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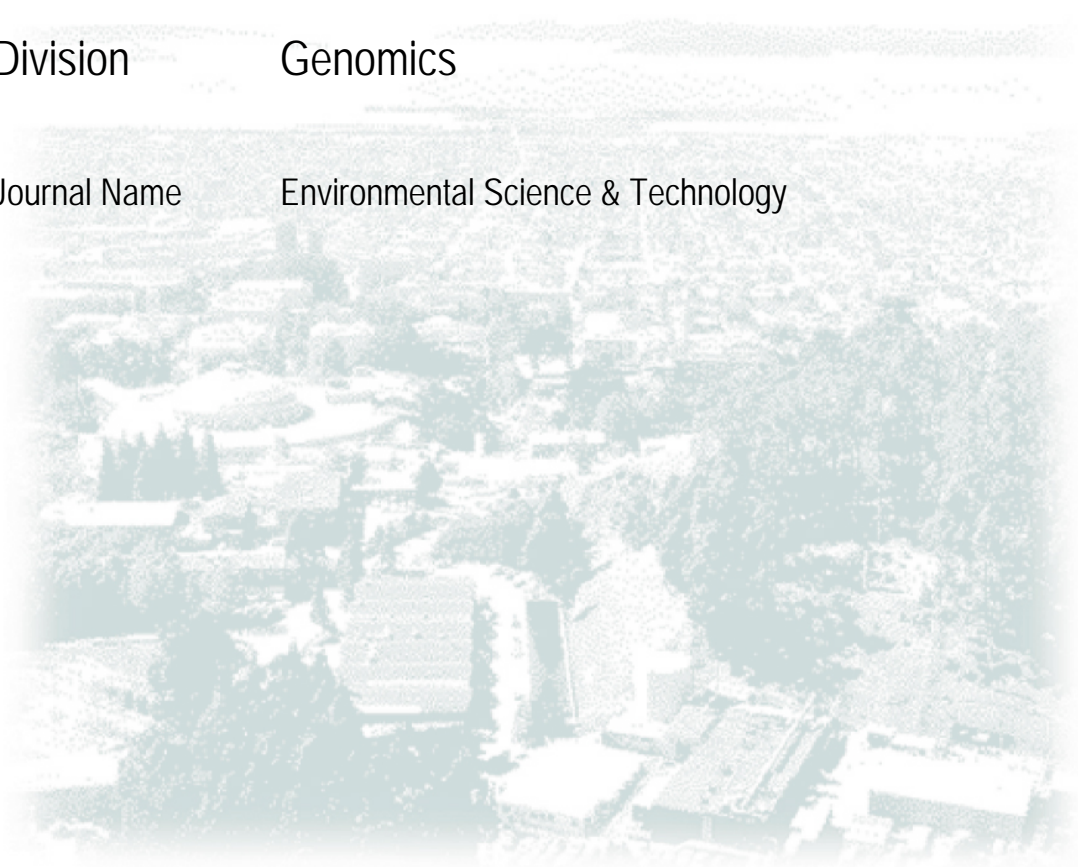
# ERNEST ORLANDO LAWRENCE BERKELEY NATIONAL LABORATORY

Title            Biocorrosive Thermophilic  
                      Microbial Communities in  
                      Alaskan North Slope Oil  
                      Facilities

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Division         Genomics

Journal Name    Environmental Science & Technology



**One-sentence summary: Anaerobic degradation of low molecular weight hydrocarbons support thriving thermophilic archaeal and bacterial communities that produce a wide variety of metabolites that can stimulate biocorrosion in Alaskan North Slope oil production facilities.**

**Title:** Biocorrosive Thermophilic Microbial Communities in Alaskan North Slope Oil Facilities

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1 **Abstract**

2 Corrosion of metallic oilfield pipelines by microorganisms is a costly but poorly  
3 understood phenomenon, with standard treatment methods targeting mesophilic sulfate-  
4 reducing bacteria. In assessing biocorrosion potential at an Alaskan North Slope oil field,  
5 we identified thermophilic hydrogen-using methanogens, syntrophic bacteria, peptide-  
6 and amino acid-fermenting bacteria, iron reducers, sulfur/thiosulfate-reducing bacteria  
7 and sulfate-reducing archaea. These microbes can stimulate metal corrosion through  
8 production of organic acids, CO<sub>2</sub>, sulfur species, and via hydrogen oxidation and iron  
9 reduction, implicating many more types of organisms than are currently targeted.  
10 Micromolar quantities of putative anaerobic metabolites of C<sub>1</sub>-C<sub>4</sub> *n*-alkanes in pipeline  
11 fluids were detected, implying that these low molecular weight hydrocarbons, routinely  
12 injected into reservoirs for oil recovery purposes, are biodegraded and provide  
13 biocorrosive microbial communities with an important source of nutrients.

14

15 **Introduction**

16 The U.S. possesses a network of over 2.3 million miles of pipelines that transmit  
17 about 75% of the nation's crude oil and 60% of refined products  
18 (<http://www.corrosioncost.com/infrastructure/gasliquid/index.htm>). Despite this  
19 importance, pipelines are not regularly considered in assessments of societal  
20 infrastructure needs ([http://www.asce.org/files/pdf/reportcard/2005\\_Report\\_Card-  
21 Full\\_Report.pdf](http://www.asce.org/files/pdf/reportcard/2005_Report_Card-Full_Report.pdf)), but there is little doubt that these facilities are vulnerable and can  
22 deteriorate over time. Through-wall breaches due to corrosion are expensive problems in  
23 the oil industry that can result in explosions, product interruptions, hazardous chemical

24 releases and environmental damage. Such was the case in the August 2006 Prudhoe Bay  
25 release on Alaska's North Slope (ANS) ([http://www.usatoday.com/news/nation/2006-08-](http://www.usatoday.com/news/nation/2006-08-06-alaskan-oil-field_x.htm)  
26 [06-alaskan-oil-field\\_x.htm](http://www.usatoday.com/news/nation/2006-08-06-alaskan-oil-field_x.htm)). The metabolic activities of microorganisms were implicated  
27 in this and other incidents of pipeline failure. In fact, it has long been known that  
28 microbes contribute to corrosion by multiple mechanisms (1-3), yet biocorrosion is not a  
29 well-understood process. There is no consensus on the identity of specific  
30 microorganisms responsible for corrosion or how they function to catalyze such  
31 incidents, resulting in poorly targeted efforts to monitor and combat biocorrosion.

32       Following the pipeline breach at Prudhoe Bay, we obtained samples from an ANS  
33 field to assess the potential for biocorrosion via metabolic indicators and microbial  
34 community analysis. The geology and geochemistry of ANS fields have previously been  
35 described (4) and subsurface conditions are well within the range for microbial  
36 communities to thrive (5). The facility produces oil, gas and water from multiple  
37 anaerobic and hot (average temperature 68°C) reservoirs and is typical of ANS oilfields  
38 that collectively have produced up to 16% of the U.S. domestic oil requirements for over  
39 30 years ([http://tonto.eia.doe.gov/oog/special/eia\\_sr\\_alaska.html](http://tonto.eia.doe.gov/oog/special/eia_sr_alaska.html)). Fluids and gases from  
40 multiple production wells are collected in a central facility, from which oil is channeled  
41 to the Trans-Alaska Pipeline System. At the facility, low molecular weight hydrocarbons  
42 (mostly methane, with lesser amounts of C<sub>2</sub>-C<sub>4</sub> *n*-alkanes) and water are reinjected into  
43 the oil-bearing formations to maintain pressure and facilitate oil recovery. Most pipelines  
44 are above ground and thermally insulated, so conditions inside the pipelines and  
45 processing facilities are anaerobic and hot. As required, seawater is treated with biocide  
46 and added to maintain formation pressures or during oil processing, thus introducing

47 seawater chemistry, a lower temperature, and potentially marine microorganisms into oil  
48 reservoirs. To assess biocorrosion potential in the ANS field, we obtained fluid samples  
49 at well heads from production wells (producing oil, gas and water), from a water  
50 reinjection well following oil processing activities in a central facility (CF), 2 locations  
51 within the CF (a 1<sup>st</sup> stage separator and a coalescer), from a pipeline carrying fluids and  
52 gas to the CF, treated seawater, and fluids and solids scraped from the inner surface of the  
53 pipeline carrying treated seawater. Samples were prepared accordingly for metabolite  
54 evaluation or for molecular community analysis (6).

55

## 56 **Bacterial Community Profiling**

57 Despite oil production from several major reservoirs with different geological  
58 histories, the facility-wide bacterial community profiles at the ANS field showed striking  
59 similarities for three of the high temperature sites. Bacterial communities from  
60 production well 2L, the 1<sup>st</sup> stage separator (PS) and the coalescer (CO) exhibited a high  
61 degree of class-level similarity (Fig. 1; table S1A), and greater levels of genetic diversity  
62 and species richness (table S2) than did the archaeal or the other two bacterial libraries.  
63 Ten "core" taxa (defined as OTUs with 97% nucleotide sequence similarity) were found  
64 at all three sites and represented over 87% of the bacterial 16S rRNA gene sequences  
65 (Fig. 2; table S3). Fig. 2 also illustrates the taxa and number of sequences shared  
66 between any two of the sites as well as those unique to each site. The most abundant of  
67 the core sequences (2L: 83%, PS: 57%, and CO: 31%) has 97-99% identity to that of  
68 *Thermovirga lienii* (7). *T. lienii* is a thermophilic anaerobe isolated from a North Sea oil  
69 well and described as a member of the Firmicute family Syntrophomonadaceae.

70 However, it has also been designated a member of the candidate division Synergistes (8)  
71 and sequences similar to those of *T. lienii* will be designated as "Synergistes" here. The  
72 type strain of *T. lienii* has an optimum growth temperature of 58°C and ferments certain  
73 amino acids, proteinaceous substrates and organic acids, producing ethanol, acetate,  
74 propionate, isovalerate/2-methylbutyrate, H<sub>2</sub>, and CO<sub>2</sub> (7). It can also reduce cystine and  
75 elemental sulfur to H<sub>2</sub>S. Synergistes-associated sequences were abundant in an  
76 extensively biodegraded mesophilic ANS oil reservoir (9). The majority were more  
77 similar to uncultured *Thermovirga* clones than to the type strain as expected from the  
78 temperature range favored by *T. lienii*.

79 The most abundant delta proteobacterial 16S rRNA gene sequences (2L: 4%, PS: 7%,  
80 CO: 18%) were similar to that of *Desulfomicrobium thermophilum* (10), a sulfate-  
81 reducing bacterium isolated from a hot spring. Sulfate-reducing bacteria (SRB) have  
82 been routinely monitored by the oil industry because of their ability to produce H<sub>2</sub>S from  
83 sulfate. However, many other core sequences were similar to those of organisms that  
84 produce sulfide through the reduction of elemental sulfur, thiosulfate, sulfate, or other  
85 sulfur oxyanions (e.g. *Thermosiphon africanus*/*T. geolei*, *Pelobacter carbinolicus*,  
86 *Desulfacinum subterraneum*, and *Thermodesulfobacterium commune*). Clone libraries  
87 based on *dsrAB* genes that code for an essential enzyme for sulfate reduction  
88 (dissimilatory (bi)sulfite reductase) were heavily dominated by sequences similar to  
89 those of the archaeal sulfate-reducer *Archaeoglobus fulgidus* (2P: 60%, PS and CO:  
90 >99%). These findings suggest that bacterial sulfate reduction makes only a minor  
91 contribution to sulfide production at the facility.

92 Core sequences similar to *Thermoanaerobacter pseudethanolicus* (and other

93 *Thermoanaerobacter* species), *Thermacetogenium phaeum*, or *P. carbinolicus* indicate  
94 the possible importance of iron-reduction and/or syntrophic metabolic interactions (11).  
95 Anaerobic iron-reducing thermophiles in deep subsurface petroleum reservoirs have  
96 been previously demonstrated (12). However, thermophilic strains of *Pelobacter* are not  
97 yet known, so its detection as a core taxon awaits further explanation.

98 In comparison to the 2L and CF samples, the production well 2P bacterial community  
99 had much lower diversity and species richness (Fig 1; table S2). The dominant (90%)  
100 bacterial 16S rRNA gene sequence is similar (97-99%) to that of moderately  
101 thermophilic, organic acid-utilizing, nitrate-reducing *Petrobacter* species (13). The  
102 second-ranked (4.7%) sequence type is similar to *T. lienii* and sequences similar to the  
103 core taxa *T. pseudethanolicus* and Thermotogales were present in low abundance (table  
104 S1A). Which specific factors are responsible for the differences are unknown; well head  
105 temperatures were similar for 2P and 2L (avg. 48°C and 49°C respectively) with little  
106 variation for 9 months prior to sampling, however the wells draw from different  
107 formations. Collectively, all well and CF samples strongly resemble anaerobic  
108 thermophilic oil reservoir and well communities.

### 109 **Archaeal Community Profiling**

110 Sequences similar to those of hyperthermophilic Archaea, notably sulfate-  
111 reducing *Archaeoglobus* species, methane-producing *Methanothermobacter*  
112 *thermautotrophicus*, and H<sub>2</sub>S-producing *Thermococcus* were abundant in the PS, CO,  
113 and 2P samples. More than 90% of the archaeal 16S rRNA gene sequences fell into the  
114 corresponding three families of Euryarchaeota (Fig. 3, tables S1B and S4).  
115 Crenarchaeota were only detected in the PS sample, which also exhibited the highest



116 diversity and species richness of the three archaeal libraries (table S2). All three  
117 Euryarchaeota groups have frequently been detected in hot oil reservoirs and production  
118 fluids (5). *Archaeoglobus* and *Thermococcus* enriched from a North Sea oil field grew at  
119 high temperatures on crude oil as the sole source of carbon and nutrients (14). The same  
120 study also found *Archaeoglobus*-like cells in hyperthermophilic cultures enriched from  
121 ANS reservoirs.

122 Sequences of methanogens (approximately ¼ of the archaeal 16S rRNA gene  
123 sequences) were less abundant than those of fermentative and sulfate-reducing archaea.  
124 Most methanogenic sequences were related to those of hydrogen-utilizing  
125 *Methanothermobacter* species. Hydrogen-utilizing methanogens have been commonly  
126 found in hot oil reservoirs (15). Approximately 12% of methanogenic sequences were  
127 99.8% similar to that of "*Methermicoccus shengliensis*" (DQ787474, Methanosaetaceae).  
128 "*M. shengliensis*" strain ZC-1 (16) was isolated from oil-production water and has  
129 optimal growth at 65°C. Strain ZC-1 is not an acetoclastic methanogen, unlike other  
130 members of the Methanosaetaceae. In contrast to our results, acetoclastic methanogens  
131 were by far the most abundant archaea in a heavily biodegraded mesophilic North Slope  
132 oil reservoir (9).

133

#### 134 **Targeted Cultivation and Seawater Pig Envelope Community Profiling**

135 In agreement with the molecular analysis, *M. thermotrophicus* was isolated as the  
136 numerically dominant (2.3/mL) hydrogen-using prokaryote from the 1<sup>st</sup> stage separator.  
137 A thermophilic *Anaerobaculum* sp. was the numerically dominant heterotroph cultured  
138 from the same sample (17). Members of the genus *Anaerobaculum* ferment organic acids

139 and peptides but also reduce thiosulfate, sulfur, and cysteine to H<sub>2</sub>S (18) and are thus  
140 directly implicated in biocorrosion. However, all populations of culturable bacteria  
141 screened (SRB, anaerobic/facultative heterotrophs, hydrogen-users) were found in low  
142 numbers (2-4 cells/mL), implying that these organisms would be missed in most routine  
143 screening procedures.

144 The seawater pig envelope sample (SW) community profile was quite different from  
145 that of the archaea-rich production wells and the CF, primarily consisting of sequences  
146 similar to those of mesophilic and psychrophilic marine bacteria. We were unsuccessful  
147 in obtaining a small subunit ribosomal archaeal RNA gene library with archaeal primers  
148 although a bacterial 16S rRNA gene sequence library was successfully obtained from the  
149 same DNA sample. Populations of culturable bacteria were 10<sup>3</sup> to 10<sup>6</sup> /mL, with the  
150 numerically dominant organism and most abundant DNA sequence from the seawater  
151 16S rRNA gene library most similar to  $\gamma$ -proteobacteria, *Pseudomonas stutzeri* and/or  
152 related species (Fig. 1, table S1). The numerically dominant culturable hydrogen-user  
153 was *Acetobacterium*. An *Acetobacterium* species has previously been isolated from  
154 marine environments (19). Sulfate-reducing bacteria were estimated at 2.4 x 10<sup>6</sup>/mL. In  
155 accord with the 16S rRNA library results, no *dsrAB* sequences similar to those of  
156 *Archaeoglobus* were obtained from the SW sample. 16S rRNA gene sequences similar to  
157 those of the deep-sea genera *Sulfurimonas* and *Arcobacter* (epsilon proteobacteria, table  
158 S1) were abundant in the SW sample but were not found in the production wells or CF  
159 samples. Only two sequences from the other four bacterial libraries, one from the PS and  
160 one from well 2L (both *Pseudomonas*) were as much as 97% similar to any of the  
161 sequences from the SW sample. Thus, mesophilic and psychrophilic marine

162 microorganisms originating from seawater seem unlikely to be responsible for  
163 biocorrosion problems at high temperature sites. However, seawater can contribute  
164 increased levels of sulfate, manganese, or organic matter that could spur increased  
165 corrosive microbial activity. For example, it was noted that H<sub>2</sub>S was not detected in one  
166 ANS oilfield until after seawater flooding was initiated (20).

167

### 168 **Metabolic Profiling**

169

170 It is well established that biocorrosive organisms form complex surface  
171 assemblages where cells are imbedded in a matrix of biologically-produced extracellular  
172 polymeric substance (EPS) that forms a protective microenvironment (1). However, the  
173 carbon source(s) supporting the formation of such surface-associated communities  
174 remain enigmatic. Clearly, the largest potentially available source of carbon to support  
175 microbial activity is the oil itself. Since hydrocarbons are known to be suitable  
176 substrates for anaerobes (21), we suspected that the more water-soluble oil components,  
177 like benzene, toluene ethylbenzene and xylene isomers (BTEX) might be preferentially  
178 metabolized to support the diverse microbial communities detected at the facility. This  
179 prospect was explored by assaying for the signature metabolites associated with  
180 anaerobic oil biodegradation (22-23). The identification of these intermediates  
181 implicates the parent hydrocarbons being metabolized.

182 Contrary to expectations, there was no evidence for the biodegradation of the  
183 most water-soluble BTEX components. However, putative low molecular weight  
184 alkylsuccinate metabolites associated with anaerobic *n*-alkane biodegradation were

185 found facility-wide. Six of the eight central facility and production well samples  
186 collected contained 0.8-2.2  $\mu\text{M}$  concentrations of low molecular weight ( $\text{C}_1\text{-C}_4$ )  
187 alkylsuccinates (Table 1). No signature hydrocarbon metabolites were found in the  
188 seawater samples. The identification of methyl-, ethyl-, propyl- and butylsuccinate  
189 suggested that the hydrocarbons routinely reinjected during normal oil recovery  
190 operations were being biologically oxidized by a fumarate addition reaction in a manner  
191 analogous to higher molecular weight *n*-alkanes (Table 1) (22). The recycling of these  
192 gases to help maintain formation pressures occurred throughout the decades-long  
193 production history of the formation and we suspect that the requisite organisms were  
194 enriched over this long time period.

195         If the analogy to higher molecular weight *n*-alkane anaerobic metabolism is  
196 accurate, we predict the formation of a series of downstream branched and straight-chain  
197 fatty acid metabolites formed as a result of the presumed carbon skeleton rearrangement  
198 and subsequent decarboxylation of the alkylsuccinate intermediates (24). Indeed, the  
199 expected downstream metabolites were also found in the same samples from the central  
200 facility, but none were found in the seawater samples (Table 1).

201         The detection of methylsuccinate in conjunction with the other low molecular  
202 weight alkylsuccinates is particularly evocative. This finding suggests that fumarate  
203 addition may represent an alternative to previously described mechanisms such as reverse  
204 methanogenesis for the anaerobic oxidation of methane (25). Consistent with an alternate  
205 hypothesis, the methanogen and  $\delta$  proteobacterial sequences were dissimilar to the genera  
206 described in other environments undergoing anaerobic methane oxidation (26).

207

## 208 **Implications for Biocorrosion**

209           Our results suggest that temperature and hydrocarbon utilization are primary  
210 factors governing microflora species composition at the ANS facility. Contrary to the  
211 emphasis placed on mesophilic bacterial SRB by standard biocorrosion monitoring  
212 procedures, many of the organisms detected using molecular techniques and targeted  
213 isolation are thermophilic bacteria capable of reducing various sulfur oxyanions or  
214 hyperthermophilic sulfate-reducing archaea that produce H<sub>2</sub>S. Methanogenic,  
215 fermentative, H<sub>2</sub>-producing and H<sub>2</sub>-utilizing physiologies were also common, unlikely to  
216 be detected using standard techniques, and could likewise stimulate corrosion. The  
217 similarity of core taxa in these samples and those from other thermophilic oil reservoirs  
218 and wells suggests that hydrocarbon-degrading, potentially corrosive microbes found in  
219 oil reservoirs will readily inoculate and proliferate in oil production facilities maintained  
220 at compatible temperatures. Such similarities also imply that pipeline integrity  
221 management programs might be able to differentially target a relatively few core taxa.

222           The detection of putative low molecular weight alkane metabolites throughout the  
223 hot oilfield facilities suggests that anaerobic hydrocarbon biodegradation is inherent and  
224 likely involved in supporting biocorrosive biofilms. Indeed the formation of relatively  
225 high concentrations of alkylsuccinates (Table 1;  $\mu\text{M}$  vs  $\text{nM}$  concentrations more typically  
226 found in fuel-contaminated aquifers, 22) allows us to postulate that such acidic  
227 intermediates can directly contribute to biocorrosion processes. The subsequent  
228 metabolism of the compounds would eventually form acetate and CO<sub>2</sub>, microbial  
229 products known to exacerbate corrosion of pipeline surfaces (3, 27). Thus, these findings

230 support the hypothesis that anaerobic hydrocarbon biodegradation processes in the  
231 oilfield environment can be an important factor in microbial influenced corrosion.

232

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274 organizing, collecting and shipping samples as well as for the use of ANS laboratory

275 facilities. The conclusions expressed in this paper are those of the authors and not  
276 necessarily shared by ConocoPhillips.

277

278 **Supporting Online Material**

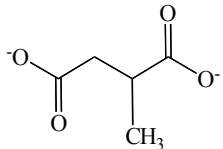
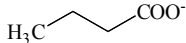
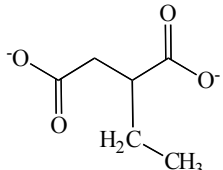
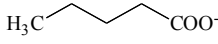
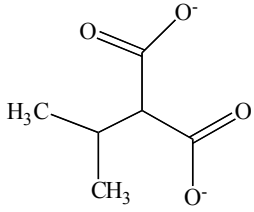
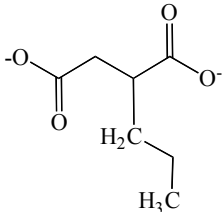
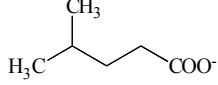
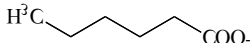
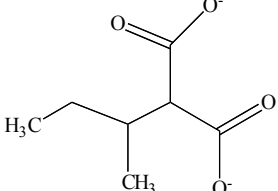
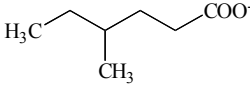
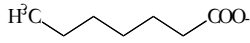
279 Materials and Methods

280 Tables S1 to S3

281 References



282 Table 1. Metabolites associated with the anaerobic biodegradation of C<sub>1</sub>-C<sub>4</sub> in Alaskan  
283 North Slope (ANS) oil field samples. Alkylsuccinates were detected in processing facility  
284 and production well samples (total=6), but not in seawater or in a pipeline transporting  
285 seawater, suggesting the anaerobic oxidation of the parent compounds methane, ethane,  
286 propane or butane. Concentrations of metabolites were in the μM range. Downstream  
287 metabolites resulting from the predicted carbon skeleton rearrangement and subsequent  
288 decarboxylation of the alkylsuccinate were also found in ANS samples. For *n*-alkanes C<sub>3</sub>  
289 or greater, a terminal and subterminal addition of fumarate (denoted with \*) are possible,  
290 resulting in two possible downstream metabolites (branched or straight chain).  
291

Parent compound	Fumarate addition metabolite	Fumarate addition metabolite concentration detected ( $\mu\text{M}$ )	Downstream metabolite (rearrangement)
<b>Methane (<math>\text{CH}_4</math>)</b>	 <p>Methylsuccinate</p>	$2.08 \pm 1.10$	 <p>Butanoic acid</p>
<b>Ethane (<math>\text{C}_2\text{H}_6</math>)</b>	 <p>Ethylsuccinate</p>	$1.77 \pm 1.54$	 <p>Pentanoic acid</p>
<b>Propane (<math>\text{C}_3\text{H}_8</math>)</b>	<p>subterminal addition:</p>  <p>terminal addition:</p>  <p>Propylsuccinate*</p>	$2.18 \pm 0.20$	 <p>4-Methylpentanoic acid</p>  <p>Hexanoic acid</p>
<b>Butane (<math>\text{C}_4\text{H}_{10}</math>)</b>	 <p>Butylsuccinate*</p>	$0.76 \pm 0.11$	 <p>4-Methylhexanoic acid</p>  <p>Heptanoic acid</p>

293 **Figure legends**

294

295 Figure 1. Relative abundances of sequences from five bacterial 16S rRNA gene libraries.

296 The RDP Project Classifier tool (<http://rdp.cme.msu.edu/classifier/classifier.jsp>) was used

297 to assign representative sequences (97% similarity) to the higher-level taxonomic groups

298 shown, except for sequences affiliated with *Thermovirga lienii* (referred to as

299 "Synergistes" in this figure), which currently are classified under "Clostridia", Incertae

300 sedis XV. N represents the total number of sequences in a library, after exclusion of

301 chimeric sequences. Origin of samples: production wells 2P and 2L (sampled at

302 wellhead), outflow from the 1<sup>st</sup> stage separator (PS) and coalescer (CO) units in a central

303 facility, and the "pig envelope" (e.g. the scraped inner surface of the pipeline) of a

304 pipeline transporting treated seawater (SW) from the Arctic Ocean to the central facility.

305 Table S1A contains the accession number of the closest match, affiliation, and the

306 relative abundance (as a percentage of the total library) of each representative sequence.

307 Temperatures at the 2P wellhead for 9 months prior to sampling ranged from 36-52°C,

308 avg. 48°C ±1.7 (1 SD); from the 2L wellhead 33-52°C, avg. 49°C ±2.1 (1 SD).

309 Temperature ranges inside the central facility were maintained at 50-55°C (1<sup>st</sup> stage

310 separator), 67-82°C (2<sup>nd</sup> stage separator) and 59-78°C (coalescer).

311 Figure 2. Distribution of bacterial sequences from 2L, PS, and CO illustrating the

312 number of sequences from taxa found in all three libraries ("2L, PS, CO: Core taxa"), 2

313 libraries ("PS and 2L", "CO and 2L", "PS and CO") or unique to one sample.

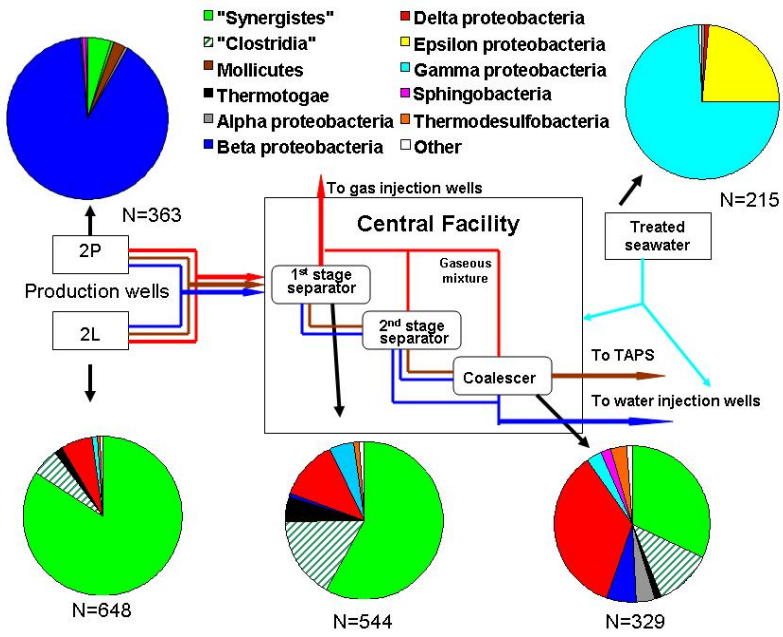
314 Figure 3. Relative abundances of sequences from three archaeal 16S rRNA gene libraries.

315 The RDP Project Classifier tool (<http://rdp.cme.msu.edu/classifier/classifier.jsp>) was used

316 to assign representative sequences (97% similarity) to the higher-level taxonomic groups

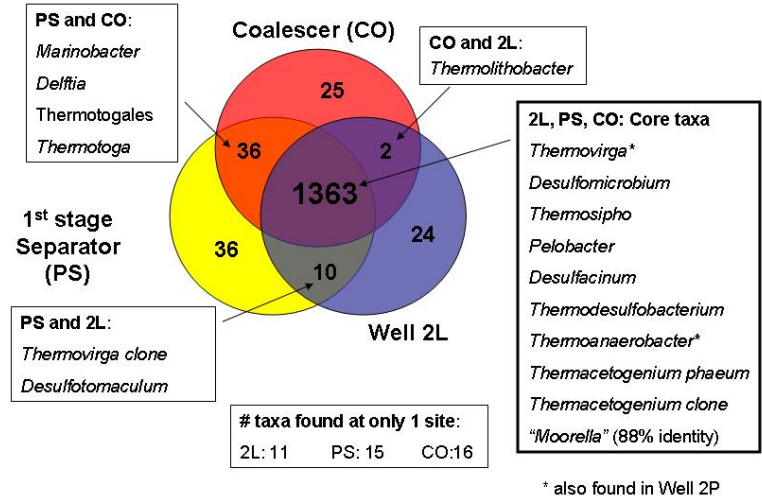
317 shown. N represents the total number of sequences in a library, after exclusion of  
318 chimeric sequences. Origin of samples: production well 2P (sampled at wellhead),  
319 outflow from the 1<sup>st</sup> stage separator (PS) and coalescer (CO) units in a central facility.  
320 Table S1B contains the accession number of the closest match, affiliation, and the  
321 relative abundance (as a percentage of the total library) of each representative sequence.  
322

323 Figure 1.  
 324



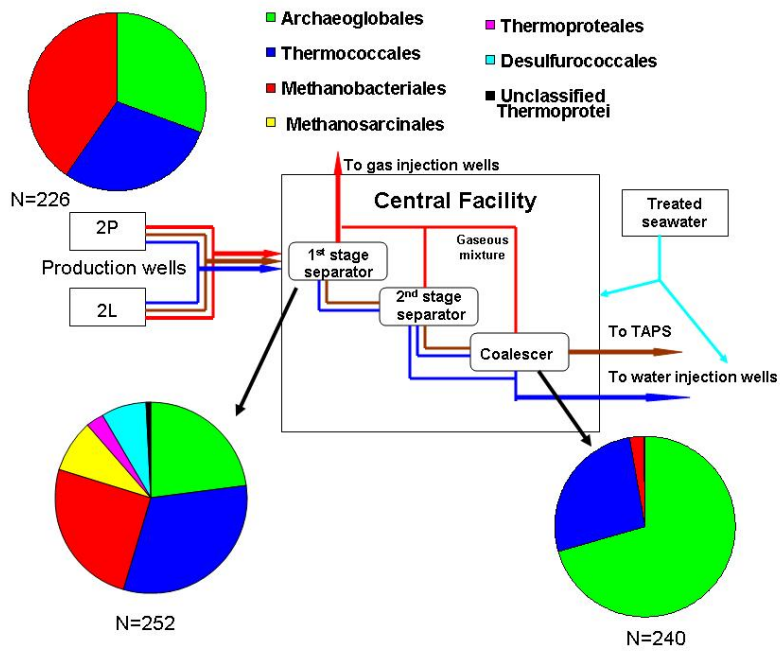
325

326 Fig. 2.  
 327



328

329 Fig. 3.  
330



331

332 **Supporting online material**

333

334 **Materials and Methods**

335 **Molecular analysis:** Samples from two production wells (2P, 2L), two locations in a  
336 central facility (CF, 1st stage separator [PS] and coalescer [CO]), and from a seawater  
337 line prior to exposure to oil (SW) were collected in 2006 from an oil field complex on the  
338 North Slope of Alaska. The seawater line sample consisted of fluids and solids from a  
339 pigging operation, whereas the other samples were fluids. Two samples (PS and SW),  
340 150 mL each) were filtered (0.45 µm) and preserved in the field by the addition of  
341 DNazol® Direct (Molecular Research Center, Inc., Cincinnati, OH) to the filter then  
342 extracted at OU using a bead-beating protocol (UltraClean™ Mega Soil DNA Isolation,  
343 MO BIO Laboratories, Inc., Carlsbad, CA). The remaining samples (20 mL) were first  
344 concentrated by ethanol precipitation and the pellet resuspended in PCR-grade water  
345 before extraction using a bead-beating protocol (PowerSoil™ DNA Isolation Kit, MO  
346 BIO). 16S rRNA primers for eubacteria (16S/18S rRNA PCR Library Creation,  
347 <http://my.jgi.doe.gov/general/index.html>, and 27F and 1492R for the seawater line (1),  
348 ARC333F and 958R for archaea (1), *dsrAB* (*dsr1F*, *dsr4R*, 2), and *mcrA* primers (ME1  
349 and ME2, 3) were used to obtain PCR products to create clone libraries (5 eubacterial  
350 16S, 3 archaeal 16S, 4 *dsrAB*, 1 *mcrA*) using the TOPO® TA Cloning Kit (Invitrogen  
351 Corp., Carlsbad, CA). A sixth, duplicate bacterial 16S library using primers 27F and  
352 1492R (1) was created from one production well sample (2P) to compare the effect of  
353 possible primer bias. Sequencing of the libraries was performed by the DOE Joint  
354 Genome Institute (Lawrence Livermore Laboratory, Walnut Creek, CA). Results for the  
355 *dsrAB* and *mcrA* libraries are briefly referred to in this work and will be reported in detail



356 later (manuscript in preparation). The duplicate bacterial 16S library created with primers  
357 27F and 1492R from sample 2P gave the same dominant *Petrobacter* sequence (87.7% of  
358 total sequences) and low sequence diversity as did the library created with 27F and 1391  
359 (90.1% of total sequences were *Petrobacter*, see tables S1A and S2) and will not be  
360 discussed further.

361         Primer binding sites were identified using the "Motifs" function in Sequencher  
362 (version 4.7, Gene Codes, Ann Arbor, MI) as a guide to trim the sequences to  
363 homologous regions, approximately 1250 bp for bacterial 16S rRNA gene sequences and  
364 600 bp for archaea. The sequences in the clone libraries were aligned using the  
365 greengenes NAST-aligner (4) and examined for chimeric sequences using the  
366 Bellerophon program (5) available through the greengenes website (version 3,  
367 [http://greengenes.lbl.gov/cgi-bin/nph-bel3\\_interface.cgi](http://greengenes.lbl.gov/cgi-bin/nph-bel3_interface.cgi)). Potential chimeric sequences  
368 identified by Bellerophon were further examined by Pintail (6) and comparing separate  
369 regions of the sequences by BLASTN (7). Distance matrices (8, greengenes, "Create  
370 distance matrix", [http://greengenes.lbl.gov/cgi-bin/nph-distance\\_matrix.cgi](http://greengenes.lbl.gov/cgi-bin/nph-distance_matrix.cgi)) were created  
371 from each library after it had been purged of chimeras. The Lane mask filter (9) was  
372 applied to limit distance matrix calculations to conserved portions of the aligned  
373 sequences. DOTUR (10) was used to create the distance matrix to produce OTUs at the  
374 97% level of similarity and calculate the Chao and ACE estimates of species richness and  
375 Shannon-Weaver and Simpson measures of diversity reported in table S2. % library  
376 coverage at the 97% level of similarity was estimated by the method of Good (11). One  
377 representative sequence was chosen from each OTU, its taxonomic affiliation determined  
378 by the RDP Classifier (12) and closest match to sequences in the GenBank database by

379 BLASTN (7; tables S1A and S1B). Distance matrices were constructed from the pooled  
380 representative sequences originating from all libraries of the same type (e.g. 5 bacterial  
381 libraries pooled into one, 3 archaeal libraries pooled into one) and DOTUR applied to  
382 produce pooled-sample OTUs at the 97% level of similarity. Correct assignment of  
383 pooled-sample OTU membership for each individual representative sequence within a  
384 pooled-sample OTU was confirmed by inspection of the taxonomic affiliation and  
385 BLASTN matches previously determined for the representative sequence.  
386 Representative bacterial sequences were deposited in GenBank under accession numbers  
387 FJ269280-FJ269403; representative archaeal sequences were assigned accession numbers  
388 FJ446497-FJ446523.

#### 389 **Enrichments and isolation**

390 General heterotrophs were enumerated in anaerobic half-strength tryptic soy broth  
391 (Becton, Dickinson and Co.) plus 1% NaCl under a N<sub>2</sub>:CO<sub>2</sub> atmosphere in a MPN assay  
392 (13). Hydrogen oxidizers were enumerated in an anaerobic, reduced basal medium under  
393 a H<sub>2</sub>:CO<sub>2</sub> atmosphere (13). SRB were enumerated in a medium designed for rapid  
394 quantitation (13).

395 **Metabolic profiling:** Fluids (1 L) from the North Slope oil field were collected from  
396 central facility (PS), production wells (2L, 2P, 2T), a pipeline carrying fluids and gas to  
397 the central facility (2U), a water reinjection well (2K) and seawater lines (biocide-treated  
398 seawater "TS", and fluids and solids scraped from the inner surface of the pipeline  
399 carrying treated seawater "SW") and immediately preserved in the field with 50% HCl  
400 (pH < 2) for metabolite analysis. Samples TS and SW were used to provide background  
401 values as they do not contain hydrocarbons. Acidified samples were kept at room

402 temperature until they were extracted with ethyl acetate, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>,  
403 and concentrated by rotary evaporation and under a stream of N<sub>2</sub>. Concentrated extracts  
404 were derivatized with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Pierce  
405 Chemical Co., Rockford, IL) to add trimethylsilyl groups for analysis by gas  
406 chromatography-mass spectrometry (GC-MS). Derivatized components were separated  
407 on a HP-5ms capillary column (30 m x 0.25 mm i.d., J&W Scientific, Folsom, CA) with  
408 a starting oven temperature of 45°C (held 5 min) increasing at 4°C/min to 270°C (held 10  
409 min) before mass spectral analysis. Metabolite identifications were made by comparison  
410 with the GC-MS features of authentic standards or with previously reported MS profiles  
411 (14-16).

412

413 Table S1A. Sequence similarity and taxonomic relationships of bacterial representative

414 small subunit partial rRNA gene sequences (OTUs at 97% similarity)

OTU	% of total	Accession number	Most similar sequences* (accession no.)	%	Class**	Source
Well 2P: total # sequences = 363, primers 27F, 1391R. 97% OTUs from DOTUR						
2P327SHNG718	90.1	FJ469286	<i>Petrobacter</i> sp. NFC7-F8 (EU250943)	99	β proteo	50°C compost
			<i>Petrobacter</i> sp. DM-3 (DQ539621)	99		Dagang oil field
2P17SHNG539	4.7	FJ469280	<i>Thermovirga lienii</i> Cas60314 (DQ071273)	99	Synergistes	North Sea oil well
2P9SHNG554	2.5	FJ469288	Uncultured clone CK06-06_Mud_MAS1B-28 (AB369171)	99	Mollicutes	Offshore drilling mud fluid
2P3SHNG611	0.8	FJ469287	Uncultured clone B5_B4 (EF025213)	99	Sphingobacteria	Turkey intestine
2P2SHNG411	0.6	FJ469285	<i>Thermoanaerobacter pseudethanolicus</i> ATCC 33223 (CP000924)	99	"Clostridia"	Octopus Springs
2P2SHNG385	0.6	FJ469284	<i>Bradyrhizobium</i> sp. JR016 (EF221629)	99	α proteo	Root nodule
2P1SHNG397	0.3	FJ469281	Uncultured Thermotogales clone bh459.f1.4.b07 (AM184116)	99	Thermotogae	Low-temp enrichment degrading polychlorinated biphenyls
2P1SHNG452	0.3	FJ469282	<i>Stenotrophomonas</i> sp. ROi7 (EF219038)	99	γ proteo	Reverse osmosis membrane
2P1SHNG731	0.3	FJ469283	<i>Ralstonia pickettii</i> 12J (CP001069)	99	β proteo	
Well 2L: total # sequences = 648, primers 27F, 1391R. 97% OTU from DOTUR.						
2L474SGXO1136	73.1	FJ469308	<i>Thermovirga lienii</i> Cas60314 (DQ071273)	99	Synergistetes	North Sea oil well
2L65SGXO482	10.0	FJ469310	<i>Thermovirga lienii</i> Cas60314 (DQ071273)	99	Synergistes	North Sea oil well
2L27SGXO638	4.2	FJ469303	<i>Desulfomicrobium thermophilum</i> P6.2 (AY464939)	98	δ proteo	Hot spring in Colombia
2L14SGXO613	2.2	FJ469289	<i>Thermoanaerobacter pseudethanolicus</i> ATCC 33223 (CP000924)	99	"Clostridia"	Octopus Springs
2L9SGXO552	1.4	FJ469316	<i>Thermosipho africanus</i> (DQ647057)	99	Thermotogae	Shallow hydrothermal system
			<i>Thermosipho</i> sp. TBA5 AF231727	99		North Sea oil field
2L7SGXO640	1.1	FJ469315	<i>Desulfacinum subterraneum</i> (AF385080)	97	δ proteo	High temp Vietnam oil field
2L6SGXO418	0.9	FJ469313	Uncultured <i>Thermovirga</i> sp. clone TCB169x (DQ647105)	95	Synergistetes	North Sea oil well
2L6SGXO1151	0.9	FJ469311	Uncultured organism clone ctg_NISA224 (DQ396164)	95	γ proteo	Deep-sea octacoral
			<i>Shewanella</i> sp. IS5	95		Diseased larval rock

2L6SGXO579	0.9	FJ469314	(AY967729) Uncultured clone Niigata-10 (AB243821)	99	$\delta$ proteo	lobster cultures Niigata (Japan) oil well
			<i>Pelobacter carbinolicus</i> (CP000142)	97		
2L6SGXO407	0.9	FJ469312	<i>Thermacetogenium phaeum</i> strain PBT (AB020336)	98	"Clostridia"	Thermophilic anaerobic methanogenic reactor
2L5SGXO560	0.8	FJ469309	<i>Clostridium</i> sp. C9 (EU862317)	99	"Clostridia"	Off-shore oil well, India
2L3SGXO643	0.5	FJ469306	<i>Thermodesulfobacterium commune</i> DSM 2178 (AF418169)	99	Thermodesulfobacteria	Yellowstone thermal spring
2L3SGXO888	0.5	FJ469307	Uncultured clone Niigata-15 (AB243826)	99	"Clostridia"	Niigata (Japan) oil well
2L2SGXO601	0.3	FJ469304	<i>Dehalococcoides</i> sp. CBDB1 (AF230641)	99	Chloroflexi Dehalococcoides	Methanogenic enrichment from Saale river sediment
2L2SGXO622	0.3	FJ469305	Gram-positive thermophile strain ODP159-02 (AY704384)	94	"Clostridia"	Ocean ridge flank crustal fluid
2L1SGXO770	0.2	FJ469299	<i>Sulfurospirillum</i> sp. NO3A (AY135396)	99	$\epsilon$ proteo	Coleville (Canada) oil field
2L1SGXO762	0.2	FJ469298	<i>Thermolithobacter thermoautotrophicus</i> KA2b (AF282254)	98	Thermolithobacteria	Yellowstone Calcite Springs
2L1SGXO814	0.2	FJ469301	Uncultured <i>Natronoanaerobium</i> sp. clone SHBZ503 (EU639010)	88	"Clostridia"	Thermophilic microbial fuel cell
			<i>Moorella thermoacetica</i> ATCC 39073 (CP000232)	88		Horse manure
2L1SGXO566	0.2	FJ469295	<i>Flexistipes</i> sp. vp180 (AF220344)	98	Deferribacteres	High temperature oil reservoir
2L1SGXO817	0.2	FJ469302	<i>Desulfotomaculum thermocisternum</i> strain ST90 (U33455)	99	"Clostridia"	Hot North Sea oil reservoir
2L1SGXO1041	0.2	FJ469292	<i>Pseudomonas stutzeri</i> strain 24a97 (AJ312172)	99	$\gamma$ proteo	Soil beneath filling station
2L1 SGXO442	0.2	FJ469293	Uncultured bacterium clone PL-25B8 (AY570610)	99	"Clostridia"	Low-temperature biodegraded Canadian oil reservoir
			<i>Acetobacterium carbinolicum</i> (AY744449)	99		
2L1SGXO751	0.2	FJ469297	Uncultured bacterium clone PL-38B5 (AY570590)	99	"Clostridia" (Anaerovorax, 100%)	Low-temperature biodegraded Canadian oil reservoir
2L1SGXO459	0.2	FJ469294	<i>Thermosipho africanus</i> strain Ob7 (DQ647057)	99	Thermotogae	
			<i>Thermosipho</i> sp. TBA5 (AF231727)	99		North Sea oil field
2L1SGXO697	0.2	FJ469296	<i>Thermovirga lienii</i> Cas60314 (DQ071273)	97	Synergistetes	North Sea oil well
2L1SGXO811	0.2	FJ469300	Uncultured <i>Thermacetogenium</i> sp. clone B11_otu13 (DQ097678)	97	"Clostridia"	High temperature Dagang oil field (China)
2L1SGXO1021	0.2	FJ469290	<i>Thermosipho geolei</i> (AJ272022)	99	Thermotogae	Siberian oil reservoir
2L1SGXO1038	0.2	FJ469291	Uncultured Spirochaetaceae clone	99		North Sea oil field

			TCB129x (DQ647164)	99		Congo oil field
			<i>Spirochaeta</i> sp. MET-E (AY800103)			
1 <sup>st</sup> stage separator: total= 544 sequences, primers 27F, 1391 R. 97% OTUs from DOTUR.						
PS313SGXI1055	57.7	FJ469337	<i>Thermovirga lienii</i> Cas60314 (DQ071273)	99	Synergistetes	North Sea oil well
PS74SGXI1247	13.6	FJ469348	<i>Thermoanaerobacter</i> <i>pseudethanolicus</i> ATCC 33223 (CP000924)	99	"Clostridia"	Octopus Springs
			<i>Thermoanaerobacter</i> strain X514(CP000923)	99		Colorado deep subsurface, iron- reducing
PS39SGXI1143	7.2	FJ469338	<i>Desulfomicrobium</i> <i>thermophilum</i> P6.2 (AY464939)	99	δ proteo	Hot spring in Colombia
PS20SGXI1921	3.7	FJ469333	Uncultured bacterium clone Niigata-10 (AB243821)	99	δ proteo	Niigata (Japan) oil well
			<i>Pelobacter carbinolicus</i> DSM 2380 (CP000142)	97		
PS10SGXI1101	1.8	FJ469317	<i>Thermosipho africanus</i> (DQ647057)	99	Thermotogae	Hot North sea oil field
PS9SGXI1270	1.7	FJ469351	<i>Thermacetogenium</i> <i>phaeum</i> (AB020336)	99	"Clostridia"	Thermophilic anaerobic methanogenic reactor
PS8SGXI1894	1.5	FJ469350	<i>Halomonas meridiana</i> strain aa-9 (EU652041)	99	γ proteo	Ocean sediment
PS8SGXI1020	1.5	FJ469349	<i>Halomonas</i> sp. A-07 (AY347310)	99	γ proteo	Tanzania soda lakes
PS6SGXI1029	1.1	FJ469345	<i>Thermodesulfobacterium</i> <i>commune</i> DSM 2178 (AF418169)	99	Thermodesulfo- bacteria	Yellowstone thermal spring
PS6SGXI904	1.1	FJ469347	<i>Thermotoga petrophila</i> RKU-1 (CP000702)	99	Thermotogae	Kubiki oil reservoir, Niigata, Japan
PS6SGXI1183	1.1	FJ469346	<i>Desulfacinum</i> <i>subterraneum</i> (AF385080)	97	δ proteo	High temp Vietnam oil field
PS5SGXI1002	0.9	FJ469344	Uncultured bacterium clone S25_271 (EF573927)	99	γ proteo	Costa Rica island
			<i>Marinobacter bacchus</i> strain FB3 (DQ282120)	99		Evaporation pond of wine wastewater
PS4SGXI1172	0.7	FJ469341	Uncultured <i>Natronoanaerobium</i> sp. clone SHBZ503 (EU639010)	87	"Clostridia"	Thermophilic microbial fuel cell
			<i>Moorella thermoacetica</i> AMP (AY884087)	87		Methanogenic sludge
PS4SGXI910	0.7	FJ469343	<i>Delftia acidovorans</i> SPH- 1 (CP000884)	99	β proteo	Sewage treatment plant
PS4SGXI1530	0.7	FJ469342	<i>Thermotoga naphthophila</i> RKU-10 (AB027017)	87	Thermotogae	Kubiki oil reservoir, Niigata, Japan
PS4SGXI1065	0.6	FJ469339	<i>Marinobacter</i> <i>hydrocarbonoclasticus</i> MARC4F (DQ768638)	99	γ proteo	Middle Atlantic Ridge Sediment
PS3SGXI1245	0.6	FJ469340	Uncultured clone MAT- CR-H3-B03 (EU245152)	85	unclassified	Hypersaline microbial mat, P.R.
PS2SGXI1098	0.4	FJ469335	<i>Petrotoga siberica</i> strain SL25T (AJ311702)	99	Thermotogae	Siberian oil reservoir
PS2SGXI1003	0.4	FJ469334	<i>Desulfotomaculum</i> <i>thermocisternum</i> (U33455)	99		Hot North Sea oil reservoir

PS2SGXI1381	0.4	FJ469336	Thermotogales TBF19.5.1 (EU980631)	99	Thermotogae	North Sea oil production fluid
PS1SGXI1064	0.2	FJ469318	<i>Thermosipho geolei</i> . DSM 13256 (AJ272022)	98	Thermotogae	Siberian oil reservoir
PS1SGXI1111	0.2	FJ469319	Uncultured bacterium clone Zplanc13 (EF602474)	93	unclassified	Zodletone Spring source sediments
PS1SGXI1133	0.2	FJ469320	Uncultured <i>Sulfurospirillum</i> sp. clone LA4-B52N (AF513952)	93	$\epsilon$ proteo	Hawaiian lake water
			<i>Sulfurospirillum carboxydovorans</i> (AY740528)	93		North Sea sediment
PS1SGXI1244	0.2	FJ469321	<i>Geotoga aestuarianus</i> strain T3B (AF509468)	99	Thermotogae	Karst sink hole thiosulfate-reducer
PS1SGXI1249	0.2	FJ469322	<i>Burkholderia multivorans</i> strain LMG 13010 <sup>T</sup> (Y18703)	99	$\beta$ proteo	Cystic fibrosis patient
PS1SGXI1260	0.2	FJ469323	Uncultured bacterium clone: HDBW-WB60 (AB237723)	99	“Clostridia”	Deep subsurface groundwater
PS1SGXI1272	0.2	FJ469324	<i>Thermovirga lienii</i> Cas60314 (DQ071273)	97	Synergistetes	North Sea oil well
PS1SGXI1281	0.2	FJ469325	Uncultured <i>Thermovirga</i> sp. clone TCB8y	97	Synergistetes	North Sea produced water
PS1SGXI1300	0.2	FJ469326	<i>Marinobacterium</i> sp. IC961 strain IC961	99	$\gamma$ proteo	Carbazole-utilizing bacterium
PS1SGXI1313	0.2	FJ469327	Uncultured bacterium a2b00 (AF419657)	92	unclassified	Hydrothermal sediments in the Guaymas Basin
PS1SGXI1413	0.2	FJ469328	Uncultured <i>Thermacetogenium</i> sp. clone B11_otu13 (DQ097678)	98	“Clostridia”	High temperature Dagang oil field (China)
PS1SGXI1848	0.2	FJ469329	<i>Pseudomonas putida</i> W619 (CP000949)	100	$\gamma$ proteo	Japan:Shizuoka, Sagara oil field
			<i>Pseudomonas</i> sp. OCR2 (AB240201)	99		
PS1SGXI1964	0.2	FJ469330	Uncultured <i>Thermovirga</i> sp. clone TCB169x (DQ647105)	96	Synergistetes	High temp North Sea oil field
PS1SGXI1984	0.2	FJ469331	Uncultured bacterium gene (AB195893)	96	Bacteroidetes	Anaerobic sludge
PS1SGXI1995	0.2	FJ469332	<i>Desulfotignum balticum</i> DSM 7044 (AF418176)	99	$\delta$ proteo	Marine coastal sediment, Baltic Sea
Coalescer: total # sequences = 329, primers 27F, 1391 R.97% OTUs from DOTUR.						
CO105SHNF404	31.9	FJ469352	<i>Thermovirga lienii</i> Cas60314 (DQ071273)	99	Synergistetes	High temp North Sea oil field
			Uncultured <i>Thermovirga</i> sp. clone TCB8y (DQ647105)	99		High temp North Sea oil field
CO60SHNF483	18.2	FJ469383	<i>Desulfocaldus</i> sp. Hobo (EF442977)	99	$\delta$ proteo	Not specified
			<i>Desulfomicrobium thermophilum</i> P6.2 (AY464939)	99	$\delta$ proteo	Hot spring in Colombia
CO46SHNF563	14.0	FJ469378	Uncultured bacterium clone: Niigata-10 (AB243821)	99	$\delta$ proteo	Niigata (Japan) oil well
			<i>Pelobacter carbinolicus</i> DSM 2380 (CP000142)	97		

CO26SHNF710	7.9	FJ469371	<i>Thermoanaerobacter pseudethanolicus</i> ATCC 33223(CP000924)	100	"Clostridia"	Octopus Springs
CO12SHNF562	3.6	FJ469354	Uncultured bacterium clone cc187 (DQ057384)	100	$\beta$ proteo	Chicken intestine
			Beta proteobacterium B7 AF035053	98		Drinking water system
CO11SHNF516	3.3	FJ469353	<i>Thermodesulfobacterium commune</i> DSM 2178 (AF418169)	99	Thermodesulfobacteria	Yellowstone thermal spring
CO7SHNF526	2.1	FJ469386	<i>Stenotrophomonas maltophilia</i> strain DN1.1 (EU034540)	99	$\gamma$ proteo	Not specified
			Uncultured bacterium clone Ana10UA-2 (EU499720)	99	$\gamma$ proteo	Freshwater sediment
CO6SHNF422	1.8	FJ469384	<i>Bradyrhizobium japonicum</i> strain SEMIA 6164 (AY904765)	99	$\alpha$ proteo	Acacia root nodule
CO6SHNF588	1.8	FJ469385	<i>Desulfacinum subterraneum</i> (AF385080)	98	$\delta$ proteo	High temp Vietnam oil field
CO5SHNF732	1.5	FJ469382	<i>Thermacetogenium phaeum</i> strain PBT (AB020336)	99	"Clostridia"	Thermophilic anaerobic methanogenic reactor
CO5SHNF565	1.5	FJ469380	<i>Ralstonia pickettii</i> 12J (CP001069)	100	$\beta$ proteo	Seafloor lavas from the Loi'hi Seamount
			Uncultured bacterium clone P7X3b4E02 (EU491068)	100	$\beta$ proteo	South Rift X3
CO5SHNF607	1.5	FJ469381	Uncultured clone B5_B4 (EF025213)	100	Sphingobacteria	Turkey intestine
			<i>Sediminibacterium salmoneum</i> (EF407879)	96		Eutrophic reservoir
CO4SHNF461	1.2	FJ469379	<i>Mesorhizobium plurifarum</i> , strain LMG 10056 (Y14161)	99	$\alpha$ proteo	Tropical tree
CO3SHNF446	0.9	FJ469375	Uncultured <i>Natronoanaerobium</i> sp. clone SHBZ503 (EU639010)	88	"Clostridia"	Microbial fuel cell
			<i>Moorella thermoacetica</i> ATCC 39073 (CP000232)	88		Horse manure
CO3SHNF573	0.9	FJ469376	Beta proteobacterium A1040 (AF236008)	99	$\beta$ proteo	Not specified
			Beta proteobacterium MB7 (AB013409)	99		Soil isolate degrading aliphatic polyesters
CO3SHNF586	0.9	FJ469377	<i>Hyphomicrobium</i> sp. P2 (AF148858)	99	$\alpha$ proteo	Portuguese soil
CO2SHNF510	0.6	FJ469373	<i>Thermosipho africanus</i> (DQ647057)	99	Thermotogae	Shallow hydrothermal system
			<i>Thermosipho</i> sp. TBA5 AF231727	99		North sea oil field
CO2SHNF508	0.6	FJ469372	<i>Solemya velum</i> symbiont (M90415)	99	$\gamma$ proteo	Sulfur-oxidizing mollusk symbiont
CO2SHNF712	0.6	FJ469374	<i>Spirochaeta thermophila</i> (X62809)	98	Spirochaetes	Kuril Island hot springs
CO1SHNF389	0.3	FJ469355	Uncultured <i>Hydrogenothermus</i> sp. clone OPPB154 (AY861874)	99	Aquificales	Yellowstone Obsidian Pool
			Aquificales bacterium	99		Yellowstone Calcite



CO1SHNF407	0.3	FJ469356	YNP-SS1 (AF507961) <i>Desulfomicrobium norvegicum</i> strain DSM 1741T (AJ277897)	99	$\delta$ proteo	Springs Oslo Harbour water
CO1SHNF410	0.3	FJ469357	<i>Marinobacter bacchus</i> strain FB3 (DQ282120)	99	$\gamma$ proteo	Wine wastewater
CO1SHNF443	0.3	FJ469358	<i>Thermotoga elfii</i> strain SM-2 (EU276416)	99	Thermotogae	Not specified
CO1SHFN484	0.3	FJ469359	Uncultured bacterium clone rRNA082 (AY958855)	98	Sphingobacteria	Oil-production water Human vaginal epithelium
			<i>Solibium</i> sp. I-32 (AM990455)	97		Ultra pure water
CO1SHNF528	0.3	FJ469360	Uncultured clone B5_F26 (EF025264)	99	Sphingobacteria	Turkey intestine
			Flavobacteria bacterium KF030 (AB269814)	94		Freshwater lake
CO1SHNF536	0.3	FJ469361	Uncultured bacterium clone PS18 (DQ984666)	93	"Clostridia"	Sulfate-reducing LCFA enrichment
			<i>Syntrophomonas palmitatica</i> (AB274040)	93		
CO1SHFN544	0.3	FJ469362	Uncultured <i>Thermacetogenium</i> sp. clone B11_otu13 (DQ097678)	98	"Clostridia"	Methanogenic sludge High temperature Dagang oil field (China)
CO1SHNF610	0.3	FJ469363	<i>Desulfovibrio aespoensis</i> clone Aspo3 (EU680957)	98	$\delta$ proteo	Aespoe hard rock
			<i>Desulfovibrio aespoensis</i> isolate Aspo2 (X95230)			
CO1SHNF612	0.3	FJ469364	<i>Thermotoga petrophila</i> RKU-1 (AJ872269)	100	Thermotogae	Kubiki oil reservoir
CO1SHNF615	0.3	FJ469365	Uncultured Termite group 1 bacterium clone HAVOmat14 (EF032762)	99	candidate division TG1	Cyanobacterial mat in Hawaiian lava cave
CO1SHNF622	0.3	FJ469366	Thermotogales bacterium 2SM-2 (EU276414)	100	Thermotogae	Oil-production water
CO1SHNF639	0.3	FJ469367	<i>Thermolithobacter thermoautotrophicus</i> clone KA2b (AF282254)	99	Thermolithobacteria	Yellowstone Calcite Springs
CO1SHNF644	0.3	FJ469368	Thermoanaerobacteriaceae clone EV818FW062101BH4M D48 (DQ079638)	95	"Clostridia"	Terrestrial subsurface fluid-filled fracture
			<i>Moorella thermoacetica</i> strain AMP (AY884087)	95		Methanogenic sludge
CO1SHNF669	0.3	FJ469369	Uncultured <i>Anaerovorax</i> sp. clone C14B-1H (EU073780)	97	"Clostridia"	Coal enrichment culture
			Clostridiaceae bacterium FH042 (AB298771)	96		Anaerobic sludge of a methanogenic reactor
CO1SHNF695	0.3	FJ469370	<i>Desulfovibrio</i> sp. X (EF442979)	99	$\delta$ proteo	Not specified
			<i>Desulfovibrio zosteriae</i> (Y18049)	95		Roots of seagrass ( <i>Zostera marina</i> )
Seawater pig envelope. Total # sequences = 215. primers 27F, 1492R. 97% OTUs from DOTUR						
SW76FGIT720	35.3	FJ469401	<i>Pseudomonas</i> sp. HZ06 (AY690706)	99	$\gamma$ proteo	Rhizosphere soil of salt marshes
SW55FGIT591	25.6	FJ469399	<i>Pseudomonas stutzeri</i> strain aa-28 (EU652047)	99	$\gamma$ proteo	Ocean sediment
SW35FGIT483	16.3	FJ469395	Uncultured proteobacterium clone	97	$\epsilon$ proteo <i>Sulfurimonas</i>	Guaymas Basin hydrothermal vent

SW12FGIT423	5.6	FJ469387	B01R008 (AY197379) <i>Pseudomonas</i> sp. EP27 (AM403529)	98	$\gamma$ proteo	sediments Deep-sea sediments
SW9FGIT664	4.2	FJ469403	Uncultured <i>Arcobacter</i> sp. clone DS172 (DQ234254)	98	$\epsilon$ proteo <i>Arcobacter</i>	Mangrove
SW7FGIT497	3.3	FJ469402	Uncultured bacterium clone W26 (AY770966)	98	$\gamma$ proteo <i>Pseudomonas</i>	Water injection well of Dagang oilfield
SW5FGIT667	2.3	FJ469400	Uncultured <i>Pseudomonas</i> sp. clone Lupin-1130m-2- MDA-pse3 (EF205269)	98	$\gamma$ proteo	Lupin gold mine fracture water
SW3FGIT405	1.4	FJ469396	<i>Pseudomonas marincola</i> (AB301071)	96	$\gamma$ proteo	Deep-sea brittle star
SW3FGIT554	1.4	FJ469397	Uncultured alpha proteobacterium clone 131582 (AY922182)	97	$\epsilon$ proteo <i>Arcobacter</i>	Grey whale bone, Pacific Ocean, depth 1674 meters
SW3FGIT592	1.4	FJ469398	Uncultured epsilon proteobacterium clone: NKB11 (AB013263)	96	$\epsilon$ proteo <i>Sulfurimonas</i>	Nankai Trough sediments
SW1FGIT389	0.5	FJ469388	"Gamma" proteobacterium IR (AF521582)	99	$\gamma$ proteo /unclassified	Not specified "Diversity of marine humics-oxidizing bacteria"
SW1FGIT424	0.5	FJ469389	Uncultured bacterium clone B8S-8 (EU652615)	88	$\delta$ proteo	Yellow Sea sediment
SW1FGIT462	0.5	FJ469390	<i>Phaeobacter arcticus</i> strain 20188 (DQ514304)	99	$\alpha$ proteo	Arctic marine sediment
SW1FGIT467	0.5	FJ469391	Uncultured bacterium clone GZKB9 (AJ853504)	97	$\epsilon$ proteo <i>Arcobacter</i>	Landfill leachate
SW1FGIT501	0.5	FJ469392	Uncultured delta proteobacterium clone d13 (AY062878)	98	$\delta$ proteo <i>Desulfuromonas</i>	Electrode surface
SW1FGIT563	0.5	FJ469393	Uncultured bacterium clone P9X2b3A09 (EU491225)	86	$\delta$ proteo /unclassified	Seafloor lavas
SW1FGIT660	0.5	FJ469394	Uncultured bacterium ARCTIC23_B_12 (EU795085)	99	Flavobacteria <i>Polaribacter</i>	Arctic

416 Table 1B. Sequence similarity and taxonomic relationships of archaeal representative  
 417 small subunit partial rRNA gene sequences (OTUs at 97% similarity)

418

OTU	% of total	Accession number	Most similar sequences (accession no.)	%	Orders	Source
Well 2P: total # sequences =226, primers ARC333 and 958R, 97% OTUs from DOTUR						
2P66FGIP571	29.2	FJ446503	Archaeon enrichment culture clone PW5.2A (EU573152)	100	Thermococcales	Ekofisk oil field
			<i>Thermococcus alcaliphilus</i> DSM 10322 (AB055121)	100		
2P66FGIP425	29.2	FJ446502	Uncultured archaeon SSE_L4_E01(EU635901)	99	Archaeoglobales	Hot spring sediment
			<i>Archaeoglobus fulgidus</i> strain L3 (DQ374392)	97		
2P64FGIP517	28.3	FJ446501	<i>Methanothermobacter thermautotrophicus</i> strain JZTM (EF100758)	99	Methanobacteriales	Jiaozhou Bay sediment
			<i>M. wolfeii</i> strain KZ24a (DQ657904)	99		Dagang oil field
2P26FGIP436	11.5	FJ446499	Uncultured Methanobacteriaceae clone A1m_OTU 3 (DQ097668)	99	Methanobacteriales	Dagang oil field
			<i>Methanothermobacter thermautotrophicus</i> strain JZTM (EF100758)	96		Jiaozhou Bay sediment
2P2FGIP540	0.9	FJ446500	Archaeon enrichment culture clone PW30.6A (EU573155)	99	Archaeoglobales	Ekofisk oil field
			<i>Archaeoglobus</i> sp. NI85-A (AB175518)	99		Deep-sea hydrothermal vent chimney
2P1FGIP407	0.4	FJ446497	Uncultured archaeon SSE_L4_E01(EU635901)	98	Archaeoglobales	Hot spring sediment
			<i>Archaeoglobus fulgidus</i> strain L3 (DQ374392)	97		
2P1FGIP710	0.4	FJ446498	Uncultured clone QHO-A15 (DQ785496)	96	Methanobacteriales	High temperature oil field in China
			<i>Methanobacterium</i> sp. F (AB302952)	96		Rice paddy soil
1 <sup>st</sup> stage separator: total # sequences =252, primers ARC333 and 958R, 97% OTUs from DOTUR						
PS70SGXN402	27.8	FJ446513	<i>Thermococcus mexicalis</i> strain GY 869 (AY099181)	99	Thermococcales	Hydrothermal deep-sea vents
			<i>Thermococcus sibiricus</i> (AJ238992)	99		Siberian high-temperature oil reservoir
PS56SGXN497	22.2	FJ446512	<i>Archaeoglobus fulgidus</i> DSM 4304 (AE000782)	99	Archaeoglobales	
			<i>Archaeoglobus fulgidus</i> strain L3 (DQ374392)	99		
PS35SGXN478	13.9	FJ446511	Uncultured archaeon clone NAK1-a1 (DQ867048)	99	Methanobacteriales	High-temperature natural gas field
			Uncultured bacterium clone QHO-A27 (DQ785508)	99		High-temperature petroleum reservoir

			<i>Methanothermobacter</i>	96		Jiaozhou Bay sediment
			<i>thermautotrophicus</i> strain JZTM (EF100758)			
PS29SGXN477	11.5	FJ446509	Uncultured archaeon clone NAK1-a1 (DQ867048)	99	Methanobacteriales	High-temperature natural gas field
			<i>Methanothermobacter</i>	99		Jiaozhou Bay sediment
			<i>thermautotrophicus</i> strain JZTM (EF100758)			
PS22SGXN482	8.7	FJ446508	Methanogenic archaeon ZC-1 (DQ787474)	99	Methanosarcinales	Oil production water
			" <i>Methermicoccus shengliensis</i> "			
PS11SGXN711	4.4	FJ446504	Uncultured Desulfurococcales YNP_SSp_A61 (DQ243776)	100	Desulfurococcales (Crenarcheota)	Yellowstone hot springs
			<i>Staphylothermus achaiicus</i> (AJ012645)	94		Geothermal vents, Greece
PS8SGXN439	3.2	FJ446514	<i>Thermococcus acidaminovorans</i> strain DSM 11906 (AY099170)	100	Thermococcales	Hydrothermal deep-sea vents, Italy
			Archaeon enrichment culture clone EA3.5 (EU573147)	99		Ekofisk oil field
PS8SGXN687	3.2	FJ446515	Uncultured archaeon clone SSE_L4_H05 (EU635920)	99	Desulfurococcales (Crenarcheota)	Nevada hot spring sediment
			<i>Thermosphaera aggregans</i> (X99556)	99		Yellowstone hot spring
PS8SGXN753	3.2	FJ446516	Uncultured archaeon G04b_L4_A09 (EU635911)	99	Thermoproteales (Crenarcheota)	Nevada hot spring sediment
			<i>Vulcanisaeta distributa</i> strain IC-065 (AB063639)	87		Japan hot spring
PS2SGXN537	0.8	FJ446510	Uncultured crenarchaeote WIP_20m_6B_A (EF420183)	99	Desulfurococcales (Crenarcheota)	Canadian oil sands tailings pond
			" <i>Desulfurococcus kamchatkensis</i> " (EU167539)	86		Kamchatka hot spring
PS1SGXN453	0.4	FJ446505	Archaeon enrichment culture clone PW30.6A (EU573155)	99	Archaeoglobales	Ekofisk oil field
			<i>Archaeoglobus profundus</i> (AF297529)	98		
PS1SGXN470	0.4	FJ446506	<i>Thermococcus mexicalis</i> strain GY 869 (AY099181)	99	Thermococcales	Hydrothermal deep-sea vents
			<i>Thermococcus sibiricus</i> (AJ238992)	99		Siberian high-temperature oil reservoir
PS1SGXN592	0.4	FJ446507	Archaeon enrichment culture clone PW15.7A (EU573156)	99	Archaeoglobales	Ekofisk oil field
			<i>Archaeoglobus profundus</i> (AF297529)	90		
			Coalescer: total # sequences = 240, primers ARC333 and 958R, 97% OTUs from DOTUR			
CO146FGIO3	60.8	FJ446519	Uncultured archaeon SSE_L4_E01(EU635901)	99	Archaeoglobales	Hot spring sediment

			Archaeon enrichment culture clone EA8.8 (EU573151)	98		Ekofisk oil field
			<i>Archaeoglobus fulgidus</i> strain L3 (DQ374392)	98		
CO64FGIO506	26.7	FJ446522	<i>Thermococcus alcaliphilus</i> DSM 10322 (AB055121)	100	Thermococales	
			Archaeon enrichment culture clone PW5.2A (EU573152)	99		Ekofisk oil field
CO12FGIO387	5.0	FJ446518	Uncultured archaeon SSE_L4_E01(EU635901)	96	Archaeoglobales	Hot spring sediment
			<i>Ferroglobus placidus</i> (AF220166)	96		
CO10FGIO395	4.2	FJ446517	Archaeon enrichment culture clone PW30.6A (EU573155)	99	Archaeoglobales	Ekofisk oil field
			<i>Archaeoglobus profundus</i> (AF297529)	98		
CO6FGIO519	2.5	FJ446523	Uncultured <i>Methanothermobacter</i> sp. clone ARCA-3F (EU073827)	99	Methanobacteriales	Coal enrichment culture
			<i>Methanothermobacter thermautotrophicus</i> strain JZTM (EF100758)	99		Jiaozhou Bay sediment
			<i>Methanothermobacter wolfeii</i> strain KZ24 (DQ657904)	99		Dagang oil field
CO1FGIO425	0.4	FJ446520	Archaeon enrichment culture clone PW30.6A (EU573155)	96	Archaeoglobales	Ekofisk oil field
			<i>Ferroglobus placidus</i> (AF220166)	96		
CO1FGIO557	0.4	FJ446521	Uncultured crenarchaeote Clone MDS-r-E06 (AB353218)	98	Desulfurococcales (Crenarchaeota)	Mesophilic digested sludge
			" <i>Desulfurococcus kamchatkensis</i> " strain 1221n (EU167539)	86		Kamchatka hot spring

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420

421 \* most similar sequence and/or isolate in Genbank, as determined by BLASTN.

422 \*\* Class affiliation, as determined by Classifier (RDP).

423

424 One sequence from each operational taxonomic unit (OTU) at the 97% level of similarity

425 as defined by DOTUR (10) was chosen from among the sequences in that OTU and its

426 taxonomic affiliation and closest GenBank match was determined. Representative

427 sequences are named with the first two letters indicating the sample origin, the following

428 numerals designate the total number of sequences within that particular OTU. The final  
429 four letters and 3-4 numbers are the JGI code identifying the sample library and location  
430 within the 384-well plate.

431 Table S2. Measures of genetic diversity and species richness.

432

Sample code	# clones	# 99.9% OTUs (%)	# 97% OTUs (%)	S <sub>Chao1</sub> (97%) (95% CI)	ACE (97%) (95% CI)	H (97%)	S (97%)	% library coverage (97%)
SWBAC	215	91	17	38 (22.1-103.3)	27.2 (19.4-59.8)	1.86 (1.71-2.01)	0.2205	97.2
2PBAC	334	35	9	10 (9.09-19.7)	13.9 (9.8-36.8)	0.47 (0.36-0.59)	0.8139	99.2
2LBAC	648	280	28	54 (34.8-127.2)	45.8 (33.7-83.7)	1.21 (1.08-1.34)	0.5474	98.0
PSBAC	544	267	35	61.3 (42.4-127.5)	53.2 (41.5-86.2)	1.76 (1.63-1.91)	0.3567	97.2
COBAC	329	60	35	65 (43.7-138.2)	56.1 (42.4-95.0)	2.36 (2.21-2.51)	0.1633	95.1
2PARC	226	82	7	7.5 (7.0-15.3)	10 (7.5-25.4)	1.41 (1.34-1.49)	0.2608	99.1
PSARC	252	83	13	14.5 (13.2-28.1)	15.9 (13.4-33.5)	2.00 (1.89-2.10)	0.1684	98.8
COARC	240	63	7	8 (7.07-20.8)	9.2 (7.3-26.5)	1.07 (0.95-1.20)	0.4418	99.2

433

434 SWBAC: Bacterial 16S rRNA library from seawater pig envelope sample.

435 2PBAC: Bacterial 16S rRNA library from well 2P sample.

436 2LBAC: Bacterial 16S rRNA library from well 2L sample.

437 PSBAC: Bacterial 16S rRNA library from 1<sup>st</sup> stage separator sample.

438 COBAC: Bacterial 16S rRNA library from coalescer sample.

439 2PARC: Archaeal 16S rRNA library from well 2P sample.

440 PSARC: Archaeal 16S rRNA library from 1<sup>st</sup> stage separator sample.

441 COARC: Archaeal 16S rRNA library from coalescer sample.

442

443 # OTUs, S<sub>Chao1</sub> (97%), ACE (97%), H (Shannon-Weaver), S (Simpson's index) were

444 estimated using DOTUR (10).

445 % library coverage was estimated by the method of Good (11) for sequences at 97%

446 similarity.

447 95% CI: 95% confidence interval values.

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450

Table S3. Core and dominant bacterial OTUs (97% similarity) and physiologies.

OTU closest match (% similarity)	%*	Physiology of closest match	2L	2P	PS	CO	SW	Total #
Bacteria: Core 2L, PS, CO								
<i>Thermovirga lienii</i> (DQ071273)	99	<sup>17***</sup> Fermentative, H <sub>2</sub> S from cystine/S <sup>0</sup>	540**	17	313	105	0	975
<i>Desulfomicrobium thermophilum</i> (AY464939)	98	<sup>18</sup> SRB	27	0	39	60	0	126
<i>Thermosiphon africanus/geolei</i> (DQ647057/AJ272022)	99	<sup>19</sup> H <sub>2</sub> S from S <sup>0</sup>	10	0	11	2	0	23
Uncultured clone/ <i>Pelobacter carbinolicus</i> (AB243821/CP000142)	97	Fermentative, Syntroph, <sup>19</sup> H <sub>2</sub> S from cystine/S <sup>0</sup> , IR	6	0	20	46	0	72
<i>Desulfacinum subterraneum</i> (AF385080)	97	<sup>20</sup> SRB	7	0	6	6	0	19
<i>Thermodesulfobacterium commune</i> (AF418169)	98	<sup>20</sup> SRB	3	0	6	11	0	20
<i>Thermoanaerobacter pseudethanolicus/X514</i> (CP000924/CP000923)	99	<sup>21</sup> Fermentative, IR (some strains), thiosulfate reduction	14	2	74	26	0	116
<i>Thermacetogenium phaeum</i> (AB020336)	98	<sup>22</sup> Acetogen, Syntroph, Sulfate/thiosulfate reduction	6	0	9	5	0	20
Uncultured <i>Thermacetogenium</i> clone B11_otu13 (DQ097678)	97	Uncultured	1	0	1	1	0	3
<i>Moorella thermoacetica</i> (AY884087)	86	Fermentative	1	0	4	3	0	8
Bacteria: dominant 2P								
<i>Petrobacter</i> sp. DM-3 (DQ539621)	99	<sup>23</sup> Fermentative NR	0	327	0	0	0	327
Bacteria: dominant SW								
<i>Pseudomonas stutzeri/putida</i>	98	<sup>24</sup> NR- and/or HC-degrading (some)	1	0	1	0	143	145

451  
452

\*: % similarity

453

\*\* : number of sequences per sample

454

\*\*\*: reference

455

SRB: sulfate-reducing bacteria

456

IR: iron reduction

457

NR: nitrate reduction



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Table S4. Dominant archaeal OTUs (97% similarity) and physiologies.

OTU closest match (% similarity)	%*	Physiology of closest match (25)	2P	PS	CO	Total #
Archaea: Core 2P, PS, CO						
<i>Archaeoglobus fulgidus</i> DSM 4304/L3 (AE000782/DQ374392)	98	SRA	66**	56	158	280
<i>Thermococcus alcaliphilus/mexicalis/sibiricus</i> (AB055121/AY099181/AJ238992)	99	Fermentative, H <sub>2</sub> S from S <sup>0</sup> , IR ( <i>T. sibiricus</i> )	66	78	64	208
<i>Methanothermobacter thermautotrophicus</i> (EF100758)	99	Methanogen H <sub>2</sub> -utilizing	64	29	6	99
Uncultured Methanobacteriaceae clone A1m_OTU 3 (DQ097668)	99	Uncultured, from Dang oil field	26	35	0	61
<i>Archaeoglobus profundus</i> (AF297529)	98	SRA	2	1	11	14

461  
462

\*: % similarity

463

\*\* : number of sequences per sample

464

SRA: sulfate-reducing archaea

465

IR: iron reduction

466

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