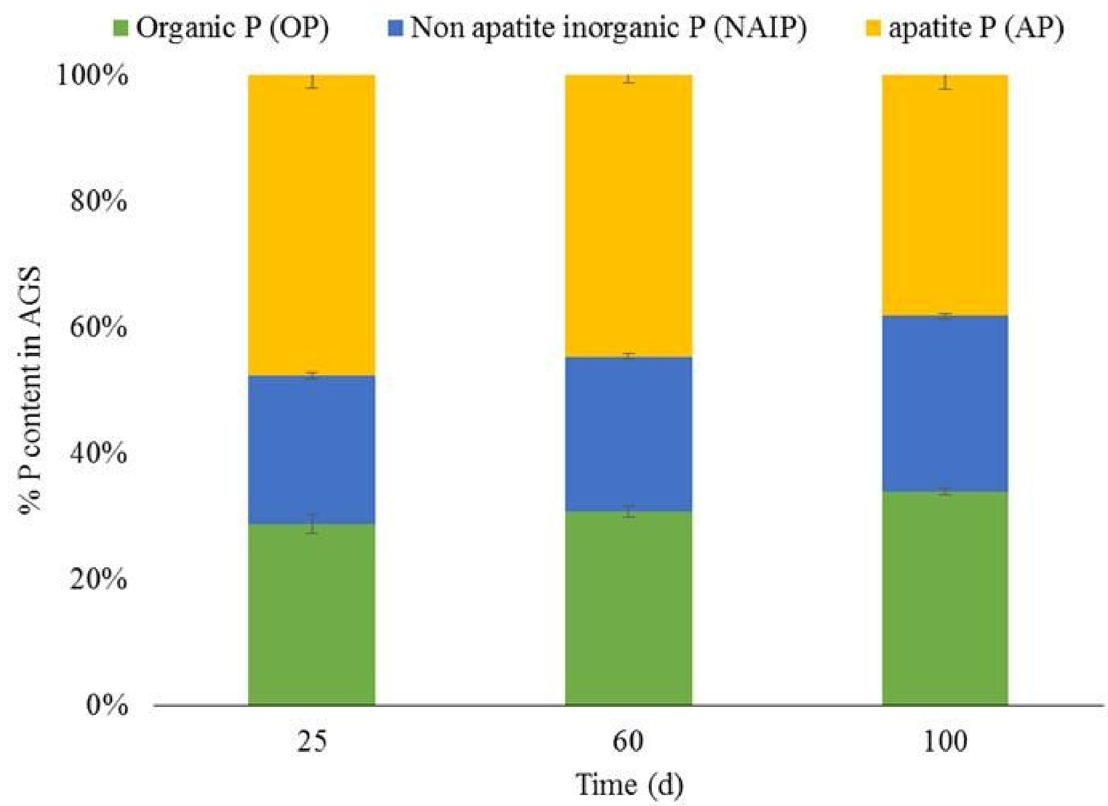

Figure S2. Changes in Phosphorus fractions in the halophilic granular sludge during granulation.

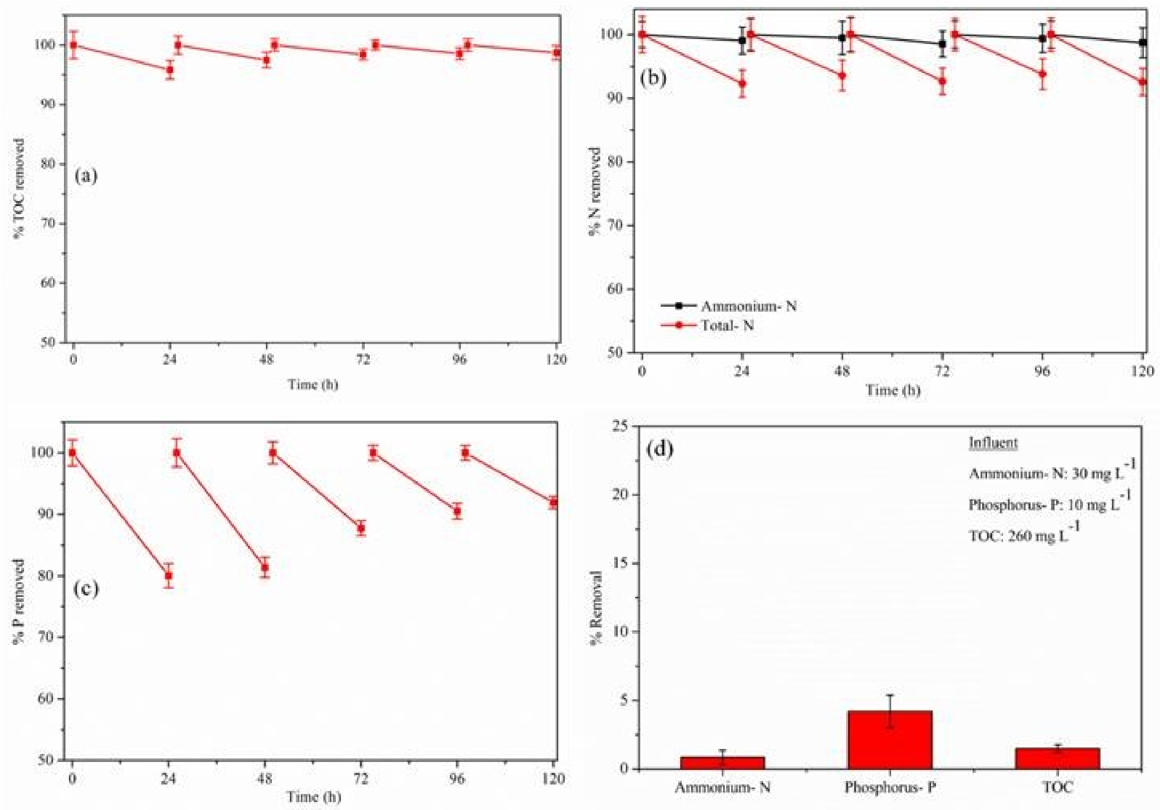

Figure S3. Carbon, nitrogen and phosphorus removal by halophilic aerobic granular sludge in fed-batch experiments at 34 ppt. a – c) Carbon, nitrogen and phosphorus removal by halophilic aerobic granular sludge. d) Carbon, nitrogen and phosphorus removal in the presence of heat-killed aerobic granules. Fed-batch experiment was carried out for 5 batches to determine the contribution of biotic/abiotic mechanisms in nutrient removal under saline conditions.

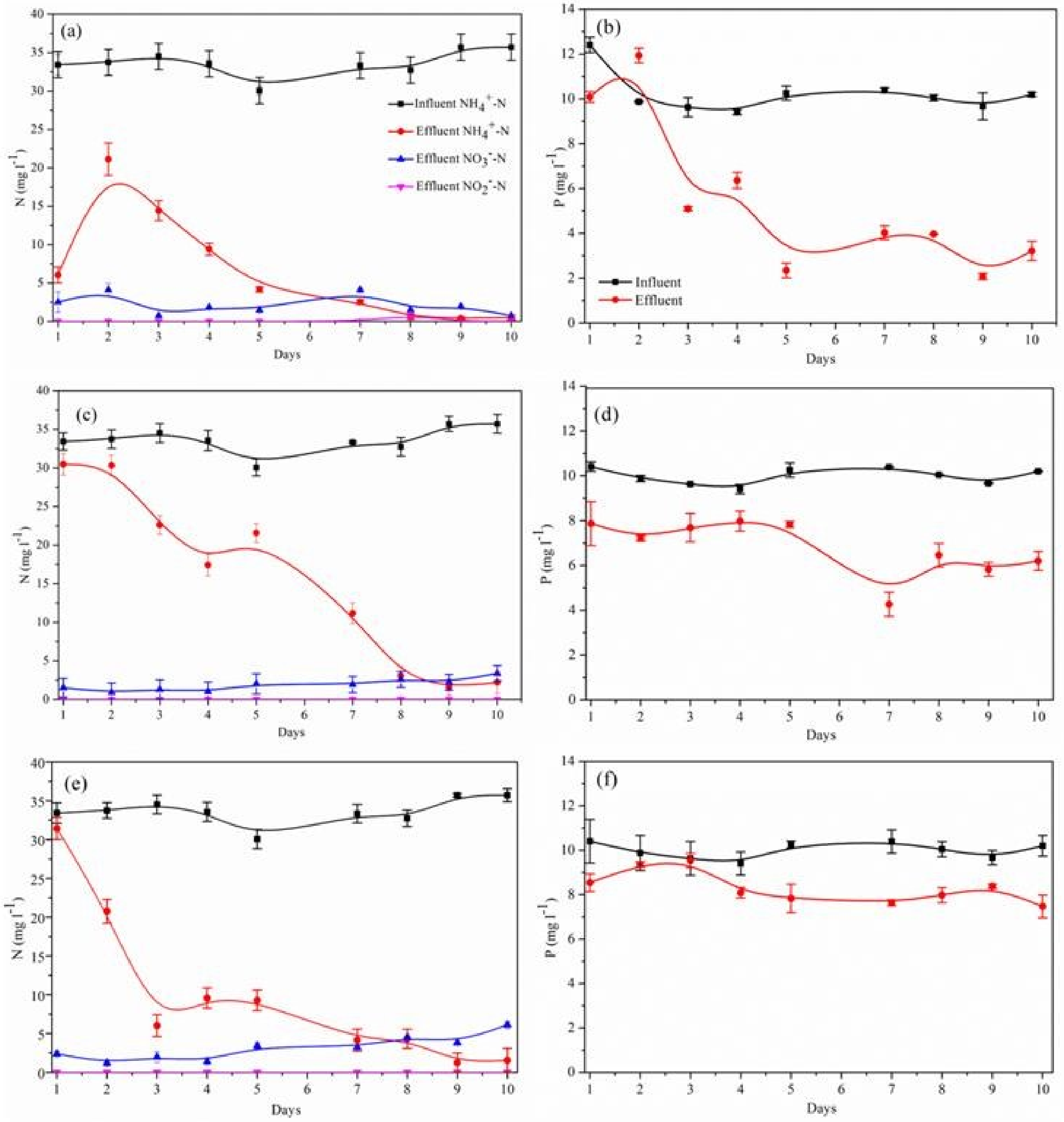

Figure S4. C, N and P removal by aerobic granular sludge at different salinities in batch experiments. a & b) 34 ppt, c & d) 25 ppt, e & f) 17 ppt.

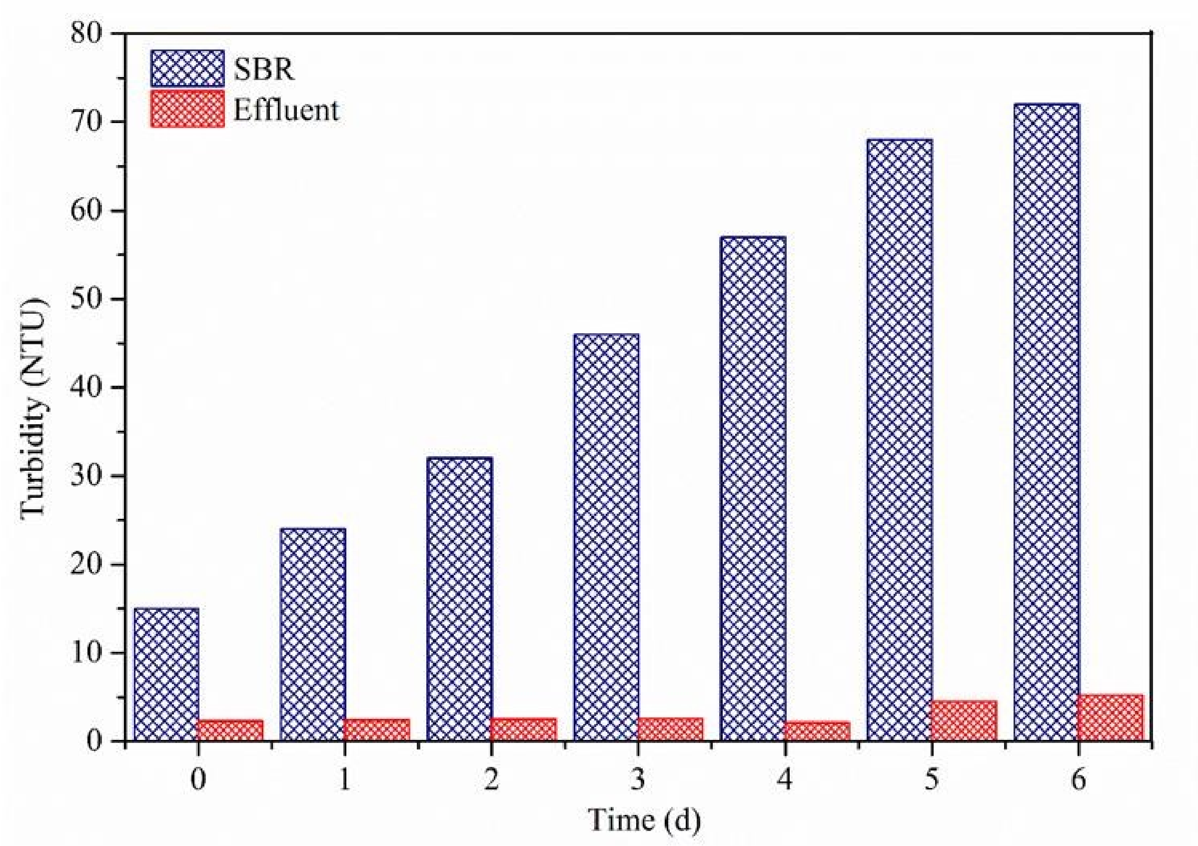

Figure S5. Microbial growth and retention monitored as turbidity during the initial days of SBR operation. Turbidity was seen only during initial days. After a week, growth was mostly in the form of aggregates.

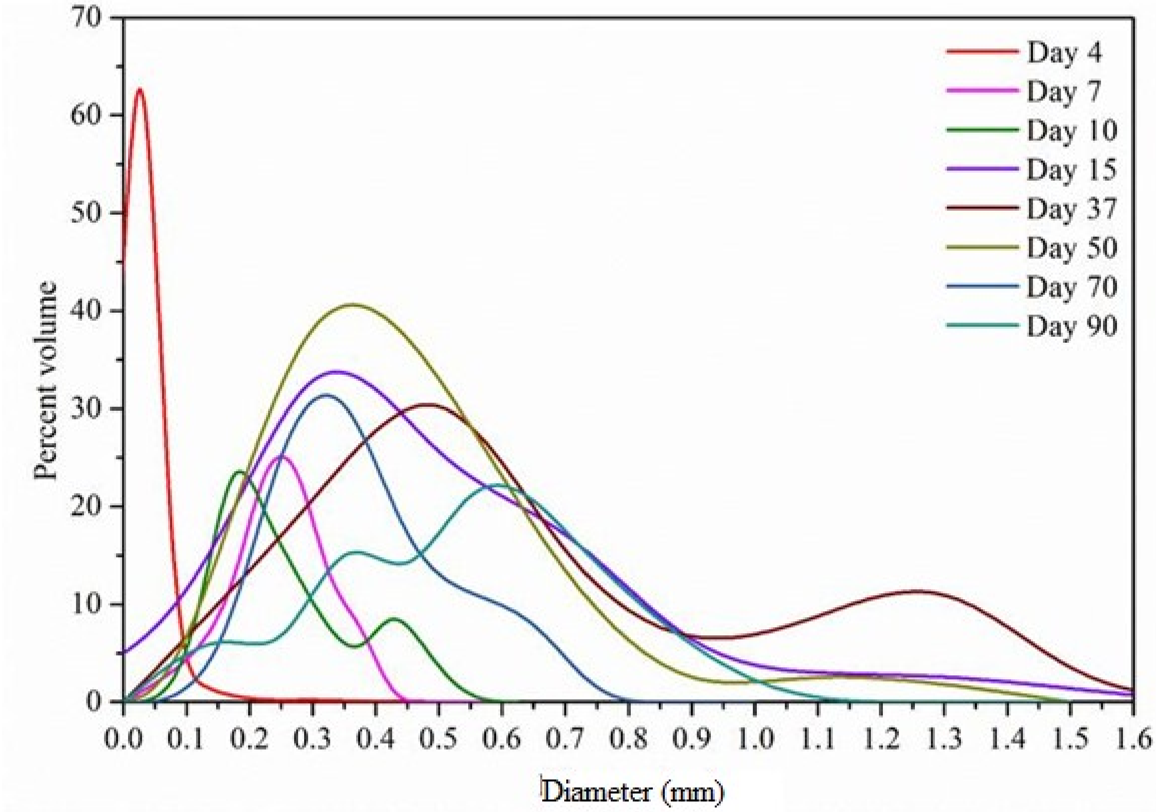

Figure S6. Particle size distribution of granules formed from seawater microbes during 100 days of SBR operation.

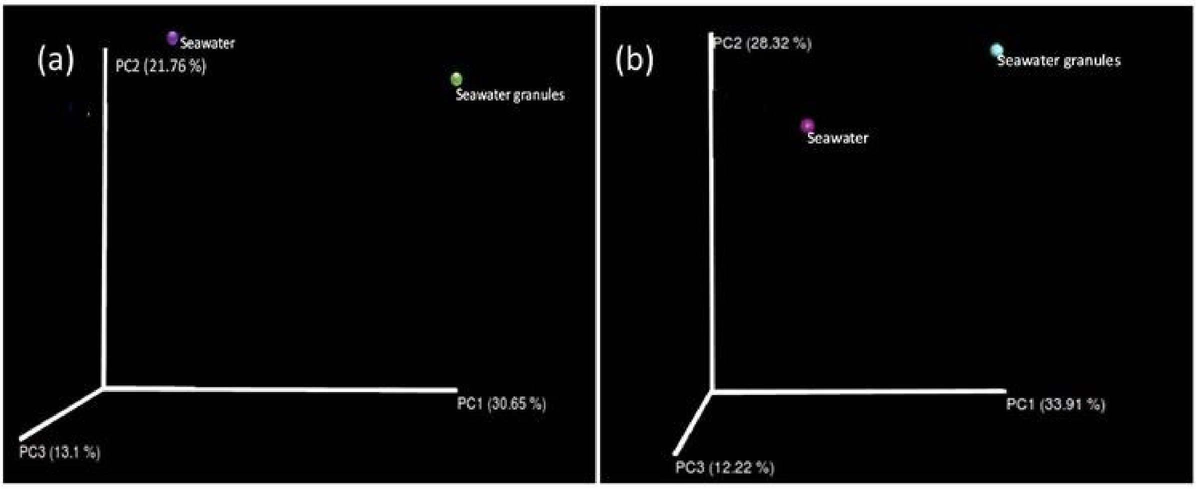

Figure S7. Principal component analysis of bacterial community of seawater and granules based on unweighted (a) and weighted (b) unifrac distance matrix methods. The axes represents the percentage of data explained by each coordinate dimension. The matrix for principal component analysis was calculated at the depth of 140000 sequences per sample.

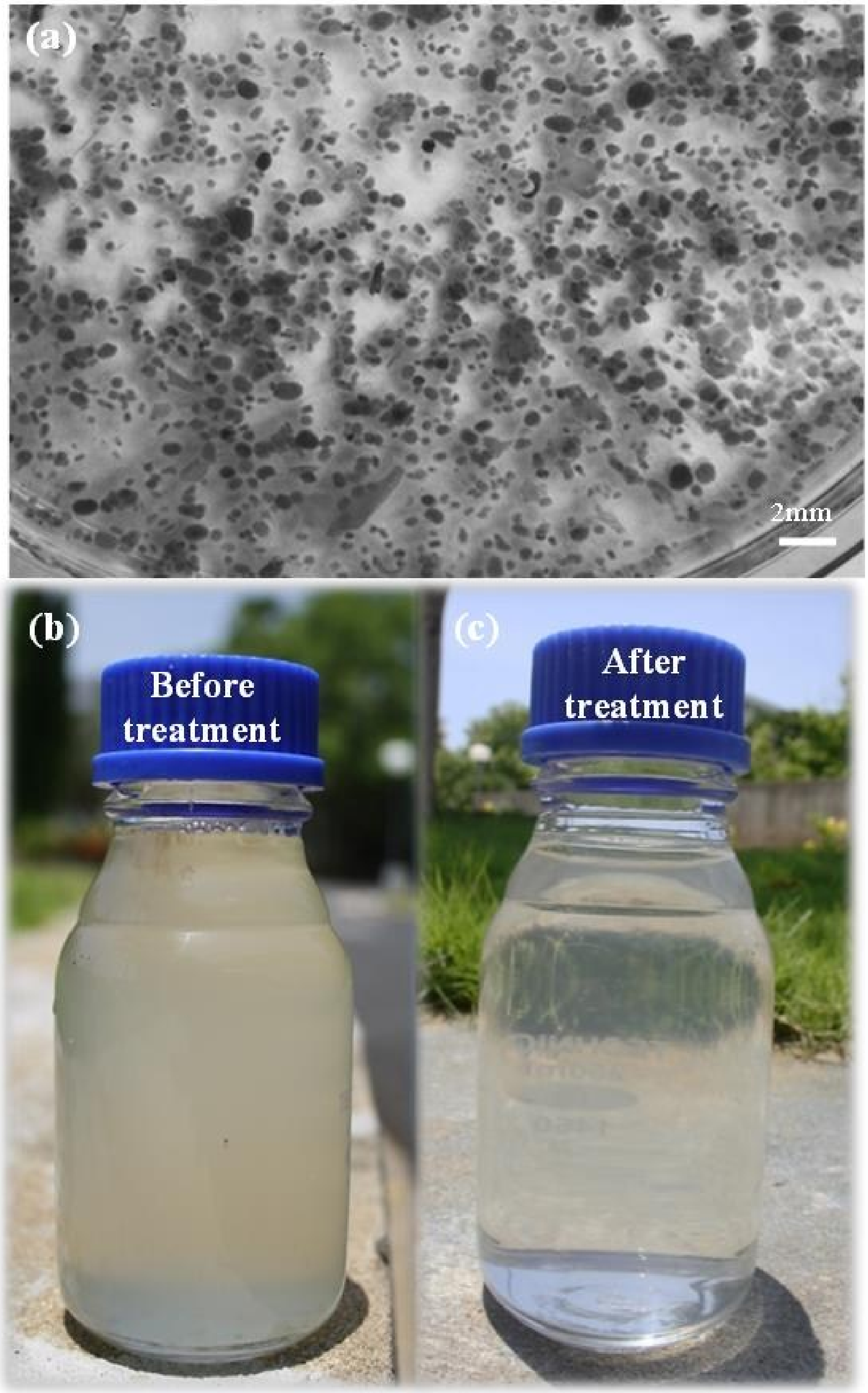

Figure S8. Photograph showing morphology of halophilic AGS (a), untreated (b) and treated (c) seafood processing wastewater.