Variations in the microbial community in biofilms under different near-wall hydraulic shear stress in agricultural irrigation systems

——Supplementary Material

Biofilms sampling and testing methods

Solid particles (SP)

After striping biofilms, the cleaned pipeline samples were weighted after dried in the oven at the constant temperature of 60 ℃ for 1 h. Thus, the SP of biofilms attached inside the pipeline were the differences between two batches of weights measured above.

(2) Phospholipid fatty acids (PLFAs)

A volume of 15 mL of mixture after scraping biofilms was collected for PLFAs test according to Pennanen et al. (1999). The chemicals used in the experiment were manufactured by Beijing Chemical Analysis Company and the deionized water was provided by Beijing KEBANZHENG YE Company. The detail testing steps were as follows:

Collect PLFAs of microorganisms: The collected liquid was mixed with chloroform, methanol, and phosphate-buffered solution at a volume ratio of 1:2:0.8. The mixture was subjected to oscillating extraction in darkness for 2-4 h and centrifugation at 7000 rpm for 15 min. The supernatant was transported to a separation funnel. Then, 10 mL phosphate buffer and 10 mL chloroform were added to perform separation at room temperature in the darkness for 2-4 h. Finally, the samples were dried in nitrogen.

Purification: The silica gel was activated in oven under 100 ℃ for 1 h. The samples loaded on activated silica gel column were eluted with 15 mL chloroform, 30 mL acetone and 15 mL methanol. The collected methanol samples were then dried in nitrogen.

Methyl esterification: Totally 1 mL methanol: toluene mixture (v: v = 1:1) and 1 mL 0.56 % (w/v) KOH were added in the samples and then incubated at 35 ℃ for 30 min. After cooling down at

room temperature, the samples were treated with acetic acid. Then, the samples were incubated with 2 mL chloroform: hexane mixture (v: v = 1:4) and ultrapure water. The hexane supernatant was collected for nitrogen drying and stored at -20 □ for later use.

Mass spectrometry (GC–MS): The extraction was dissolved in the solution containing 331 $g.mL^{-1}$ nonadecanoic acid methyl ester and internal standard of chloroform: n-hexane mixture (v: v = 1:4). The HP6890 gas chromatography–HP5973 mass spectrometer (GC–MS) was used for the test at the temperature of 280℃. Highly pure helium (1 $mL.min^{-1}$) was used as the carrier gas, and the electron ionization (EI) mode was used as the electron energy of 70 eV.

Biomass evaluation: PLFA as a biomarker for labeling microorganisms were shown in Table S1.

Table S1 PLFAs biomarkers of indicated microorganisms

Microorganisms	PLFAs biomarkers
Bacteria	Containing saturated fatty acids or monounsaturated fatty acids connect witherspoon ether chain and glycerin (estimated by 15:0, a15:0, i15:0, i16:0, 16:1x7t, 16:1x9t, 17:0, i17:0, a17:0, cy17:0, 18:1x5, 18:1x7 and cy19:0) (Chinalia and Killham 2006; Frostegard et al. 1993)
Aerobic bacteria	15:0, a15:0, i15:0, 16:0, i16:0, 16:1x7t, 16:1x9t, 17:0, i17:0, a17:0, 18:0, 18:1x7t, 19:0
Anaerobic bacteria	18:1x7c, cy19:0, cy17:0
Gram positive bacteria	iso-, anteiso- branched-chain fatty acids (estimated by 16:0 (Me), 17:0 (Me), 18:0 (Me), 15:0, a15:0, i15:0, i16:0, i17:0 and a17:0) (O'Leary and Wilkinson 1998)
Gram negative bacteria	Monoenoic fatty acid, propyl-ring fatty acids (estimated by 16:1x7t, 16:1x9t, cy17:0, 18:1x5, 18:1x7 and cy19:0) (Wilkinson 1988)
Fungus	Containing unique phospholipids fatty acids (estimated by 18:2x6, 9) (Frostegard et al.1993)
Sulfate-reducing bacteria	10Me16:0, i17:1x7, 17:1x6
Methane-oxidizing acteria	16:1x8c, 16:1x8t, 16:1x5c, 18:1x8c, 18:1x8t, 18:1x6c, 16:1x6c
Flavobacterium	i17:1x7
Bacillus	Containing various branch chain fatty acids
Actinomycetes	10Me16:0, 10Me17:0, 10Me18:0, etc.
Cyanobacteria	Containing various polyunsaturated phospholipid fatty acids (like 18:2x6)
Microalgae	16:3x3
Desulfurizing bacteria	cy18:0x (7,8)

Sulfur bacteria	10Me18:1x6, i17:1x5, 11Me18:1x6
Desulfovibrio	i17:1x7c, i15:1x7c, i19:1x7c
Desulfobulbus	17:1x6, 15:1

Chinalia FA, Killham KS. 2006. 2, 4-Dichlorophenoxyacetic acid (2,4-D) biodegradation in river sediments of Northeast-Scotland and its effect on the microbial communities (PLFA and DGGE). Chemosphere. 64: 1675-1683

Frostegard A, Tunlid A, Baath E. 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl Environ Microbiol. 59: 3605-3617

O'Leary WM, Wilkinson SG. 1998. Gram-positive bacteria. In: Ratledge C, Wilkinson SG (eds) Microbial lipids. Academic Press, London

Pennanen T, Liski J, Baath E, Kitunen V, Uotila J, Westman CJ, Fritze H. 1999. Structure of the microbial communities in coniferous forest soils in relation to site fertility and stand development stage. Microb Ecol. 38: 168-179

Wilkinson SG. 1988. Gram-negative bacteria. In: Ratledge C, Wilkinson SG (eds) Microbial lipids. Academic Press, London

Extracellular polymeric substances (EPS)

The EPS of biofilms attached on the pipeline mainly included extracellular polysaccharides (EPO) and extracellular proteins (EPR). The EPO was tested through the phenol–sulfuric acid method, and the EPR was determined by Lowry method (Lowry et al. 1951; Nocker et al. 2007). As for the chemicals used in the experiment, NaOH and Na_2CO_3 were manufactured by Beijing Chemical Analysis Company, $CuSO_4$ and Na-tartrate were manufactured by Sinopharm Chemical Regent Co. Ltd, standard BSA liquid was offered by Beijing Tiandz Inc, and forint-phenol reagent was

manufactured by Beijing Solarbio Inc. The specific testing procedures were as follows:

A volume of 15 mL of mixture after scraping biofilms was subjected to centrifugation at the speed of 12000 rpm for 15 min. The suspended solids were collected into a 1.5 mL tube and the suspended solids were resuspended in sterile water.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ 1951. Protein measurement with the Folin phenol reagent. J Biol Chem. 193: 265-275.

Nocker A, Lepo JE, Martin LL, Snyder RA. 2007. Response of estuarine biofilm microbial community development to changes in dissolved oxygen and nutrient concentrations. Microb Ecol. 54(3): 532-542

2. Molecular microbiological analysis methods

Extraction of genome DNA

Total genome DNA from samples was extracted using CTAB/SDS method. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1 $ng.\mu L^{-1}$ using sterile water.

Amplicon Generation

16S rRNA genes of distinct regions (16S V4) were amplified used specific primer (16S V4) with the barcode. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs).

PCR Products Mixing and Purification

Mix same volume of 1×loading buffer (contained SYB green) with PCR products and operate electrophoresis on 2% agarose gel for detection. PCR products was mixed in equidensity ratios. Then, mixture PCR products was purified with GeneJETTM Gel Extraction Kit (Thermo Scientific).

Library preparation and sequencing

Sequencing libraries were generated using Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific) following manufacturer's recommendations. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific). At last, the library was sequenced on an Ion S5TM XL platform and 400 bp/600 bp single-end reads were generated.

Variation of PLFAs under different hydraulic shear forces

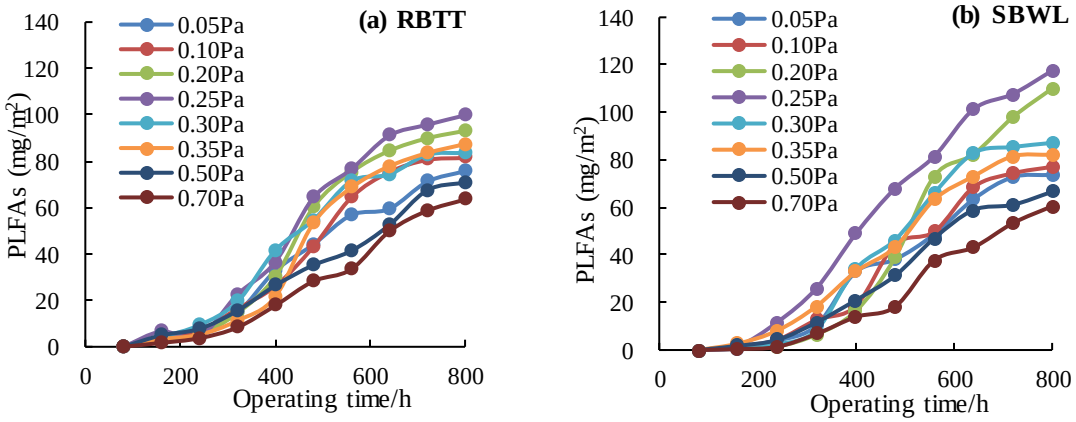

Fig. S1 Variation of PLFAs contents under different hydraulic shear forces

The relative abundances of different microorganisms

Table S2 Relative abundances of the top 5 microorganisms in SBWL reclaimed water (unit: %)

Water shear force treatments/Pa	Bacillus	Paenibacillus	Leptolyngbya	Alkaliphilus	Cronobacter
0.05	25.90	13.19	9.89	5.11	4.08
0.10	6.30	2.80	26.76	1.26	0.72
0.20	18.96	9.84	14.31	3.77	3.26
0.25	19.62	10.19	19.92	3.83	3.23
0.30	29.18	16.25	11.61	6.14	5.85
0.35	16.18	8.07	12.78	2.97	2.43
0.50	26.57	14.08	10.48	5.07	5.15
0.70	38.42	19.57	1.42	8.46	8.60

Table S3 Relative abundances of the top 5 microorganisms in RBTT reclaimed water (unit: %)

Water shear force treatments (Pa)	SubsectionI_Family I_unclassified	Bacillus	Paenibacillus	Leptolyngbya	Alkaliphilus
0.05	15.62	14.94	7.88	6.57	2.90
0.10	14.71	13.37	6.67	8.16	2.42
0.20	1.19	23.37	10.46	9.45	3.95
0.25	0.76	35.59	18.80	3.95	7.07
0.30	0.07	25.36	13.38	12.76	5.40
0.35	0.18	4.90	2.20	32.15	0.90