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Electronic Supporting Information for

Higher Microbial Diversity in Two-Phase Anaerobic Digestion Improves Process Performance

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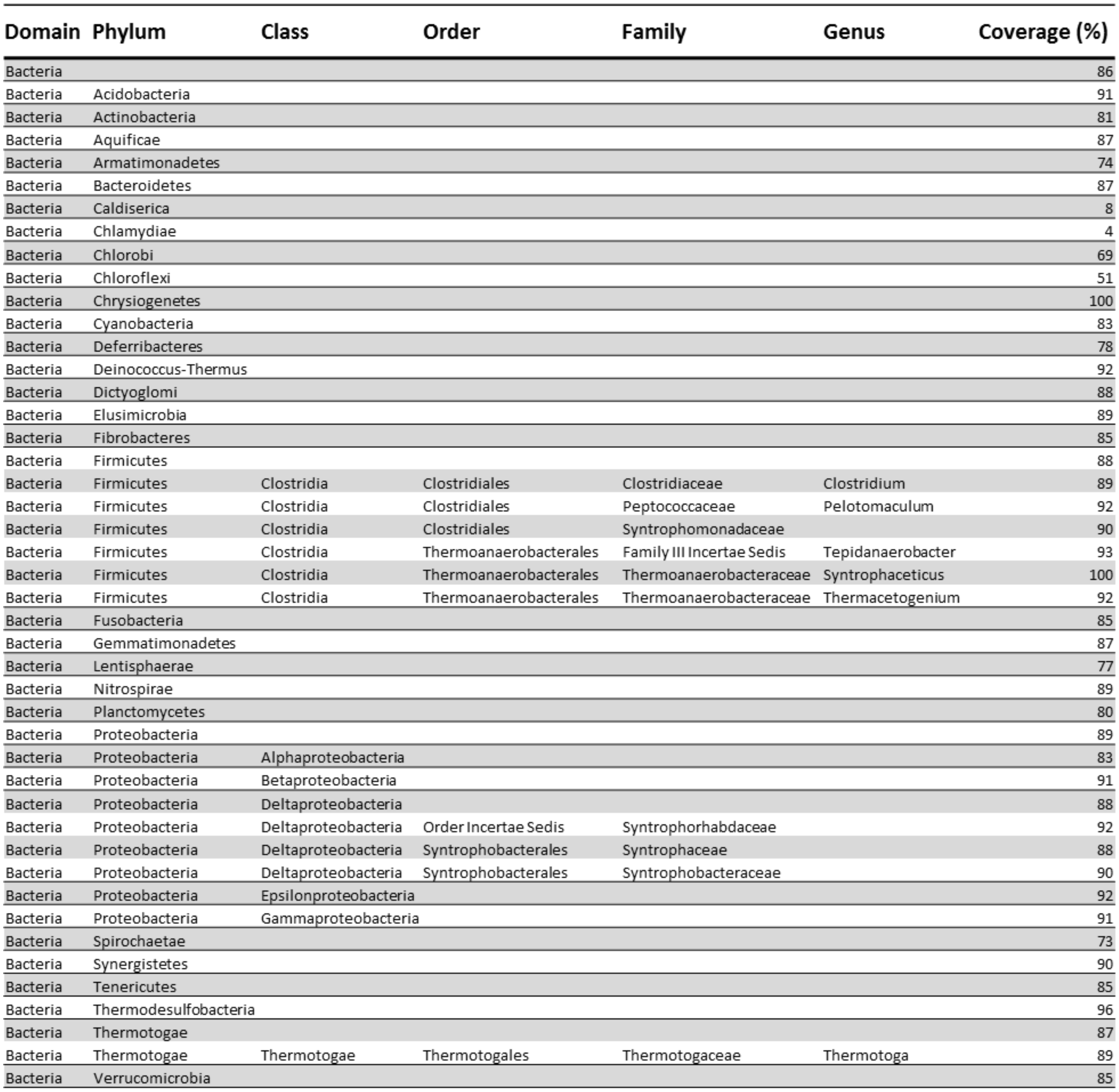
Table S1. Primer coverage of *Archaea* for 16S rRNA gene primers F515 (GTGCCAGCMGCCGCGGTAA) and R806 (GGACTACHVGGGTWTCTAAT) targeting the V4 region (Caporaso et al. 2011) according to TestPrime 1.0. TestPrime 1.0 evaluates the coverage of primer pairs by running an *in silico* PCR using the SILVA databases. Zero primer mismatches were allowed.

Domain	Phylum	Class	Order	Family	Genus	Coverage (%)
Archaea						51
Archaea	Crenarchaeota					0
Archaea	Euryarchaeota					82
Archaea	Euryarchaeota	Methanobacteria				93
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales			93
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae		93
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobacterium	89
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanobrevibacter	95
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanosphaera	82
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanobacteriaceae	Methanothermobacter	89
Archaea	Euryarchaeota	Methanobacteria	Methanobacteriales	Methanothermaceae	Methanothermus	100
Archaea	Euryarchaeota	Methanococci				83
Archaea	Euryarchaeota	Methanococci	Methanococcales			83
Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanocaldococcaceae		74
Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanocaldococcaceae	Methanocaldococcus	92
Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanocaldococcaceae	Methanotorris	39
Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanococcaceae	Methanococcus	96
Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanococcaceae	Methanothermococcus	90
Archaea	Euryarchaeota	Methanomicrobia				88
Archaea	Euryarchaeota	Methanomicrobia	Methanocellales			88
Archaea	Euryarchaeota	Methanomicrobia	Methanocellales	Methanocellaceae		89
Archaea	Euryarchaeota	Methanomicrobia	Methanocellales	Methanocellaceae	Methanocella	94
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales			90
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Family Incertae Sedis	Methanocalculus	97
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanocorpusculaceae		82
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanocorpusculaceae	Methanocorpusculum	82
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae		90
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanoculleus	93
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanofollis	89
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanogenium	97
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanolacinia	100
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanomicrobium	76
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanomicrobiaceae	Methanoplanus	100
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae		93
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanolinea	94
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanoregula	92
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Methanosphaerula	100
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanospirillaceae		83
Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanospirillaceae	Methanospirillum	83
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales			88
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosaetaceae		89
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosaetaceae	Methanosaeta	89
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae		88
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanimicrococcus	93
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanococcoides	86
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanohalobium	100
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanohalophilus	89
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanolobus	91
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanomethylovorans	87
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanosalsum	100
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosarcinaceae	Methanosarcina	89
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methermicoccaceae		73
Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methermicoccaceae	Methermicoccus	73
Archaea	Euryarchaeota	Methanopyri				0
Archaea	Korarchaeota					47
Archaea	Nanoarchaeota					0
Archaea	Thaumarchaeota					8

Table S2. Primer coverage of *Bacteria* for 16S rRNA gene primers targeting the V4 region according to TestPrime 1.0 (see Table S1 legend for additional details). The coverage of taxa in which known fatty acid-oxidizing syntrophic bacteria group is specified down to the genus or family levels.



Domain	Phylum	Class	Order	Family	Genus	Coverage (%)
Bacteria						86
Bacteria	Acidobacteria					91
Bacteria	Actinobacteria					81
Bacteria	Aquificae					87
Bacteria	Armatimonadetes					74
Bacteria	Bacteroidetes					87
Bacteria	Caldiserica					8
Bacteria	Chlamydiae					4
Bacteria	Chlorobi					69
Bacteria	Chloroflexi					51
Bacteria	Chrysiogenetes					100
Bacteria	Cyanobacteria					83
Bacteria	Deferribacteres					78
Bacteria	Deinococcus-Thermus					92
Bacteria	Dictyoglomi					88
Bacteria	Elusimicrobia					89
Bacteria	Fibrobacteres					85
Bacteria	Firmicutes					88
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	89
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Pelotomaculum	92
Bacteria	Firmicutes	Clostridia	Clostridiales	Syntrophomonadaceae		90
Bacteria	Firmicutes	Clostridia	Thermoanaerobacterales	Family III Incertae Sedis	Tepidanaerobacter	93
Bacteria	Firmicutes	Clostridia	Thermoanaerobacterales	Thermoanaerobacteraceae	Syntrophaceticus	100
Bacteria	Firmicutes	Clostridia	Thermoanaerobacterales	Thermoanaerobacteraceae	Thermacetogenium	92
Bacteria	Fusobacteria					85
Bacteria	Gemmatimonadetes					87
Bacteria	Lentisphaerae					77
Bacteria	Nitrospirae					89
Bacteria	Planctomycetes					80
Bacteria	Proteobacteria					89
Bacteria	Proteobacteria	Alphaproteobacteria				83
Bacteria	Proteobacteria	Betaproteobacteria				91
Bacteria	Proteobacteria	Deltaproteobacteria				88
Bacteria	Proteobacteria	Deltaproteobacteria	Order Incertae Sedis	Syntrophorhabdaceae		92
Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae		88
Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae		90
Bacteria	Proteobacteria	Epsilonproteobacteria				92
Bacteria	Proteobacteria	Gammaproteobacteria				91
Bacteria	Spirochaetae					73
Bacteria	Synergistetes					90
Bacteria	Tenericutes					85
Bacteria	Thermodesulfobacteria					96
Bacteria	Thermotogae					87
Bacteria	Thermotogae	Thermotogae	Thermotogales	Thermotogaceae	Thermotoga	89
Bacteria	Verrucomicrobia					85

Supplementary Figures

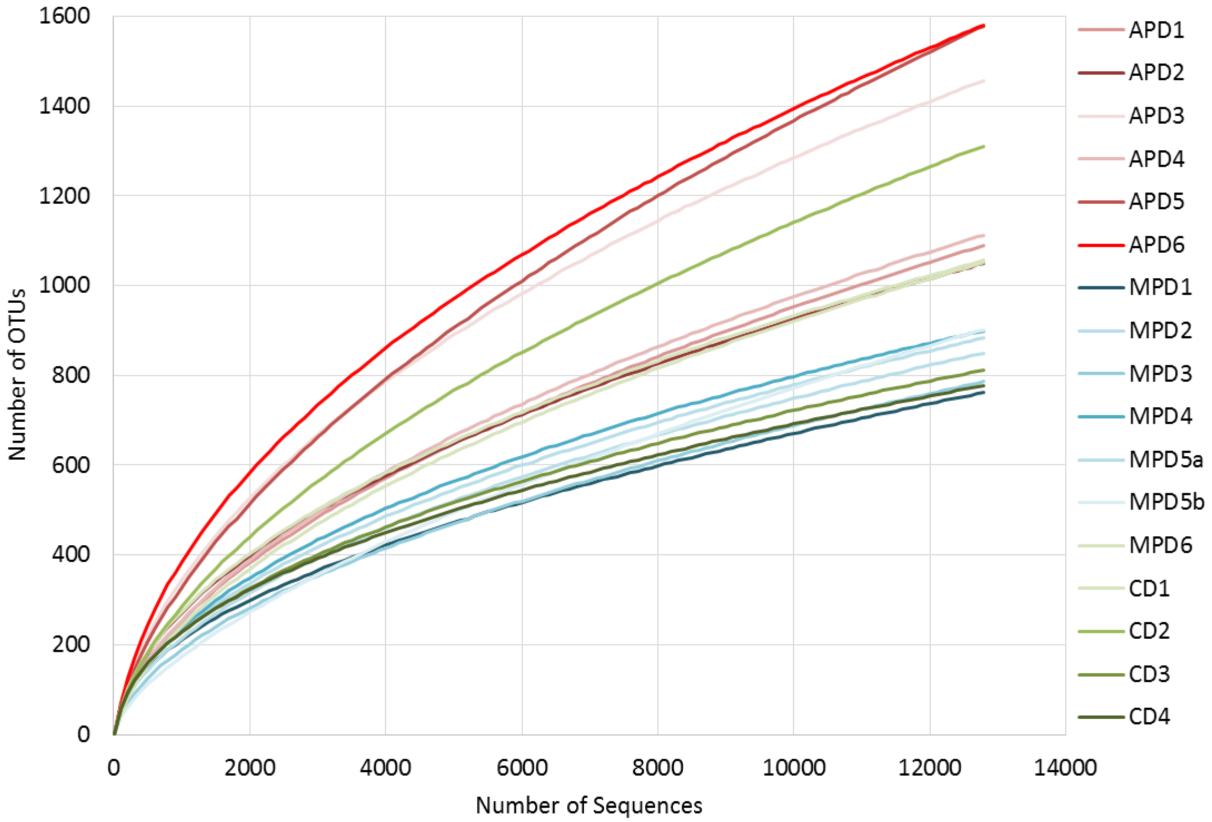


Figure S1. Rarefaction curves from each digester.

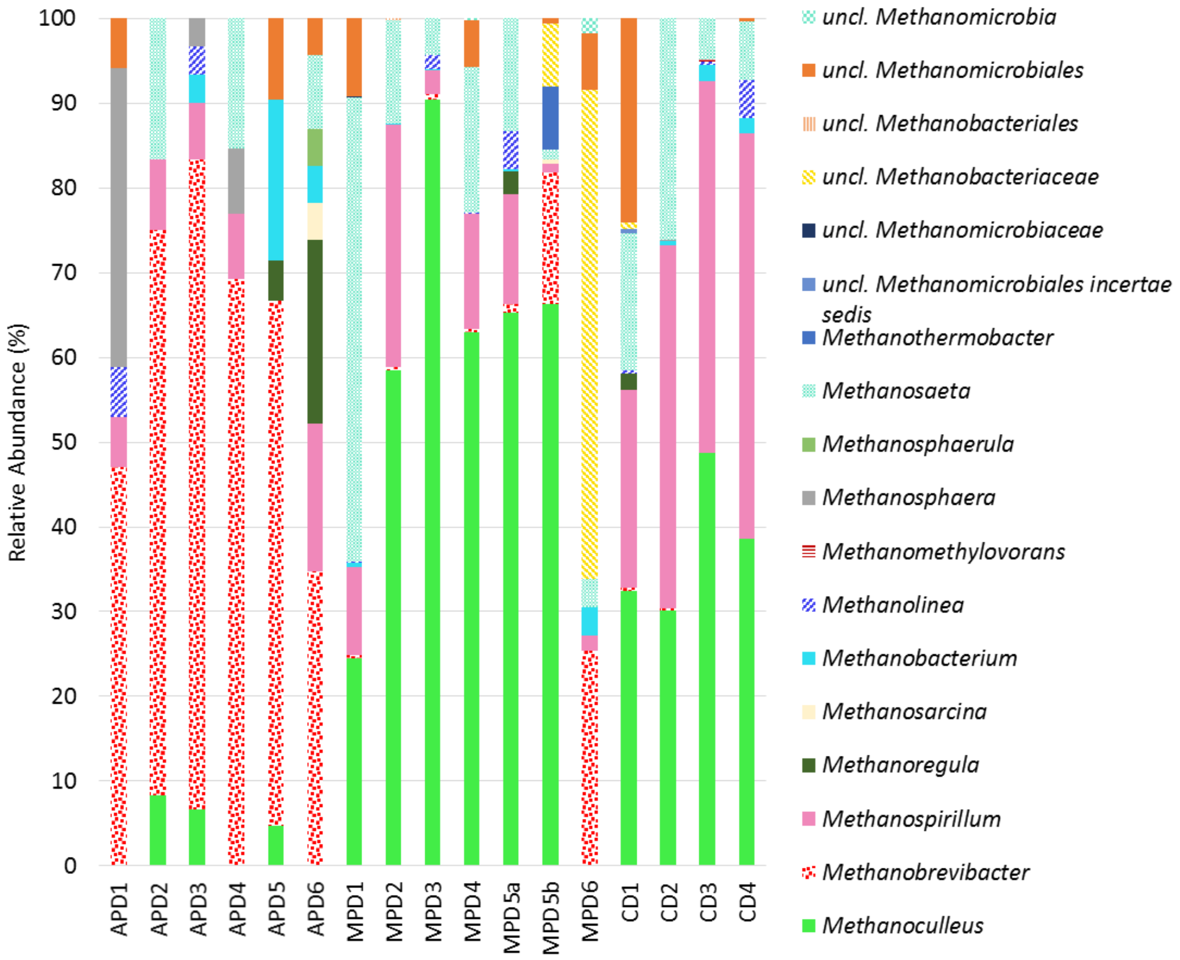


Figure S2. Relative abundance of methanogens identified to the genus level based on 16S rRNA gene sequencing. Data are expressed as percentages and were normalized using the total number of 16S rRNA gene sequences classified as known methanogens.

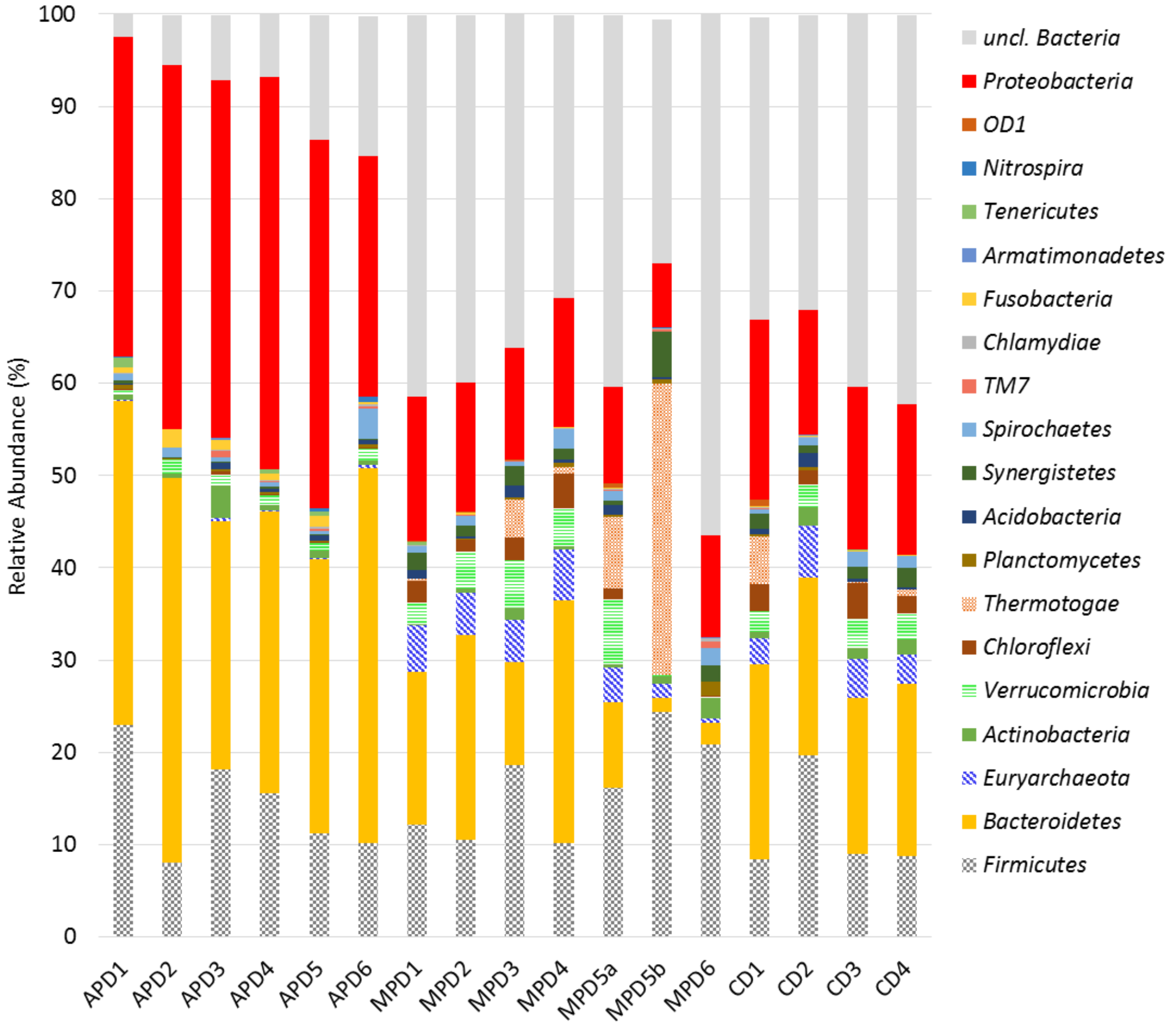


Figure S3. Relative abundance at the phylum level based on 16S rRNA gene sequencing. Data are expressed as percentages and were normalized using the total number of 16S rRNA gene sequences (including *Bacteria* and *Archaea*).

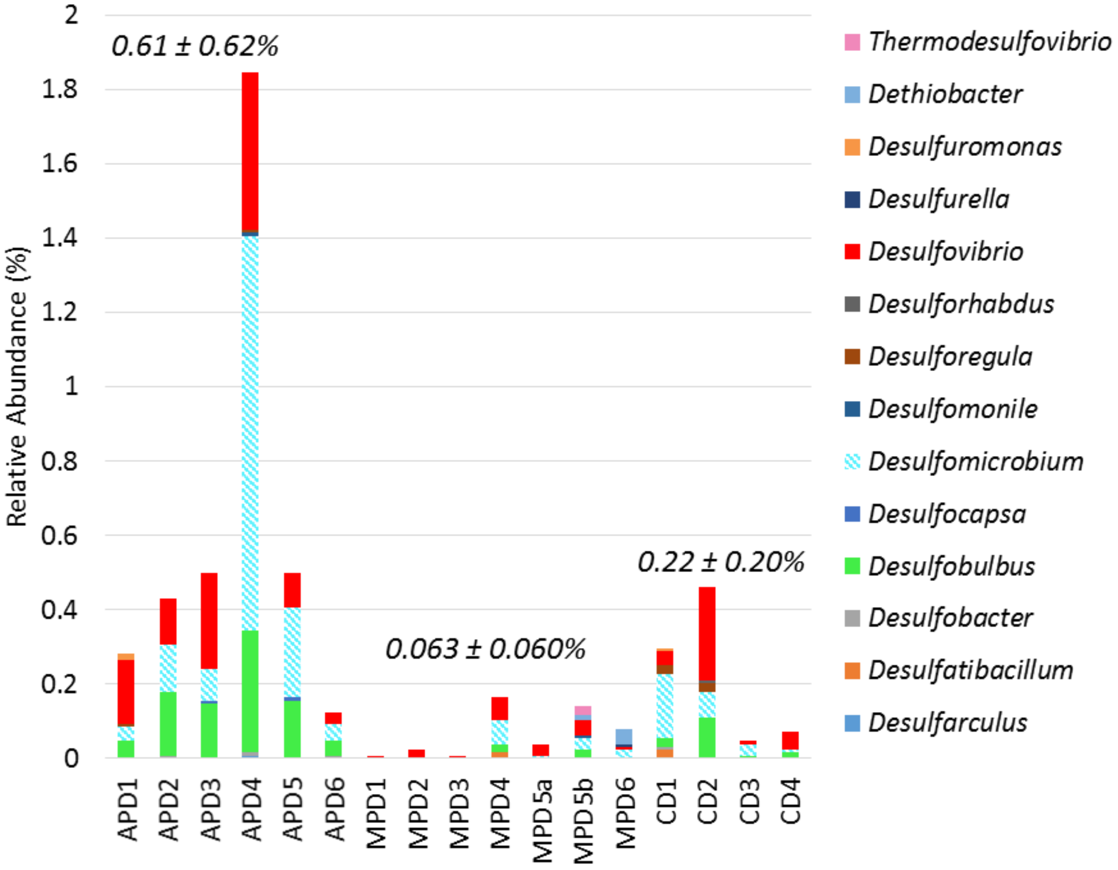


Figure S4. Relative abundance of known sulfate reducing bacteria identified to the genus level based on 16S rRNA gene sequencing. Data are expressed as percentages and were normalized using the total number of 16S rRNA gene sequences (including *Bacteria* and *Archaea*). A truncated y-axis (0 to 2%) is shown to accentuate differences in relative abundance.