

Supplementary Data

Table S1: Mean SCFA and NH₃ (mmol/L), grouped by substrate and time. TotBrCh refers to the sum of *iso*-butyric, *iso*-valeric and valeric acid concentrations (as an associated marker for protein fermentation).

Time	Acetic acid		Propionic acid		Butyric acid		Total SCFA		TotBrCh		NH ₃	
	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev
ACW												
0	4.1	0.71	1.2	0.28	0.6	0.14	6.5	1.06	0.6	0.07	10.4	2.05
2	3.8	1.20	1.1	0.42	0.55	0.21	5.9	2.05	0.5	0.14	11.9	-
4	6.2	0.28	1.85	0.21	1	0	9.9	0.49	0.8	0	14.6	0.07
6	8.7	0	2.7	0	1.3	0	14.2	0	1.6	0	17.8	0.85
9	14.2	0.78	4.1	0.14	1.6	0.14	22	1.27	2.1	0.28	17.4	0.64
12	19.9	0.14	5.65	0.07	2.0	0	30	0.14	2.5	0	15.2	0.14
24	26	0.07	6.6	0.14	2.4	0	37.7	0.07	2.6	0	15.5	0.56
48	27.5	0.85	6.9	0.28	2.8	0.07	40.1	1.13	3.0	0	17.9	0.14
72	28.1	0.71	7.6	0.12	2.9	0.08	42	0.8	3.3	0	18.8	0.36
ACW+TM												
0	3.6	0.07	1	0	0.5	0	5.5	0.14	0.45	0.07	6.1	0.14
2	5.7	0.92	1.6	0.28	0.8	0.14	9.2	2.1	1.15	0.92	6.5	1.20
4	8.1	1.56	2.2	0.35	1.1	0.14	12.3	2.4	1	0.28	9.0	1.55
6	10.2	0.49	2.5	0.14	1.4	0.07	15.4	0.64	1.3	0	15.6	1.20
9	14	0.99	3.6	0.42	1.7	0.07	21.3	1.7	2	0.14	19.6	0.28
12	18.1	1.2	4.7	0.14	2.0	0.07	27.2	1.4	2.5	0	20.1	1.27
24	24.8	0.71	6.4	0.21	2.3	0	36.1	0.85	2.6	0	21.9	0.92
48	27.1	0	6.5	0.14	2.6	0	39.2	0.28	3.0	0.07	23.1	0.21
72	27	0.29	7	0.12	2.8	0.06	39.8	0.22	3.2	0.1	22.9	0.36

Time	Acetic acid		Propionic acid		Butyric acid		Total SCFA		TotBrCh		NH ₃	
	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev
ACW+CAT												
0	3.4	0.07	0.9	0	0.5	0	5.2	0.14	0.4	0	8.6	0.14
2	4.7	0.14	1.4	0.07	0.65	0.07	7.3	0.35	0.5	0	11.4	0.64
4	7.3	0.14	2.1	0	1.05	0.07	11.3	0.21	0.8	0	13.7	0.99
6	10.1	0.14	2.9	0.07	1.35	0.07	15.7	0.28	1.4	0.07	13.4	0.56
9	14	0.07	3.9	0.07	1.6	0	21.7	0.21	2.2	0.07	12.9	2.75
12	17.5	0.49	5.05	0.21	1.85	0.07	27	0.92	2.6	0.21	13.6	0.92
24	26.3	0.85	6.5	0	2.4	0.14	38	1.06	2.7	0.07	15	2.83
48	27.6	0.49	6.8	0.21	2.8	0	40.1	0.71	2.9	0	19.1	0.35
72	28.1	0.46	7.7	0.14	2.95	0.13	42.1	0.41	3.3	0.1	19.1	1.37
ACW+CYAN												
0	3.7	.	1.0	-	0.5	-	5.6	-	0.5	-	8.0	-
2	4.8	0.14	1.4	0	0.7	0	7.4	0.14	0.5	0	11.1	0.14
4	7.3	0.28	2.1	0.07	1.1	0.07	11.3	0.35	0.8	0	14.7	0.42
6	9.6	0.14	2.7	0.07	1.4	0	15	0.14	1.3	0	13.0	1.41
9	14.7	0.14	4.3	0.07	1.8	0.07	23.1	0.14	2.4	0.07	19.4	0.14
12	15.4	1.63	4.5	0.64	1.7	0.14	24.1	2.55	2.6	0.14	20.3	0.49
24	25.9	0.21	6.7	0.07	2.5	0	37.7	0.35	2.8	0.21	17.8	0.77
48	28.0	0.78	7.2	0	2.9	0.07	41	0.71	3.1	0.07	19.9	0.07
72	28.0	0.35	7.9	0.06	2.9	0.06	42.1	0.40	3.3	0.06	20.1	0.40

Time	Acetic acid		Propionic acid		Butyric acid		Total SCFA		TotBrCh		NH ₃	
	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev	Mean	StdDev
ACW+FER												
0	3.5	0.07	0.95	0.07	0.5	0	5.4	0.14	0.5	0	10.1	0.07
2	4.6	0.42	1.3	0.14	0.65	0.07	7.1	0.71	0.5	0	11.8	0.42
4	6.7	0.07	1.8	0	0.95	0.07	10.2	0.07	0.8	0	15.4	0.21
6	9.5	0.21	2.4	0.07	1.3	0	14.4	0.42	1.35	0.07	18.9	0.42
9	13.2	1.41	3.5	0.21	1.5	0.14	20.2	1.98	2.05	0.21	18.9	0.63
12	17.9	1.27	4.7	0.28	1.9	0.07	27	1.7	2.6	0	18.2	0.42
24	27	0.07	6.5	0.14	2.4	0	38.6	0	2.7	0	17.3	0.28
48	28.3	1.41	6.5	0.07	2.9	0.07	40.4	1.63	2.8	0	20.7	0
72	28.1	1	7.4	0.21	2.8	0.08	41.4	0.85	3.18	0.05	19.7	0.31
Blank+TM												
0	3.4	0.07	0.8	0	0.4	0	5.0	0	0.4	0	6.1	0.14
2	3.9	0.14	1.0	0	0.5	0	5.9	0.14	0.5	0	6.5	1.20
4	5.0	1.07	1.2	0	0.7	0	7.5	0	0.5	0	9.0	1.55
6	7.5	0.92	1.6	0.07	1.1	0.14	11.2	1.34	1.0	0.14	15.6	1.20
9	12.3	0.42	2.0	0	1.4	0	17.5	0.49	1.9	0.07	19.6	0.28
12	12.6	0.49	2.1	0.21	1.5	0.07	17.9	1.20	1.8	0.49	20.1	1.27
24	12.6	0	2.5	0.07	1.6	0	19.3	0.14	2.7	0	22.0	0.92
48	13.0	0.14	2.6	0.07	1.6	0	20.0	0.35	2.8	0.07	23.1	0.21
72	13.3	0.17	2.7	0.05	1.7	0.06	20.6	0.13	3.0	0.05	23.0	0.36
Blank*												
72	7.9	0.49	2.4	0.07	1.5	0.07	14.7	0.64	3.0	0	22.9	0.35

*This is the blank value for medium & inoculum, but no substrate.

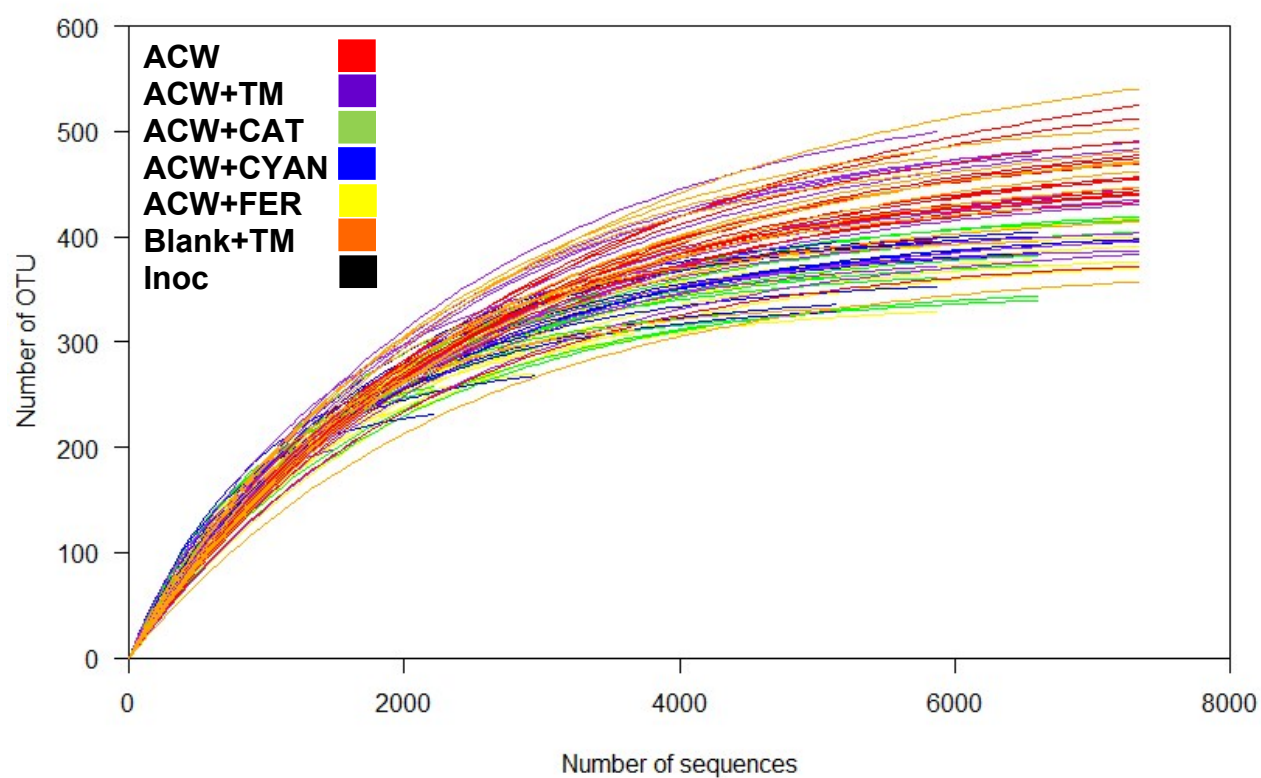


Figure S1: Rarefaction analysis of number of OTU (observed species) plotted against number of sequences per sample. Each coloured line represents the depth of coverage of an individual serum bottle sample. Samples are coloured by substrate.

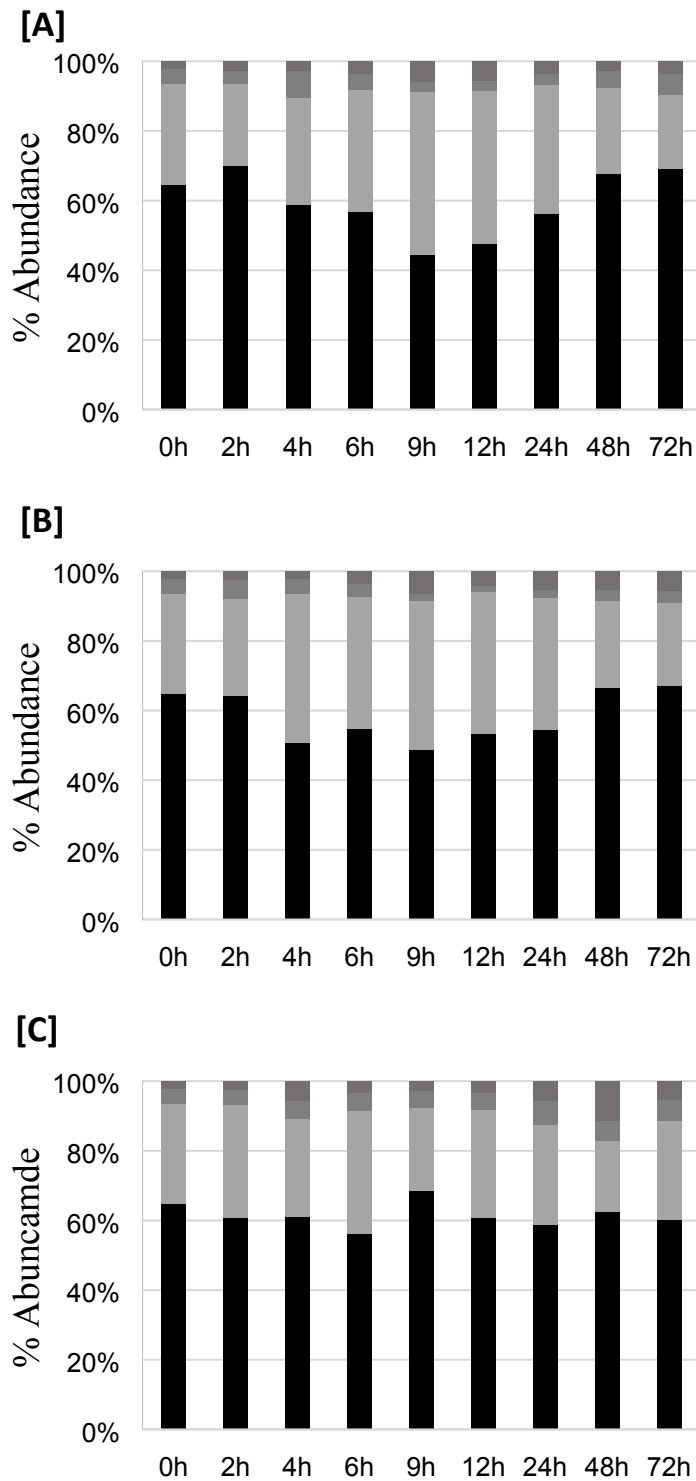
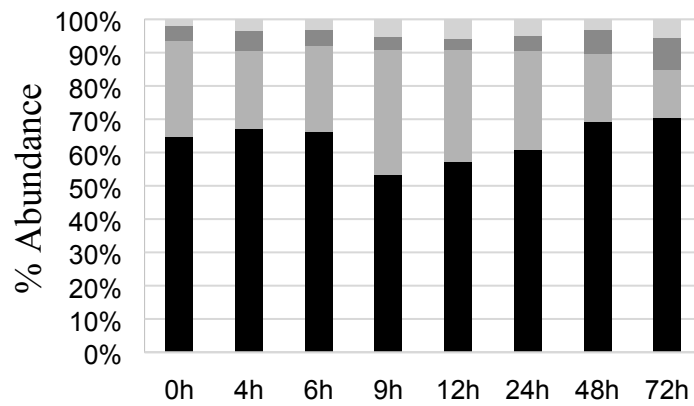
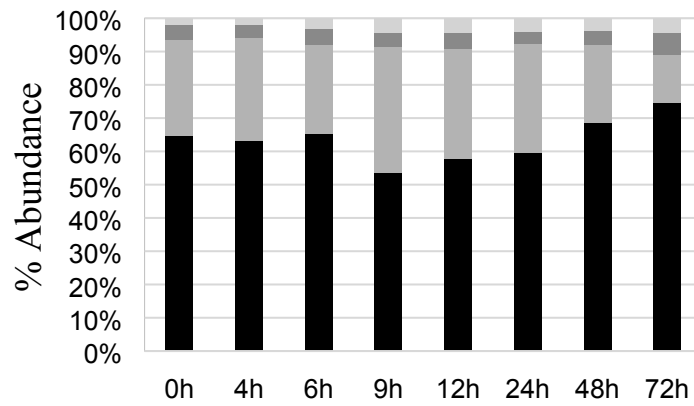


Figure S2: Major phyla ratios of ACW [A], ACW+TM [B] and Blank+TM [C], at different collection time points. Bars are coloured by phylum where; ■ refers to Firmicutes, ■ Bacteroidetes, ■ Proteobacteria and ■ Other. The original inoculum is represented by 0 h.

[A]



[B]



[C]

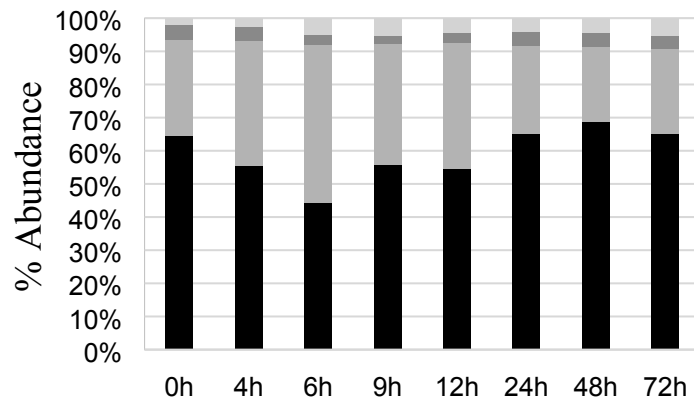


Figure S3: Major phyla ratios of ACW+FER [A], ACW+CAT [B] and ACW+CYAN [C], at different collection time points. Bars are coloured by phylum where: ■ refers to Firmicutes, ■ Bacteroidetes, ■ Proteobacteria and ■ Other. The original inoculum is represented by 0 h.

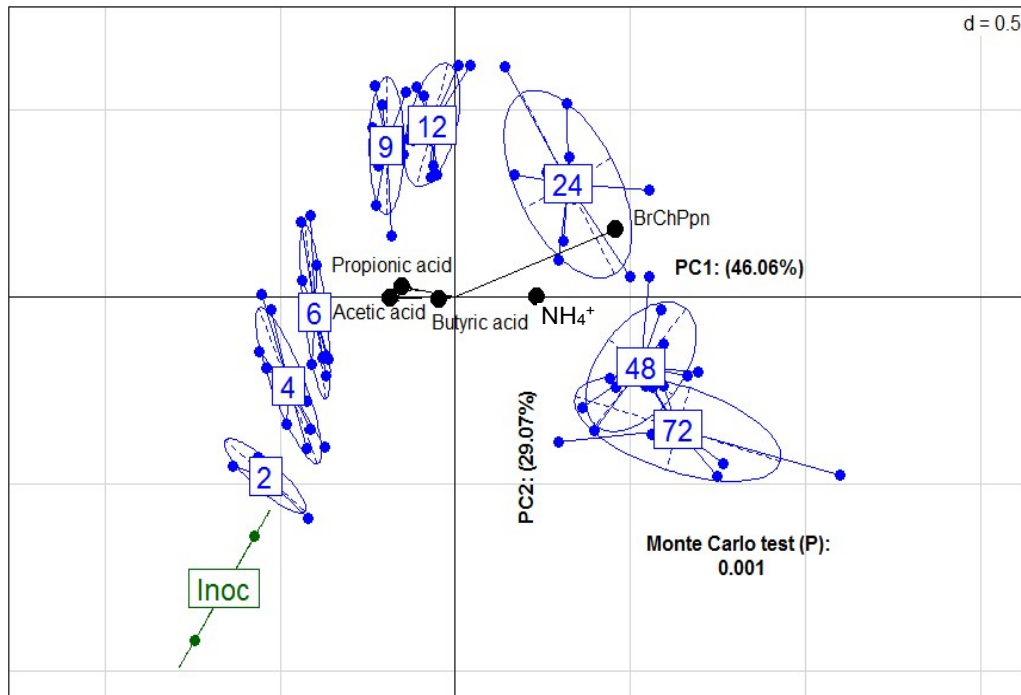


Figure S4: PCoA plot showing relationship between overall bacterial community structures of bacteria at different removal times, based on 16S rRNA sequences of collected biomass. Each point • represents an individual sample, and each ellipsoid encapsulates sample groups (time removal in hours). Inoc refers to the starting community of the initial inoculum. Larger points represent bacterial fermentation end-products and their positioning shows association with sample groups. BrChPpn refers to the branched-chain acid proportion, including valeric acid. Values are for all substrates including ACW, but not Blanks.