Supporting information

Two-phase improves performance of anaerobic membrane bioreactor treatment of food waste at high organic loading rates

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SI Tables

Table S1. Experimental conditions for different organic loading rates (OLRs).

Experimental Phase	FW (g COD L^{-1})	SP		TP-AP	TP-MP	
		HRT (days)	SRT (days)	HRT/SRT (days)	HRT (days)	SRT (days)
2.5[+] g COD·L day^{-1}	62 ± 11	24	140	3	24	140
3.5 g COD·L day^{-1}	73 ± 2	20	140	3	20	140
5 g COD·L day^{-1}	122 ± 5	24	100	3	24	100
10 g COD·L day^{-1}	120 ± 12	12	33	3	12	43
15 g COD·L day^{-1}	157 ± 14	10	29	3	10	29

[+]initial identical SP AnMBRs (SP1/SP2) also run at same conditions
FW= food waste; SRT=sludge retention time; HRT= hydraulic retention time; SP=single-phase; TP-AP= two-phase acid-phase; TP-MP= two-phase methane-phase

Table S2. FW characterization.

Item	Value	units
pH	3.5	
Acetate	3980 ± 550	mg L^{-1}
Propionate	1810 ± 680	mg L^{-1}
Formate	5.5 ± 0.9	mg L^{-1}
Phosphate	426 ± 12	mg L^{-1}
Sulfate	85.8 ± 15.7	mg L^{-1}
Chloride	956 ± 47	mg L^{-1}
Nitrate	50.5 ± 13.9	mg L^{-1}
TS	65.8 ± 1.0	g L^{-1}
TVS	60.1 ± 0.9	g L^{-1}
COD	123 ± 6.6	g L^{-1}
Protein	1	Wt%
Fat	1.5	Wt%
Carbohydrates	3.5	Wt%
Moisture	94	%
C:N Ratio	16	

TS=total solids; TVS=total volatile solids; COD=chemical oxygen demand

SI Figures

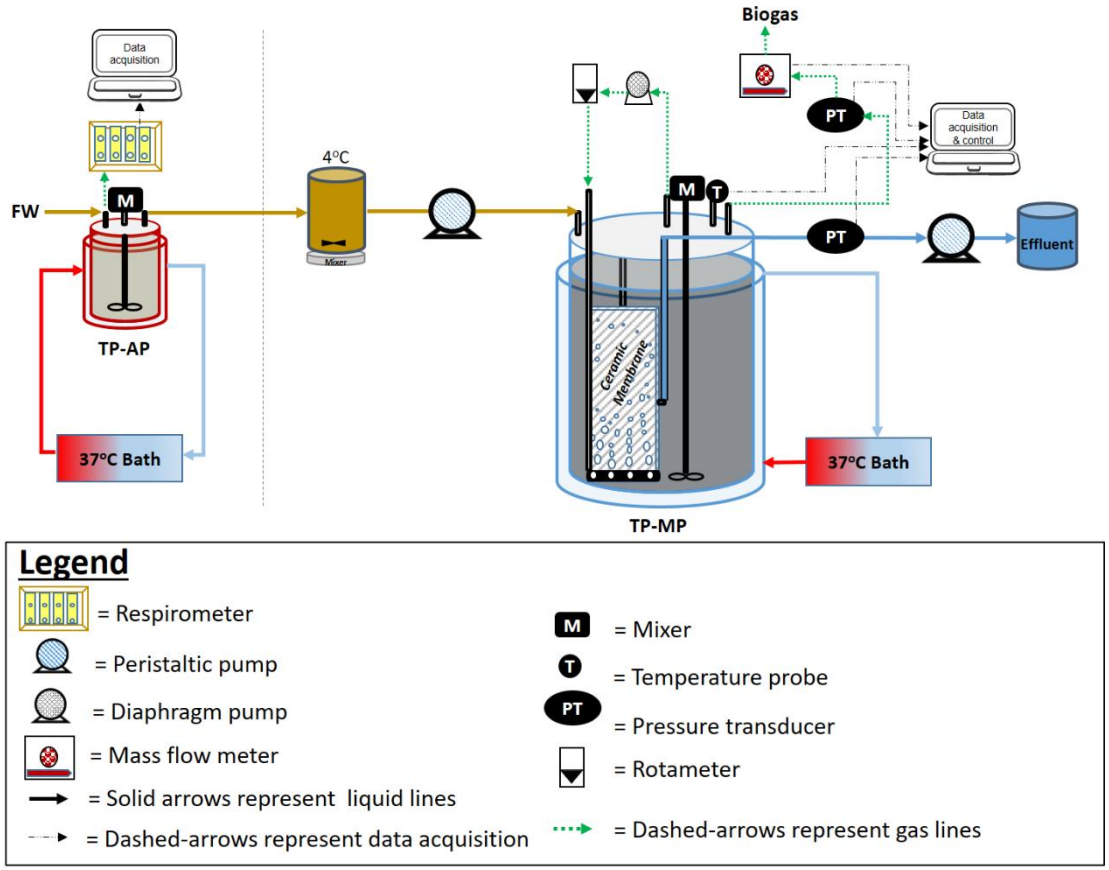

Figure S1. Schematic of TP system consisting of TP-AP and TP-MP. In SP, FW was directly fed (no acid-phase) and it consisted of only the components to the right of the vertical dashed line.

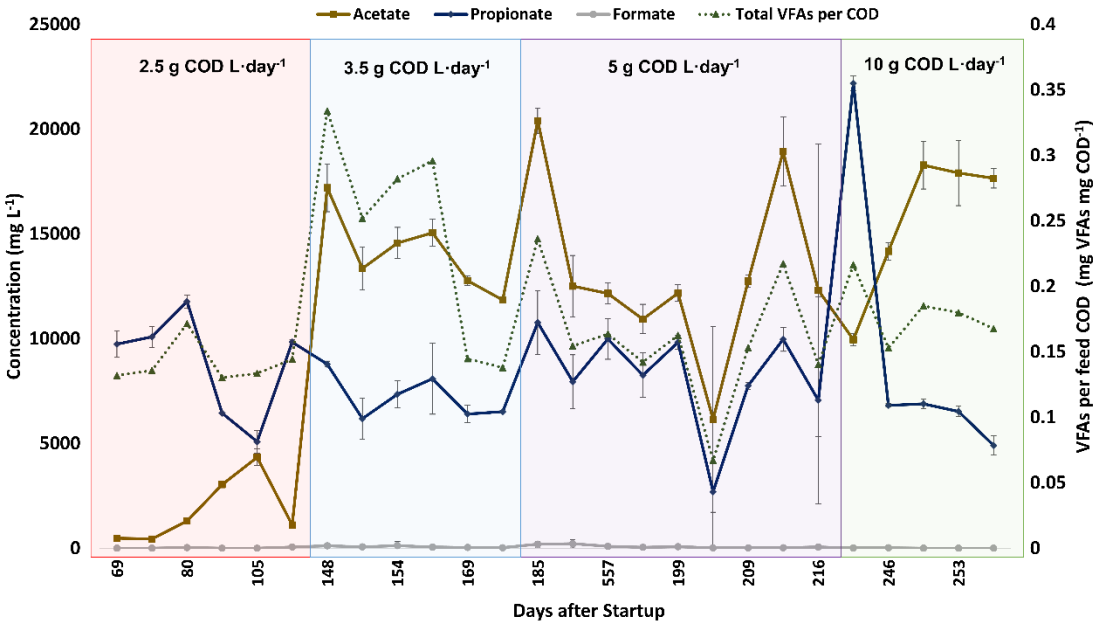

Figure S2. Volatile fatty acids (VFAs) concentrations in TP-AP at different OLRs. The solid line represents VFA concentration (primary y-axis) and the dashed line represents ratio of VFAs (acetic acid equivalent) to COD in the FW (secondary y-axis). Error bars for VFA concentrations represent the standard deviation for triplicate samples for each sampling point.

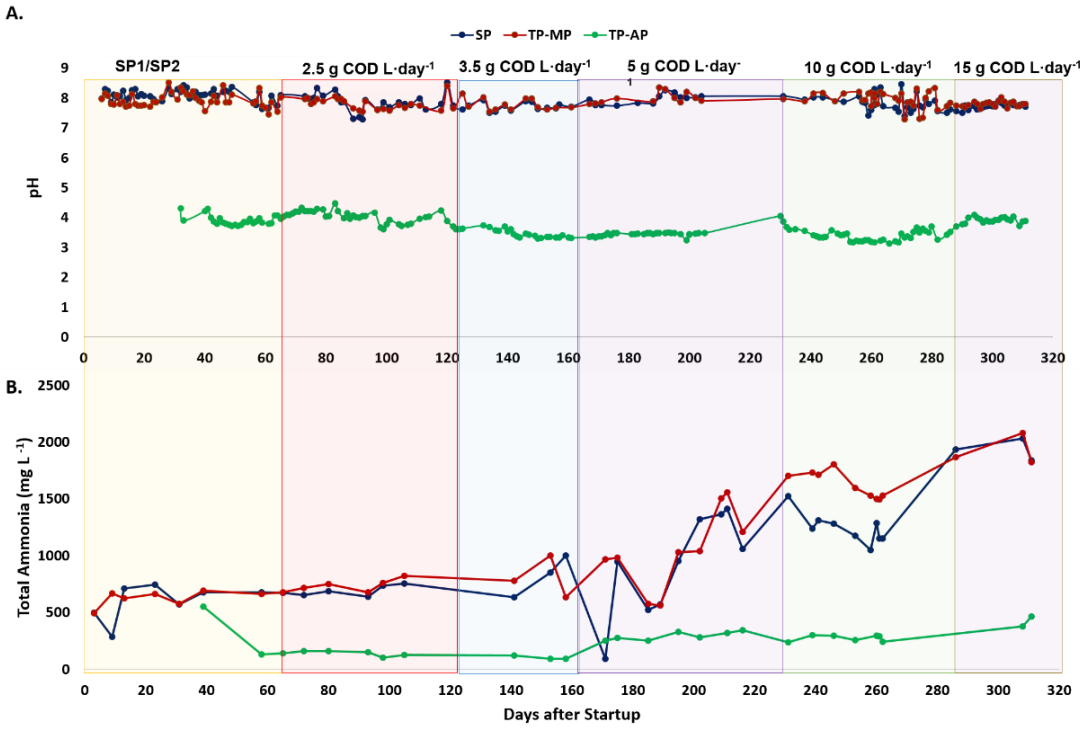

Figure S3 (A) pH and **(B)** total ammonia-nitrogen concentration in SP, TP-MP, and TP-AP at different OLRs.

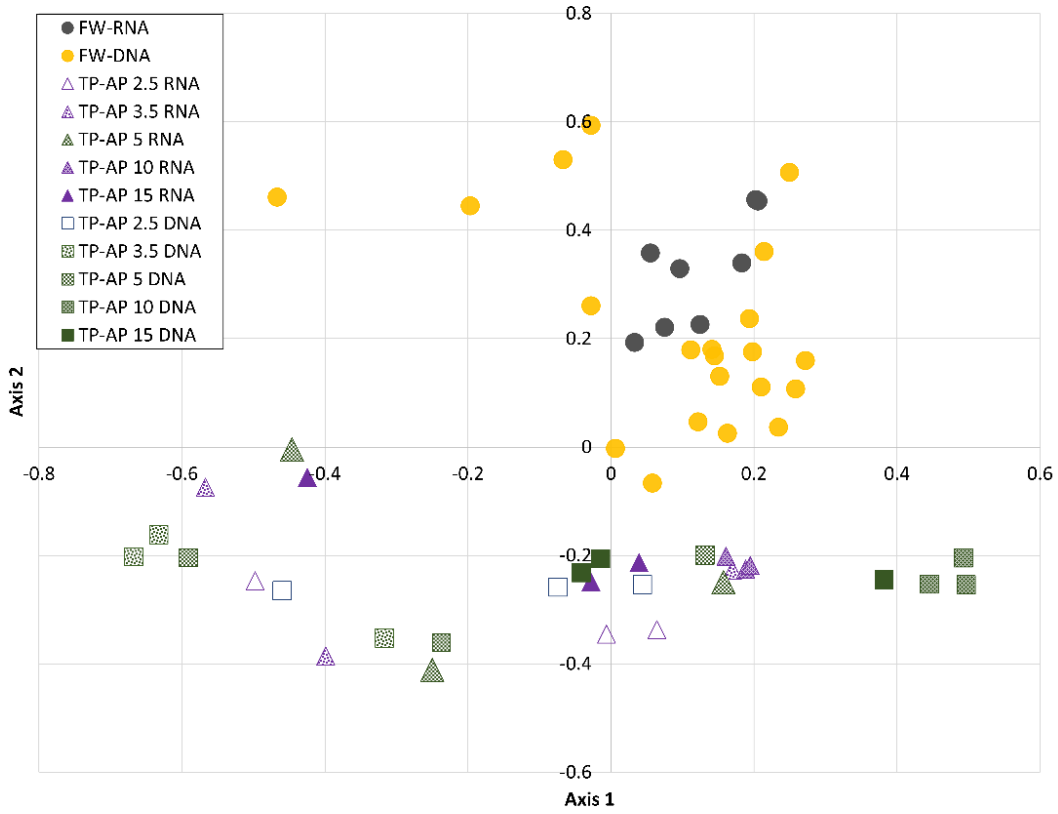

Figure S4. Non-metric multi-dimensional analysis (NMDS) plot showing ordination of FW and TP-AP samples (at different OLRs) analyzed using DNA- and RNA-based sequencing. The different patterns in the TP-AP RNA and TP-AP DNA samples signify the OLRs. The stress values for the NMDS at 2 and 3 dimension analysis were 0.18 and 0.09, respectively.

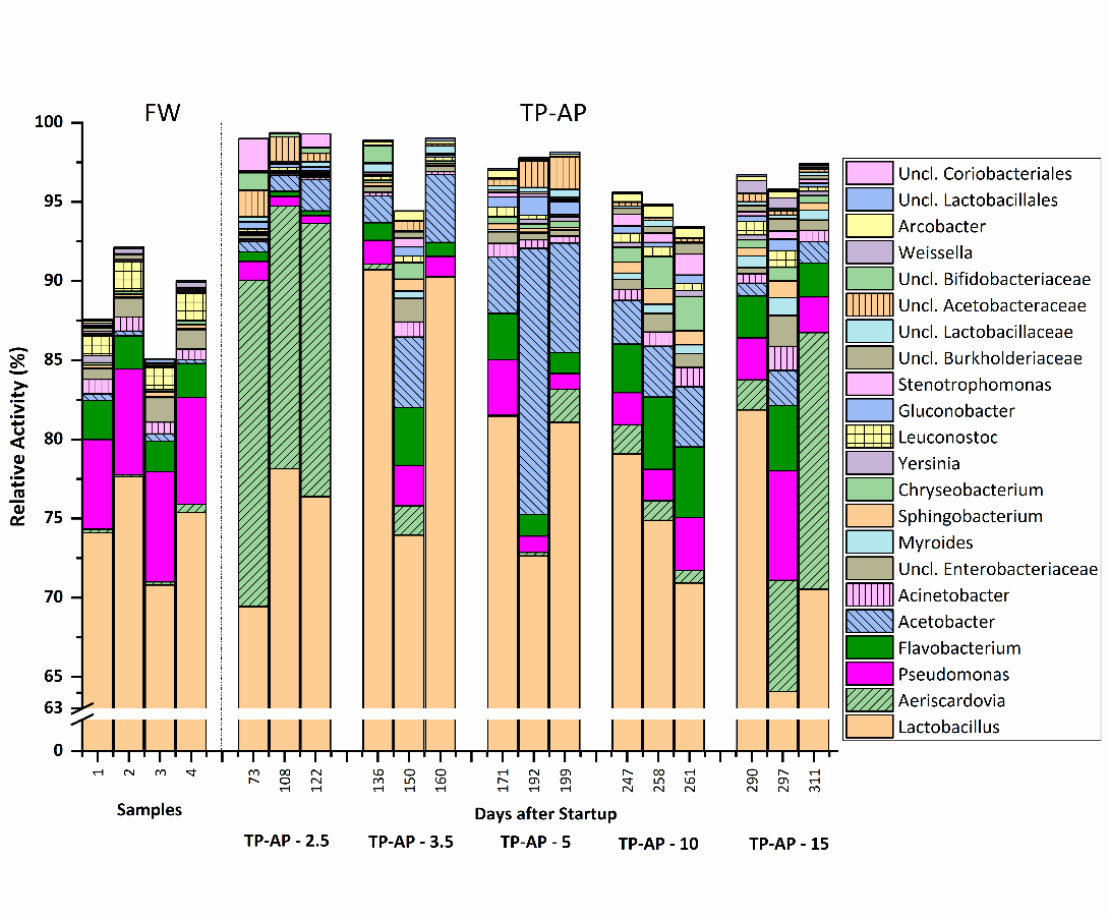

Figure S5. Relative activity of microbial communities in FW and TP-AP based on 16S rRNA sequencing, identified to the genus level where possible. FW samples were retrieved from the full-scale AnMBR operated by Divert Inc. weekly in December, 2017 (denoted as Sample 1-4). TP-AP samples were from the different OLRs at the bench-scale and the numbers on the x-axis represent days after startup of the AnMBR. All data are expressed as a percentage normalized using total 16S rRNA sequences (*Bacteria* and *Archaea*). A y-axis break (at 63%) was used to accentuate differences in lower activity populations.

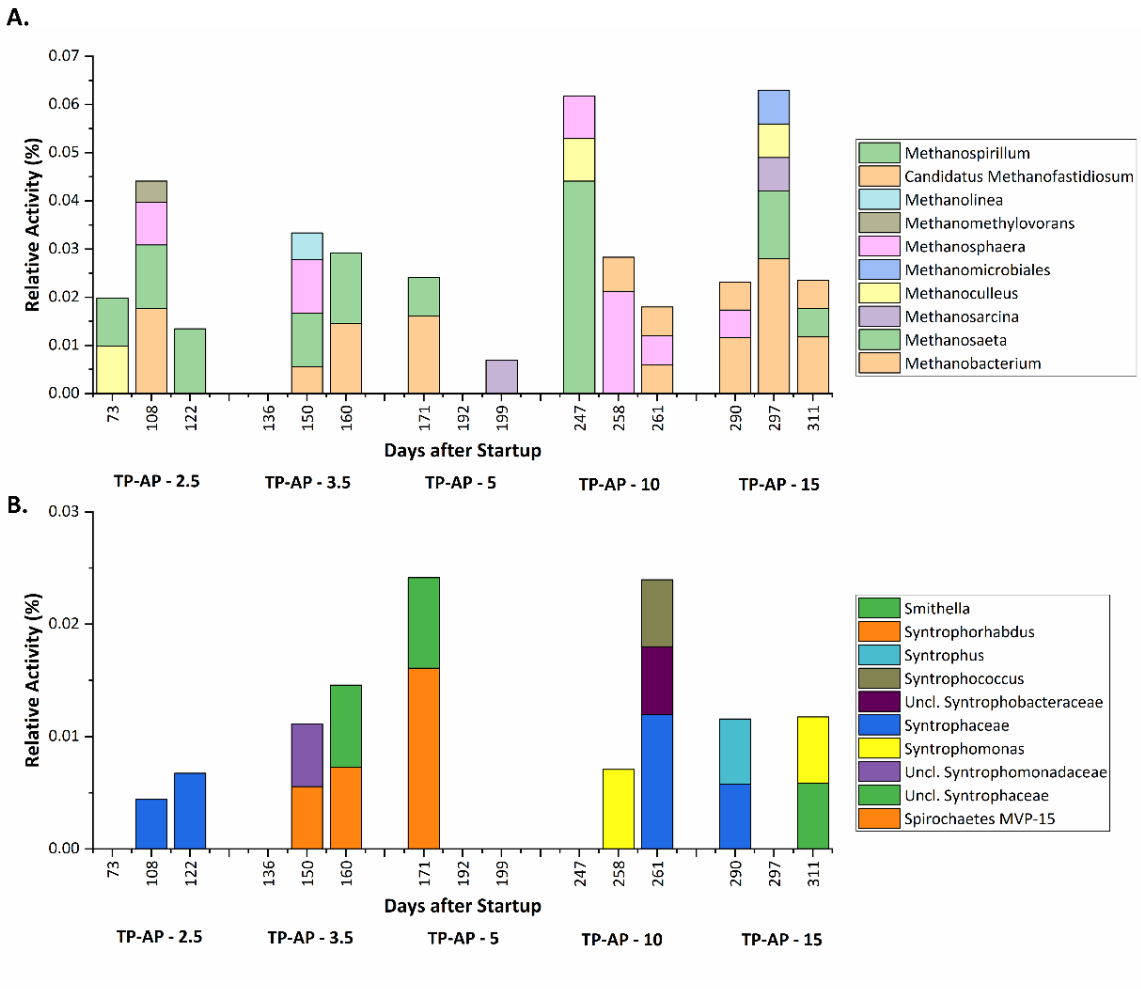

Figure S6. (A) Relative activity of methanogens identified at the genus level where possible based on 16S rRNA sequencing and **(B)** relative activity of syntrophic fatty-acid oxidizers identified at the genus level where possible using 16S rRNA sequencing for TP-AP samples. Results are expressed as a percentage normalized using total of 16S rRNA sequences (*Bacteria* and *Archaea*). Truncated y-axes (0 to 0.07% and 0 to 0.03% on figure A and B, respectively) are shown to accentuate differences in abundance.

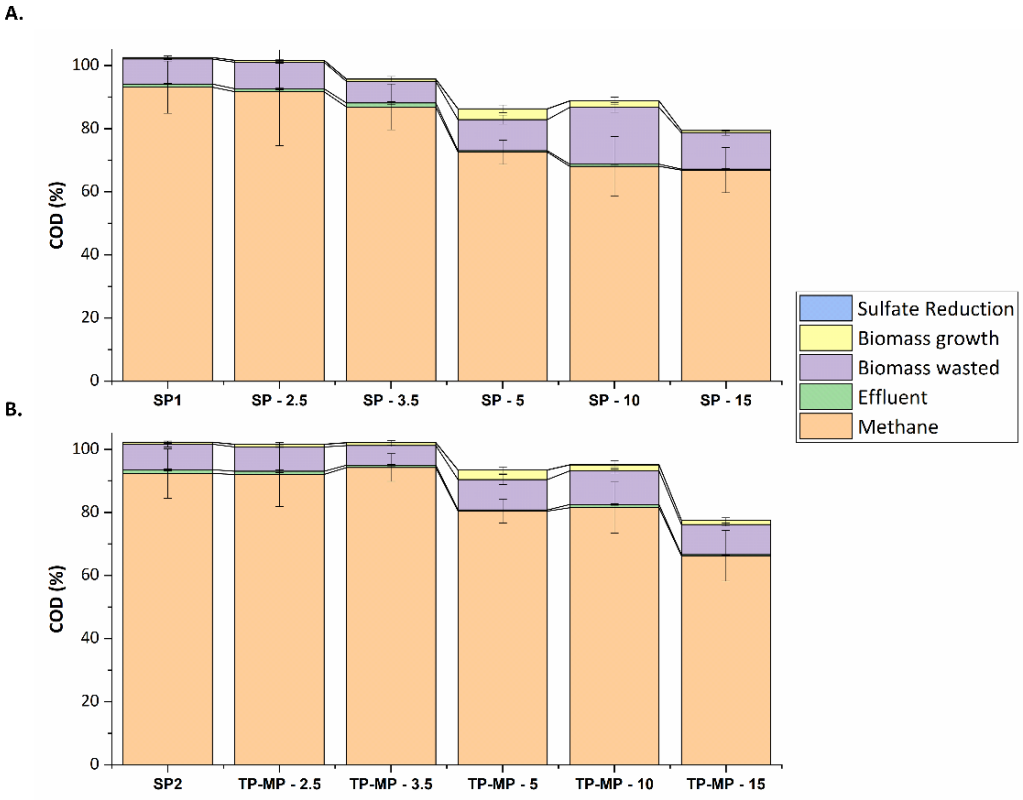

Figure S7 (A) Mass-balance analysis for SP and **(B)** TP-MP based on COD allocation of output relative to input COD (%), at different OLRs. Complete sulfate reduction was assumed based on influent sulfate concentration. Sulfate was measured in the FW and then the stoichiometric COD removal associated with sulfate reduction was accounted for in the COD mass balance. COD accumulation at high OLR resulted in select bars being <100%. The main reason for the inaccuracy or unaccounted COD is the accumulation of FW in the AnMBRs. The COD to TVS ratio for the FW is approximately 2 g COD $gTVS^{-1}$ based on our measurements. At higher OLR we observed that untreated FW was accumulating in the reactors, particularly at 10 g COD $L{\cdot}d^{-1}$ for SP and 15 g COD $L{\cdot}d^{-1}$ for both reactors. Visually, we also observed that the sludge changed from dark black to yellowish color at these respective OLRs. We confirmed that g COD $gTVS^{-1}$ in the sludge was also more than 1.42 g COD $gTVS^{-1}$ at increasing OLRs (the theoretical value for biomass). For example, at 10 g COD $L{\cdot}d^{-1}$, the COD to TVS ratio in SP and TP-MP was 1.73 and 1.55, respectively (one sampling time-point). Similarly, at 15 g COD $L{\cdot}d^{-1}$ condition, this ratio was 1.56 ± 0.02 for SP and 1.54 ± 0.07 for TP-MP (two sampling time-points). We did not continuously monitor the COD concentration of the biomass, therefore, we used a general conversion 1.42 g COD g TVS^{-1} for the COD mass balance, to account for wasted biomass and growth. We theorize that as undigested FW was accumulating in the reactor, there was increase in unaccounted COD with the wasted biomass and accumulated COD within the reactor.

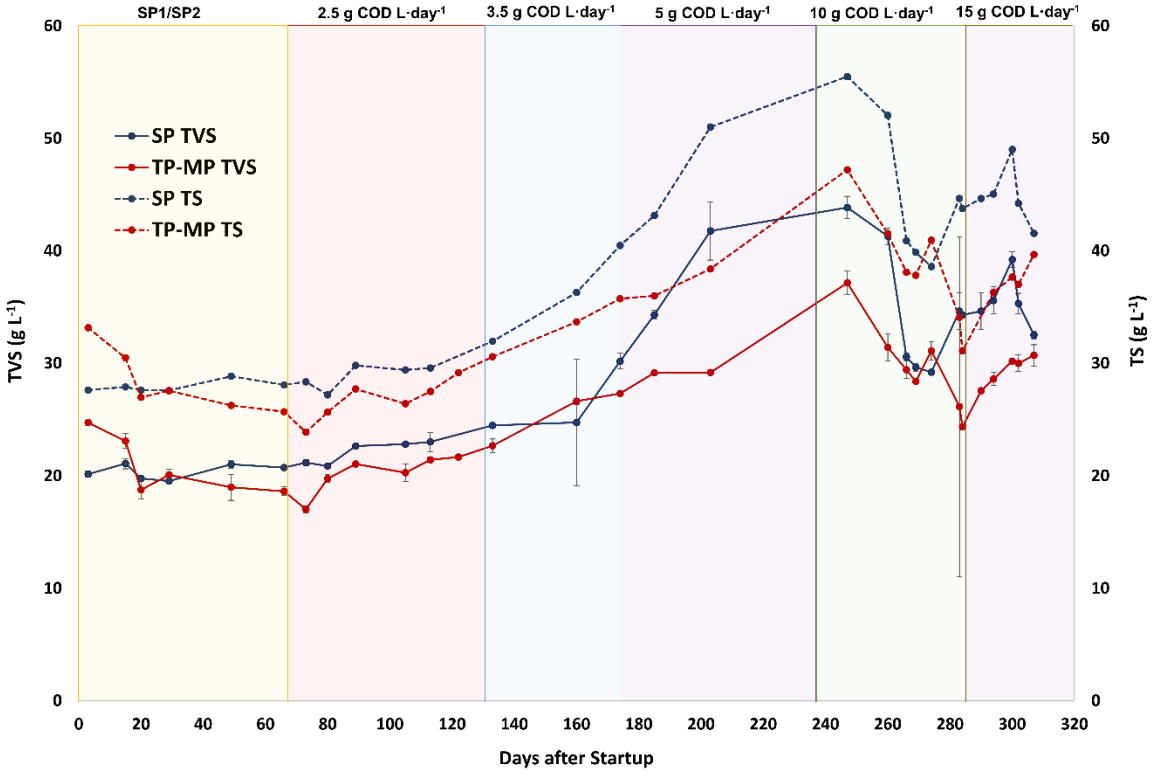

Figure S8. TVS concentration (primary y-axis), signified by solid line, and TS concentration (secondary y-axis) signified by broken line for SP and TP-MP at different OLRs. Error bars for TVS and TS concentrations represent the standard deviation for duplicate samples.

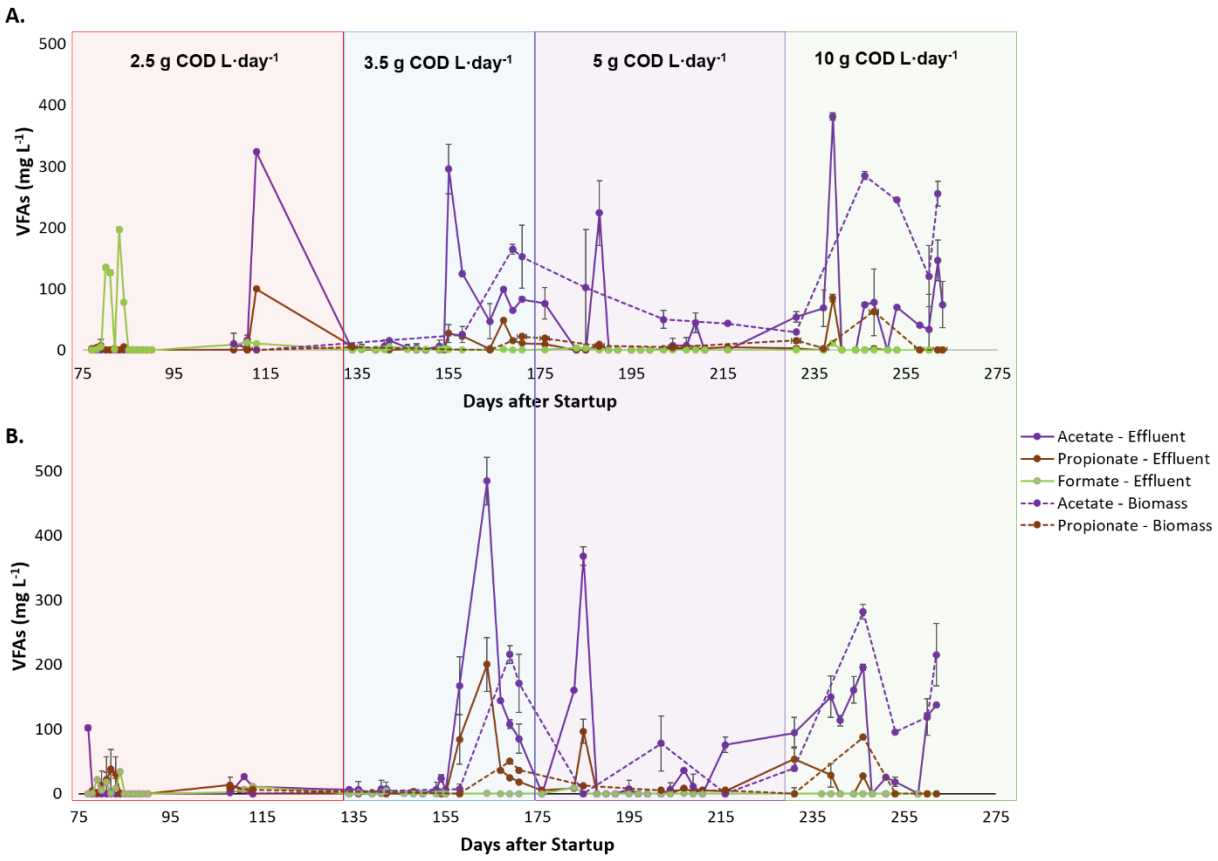

Figure S9. (A) VFAs concentration in SP, and **(B)** TP-MP for effluent (solid-line) and biomass (dashed-line) samples at different OLRs. Error bars for VFAs concentration represent the standard deviation for triplicate samples.

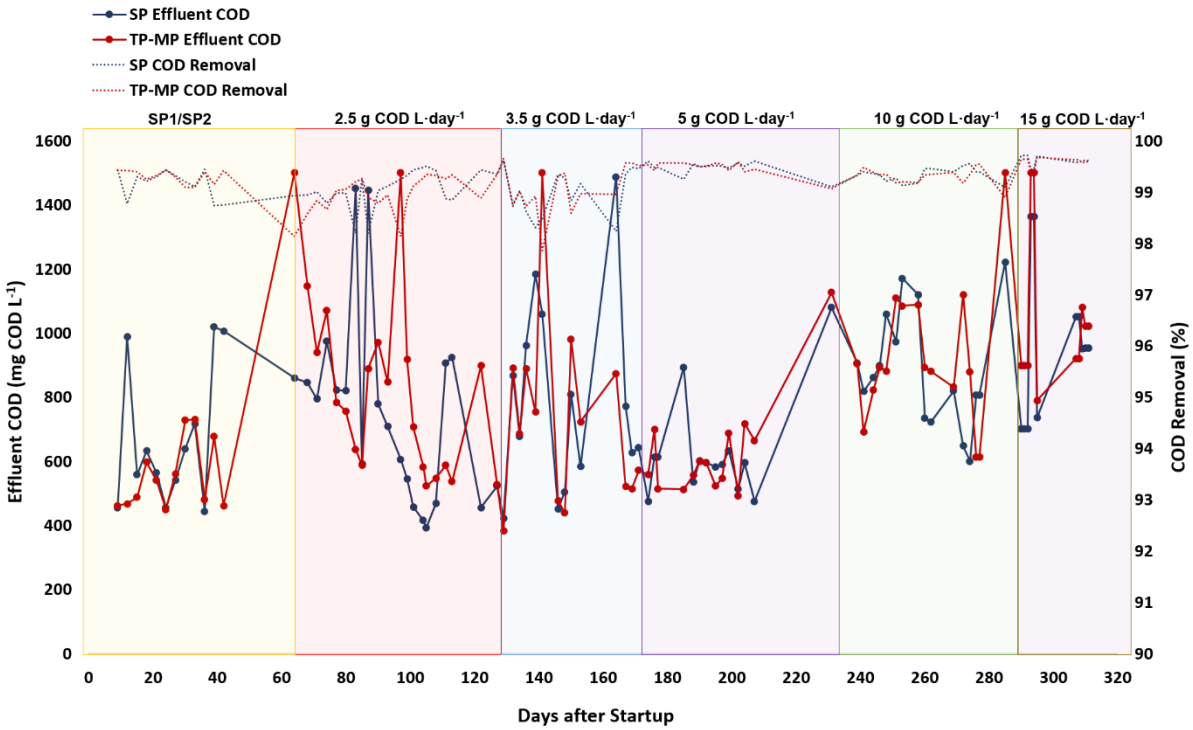

Figure S10. COD concentration in effluent (primary y-axis) and COD removal efficiency (secondary y-axis) for different OLRs for SP and TP-MP. The solid line show effluent concentration and dashed lines indicated COD removal efficiency based on influent COD.

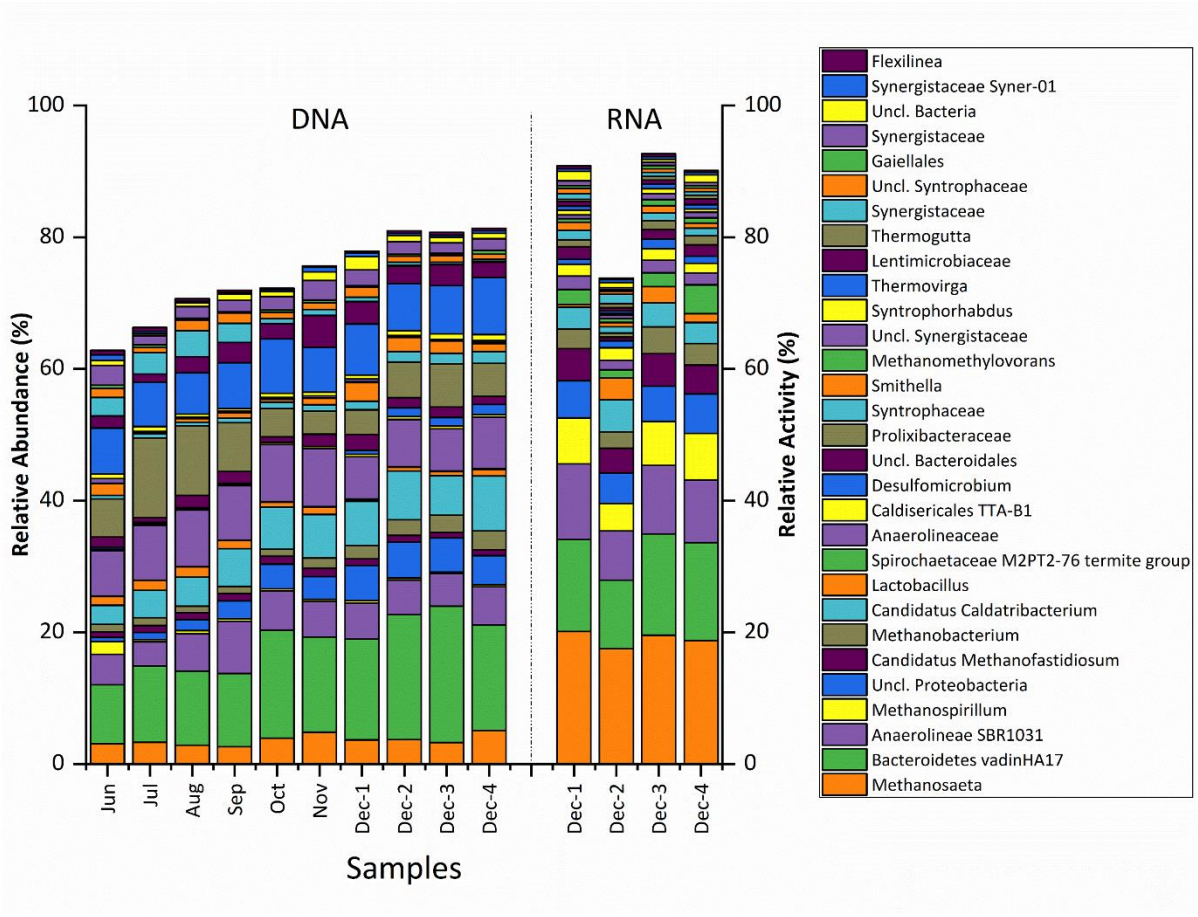

Figure S11. Relative abundance (primary y-axis) and relative activity (secondary y-axis) of microbial communities in mixed liquor (ML) samples from the full-scale AnMBR (Divert, Inc.) based on 16S rRNA gene and 16S rRNA sequencing, respectively. The microbial communities were identified to the genus level where possible. ML samples were retrieved monthly (June-November, 2017) and weekly in December, 2017 (denoted as Sample 1-4). Monthly relative abundance data are average values for triplicate samples. All data are expressed as a percentage normalized using total 16S rRNA gene sequences (*Bacteria* and *Archaea*) and 16S rRNA sequences (*Bacteria* and *Archaea*) for relative abundance and relative activity data, respectively.

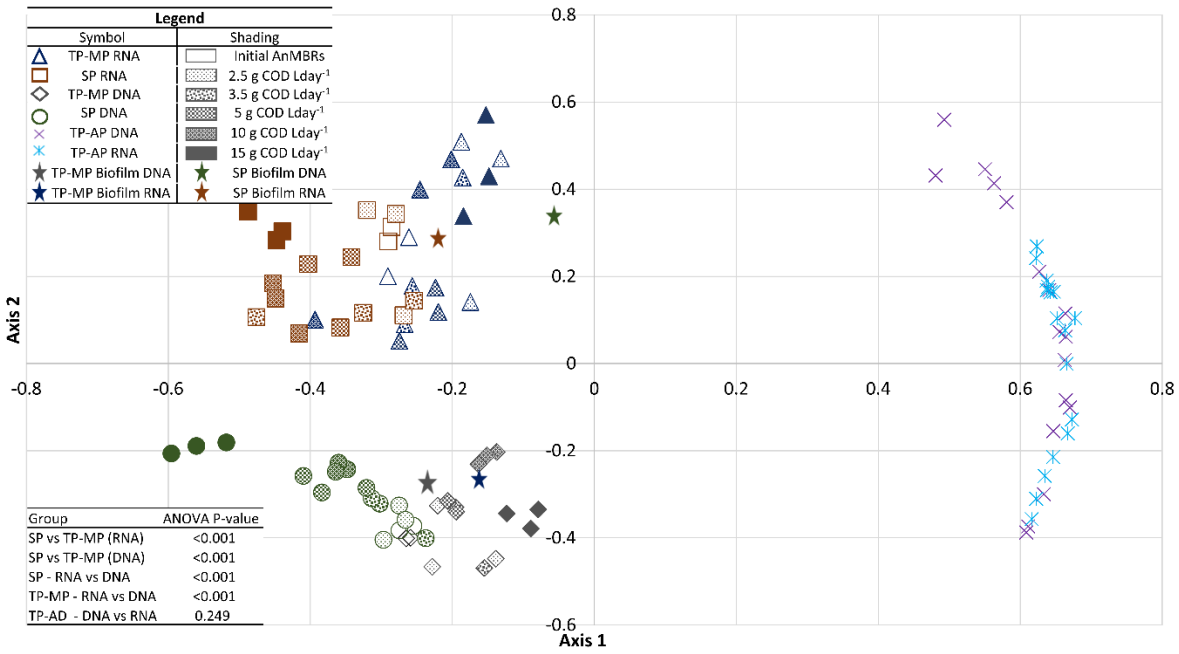

Figure S12. NMDS plot showing ordination of microbial community structure (DNA-based) and activity (RNA-based) for SP, TP-MP, and TP-AP samples. The different fills for SP and TP-MP samples indicate different OLRs. The star symbols signify biofilm samples from SP and TP-MP. The table shows two-tailed t-test to test the hypothesis that the clustering of ordination between different groups is significant, where the p-value<0.05 indicates significant differential clustering. The stress values for the NMDS at 2 and 3 dimension analysis were 0.19 and 0.11, respectively.

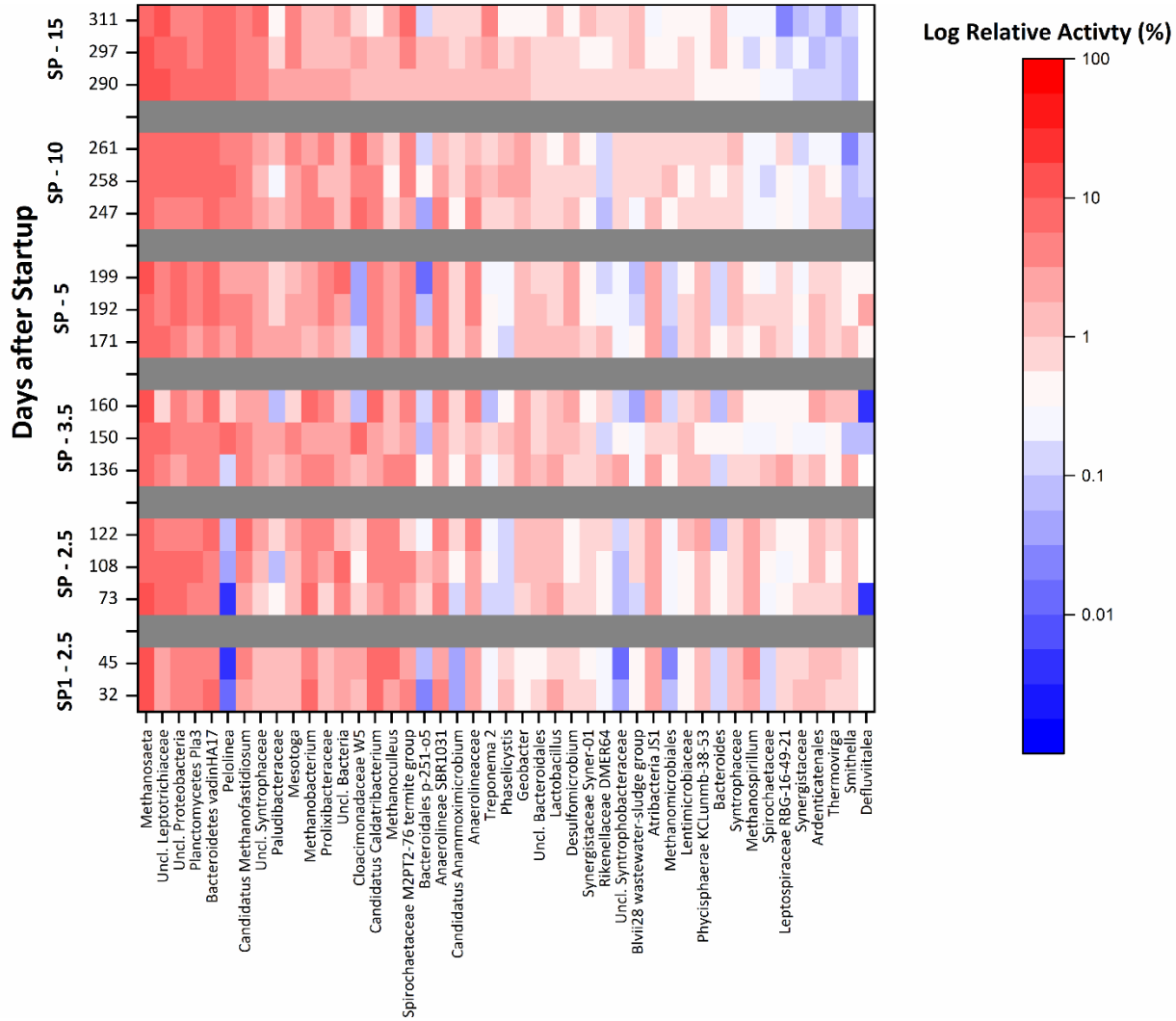

Figure S13. Heat-map showing log relative activity (%) of microbial communities in SP based on 16S rRNA sequencing, identified to the genus level where possible. All data are expressed as a percentage normalized using total 16S rRNA sequences (*Bacteria* and *Archaea*). Only groups that showed ≥ 0.5% relative activity in at least one sample are shown here.

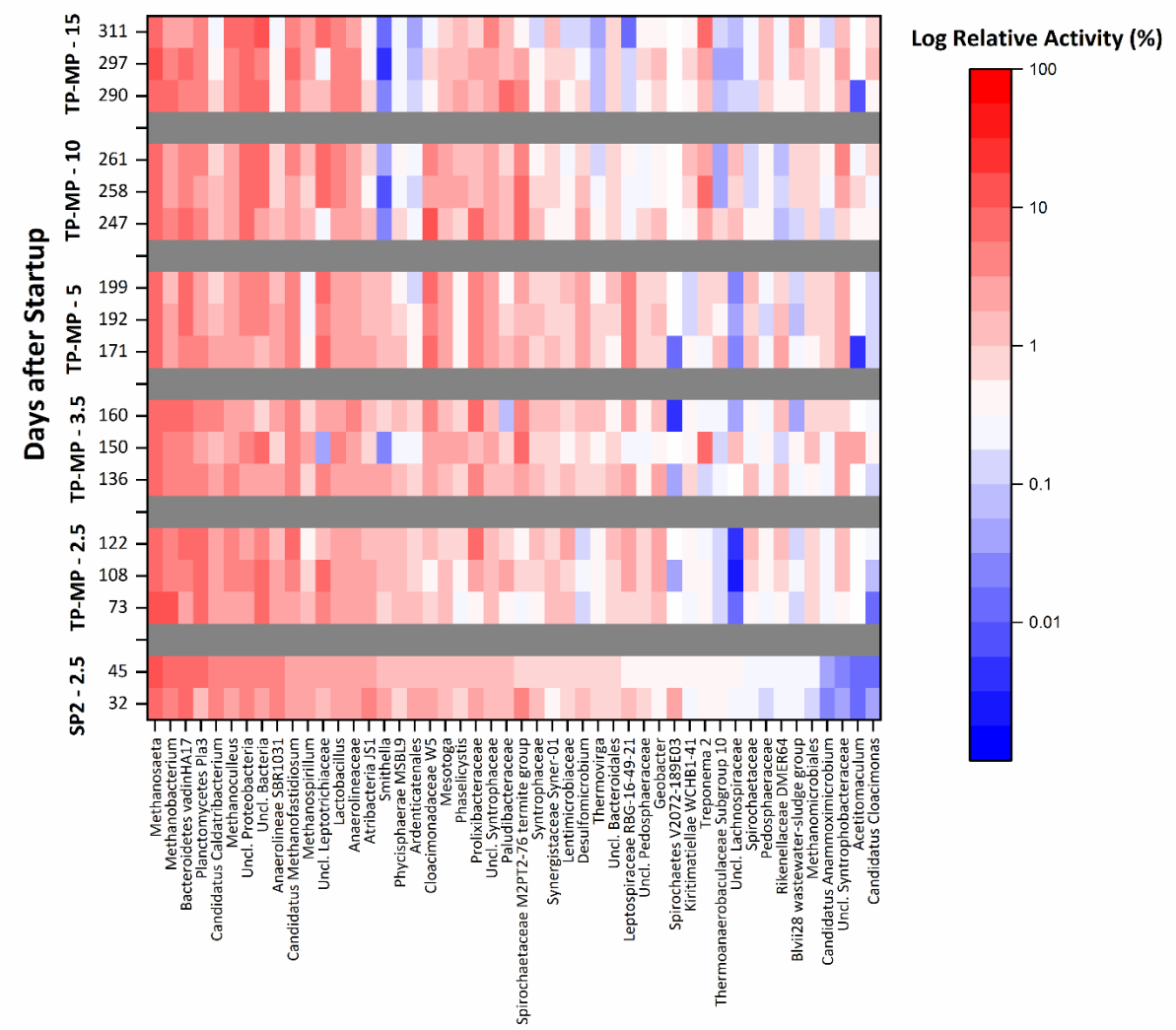

Figure S14. Heat-map showing log relative activity (%) of microbial communities in TP-MP based on 16S rRNA sequencing, identified to the genus level where possible. All data are expressed as a percentage normalized using total 16S rRNA sequences (*Bacteria* and *Archaea*). Only groups that showed ≥ 0.5% relative activity in at least one sample are shown here.

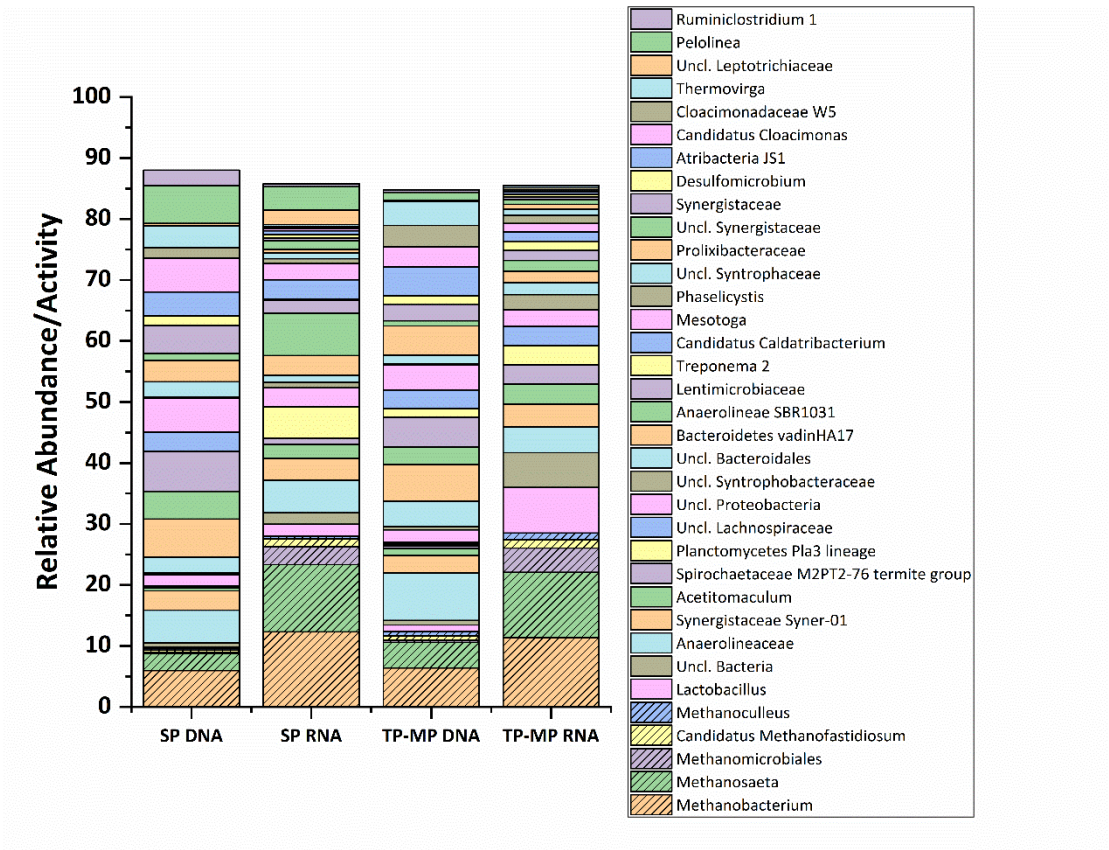

Figure S15. Relative abundance (for DNA) and relative activity (for RNA) of biofilm communities in SP and TP-MP at an OLR of 10 g COD L·day^{-1} based on 16S rRNA sequencing, identified to the genus level where possible. All data are expressed as a percentage normalized using total 16S rRNA sequences (*Bacteria* and *Archaea*).