Supporting Information

Impacts of hydrodynamic conditions and surface roughness on the critical condition to develop and thickness of *Pseudomonas putida* biofilms

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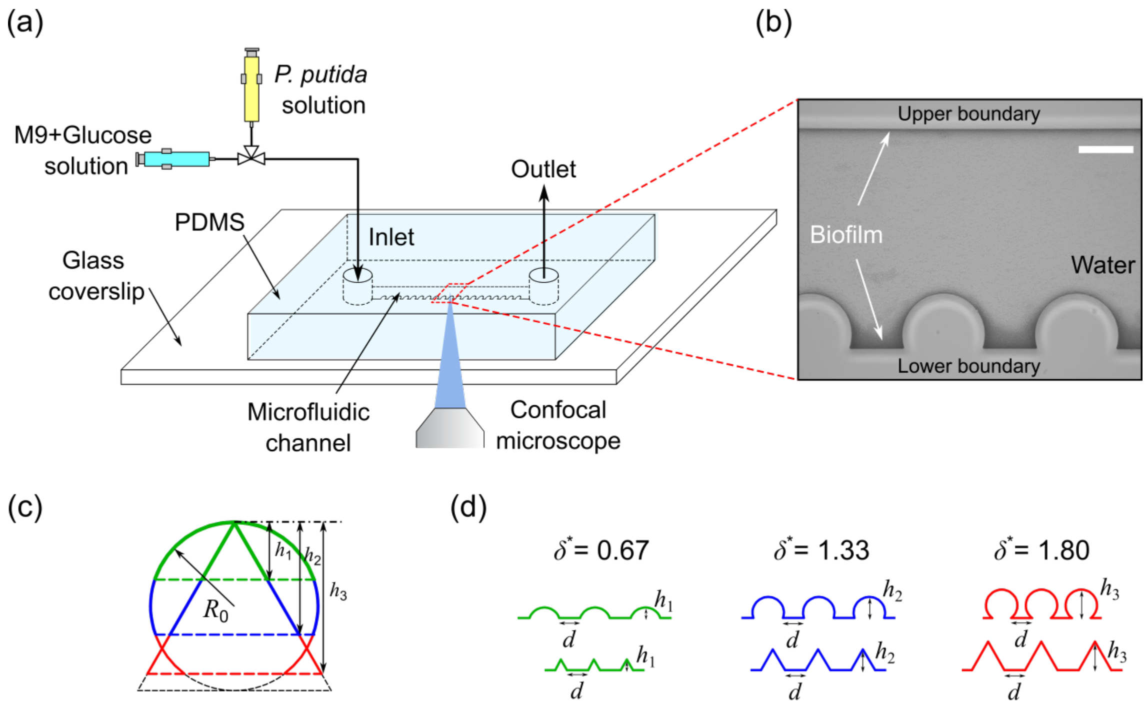

Figure S1. (a) Schematic diagram of the experimental set up. (b) Cross-sectional image of the microfluidic channel. Biofilms (gray color) accumulate at the upper and lower boundaries of the channel. The scale bar is 100 μm. (c) The relative roughness height δ^* is defined as h/R_0. R_0 = 75 μm denotes the radius of the circle and half of the triangle height. h denotes the height of each roughness on the surface: h_1 = 50 μm, h_2 = 100 μm, h_3 = 135 μm. (d) Six types of rough surfaces were used in this study with three relative heights (δ^* = 0.67, 1.33, 1.80) and two types of roughness shape (round and angular) at the lower boundary. The distance between the neighboring roughness elements for all cases is the same, i.e., d = 100 μm.

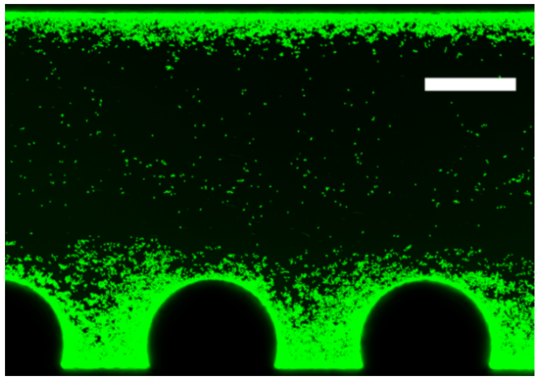

Figure S2. Confocal image of the biofilm stained with EPS dyes after the 14-hour experiments at flow rate of 5 μL/min, $\delta^* = 1.33$. EPS is visualized with green fluorescent dyes[1]. The EPS stains included 5 μM of SYTO-9 green fluorescent nucleic acid stain (Thermo Fisher Scientific, USA), 20 μg/mL fluorescein isothiocyanate (FITC) conjugated Concanavalin A from *Canavalia ensiformis* (Sigma), and 20 μg/mL FITC conjugated lectin from *Triticum vulgaris* (Sigma). The scale bar is 100 μm.

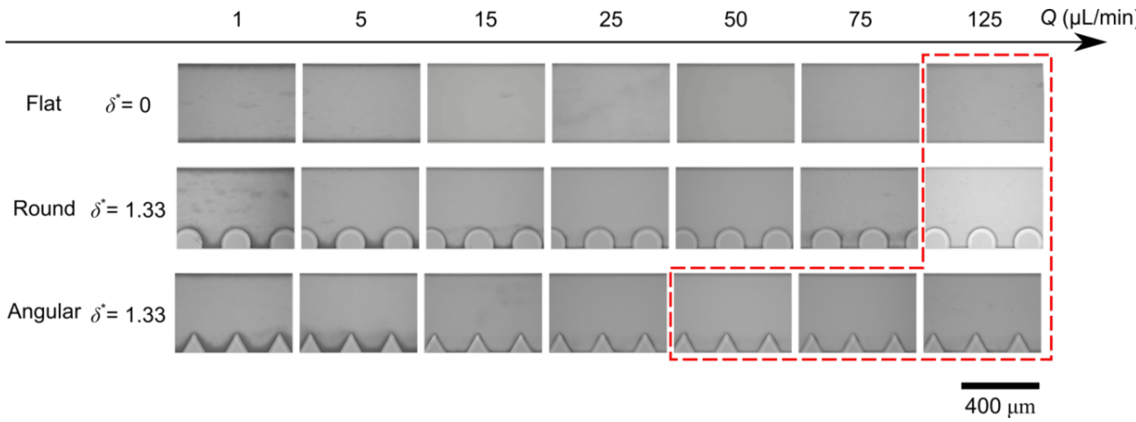

Figure S3. Cross-sectional images of the biofilms at 14-hour growth time in channels with flat boundaries and boundaries with round and angular roughness (both with roughness relative height δ^* = 1.33) at seven flow rates. Red dashed line represents cases with no biofilm growth.

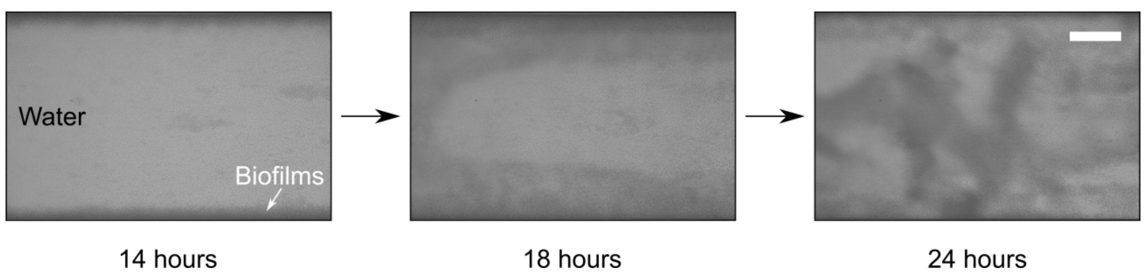

Figure S4. At low-flow conditions (<5 μL/min), biofilms in microfluidic channels continue growing after 14 hours and start to clog the channel after 24 hours. The scale bar is 100 μm.

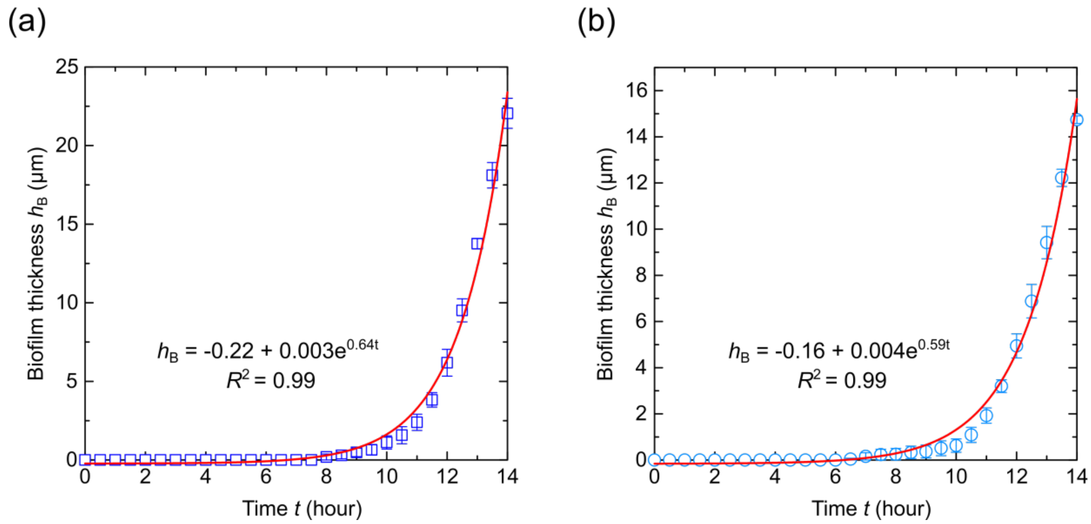

Figure S5. The biofilm growth follows exponential law under low flow rates: 1 μL/min (a) and 5 μL/min (b).

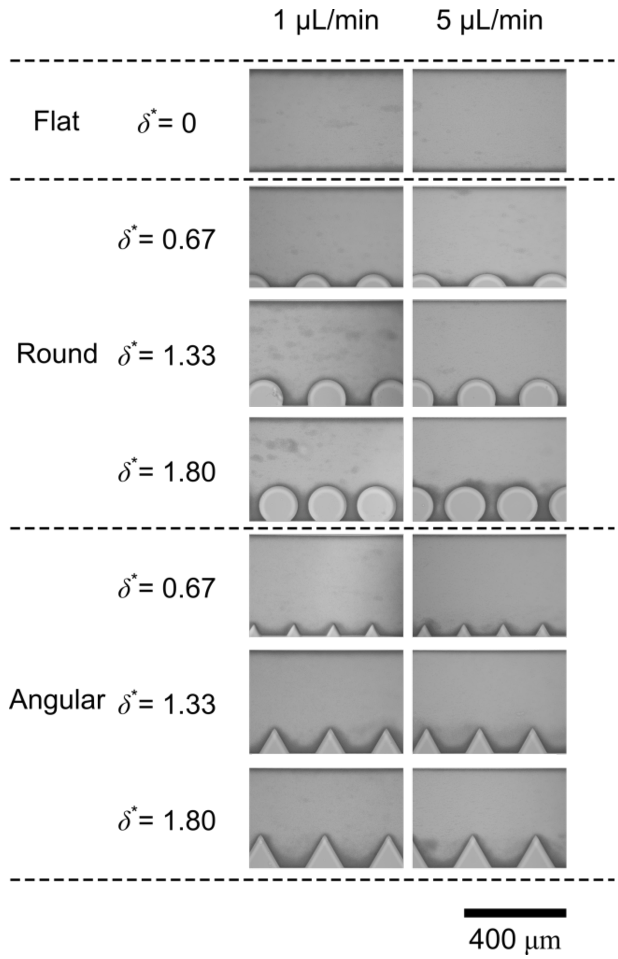

Figure S6. Cross-sectional images of the biofilms at 14-hour growth time in channels with flat boundaries and boundaries with round and angular roughness at two different flow rates and three roughness heights.

Table S1. Parameters used in COMSOL simulation.

Flow conditions	Laminar flow Incompressible flow
Density	995.6 kg/m^3
Dynamic viscosity	0.001 Pa·s
Average inflow velocity	0.09 m/s 0.05 m/s 0.03 m/s 0.02 m/s 0.01 m/s 0.003 m/s 0.0007 m/s
Temperature	303.15 K

Table S2. The 90% confidence interval of critical shear stress τ_{crit}, p value, and R^2 were calculated by regression analysis using MATLAB.

	90% confidence interval	p	R^2
Flat	0.19 ~ 0.48	1.0×10^{-3}	0.95
Round	0.59 ~ 1.44	1.1×10^{-4}	0.96
Angular	0.61 ~ 1.84	2.2×10^{-4}	0.95

References

(1) Drescher, K.; Shen, Y.; Bassler, B.L.; Stone, H.A. Biofilm streamers cause catastrophic disruption of flow with consequences for environmental and medical systems. *Proceedings of the National Academy of Sciences*. 2013, *110*(11), 4345-4350.