

Supplementary Material

In anaerobic reactors the microbial community structure depends on feed type, with no “keystone” species tied to COD removal

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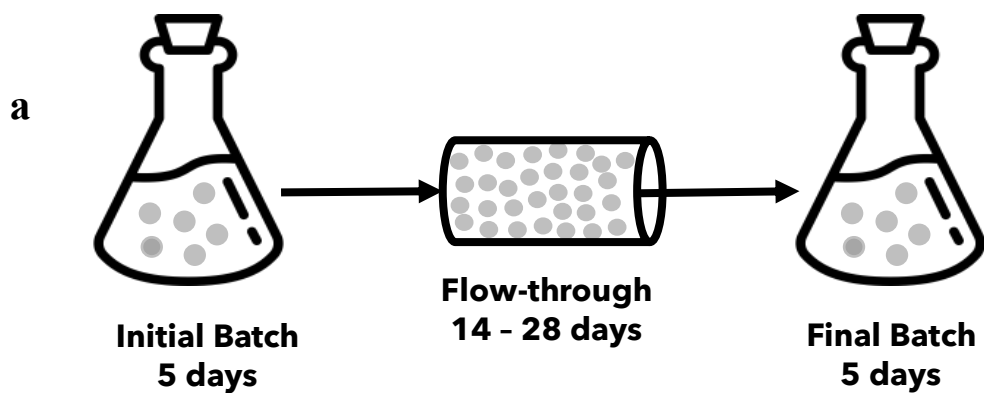
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Supplemental Methods

Table S1. Synthetic wastewater recipes used in the experiment

Wastewater Type	Quantities (g) added to water to a final volume of 1 L						
	Polysorbate 80	Gelatin	Meat Extract	Casamino Acids	Starch	Yeast Extract	Rapeseed Oil
Starch-Rich	0.96	1.5	-	0.08	4.0	0.08	-
Protein-Rich	0.96	3.5	1.0	0.08	1.0	0.08	-
Lipid-Rich	0.96	2.32	-	0.08	1.18	0.08	2.0



b



Figure S1. (a) Schematic showing the three-phase system including an initial batch reactor, a flow-through reactor and final batch reactor. After 5 days, beads were removed from the batch reactor and placed in the flow-through reactor. After 14 days in the flow-through reactor, they were returned to batch reactor to assess changes in VFA production and sCOD degradation over a final 5-day batch period. **(b)** Image of beads made from 2nd-stage methanogenic cultures.

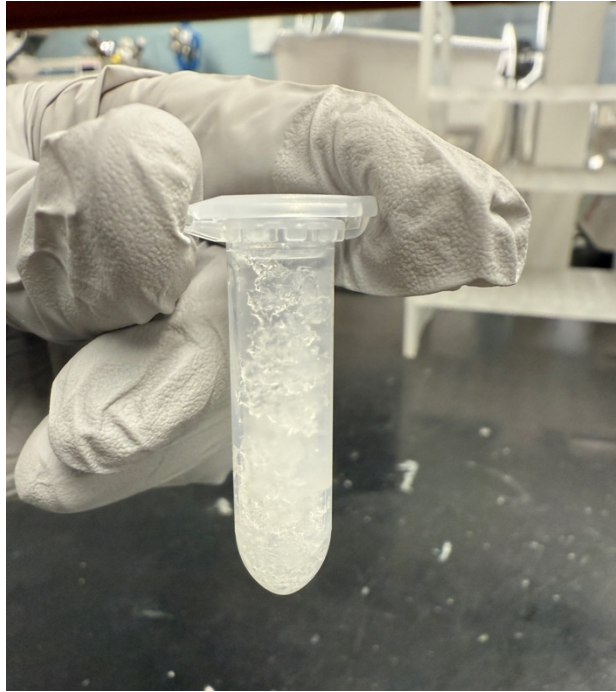


Figure S2. Photo of crushed bead used for DNA extraction.

Supplemental Results

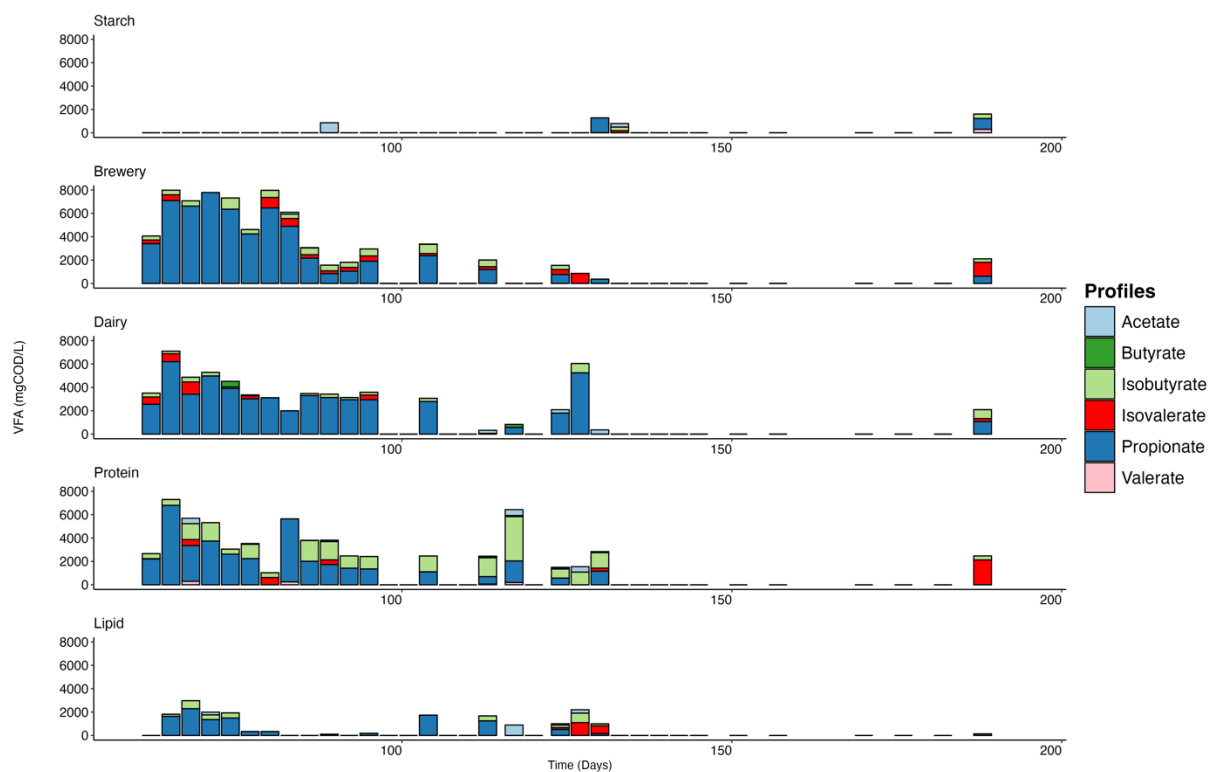


Figure S3. VFA production over time in the 1st-stage reactors fed different wastewaters. The wastewater feed type is shown for each panel. On the days with no results shown, no VFAs were detected in samples.

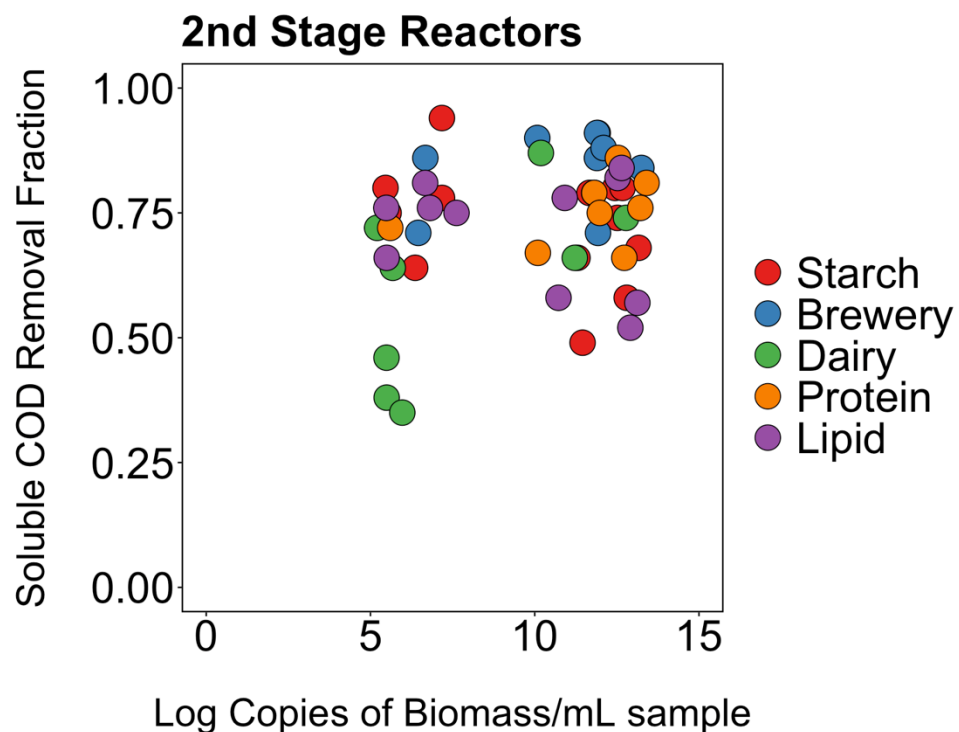
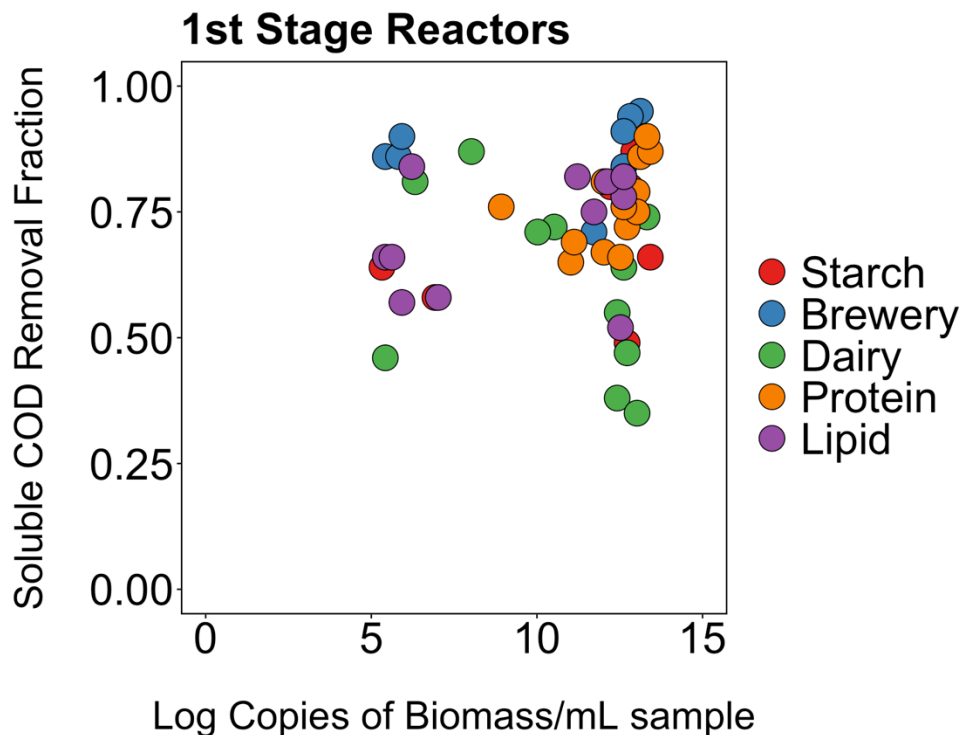
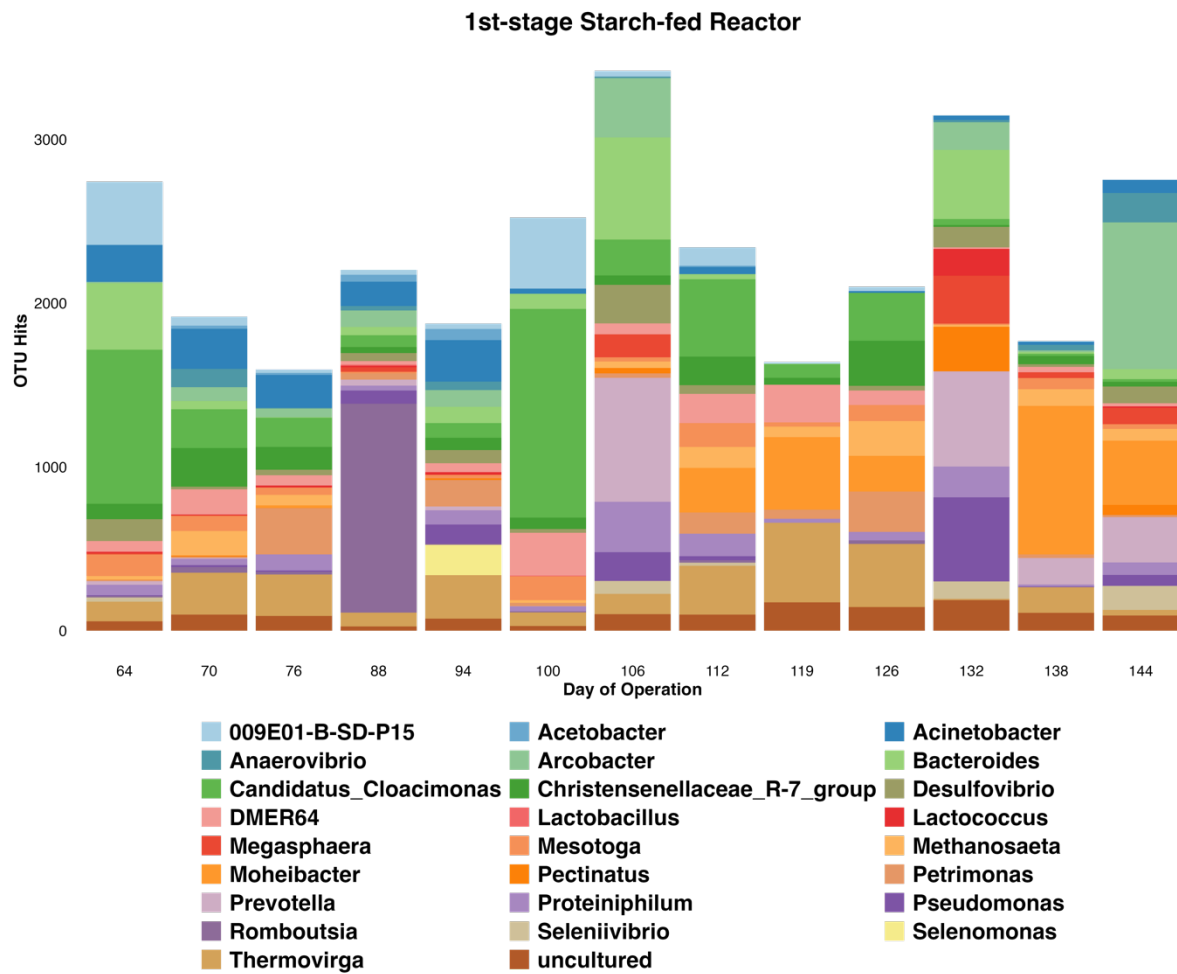
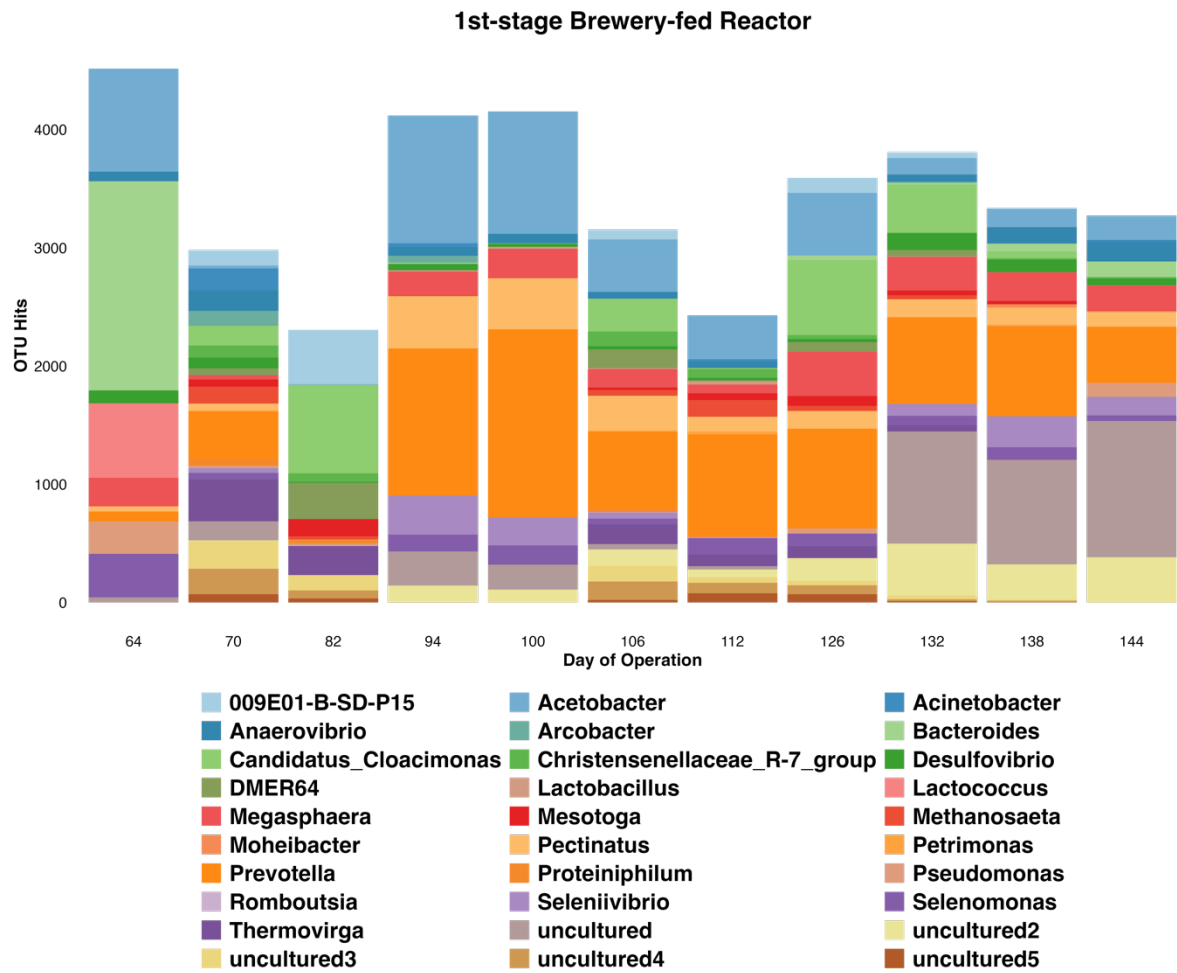
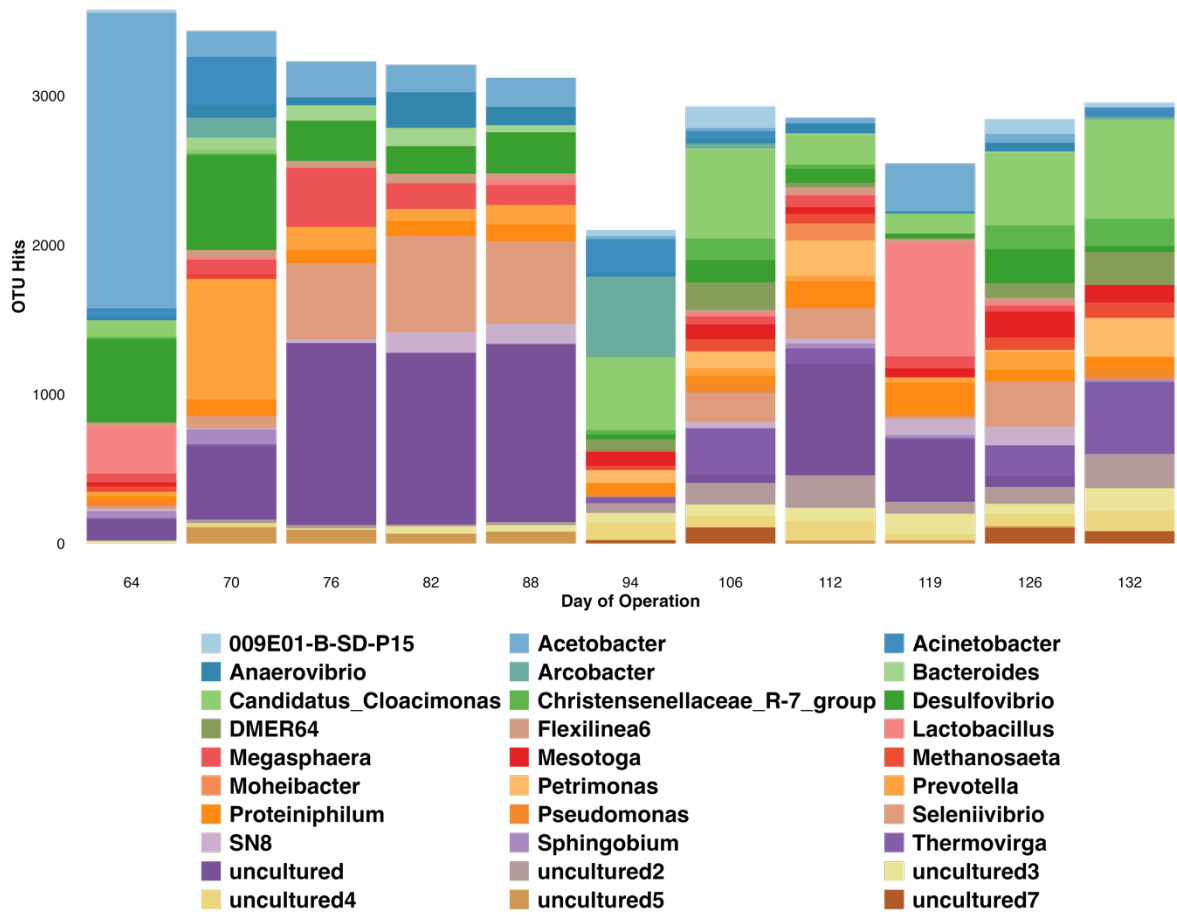


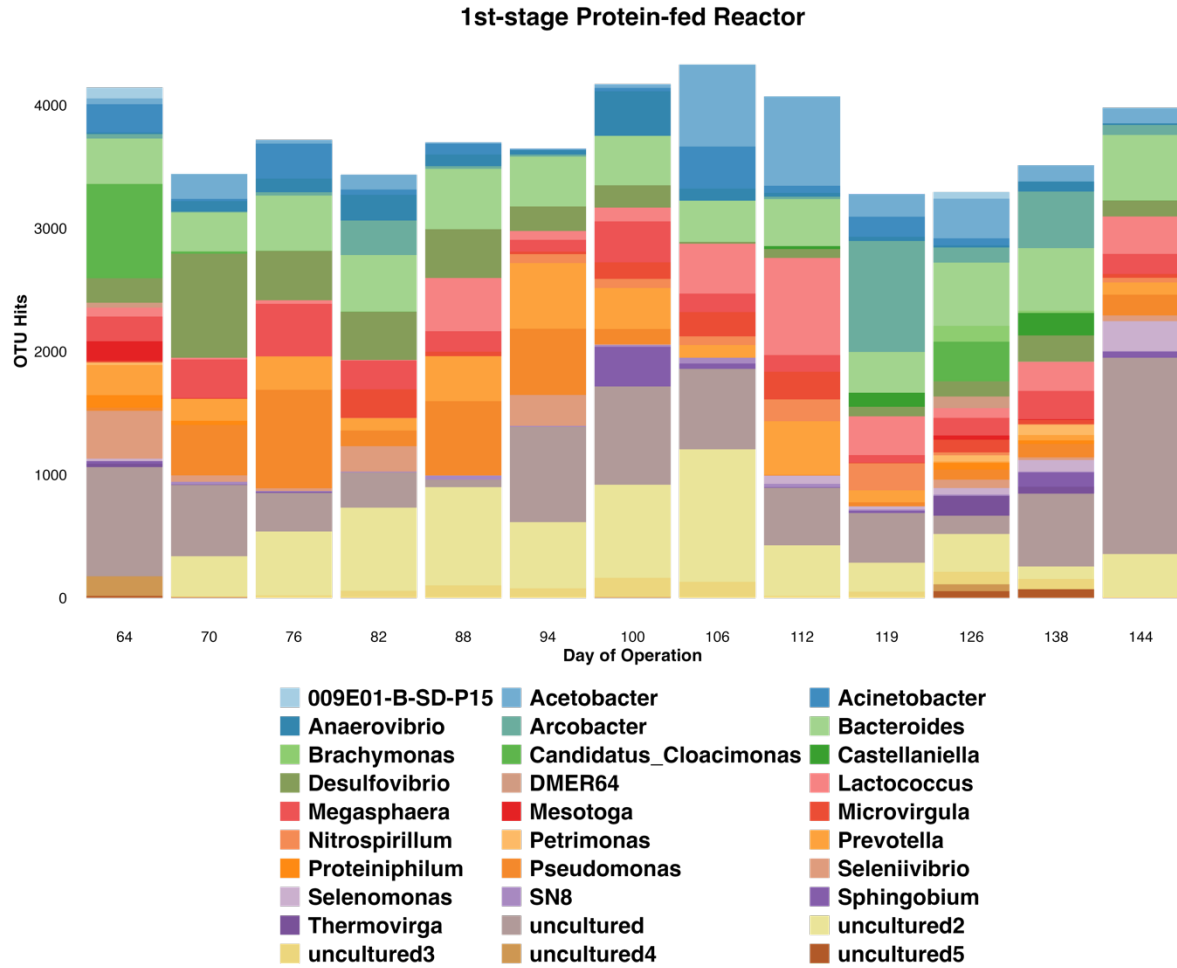
Figure S4. Total two-stage system COD removal as a function of total biomass concentrations (measured as total 16S rRNA gene copies/mL reactor) present in the 1st-stage and 2nd-stage reactors. Correlations between COD removal and log copy of biomass/mL reactor were not statistically significant, with p-values of 0.75 for the 1st-stage, 0.53 for the 2nd-stage.





1st-stage Dairy-fed Reactor





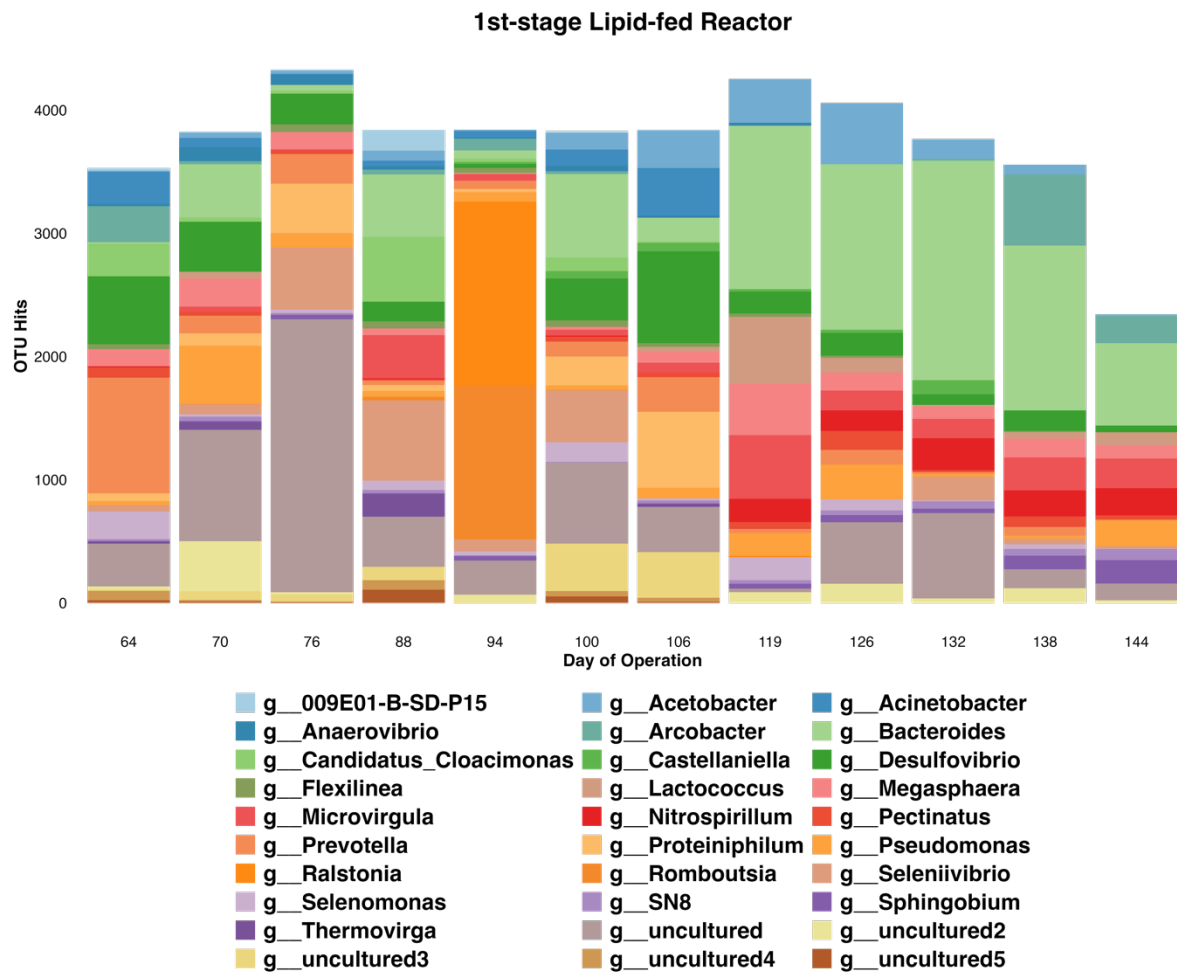
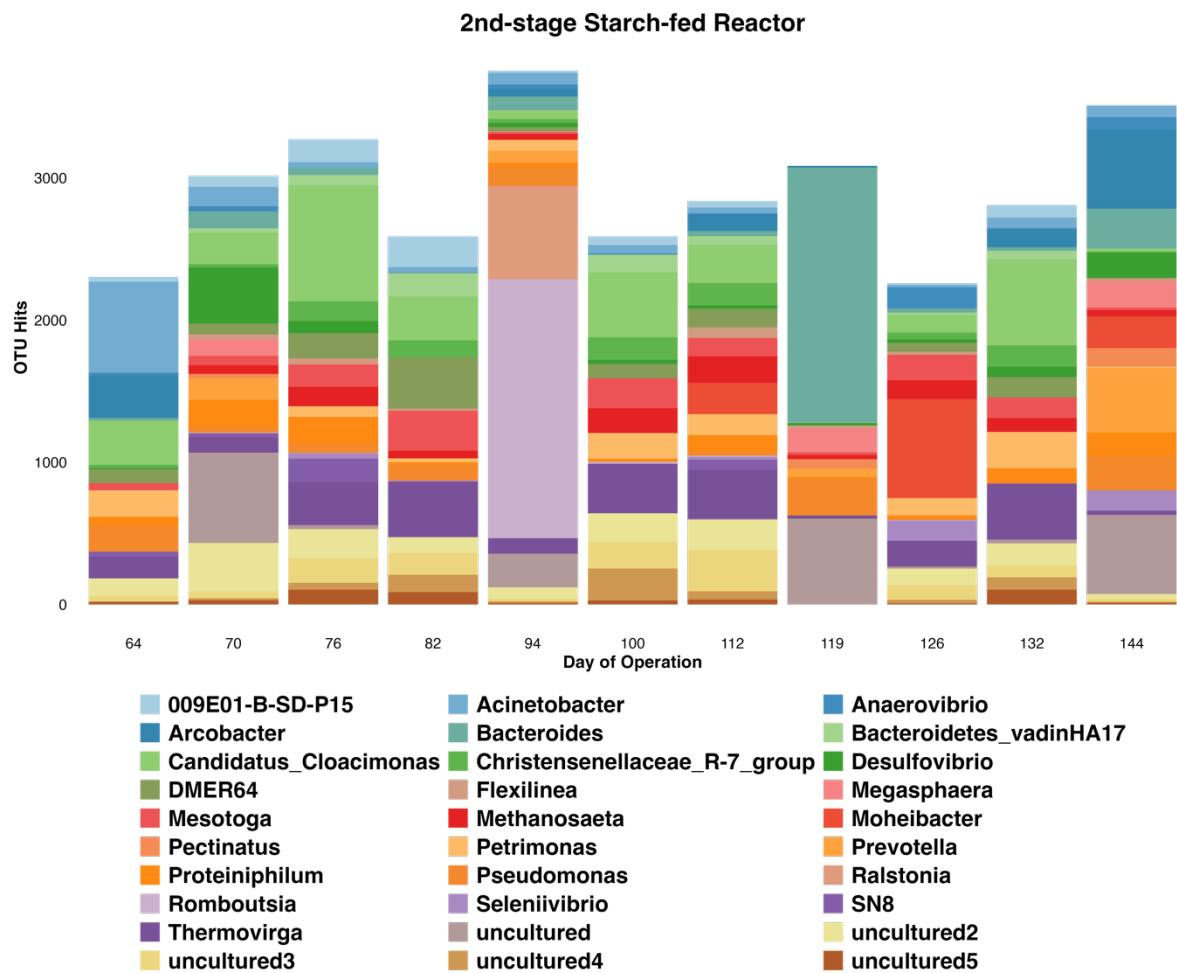
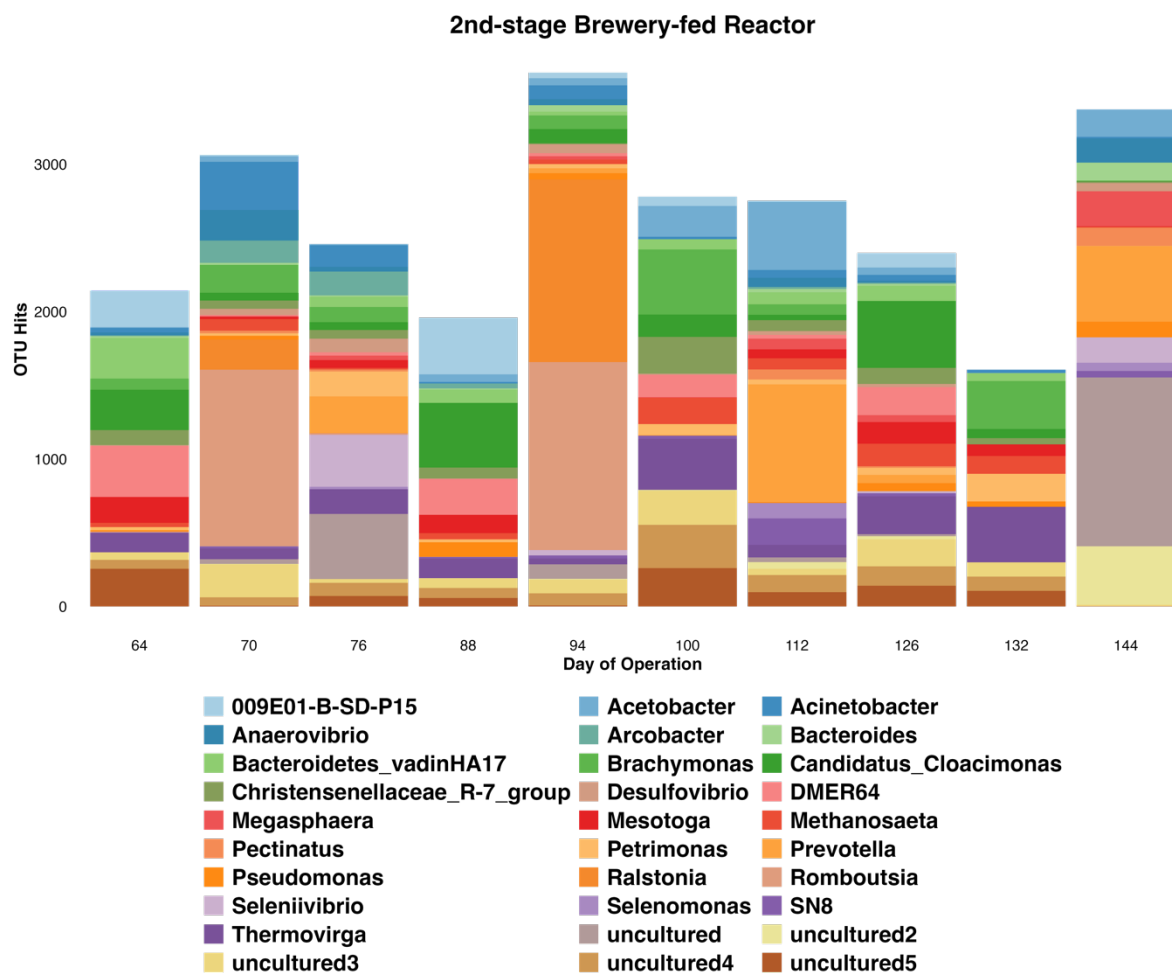
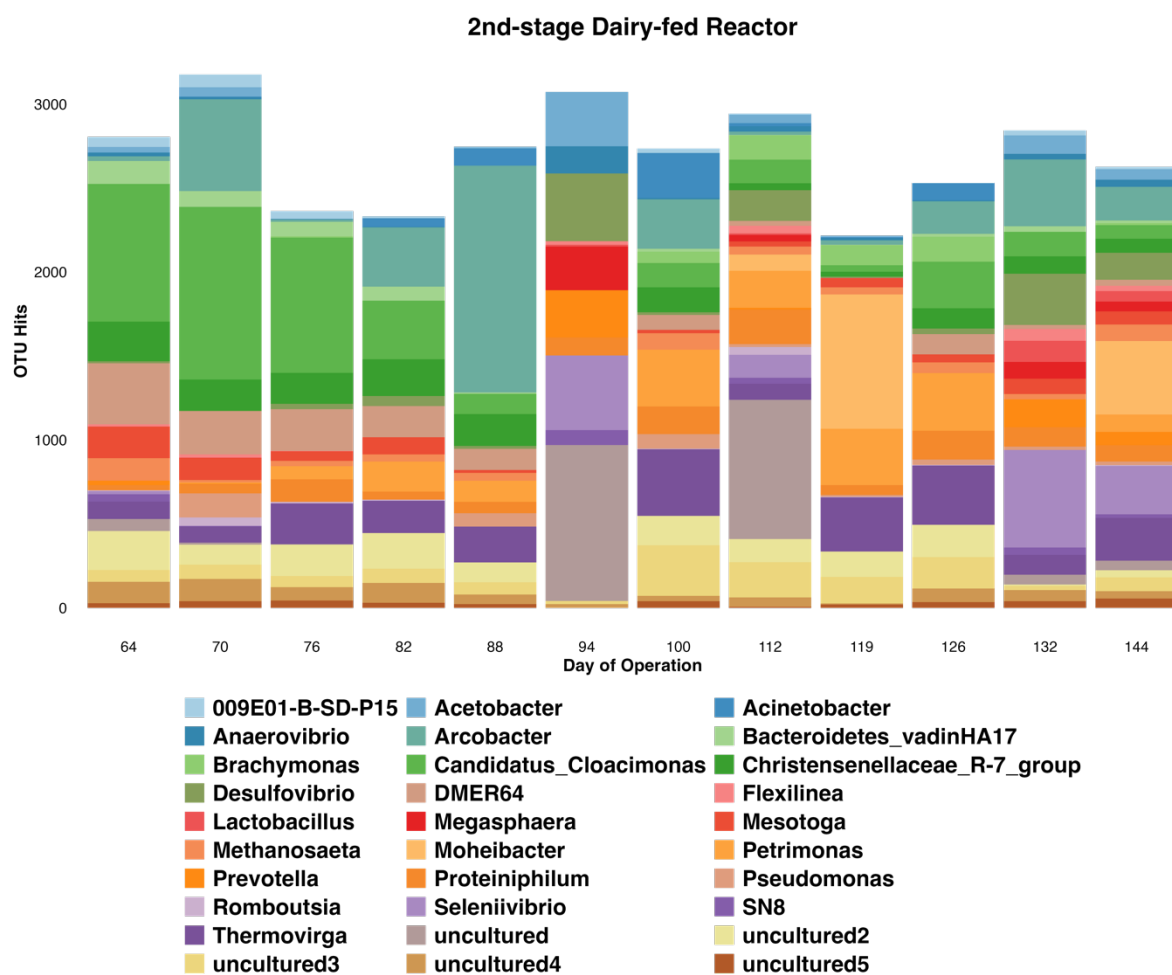
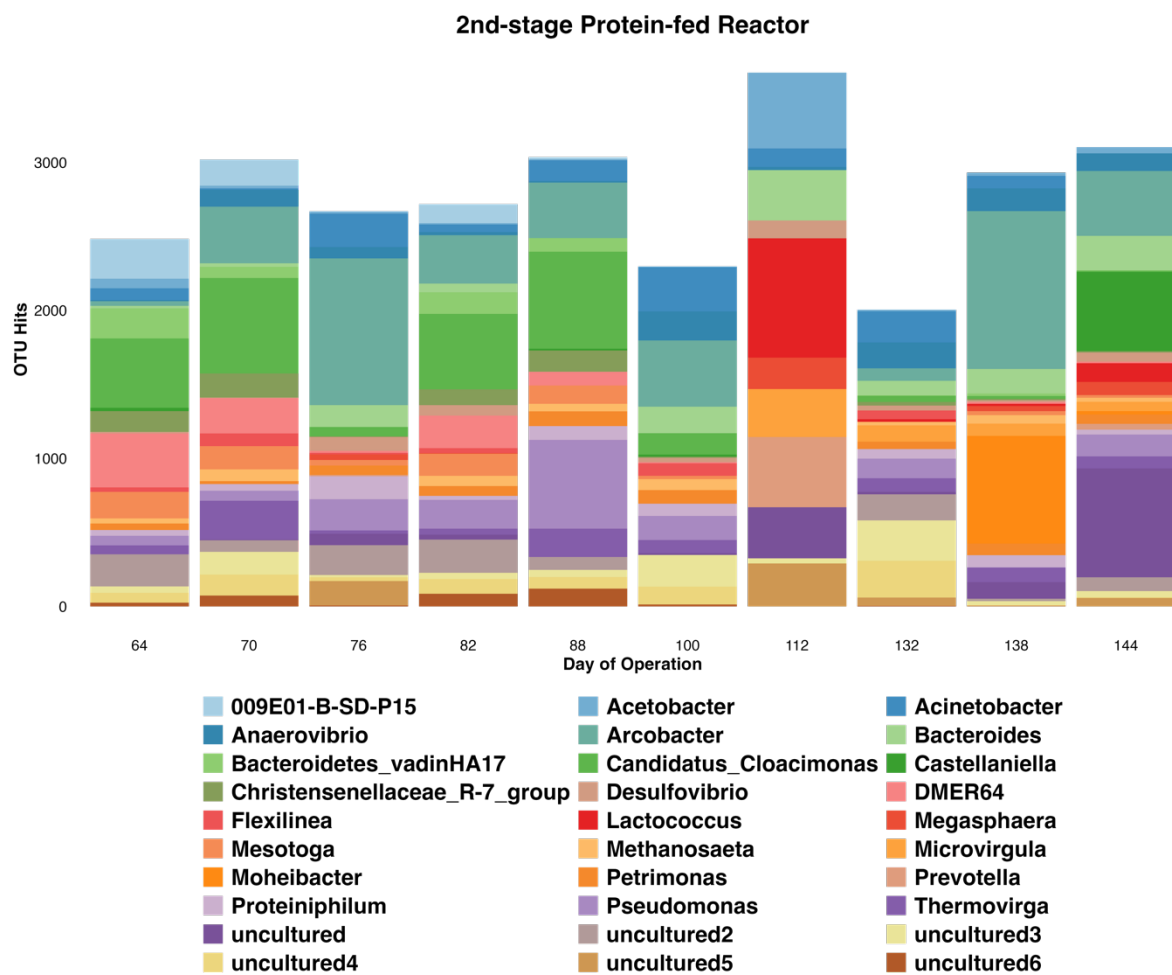


Figure S5. The top 30 OTUs identified in the 1st-stage reactors fed the five different feeds.









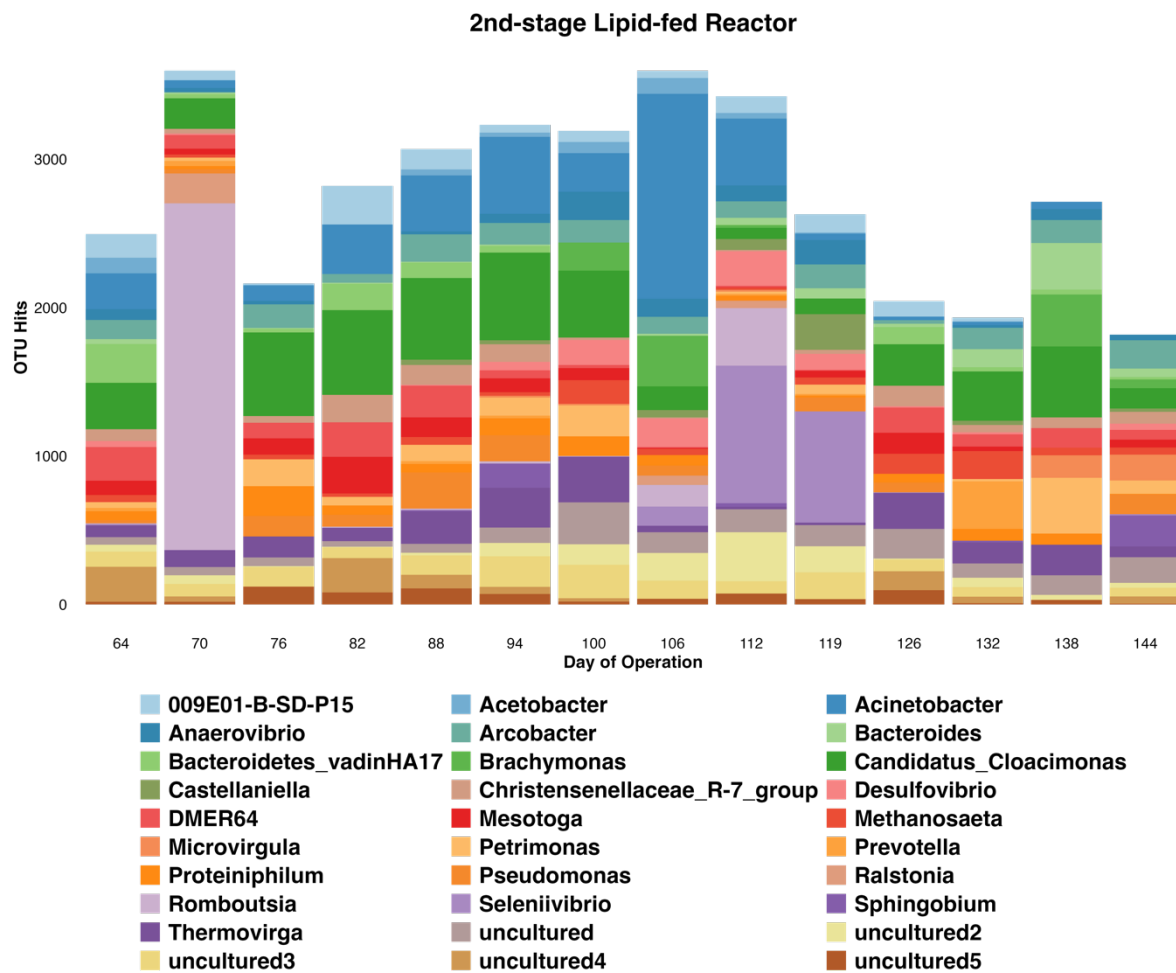


Figure S6. Top 30 OTUs identified in the 2nd-stage reactors receiving effluent from the 1st-stage reactors.

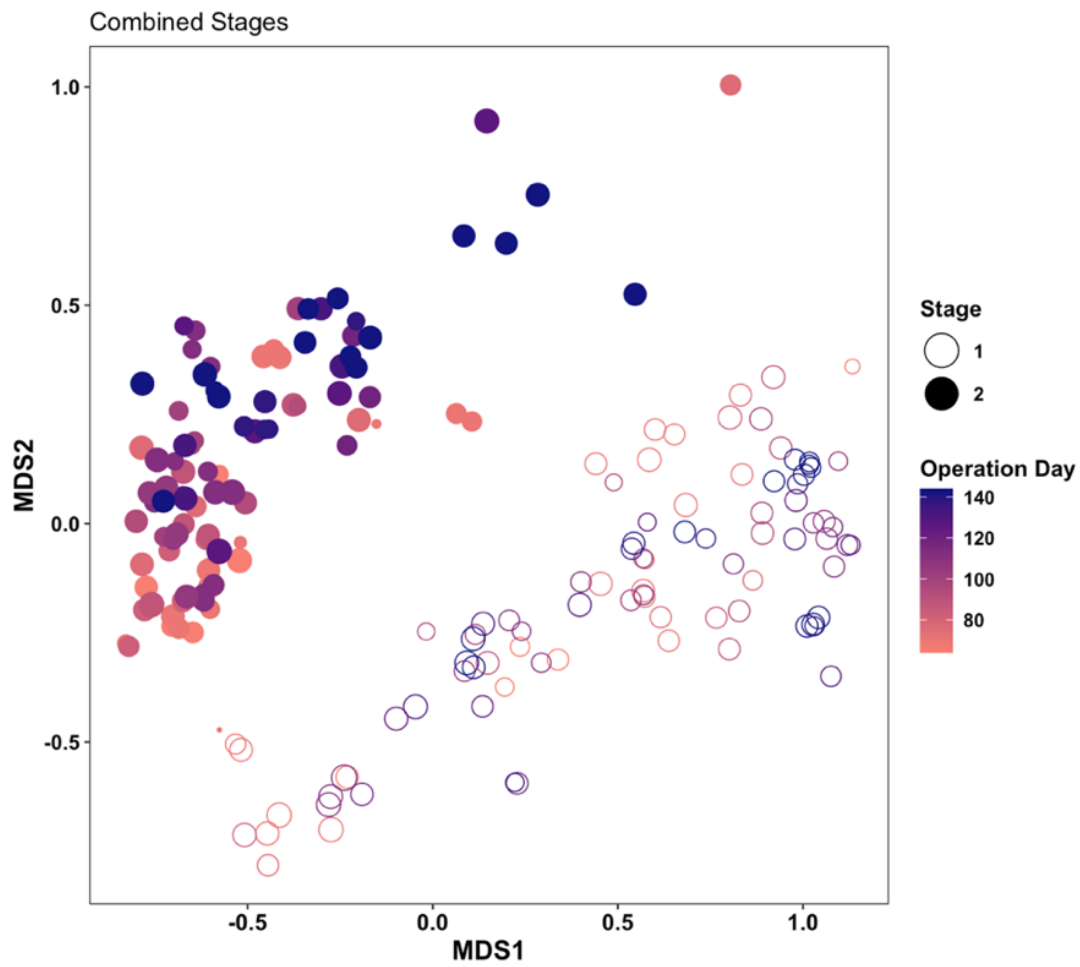


Figure S7. Non-multidimensional Scaling (MDS) using Bray-Curtis dissimilarity, showing the differences in the microbial community structure in both reactor stages over time.

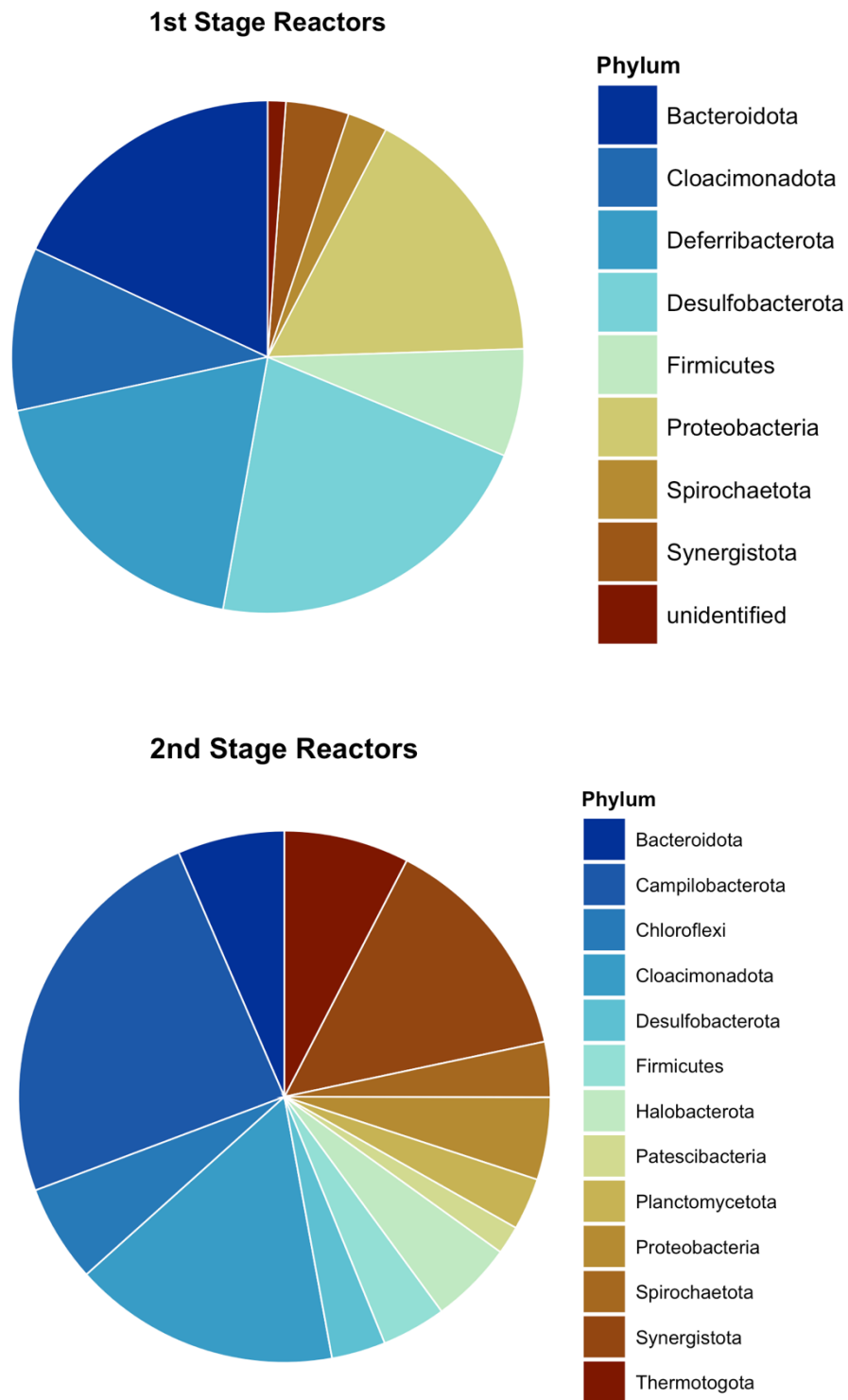


Figure S8. Core communities on the phylum level common to all feeds in the (a) 1st-stage and (b) the 2nd-stage reactor samples.

Table S2. Core genera identified in the 1st and 2nd-stage reactors

1st -stage Core Genera	2nd-stage Core Genera
Uncultured	Uncultured
<i>Prevotella</i>	<i>Bacteroides</i>
<i>Bacteroides</i>	<i>Acetobacter</i>
<i>Acetobacter</i>	<i>Candidatus Cloacimonas</i>
<i>Candidatus Cloacimonas</i>	<i>Pseudomonas</i>
<i>Pseudomonas</i>	<i>Arcobacter</i>
<i>Desulfovibrio</i>	<i>Desulfovibrio</i>
<i>Megasphaera</i>	<i>Thermovirga</i>
Uncultured	Uncultured
Uncultured	<i>Acinetobacter</i>
<i>Seleniivibrio</i>	Uncultured
<i>Lactococcus</i>	DMER64
<i>Proteiniphilum</i>	<i>Proteiniphilum</i>
<i>Anaerovibrio</i>	<i>Anaerovibrio</i>
<i>Pectinatus</i>	<i>Christensenellaceae R-7 group</i>
Uncultured	<i>Petrimonas</i>
SN8	<i>Mesotoga</i>
<i>Nitrospirillum</i>	<i>Methanosaeta</i>
<i>Macellibacteroides</i>	009E01-B-SD-P15
<i>Acidaminococcus</i>	Uncultured
UCG-009	Uncultured
Unidentified_1	<i>Flexilinea</i>
Unidentified_2	W5
Unidentified_3	<i>Macellibacteroides</i>
	<i>Fluviicola</i>
	<i>Fermentimonas</i>
	<i>Sphaerochaeta</i>
	<i>Dechlorobacter</i>
	<i>Leptolinea</i>

	<i>Lentimicrobium</i>
	<i>Sedimentibacter</i>
	<i>Methanospirillum</i>
	Uncultured
	Syner-01
	<i>Saccharimonadales</i>
	<i>Thauera</i>
	<i>Pirellula</i>
	[Eubacterium] coprostanoligenes group
	Uncultured
	RBG-13-54-9
	<i>Dysgonomonadaceae</i>
	<i>Devosia</i>