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Supplementary Material

Effects of Estrone and Organic Carbon Exposure on the Transformation of Estrone

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DETAILED PROCEDURES FOR DNA AND E1 ANALYSIS

DNA Collection, Processing, and Analysis All samples for DNA analysis were collected in triplicate and subsequently processed. Reactor liquor (1.5 mL) was centrifuged and decanted, after which the pellet underwent three consecutive freeze-thaw cycles and incubation at 70°C for 90 min to lyse cells. DNA was extracted from lysed cells with the FastDNA spin kit (MP Biomedicals, Solon, OH) and stored at -20°C until further processing.

Illumina The V3 region of the 16S rDNA was amplified via PCR using the primers described by Muyzer et al,[1] according to the method described by Bartram et al.[2] The primer sequences were as follows: forward primer: 5'-ID-CCTACGGGAGGCAGCAG-3'; and reverse primer: 5'-ID-ATTACCGCGGCTGCTGG-3', where ID is the Illumina adapter sequence containing a six base identification barcode for sample identification.[2] PCR products were screened using 2% agarose gels, pooled from duplicate PCR reactions, and purified using the PCR Purification kit (Qiagen; Valencia, Calif.) per manufacturer's instructions. Purified DNA was run on gels for quantification, and equal amounts of each sample were pooled and paired-end sequenced (2 × 150) on an Illumina MiSeq platform at the University of Minnesota Genomics Center (UMGC; Saint Paul, MN, USA).

DNA sequences from Illumina were binned based on the barcode sequencing by UMGC. Illumina sequence reads were processed using Quantitative Insights Into Microbial Ecology (QIIME),[3] generating paired reads of 125 base-pairs. Sequences were screened to exclude the following: sequences containing more than one mismatch in the barcoded primer, sequence lengths of less than 50 bp or more than 125 bp, and sequences with a Q-score of less than 35. Primers and barcodes were trimmed from the screened sequence reads. Sequences were clustered into de novo operational taxonomic units (OTUs) using uclust at 97% similarity.[4] Reference sequences for each OTU cluster were selected based on the most numerous sequence and compared to the Greengenes reference database to determine taxonomy.[5] Reference sequences were aligned using PyNAST[6] and a phylogenetic tree was constructed using FastTree.[7] UNIFRAC was used for principle coordinate analysis of communities.[5]

E1 Sample Extraction and Cleanup Solid phase extraction (SPE) and silica gel clean-up procedures were adapted from Ternes et al.[8] Briefly, samples of 50 mL or 100 mL were collected for E1 analysis, acidified to pH 3 with concentrated sulfuric acid, and amended with 80 ng of a labeled surrogate, (2,4,16,16-D_4-estrone). Biomass was not separated from the liquid sample. Resprep Bonded Reversed Phase SPE cartridges (6 mL, Restek) were preconditioned with two column volumes each of acetone and Milli-Q water. Samples were then loaded onto the cartridges at a flowrate of ~3 mL/min. Samples were eluted from the column with two column volumes of acetone. Controls with non-E1 degrading biomass show that this procedure is capable of recovering E1 that may be sorbed to the biomass.

Eluted samples were blown down to dryness with nitrogen and resuspended in 2 mL of hexane for silica gel cleanup. Silica gel columns were prepared by packing silica gel to a height of 3 cm (~ 1 mL) into pasture pipettes and then washing with two column volumes of hexane. Samples were then loaded onto the column and eluted with three column volumes of a 65:35 mixture of acetone and hexane (v/v), blown down to dryness with nitrogen, and resuspended in a 60:40 mixture of methanol and water (v/v) containing 100 ng (a concentration of 250 µg/L) of an internal standard (13,14,15,16,17,18-$^{13}C_6$-estrone). The sample was then stored at 4°C until analysis via liquid chromatography-mass spectrometry (LC-MS) (described in the Supporting Information). Average sample recovery was 52% with a standard deviation of 19%.

LC-MS Analysis E1 samples were quantified via LC-MS using an HP 1050-series LC coupled to an Agilent/HP 1100 Series G1946D mass spectrometer detector or an Agilent 1100 series LC coupled to a 4000 QTRAP triple quadrupole mass spectrometer. E1 was separated on a Synergi 4u Polar-RP 80A 150 × 2.00 mm 4 μm particle size column (Phenomenex).

For LC-MS analysis, a binary gradient consisting of a pH 4 ammonium acetate buffered solution (10 mM) in 90% water and 10% acetonitrile (A) and 100% acetonitrile (B) at a flow rate of 0.2 mL/min was used. The gradient was as follows: 35% B for 17 min, followed by a linear increase to 100% B over 3 min, held at 100% B for 5 min, and stepped down to 35% B for equilibration over 5 min. The mass spectrometer was operated in negative ion, selected ion monitoring mode with m/z ratios of 269, 273, and 275 for the detection of estrone, the surrogate, and the internal standard, respectively.

Tandem mass spectrometry analysis methods were adapted from Di Carro et al.[9] A binary gradient consisting of a pH 5.5 ammonium acetate buffered solution (10 mM) in 90% water and 10% acetonitrile (A) and 100% acetonitrile (B) at a flow rate of 0.2 mL/min was used. The gradient was as follows: 30% B for 2 min, followed by a linear increase to 95% B over 8 min, held at 95% B for 1 min, and stepped down to 30% B for equilibration over 4 min. The mass spectrometer was operated in negative ion, selected reaction monitoring mode, using the two most abundant ion pairs for quantification and confirmation, respectively. The *m/z* pairs were:

269→145 and 269→143 for estrone; 273→147 and 273→187 for the surrogate; and 275→145 and 275→186 for the internal standard.

Standard curves of at least seven points were used in sample quantification. Blanks of 40:60 methanol and water, as well as method blanks were run at the beginning of each sample analysis, as well as intermittently between samples. Typical instrument quantification limits were 10 µg/L (sample quantification limits of 40 ng/L) for the HP 1100 Series G1946D mass spectrometer and 1 µg/L (sample quantification limits of 4 ng/L) for the 4000 QTRAP model. In-vial concentrations of E1 and surrogate were corrected by the internal standard. Sample concentrations of E1 were further corrected using surrogate recovery.

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TAXONOMIC ANALYSIS OF ILLUMINA SEQUENCING

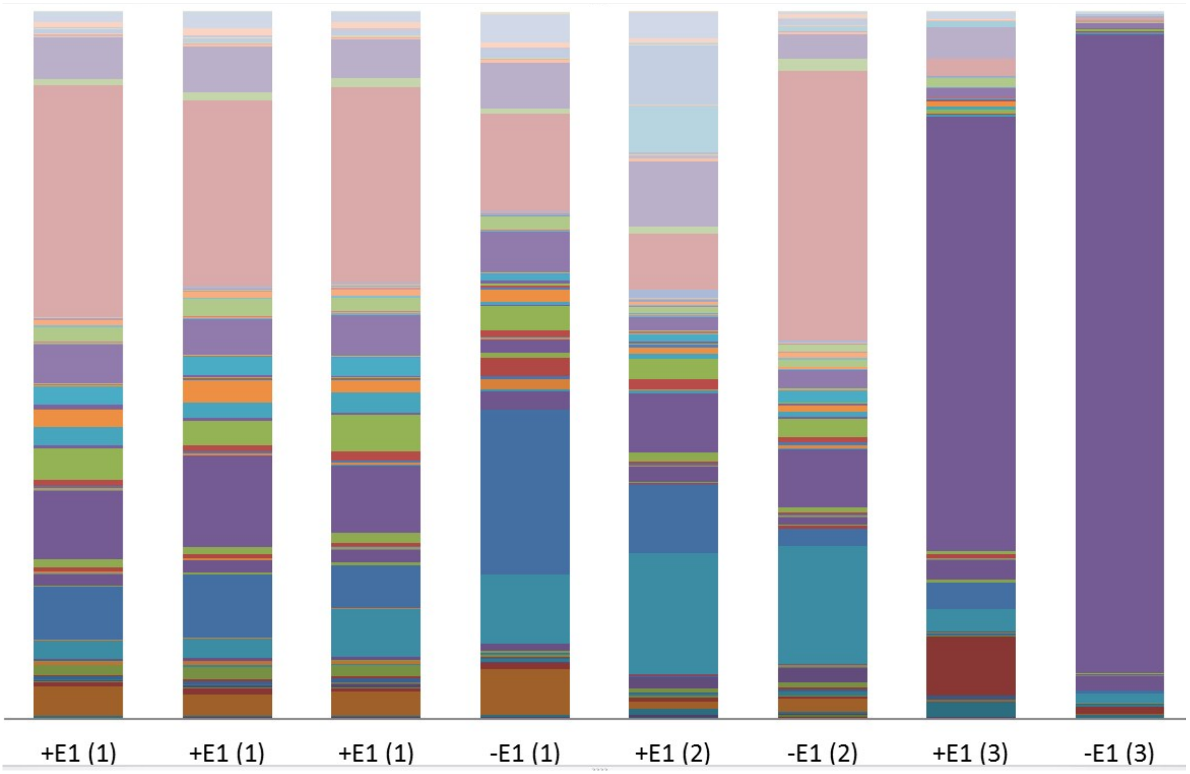

Figure S1: Visual representation of taxonomy for reactors with and without exposure to E1 as analyzed by Illumina, with details reported in the table below. Analysis was done at the genus level. Only genera comprising more than 0.1% of at least one reactor population are shown here for brevity.

	+E1 (1)	+E1 (1)	+E1 (1)	-E1 (1)	+E1 (2)	-E1 (2)	+E1 (3)	-E1 (3)
k__Bacteria;p__Acidobacteria;c__[Chloracidobacteria];o__RB41;f__Ellin6075;g__	0.0002	0.0002	0.0002	0.0041	0.0002	0.0000	0.0001	0.0000
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__	0.0006	0.0007	0.0006	0.0007	0.0011	0.0010	0.0000	0.0000
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Agromyces	0.0143	0.0227	0.0143	0.0392	0.0359	0.0024	0.0110	0.0032
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;g__Microbacterium	0.0071	0.0098	0.0074	0.0060	0.0058	0.0058	0.0003	0.0001
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Microbacteriaceae;Other	0.0003	0.0005	0.0004	0.0005	0.0013	0.0006	0.0001	0.0001
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__	0.0015	0.0018	0.0014	0.0022	0.0013	0.0037	0.0000	0.0000
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Arthrobacter	0.0006	0.0012	0.0006	0.0004	0.0009	0.0004	0.0000	0.0000

k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Propionibacteriaceae;g__Propionibacterium	0.0000	0.0000	0.0015	0.0000	0.0001	0.0000	0.0000	0.0005
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__	0.0062	0.0059	0.0067	0.0113	0.0827	0.0052	0.0004	0.0002
k__Bacteria;p__Bacteroidetes;c__[Saprospirae];o__[Saprospirales];f__Chitinophagaceae;g__Niabella	0.0007	0.0005	0.0007	0.0005	0.0005	0.0017	0.0000	0.0001
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Emticicia	0.0000	0.0000	0.0000	0.0000	0.0656	0.0071	0.0000	0.0000
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Larkinella	0.0002	0.0002	0.0005	0.0005	0.0030	0.0004	0.0000	0.0000
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Rudanella	0.0008	0.0013	0.0015	0.0014	0.0002	0.0000	0.0000	0.0000
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;g__Spirosoma	0.0009	0.0010	0.0011	0.0003	0.0021	0.0003	0.0000	0.0000
k__Bacteria;p__Bacteroidetes;c__Cytophagia;o__Cytophagales;f__Cytophagaceae;Other	0.0008	0.0006	0.0007	0.0010	0.0029	0.0006	0.0004	0.0000
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__Chryseobacterium	0.0015	0.0020	0.0016	0.0043	0.0046	0.0025	0.0002	0.0000
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__[Weeksellaceae];g__Wautersiella	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0099	0.0022
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium	0.0587	0.0646	0.0529	0.0632	0.0899	0.0343	0.0432	0.0026
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__	0.0094	0.0113	0.0129	0.0072	0.0088	0.0161	0.0007	0.0003
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Pedobacter	0.3223	0.2599	0.2723	0.1376	0.0780	0.3753	0.0243	0.0027
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;g__Sphingobacterium	0.0023	0.0026	0.0028	0.0020	0.0112	0.0033	0.0003	0.0001
k__Bacteria;p__Bacteroidetes;c__Sphingobacteriia;o__Sphingobacteriales;f__Sphingobacteriaceae;Other	0.0013	0.0009	0.0014	0.0007	0.0011	0.0018	0.0001	0.0000
k__Bacteria;p__Cyanobacteria;c__ML635J-21;o__;f__;g__	0.0000	0.0000	0.0001	0.0000	0.0013	0.0001	0.0000	0.0000
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	0.0007	0.0008	0.0011	0.0002	0.0012	0.0004	0.0002	0.0002
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Paenibacillaceae;g__Cohnella	0.0009	0.0023	0.0015	0.0000	0.0010	0.0102	0.0001	0.0000
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae;g__	0.0010	0.0008	0.0026	0.0008	0.0000	0.0012	0.0008	0.0017
k__Bacteria;p__Firmicutes;c__Clostridia;o__Thermoanaerobacterales;f__Thermoanaerobacteraceae;g__Thermacetogenium	0.0001	0.0000	0.0001	0.0001	0.0014	0.0000	0.0001	0.0000
k__Bacteria;p__Gemmatimonadetes;c__Gemmatimonadetes;o__Gemmatimonadales;f__Gemmatimonadaceae;g__Gemmatimonas	0.0076	0.0087	0.0084	0.0000	0.0037	0.0075	0.0000	0.0000
k__Bacteria;p__Planctomycetes;c__Planctomycetia;o__Gemmatales;f__Isosphaeraceae;g__	0.0024	0.0018	0.0020	0.0020	0.0019	0.0025	0.0004	0.0001
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__;f__;g__	0.0003	0.0002	0.0008	0.0006	0.0012	0.0004	0.0004	0.0008
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__	0.0203	0.0239	0.0180	0.0197	0.0077	0.0094	0.0135	0.0010
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Brevundimonas	0.0004	0.0006	0.0010	0.0005	0.0013	0.0006	0.0003	0.0001
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Caulobacter	0.0001	0.0004	0.0011	0.0003	0.0008	0.0005	0.0001	0.0001
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;g__Phenylobacterium	0.0020	0.0016	0.0013	0.0004	0.0012	0.0027	0.0002	0.0000
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Caulobacterales;f__Caulobacteraceae;Other	0.0016	0.0013	0.0029	0.0013	0.0026	0.0012	0.0007	0.0003
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__;g__	0.0543	0.0501	0.0559	0.0575	0.0184	0.0251	0.0127	0.0058
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bartonellaceae;g__	0.0016	0.0013	0.0015	0.0009	0.0019	0.0018	0.0001	0.0002
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Bradyrhizobiaceae;g__	0.0008	0.0005	0.0005	0.0005	0.0012	0.0003	0.0009	0.0002
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Brucellaceae;g__Ochrobactrum	0.0003	0.0003	0.0006	0.0003	0.0012	0.0002	0.0001	0.0001
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Brucellaceae;Other	0.0007	0.0006	0.0010	0.0005	0.0013	0.0004	0.0001	0.0001
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Devosia	0.0269	0.0254	0.0251	0.0085	0.0098	0.0174	0.0018	0.0003
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Hyphomicrobiaceae;g__Hyphomicrobium	0.0031	0.0034	0.0017	0.0046	0.0037	0.0003	0.0008	0.0003
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__	0.0010	0.0012	0.0010	0.0027	0.0011	0.0013	0.0008	0.0002
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;g__Mesorhizobium	0.0009	0.0009	0.0008	0.0033	0.0004	0.0012	0.0012	0.0003
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Phyllobacteriaceae;Other	0.0013	0.0013	0.0019	0.0016	0.0036	0.0013	0.0004	0.0003
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__	0.0249	0.0314	0.0171	0.0172	0.0089	0.0086	0.0071	0.0008
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Agrobacterium	0.0251	0.0216	0.0278	0.0052	0.0062	0.0071	0.0033	0.0002
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;g__Kaistia	0.0050	0.0050	0.0039	0.0010	0.0009	0.0032	0.0006	0.0000
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae;Other	0.0431	0.0340	0.0497	0.0348	0.0280	0.0252	0.0059	0.0019
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;Other;Other	0.0084	0.0080	0.0132	0.0086	0.0137	0.0066	0.0011	0.0012
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__	0.0035	0.0028	0.0031	0.0010	0.0004	0.0052	0.0002	0.0001
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Acetobacteraceae;g__Roseococcus	0.0025	0.0022	0.0024	0.0022	0.0016	0.0038	0.0002	0.0000
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__	0.0010	0.0008	0.0019	0.0011	0.0041	0.0011	0.0021	0.0040
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhodospirillales;f__Rhodospirillaceae;g__Azospirillum	0.0958	0.1278	0.0934	0.0180	0.0809	0.0810	0.6104	0.8947
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rickettsiales;f__;g__	0.0124	0.0096	0.0145	0.0066	0.0135	0.0066	0.0046	0.0022
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__	0.0048	0.0055	0.0037	0.0259	0.0027	0.0035	0.0053	0.0010
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;g__Sphingomonas	0.0009	0.0010	0.0017	0.0044	0.0019	0.0013	0.0007	0.0005
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;f__Sphingomonadaceae;Other	0.0020	0.0019	0.0023	0.0137	0.0018	0.0020	0.0018	0.0005
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Sphingomonadales;Other;Other	0.0015	0.0008	0.0009	0.0031	0.0006	0.0007	0.0007	0.0001
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;Other;Other;Other	0.0155	0.0167	0.0179	0.0259	0.0214	0.0094	0.0279	0.0201
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Achromobacter	0.0022	0.0030	0.0036	0.0006	0.0012	0.0022	0.0036	0.0001

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Pigmentiphaga	0.0001	0.0002	0.0002	0.0000	0.0005	0.0042	0.0002	0.0000
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__	0.0739	0.0884	0.0590	0.2300	0.0958	0.0233	0.0368	0.0045
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Comamonas	0.0007	0.0011	0.0018	0.0000	0.0002	0.0000	0.0000	0.0000
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Delftia	0.0253	0.0269	0.0666	0.0983	0.1683	0.1646	0.0321	0.0128
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Variovorax	0.0040	0.0043	0.0045	0.0087	0.0002	0.0006	0.0003	0.0005
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Oxalobacteraceae;g__	0.0003	0.0003	0.0005	0.0033	0.0007	0.0003	0.0001	0.0002
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;Other;Other	0.0000	0.0000	0.0000	0.0001	0.0018	0.0000	0.0000	0.0000
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Bdellovibrionales;f__Bacteriovoracaceae;g__	0.0002	0.0003	0.0002	0.0011	0.0004	0.0005	0.0000	0.0001
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__;g__	0.0042	0.0053	0.0044	0.0001	0.0003	0.0035	0.0002	0.0001
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Myxococcaceae;g__Myxococcus	0.0008	0.0029	0.0010	0.0011	0.0002	0.0004	0.0000	0.0000
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Myxococcales;f__Nannocystaceae;g__Nannocystis	0.0000	0.0000	0.0000	0.0000	0.0155	0.0200	0.0000	0.0000
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__	0.0134	0.0173	0.0163	0.0042	0.0051	0.0080	0.0020	0.0003
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Enterobacter	0.0021	0.0024	0.0033	0.0004	0.0007	0.0019	0.0004	0.0008
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;Other	0.0039	0.0042	0.0054	0.0014	0.0007	0.0031	0.0006	0.0005
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Halomonadaceae;g__Halomonas	0.0007	0.0007	0.0017	0.0005	0.0001	0.0008	0.0005	0.0012
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae;g__Acinetobacter	0.0007	0.0013	0.0015	0.0047	0.0028	0.0045	0.0012	0.0016
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas	0.0012	0.0023	0.0029	0.0007	0.0010	0.0009	0.0004	0.0001
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Sinobacteraceae;g__	0.0017	0.0020	0.0015	0.0009	0.0022	0.0011	0.0007	0.0002
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__	0.0062	0.0086	0.0054	0.0078	0.0049	0.0024	0.0836	0.0107
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Dokdonella	0.0000	0.0000	0.0000	0.0004	0.0006	0.0003	0.0052	0.0001
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Pseudoxanthomonas	0.0413	0.0288	0.0326	0.0633	0.0094	0.0186	0.0029	0.0005
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;g__Stenotrophomonas	0.0021	0.0022	0.0022	0.0031	0.0087	0.0026	0.0216	0.0043
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae;Other	0.0005	0.0009	0.0014	0.0016	0.0027	0.0018	0.0019	0.0012
k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae;g__Luteolibacter	0.0008	0.0008	0.0007	0.0005	0.0001	0.0028	0.0002	0.0000
k__Bacteria;p__WPS-2;c__;o__;f__;g__	0.0000	0.0000	0.0000	0.0000	0.0000	0.0014	0.0000	0.0000
Unassigned;Other;Other;Other;Other;Other	0.0005	0.0007	0.0004	0.0009	0.0029	0.0011	0.0003	0.0004

FIRST-ORDER KINETICS OF E1 DEGRADATION IN FEEDING CYCLE EXPERIMENTS

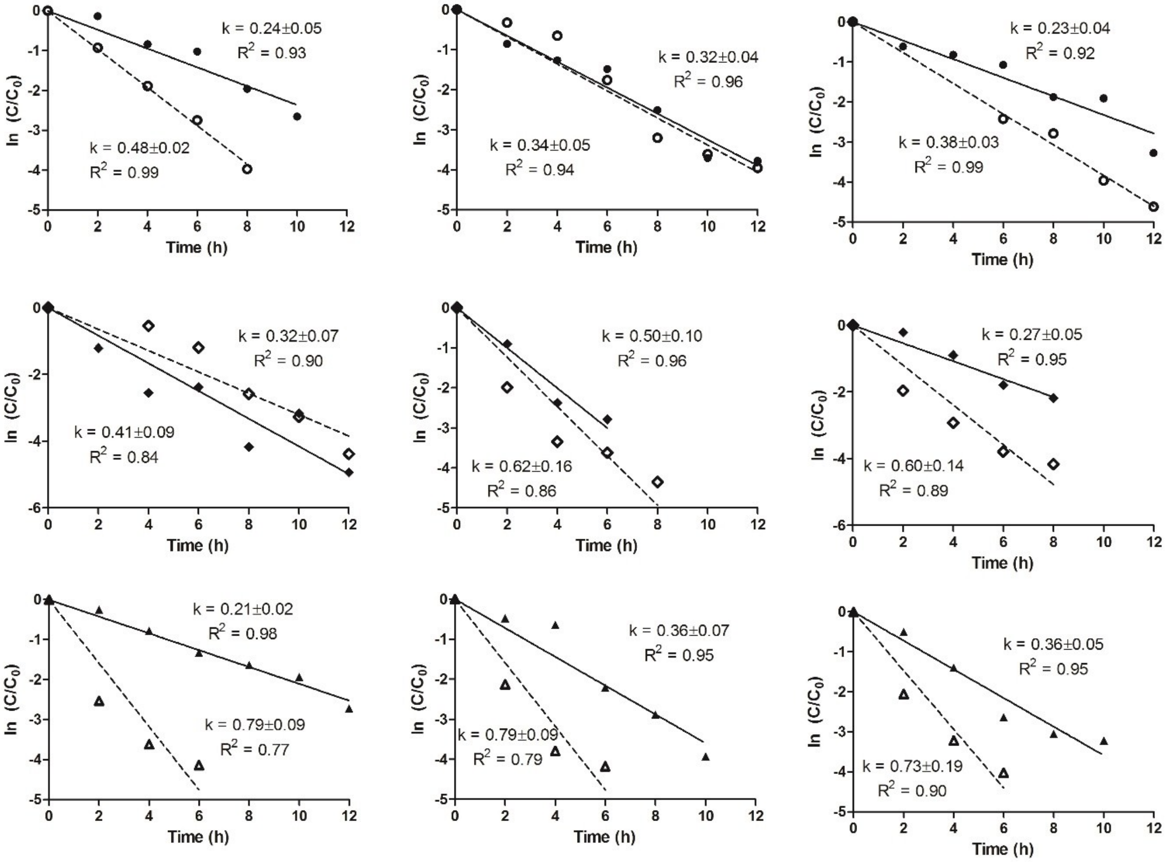

Figure S2: First-order degradation rates of E1 on Days 6 and 12 in feeding cycle experiments with high carbon loads. Closed symbols represent degradation at Day 6 and open symbols represent degradation at Day 12. Circles represent reactors with a daily feed cycle, diamonds represent reactors fed every 3 d, and triangles represent reactors fed every 6 d. Error terms represent 95% confidence intervals from regression.

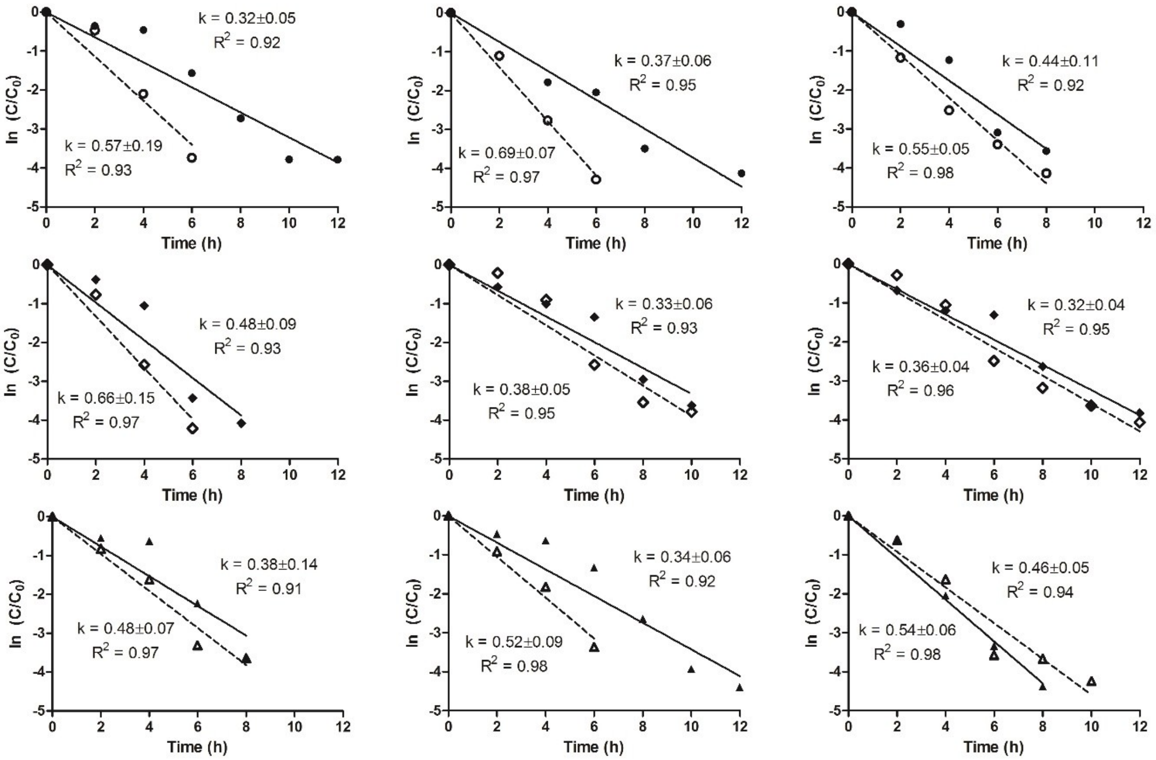

Figure S3: First-order degradation rates of E1 on Days 6 and 12 in feeding cycle experiments with low carbon loads. Closed symbols represent degradation at Day 6 and open symbols represent degradation at Day 12. Circles represent reactors with a daily feed cycle, diamonds represent reactors fed every 3 d, and triangles represent reactors fed every 6 d. Error terms represent 95% confidence intervals from regression.

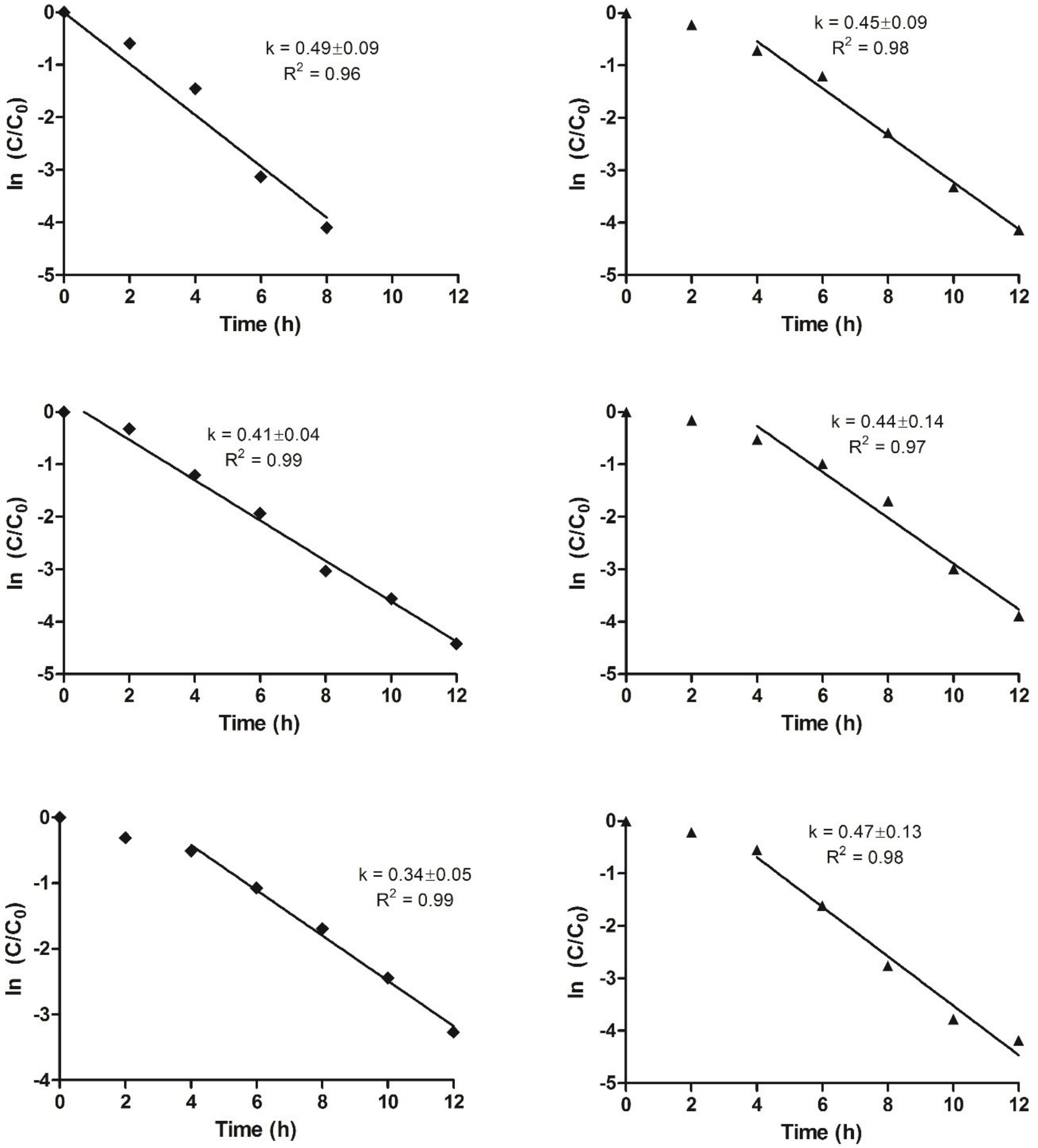

Figure S4: First-order degradation rates of E1 on Day 11 in feeding cycle experiments with low carbon loads. Data is plotted separately from Figure S3 to clearly show lag phase effects. Diamonds represent reactors fed every 3 d, and triangles represent reactors fed every 6 d. Error terms represent 95% confidence intervals from regression.

GROWTH YIELDS

Table S1: Microbial growth as measured via Volatile Suspended Solids (VSS) and calculated growth yields. Error terms are standard deviations from triplicate reactors.

COD Loading	Low			High		
Feed Interval	1d	3d	6d	1d	3d	6d
VSS at day 6, X_6 (mg/L)	101 ± 10	88 ± 10	85 ± 16	189 ± 21	143 ± 12	144 ± 14
VSS at day 12, X_{12} (mg/L)	161 ± 2	135 ± 2	168 ± 4	287 ± 23	224 ± 7	249 ± 61
ΔX ($X_{12} - X_6$) (mg/L)	60 ± 16	47 ± 12	83 ± 26	97 ± 2	81 ± 8	105 ± 5
COD fed over a 6 day period (mg/L)	180	180	180	600	600	600
Yield (mg X/mg COD)	0.33 ± 0.09	0.26 ± 0.07	0.46 ± 0.14	0.161 ± 0.003	0.135 ± 0.003	0.175 ± 0.003