**Supporting Information for:**

# Effects of Pharmaceutically Active Compounds on Activated Sludge Microorganisms

Shuyi Wang, Ryan Holzem[†], Claudia.K. Gunsch[*]

Department of Civil and Environmental Engineering, Duke University, Box 90287, Durham, NC 27708, USA

Number of pages: 5

Number of tables: 1

Number of figures: 0

[*]Correspondence author phone: 919-660-5208; fax: 919-660-5219; e-mail: ckgunsch@duke.edu

[†] Present address: Department of Environmental Engineering, University of Wisconsin, 1415 Engineering Dr, Madison, WI 53706

**Relationship Between $OD_{600}$ and Cell Mass.** Cell mass measurements were derived from $OD_{600}$ measurements using the standard curve shown in Figure S1. The standard curve was prepared by vacuum filtering 10 mL cell cultures with known $OD_{600}$ values through dry Whatman filter membranes (0.2 μM). Membranes were placed in aluminum planchettes, dried at 105 ºC for 4 hours, cooled to room temperature in a desiccator and weighed on a four-place balance. All measurements were performed in triplicates.

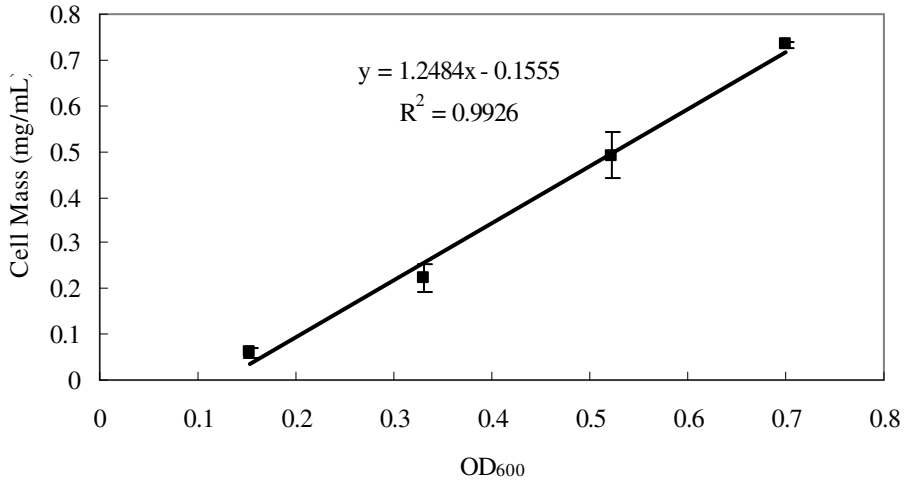


Figure S1. Standard curve of OD600 and cell mass.

**Specific Oxygen Uptake Rate (SOUR) Measurements.** SOUR measurements were obtained using standard methods (*1*). Briefly, 10 mL of inoculum sludge (i.e., Day 0) and 10 μM PhACs were added in 300-mL bottles filled with basal medium. The DO levels were measured using a pre-inserted DO probe (YSI model 50B, Yellow Springs, Ohio) over a 20 min period. VSS was measured using standard methods (*1*). SOUR measurements were performed at a final PhAC concentration of 10 μM in 0.2% (v/v)

ethanol. Triplicate tests were performed for each PhAC.

**DNA Extraction.** One hundred µL biomass samples were obtained by centrifuging 1 min at 16,300×g RCF at room temperature using a Spectrafuge 24D microcentrifuge (Eppendorf, Inc., Woodbridge, New Jersey). If DNA extraction was not performed immediately, the cell pellets were stored at -80 ºC in a Revco Ultima Ultra-Cold Freezer (Thermo Electron Corporation, Marietta, Ohio). DNA was extracted using Mo Bio Ultra Clean™ microbial DNA kits (Carlsbad, California). The extraction was performed following the manufacturer's instructions without any modifications. The purified DNA was eluted in sterile water and stored at -20ºC (GE® Upright Freezer, Louisville, Kentucky) for PCR amplification. DNA quality was assessed by measuring the $A_{260}/A_{280}$ ratio using a ND-1000 Spectrophotometer (Nanodrop®, Wilmington, Delaware). Only samples with ratios between 1.8 and 1.9 were used (*2*).

**Polymerase Chain Reaction (PCR).** The first PCR product was amplified using the bacterial forward primer 8F and the universal reverse primer 1492R (*3*). The final PCR amplicon was obtained using the I-341-fGC and I-533r primers (*4*). A summary of the primers sequences is shown in Table 2. All PCR amplifications were performed using a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA). All reagents were obtained from the Eppendorf Master*Taq*® kit (Hamburg, Germany) and used following the manufacturer's instructions. The program for the first PCR amplification consisted of a 30-sec denaturation step at 94°C, followed by a 30-sec

annealing step at 52°C and a 30-sec extension step at 72°C (30 cycles). Touch-down PCR was used for the second PCR amplification. The first two cycles consisted of a 30-sec denaturation step at 94°C, a 30-sec annealing step at 52°C and a 30-sec extension step at 72°C. The next 30 cycles consisted of a 30-sec denaturation step at 94°C, a 30-sec annealing step at 47°C and a 30-sec extension step at 72°C.

Table S1. Primer sequences for DGGE analysis. [a] Numbers correspond to the nucleotide positions in *Escherichia.coli*; F or f, forward primer; R or r, reverse primer. [b] I, inosine.

| **Primer**[a] | **Sequence (5'-3')**[b] |
|---|---|
| 8F | AGAGTTTGATCCTGGCTCAG |
| 1492R | GGTTACCTTGTTACGACTT |
| I-341-fGC | CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGIGGCIGCA |
| I-533r | TIACCGIIICTICTGGCAC |

**Denaturing Gradient Gel Electrophoresis (DGGE).** All PCR amplifications were loaded on an 8% (w/v) polyacrylamide gel with a denaturing gradient ranging from 20 to 55% urea-formamide (100% denaturing solution corresponded to 40% (v/v) deionized formamide and 7 M urea). The gel was run at 57°C under a voltage of 50 V for 18 h and then stained with 1X SYBR Gold (Molecular Probes, Eugene, OR). Sequencing was performed on excised bands using the I-533r primer. DNA sequence analysis was performed using the BLAST server of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

References:


(1) Eaton A.D.; Clesceri L.S.; Rice E.W.; A.E., G., Standard methods for the examination of water and wastewater (21st Edition). **2005**, 2:79-80.

(2) Glasel, J. A., Validity of Nucleic Acid Purities Monitored by A260/A280 Absorbance Ratios. *Biotechniques* **1995,** *18*, (62-63).

(3) Weisburg, W. G.; Barns, S. M.; Pelletier, D. A.; Lane, D. J., 16s Ribosomal DNA Amplification for Phylogenetic Study. *Journal of Bacteriology* **1991,** *173*, (2), 697-703.

(4) Watanabe, K.; Kodama, Y.; Harayama, S., Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting. *Journal of Microbiological Methods* **2001,** *44*, (3), 253-262.