Supporting Information for

Quorum Sensing: A New Biofouling Control Paradigm in a Membrane Bioreactor for Advanced Wastewater Treatment

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AHL detection mechanism of *A.tumefaciens* A136 reporter strain. *A. tumefaciens* (Ti^-)(pCF218)(pCF372) is a genetically modified microorganism for the detection of exogenous AHL signal molecules. Its AHL detection mechanism is depicted in Figure S2. This microorganism has the three following genetic characteristics: (1) knockout of Ti plasmid (Ti^-); (2) pCF 218, which codes for *traR*; and (3) pCF 372, which contains *traI-lacZ* fusion, which is under *traR* regulation. *A. tumefaciens* A136 cannot produce the AHL autoinducers because the Ti plasmid on which regulatory components of the *A. tumefaciens* quorum sensing system are located has been genetically removed. Instead, when exogenous AHL diffuses into *A. tumefaciens* A136, it makes a complex with TraR protein from the pCF 218. TraR is an AHL-responsive transcription factor that recognizes *N*-3-(oxooctanoyl)-L-homoserine lactone (AHL of *A. tumefaciens*) as well as a wide range of related AHLs. This AHL-TraR complex activates *traI-lacZ* on pCF 372 and induces the production of beta-

galactosidase, which degrades X-gal and develops a blue color. We can therefore detect AHL in a sample based on blue color development on an agar plate covered with X-gal.

The composition of the synthetic wastewater. 1.0 g/L glucose, 0.05 g/L yeast extract, 0.05 g/L bactopeptone, 0.5 g/L $(NH_4)_2SO_4$, 0.3 g/L $K_2H_2PO_4$, 0.3 g/L KH_2PO_4, 0.009 g/L $MgSO_4$, 0.0002 g/L $FeCl_3$, 0.007 g/L NaCl, 0.0002 g/L $CaCl_2$, 0.0024 g/L $CoCl_2$ and 0.15 g/L $NaHCO_3$.

TABLE S1. Continuous MBR Operating conditions.

Working volume	1 L
Mixed liquor suspended solids	12,000 (±500) mg/L
Hydraulic retention time	10 h
Solids retention time	50 days
Membrane area	0.008 m^2
Flux	15 $L/m^2/h$
Chemical oxygen demand (COD)removal efficiency	98 %

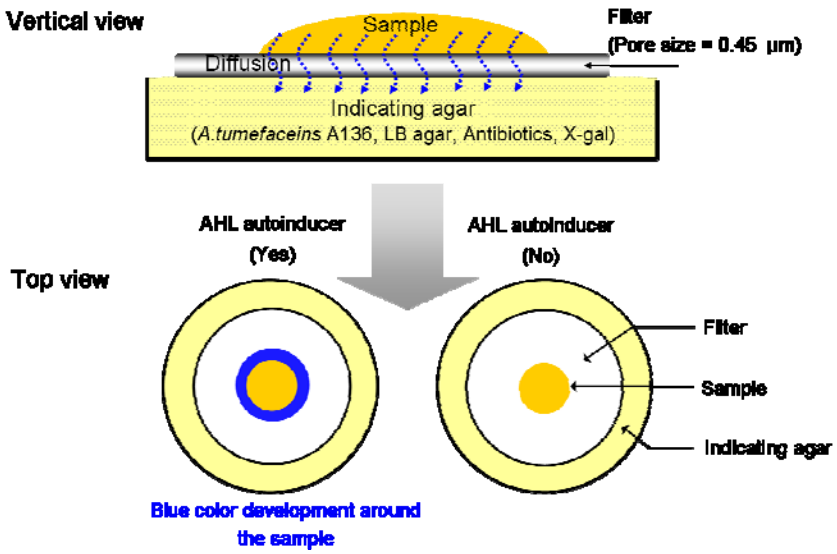


FIGURE S1. Schematic representation of *A. tumefaciens* A136 bioassay for *in situ* detection of total AHL

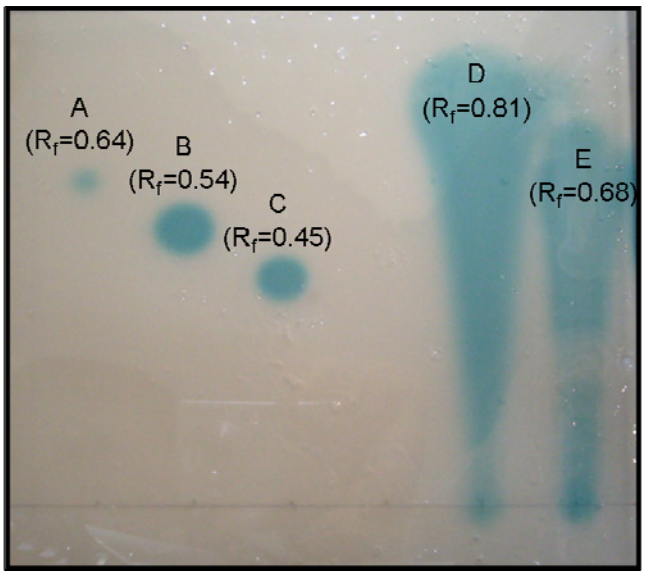
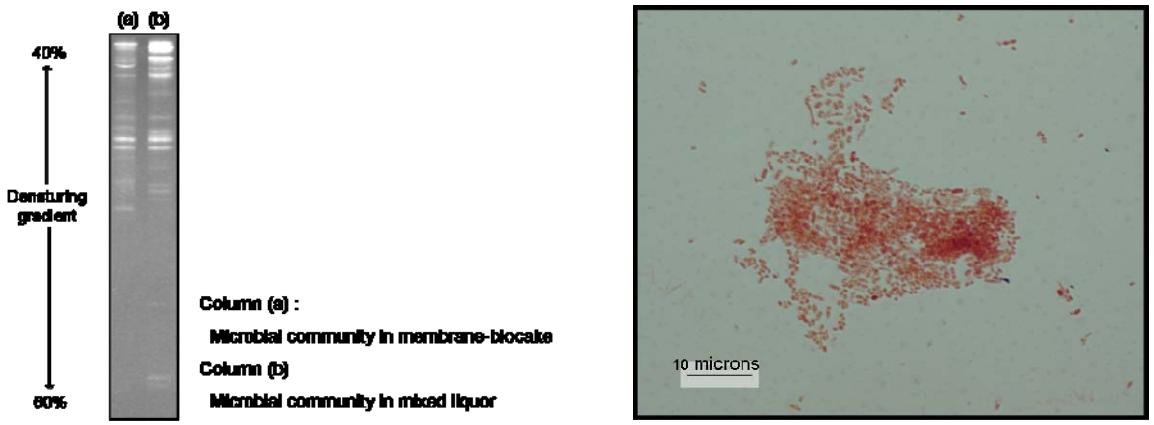


FIGURE S2. TLC chromatogram and R_f value of AHL standards:

A: *N*-hexanoyl-DL-homoserine lactone, B: *N*-heptanoyl-DL-homoserine lactone,

C: *N*-octanoyl-DL-homoserine lactone, D: *N*-(3-oxo-hexanoyl)-DL-homoserine lactone,

E: *N*-(3-oxo-octanoyl)-L homoserine lactone



(a) (b)

FIGURE S3. Characterization of bacterial community in MBR. (a) DGGE of bacteria 16S rDNA PCR products. (b) Gram-stained image of biocake detached from membrane module. Both 16S rDNA for PCR-DGGE and biocake for gram staining were obtained at the operation time of 72 h.

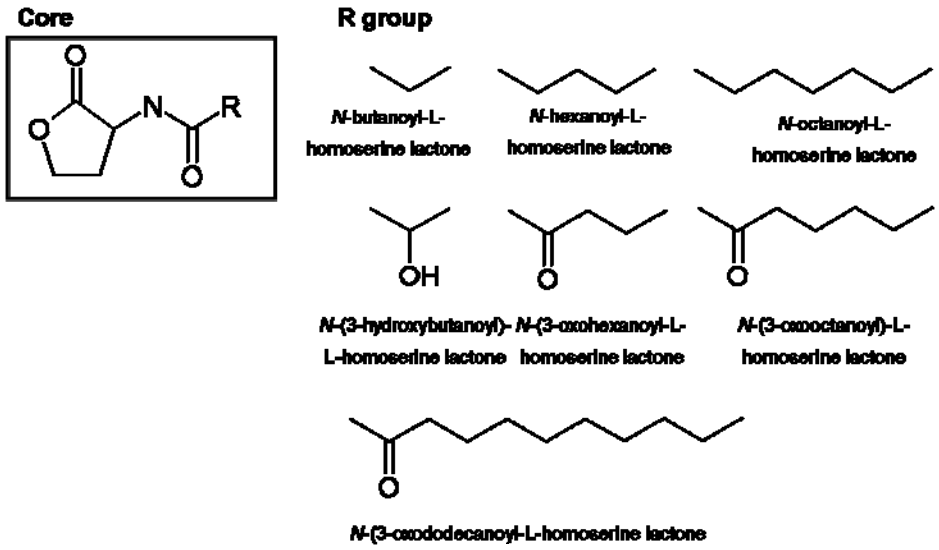


FIGURE S4. Molecular structure of AHL autoinducers.

Measurement of AHL activity using bioassay. Standard solutions of C8-HSL, which was identified to be a major autoinducer in this study, were prepared with the concentration range of 0.5 ~ 20 mg/L. 2 μL of each standard solution was added to the bioassay agar and incubated overnight. Based on the length of blue color developed at each AHL concentration, the following formula was obtained and used for the quantitative estimation of AHL levels (ng C8-HSL equivalent) in samples to be tested.

$$\text{AHL level (ng C8-HSL equivalent)} = 0.1204\, e^{0.164X} \quad (r^2=0.998)$$

X: length of blue color on bioassay agar (mm)

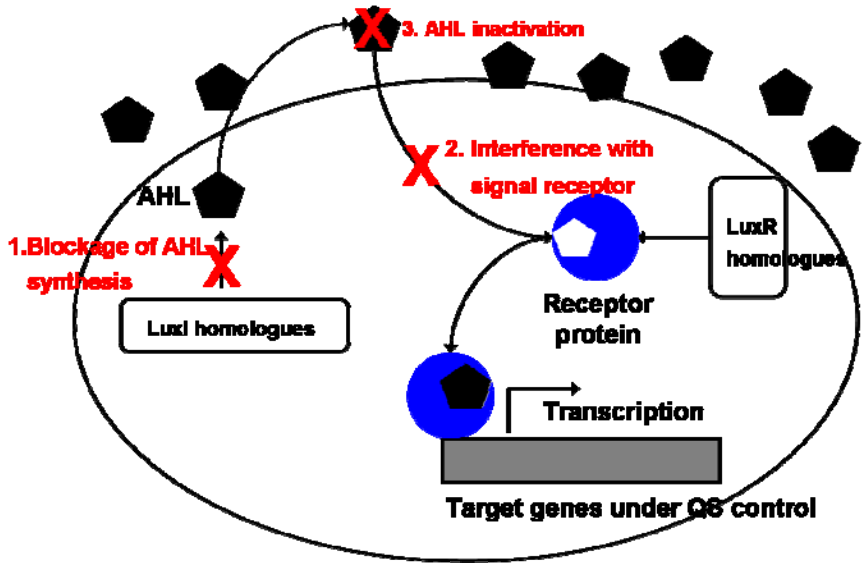


FIGURE S5. Basic AHL QS control strategies: (1) Blockage of AHL synthesis, (2) Interference with signal receptor and (3) AHL inactivation

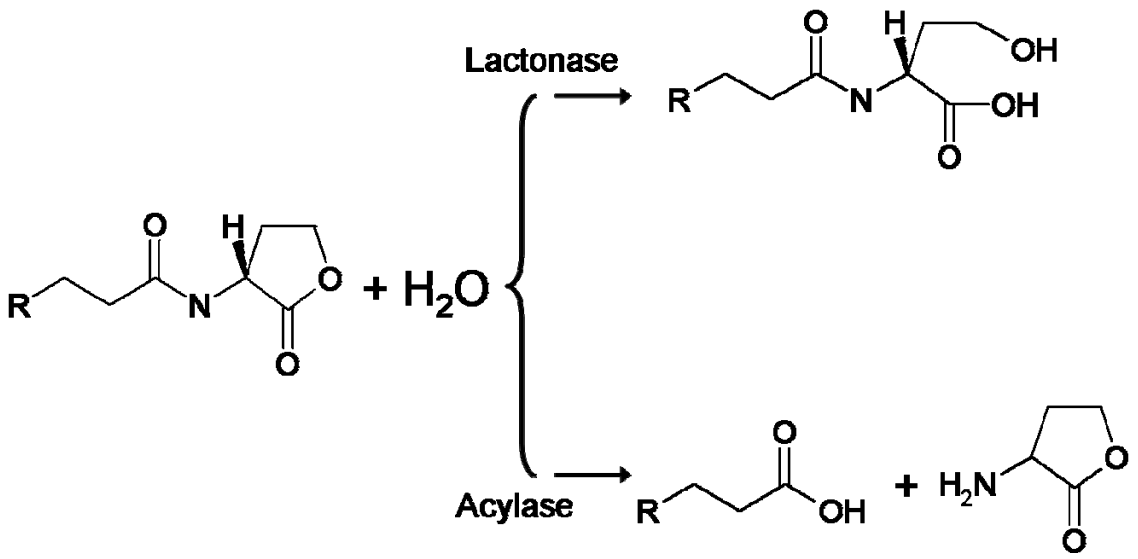


Figure S6. Quorum-quenching reaction of lactonase and acylase.