

New insights to the difference in microbial composition and interspecies interactions between fouling layer and mixed liquor in membrane bioreactor

Revised Supplementary Information

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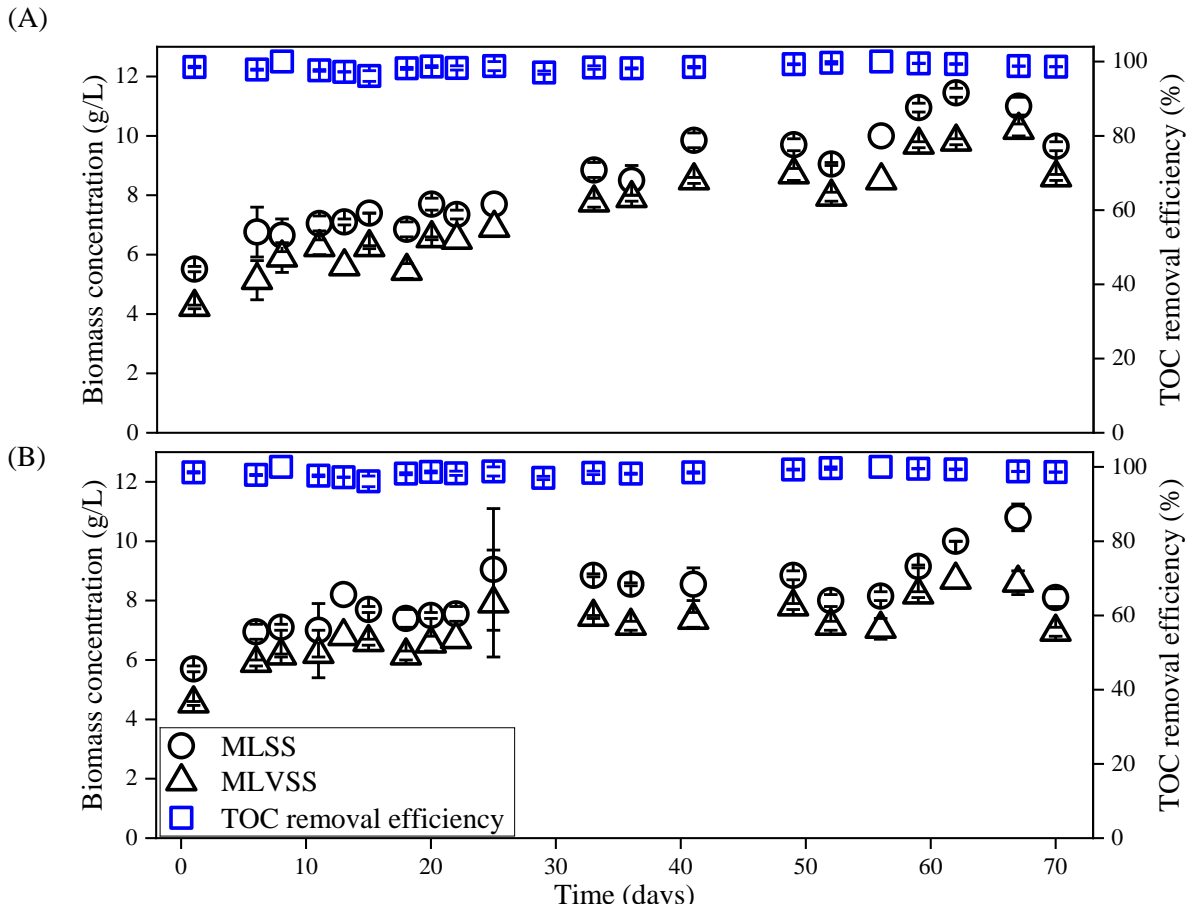


Figure S1. Biomass quality in terms of mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and total organic carbon (TOC) removal efficiency in the two “parent” MBR systems from which the inoculum was taken.

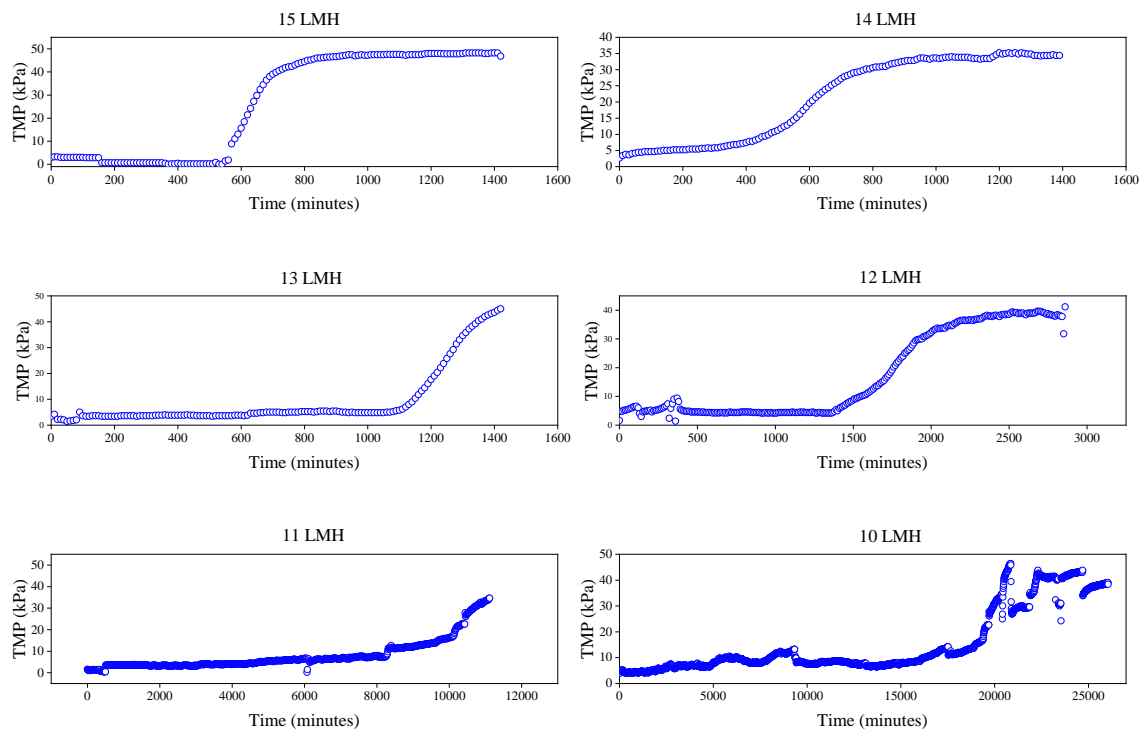


Figure S2. Transmembrane pressure profile of the MBR during preliminary fouling runs in comparison with first biomass collection phases.

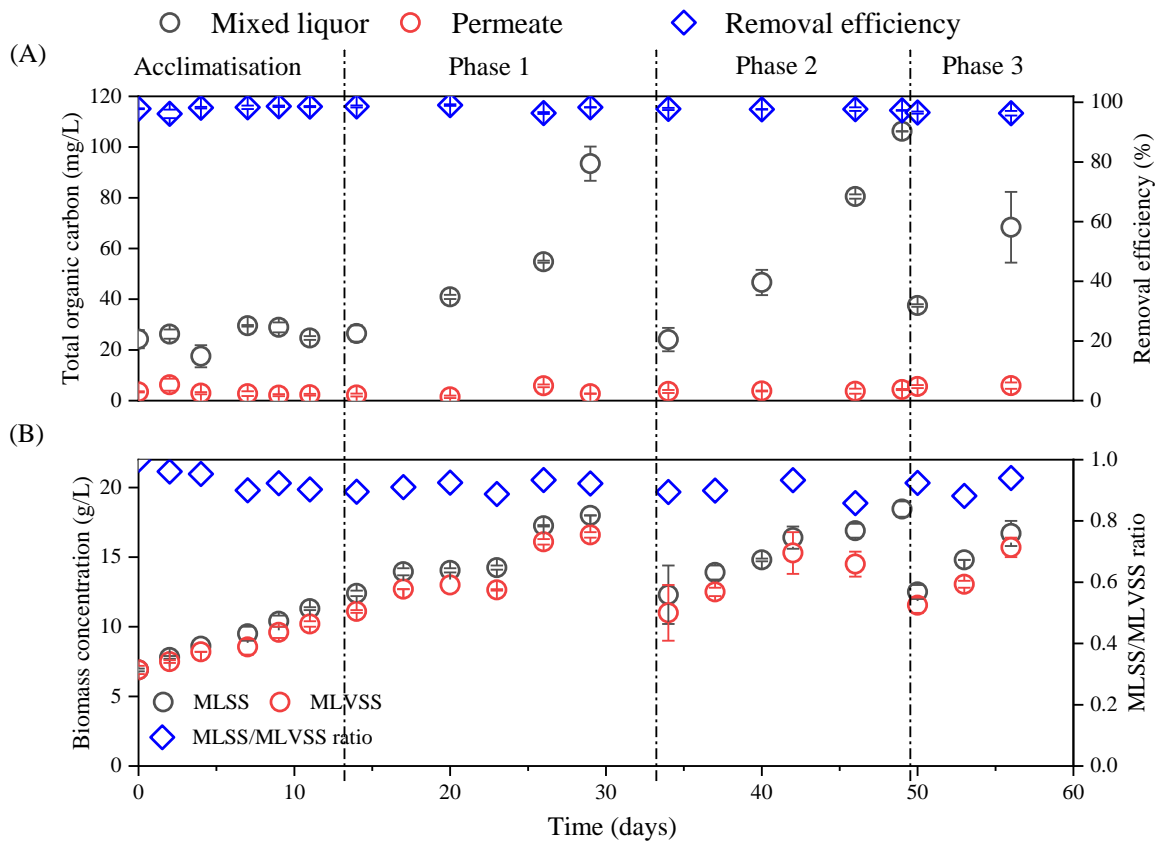


Figure S3. Total organic carbon concentration and removal efficiency (A) and biomass concentration (B) during the experiment. The error bar represents the standard deviation from duplicate samples.

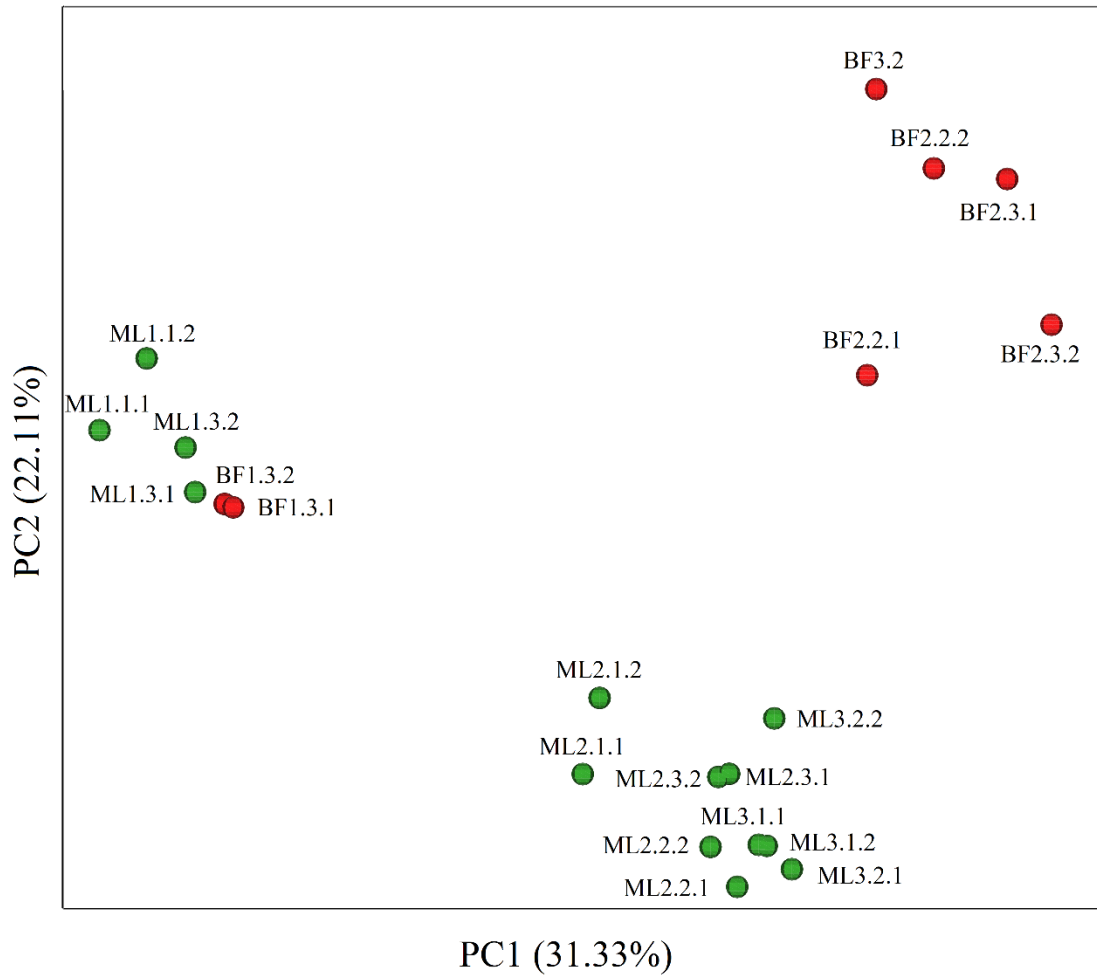


Figure S4. Principal coordinates analysis showing similarity/dissimilarity between mixed liquor (ML) and biofilm (BF) samples microbial community composition.

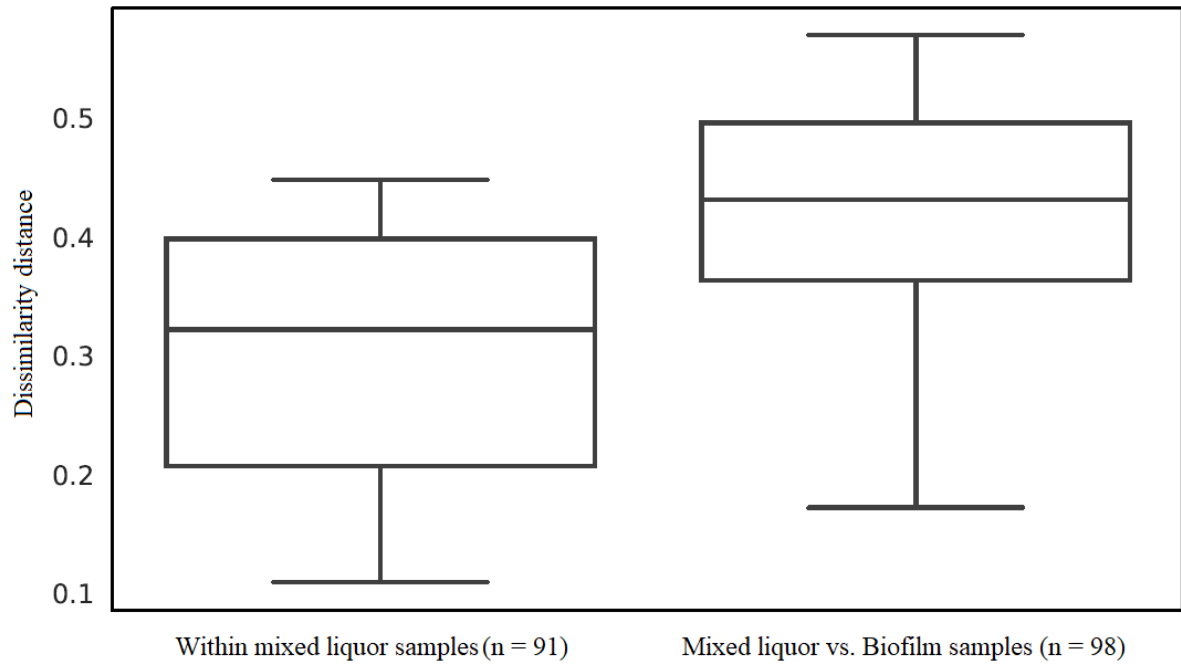


Figure S5. Bray-Curtis dissimilarity distance within mixed liquor samples and between mixed liquor and biofilm samples. The whiskers of the box represent the minimum and maximum values. The bottom and top of the box are the first and third quartiles, respectively, and the line inside the box denotes the median.

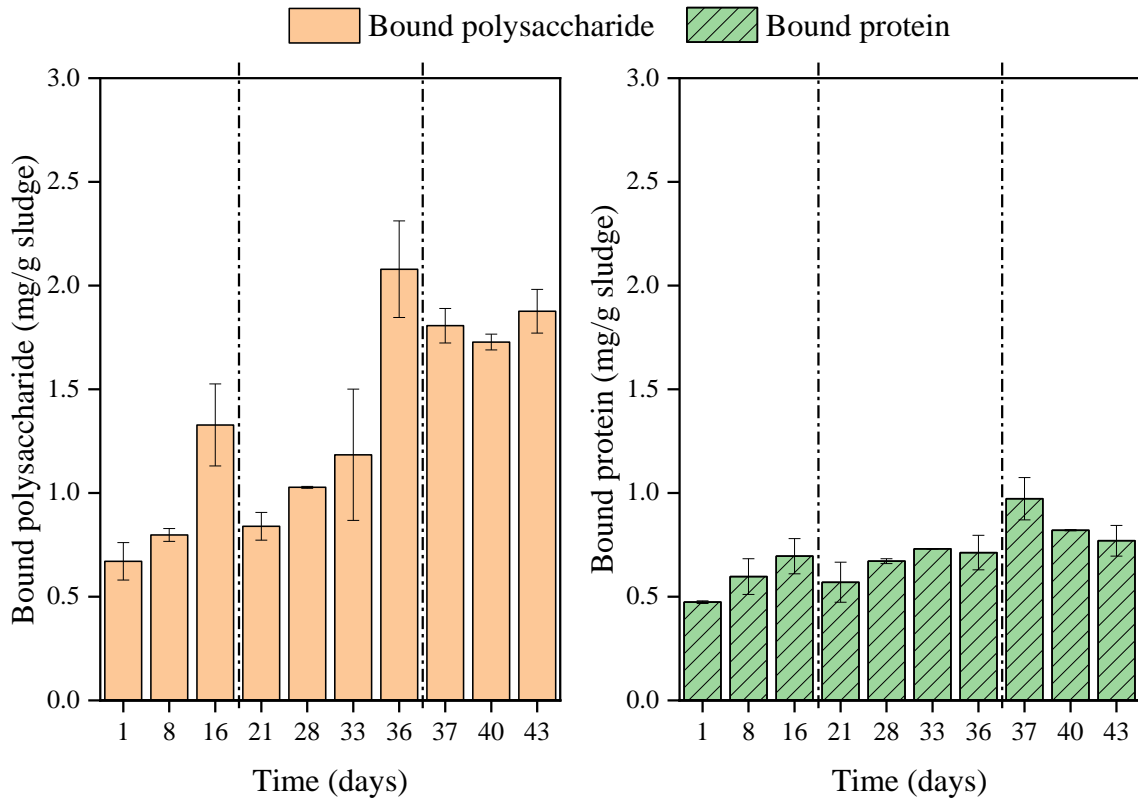


Figure S6. Extracellular polymeric substances (EPS) concentration in the mixed liquor during the experiment normalized to biomass concentration. The error bar represents the standard deviation from duplicate samples.

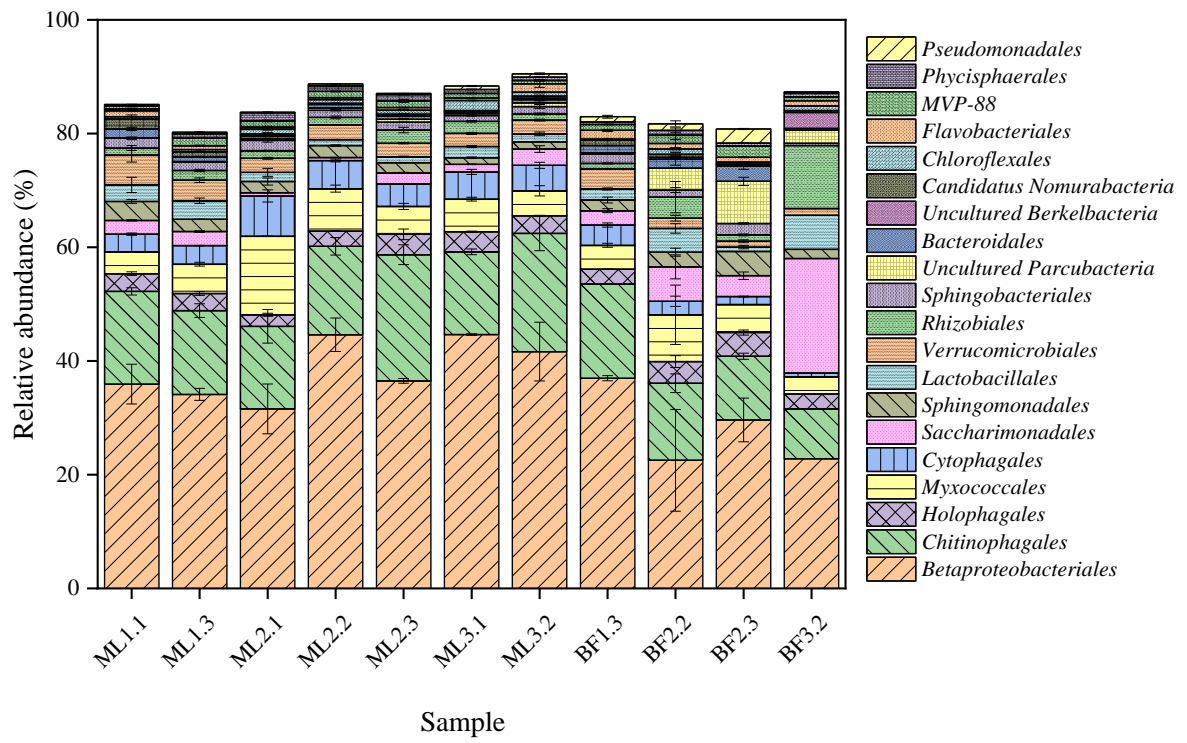


Figure S7. Relative abundances of top 10 orders in sample (a total of 20 orders) of mixed liquor (ML) and biofilm (BF).

Table S1. Pearson correlation between microbial orders in the mixed liquor with fouling indicators. Taxa that were identified as network connectors/module hubs are marked with an asterisk. Taxa that showed strong correlations with fouling indicator (>0.6) are marked with two asterisks.

Taxa	SMPc	SMPp	EPSc	EPSp
<i>Absconditabacteriales SRI</i>	0.03	0.177	0.062	0
<i>Acetobacterales</i>	0.001	0.452	0.212	0.179
<i>Anaerolinea 1-20</i>	0.025	0.36	0.113	0
<i>Anaerolineales</i>	0.129	0.075	0.001	0.003
<i>Armatimonadales</i>	0.489	0.446	0.27	0.139
<i>Azospirillales</i>	0.585	0.214	0.011	0.103
<i>Babeliales**</i>	0.615	0.118	0.032	0.021
<i>Bacteroidales</i>	0.027	0.008	0.16	0.223
<i>Bdellovibrionales</i>	0.359	0.047	0.152	0.176
<i>Betaproteobacteriales</i>	0.016	0	0.002	0.026
<i>Blastocatellales**</i>	0.736	0.136	0.056	0.004
<i>Blastocatellia 11-24</i>	0.084	0.212	0.23	0.128
<i>Caedibacterales**</i>	0.906	0.588	0.072	0.284
<i>Caldilineales</i>	0.071	0.065	0.115	0.033
<i>Candidatus Falkowbacteria**</i>	0.135	0	0.908	0.769
<i>Candidatus Magasanikbacteria</i>	0.074	0.146	0.15	0.088
<i>Candidatus Nomurabacteria</i>	0.096	0.001	0.216	0.265
<i>Candidatus Pacebacteria</i>	0.049	0.352	0.024	0.076
<i>Caulobacterales</i>	0.073	0.055	0.216	0.187
<i>CCM19a</i>	0.181	0.047	0.09	0.339
<i>Chitinophagales</i>	0.007	0.455	0.26	0.114
<i>Chlamydiales</i>	0.054	0.111	0.086	0.041
<i>Chloroflexales</i>	0.057	0.004	0.004	0.059
<i>Chthoniobacterales</i>	0	0	0.139	0.115
<i>Chthonomonadales</i>	0.08	0.001	0.017	0.006
<i>Clostridiales</i>	0.283	0.007	0.028	0.025
<i>Cytophagales</i>	0.13	0	0.082	0.051
<i>Desulfarculales</i>	0.47	0.291	0.034	0.166
<i>Desulfobacterales</i>	0.015	0.335	0.093	0.008
<i>Desulfovibrionales</i>	0.31	0.037	0.183	0.193
<i>Desulfuromonadales</i>	0.2	0.008	0.001	0.001
<i>Diplorickettsiales**</i>	0.767	0.255	0	0.033
<i>Dongiales**</i>	0.093	0.034	0.634	0.805
<i>Erysipelotrichales**</i>	0.086	0.057	0.945	0.717
<i>Fibrobacterales**</i>	0.002	0.741	0.26	0.085
<i>Fimbriimonadales</i>	0.201	0.073	0.005	0.026
<i>Flavobacteriales</i>	0.053	0.326	0.006	0
<i>Frankiales</i>	0.065	0.004	0.15	0.139
<i>Gammaproteobacteria Incertae Sedis</i>	0	0.025	0.062	0.09
<i>Gemmatales</i>	0.033	0.038	0.377	0.281
<i>Gemmatimonadales</i>	0.309	0.089	0.18	0.103
<i>Holophagales</i>	0.096	0.075	0.039	0.034

<i>Holosporales**</i>	0.682	0.007	0.372	0.399
<i>Hydrogenedentiales</i>	0.318	0.161	0.17	0.024
<i>Isosphaerales</i>	0.06	0.297	0.097	0.001
<i>Lactobacillales</i>	0.338	0.041	0.129	0.062
<i>Legionellales**</i>	0.506	0.351	0.993	0.768
<i>Leptospirales</i>	0.054	0.002	0	0.021
<i>Micrococcales</i>	0.149	0.196	0.006	0.009
<i>Microtrichales</i>	0.383	0.448	0.475	0.384
<i>MVP-88</i>	0.148	0.269	0.018	0.044
<i>Myxococcales</i>	0.035	0.049	0.228	0.196
<i>NBIj</i>	0.12	0.123	0.01	0
<i>Nitrospirales</i>	0.002	0.117	0.056	0.128
<i>Obscuribacterales</i>	0.106	0.002	0.198	0.273
<i>Oligoflexales</i>	0.531	0.049	0.19	0.16
<i>OPB56</i>	0.023	0.005	0.243	0.28
<i>Opitales</i>	0.131	0.004	0.006	0.002
<i>Paracaedibacterales**</i>	0.802	0.258	0.005	0.061
<i>Pedosphaerales</i>	0.085	0.107	0.151	0.161
<i>Phycisphaeraemle18</i>	0.312	0.106	0	0.026
<i>Phycisphaerales</i>	0.037	0.034	0.029	0.061
<i>Pirellulales</i>	0.005	0.001	0.146	0.2
<i>Planctomycetales</i>	0.035	0.002	0.316	0.398
<i>Propionibacteriales</i>	0.023	0.006	0.294	0.252
<i>Pseudomonadales</i>	0.002	0.07	0.087	0.162
<i>RBG13549</i>	0.111	0.003	0	0.007
<i>Reyranelles</i>	0.294	0.178	0.095	0.079
<i>Rhizobiales</i>	0	0.019	0.174	0.167
<i>Rhodobacterales</i>	0.536	0.22	0.063	0.025
<i>Rhodospirillales</i>	0	0.16	0.03	0.022
<i>Rickettsiales**</i>	0.708	0.264	0.018	0
<i>Saccharimonadales</i>	0.299	0.217	0.086	0.039
<i>SAR324 clade Marine group B</i>	0.027	0.057	0.079	0.157
<i>SBR1031</i>	0.024	0.097	0.489	0.483
<i>Selenomonadales</i>	0.11	0.107	0.042	0.045
<i>SMIA07</i>	0	0.236	0.019	0.078
<i>Solibacterales</i>	0.183	0.131	0.097	0.068
<i>Sphingobacteriales</i>	0.014	0.015	0.188	0.292
<i>Sphingomonadales</i>	0.011	0.009	0.279	0.372
<i>Spirochaetales</i>	0.018	0.048	0	0.046
<i>Steroidobacterales</i>	0.479	0.08	0.341	0.243
<i>Tepidisphaerales</i>	0.389	0.002	0.014	0.004
<i>Thermales</i>	0.377	0.322	0.022	0.004
<i>Thermoanaerobaculales</i>	0.428	0.219	0.005	0.001
<i>Thermomicrobiales</i>	0	0.278	0.218	0.174
<i>Tistrellales</i>	0.161	0.278	0.205	0.119
<i>Unassigned Acidobacteria</i>	0.165	0.133	0.005	0.03

<i>Unassigned Alphaproteobacteria**</i>	0.778	0.223	0	0.028
<i>Unassigned Bacteroidia</i>	0.016	0.185	0.182	0.2
<i>Unassigned Chloroflexi</i>	0	0.1	0.2	0.077
<i>Unassigned Deltaproteobacteria</i>	0.002	0.102	0.03	0.037
<i>Unassigned Elusimicrobia</i>	0.076	0.18	0.013	0.014
<i>Unassigned LineageIIa</i>	0.357	0.06	0.003	0.016
<i>Unassigned Microgenomatia</i>	0.135	0.513	0.391	0.143
<i>Unassigned microorganism</i>	0.337	0.525	0.005	0.024
<i>Unassigned OM190</i>	0.024	0.015	0.008	0.049
<i>Unassigned Subgroup 17**</i>	0.537	0	0.592	0.653
<i>Unassigned Subgroup 6</i>	0.183	0.058	0.013	0.065
<i>UnassignedBacteria</i>	0.044	0.135	0.313	0.498
<i>Uncultured Alphaproteobacteria</i>	0.219	0.091	0.055	0.071
<i>Uncultured Berkelbacteria*</i>	0.378	0.617	0.483	0.466
<i>Uncultured LCP89**</i>	0.008	0	0.592	0.644
<i>Uncultured OM190</i>	0	0.09	0.04	0
<i>Uncultured Parcubacteria*</i>	0.13	0.322	0.202	0.09
<i>Uncultured Pelobacter sp.</i>	0.396	0.149	0.168	0.063
<i>Uncultured Woesearchaeia</i>	0.338	0.065	0.15	0.081
<i>Uncultured WS6 Dojkabacteria</i>	0.015	0.205	0.38	0.091
<i>Verrucomicrobiales</i>	0.074	0	0.215	0.229
<i>Victivallales*</i>	0.026	0.006	0.669	0.764
<i>WD260**</i>	0.57	0.725	0.395	0.352
<i>Xanthomonadales</i>	0.014	0.004	0.044	0.151

Table S2. Pearson correlation between microbial orders in the biofilm with fouling indicators. Taxa that were identified as network connectors/module hubs are marked with an asterisk. Taxa that showed strong correlations with fouling indicator (>0.6) are marked with two asterisks.

Taxa	SMPc	SMPp	EPSc	EPSp
<i>Abconditabacteriales SRI**</i>	0.279	0.701	0.465	0.074
<i>Acetobacterales</i>	0.163	0.25	0.516	0.116
<i>Anaerolineales**</i>	0.221	0.06	0.491	0.928
<i>Azospirillales**</i>	0.39	0.639	0.918	0.254
<i>Babeliales</i>	0.102	0.002	0.093	0.027
<i>Bacteroidales</i>	0.04	0.037	0.079	0.004
<i>Bdellovibrionales</i>	0.515	0.202	0	0.006
<i>Betaproteobacteriales</i>	0.165	0.074	0.024	0.015
<i>Blastocatellales</i>	0.529	0.269	0.026	0.001
<i>Blastocatellia 11-24*</i>	0.728	0.44	0.123	0.008
<i>Brevinematales*</i>	0.075	0	0.316	0.8
<i>Caedibacterales**</i>	0.936	0.153	0	0.042
<i>Caldilineales</i>	0.104	0	0.095	0.023
<i>Candidate division WOR1 bacterium RIFOXYB2FULL4235**</i>	0.499	0.969	0.535	0.4
<i>Candidatus Collierbacteria</i>	0.039	0.297	0.346	0.204
<i>Candidatus Falkowbacteria</i>	0.2	0.011	0.04	0.328

<i>Candidatus Nomurabacteria</i>	0.312	0.49	0.289	0.114
<i>Candidatus Pacebacteria</i>	0	0.029	0.124	0.194
<i>Candidatus Zambryskibacteria</i>	0.082	0.07	0.095	0.004
<i>Caulobacterales**</i>	0.017	0.14	0.613	0.997
<i>Chitinophagales</i>	0.164	0.484	0.182	0.172
<i>Chlamydiales</i>	0.004	0.011	0	0.035
<i>Chloroflexales</i>	0.449	0.004	0.255	0.408
<i>Chthonomonadales**</i>	0.645	0.556	0.093	0.033
<i>Clostridiales</i>	0.016	0.114	0.369	0.085
<i>Coxiellales</i>	0.019	0.025	0.035	0.045
<i>Cytophagales**</i>	0.343	0.628	0.245	0.099
<i>Desulfarculales**</i>	0.579	0.199	0.78	0.54
<i>Desulfobacterales</i>	0	0.197	0.26	0.011
<i>Desulfovibrionales</i>	0.036	0.537	0.576	0.099
<i>Desulfuromonadales</i>	0	0.436	0.064	0.02
<i>Diplorickettsiales</i>	0.134	0.201	0.136	0.009
<i>Fibrobacterales</i>	0.317	0.056	0.232	0.008
<i>Fimbriimonadales</i>	0.451	0.412	0.016	0
<i>Flavobacteriales</i>	0.14	0.195	0.088	0.093
<i>Frankiales</i>	0.01	0.257	0.016	0.023
<i>Gammaproteobacteria Incertae Sedis</i>	0.104	0.035	0.065	0.121
<i>Gemmatales</i>	0.038	0.168	0.327	0.065
<i>Gemmatimonadales</i>	0.537	0.445	0.122	0.004
<i>Holophagales</i>	0.392	0.025	0.094	0
<i>Holosporales</i>	0.278	0.551	0.248	0.126
<i>Isosphaerales**</i>	0.135	0.259	0.677	0.163
<i>Lactobacillales</i>	0.061	0.005	0.334	0.304
<i>Leptospirales</i>	0.427	0.03	0.211	0.086
<i>Micrococcales**</i>	0.002	0.215	0.632	0.327
<i>MVP-88</i>	0.123	0.036	0.129	0.002
<i>Myxococcales</i>	0.126	0.071	0.098	0.12
<i>NBIj**</i>	0.002	0.946	0.708	0.333
<i>Nitrospirales**</i>	0.706	0.097	0.056	0.023
<i>Obscuribacterales</i>	0.147	0.389	0.243	0.165
<i>Oligoflexales</i>	0.016	0	0	0.035
<i>OPB56</i>	0.024	0.005	0.108	0.004
<i>Opitutales</i>	0.328	0.033	0.075	0.085
<i>Paracaedibacterales</i>	0.768	0.272	0.013	0.016
<i>Pedosphaerales</i>	0.459	0.377	0.094	0.016
<i>Phycisphaerales</i>	0.199	0.003	0.112	0.152
<i>Pirellulales*</i>	0.013	0.011	0.169	0.038
<i>Planctomycetales</i>	0.012	0.249	0.473	0.274
<i>Pseudomonadales</i>	0.102	0.056	0.242	0.017
<i>RBG13549</i>	0.383	0.119	0.006	0.166
<i>Reyranellales</i>	0.007	0.389	0.423	0.276
<i>Rhizobiales</i>	0.075	0.134	0.014	0.034
<i>Rhodobacterales</i>	0.006	0.134	0.102	0.062
<i>Rhodospirillales</i>	0.232	0.2	0.059	0.034

<i>Rickettsiales**</i>	0.704	0.2	0.002	0.013
<i>Saccharimonadales</i>	0.064	0.303	0	0.011
<i>SAR324 clade Marine group B**</i>	0.1	0.636	0.352	0.015
<i>SBR1031</i>	0.109	0.004	0.321	0.526
<i>Selenomonadales</i>	0.134	0.378	0.158	0.001
<i>Solibacterales**</i>	0.798	0.378	0.041	0.012
<i>Sphingobacteriales</i>	0.005	0.009	0.121	0.021
<i>Sphingomonadales</i>	0.195	0.158	0.305	0.035
<i>Spirochaetales**</i>	0.097	0.943	0.725	0.19
<i>Steroidobacterales</i>	0.341	0.004	0.059	0.052
<i>Synergistales**</i>	0.599	0.77	0.691	0.496
<i>Tepidisphaerales*</i>	0.82	0.083	0.997	0.956
<i>Thermales**</i>	0.704	0.174	0.01	0.104
<i>Thermoanaerobaculales*</i>	0.589	0.629	0.291	0.035
<i>Tistrellales</i>	0.151	0.002	0.188	0.151
<i>Unassigned Acidobacteria</i>	0.325	0.01	0.204	0.155
<i>Unassigned Alphaproteobacteria**</i>	0.889	0.206	0.01	0.019
<i>Unassigned Bacteria**</i>	0.402	0.88	0.709	0.147
<i>Unassigned Bacteroidia*</i>	0.173	0.325	0.495	0.509
<i>Unassigned Chloroflexi</i>	0.314	0.023	0.038	0.01
<i>Unassigned Deltaproteobacteria</i>	0.04	0.746	0.241	0.025
<i>Unassigned LineageIIa</i>	0.371	0.271	0.545	0.344
<i>Unassigned Margulisbacteria*</i>	0.435	0.976	0.856	0.512
<i>Unassigned Microgenomatia**</i>	0.282	0.603	0.276	0.002
<i>Unassigned microorganism**</i>	0.23	0.639	0.759	0.172
<i>Unassigned OM190</i>	0.228	0.119	0.155	0.112
<i>Unassigned Parcubacteria**</i>	0.626	0.164	0.018	0.268
<i>Unassigned Subgroup 6</i>	0.332	0.426	0.27	0.072
<i>Unassigned WS6 Dojkabacteria</i>	0.597	0.561	0.111	0.124
<i>Uncultured Alphaproteobacteria**</i>	0.686	0	0.227	0.175
<i>Uncultured Berkelbacteria</i>	0.421	0.303	0.009	0.039
<i>Uncultured OM190*</i>	0.083	0.222	0.633	0.311
<i>Uncultured Parcubacteria</i>	0.421	0.272	0.212	0
<i>Uncultured Woesearchaeia</i>	0.017	0.29	0.13	0
<i>Uncultured WS6 Dojkabacteria</i>	0.345	0.084	0.014	0.046
<i>Verrucomicrobiales</i>	0.583	0.566	0.163	0.007
<i>Victivallales</i>	0.235	0.048	0.05	0.007
<i>Xanthomonadales</i>	0.3	0.188	0.159	0.171