Supplementary material

for

Disintegration of aerobic granules during prolonged operation

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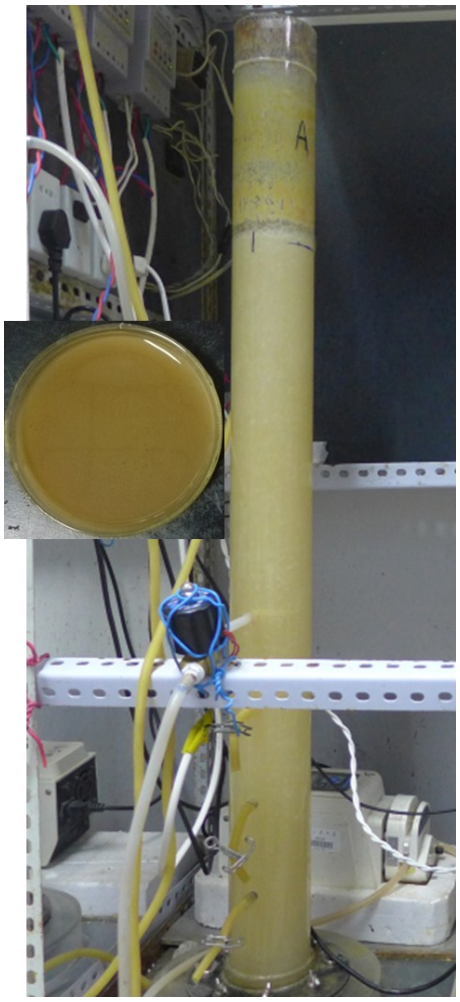

Fig. S1 Sequencing batch reactor and seed sludge.

Table S1 Basic element and trace element of synthetic wastewater

Basic element	Concentration (mg/L)	Trace element	Concentration (mg/L)
$C_6H_{12}O_6$	468.7	$MnCl_2 \cdot 4H_2O$	0.12
CH_3COONa	640.6	H_3BO_3	0.15
NH_4Cl	382.1	$CuSO_4 \cdot 5H_2O$	0.03
$CaCl_2$	166.5	KI	0.03
KH_2PO_4	22.1	$ZnCl_2$	0.12
$MgSO_4 \cdot 7H_2O$	25.3	$CoCl_2 \cdot 6H_2O$	0.06
$FeSO_4 \cdot 7H_2O$	20.3	$Na_2MoO_4 \cdot 2H_2O$	0.15
EDTA	20.4		
$FeCl_3 \cdot 6H_2O$	1.5		

Table S2 Stains used in staining operation

Dyes	Excitation (nm)	Emission (nm)	Targets	Staining time (h)
SYTO 9	488	530	Live cells	1
Propidium iodide	488	630	Dead cells	1
SYPRO orange	488	570	proteins	4
Con A with Alexa Flour 633 conjugates	633	647	Polysaccharides (α-Mannose, α-Glucose)	4

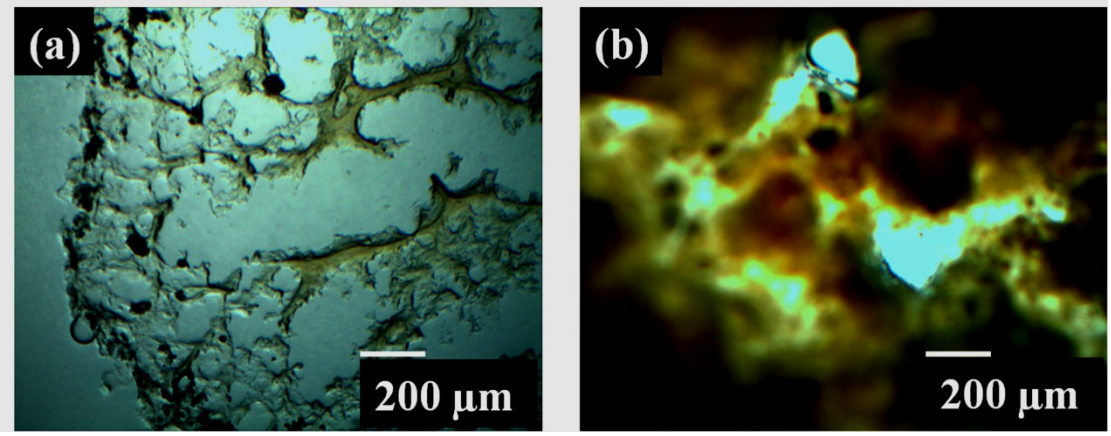

Fig. S2 Graphs of (a) edge section (thickness of 50 μm) and (b) inner core section (thickness of 200 μm) of intact aged granules (IG_A).

Table S3 Percentage of Ca precipitation in different part of intact young granules (IG_Y) and intact aged granules (IG_A) (Part 1: outer zones of cross-section in intact granules; Part 2: middle zones of cross-section in intact granules; Part 3: core of cross-section in intact granules)

Ca (%)	Part 1	Part 2	Part 3
IG_Y	1.09 ± 0.43	6.92 ± 0.56	13.72 ± 2.29
IG_A	6.92 ± 0.56	9.05 ± 0.32	4.19 ± 0.90

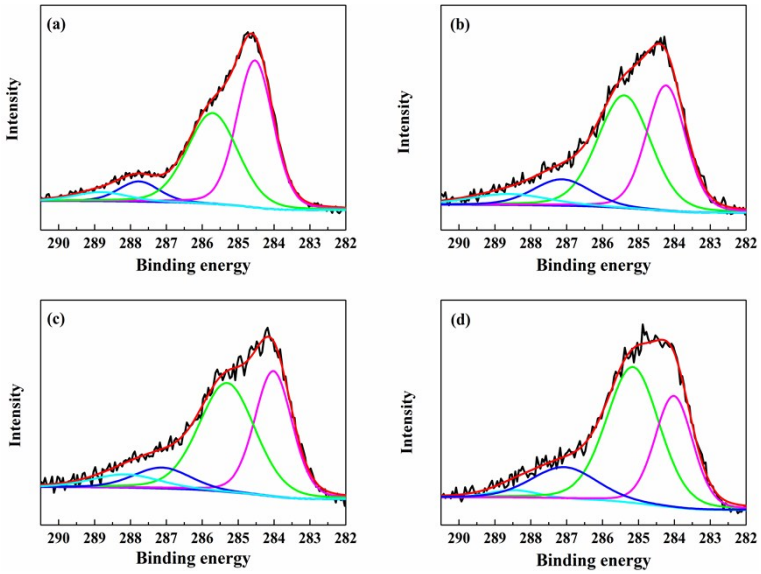

Fig. S3 High-resolution C 1s XPS spectra of EPS from (a) intact young granules (IG_Y), (b) intact aged granules (IG_A), (c) crannied granules (CG) and (d) broken granules (BG).

Table S4 Microbial community diversity and richness of intact young granules (IG_Y), intact aged granules (IG_A), crannied granules (CG) and broken granules (BG)

Samples	Level	OTUs	Chao index	Shannon index	Coverage
IG_Y	97%	1216	1171	6.68	99.91%
IG_A		1292	1035	5.44	99.83%
CG		1301	1132	5.85	99.94%
BG		1289	1129	5.75	99.93%

Chao index: Community richness. A higher number represented more richness.
Shannon index: Community diversity. A higher number represented more diversity.

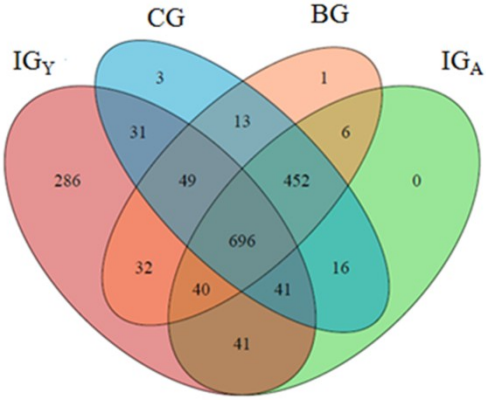

Fig. S4 Venn diagram of intact young granules (IG_Y), intact aged granules (IG_A), crannied granules (CG) and broken granules (BG).

Table S5 Shared OTUs percentage of intact young granules (IG_Y), aged young granules (IG_A), crannied granules (CG) and broken granules (BG).

samples	Shared OTUs percentage (%)
All	13.66
IG_Y, IG_A & CG	19.35
IG_Y, IG_A & BG	19.38
IG_Y, CG &BG	19.57
IG_A, CG & BG	29.57
IG_Y & IG_A	32.67
IG_Y & CG	32.46
IG_Y & BG	32.61
IG_A & CG	46.47
IG_A & BG	46.26
CG & BG	46.72

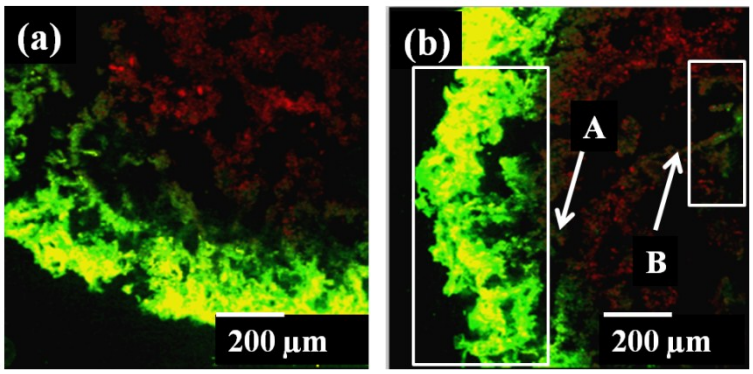

Fig. S5 CLSM image of live/dead cell spatial distribution over section of (a) intact aged granules (IG_A), (b) broken granules (BG) (A: outer side; B: broken surface) (Live cells were stained with green; dead cells were stained with red).