

The effect of biofilms on turbulent flow over permeable beds

Kazemifar, F; Blois, G; Aybar, M; Perez Calleja, P; Nerenberg, R; Sinha, S; Hardy, RJ; Best, JL; Sambrook-Smith, Greg; Christensen, KT

DOI:

[10.1029/2019WR026032](https://doi.org/10.1029/2019WR026032)

License:

None: All rights reserved

Document Version

Peer reviewed version

Citation for published version (Harvard):

Kazemifar, F, Blois, G, Aybar, M, Perez Calleja, P, Nerenberg, R, Sinha, S, Hardy, RJ, Best, JL, Sambrook-Smith, G & Christensen, KT 2021, 'The effect of biofilms on turbulent flow over permeable beds', *Water Resources Research*, vol. 57, no. 2, e2019WR026032. <https://doi.org/10.1029/2019WR026032>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

An edited version of this paper was published by AGU. Copyright 2020 American Geophysical Union.

Kazemifar, F., Blois, G., Aybar, M., Perez Calleja, P., Nerenberg, R., Sinha, S., et al. (2021). The effect of biofilms on turbulent flow over permeable beds. *Water Resources Research*, 57, e2019WR026032. To view the published open abstract, go to: <https://doi.org/10.1029/2019WR026032>

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

The Effect of Biofilms on Turbulent Flow over Permeable Beds

Farzan Kazemifar¹, Gianluca Blois², Marcelo Aybar^{3*}, Patricia Perez Calleja³, Robert Nerenberg³, Sumit Sinha^{4‡}, Richard J. Hardy⁴, James L. Best⁵, Gregory H. Sambrook Smith⁶, Kenneth T. Christensen^{2,3,7}

¹Department of Mechanical Engineering, San Jose State University, USA. ²Department of Aerospace and Mechanical Engineering, University of Notre Dame, USA. ³Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame, USA. ⁴Department of Geography, Durham University, UK. ⁵Departments of Geology, Geography and GIS, Mechanical Science and Engineering and Ven Te Chow Hydrosystems Laboratory, University of Illinois at Urbana-Champaign, USA. ⁶School of Geography, Earth and Environmental Sciences, University of Birmingham, UK. ⁷International Institute for Carbon Neutral Energy Research (I²CNER), Kyushu University, Japan.

Corresponding author: Kenneth Christensen (christensen.33@nd.edu)

*Current address: Department of Civil Engineering, Universidad de Concepción, Chile.

‡Current address: School of Earth and Environment, University of Leeds, UK.

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
11 conditions using particle image velocimetry. The wall shear stress and friction velocity obtained
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
36 studied. Four important modifications have been highlighted compared to flows over impermeable
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
44 biomass, and hence porosity, of the interface may change with the seasons, or at much smaller

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
61 as influencing the generation of bedforms (Lichtman et al. 2018; Malarkey et al. 2015; Parsons et
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,
64 biofilm growth can affect the hydrodynamics of flow and hyporheic exchange by modifying the
65 geometry of the bed interface and the connectivity of subsurface pore spaces. This can, in turn,
66 affect the concentration of oxygen, organic carbon, and other electron acceptors, as well as the
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
77 relationship between biofilm attachment strength and shear stress during biofilm growth. Walker
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
86 al., 2016; Carrel et al., 2018; Roche et al., 2017).

87
88 Thus, while biofilms are ubiquitous in aquatic environments and exist on almost all wet surfaces,
89 their effect on flow over permeable walls is poorly understood. This lack of direct measurements
90 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**

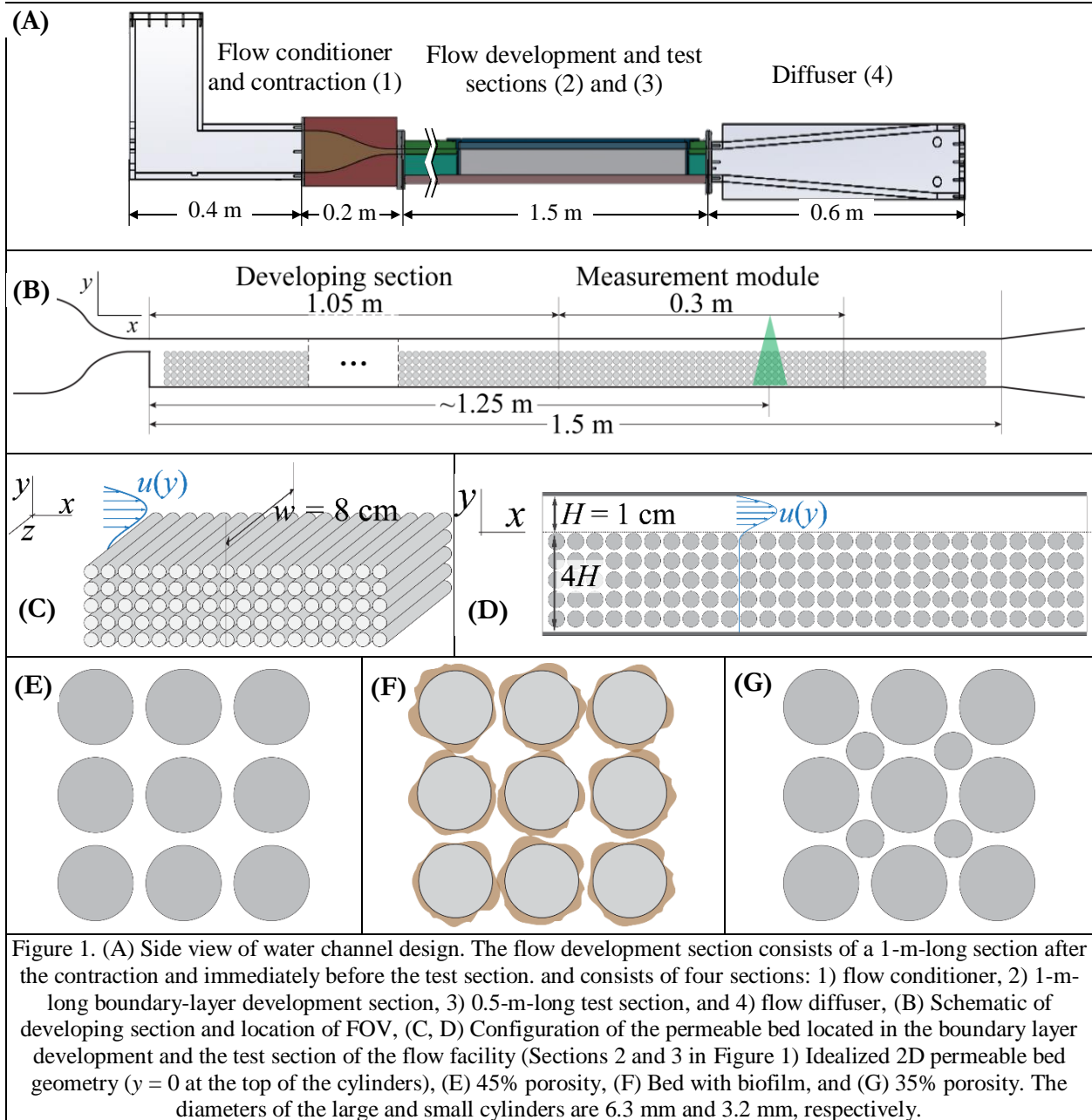
98 A special flow facility was designed and built to accommodate the use of permeable beds with
99 different geometries. The flow facility was a recirculating closed water channel with rectangular
100 cross-section (Figure 1A and B), and consisted of four sections: 1) flow conditioner, 2) boundary-
101 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 acrylic rods were secured to two machined perforated acrylic plates with holes
112 for the rods (similar to a peg board). The bed consisted of four 0.30-m-long individual sections
113 plus two 0.15-m-long sections to accommodate the transition after the contraction and before the
114 diffuser. The design of these perforated plates allowed the rods to be mounted in two different
115 arrangements and thus two different porosities of 35% and 45%. As shown in Figure 1, the higher
116 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 ~10%. The value for the lower porosity was primarily
119 determined based on cost and manufacturability constraints, yet its porosity was also representative
120 of 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.



133

2.2.2 Biofilm Development Protocol

134

135

136

137

138

139

140

141

142

143

Figure 2A shows schematically the configuration of the reactor. A multi-cassette Cole Parmer 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 (KCH₃COO) was used as the electron donor and tap water was used as the growth medium due to its high mineral concentration. A Cole Parmer gear pump (P2) was used for recirculation of the fluids at 40× the combined influent and effluent flow rate (~320 ml/min) to establish fully mixed 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 peristaltic pump was used to extract the effluent from the aeration bottle and maintain a constant fluid level therein. An aeration stone inside the aeration bottle was connected to a low-pressure air

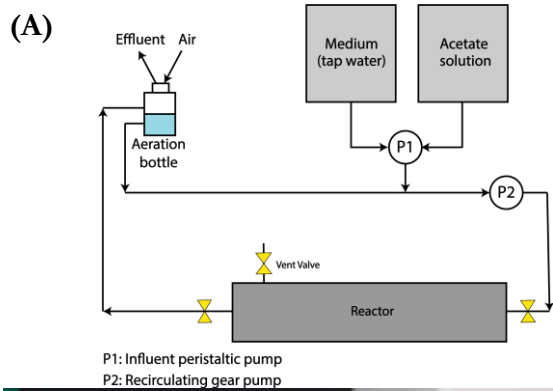
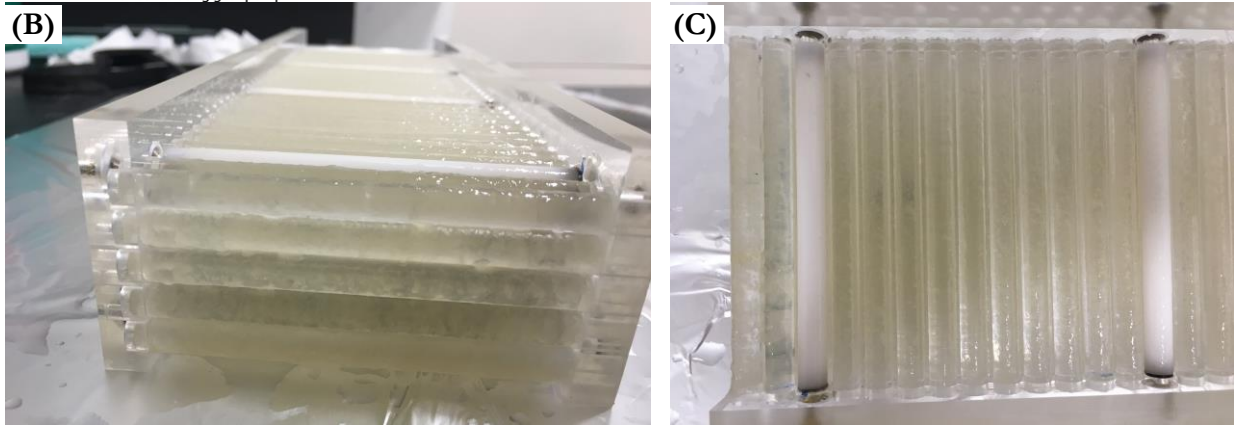


Figure 2. Flow diagram for the recirculating biofilm reactor (A). Biofilm bed section removed from the reactor before being transferred to the flow facility (B), (C). A multi-cassette Cole Parmer MasterFlex L/S peristaltic pump (P1) was used to deliver the electron donor and growth medium solutions. A Cole Parmer gear pump (P2) was used for recirculation of the fluids to establish fully-mixed conditions in the reactor.



144 supply line to provide oxygen to the reactor. In this manner, the dissolved oxygen levels were
 145 maintained close to saturation and thus compensated for oxygen consumption by biofilm activity.
 146

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
 150 approximately 6–10 hours each day. Based on the inoculum and growth conditions, the dominant
 151 members in this biofilm were heterotrophic and nitrifying bacteria whose growth is relatively
 152 insensitive to light conditions. The reactor was maintained at the room temperature of 20–22°C.
 153 After the 14-day growth period, the biofilm-covered measurement module was removed from the
 154 reactor and its sidewalls above the top layer of the cylinders were cleaned carefully without
 155 disturbing the biofilm to ensure unhindered optical access. This module was then transferred to
 156 the flow facility for flow experiments. Figure 2B and C show the biofilm-covered bed before being
 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
 164 (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 (2560×2160 -pixel array) sensor and a pixel size
 168 of $6.5 \mu\text{m}$ 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 $\sim 7 \mu\text{m}/\text{pixel}$, and a resolution of $\sim 40 \mu\text{m}$ at 550 nm wavelength. The field of view of the imaging
 172 setup was $\sim 18 \times 14 \text{ mm}^2$. The FOV covered up to $\sim 7 \text{ mm}$ above the cylinder tops, in order to avoid
 173 error from optical aberrations observed near the top of the FOV. Fluorescent particles $\sim 10\text{-}15\mu\text{m}$
 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
 176 using a sequential cross-correlation algorithm with a final interrogation window size of 64^2 pixels
 177 with 50% overlap, resulting in a vector field resolution of $\sim 0.44 \text{ mm}$ and a vector grid spacing of
 178 $\sim 0.22 \text{ mm}$ ($0.035d$).

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

Table 1. Dataset Summary

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

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

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

195 where A_f is the area occupied by fluid at any vertical position, y , and is determined based on the
 196 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 \bar{u} contours
 200 and streamlines (Figure 3A), in addition to the corresponding double-averaged velocity profile,
 201 $\langle \bar{u} \rangle(y)$, (Figure 3B). Due to flow detachment, a recirculation region exists between the cylinders
 202 that results in negative streamwise velocities with a velocity magnitude of $\sim 0.1 U_{max}$. Similar to
 203 observations by Breugem et al., (2006), the line-averaged velocity profile, $\langle \bar{u} \rangle$, exhibits an
 204 inflection point just below the interface, denoted as y_{inflec} . The PIV uncertainty in particle
 205 displacement calculated from the images is 5% of particle image diameter, or ~ 0.1 pixels ($\sim 1.4 \times 10^{-3}$
 206 mm in the measurement plane). For the different laser pulse time delays used herein, this equates
 207 to a velocity uncertainty of $\sim 0.4\%$ of U_{max} in each case.
 208

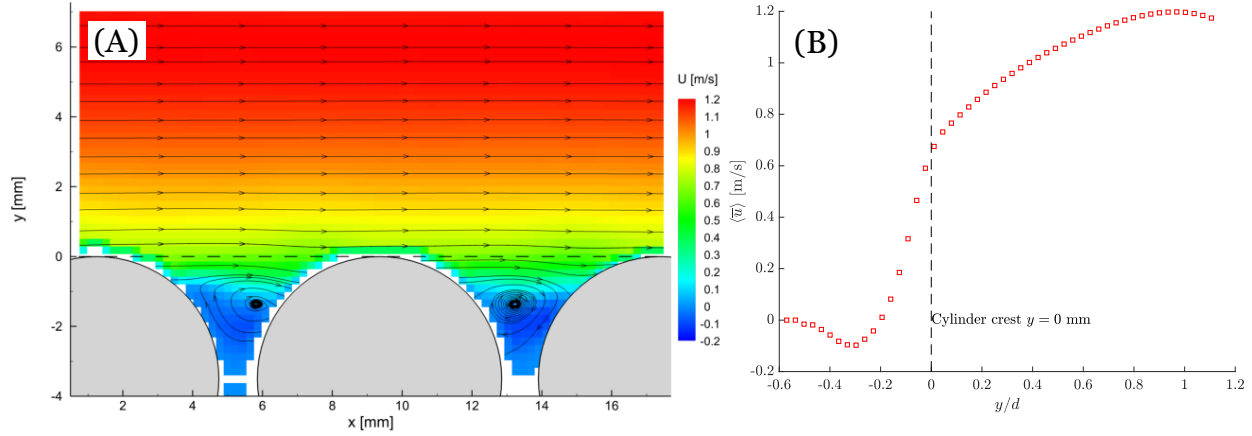


Figure 3. (A) Time-averaged streamwise velocity, \bar{u} , (B) double-averaged velocity profile at $Re \approx 7000$. Dashed line indicates the top of the cylinders at $y = 0$ mm, and $d = 6.3$ mm is the cylinder diameter.

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

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

211 Figure 4 presented the total shear stress as well
 212 as the individual terms from Eq. (2),
 213 normalized with wall shear stress τ_w^p . The shear
 214 stress does not include the drag term below the
 215 cylinder crest ($y/d < 0$). The shear stress at the
 216 permeable wall, τ_w^p , was calculated by
 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 $\mu = 0.9321 \times 10^{-3} \text{ Pa}\cdot\text{s}$) near the channel
 221 centerline to the crest location ($y = 0$).
 222

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

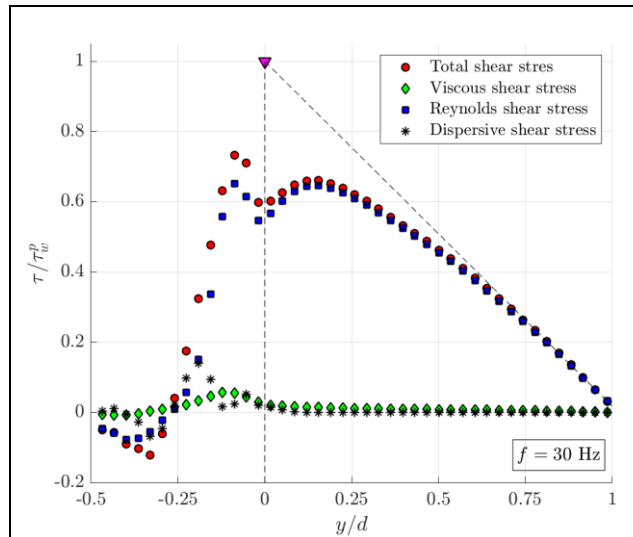


Figure 4. Example of contributions of individual terms to total shear stress and extrapolation for wall shear stress calculation.

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

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

229 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
 230 of water, and U_b is the bulk velocity calculated as the average velocity for $y \in [0, H]$, as defined by
 231 Breugem et al. (2006). Since the velocity field is resolved up to $y \approx 7 \text{ mm}$, a fourth order
 232 polynomial curve fit is used to approximate the velocity profile up to the top wall for calculation
 233 of U_b . The friction velocity (u_τ^p) and friction Reynolds number (Re_τ^p) are defined as:

$$u_\tau^p = \sqrt{\tau_w^p / \rho}, \quad (4)$$

234

$$Re_\tau^p = \frac{u_\tau^p H}{\nu}. \quad (5)$$

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

$$Re_K = \frac{u_\tau^p \sqrt{K}}{\nu} \quad (6)$$

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

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

238 where ϕ is porosity and d_h is the pore hydraulic diameter. This yields a permeability of 0.087 mm^2
 239 and 0.051 mm^2 for the bed with 45% and 35% porosity, respectively. As a first approximation for
 240 the bed with biofilm, we used the same permeability value as the low-porosity bed.

241

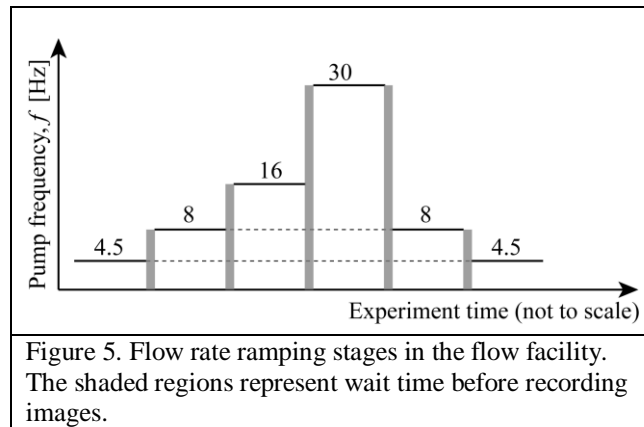
242 For sufficiently high Reynolds number, a logarithmic layer is expected in the boundary layer above
 243 the permeable bed. A common parametrization of the log law is

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

244 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;
 245 Fang et al. 2018). Also, y^+ is the vertical distance above the cylinder crest, y_0^+ is the zero-
 246 displacement height, and k_s^+ is the equivalent roughness height, all normalized with viscous length
 247 scale $y^* = \nu / u_\tau^p$.

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 $f = 4.5 \text{ Hz}$ and 8 Hz ($Q \approx 0.18$ and 0.28 L/s),
 253 before reaching the target of 16 Hz and 30 Hz
 254 ($Q \approx 0.54$ and 0.95 L/s). After recording data
 255 for the highest flow rate, the pump frequency
 256 was reduced, and data was recorded
 257 sequentially at $f = 8 \text{ Hz}$ and 4.5 Hz
 258 ($Q \approx 2.8 \text{ L/s}$ 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



261 schematically. The number of image pairs for each flow setting and the corresponding number of
 262 flow-through times is given in Table 3. The flow-through time is calculated as the ratio of the
 263 streamwise length (18 mm) of the field of view to the bulk velocity (U_b). After each stage, there
 264 was at least a 2-minute wait time before recording images. The flow characteristics during ramp
 265 up and ramp-down, referred to as *before detachment* (BD) and *after detachment* (AD),
 266 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_K 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.

Dataset	f [Hz]	Q [L/s]	U_b [m/s]	U_{max} [m/s]	y_{max} [mm]	$u_{\tau}^p \times 10^{-3}$ [m/s]	Re	Re_{τ}^p	Re_K	k_s^+	y_0^+	κ	
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	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
BF2	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, f [Hz]	Ensemble size	Number of flow- 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

271 Despite maintaining very similar experimental conditions in the three flow experiments conducted
 272 in the presence of biofilm, due to unavoidable differences in biofilm growth, the flow was
 273 inherently subject to some degree of variability. Under these conditions, data repeatability was

274 difficult to achieve. As such, the first step in the present analysis was to statistically characterize
 275 the boundary layer by computing the ensemble-averaged profiles and quantifying the variability
 276 between the three different sets of experiments. The results presented from biofilm experiments,
 277 unless noted otherwise, are the average of the three separate experimental datasets: *BF1*, *BF2* and
 278 *BF3* in Table 2, and the error bars for flow over the biofilm beds represent the range of
 279 measurements. Individual results are provided as Supporting Information for completeness.

280 3 Results

281 The results and analysis presented in the following sections are focused on $f = 4.5$ Hz
 282 ($Re = 1700 \pm 100$) and $f = 30$ Hz ($Re = 11150 \pm 700$), selected to illustrate the main trends observed
 283 in the data. Similar behavior was observed for the two intermediate flow rates, whose results are
 284 presented as Supplementary Information. In order to facilitate comparison, flow and turbulence
 285 variables are presented in dimensionless form; streamwise and wall-normal velocity components
 286 are normalized with bulk velocity, U_b , and Reynolds and form-induced stresses are normalized
 287 with $(u_t^p)^2$.

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
 301 pixel, the maximum and minimum pixel
 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 where tracer particles were present
 311 intermittently. The range image for the highest
 312 pump frequency, $f = 30$ Hz ($Re = 11150 \pm 700$),
 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
 317 in the top layer was nearly non-existent. The

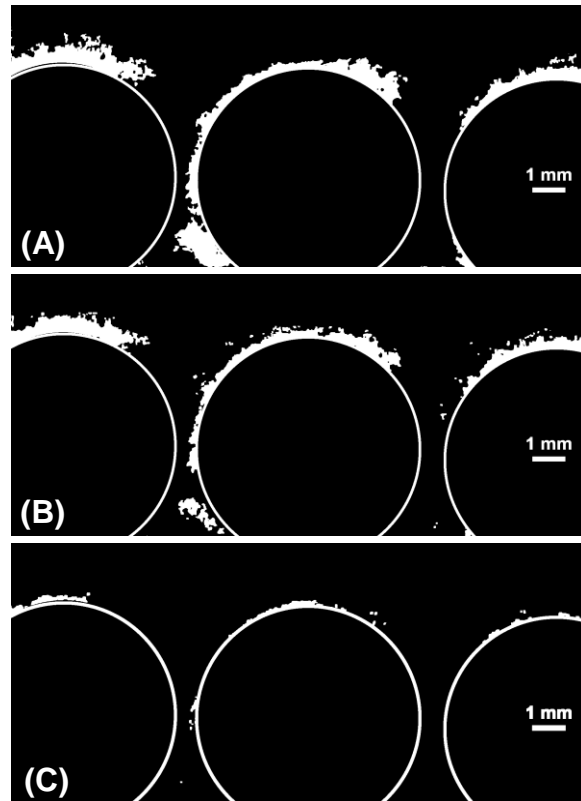


Figure 6. Biofilm coverage at different Re during the ramp-up stage (A) $f = 4.5$ Hz ($Re = 1700 \pm 100$), (B) $f = 8$ Hz ($Re = 3000 \pm 200$), (C) $f = 16$ Hz ($Re = 5800 \pm 400$). The cylinders are outlined in white and a 1-mm scale bar is shown for reference.

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 ($Re = 1700 \pm 100$), 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
 325 of biofilm detachment was observed (Figure 6B). Finally, at the 2nd highest pump frequency of
 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
 328 raw PIV images. Nevertheless, this method does yield a qualitative picture of the biofilm coverage,
 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

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

350

351

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

356

357 The dimensionless wall-normal Reynolds normal stress, $\langle \overline{v'v'} \rangle / (u_\tau^p)^2$, at constant pump frequency
 358 (Figure 8B) collapsed for $0.4 \lesssim y/d \lesssim 1$. Close to the bed, for $0 \lesssim y/d \lesssim 0.4$, similar to the
 359 streamwise Reynolds normal stress, the non-biofilm bed had the highest stress, however, there was
 360 very minor difference between the before-detachment (BF-BD) and after-detachment
 361 configurations (BF-AD). For nearly all vertical positions, the before and after-detachment
 362 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

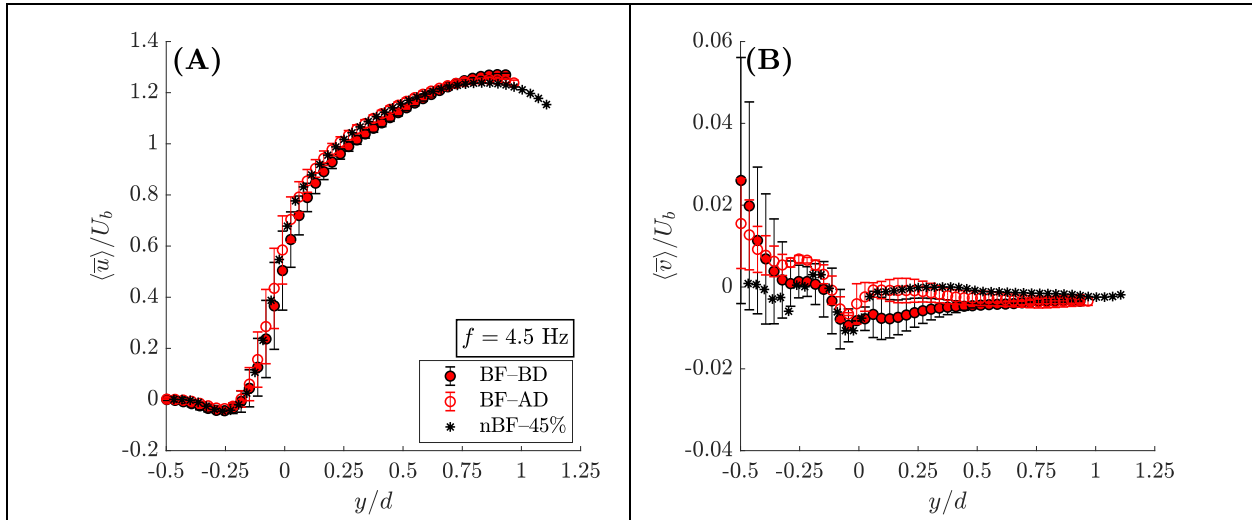


Figure 7. Effect of biofilm detachment on (A) streamwise, and (B) wall-normal velocity. Biofilm before detachment (BF-BD, filled red circle), and after detachment (BF-AD, open red circle), no biofilm (black asterisk). The error bars indicate the range of data for biofilm datasets. The biofilm data (“BF-BD” 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
 367 Similar to the other components of Reynolds stress presented herein, the dimensionless Reynolds
 368 shear stress, $-\langle \bar{u}'\bar{v}' \rangle / (u_t^p)^2$, was highest for the bed without biofilm in the range $0 \lesssim y/d \lesssim 0.5$
 369 (Figure 8C). The profiles collapsed for $0.5 \lesssim y/d \lesssim 1$, although within this range, the BF-BD had
 370 slightly higher stress values. The biofilm before and after detachment had highly similar profiles,
 371 except in the $-0.1 \lesssim y/d \lesssim 0.3$ range where the normalized RSS after detachment (AD) was
 372 higher by approximately 10%. The peak for nBF-45% was at $y/d \approx 0$, whereas the peak for both
 373 BF-BD and BF-AD occurred at $y/d \approx 0.18$.
 374
 375

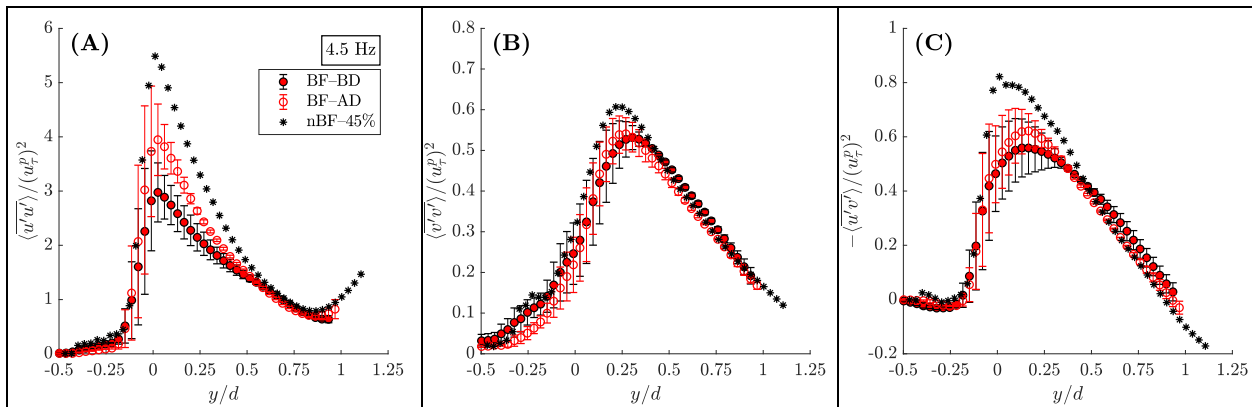


Figure 8. Effect of biofilm presence and subsequent detachment on dimensionless Reynolds stresses. (A) streamwise Reynolds normal stress, (B) wall-normal Reynolds normal stress, and (C) Reynolds shear stress. Biofilm before detachment (BF-BD, filled red circle), biofilm after detachment (BF-AD, open red circle), no biofilm (nBF, black asterisk). The error bars indicate the range of data for biofilm datasets.

3.2 Biofilm and Reduced Bed Porosity

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

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

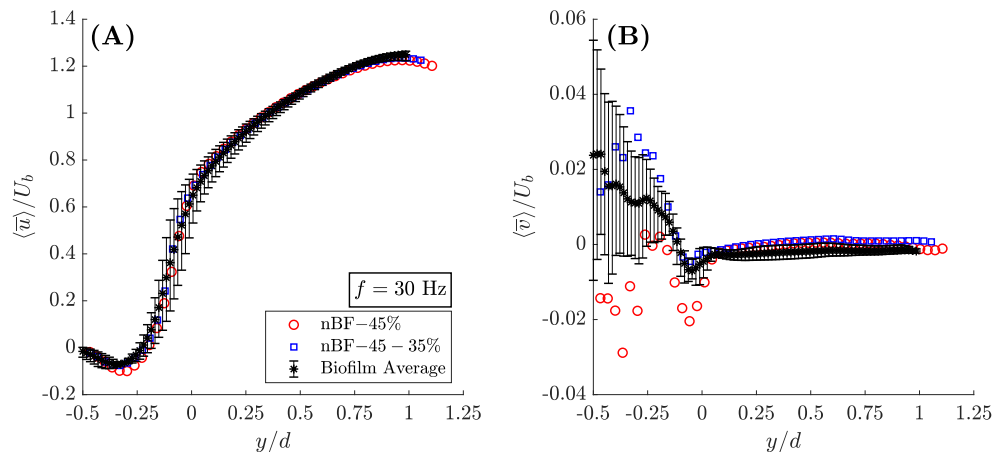


Figure 9. Biofilm effect comparison with reduced bed porosity on streamwise velocity (A, B), and wall-normal velocity (C, D). No biofilm with 45% bed porosity (red circle), no biofilm with 45% porosity in the flow developing section and 35% in the test section (blue square), biofilm (black asterisk).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

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 ($y/d < 0$) 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)
 427 normalized with $(u_\tau^p)^2$ collapse for all bed
 428 configurations in the outer layer 0.25 –
 429 $0.4 \lesssim y/d \lesssim 1$. Near the permeable bed
 430 ($0 \lesssim y/d \lesssim 0.25$) 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 $0.25 \lesssim y/d \lesssim 1$
 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 $-0.1 \lesssim y/d \lesssim 0.25$ 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 $y/d \gtrsim 0.4$ (Figure 11B). For $-0.5 \lesssim y/d \lesssim 0.3$, 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

448 The dimensionless Reynolds shear stress profiles, $-\langle \overline{u'v'} \rangle / (u_\tau^p)^2$, demonstrate a trend similar to the
 449 other Reynolds stress components, where the profiles collapsed for $y/d \gtrsim 0.4$ (Figure 11C). For
 450 $-0.2 \lesssim y/d \lesssim 0.4$, nBF-45% had the highest stress compared to the two other datasets. Moreover,

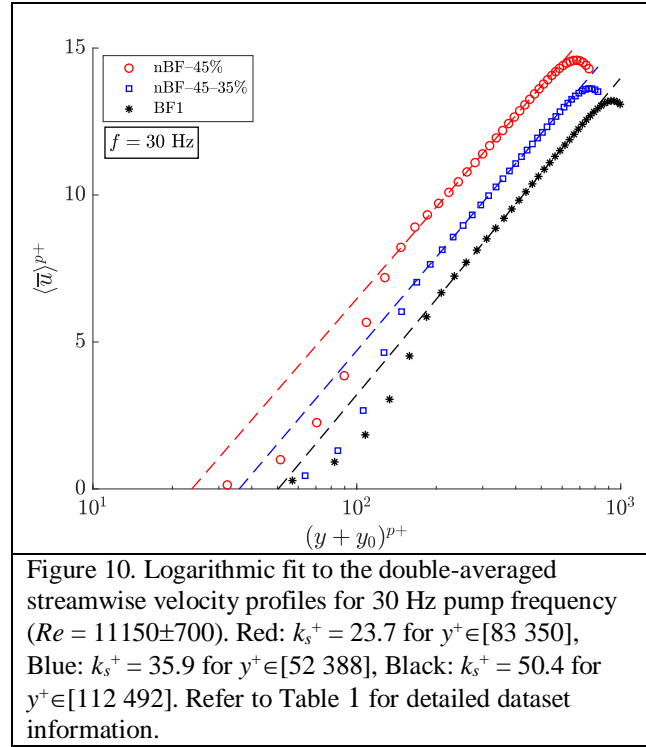


Figure 10. Logarithmic fit to the double-averaged streamwise velocity profiles for 30 Hz pump frequency ($Re = 11150 \pm 700$). Red: $k_s^+ = 23.7$ for $y^+ \in [83 \ 350]$, Blue: $k_s^+ = 35.9$ for $y^+ \in [52 \ 388]$, Black: $k_s^+ = 50.4$ for $y^+ \in [112 \ 492]$. Refer to Table 1 for detailed dataset information.

451 while the reduced porosity non-biofilm bed (nBF-45-35%) and the biofilm bed had relatively flat
 452 profiles for $-0.1 \lesssim y/d \lesssim 0.25$ 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

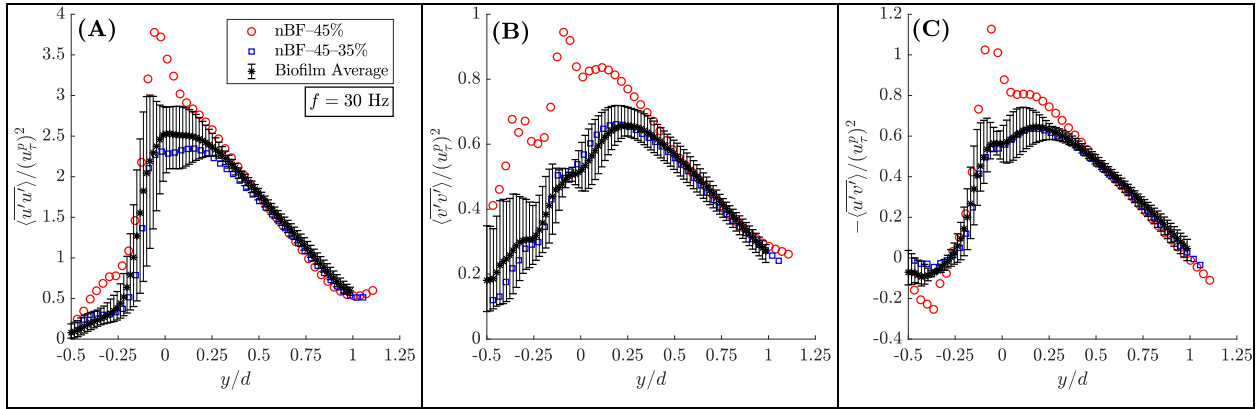


Figure 11. Biofilm effect comparison with reduced bed porosity for $f = 30$ Hz ($Re = 11150 \pm 700$). (A) streamwise Reynolds normal stress, (B) wall-normal Reynolds normal stress, and (C) Reynolds shear stress. No biofilm with 45% bed porosity (red circle), no biofilm with 45% porosity in the flow 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
 456 The form-induced (dispersive) stresses are presented in Figure 12. In all cases, the form-induced
 457 stresses are very small right above the cylinder crests ($0 \lesssim y/d \lesssim 0.25$) and practically zero for
 458 $y/d \geq 0.25$. Comparing Figures 11 and 12 shows that above the cylinder crests, the form-induced
 459 stresses are negligible compared to Reynolds stresses. However, below the crests ($-0.5 \lesssim y/d \lesssim 0$)
 460 the form-induced stresses are comparable to Reynolds stresses in magnitudes and cannot be
 461 neglected.
 462

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

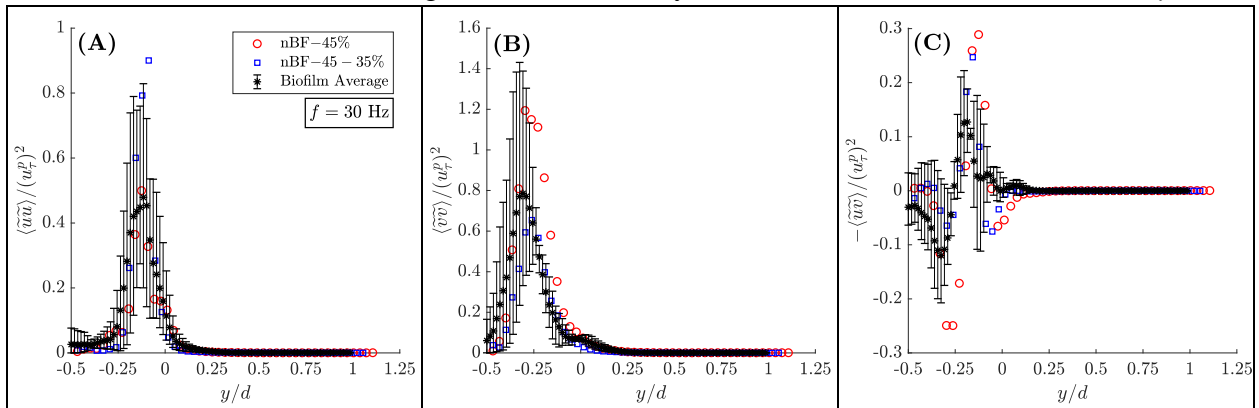


Figure 12. Biofilm effect comparison with reduced bed porosity for $f = 30$ Hz ($Re = 11150 \pm 700$). (A) streamwise form-induced normal stress, (B) wall-normal form-induced normal stress, and (C) form-induced shear stress. No biofilm with 45% bed porosity (red circle), no biofilm with 45% porosity 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 datasets
 469 demonstrate a broad range of variation that was not observed in Reynolds stresses.

470

471 The correlation coefficient ($-\langle u'v' \rangle / u'_{\text{rms}} v'_{\text{rms}}$)
 472 of the velocity fluctuations u' and v' , is a
 473 measure of the efficiency of wall-normal
 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 $0 \lesssim y/d \lesssim 0.6$, 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 Quadrant analysis of the instantaneous velocity
 484 fields was performed to gain further insight
 485 into trends observed in the RSS (Wallace
 486 2016). In this approach, $u'v'$ is classified into
 487 four categories, termed quadrants (Q), 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 Q4
 490 ($u' > 0, v' < 0$), where Q2 and Q4 events are
 491 referred to as ejections and sweeps, respectively, and Q1 and Q3 events are called outward and
 492 inward interactions. The hyperbolic hole size method of Lu and Willmarth (1973), with a hole size
 493 of $\eta = 4$ has been applied herein. Thus, the intense Reynolds-stress-producing events above the
 494 $\eta = 4$ threshold have been considered. In this configuration, contributions from Q2 and Q4 were
 495 stronger than that of Q1 and Q3 by a factor of >10 , so only data for Q2 and Q4 are considered
 496 herein. For the flow with biofilm, only one of the three separate datasets has been considered
 497 (BF1).

498

499 The contributions from Q2 and Q4 to the Reynolds shear stress, non-dimensionalized with $(u'_\tau)^2$
 500 and in absolute units are presented in Figure 14A and B, respectively. For the biofilm bed the Q2
 501 and Q4 contributions shift in +y-direction compared to the non-biofilm datasets. The crossover
 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
 505 occurred closer to the cylinder crests at $y/d \approx -0.05$. The peak for contributions from Q2 events
 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.
 507 In absolute units, the Q4 contributions (Figure 14B) for the two non-biofilm beds were in relatively
 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 $0.25 \lesssim y/d \lesssim 1$. 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 $0 \lesssim y/d \lesssim 0.2$ where nBF-
 512 45% had a higher Q2 Reynolds shear stress contribution.

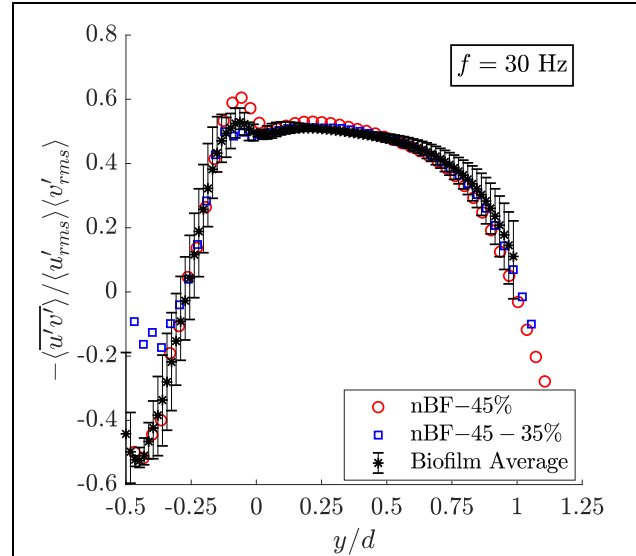


Figure 13. Biofilm effect comparison with reduced bed porosity on correlation coefficient of velocity fluctuations for $f = 30$ Hz ($Re = 11150 \pm 700$). No biofilm with 45% bed porosity (red circle), no biofilm with 45% porosity in the flow developing section and 35% in the test section (blue square),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

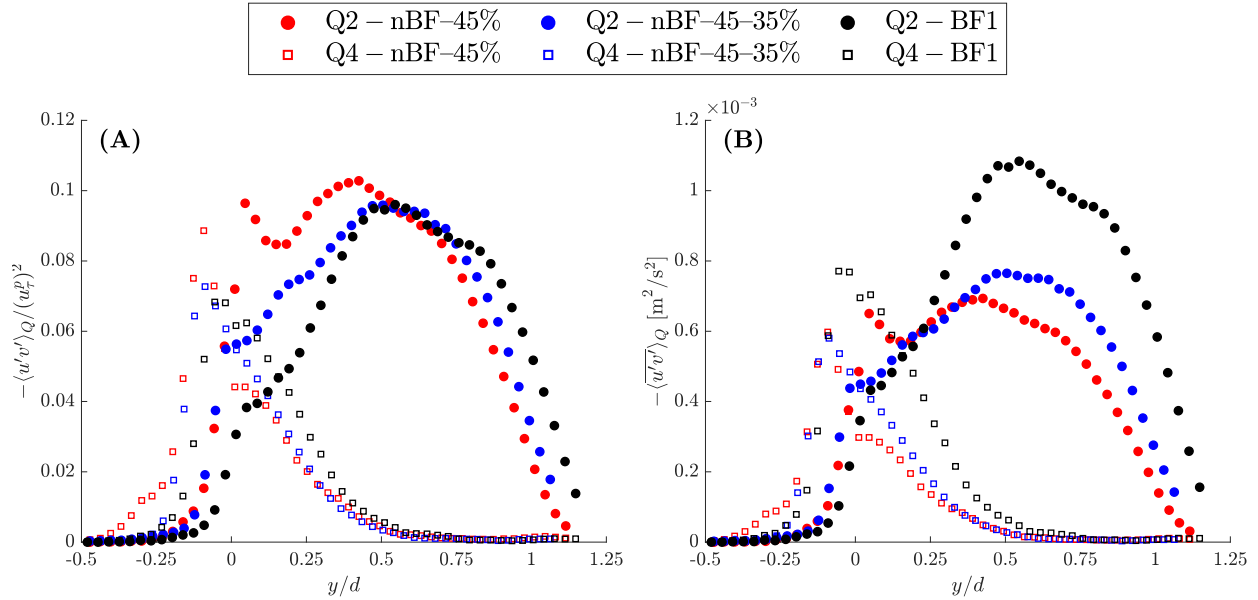
513
514

Figure 14. Quadrant analysis showing the distribution of high-intensity Q2 and Q4 (Hole size, $\eta = 4$) for $f = 30$ Hz ($Re = 11150 \pm 700$). (A) Normalized with $(u_{\tau}^p)^2$, and (B) in absolute units.

515

516 4 Discussion

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
 521 non-biofilm beds (Figure 7A and Figure 9A). The increase in U_b in the presence of biofilm is
 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 $y/d \gtrsim 0.25$,
 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 $-0.5 \lesssim y/d \lesssim -0.1$ (Figure 7B and Figure 9B). The occurrence of the upward motion
 530 was likely due to the experimental configuration with a transition in bed porosity at the test section,
 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
 538 consistent increase in k_s^+ and y_0^+ in the presence of biofilm. Moreover, for all flow conditions y_0^+
 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
 553 Reynolds stresses, scaled with $(u_p^p)^2$ for $0.4 \lesssim y/d \gtrsim 1$ (outer layer). Close to the bed surface
 554 $0 \lesssim y/d \lesssim 0.4$ (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 $0 \lesssim y/d \lesssim 0.5$, 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
 573 flow depth can be taken as y_{max} (i.e. distance of maximum $\langle \bar{u} \rangle$ from the cylinder crest). Hence, the
 574 d/y_{max} ratio in the present study is ~ 1 . Whereas the flow depth to roughness height 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 $-0.5 \lesssim y/d \lesssim 0$, 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
 583 to that of flow observed at the top of a vegetation canopy. Notably, while there is not a universal
 584 profile for correlation coefficient for different vegetation canopies, the value at the canopy top
 585 from studies in different configuration all fall within the range 0.4–0.5 (Finnigan, 2000).

586 Moreover, the decreased correlation coefficient for flow over a biofilm bed as compared to case
587 without biofilm was similar to the effect expected with reduced bed porosity (Breugem et al.,
588 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
618 impermeable in these previous studies, which is not representative of natural gravel-bed rivers.
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-mm-
628 deep beds consisting of beads/grains 1–2.2 mm in diameter, and with a free flow depth of 30–
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

636 Figure 15 depicts the key processes involved in flow over a permeable bed with biofilm as studied
 637 herein. The main question which we attempted to answer was how the reduced bed porosity and
 638 change in roughness combine to modify flow the bed.

639

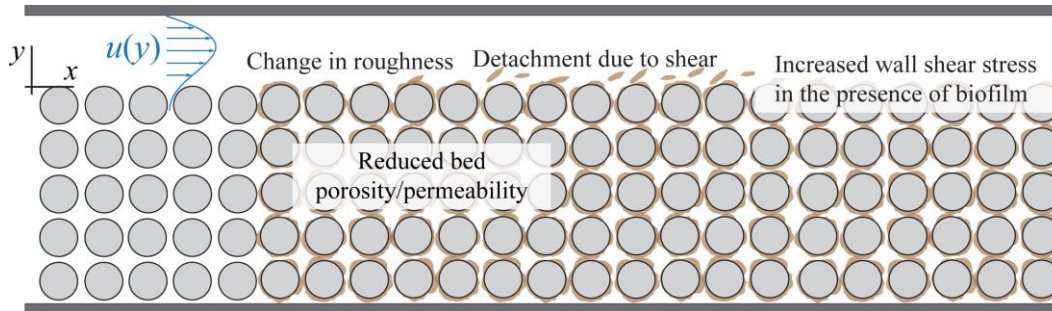


Figure 15. Processes involved in flow over a permeable bed with biofilm as investigated in the present work.

640

641 5 Limitations and Implications of the Flow Apparatus

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

649

650 Another point to mention is that biofilm coverage in the flow channel was limited to a 0.30-m long
 651 portion in the test section as biofilm was developed in a standalone recirculating reactor (c.f. §2.2).
 652 This was done to avoid undesired biofilm growth in portions of the water channel with limited
 653 access for cleaning. Thus, there was a transition in bed geometry at the beginning of the test
 654 section. As a result, in the cases of flow with biofilm (BF datasets) and reduced-porosity-bed (nBF-
 655 45-35%) the flow was likely not fully developed.

656

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
 660 growth stage. Vignaga et al. (2013) used a similar approach for part of their experiments to test
 661 biofilm at different stages of development. In other studies, however, the biofilm development and
 662 growth were carried out under same flow conditions (i.e. Re and shear) as the flow experiments.
 663 The significance of this difference is increased attachment and cohesive strength of biofilms
 664 developed under high shear (Stoodley et al., 2002), resulting in a denser biofilm compared to the
 665 present study. As a result, in the present study, progressive biofilm detachment occurred as the
 666 flow rate was increased. This particular scenario may be similar to that during flood events in

667 natural channels, where biofilm experience abrupt increases and decreases in flow-induced shear
 668 during unsteady flow conditions.

669
 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 Re_τ^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
 688 is highly complicated and full understanding of the two-way interaction between flow and biofilms
 689 requires further investigations. The following main conclusions can be drawn from the present
 690 study:

- 691 1. The wall shear stress and friction velocity obtained from the total shear stress increased in
 692 the presence of biofilm, and decreased as a result of biofilm detachment, when compared
 693 at constant pump frequency.
- 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 $0 \lesssim y/d \lesssim 0.25$ (inner layer).
- 702 4. The quadrant analysis (hole size, $\eta = 4$) suggests that the reduction in dimensionless
 703 Reynolds shear stresses in the inner layer in the presence of biofilm is primarily due to a
 704 reduction in strong Q2 contributions.

705 Turbulence plays a major role in mass and momentum exchange across the bed interface between
 706 the free and subsurface flow in a wide range of geophysical flows. Our results suggest that models
 707 for flow and transport over such permeable media in aquatic environments cannot neglect the role
 708 of biofilms in modifying turbulence. In light of observations reported herein, the following are
 709 important areas that were not considered in the present study, but we believe must be considered
 710 in future investigations:

- 711 1. In-situ imaging and quantification of biofilm morphology can help elucidate the two-way
712 coupling between turbulence and biofilm growth/detachment. Distribution of biomass in
713 the span-wise and depth directions of the bed may create non-negligible three-dimensional
714 effects in flow.
- 715 2. The interaction between free and subsurface flow in the presence of biofilm is worthy of
716 further investigation. However, non-intrusive measurement and optical access are expected
717 to be major obstacles to investigating flow inside the bed.

718 **Acknowledgment**

719 We thank the UK Natural Environment Research Council for funding this work (NE/K012819/1),
720 and Monica Ochoa for her help in running and maintaining the biofilm reactor. We are also
721 thankful to the anonymous reviewers whose comments helped improve the paper. All experiments
722 were undertaken in the Department of Aerospace and Mechanical Engineering at the University
723 of Notre Dame. We note that data used in the manuscript is available for download at
724 <https://doi.org/10.3886/E118207V1>.

725 **References**

- 726 Aubeneau, A. F., Brittany Hanrahan, Diogo Bolster, and Jennifer Tank. 2016. "Biofilm Growth in
727 Gravel Bed Streams Controls Solute Residence Time Distributions." *Journal of Geophysical*
728 *Research: Biogeosciences* 121(7):1840–50.
- 729 Battin, Tom J., Louis A. Kaplan, J. Denis Newbold, and Claude M. E. Hansen. 2003.
730 "Contributions of Microbial Biofilms to Ecosystem Processes in Stream Mesocosms." *Nature*
731 426(6965):439–42.
- 732 Battin, Tom J., William T. Sloan, Staffan Kjelleberg, Holger Daims, Ian M. Head, Tom P. Curtis,
733 and Leo Eberl. 2007. "Microbial Landscapes: New Paths to Biofilm Research." *Nature*
734 *Reviews. Microbiology* 5(1):76–81.
- 735 Blois, G., G. H. Sambrook Smith, J. L. Best, R. J. Hardy, and J. R. Lead. 2011. "Quantifying the
736 Dynamics of Flow within a Permeable Bed Using Time-Resolved Endoscopic Particle
737 Imaging Velocimetry (EPIV)." *Experiments in Fluids* 53(1):51–76.
- 738 Blois, G., G. H. Sambrook Smith, J. L. Best, R. J. Hardy, and J. R. Lead. 2012. "Quantifying the
739 Dynamics of Flow within a Permeable Bed Using Time-Resolved Endoscopic Particle
740 Imaging Velocimetry (EPIV)." *Experiments in Fluids* 53(1):51–76.
- 741 Blois, Gianluca, James L. Best, Gregory H. Sambrook Smith, and Richard J. Hardy. 2014. "Effect
742 of Bed Permeability and Hyporheic Flow on Turbulent Flow over Bed Forms." *Geophysical*
743 *Research Letters* 41(18):6435–42.
- 744 Boano, F., J. W. Harvey, a. Marion, a. I. Packman, R. Revelli, L. Ridolfi, and a. Wörman. 2014.
745 "Hyporheic Flow and Transport Processes: Mechanisms, Models, and Biogeochemical
746 Implications." *Reviews of Geophysics* 1–77.
- 747 Breugem, W. P. and B. J. Boersma. 2005. "Direct Numerical Simulations of Turbulent Flow over

- 748 a Permeable Wall Using a Direct and a Continuum Approach.” *Physics of Fluids*
 749 17(2):025103.
- 750 Breugem, Wim-Paul, B. J. Boersma, and R. E. Uittenbogaard. 2006. “The Influence of Wall
 751 Permeability on Turbulent Channel Flow.” *Journal of Fluid Mechanics* 562:35.
- 752 Cardenas, M. Bayani. 2015. “Hyporheic Zone Hydrologic Science: A Historical Account of Its
 753 Emergence and a Prospectus.” *Water Resources Research* n/a-n/a.
- 754 Carrel, M., V. L. Morales, M. Dentz, N. Derlon, E. Morgenroth, and M. Holzner. 2018. “Pore-
 755 Scale Hydrodynamics in a Progressively Bioclogged Three-Dimensional Porous Medium: 3-
 756 D Particle Tracking Experiments and Stochastic Transport Modeling.” *Water Resources*
 757 *Research* 1–16.
- 758 Dzubakova, Katharine, Hannes Peter, Enrico Bertuzzo, Carmelo Juez, Mário J. Franca, Andrea
 759 Rinaldo, and Tom J. Battin. 2018. “Environmental Heterogeneity Promotes Spatial Resilience
 760 of Phototrophic Biofilms in Streambeds.” *Biology Letters* 14(10):0–3.
- 761 Fang, Hongwei, Xu Han, Guojian He, and Subhasish Dey. 2018. “Influence of Permeable Beds on
 762 Hydraulically Macro-Rough Flow.” *Journal of Fluid Mechanics* 847:552–90.
- 763 Finnigan, John. 2000. “Turbulence in Plant Canopies.” *Annual Review of Fluid Mechanics*
 764 32(1):519–71.
- 765 Graba, Myriam, Frédéric Y. Moulin, Stéphanie Boulêtreau, Frédéric Garabétian, Ahmed Kettab,
 766 Olivier Eiff, José Miguel Sánchez-Pérez, and Sabine Sauvage. 2010. “Effect of Near-Bed
 767 Turbulence on Chronic Detachment of Epilithic Biofilm: Experimental and Modeling
 768 Approaches.” *Water Resources Research* 46(11):n/a-n/a.
- 769 Graba, Myriam, Sabine Sauvage, Frédéric Y. Moulin, Gemma Urrea, Sergi Sabater, and José
 770 Miguel Sanchez-Pérez. 2013. “Interaction between Local Hydrodynamics and Algal
 771 Community in Epilithic Biofilm.” *Water Research* 47(7):2153–63.
- 772 Grant, Stanley B., Jesus D. Gomez-Velez, and Marco Ghisalberti. 2018. “Modeling the Effects of
 773 Turbulence on Hyporheic Exchange and Local-to-Global Nutrient Processing in Streams.”
 774 *Water Resources Research* (1):5883–89.
- 775 Kim, Taehoon, Gianluca Blois, James L. Best, and Kenneth T. Christensen. 2018. “Experimental
 776 Study of Turbulent Flow over and within Cubically Packed Walls of Spheres: Effects of
 777 Topography, Permeability and Wall Thickness.” *International Journal of Heat and Fluid*
 778 *Flow* 73(May):16–29.
- 779 Kim, Taehoon, Gianluca Blois, James L. Best, and Kenneth T. Christensen. 2019. “PIV
 780 Measurements of Turbulent Flow Overlying Large, Cubic- and Hexagonally-Packed
 781 Hemisphere Arrays.” *Journal of Hydraulic Research* 1686.
- 782 Kim, Taehoon, Gianluca Blois, James L. Best, and Kenneth T. Christensen. 2020. “Experimental
 783 Evidence of Amplitude Modulation in Permeable-Wall Turbulence.” *Journal of Fluid*

784 *Mechanics.*

- 785 Labiod, C., R. Godillot, and B. Caussade. 2007. “The Relationship between Stream Periphyton
786 Dynamics and Near-Bed Turbulence in Rough Open-Channel Flow.” *Ecological Modelling*
787 209(2–4):78–96.
- 788 Lichtman, Ian D., Jaco H. Baas, Laurent O. Amoudry, Peter D. Thorne, Jonathan Malarkey, Julie
789 A. Hope, Jeffrey Peakall, David M. Paterson, Sarah J. Bass, Richard D. Cooke, Andrew J.
790 Manning, Alan G. Davies, Daniel R. Parsons, and Leiping Ye. 2018. “Bedform Migration in
791 a Mixed Sand and Cohesive Clay Intertidal Environment and Implications for Bed Material
792 Transport Predictions.” *Geomorphology* 315:17–32.
- 793 Lu, S. S. and W. W. Willmarth. 1973. “Measurements of the Structure of the Reynolds Stress in a
794 Turbulent Boundary Layer.” *Journal of Fluid Mechanics* 60(03):481.
- 795 Malarkey, Jonathan, Jaco H. Baas, Julie A. Hope, Rebecca J. Aspden, Daniel R. Parsons, Jeff
796 Peakall, David M. Paterson, Robert J. Schindler, Leiping Ye, Ian D. Lichtman, Sarah J. Bass,
797 Alan G. Davies, Andrew J. Manning, and Peter D. Thorne. 2015. “The Pervasive Role of
798 Biological Cohesion in Bedform Development.” *Nature Communications* 6:1–6.
- 799 Manes, C., D. Poggi, and L. Ridolfi. 2011. “Turbulent Boundary Layers over Permeable Walls:
800 Scaling and near-Wall Structure.” *Journal of Fluid Mechanics* 687:141–70.
- 801 Manes, C., D. Pokrajac, V. I. Nikora, L. Ridolfi, and D. Poggi. 2011. “Turbulent Friction in Flows
802 over Permeable Walls.” *Geophysical Research Letters* 38(3):n/a-n/a.
- 803 Manes, Costantino, Dubravka Pokrajac, Ian McEwan, and Vladimir Nikora. 2009. “Turbulence
804 Structure of Open Channel Flows over Permeable and Impermeable Beds: A Comparative
805 Study.” *Physics of Fluids* 21(12):1–12.
- 806 Nakayama, A., F. Kuwahara, and Y. Sano. 2007. “Concept of Equivalent Diameter for Heat and
807 Fluid Flow in Porous Media.” *AIChE Journal* 53(3):732–36.
- 808 Nezu, Iehisa. 2005. “Open-Channel Flow Turbulence and Its Research Prospect in the 21st
809 Century.” *Journal of Hydraulic Engineering* 131(4):229–46.
- 810 Nikora, V. I., D. G. Goring, and B. J. F. Biggs. 2002. “Some Observations of the Effects of Micro-
811 Organisms Growing on the Bed of an Open Channel on the Turbulence Properties.” *Journal*
812 *of Fluid Mechanics* 450:317–41.
- 813 Nikora, Vladimir, Derek Goring, Ian McEwan, and George Griffiths. 2001. “Spatially Averaged
814 Open-Channel Flow over Rough Bed.” *Journal of Hydraulic Engineering* 127(2):123–33.
- 815 Packman, Aaron I. and K. E. Bencala. 2000. “Modeling Surface-Subsurface Hydrological
816 Interactions.” Pp. 45–80 in *Streams and Ground Waters*.
- 817 Parsons, Daniel R., Robert J. Schindler, Julie A. Hope, Jonathan Malarkey, Jaco H. Baas, Jeffrey
818 Peakall, Andrew J. Manning, Leiping Ye, Steve Simmons, David M. Paterson, Rebecca J.

- 819 Aspden, Sarah J. Bass, Alan G. Davies, Ian D. Lichtman, and Peter D. Thorne. 2016. “The
820 Role of Biophysical Cohesion on Subaqueous Bed Form Size.” *Geophysical Research Letters*
821 43(4):1566–73.
- 822 Pokrajac, Dubravka, Lorna Jane Campbell, Vladimir Nikora, Costantino Manes, and Ian McEwan.
823 2007. “Quadrant Analysis of Persistent Spatial Velocity Perturbations over Square-Bar
824 Roughness.” *Experiments in Fluids* 42(3):413–23.
- 825 Pokrajac, Dubravka and Costantino Manes. 2009. “Velocity Measurements of a Free-Surface
826 Turbulent Flow Penetrating a Porous Medium Composed of Uniform-Size Spheres.”
827 *Transport in Porous Media* 78(3):367–83.
- 828 Roche, K. R., G. Blois, J. L. Best, K. T. Christensen, A. F. Aubeneau, and A. I. Packman. 2018.
829 “Turbulence Links Momentum and Solute Exchange in Coarse-Grained Streambeds.” *Water*
830 *Resources Research* 54(5):3225–42.
- 831 Roche, K. R., J. D. Drummond, F. Boano, A. I. Packman, T. J. Battin, and W. R. Hunter. 2017.
832 “Benthic Biofilm Controls on Fine Particle Dynamics in Streams.” *Water Resources*
833 *Research* 53(1):222–36.
- 834 Rosti, Marco E., Luca Cortelezzi, and Maurizio Quadrio. 2015. “Direct Numerical Simulation of
835 Turbulent Channel Flow over Porous Walls.” *Journal of Fluid Mechanics* 784(2015):396–
836 442.
- 837 Salant, Nira L. 2011. “‘Sticky Business’: The Influence of Streambed Periphyton on Particle
838 Deposition and Infiltration.” *Geomorphology* 126(3–4):350–63.
- 839 Schultz, M. P., J. M. Walker, C. N. Steppe, K. A. Flack, J. M. Walker, C. N. Steppe, and K. A.
840 Flack Impact. 2015. “Impact of Diatomaceous Biofilms on the Frictional Drag of Fouling-
841 Release Coatings.” *Biofouling* 31(9–10):759–73.
- 842 Sinha, Sumit, Richard J. Hardy, Gianluca Blois, James L. Best, and Gregory H. Sambrook Smith.
843 2017. “A Numerical Investigation into the Importance of Bed Permeability on Determining
844 Flow Structures over River Dunes.” *Water Resources Research* 53(4):3067–86.
- 845 Sternecker, Katharina, Romy Wild, and Juergen Geist. 2013. “Effects of Substratum Restoration
846 on Salmonid Habitat Quality in a Subalpine Stream.” *Environmental Biology of Fishes*
847 96(12):1341–51.
- 848 Stoesser, Thorsten and Wolfgang Rodi. 2007. “Large Eddy Simulation of Open-Channel Flow
849 Over Spheres.” Pp. 321–30 in *High Performance Computing in Science and Engineering’06*.
850 Springer Berlin Heidelberg.
- 851 Stoodley, P., R. Cargo, C. J. Rupp, S. Wilson, and I. Klapper. 2002. “Biofilm Material Properties
852 as Related to Shear-Induced Deformation and Detachment Phenomena.” *Journal of Industrial*
853 *Microbiology & Biotechnology* 29(6):361–67.
- 854 Suga, K., Y. Matsumura, Y. Ashitaka, S. Tominaga, and M. Kaneda. 2010. “Effects of Wall

- 855 Permeability on Turbulence.” *International Journal of Heat and Fluid Flow* 31(6):974–84.
- 856 Suga, K., M. Mori, and M. Kaneda. 2011. “Vortex Structure of Turbulence over Permeable Walls.”
857 *International Journal of Heat and Fluid Flow* 32(3):586–95.
- 858 Suga, Kazuhiko, Yuka Nakagawa, and Masayuki Kaneda. 2017. “Spanwise Turbulence Structure
859 over Permeable Walls.” *J. Fluid Mech* 822:186–201.
- 860 Vignaga, Elisa, David M. Sloan, Xiaoyu Luo, Heather Haynes, Vernon R. Phoenix, and William
861 T. Sloan. 2013. “Erosion of Biofilm-Bound Fluvial Sediments.” *Nature Geoscience*
862 6(9):770–74.
- 863 Walker, J. M., J. E. Sargison, and A. D. Henderson. 2013. “Turbulent Boundary-Layer Structure
864 of Flows over Freshwater Biofilms.” *Experiments in Fluids* 54(12):1628.
- 865 Wallace, James M. 2016. “Quadrant Analysis in Turbulence Research: History and Evolution.”
866 *Annual Review of Fluid Mechanics* 48(1):131–58.
- 867