frontiers

Supplementary Material

Table S1 – Main QIA variables considered for PCA

Descriptor group	Parameter	Description	Calculation
Size	D_{eq} (µm)	Aggregates average equivalent diameter of IG (intermediate granules) and LG (large granules)	$Deq = 2F_{Cal}\sqrt{\frac{2}{\pi}}$
	%Nb	Number percentage of aggregates of IG (intermediate granules) and LG (large granules)	$\%\ Nb = \frac{\sum_{i=1}^{Nclass} N_i}{T_{Nb}}$
Morphology	Conv	Aggregates average convexity of IG (intermediate granules) and LG (large granules)	$Conv = \frac{P_{Conv}}{P}$
	Rob	Aggregates average robustness of IG (intermediate granules) and LG (large granules)	$Rob = \frac{2er_{obj}}{\sqrt{A}}$
	Round	Aggregates average roundness of IG (intermediate granules) and LG (large granules)	$Round = \frac{4\pi A}{P_{Conv}{}^2}$

F_{cal} is the calibration factor (µm per pixel); N_i is number of each aggregate belonging to the i size class; T_{Nb} is the total number of aggregates; P is the perimeter; P_{conv} is the convex envelop perimeter; er_{obj} is the number of erosions needed to delete an object; A is the area of the aggregates.

Table S2 – QIA data used for PCA analysis

Days	Deq_IG (µm)	Deq_LG (µm)	Rob_IG	Rob_LG	Conv_IG	Conv_LG	Round_IG	Round_LG	%Nb_IG	%Nb_LG
0	391.50858	3443.72472	0.66758	0.79772	0.92865	0.78851	0.68165	0.80430	63.95583	27.06858
6	517.44135	2601.84354	0.73573	0.79519	0.94389	0.88903	0.77149	0.84087	76.60471	23.39529
13	450.08291	2652.06122	0.75728	0.79470	0.94312	0.90772	0.80047	0.84714	81.05330	18.94670
21	479.23932	2387.95589	0.73332	0.78149	0.92326	0.87421	0.75322	0.82772	91.51091	8.48909
34	419.13176	2588.45131	0.71769	0.77620	0.92827	0.88479	0.74355	0.82639	85.82419	14.17581
44	332.86734	2733.90309	0.69573	0.73388	0.92046	0.81389	0.71186	0.76679	94.74487	5.25513
61	375.54310	2542.87275	0.74043	0.76093	0.93639	0.88969	0.77139	0.81764	85.63309	14.36691
72	548.06740	2284.22503	0.75636	0.77914	0.93582	0.89920	0.79290	0.82695	86.94826	13.05174
86	549.64829	2246.71776	0.75483	0.76758	0.93300	0.89468	0.79004	0.83021	85.74209	14.25792
96	475.65219	2429.32969	0.74704	0.77129	0.93816	0.90111	0.77754	0.83245	83.40140	16.59860
107	495.33599	1667.12378	0.76563	0.77257	0.93825	0.91169	0.80367	0.82393	88.64158	11.35842

Table S3 – Operational physicochemical data used for PCA analysis

Days	OLR	COD_inlet	COD_in	COD_out	Ntotal_in	NH4+_in	NH4+_out	NO3_out	NO2_out	pH_out	SST_out	Bed volume	Cl-_out
0	1.32	800.00	200.00	16.00	45	20.5	12.4	11.6	0.98	7.43	0.974	26	2100
6	0.91	600.00	140.00	48.00	38.5	24.2	1.1	18.6	0.42	7.14	0.198	12.5	1500
13	0.87	582.52	310.68	77.67	53.5	27.1	14	8.4	0.84	7.98	0.352	12.5	2070
21	1.19	769.23	211.54	92.31	37.5	31	36.8	4.3	0.84	8.1	0.276	11.5	1900
34	1.60	1018.87	198.11	60.38	54.5	41.5	51.5	3	0.56	8.26	0.269	15	1650
44	1.35	844.04	183.49	58.72	45.50	18.25	42.5	3	0.46	8.4	0.53	20.5	1490
61	0.37	226.42	105.66	67.92	47.00	40	48.5	3.8	0.78	8.23	0.228	26	2540
72	0.26	130.84	52.34	22.43	23.60	14.5	14.4	9.3	1.98	7.85	0.137	12.5	1560
86	0.60	403.85	84.62	84.62	32.40	10.3	12.3	14.8	2.75	5.55	0.091	9.25	2320
96	0.43	103.70	59.26	66.67	30.3	25.8	11.1	14.4	7.4	6.4	0.093	8.5	2200
107	0.26	162.96	51.85	51.85	32.40	24.8	10	13.4	9.6	5.38	0.141	8	2300

Table S4 – One-way ANOVA analysis results obtained for the comparison of total EPS and EPS components (Carbohydrates – CH, Proteins, Humic Acids – HA and Proteins to Polysaccharides ratio – PNPS) values during the three operational phases

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
CH	Between Groups	5897.511	2	2948.756	60.027	<.001
	Within Groups	736.852	15	49.123		
	Total	6634.363	17			
Proteins	Between Groups	1799.785	2	899.892	2.645	.104
	Within Groups	5103.407	15	340.227		
	Total	6903.192	17			
HA	Between Groups	29165.858	2	14582.929	55.130	<.001
	Within Groups	3967.803	15	264.520		
	Total	33133.661	17			
PNPS	Between Groups	.184	2	.092	140.332	<.001
	Within Groups	.010	15	.001		
	Total	.193	17			
EPS	Between Groups	15379.461	2	7689.731	6.427	.010
	Within Groups	17945.717	15	1196.381		
	Total	33325.178	17			

Table S5 – Tukey's post-hoc test results (Multiple comparisons) obtained for the comparison of total EPS and EPS components (Carbohydrates – CH, Proteins, Humic Acids – HA and Proteins to Polysaccharides ratio – PNPS) during the three operational phases

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Phases	(J) Phases	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
CH	1.00	2.00	-44.27765*	4.04654	<.001	-54.7884	-33.7669
		3.00	-20.14003*	4.04654	<.001	-30.6508	-9.6293
	2.00	1.00	44.27765*	4.04654	<.001	33.7669	54.7884
		3.00	24.13762*	4.04654	<.001	13.6269	34.6484
	3.00	1.00	20.14003*	4.04654	<.001	9.6293	30.6508
		2.00	-24.13762*	4.04654	<.001	-34.6484	-13.6269
Proteins	1.00	2.00	19.55583	10.64937	.192	-8.1056	47.2173
		3.00	-2.99405	10.64937	.957	-30.6555	24.6674
	2.00	1.00	-19.55583	10.64937	.192	-47.2173	8.1056
		3.00	-22.54989	10.64937	.120	-50.2113	5.1115
	3.00	1.00	2.99405	10.64937	.957	-24.6674	30.6555
		2.00	22.54989	10.64937	.120	-5.1115	50.2113
HA	1.00	2.00	94.80908*	9.39007	<.001	70.4186	119.1995
		3.00	70.85435*	9.39007	<.001	46.4639	95.2448
	2.00	1.00	-94.80908*	9.39007	<.001	-119.1995	-70.4186
		3.00	-23.95472	9.39007	.055	-48.3451	.4357
	3.00	1.00	-70.85435*	9.39007	<.001	-95.2448	-46.4639
		2.00	23.95472	9.39007	.055	-.4357	48.3451
PNPS	1.00	2.00	-.24357*	.01477	<.001	-.2819	-.2052
		3.00	-.08417*	.01477	<.001	-.1225	-.0458
	2.00	1.00	.24357*	.01477	<.001	.2052	.2819
		3.00	.15940*	.01477	<.001	.1210	.1978
	3.00	1.00	.08417*	.01477	<.001	.0458	.1225
		2.00	-.15940*	.01477	<.001	-.1978	-.1210
EPS	1.00	2.00	70.08726*	19.96982	.008	18.2162	121.9583
		3.00	47.72027	19.96982	.074	-4.1507	99.5913
	2.00	1.00	-70.08726*	19.96982	.008	-121.9583	-18.2162
		3.00	-22.36699	19.96982	.517	-74.2380	29.5040
	3.00	1.00	-47.72027	19.96982	.074	-99.5913	4.1507
		2.00	22.36699	19.96982	.517	-29.5040	74.2380

*. The mean difference is significant at the 0.05 level.

Table S6 – One-way ANOVA analysis results obtained for the comparison between COD, OLR, NH_4^+, TN, TOC, C/N ratio and NaCl present in the wastewater, during the three operational phases

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
COD	Between Groups	2737760.227	2	1368880.113	89.781	<.001
	Within Groups	381170.833	25	15246.833		
	Total	3118931.060	27			
OLR	Between Groups	6.691	2	3.346	91.054	<.001
	Within Groups	.919	25	.037		
	Total	7.610	27			
ammonium	Between Groups	1466.475	2	733.237	11.753	<.001
	Within Groups	1497.310	24	62.388		
	Total	2963.785	26			
TN	Between Groups	13752.964	2	6876.482	57.964	<.001
	Within Groups	2965.854	25	118.634		
	Total	16718.819	27			
Cl	Between Groups	4584329.042	2	2292164.521	13.346	<.001
	Within Groups	4293742.262	25	171749.690		
	Total	8878071.304	27			
TOC	Between Groups	1260582.223	2	630291.111	111.962	<.001
	Within Groups	135108.577	24	5629.524		
	Total	1395690.799	26			
CN	Between Groups	134.307	2	67.154	98.983	<.001
	Within Groups	15.604	23	.678		
	Total	149.911	25			

Table S7 – Tukey's post-hoc test results (Multiple comparisons) obtained for the comparison between COD, OLR, NH_4^+, TN, TOC, C/N ratio and NaCl present in the wastewater, during the three operational phases

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Phases	(J) Phases	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
COD	1.00	2.00	-282.26757*	66.00180	<.001	-446.6668	-117.8683
		3.00	451.54751*	57.15924	<.001	309.1736	593.9215
	2.00	1.00	282.26757*	66.00180	<.001	117.8683	446.6668
		3.00	733.81508*	57.15924	<.001	591.4411	876.1890
	3.00	1.00	-451.54751*	57.15924	<.001	-593.9215	-309.1736
		2.00	-733.81508*	57.15924	<.001	-876.1890	-591.4411
OLR	1.00	2.00	-.43179*	.10246	<.001	-.6870	-.1766
		3.00	.71291*	.08873	<.001	.4919	.9339
	2.00	1.00	.43179*	.10246	<.001	.1766	.6870
		3.00	1.14470*	.08873	<.001	.9237	1.3657
	3.00	1.00	-.71291*	.08873	<.001	-.9339	-.4919
		2.00	-1.14470*	.08873	<.001	-1.3657	-.9237
ammonium	1.00	2.00	-15.57262*	4.39438	.005	-26.5466	-4.5986
		3.00	2.87857	3.65634	.714	-6.2524	12.0095
	2.00	1.00	15.57262*	4.39438	.005	4.5986	26.5466
		3.00	18.45119*	3.85412	<.001	8.8263	28.0760
	3.00	1.00	-2.87857	3.65634	.714	-12.0095	6.2524
		2.00	-18.45119*	3.85412	<.001	-28.0760	-8.8263
TN	1.00	2.00	-4.42857	5.82198	.730	-18.9301	10.0730
		3.00	42.00000*	5.04198	<.001	29.4413	54.5587
	2.00	1.00	4.42857	5.82198	.730	-10.0730	18.9301
		3.00	46.42857*	5.04198	<.001	33.8698	58.9873
	3.00	1.00	-42.00000*	5.04198	<.001	-54.5587	-29.4413
		2.00	-46.42857*	5.04198	<.001	-58.9873	-33.8698
Cl	1.00	2.00	257.50000	221.52052	.486	-294.2700	809.2700
		3.00	-659.76190*	191.84240	.006	-1137.6087	-181.9151
	2.00	1.00	-257.50000	221.52052	.486	-809.2700	294.2700
		3.00	-917.26190*	191.84240	<.001	-1395.1087	-439.4151
	3.00	1.00	659.76190*	191.84240	.006	181.9151	1137.6087
		2.00	917.26190*	191.84240	<.001	439.4151	1395.1087
TOC	1.00	2.00	-283.60143*	40.10530	<.001	-383.7559	-183.4470
		3.00	239.31323*	35.17468	<.001	151.4720	327.1545
	2.00	1.00	283.60143*	40.10530	<.001	183.4470	383.7559
		3.00	522.91466*	35.17468	<.001	435.0734	610.7559
	3.00	1.00	-239.31323*	35.17468	<.001	-327.1545	-151.4720
		2.00	-522.91466*	35.17468	<.001	-610.7559	-435.0734
CN	1.00	2.00	-3.39961*	.44027	<.001	-4.5022	-2.2970
		3.00	2.11141*	.39173	<.001	1.1304	3.0924
	2.00	1.00	3.39961*	.44027	<.001	2.2970	4.5022
		3.00	5.51103*	.39173	<.001	4.5300	6.4921
	3.00	1.00	-2.11141*	.39173	<.001	-3.0924	-1.1304
		2.00	-5.51103*	.39173	<.001	-6.4921	-4.5300

*. The mean difference is significant at the 0.05 level.

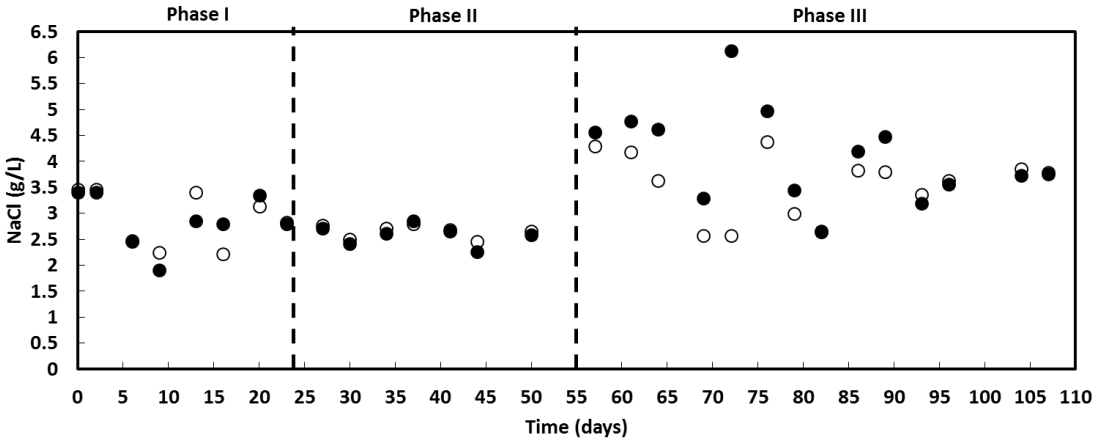

Figure S1. NaCl concentrations along AGS-SBR operation. Concentration in the wastewater (●) and outlet (○) are shown.

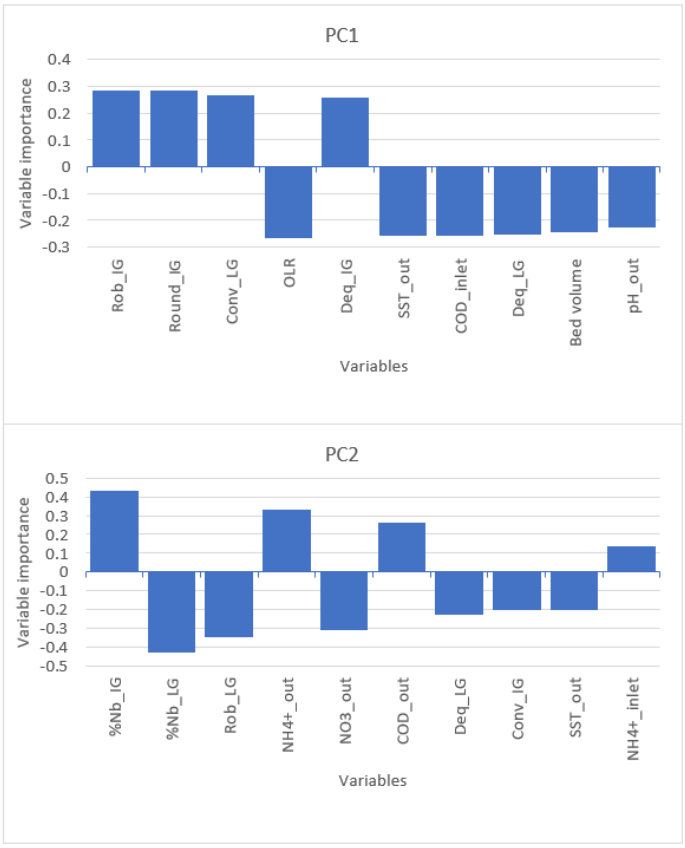

Figure S2 - Variable importance for PC1 and PC2, regarding the PCA analysis of Figure 3.

	Phase I			Phase II	Phase III			
	2.1	2.2	2.6	2.4	2.8	2.11		0-2.5
Family	d0	d6	d23	d44	d72	d107		2.5-5
f__Haliscomenobacteraceae	10.88531	1.128281	0.222577	0	0	1.916916		5-10
f__Comamonadaceae	8.70411	7.951696	8.669362	5.758168	4.142954	3.48196		10-20
f__Chitinophagaceae	7.888746	4.715243	1.398524	5.962198	4.340518	9.138176		20-100
f__Sphingomonadaceae	7.379661	6.961572	1.932708	2.638216	2.246926	3.219369		
f__Methylococcaceae	6.038657	3.172491	1.91045	5.673156	0.978976	0		
f__Candidatus Competibacte	5.695129	5.567211	2.140446	4.414974	1.595258	0.572449		
f__Flavobacteriaceae	4.478291	26.58752	44.14067	45.3144	39.98762	28.071		
f__Cytophagaceae	4.44518	2.993399	3.216233	5.724163	5.537699	7.189748		
f__Caulobacteraceae	3.753984	4.10633	2.908335	2.408683	4.744493	5.540675		
f__Zoogloeaceae	3.464261	3.305531	9.682086	4.128765	6.557957	6.507011		
f__Rhodobacteraceae	2.93862	6.493374	5.382646	4.721018	4.293339	4.091172		
f__Burkholderiaceae	2.061173	1.110372	0.051935	0.06801	3.287825	3.53973		
f__Gemmatimonadaceae	0.856753	0	0.638053	0.799116	1.79872	3.156347		

Figure S3. Heatmap presenting the evolution in dominant bacterial families in the AGS biomass along the reactor operational phases, considering the first twelve dominant bacterial genera from each biomass sample.

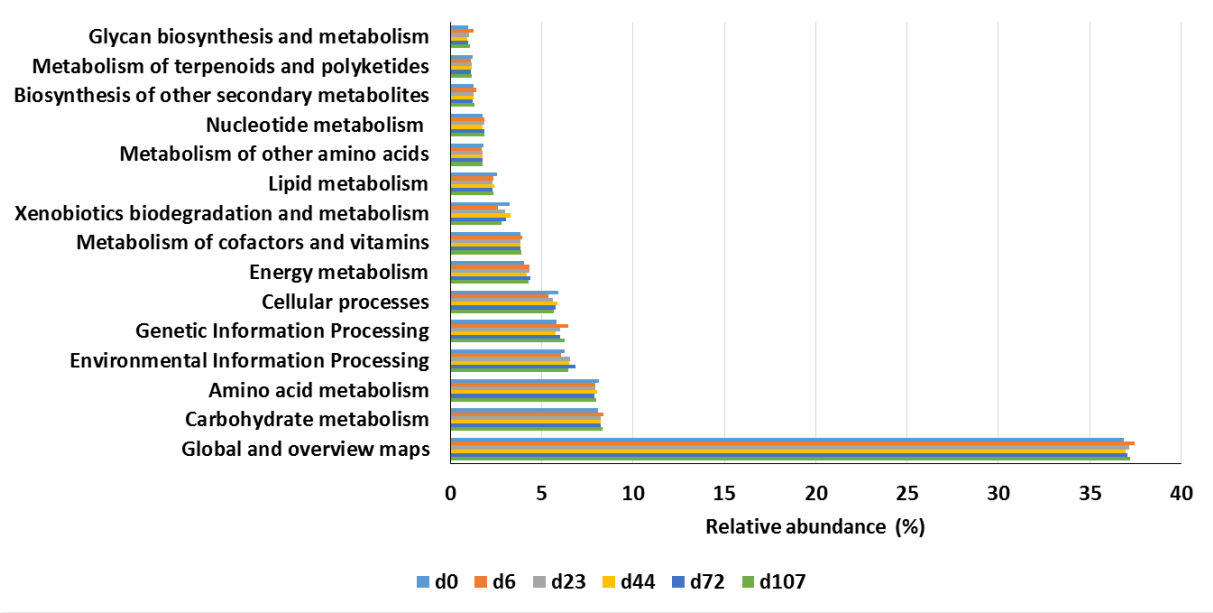

Figure S4. Relative abundance of main functional categories predicted in the microbiome from the biomass samples (sampling days) analyzed along reactor operation.