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The Effect of Biofilms on Turbulent Flow over Permeable Beds

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Key Points:

- The effect of presence of biofilm on turbulence in the free flow is due to a combination of reduced bed porosity and change in geometry and roughness.
- Presence of biofilm increases Reynolds stresses in the outer layer scaling with wall shear stress.
- Presence of biofilm dampens dimensionless Reynolds stresses in the vicinity of the permeable bed.

1 Abstract

2 In nearly all aquatic, and many industrial environments, colonization of bacteria on solid surfaces 3 results in microbial growth in the form of biofilms, consisting of a collection of microscopic 4 organisms living in a self-secreted polymeric matrix. The growth and detachment of biofilms are 5 coupled to flow hydrodynamics and turbulence. In alluvial channels, a typical substrate consists 6 of a rough permeable bed where biofilm presence modifies both bed porosity and surface 7 roughness, thereby altering mass and momentum exchange at the bed interface. While there is 8 literature detailing turbulent flow over permeable media, little is known concerning how such flow 9 may be affected by the presence of biofilms. This paper addresses this challenge by quantifying 10 the effects of biofilms on flow over laboratory permeable beds with idealized geometry and conditions using particle image velocimetry. The wall shear stress and friction velocity obtained 11 12 from the total shear stress increased in the presence of biofilm, and decreased as a result of biofilm 13 detachment, when compared at constant pump frequency. The dimensionless Reynolds stresses, at 14 constant pump frequency, collapsed for different bed configurations in the outer layer, while for 15 the inner layer, the presence of biofilm led to a decrease in dimensionless Reynolds stress. 16 Quadrant analysis showed that this decrease was primarily due to a reduction in strong Q2 17 contributions. These results suggest that models for flow and transport over permeable media in 18 aquatic environments cannot neglect the role of biofilms in modifying turbulence.

19 **1** Introduction

20 Flows over porous media are central to key biogeochemical processes occurring in many natural 21 and industrial aquatic systems. In particular, exchange of mass, momentum, nutrients and heat in 22 the hyporheic zone is central to many hydrologic systems (Cardenas 2015; Packman and Bencala 23 2000; Sternecker, Wild, and Geist 2013). In alluvial channels, for example, recent studies have 24 shown that bed porosity results in the generation of a diverse mosaic of turbulent suction and 25 ejection events that are fundamentally different from those occurring over impermeable walls 26 (Blois et al. 2011, 2014; Kim et al. 2018; Manes, Pokrajac, et al. 2011; Sinha et al. 2017; Stoesser 27 and Rodi 2007; Suga et al. 2010). Knowledge of these dynamics, at a range of scales, is important 28 due to their critical role in sedimentation, as well as transport of nutrients, pollutants, and dissolved 29 oxygen (Boano et al., 2014; Grant, Gomez-Velez, and Ghisalberti 2018; Roche et al., 2018). 30 Turbulent flow over permeable walls has been studied using both experiments (Blois et al., 2012; 31 Kim et al., 2018, 2019, 2020; Manes et al., 2009; Manes et al., 2011a; Manes et al., 2011b; Pokrajac 32 and Manes 2009; Roche et al., 2018; Suga et al., 2010; Suga et al., 2017) and numerical simulations 33 (Breugem and Boersma 2005; Breugem et al., 2006; Rosti et al., 2015; Sinha et al., 2017). In these 34 studies, the effects of wall permeability on the structure and dynamics of turbulence across the 35 permeable interface, as well as the link between turbulence inside and outside the wall, have been studied. Four important modifications have been highlighted compared to flows over impermeable 36 37 walls with similar interfacial topography: 1) an earlier transition to turbulence (Suga et al., 2010), 38 2) increased bulk flow resistance, 3) increased Reynolds shear stress (RSS) contributions from 39 sweep events in the immediate vicinity of the permeable wall (Suga et al., 2011), and 4) enhanced 40 turbulence due to bed permeability. While these studies provide a wealth of new understanding 41 concerning the physics of turbulent flows overlying permeable walls, they have focused on a static 42 wall geometry rather than a dynamic wall interface, where the geometry can be altered by 43 processes such as microorganism colonization over a range of timescales. For example, the total biomass, and hence porosity, of the interface may change with the seasons, or at much smaller 44

45 scales as individual biofilm streamers move in response to turbulence and that can lead to short-

- 46 lived fluctuations in the interface porosity.
- 47

48 In almost all aquatic environments, as a result of attachment and colonization of bacteria on solid 49 surfaces, microbial biomass exists in the form of biofilms consisting of a collection of different 50 microscopic organisms living in a self-secreted polymeric matrix (Battin et al., 2007), and that can 51 have profound effects on the flow dynamics. The presence of biofilms can alter flow structure in 52 a number of ways, such as reduced bed porosity due to the presence of biomass in the pores and 53 throats of the porous bed. Previous studies have shown decreased velocity fluctuations and 54 Reynolds shear stresses with reduced bed porosity (Breugem et al., 2006; Rosti et al., 2015). In 55 addition, the biofilm can modify the geometry of the porous matrix, which is particularly important 56 at the interface between the subsurface and free-flow where changes in surface roughness can 57 affect the flow structure. The combined effects from these factors result in a modified flow 58 structure for flow over biofilm-covered permeable beds.

59

60 Biofilm growth may stabilize sediments and alter the mechanism of sediment entrainment, as well as influencing the generation of bedforms (Lichtman et al. 2018; Malarkey et al. 2015; Parsons et 61 62 al. 2016). Vignaga et al. (2013) showed that bio-stabilized sediment (i.e. biofilm-bound sediment) 63 acts more like a stretched membrane than a collection of loose particles. In gravel-bed rivers, biofilm growth can affect the hydrodynamics of flow and hyporheic exchange by modifying the 64 geometry of the bed interface and the connectivity of subsurface pore spaces. This can, in turn, 65 affect the concentration of oxygen, organic carbon, and other electron acceptors, as well as the 66 67 biogeochemical reactions occurring inside the bed (Battin et al. 2003; Boano et al. 2014; 68 Dzubakova et al. 2018). However, a limited number of studies have addressed quantitatively the 69 effects of biofilm on flow characteristics, particularly for flow over permeable surfaces. Graba et 70 al. (2010) quantified biomass dynamics during growth and subsequent detachment stages of 71 epilithic biofilms on impermeable rough beds with 20-mm-high hemispherical artificial cobbles in 72 a turbulent flow. They reported a modest decrease in bed shear stress and a decrease in equivalent 73 sand grain roughness due to smoothing of bottom roughness from biofilm growth. In a follow-up 74 study, Graba et al. (2013) investigated the effect of flow rate and wall shear stress on biomass 75 dynamics and the algal composition of biofilm during growth and detachment stages. They 76 observed a similar trend in wall shear stress with biofilm growth and also reported a direct relationship between biofilm attachment strength and shear stress during biofilm growth. Walker 77 78 et al. (2013) studied the modification of a turbulent boundary layer developing over a biofilm-79 covered smooth impermeable surface, such as would relate to the drag on a ship hull. They reported 80 increased skin friction due to biofilm growth on smooth walls. Moreover, a notable finding from 81 their study was that the effective equivalent sand grain roughness of biofilms was greater than their 82 physical roughness, owing to their compliant structure and resulting motion under turbulent flow 83 conditions. In another group of studies, the effect of the presence and growth of biofilm on 84 transport phenomena over and inside porous media has been investigated, showing increased 85 dispersion, longer retention times, and non-Fickian transport with biofilm growth (Aubeneau et al., 2016; Carrel et al., 2018; Roche et al., 2017). 86

87

88 Thus, while biofilms are ubiquitous in aquatic environments and exist on almost all wet surfaces,

their effect on flow over permeable walls is poorly understood. This lack of direct measurements represents a knowledge gap that limits our ability to develop reliable predictive models of flow 91 over environmental systems such as gravel bed rivers, which are highly permeable and typically a

92 location for biofilm growth. In the present study, we aim to investigate experimentally the effects

93 of presence of biofilm on flow over idealized permeable beds. To this end, we use particle image

- 94 velocimetry (PIV) to quantify the characteristics of transitional and turbulent flow over permeable
- 95 beds with and without biofilms.

96 2 Experimental Apparatus

97 **2.1 Flow Facility**

98 A special flow facility was designed and built to accommodate the use of permeable beds with 99 different geometries. The flow facility was a recirculating closed water channel with rectangular 100 cross-section (Figure 1A and B), and consisted of four sections: 1) flow conditioner, 2) boundary-101 layer development section, 3) test section, and 4) flow diffuser. In order to reduce the turbulence 102 intensity of the incoming flow, the flow conditioner was equipped with a perforated plastic sheet, 103 3 mesh screens, and a two-dimensional contraction section with inlet to outlet height ratio of 8:1. 104 The boundary-layer development section was 1 m in length followed by the 0.50-m-long test 105 section equipped with glass windows on the two side walls as well as the top wall for optical 106 access. Flow rate was controlled by setting the pump frequency, f, using a variable frequency drive, 107 and discharge was measured using a SeaMetrics EX810P insertion electromagnetic flow meter.

108

109 The permeable bed consisted of a regular array of acrylic rods spanning the width of the channel

110 covering both the boundary layer development section and the test section (sections (2) and (3) in 111 Figure 1A). The correlic rede were covered to two modified performed correlic related with heles

Figure 1A). The acrylic rods were secured to two machined perforated acrylic plates with holes for the rods (similar to a peg board). The bed consisted of four 0.30-m-long individual sections

plus two 0.15-m-long sections to accommodate the transition after the contraction and before the

diffuser. The design of these perforated plates allowed the rods to be mounted in two different arrangements and thus two different porosities of 35% and 45%. As shown in Figure 1, the higher porosity was achieved using 6.3 mm (0.25 in) acrylic rods mounted in a square array, while the

117 lower porosity was achieved by adding 3.2 mm (0.125 in) acrylic rods to the center of each square

118 cell, thus reducing the porosity by $\sim 10\%$. The value for the lower porosity was primarily 119 determined based on cost and manufacturability constraints, yet its porosity was also representative

determined based orof gravel riverbeds.

121 **2.2 Biofilm Growth Reactor**

122 **2.2.1 Biofilm Reactor**

123 A dedicated recirculating reactor was designed and built to grow biofilm on the measurement 124 module of the permeable bed under controlled conditions. The measurement module was a 0.30-125 m-long section of the permeable bed that was transferred to the water channel and placed in the 126 test section for flow experiments after biofilm growth. The decision to develop biofilm outside 127 the flow facility, and in a standalone biofilm reactor, was made to avoid undesired biofilm growth 128 in portions of the water channel with limited access for cleaning. In this regard, biofilm growth on 129 the top wall in the boundary layer development section could have had unpredictable effects on 130 the flow characteristics in the channel. Due to this choice, there was a transition in bed porosity at 131 the beginning of the test section that resulted in developing flow over the biofilm bed in the test 132 section.

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133

2.2.2 Biofilm Development Protocol

134 Figure 2A shows schematically the configuration of the reactor. A multi-cassette Cole Parmer 135 MasterFlex L/S peristaltic pump (P1) was used to deliver the electron donor and growth medium at fixed equal flow rates of 4 ml/min. A 250-g/m³ aqueous solution of potassium acetate 136 137 (KCH₃COO) was used as the electron donor and tap water was used as the growth medium due to 138 its high mineral concentration. A Cole Parmer gear pump (P2) was used for recirculation of the 139 fluids at 40× the combined influent and effluent flow rate (~320 ml/min) to establish fully mixed 140 conditions in the reactor. The resulting average streamwise flow velocity in the reactor, based on the volumetric flow rate and the cross-sectional area, was ~4 mm/s. A third cassette on the 141 142 peristaltic pump was used to extract the effluent from the aeration bottle and maintain a constant 143 fluid level therein. An aeration stone inside the aeration bottle was connected to a low-pressure air



supply line to provide oxygen to the reactor. In this manner, the dissolved oxygen levels were maintained close to saturation and thus compensated for oxygen consumption by biofilm activity.

146 147

147 The reactor, with the measurement module of the permeable bed placed inside, was inoculated 148 with activated sludge from a local wastewater treatment plant. Biofilm was allowed to grow on the 149 cylinders for 14 days. During this time period, the biofilm was exposed to room light for approximately 6–10 hours each day. Based on the inoculum and growth conditions, the dominant 150 151 members in this biofilm were heterotrophic and nitrifying bacteria whose growth is relatively insensitive to light conditions. The reactor was maintained at the room temperature of 20-22°C. 152 After the 14-day growth period, the biofilm-covered measurement module was removed from the 153 reactor and its sidewalls above the top layer of the cylinders were cleaned carefully without 154 155 disturbing the biofilm to ensure unhindered optical access. This module was then transferred to the flow facility for flow experiments. Figure 2B and C show the biofilm-covered bed before being 156 157 placed in the water channel. It is worth noting that the biofilm growth was limited to the reactor-158 processed portion of the bed section, and thus a sharp transition in terms of bed porosity and 159 roughness geometry existed at the ends of this section. The effect of this transition on flow 160 development is discussed in §5.

161 **2.3 PIV Setup**

162 A dual-head, pulsed, Evergreen Nd:YAG laser with maximum energy of 200 mJ/pulse was used 163 to form a ~1-mm-thick laser sheet to illuminate the tracer particles in the streamwise–wall-normal

- (x-y) plane. The longitudinal position of the light sheet was 1.25 m downstream of the channel
- 165 inlet and approximately 0.20 m from the beginning of the measurement module (Figure 1B), and

166 its spanwise (z-direction, Figure 1C) position was equidistant from the two side walls. A 16-bit, 167 Andor Neo sCMOS camera with 5.5-megapixel $(2560 \times 2160$ -pixel array) sensor and a pixel size 168 of 6.5 µm was used to capture image pairs at a rate of 10 Hz. The camera was coupled with a 169 Navitar long-distance microscope with numerical aperture (NA) of ~0.012, consisting of a $0.25 \times$ 170 objective, a $2\times$ adapter, and a zoom lens set at $\sim 2\times$ resulting in a magnification of $\sim 1.1\times$ translating 171 to $\sim 7 \,\mu$ m/pixel, and a resolution of $\sim 40 \,\mu$ m at 550 nm wavelength. The field of view of the imaging 172 setup was $\sim 18 \times 14$ mm². The FOV covered up to ~ 7 mm above the cylinder tops, in order to avoid 173 error from optical aberrations observed near the top of the FOV. Fluorescent particles ~10-15µm 174 in diameter were introduced into the flow as tracers and their fluoresced light was recorded using 175 a long-pass 550 nm filter. Raw PIV images were processed with LaVision DaVis 8.2 software using a sequential cross-correlation algorithm with a final interrogation window size of 64^2 pixels 176 177 with 50% overlap, resulting in a vector field resolution of ~0.44 mm and a vector grid spacing of 178 ~0.22 mm (0.035*d*).

179 **2.4 Experiments**

180 Five sets of experiments were conducted - two without biofilm (*nBF*) and three with biofilm-181 covered bed sections (BF). The reference case is taken as flow over the bed with 45% porosity 182 throughout the channel without biofilm (nBF-45%). The other dataset without biofilm was nBF-183 45-35% where the bed porosity is 45% in the developing section leading to a 0.30-m-long section 184 with 35% porosity. Lastly, the BF datasets correspond to experiments on different batches of 185 biofilm developed independently at different times. The bed porosity in the channel was 45% 186 leading to 30-m-long biofilm-covered section, whose bed porosity was presumably smaller than 187 45% due to presence of biomass. Details of the experimental beds are presented in Table 1.

188	
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Table 1. Dataset Summary						
Dataset	Biofilm	Description				
nBF-45% (reference)	No	45% bed porosity in the boundary-layer development and test section				
nBF-45-35%	No	45% bed porosity in the boundary-layer development section, and 35% porosity in the test section.				
BF1	Yes	45% bed porosity in clean conditions (before biofilm growth; the				
BF2	Yes	actual porosity in the biofilm section is likely lower due to presence				
BF3	Yes	of biofilm). These correspond to three different batches of biofilm developed independently at different times in the same reactor.				

189

190 Temporal decomposition of the velocity field, $\mathbf{u}(x,y,t) = (u(x,y,t), v(x,y,t))$, was performed as 191 $u = \bar{u} + u'$, where the overbar denotes time-averaged (Reynolds-averaged or mean) values and 192 the prime denotes the deviation from the time-averaged value. Line averaging in the streamwise 193 direction (x-direction) at any vertical position y, was performed according to the definition given 194 by Nilsen et al. (2001). For any particular for mean streamwise sub-site \bar{u}

by Nikora et al. (2001). For example, for mean streamwise velocity, \bar{u} :

$$\langle \bar{u} \rangle (y) = \left(\int_{A_f} \bar{u}(x', y) dx' \right) / A_f \tag{1}$$

where A_f is the area occupied by fluid at any vertical position, y, and is determined based on the number of vectors at each y location. Also, spatial deviation is calculated as the difference between

197 the time-averaged and doubled-averaged quantities. $\tilde{u} = \bar{u} - \langle \bar{u} \rangle$.

198

199 Figure 3 shows a representative time-averaged velocity field, with streamwise velocity \overline{u} contours 200 and streamlines (Figure 3A), in addition to the corresponding double-averaged velocity profile, $\langle \bar{u} \rangle$ (y), (Figure 3B). Due to flow detachment, a recirculation region exists between the cylinders 201 202 that results in negative streamwise velocities with a velocity magnitude of $\sim 0.1 U_{max}$. Similar to observations by Breugem et al., (2006), the line-averaged velocity profile, $\langle \bar{u} \rangle$, exhibits an 203 204 inflection point just below the interface, denoted as yinflec. The PIV uncertainty in particle 205 displacement calculated from the images is 5% of particle image diameter, or ~0.1 pixels (~ 1.4×10^{-1} 206 3 mm in the measurement plane). For the different laser pulse time delays used herein, this equates to a velocity uncertainty of ~0.4% of U_{max} in each case. 207

208



Figure 3. (A) Time-averaged streamwise velocity, \bar{u} , (B) double-averaged velocity profile at $Re \approx 7000$. Dashed line indicates the top of the cylinders at y = 0 mm, and d = 6.3 mm is the cylinder diameter.

209 The total shear stress, τ , is calculated as the sum of viscous, Reynolds, and dispersive (forminduced) shear stresses: 210

$$\tau = \mu \frac{d\langle \bar{u} \rangle}{dv} - \rho \langle \overline{u'v'} \rangle - \rho \langle \tilde{u}\tilde{v} \rangle.$$
⁽²⁾

211 Figure 4 presented the total shear stress as well 212 as the individual terms from Eq. (2), normalized with wall shear stress τ_w^p . The shear 213 stress does not include the drag term below the 214 215 cylinder crest (y/d < 0). The shear stress at the permeable wall, $\tau_w^{p'}$, was calculated by 216 217 extrapolating the linear segment of the total 218 shear stress profile (Eq. (2) with density, 219 $\rho = 997.5 \text{ kg/m}^3$ and viscosity. 220 $\mu = 0.9321 \times 10^{-3}$ Pa.s) near channel the 221 centerline to the crest location (y = 0).

222

223 Table 2 presents details of the experimental conditions for different runs and datasets. 224 225 Discharge, Q, was directly measured by the 226 flowmeter, while U_{max} , y_{max} and were obtained





from the double-averaged velocity profiles. The bulk Reynolds number, Re, for this flow configuration is defined as:

$$Re = \frac{U_b H}{v},$$
(3)

where *H* is the channel height (0.01 m for all cases), $\nu = 0.934 \times 10^{-3} \text{ m}^2/\text{s}$ is the kinematic viscosity

of water, and U_b is the bulk velocity calculated as the average velocity for $y \in [0 \text{ H}]$, as defined by Breugem et al. (2006). Since the velocity field is resolved up to $y \approx 7 \text{ mm}$, a fourth order

polynomial curve fit is used to approximate the velocity profile up to $y \approx 7 \text{ min}$, a routil order

233 of U_b . The friction velocity (u_τ^p) and friction Reynolds number $(\operatorname{Re}_\tau^p)$ are defined as:

$$u_{\tau}^{p} = \sqrt{\tau_{w}^{p}/\rho} , \qquad (4)$$

234

$$\operatorname{Re}_{\tau}^{p} = \frac{u_{\tau}^{p}H}{\nu}.$$
(5)

235 The permeability Reynolds number (Re_K) is calculated as,

$$\operatorname{Re}_{\mathrm{K}} = \frac{u_{\tau}^{p}\sqrt{K}}{\nu} \tag{6}$$

where K is the bed permeability estimated from the Kozeny-Carman equation for cylinders in cross-flow (Nakayama, Kuwahara, and Sano 2007),

$$K = \frac{\phi^3 d_h^2}{144(1-\phi)^2} \tag{7}$$

where ϕ is porosity and d_h is the pore hydraulic diameter. This yields a permeability of 0.087 mm² and 0.051 mm² for the bed with 45% and 35% porosity, respectively. As a first approximation for

the bed with biofilm, we used the same permeability value as the low-porosity bed.

- 241
- For sufficiently high Reynolds number, a logarithmic layer is expected in the boundary layer above
- the permeable bed. A common parametrization of the log law is

$$\langle \bar{u} \rangle^+ = \frac{1}{\kappa} \ln\left(\frac{y^+ + y_0^+}{k_s^+}\right),\tag{8}$$

where $\langle \bar{u} \rangle^+ = \langle \bar{u} \rangle / u_{\tau}^p$, and κ is the equivalent to the von Karman constant (Breugem et al. 2006; Fang et al. 2018). Also, y^+ is the vertical distance above the cylinder crest, y_0^+ is the zerodisplacement height, and k_s^+ is the equivalent roughness height, all normalized with viscous length scale $y^* = v / u_{\tau}^p$.

248

249 In experiments with biofilm, the pump 250 frequency, f, and channel flow rate, Q, were 251 increased in steps, and data were recorded for 252 f = 4.5 Hz and 8 Hz ($Q \approx 0.18$ and 0.28 L/s), 253 before reaching the target of 16 Hz and 30 Hz 254 $(Q \approx 0.54 \text{ and } 0.95 \text{ L/s})$. After recording data 255 for the highest flow rate, the pump frequency 256 was reduced, and data was recorded 257 sequentially at f = 8 Hz4.5 Hz and 258 $(Q \approx 2.8 \text{ L/s} \text{ and } Q \approx 0.18 \text{ L/s})$ (with the 259 exception of the BF3 dataset). Figure 5 260 illustrates the flow rate ramping stages



schematically. The number of image pairs for each flow setting and the corresponding number of flow-through times is given in Table 3. The flow-through time is calculated as the ratio of the streamwise length (18 mm) of the field of view to the bulk velocity (U_b). After each stage, there was at least a 2-minute wait time before recording images. The flow characteristics during ramp up and ramp-down, referred to as *before detachment* (BD) and *after detachment* (AD), respectively, are compared later to illustrate the effect of biofilm detachment on flow behavior.

267

Table 2. Experimental conditions: *f* is pump frequency, *Q* is volumetric flow rate, U_b is bulk velocity, U_{max} is maximum streamwise velocity, y_{max} is the coordinate where U_{max} occurs, u_{τ}^p is the friction velocity at the permeable wall (Eq. 4), Re is the bulk Reynolds number (Eq. 2), and Re_{τ}^p is the friction Reynolds number at the permeable wall (Eq. 5), and Re_{κ} is the permeability Reynolds number (Eq. 6). k_s^+ , y_0^+ and κ are the equivalent roughness height and zero displacement height (normalized with viscous wall units y^*) and von Karman constant, respectively, from the logarithmic fit to velocity profile (Eq. 8). BD and AD indicate "before detachment" and "after detachment", respectively.

Dat	aset	f	Q	U_b	U_{max}	<i>Y</i> _{max}	$u_{\tau}^p \times 10^{-3}$	Re	$\operatorname{Re}_{\tau}^{p}$	Re _K	k_s^+	y_0^+	κ
		[Hz]	[L/s]	[m/s]	[m/s]	[mm]	[m/s]		Ū				
nBF-45%		4.5	0.173	0.150	0.186	5.3	14.1	1610	150	4.4	0.4	4.5	0.40
		8	0.293	0.259	0.315	5.9	24.5	2770	262	7.7	2.9	28.3	0.31
		16	0.534	0.503	0.615	6.2	46.7	5390	500	14.8	13.4	81.0	0.25
		30	0.971	0.977	1.198	6.2	82.1	10460	879	26.0	23.7	141	0.22
nBF-45-35%		4.5	0.177	0.152	0.191	5.2	14.3	1630	153	3.5	0.9	13.2	0.33
		8	0.293	0.267	0.322	5.6	23.2	2860	249	5.6	3.2	33.8	0.28
		16	0.536	0.521	0.639	6.0	46.6	5580	499	11.2	13.1	88.8	0.24
		30	0.953	0.985	1.217	6.0	89.3	10540	956	21.6	35.9	180	0.22
BF1 A	BD	4.5	0.177	0.167	0.213	5.9	20.1	1790	215	4.9	5.5	28.4	0.31
	BD	8	0.284	0.296	0.379	6.6	35.2	3170	377	8.5	14.0	52.7	0.28
		16	0.543	0.582	0.757	6.8	69.1	6230	739	16.7	60.9	153	0.21
		30	0.953	1.105	1.403	6.6	106	11840	1137	25.6	50.4	173	0.21
	AD	8	0.291	0.297	0.368	6.3	29.3	3180	314	7.1	4.1	30.7	0.31
	AD	4.5	0.173	0.166	0.209	5.7	15.9	1780	170	3.8	0.4	3.4	0.41
BD BD BF2 AD AD	BD	4.5	0.178	0.162	0.205	5.6	17.7	1730	189	4.3	4.3	29.9	0.29
	BD	8	0.281	0.283	0.355	6.0	32.0	3030	343	7.7	13.6	68.6	0.26
		16	0.536	0.563	0.701	6.3	58.7	6030	629	14.2	39.6	165	0.21
		30	0.953	1.029	1.296	6.3	102	11020	1093	24.6	44.4	208	0.23
	AD	8	0.287	0.280	0.344	5.8	26.2	3000	281	6.3	5.9	51.6	0.27
	AD	4.5	0.178	0.158	0.198	5.2	15.3	1690	163	3.7	0.8	14.4	0.36
BF3		16	0.539	0.567	0.685	5.9	51.9	6070	555	12.5	8.5	92.1	0.29
		30	0.946	1.031	1.263	6.1	91.6	11040	981	22.1	28.8	194	0.23

268 269

Table 3. PIV ensemble size (number of image pairs)							
Pump frequency,	Ensemble	Number of flow-					
f [Hz]	size	through times					
4.5	750	~650					
8	1000	~1500					
16	3000	~8670					
30	4000	~21780					

270 **2.5 Flow Characteristics and Variability due to Biofilm Presence**

Despite maintaining very similar experimental conditions in the three flow experiments conducted in the presence of biofilm, due to unavoidable differences in biofilm growth, the flow was inherently subject to some degree of variability. Under these conditions, data repeatability was difficult to achieve. As such, the first step in the present analysis was to statistically characterize the boundary layer by computing the ensemble-averaged profiles and quantifying the variability between the three different sets of experiments. The results presented from biofilm experiments, unless noted otherwise, are the average of the three separate experimental datasets: *BF*1, *BF*2 and *BF*3 in Table 2, and the error bars for flow over the biofilm beds represent the range of measurements. Individual results are provided as Supporting Information for completeness.

280 3 Results

The results and analysis presented in the following sections are focused on f = 4.5 Hz ($Re = 1700\pm100$) and f = 30 Hz ($Re = 11150\pm700$), selected to illustrate the main trends observed in the data. Similar behavior was observed for the two intermediate flow rates, whose results are presented as Supplementary Information. In order to facilitate comparison, flow and turbulence variables are presented in dimensionless form; streamwise and wall-normal velocity components are normalized with bulk velocity, U_b , and Reynolds and form-induced stresses are normalized with $(u_{\tau}^p)^2$.

288

289 **3.1 Biofilm Detachment**

290 **3.1.1 Imaging Analysis**

291 The flow experiments in the water channel 292 were carried out at different flow rates where 293 the biofilm was subjected to increased shear 294 stress in several incremental stages (Figure 5). 295 This led to progressive detachment of the 296 biofilm from the solid surfaces. For each flow 297 rate, the extent of biofilm that remained 298 attached to the cylinders was measured using 299 a semi-quantitative image processing method 300 using the raw PIV images. At each image pixel, the maximum and minimum pixel 301 302 intensity within each time series was 303 identified. Next, a map of the difference, or 304 range, between these two values at each pixel 305 (range = max - min) was generated. The low 306 values in this map correspond to portions of 307 the image where particles cannot be present; 308 i.e. the solid cylinder and biofilm, whereas the 309 high values correspond to regions of the image 310 particles where tracer were present 311 intermittently. The range image for the highest pump frequency, f = 30 Hz ($Re = 11150 \pm 700$), 312 313 in each dataset was taken as the reference. 314 This was done because after this flow stage 315 nearly all biofilm on the top row of cylinders 316 was detached and its visible biofilm coverage in the top layer was nearly non-existent. The 317



Figure 6. Biofilm coverage at different Re during the ramp-up stage (A) f = 4.5 Hz ($Re = 1700\pm100$), (B) f = 8 Hz ($Re = 3000\pm200$), (C) f = 16 Hz ($Re = 5800\pm400$). The cylinders are outlined in white and a 1-mm scale bar is shown for reference.

318 difference calculated with respect to this reference image, and binarized subsequently, depicts the

- 319 biofilm coverage on the cylinders.
- 320

321 Figure 6 shows the biofilm obtained using this method for a representative dataset. At the 322 beginning of the experiment with the lowest flow rate, f = 4.5 Hz ($Re = 1700\pm100$), the biofilm 323 was in a nearly-pristine condition (Figure 6A) as wall shear stress was low and hence detachment 324 was insignificant. As the flow rate was increased, at f = 8 Hz ($Re = 3000 \pm 200$), a modest amount of biofilm detachment was observed (Figure 6B). Finally, at the 2nd highest pump frequency of 325 326 f = 16 Hz ($Re = 5800 \pm 400$), the biofilm coverage was dramatically reduced (Figure 6C). It should 327 be noted that in this way, the biofilm was visualized *indirectly* using the fluorescent particles in raw PIV images. Nevertheless, this method does yield a qualitative picture of the biofilm coverage, 328 329 since at the end of the experiment biofilm coverage on the top of the cylinders appeared minimal 330 and barely visible to the naked eye. Based on the images, the biofilm thickness on the cylinder 331 crests can be estimated roughly as 0.5-1 mm, 0.25-0.75 mm, and 0.1-0.25 mm at 4.5 Hz, 8 Hz, 332 and 16 Hz, respectively.

333 **3.1.2 Effect of Biofilm Detachment on Flow Statistics**

334 Results for flow over beds with different biofilm conditions were compared to quantify the effect 335 of presence of biofilm and biofilm detachment. Comparisons were made at the pump frequency of 336 f = 4.5 ($Re = 1700 \pm 100$) at the beginning and end of each run, corresponding to ramp-up and ramp-337 down stages, respectively (c.f. Figure 5). The lowest pump frequency was selected for this purpose 338 because of the near-pristine condition of the biofilm at the beginning of the experiment; hence, the 339 biggest difference in biofilm coverage between before and after detachment cases. Figures 7 and 340 8 compare double-averaged flow statistics for three cases: no biofilm (nBF-45%), biofilm before 341 detachment (BF-BD), and biofilm after detachment (BF-AD). The biofilm data is the average of 342 two independent runs (BF1 and BF2), and the error bars indicate the data range.

343

Double averaged streamwise and wall-normal velocity profiles, normalized with bulk velocity, U_b are presented (Figure 7). The streamwise velocity profile for flow over the beds with and without biofilm are highly similar. One difference though is the slight increase in the maximum streamwise velocity (U_{max}/U_b) in the presence of biofilm of the free flow at constant flow rate (Figure 7A). The wall normal velocity (Figure 7B), showed an upward motion below the cylinder crests for both BD and AD which was not observed for nBF.

350

The dimensionless streamwise Reynolds normal stress, $\langle \overline{u'u'} \rangle / (u_{\tau}^p)^2$, at constant pump frequency (Figure 8A) collapsed for $0.5 \le y/d \le 1$. Closer to the bed interface, for $0 \le y/d \le 0.5$, the stress was highest for the bed without biofilm (nBF) and lowest for biofilm before detachment (BF–BD). The maximum stress occurred at $y/d \approx 0$ for all configurations.

356

The dimensionless wall-normal Reynolds normal stress, $\langle \overline{v'v'} \rangle / (u_\tau^p)^2$, at constant pump frequency (Figure 8B) collapsed for $0.4 \leq y/d \leq 1$. Close to the bed, for $0 \leq y/d \leq 0.4$, similar to the streamwise Reynolds normal stress, the non-biofilm bed had the highest stress, however, there was very minor difference between the before-detachment (BF–BD) and after-detachment configurations (BF–AD). For nearly all vertical positions, the before and after-detachment configurations (BF–BD and BF–AD) had nearly identical profiles. The peak stress for nBF and 363 BF–AD occurred at $y/d \approx 0.25$, while the peak for BF–BD occurred at a slightly higher coordinate 364 at $y/d \approx 0.3$.

365



Figure 7. Effect of biofilm detachment on (A) streamwise, and (B) wall-normal velocity. Biofilm before detachment (BF–BD, filled red circle), and after detachment (BF–AD, open red circle), no biofilm (black asterisk). The error bars indicate the range of data for biofilm datasets. The biofilm data ("BF–BD" and "BF–AD") are from *BF1* and *BF2* datasets. Values of U_{max} for the presented data are, nBF-45: 0.19 m/s; BF–BD: 0.21 m/s and 0.21 m/s; BF–AD: 0.20 m/s and 0.21 m/s.

366

Similar to the other components of Reynolds stress presented herein, the dimensionless Reynolds shear stress, $-\langle \overline{u'v'} \rangle / (u_\tau^p)^2$, was highest for the bed without biofilm in the range $0 \le y/d \le 0.5$ (Figure 8C). The profiles collapsed for $0.5 \le y/d \le 1$, although within this range, the BF–BD had slightly higher stress values. The biofilm before and after detachment had highly similar profiles, except in the $-0.1 \le y/d \le 0.3$ range where the normalized RSS after detachment (AD) was higher by approximately 10%. The peak for nBF-45% was at $y/d \approx 0$, whereas the peak for both BF–BD and BF–AD occurred at $y/d \approx 0.18$.

375



biofilm (nBF, black asterisk). The error bars indicate the range of data for biofilm datasets.

376 **3.2 Biofilm and Reduced Bed Porosity**

377 One of the potential effects of biofilm growth in a porous bed is a reduction of porosity likely 378 concomitant with reduction in permeability due to blocking of the pores. As described in the previous section, biofilm detachment occurred due to flow shear during experiments. Since shear 379 380 stress above the bed is larger than stress within the bed, detachment is expected to occur mostly in 381 the top layers of the bed while biomass in the bottom layers of the bed is less affected. It is 382 reasonable to conjecture that the effects described in §3.1.2 were, at least in part, induced by this 383 reduction in pore space size that was maintained even after the ramp-up phase of the experiment, 384 as qualitatively observed in §3.1.1. In an attempt to replicate the effect of reduced bed porosity 385 associated with biofilm presence, and thus test this hypothesis, flow measurements were performed 386 using a permeable bed section with no biofilm but with a lower porosity. In this manner, the 387 experiments aimed to decouple any changing bed porosity from other effects arising from the 388 presence of biofilms, such as roughness and dynamic geometry. Given that the porosity of the bed 389 with biofilm was not quantified, only partial decoupling was possible. It should be noted that, in 390 order to maintain the incoming flow configuration as close to the case of flow with biofilm as 391 possible, the porosity of boundary layer development section of the bed was kept at 45%, and only 392 in the test section was porosity reduced to 35%. This created a transition at the beginning of the 393 test section resulting in developing flow. 394

395 The double-averaged profiles of streamwise and wall-normal velocity for the two non-biofilm 396 datasets (nBF-45% and nBF-45-35%), and the biofilm averaged data are presented (Figure 9). The 397 streamwise velocity profiles are highly similar and collapse in the outer layer ($0.5 \leq v/d \leq 1$) for 398 both biofilm and non-biofilm beds. Variations are evident among the biofilm datasets in the inner 399 layer and slightly below the cylinder crest for $-0.2 \leq y/d \leq 0.5$. One notable feature as was 400 observed in the before and after detachment cases (Figure 7A) was the slight increase in the 401 U_{max}/U_b ratio for the biofilm datasets. A similar, albeit smaller increase is also observed for the 402 non-biofilm reduced porosity bed.

403



Figure 9. Biofilm effect comparison with reduced bed porosity on streamwise velocity (A, B), and wall-normal velocity (C, D). No biofilm with 45% bed porosity (red circle), no biofilm with 45% porosity in the flow developing section and 35% in the test section (blue square), biofilm (black asterisk). The error bars indicate the range of data for biofilm datasets. Values of U_{max} for the presented data are, <u>nBF-45%</u>: 1.20 m/s; <u>nBF-45-35%</u>: 1.22 m/s; <u>BF</u>: 1.40 m/s, 1.30 m/s, and 1.26 m/s. (*Re* = 11150±700),

404

405 The wall-normal velocity above the cylinder crest (y/d > 0) for all datasets show a near zero 406 velocity, indicating purely horizontal flow. However, there was an upward velocity below the 407 cylinder-tops (v/d < 0) for both the reduced porosity bed (nBF-45-35%) and the biofilm bed. This 408 behavior was also observed comparing before and after-detachment at f = 4.5 Hz (Figure 7B).

409

410 The log law (Eq. (8)) was fitted to the double-411 averaged streamwise velocity profile 412 following the method by Fang et al. (2018) 413 (Figure 10). The fitting parameters for all 414 datasets are provided in Table 2. The results 415 show increased equivalent wall roughness 416 height k_s^+ and y_0^+ for the biofilm bed 417 compared to the two no biofilm cases.

418

419 Figure 11 presents the double-averaged 420 dimensionless Reynolds stresses for the two 421 datasets without biofilm (nBF-45% and nBF-422 45-35%), along with the biofilm average. A 423 common feature of the profiles presented 424 herein is that at fixed pump frequency, 425 individual Reynolds stress components 426 (streamwise, wall-normal, and shear) normalized with $(u_{\tau}^{p})^{2}$ collapse for all bed 427 428 configurations in the outer layer 0.25-429 $0.4 \leq y/d \leq 1$. Near the permeable bed 430 $(0 \le v/d \le 0.25)$ the reference non-biofilm



431 bed (nBF-45%) has the highest stress. Moreover, the non-biofilm reduced porosity bed (nBF-45-432 35%) and the biofilm average had highly similar profiles at nearly all y positions.

433

434 The dimensionless streamwise Reynolds normal stress, $\langle \overline{u'u'} \rangle / (u_\tau^p)^2$, collapsed for $0.25 \leq y/d \leq 1$ 435 for the three bed configurations (Figure 11A). Also, the maximum streamwise Reynolds normal 436 stress occurred slightly below the crest at $y/d \approx -0.1$ for all flow configurations. However, while 437 the reference non-biofilm bed (nBF-45%) had a prominent peak, the reduced porosity non-biofilm 438 bed (nBF-45-35%) and the biofilm bed (BF) had nearly flat and highly similar profiles within the 439 $-0.1 \leq y/d \leq 0.25$ range. Within this range, the nBF-45% bed had the highest value of $\langle \overline{u'u'} \rangle / (u_\tau^p)^2$. 440

441 The dimensionless wall-normal Reynolds normal stress, $\langle \overline{v'v'} \rangle / (u_{\tau}^p)^2$, for the three bed 442 configurations collapsed for $y/d \ge 0.4$ (Figure 11B). For $-0.5 \le y/d \le 0.3$, the reference non-443 biofilm bed (nBF-45%) had the highest stress values. Also, nBF-45% had a maximum below the 444 cylinder crests at $y/d \approx -0.1$, while BF and nBF-45-35% attained their maximum above the bed at 445 $y/d \approx 0.2$. The non-biofilm reduced porosity bed (nBF-45-35%) and the biofilm average had nearly 446 identical profiles for all y positions.

447

The dimensionless Reynolds shear stress profiles, $-\langle \overline{u'v'} \rangle / (u_{\tau}^p)^2$, demonstrate a trend similar to the 448

449 other Reynolds stress components, where the profiles collapsed for $y/d \ge 0.4$ (Figure 11C). For 450 $-0.2 \leq v/d \leq 0.4$, nBF-45% had the highest stress compared to the two other datasets. Moreover,

451 while the reduced porosity non-biofilm bed (nBF-45-35%) and the biofilm bed had relatively flat 452 profiles for $-0.1 \le y/d \le 0.25$ and a maximum near $y/d \approx 0.2$, the reference non-biofilm bed (nBF-

453 45%) had a prominent peak at $y/d \approx -0.1$.

454



Figure 11. Biofilm effect comparison with reduced bed porosity for f = 30 Hz ($Re = 11150 \pm 700$). (A) streamwise Reynolds normal stress, (B) wall-normal Reynolds normal stress, and (C) Reynolds shear stress. No biofilm with 45% bed porosity (red circle), no biofilm with 45% porosity in the flow developing section and 35% in the test section (blue square), biofilm (black asterisk). The error bars indicate the range of data for biofilm datasets.

455

The form-induced (dispersive) stresses are presented in Figure 12. In all cases, the form-induced stresses are very small right above the cylinder crests ($0 \le y/d \le 0.25$) and practically zero for $y/d \ge 0.25$. Comparing Figures 11 and 12 shows that above the cylinder crests, the form-induced stresses are negligible compared to Reynolds stresses. However, below the crests ($-0.5 \le y/d \le 0$) the form-induced stresses are comparable to Reynolds stresses in magnitudes ad cannot be neglected.

462

The streamwise form-induced normal stresses attain their peak at $y/d \approx -0.12$, with a magnitude comparable to that of the corresponding local Reynolds stress (Figure 12A). The wall-normal form-induced normal stresses attain their maximum at $y/d \approx -0.3$, where the peak value is even greater than the corresponding local Reynolds stress (Figure 12B). Lastly, the form-induced shear stress showed a behavior and range similar to the Reynolds shear stress within the $-0.5 \leq y/d \leq 0$



Figure 12. Biofilm effect comparison with reduced bed porosity for f = 30 Hz ($Re = 11150 \pm 700$). (A) streamwise form-induced normal stress, (B) wall-normal form-induced normal stress, and (C) form-induced shear stress. No biofilm with 45% bed porosity (red circle), no biofilm with 45% porosity in the flow developing section and 35% in the test section (blue square), biofilm (black asterisk). The error bars indicate the range of data for biofilm datasets. ($Re = 11150 \pm 700$),

468 range (Figure 12C). For all components of the form-induced stress, the biofilm datasets469 demonstrate a broad range of variation that was not observed in. Reynolds stresses.

470

The correlation coefficient $(-\langle \overline{u'v'} \rangle / u'_{\rm rms} v'_{\rm rms})$ 471 472 of the velocity fluctuations u' and v', is a measure of the efficiency of wall-normal 473 474 motion in transporting streamwise momentum 475 (Figure 13A). The results indicate that at both 476 *Re*, the coefficient has a nearly constant value 477 of ~0.5 for $0 \leq y/d \leq 0.6$, and a peak at $y/d \approx -$ 478 0.1. The value of the peak decreases with 479 porosity and is nearly flat for both the biofilm 480 average and the non-biofilm reduced-porosity 481 bed (*nBF*-45-35%).

482 **3.3 Quadrant Analysis**

483 Ouadrant analysis of the instantaneous velocity 484 fields was performed to gain further insight 485 into trends observed in the RSS (Wallace 2016). In this approach, u'v' is classified into 486 487 four categories, termed quadrants (O), based 488 on the sign of u' and v': Q1 (u' > 0, v' > 0), Q2 489 (u' < 0, v' > 0), Q3 (u' < 0, v' < 0), and Q4490 (u' > 0, v' < 0), where Q2 and Q4 events are



Figure 13. Biofilm effect comparison with reduced bed porosity on correlation coefficient of velocity fluctuations for f = 30 Hz ($Re = 11150 \pm 700$). No biofilm with 45% bed porosity (red circle), no biofilm with 45% porosity in the flow developing section and 35% in the test section (blue square), biofilm (black asterisk).

referred to as ejections and sweeps, respectively, and Q1 and Q3 events are called outward and inward interactions. The hyperbolic hole size method of Lu and Willmarth (1973), with a hole size of $\eta = 4$ has been applied herein. Thus, the intense Reynolds-stress-producing events above the $\eta = 4$ threshold have been considered. In this configuration, contributions form Q2 and Q4 were stronger than that of Q1 and Q3 by a factor of >10, so only data for Q2 and Q4 are considered herein. For the flow with biofilm, only one of the three separate datasets has been considered (BF1).

498

499 The contributions from Q2 and Q4 to the Reynolds shear stress, non-dimensionalized with $(u_{\tau}^{p})^{2}$ 500 and in absolute units are presented in Figure 14A and B, respectively. For the biofilm bed the Q2 and Q4 contributions shift in +y-direction compared to the non-biofilm datasets. The crossover 501 502 between Q2 and Q4 contributions for the two non-biofilm beds (nBF-45% and nBF-45-35%) 503 occurred at $y/d \approx 0$, while for the biofilm bed (BF1) the crossover occurred at $y/d \approx 0.2$. Similarly, 504 while Q4 contributions had a maximum near $y/d \approx -0.1$ for the non-biofilm beds, the peak for BF1 occurred closer to the cylinder crests at $y/d \approx -0.05$. The peak for contributions from Q2 events 505 506 occurred at $y/d \approx 0.4$ for the nBF-45% dataset and at $y/d \approx 0.5$ for nBF-45-35% and BF1 datasets. In absolute units, the Q4 contributions (Figure 14B) for the two non-biofilm beds were in relatively 507 508 close agreement, while BF1 had a wider and taller peak compared to the two non-biofilm datasets. 509 Also, the Q2 contributions for flow over the biofilm bed were stronger than both non-biofilm beds 510 for $0.25 \leq y/d \leq 1$. Thus, for nearly all vertical positions, the intense Q2 and Q4 Reynolds shear 511 stresses for BF1 were stronger than the two nBF datasets, except for $0 \le y/d \le 0.2$ where nBF-512 45% had a higher Q2 Reynolds shear stress contribution.



Figure 14. Quadrant analysis showing the distribution of high-intensity Q2 and Q4 (Hole size, $\eta = 4$) for f = 30 Hz $(Re = 11150\pm700)$. (A) Normalized with $(u_{\tau}^{p})^{2}$, and (B) in absolute units.

515

Discussion 516 4

517 In comparing flow with and without biofilm at constant pump frequency, f, the present results 518 show that flow over a bed with biofilm had higher maximum double-averaged streamwise velocity 519 (U_{max}) above the bed compared to the no biofilm case. This increase in U_{max} was concomitant with 520 an increase in bulk velocity, U_b , resulting in highly similar velocity profiles for the biofilm and non-biofilm beds (Figure 7A and Figure 9A). The increase in U_b in the presence of biofilm is 521 522 expected based on the reduction in bed porosity while the total volumetric flow rate, Q, measured 523 by the flow meter, remained nearly constant.

524

525 The double-averaged wall-normal velocity, $\langle \bar{v} \rangle$, in all cases, was very close to zero, for $v/d \gtrsim 0.25$, 526 indicating a purely horizontal flow, as would be expected. Also, the before and after detachment 527 (BF-BD and BF-AD) profiles were qualitatively similar. In all cases of flow over the biofilm bed 528 and the non-biofilm reduced porosity bed (nBF-45-35%), the profile showed a weak upward 529 velocity for $-0.5 \leq v/d \leq -0.1$ (Figure 7B and Figure 9B). The occurrence of the upward motion was likely due to the experimental configuration with a transition in bed porosity at the test section, 530 531 as explained in §2.1. This apparent channeling of flow from the permeable bed to the free flow 532 was observed in all cases except for the nBF-45% case where the entire bed had constant porosity.

533

534 The logarithmic fit to the streamwise velocity profile, Eq. (8), yielded dimensionless equivalent 535 roughness height (k_s^+) and zero displacement height (y_0^+) that are smaller for the biofilm after

536 detachment (BF-AD) compared to the before detachment (BF-BD) case, at constant pump

537 frequency. In a similar manner, comparing flow with and without biofilm (Figure 10), revealed

consistent increase in k_s^+ and y_0^+ in the presence of biofilm. Moreover, for all flow conditions y_0^+ 538

539 increased with Re_K consistent with previous results by Suga et al. (2010) and Fang et al. (2018). 540 The von Karman constant for the logarithmic fit, κ , also decreased with increasing Re_{*K*} with and 541 without biofilm, consistent with previous studies (Fang et al. 2018; Nezu 2005; Suga et al. 2010). 542 However, we should point out there are a few exceptions to this trend. Specifically, when 543 comparing the flow without biofilm over the reduced porosity bed (nBF-45-35%) and the reference 544 flow (nBF-45%) at constant pump frequency, y_0^+ and k_s^+ increased and κ decreased with 545 decreasing bed permeability. Although we cannot determine the exact reason for this behavior, we 546 speculate this effect to be due to the developing nature of flow in nBF-45-35%.

547

548 The biofilm coverage depicted in Figure 6 shows a gradual decrease in biofilm coverage due to 549 detachment from flow shear. Unlike the velocity profiles that were not affected significantly by 550 biofilm detachment, the Reynolds stresses experienced a more pronounced impact. Taking 551 together the results from flow over the biofilm bed before and after detachment (Figure 8) as well 552 as with and without biofilm (Figure 11), it is observed that at constant pump frequency, the Reynolds stresses, scaled with $(u_r^p)^2$ for $0.4 \leq y/d \geq 1$ (outer layer). Close to the bed surface 553 554 $0 \leq y/d \leq 0.4$ (inner layer) this scaling fell apart, where in all cases, the dimensionless Reynolds 555 stresses, were dampened in the presence of biofilm. Moreover, for flow over the biofilm bed, as 556 well as the reduced porosity bed (nBF-45-35%), the maximum Reynolds shear stress (Figure 8C) 557 occurred higher above the cylinder crest at $y/d \approx 0.1$, compared to $y/d \approx 0$ for the reference non-558 biofilm bed. This trend was consistent with the effect of decreased bed porosity observed in 559 previous studies (Breugem et al., 2006).

560

561 The quadrant analysis of Reynolds shear stress contributions with a hole size of $\eta = 4$ showed that 562 for all bed configurations, sweep (Q4) events were strongest near the bed, while ejection events 563 (Q2) were dominant away from the bed surface, for all flow configurations (Figure 14). This was 564 expected from previous studies of flow over permeable beds (Suga et al. 2011). The quadrant 565 analysis reveals that the decrease in dimensionless Reynolds shear stress in the inner layer, in the 566 presence of biofilm, stems primarily from a reduction in Q2 contributions for $0 \le y/d \le 0.5$, offset 567 by a modest increase in Q4 contributions.

568

569 The form-induced stresses were all nearly zero and practically negligible above the cylinder crests 570 for all datasets. This is in contrast to the findings from (Fang et al. 2018; Manes et al. 2009; 571 Pokrajac et al. 2007). This effect is likely due to the shallow submergence ratio of the roughness 572 elements to the flow depth. To facilitate comparison with studies in open channels, the effective flow depth can be taken as y_{max} (i.e. distance of maximum $\langle \bar{u} \rangle$ from the cylinder crest). Hence, the 573 574 d/y_{max} ratio in the present study is ~1. Whereas the flow depth to roughness heigh ratio was 3.5 in 575 Fang et al. (2018), 6–15 in Pokrajac et al. (2007) and 1.67–3.5 in Manes et al. (2009). Below the 576 cylinder crests, for $-0.5 \leq y/d \leq 0$, the form-induced stress terms were significant and, in some 577 instances, greater than their Reynolds stress counterparts. The biofilm datasets had a wide range 578 of variation that can translate to increase or decrease in form-induced stresses relative to the non-579 biofilm beds. This illustrates the fact that, compared to Reynolds stresses, form-induced stresses 580 are more sensitive to inter-dataset variations.

581

582 The peak value of the correlation coefficient (Figure 13) of ~0.5 near the cylinder crest was similar

to that of flow observed at the top of a vegetation canopy. Notably, while there is not a universal profile for correlation coefficient for different vegetation canopies, the value at the canopy top

from studies in different configuration all fall within the range 0.4–0.5 (Finnigan, 2000).

Moreover, the decreased correlation coefficient for flow over a biofilm bed as compared to case
without biofilm was similar to the effect expected with reduced bed porosity (Breugem et al.,
2006).

589

590 In assessing the impact of biofilm presence on flow over a permeable bed with a given geometry, 591 at least two competing effects may be considered: 1) reduced bed porosity concomitant with a 592 likely reduction in bed permeability, and 2) increased roughness and change in geometry of the 593 top of the cylinders. Reduced bed porosity/permeability has a damping effect on Reynolds stresses 594 (Breugem et al., 2006; Suga et al., 2010) while increased roughness has the opposite effect. Studies 595 on flow over smooth impermeable walls have shown that the equivalent roughness for biofilm is 596 larger than its physical roughness, owing to its motion in the flow (Schultz et al., 2015). To test 597 the impact of reduced bed porosity, but without any roughness effect, we used a permeable test 598 module with reduced porosity (35% instead of 45%) but with the same roughness using the same 599 arrangement of cylinders in the top layer (Figure 1G). Interestingly, at constant pump frequency 600 of f = 30 Hz, the dimensionless Reynolds stress profiles (Figure 11) showed close agreement 601 between the non-biofilm reduced porosity bed (nBF-45-35%) and the biofilm average, whereas 602 this was not the case at lower frequencies. This is in part due to the fact that at the highest tested 603 flow rate the biofilm coverage on cylinder crests was mostly detached, while some biofilm remain 604 attached at lower pumping frequencies (c.f. Figure 6). Unlike the Reynolds stresses, the form-605 induced stresses (Figure 12), which are dominant below the cylinder crests, showed significant 606 difference between the non-biofilm reduced porosity bed (nBF-45-35%) and the biofilm beds.

607

608 An important point to note here is that comparison of different studies is not a straightforward task, 609 primarily due to the unique nature of different experimental configurations in terms of bed 610 configuration (porosity, grain size) as well as biofilm coverage and growth method. In particular, 611 there are very few studies with velocity measurements over *permeable* beds with biofilm. Our 612 results indicate higher wall shear stress as a result of presence of biofilm in flow over a permeable 613 bed (Table 2). However, in open channel flow over rough impermeable beds with 20-mm tall 614 hemispheres, Graba et al. (2010) showed that biofilm growth results in slightly lower wall shear 615 stress. Nikora et al. (2002) used a nearly identical rough impermeable geometry as that used by 616 Graba et al. (2010) and reported no change in wall shear stress. One major difference between the 617 present study and both Graba et al. (2010) and Nikora et al. (2002) is that the rough wall was impermeable in these previous studies, which is not representative of natural gravel-bed rivers. 618 619 Another factor worthy of note is the extent of biofilm growth and coverage. In the study of Graba 620 et al. (2010), the biofilm occupied the space between the hemispherical roughness elements 621 (pebbles), whilst in Nikora et al. (2002) biofilm covered the entire bed as a thick mat, thus 622 completely changing the nature of the roughness and the bed. However, in the current study the 623 biofilm remained as a heterogenous film covering the cylinders but without connecting the 624 adjacent cylinders (at least in the top row which was easily visible). Moreover, the increased wall-625 normal Reynolds normal stress in absolute units in the presence of biofilm reported herein are in 626 contrast to results from Vignaga et al. (2013). This discrepancy can be attributed to the 627 experimental configuration, as Vignaga et al. (2013) conducted their experiments with 20-mmdeep beds consisting of beads/grains 1-2.2 mm in diameter, and with a free flow depth of 30-628 629 40 mm. Their observed decrease in wall-normal velocity fluctuations (no results on streamwise 630 fluctuations or RSS were reported) can be in part attributed to the shallow depth of the bed relative 631 to free flow, as suggested by results of Kim et al. (2018). Moreover, the reduced velocity

632 fluctuations reported by Vignaga et al. (2013), may be a result of biofilm smoothing the surface,

633 similar to that observed by Graba et al. (2010) and Nikora et al. (2002) as the biofilm growth 634 resulted in a sediment-biofilm composite material.

635

Figure 15 depicts the key processes involved in flow over a permeable bed with biofilm as studied

- herein. The main question which we attempted to answer was how the reduced bed porosity andchange in roughness combine to modify flow the bed.
- change in roughness combine to modify flow the b
- 639



Figure 15. Processes involved in flow over a permeable bed with biofilm as investigated in the present work.

640

6415Limitations and Implications of the Flow Apparatus

The results of this study are inevitably impacted by the choices in the design of the experimental apparatus and protocol. There were potentially important parameters whose impact has not been studied herein. One such effect is the spanwise variations for the BF datasets. While the incoming flow conditions during biofilm development were maintained as uniform as possible in the spanwise direction, there could have been some non-uniformity in biofilm growth and biomass accumulation in the spanwise direction. Specifically, near the side walls the distribution of biomass could be different from the mid-plane.

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Another point to mention is that biofilm coverage in the flow channel was limited to a 0.30-m long portion in the test section as biofilm was developed in a standalone recirculating reactor (c.f. §2.2). This was done to avoid undesired biofilm growth in portions of the water channel with limited access for cleaning. Thus, there was a transition in bed geometry at the beginning of the test section. As a result, in the cases of flow with biofilm (BF datasets) and reduced-porosity-bed (nBF-45-35%) the flow was likely not fully developed.

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657 It is also worthy of consideration whether the method of biofilm development (i.e. in a separate 658 reactor at low Re and shear) had an impact on the results. Due to this approach, the shear stress 659 experienced by the biofilm was significantly higher during the flow experiments than during the growth stage. Vignaga et al. (2013) used a similar approach for part of their experiments to test 660 661 biofilm at different stages of development. In other studies, however, the biofilm development and growth were carried out under same flow conditions (i.e. *Re* and shear) as the flow experiments. 662 The significance of this difference is increased attachment and cohesive strength of biofilms 663 664 developed under high shear (Stoodley et al., 2002), resulting in a denser biofilm compared to the present study. As a result, in the present study, progressive biofilm detachment occurred as the 665 flow rate was increased. This particular scenario may be similar to that during flood events in 666

natural channels, where biofilm experience abrupt increases and decreases in flow-induced shearduring unsteady flow conditions.

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670 Finally, the flow facility used in the present study consisted of a closed channel, whereas in past 671 studies, (e.g. Graba et al. 2010; Nikora et al. 2002; Vignaga et al. 2013) an open channel was 672 employed. We adopted use of a closed channel with a smooth impermeable top wall herein for the 673 purpose of optical access and better control over boundary conditions. Although the top smooth 674 impermeable wall can dampen pressure fluctuations compared to the case of an open channel flow, 675 turbulence effects are still dominated by the rough permeable bottom wall. Thus, our results can 676 provide insight into the effect of biofilm on flow over a permeable bed. In this regard, we expect 677 the trends observed in Reynolds stresses in the presence of biofilm, as well as before and after 678 biofilm detachment to be applicable (at least qualitatively) to open channel flow with similar Re_K 679 and $\operatorname{Re}_{\tau}^{p}$.

680 6 Summary and Conclusions

681 The effect of biofilms on flow structure over a permeable bed was quantified using PIV 682 measurements in a closed-top recirculating channel flow. In order to account for the variability in 683 biofilm growth, three separate biofilm batches were developed and tested. One of the ways that 684 biofilm presence can affect flow over a permeable bed is through a reduction in bed porosity by 685 biomass occupying the pore spaces. This effect was investigated by testing flow over a reduced-686 porosity bed without biofilm. The results showed that while certain aspects of the effect of biofilm 687 presence on flow can be replicated with reduced bed porosity without biofilm, the effect of biofilm is highly complicated and full understanding of the two-way interaction between flow and biofilms 688 689 requires further investigations. The following main conclusions can be drawn from the present 690 study:

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 1. The wall shear stress and friction velocity obtained from the total shear stress increased in 692
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- 694 2. At constant pump frequency, the equivalent roughness height, k_s^+ , and zero displacement 695 height, y_0^+ , were lower for biofilm after detachment (BF–AD) compared to before 696 detachment (BF–BD). Also, k_s^+ and y_0^+ were higher in the presence of biofilm compared 697 to the no biofilm datasets.
- 698 3. In all flow configuration studied herein (with and without biofilm) at constant pump 699 frequency, the individual components of Reynolds stresses scaled with $(u_{\tau}^{p})^{2}$ for 700 $\sim 0.4 < y/d < 1$ (outer layer), while the dimensionless Reynolds stresses decreased in the 701 presence of biofilm for $0 \le y/d \le 0.25$ (inner layer).
- 7024. The quadrant analysis (hole size, $\eta = 4$) suggests that the reduction in dimensionless703Reynolds shear stresses in the inner layer in the presence of biofilm is primarily due to a704reduction in strong Q2 contributions.

Turbulence plays a major role in mass and momentum exchange across the bed interface between the free and subsurface flow in a wide range of geophysical flows. Our results suggest that models for flow and transport over such permeable media in aquatic environments cannot neglect the role of biofilms in modifying turbulence. In light of observations reported herein, the following are important areas that were not considered in the present study, but we believe must be considered in future investigations: In-situ imaging and quantification of biofilm morphology can help elucidate the two-way coupling between turbulence and biofilm growth/detachment. Distribution of biomass in the span-wise and depth directions of the bed may create non-negligible three-dimensional effects in flow.

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2. The interaction between free and subsurface flow in the presence of biofilm is worthy of
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