# Appendix A: Supplementary Material

# Effects of influent municipal wastewater microbial community and antibiotic resistance gene profiles on anaerobic membrane bioreactor effluent

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**Table S1** Synthetic wastewater composition used prior to treating municipal wastewater.

| Concentrate solution | | Dilution water | |
|---|---|---|---|
| Reagent | Conc. (mg/L) | Reagent | Conc. (mg/L) |
| Ammonium Chloride | 12 | Sodium Bicarbonate | 420 |
| Calcium Chloride | 12 | Sodium Carbonate | 420 |
| Iron Sulfate | 6.08 | Potassium Phosphate | 24.2 |
| Sodium Sulfate | 12 | | |
| Sodium Acetate | 321 | | |
| Peptone | 42.5 | | |
| Yeast | 127.5 | | |
| Milk Powder | 283.3 | | |
| Starch | 297.5 | | |
| Copper Chloride | 4.2 | | |
| Manganese Sulfate | 0.83 | | |
| Lead Chloride | 3.33 | | |

**qPCR methods for gene quantification**

For standard preparation, PCR was conducted in 20 µL reactions using 4 µL of 5x FIREPol Master Mix (Solis BioDyne, USA), 1 µL each of forward and reverse primers at 5 µM, 1 µL DNA template, and 13 µL molecular-grade water. PCR products were run on a 1.5 % agarose gel and detected on a ChemiDoc Touching Imaging System (Bio-Rad Laboratories, USA). A sterile scalpel was used to excise bands, which were then purified using the GenElute Gel Extraction Kit (Sigma-Aldrich, USA) according to the manufacturer's protocol. Concentrations of gel extracts were quantified using the AccuGreen™ High Sensitivity dsDNA Quantitation Kit (Biotium) with a Qubit 2.0 Fluorometer (Thermo Fisher, USA). Thermocycling conditions for each primer set used in standard preparation are as shown in Table S2, but with the addition of a final elongation step. For qPCR, each reaction consisted of 20 µL using 10 µL of the Biotium Forget-Me-Not qPCR Master Mix, 1 µL of 5 µM each for forward and reverse primers, 1 µL of the template, and 7 µL of molecular-grade water. Standard curves were generated during qPCR using serial dilutions of the prepared standards at $10^{-2}$ to $10^{-8}$ of the stock concentration.

**Table S2** Primer sequences, thermocycling conditions, and amplicon sizes for all genes assessed by qPCR.

| Gene | Primers (5'-3') | Preincubation | Amplification | Cycles | Amplicon (bp) | Reference |
|---|---|---|---|---|---|---|
| *sul1* | F- CGCACCGGAAACATCGCTGCAC<br>R- TGAAGTTCCGCCGCAAGGCTCG | 95°C for 5 min | 95°C for 30 s, 55 °C for 30 s, 72°C for 60 s | 40 | 163 | (Pei et al., 2006) |
| *sul2* | F- TCCGGTGGAGGCCGGTATCTGG<br>R- CGGGAATGCCATCTGCCTTGAG | 95°C for 5 min | 95°C for 15 s, 56 °C for 30 s, 72℃ for 40s | 40 | 191 | (Pei et al., 2006) |
| *tetC* | F-GCGGGATATCGTCCATTCCG<br>R-GCGTAGAGGATCCACAGGACG | 95°C for 5 min | 95°C for 30 s, 55 °C for 30 s, 72°C for 60s | 40 | 207 | (Naas et al., 2011) |
| *tetQ* | F- AGAATCTGCTGTTTGCCAGTG<br>R- CGGAGTGTCAATGATATTGCA | 95°C for 5 min | 95°C for 30 s, 55 °C for 30 s, 72°C for 60 s | 40 | 124 | (Naas et al., 2011) |
| *ampC* | F- CCTCTTGCTCCACATTTGCT<br>R- ACAACGTTTGCTGTGTGACG | 95°C for 5 min | 95°C for 45 s, 58 °C for 60 s, 72°C for 60s | 40 | 189 | (Szczepanowski et al., 2009) |
| $bla_{TEM}$ | F-TTCCTGTTTTTGCTCACCCAG<br>R-CTCAAGGATCTTACCGCTGTTG | 95°C for 5 min | 95°C for 45 s, 58 °C for 60 s, 72°C for 60s | 40 | 445 | (Bibbal et al., 2007) |
| *intI1* | F- CTGGATTTCGATCACGGCACG<br>R- ACATGCGTGTAAATCATCGTCG | 95°C for 5 min | 95°C for 30 s, 60 °C for 60 s, 72°C for 60s | 40 | 196 | (Barlow et al., 2004) |
| *rpoB* | F- AACATCGGTTTGATCAAC<br>R- CGTTGCATGTTGGTACCCAT | 94°C for 5 min | 94°C for 30 s, 50 °C for 90 s, 72°C for 90s | 40 | 381 | (Dahllöf et al., 2000) |

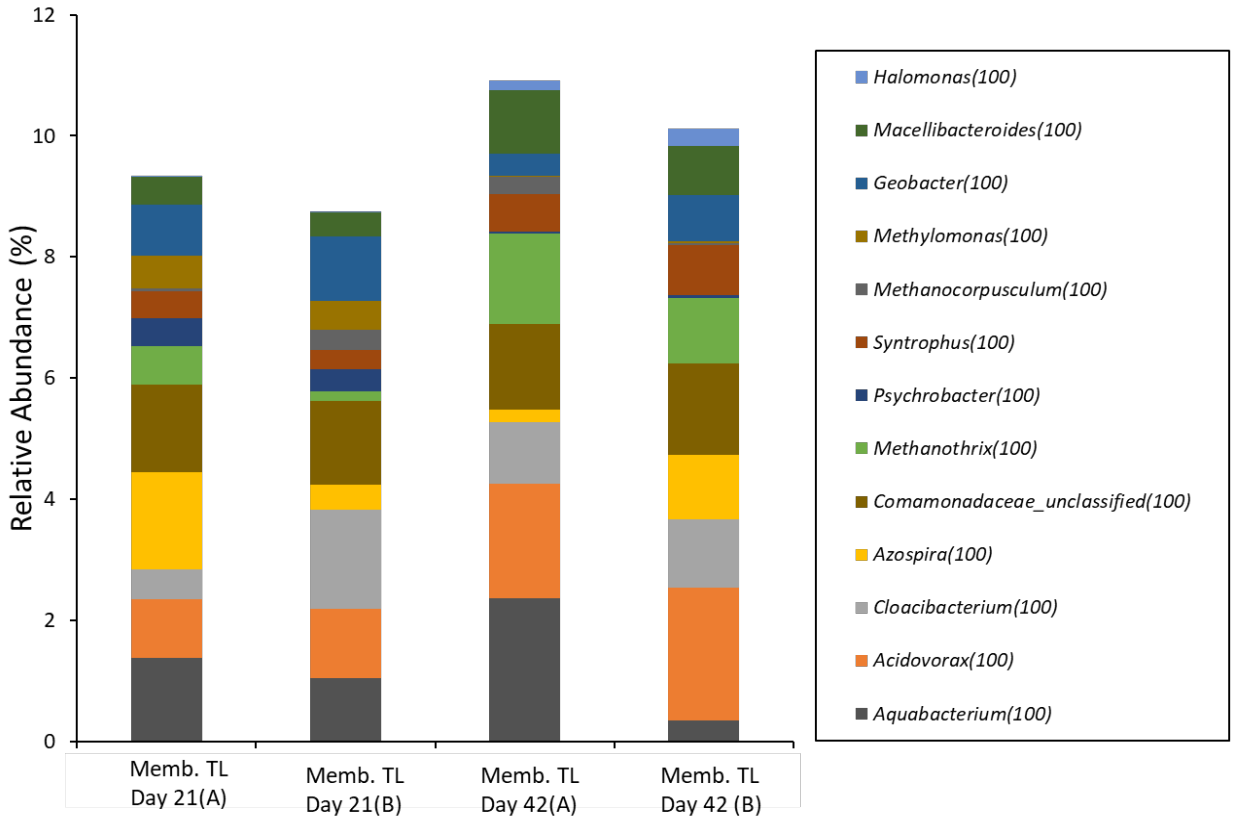


**Figure S1** Relative abundance (%) of microbial communities of the tightly-bound membrane biofilm layer (TL) from membranes harvested on Day 21 and Day 42 of the experiment. Groups shown represent microbial groups of relative abundance greater than 0.5% and less than 3% to illustrate methanogenic genera.

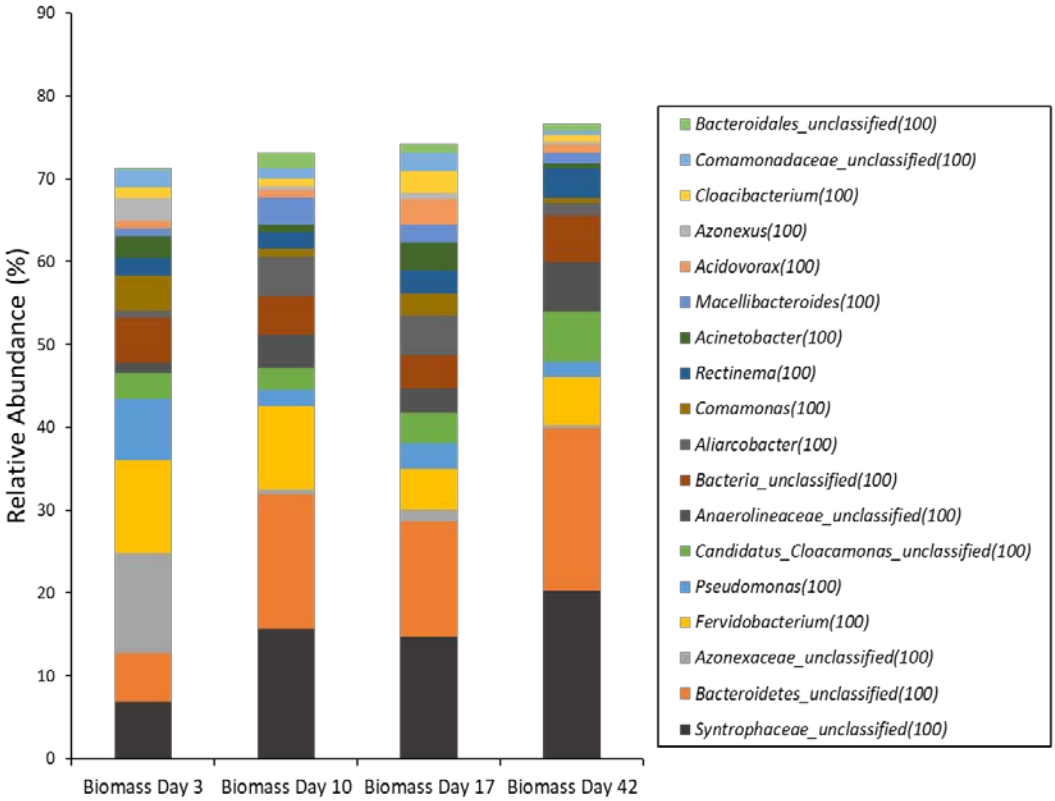


**Figure S2** Relative abundance (%) of microbial communities of the AnMBR reactor biomass on Days 3, 10, 17, and 42 of the experiment.

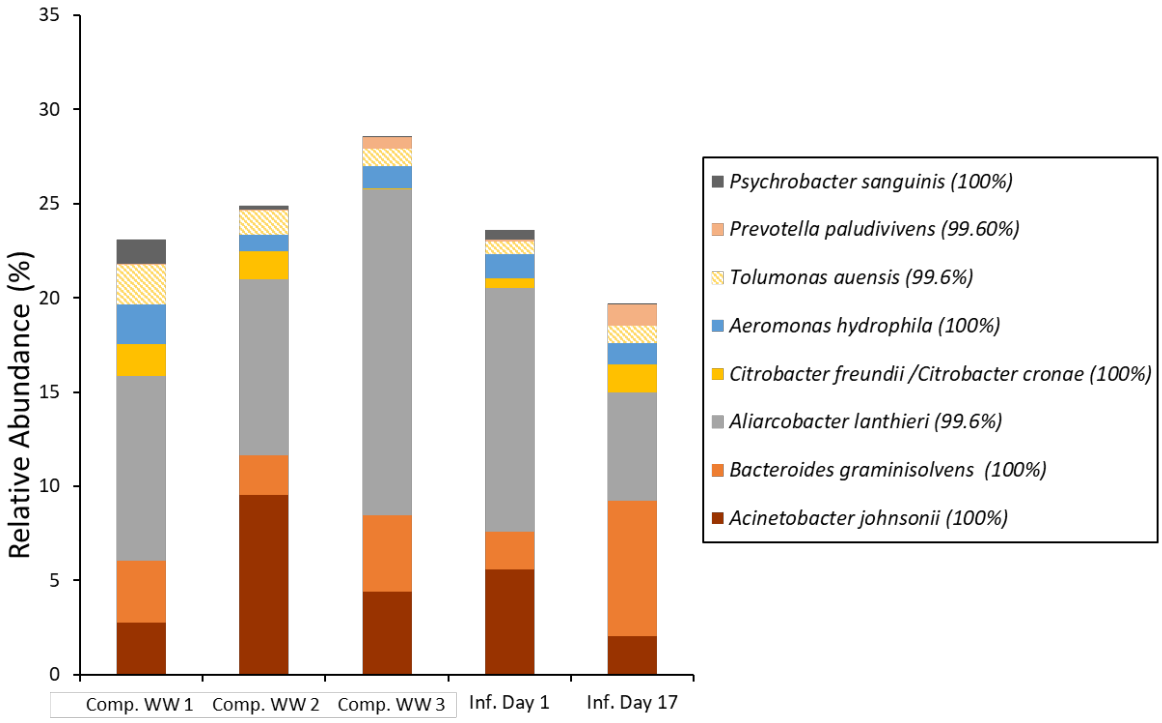


**Figure S3** 2 Relative abundance (%) of microbial community operational taxonomic units (OTUs) in the influent wastewater associated with potentially pathogenic species from Day 1 and Day 17 of the experiment and three other municipal wastewater samples collected from the same seasonal timeframe.

## References

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