Biochemical Engineering Journal 106 (2016) 48–60

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

A fluid dynamic gauging device for measuring biofilm thickness on cylindrical surfaces

M. Lemos[a,b], S. Wang[a], A. Ali[a], M. Simões[b], D.I. Wilson[a,*]

[a] Department of Chemical Engineering & Biotechnology, New Museums Site, Pembroke Street Cambridge, CB2 3RA, UK
[b] LEPABE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, R Dr Roberto Frias, s/n, 4200-465 Porto, Portugal

ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form 2 November 2015
Accepted 9 November 2015
Available online 14 November 2015

Keywords:
Biofilms
Bioreactors
Fluid mechanics
Fouling
Instrumentation
Pseudomonas flourescens

ABSTRACT

Many industrial processes are susceptible to biofouling. The thickness and structure of such biofilms are key factors in the design of effective cleaning strategies. A novel method based on fluid dynamic gauging has been developed for measuring the thickness and the shear stress needed for removal of the biofilms formed on cylindrical surfaces. The device operates with the test cylinder immersed in liquid: liquid is withdrawn or ejected from a nozzle located near the biofilm surface. There is no net change of liquid volume, making it ideal for sterile and aseptic operation and for studies using valuable liquids. Biofilm removal may also be tested by using appropriate hydrodynamic conditions.

Calibration tests using ejection and suction flows in the laminar regime (Reynolds number around 100) indicated a measurement accuracy of ±19 μm and showed good agreement with computational fluid dynamics simulations. The device was commissioned in tests on *Pseudomonas fluorescens* biofilms formed on high density polyethylene (HDPE) and stainless steel (SS) cylinders of diameter 25 mm under conditions of mild shear stress (around 2 Pa in these tests). The biofilm thickness was not uniform to the eye and measurements made over the surface of the test cylinders confirmed this: layer thicknesses ranged from effectively 0–300 μm. The biofilms formed on HDPE were thicker than those formed on SS.

1. Introduction

Biofouling is a costly challenge for many industries. Biofilm accumulation in pipelines causes reduced flow area, requiring more work to pump liquids and causing reduced heat transfer rates. Biofouling can also lead to product contamination and corrosion (*e.g.* pitting). Failure to control biofilms results in increased energy consumption, additional maintenance and operational costs, and in several cases product losses [1,2]. In water treatment and manufacturing plants this problem is mainly countered using biocides, which aim to kill organisms and disinfect surfaces [3].

Biofilms grow when there are sufficient amounts of water and nutrients. There are several mechanisms affecting the events at the surface. Particle deposition, controlled essentially by shear stress and temperature, conditions the surface and facilitates the attachment of bacteria. Biological aspects such as species diversity, their ability to secrete extracellular polymeric substances (EPS), motility and quorum sensing mechanisms also influence the adsorption rate and irreversible adhesion of bacteria to surfaces [4,5]. The EPS matrix acts as a physical barrier to aggressors and delays the diffusion of nutrients and oxygen as well as antimicrobial agents [6].

The cells in biofilms differ from their planktonic counterparts: the cells exist in different metabolic states, are less susceptible to pH and temperature variations [7,8], and experience alterations in their gene regulation following irreversible adhesion [9]. Biofilms adopt complex structures, with viscoelastic properties, that are resilient to physical changes and chemical agents [7,10]. Biofilms interact with their surroundings, both the liquid environment and the surfaces around them, and the products of sessile cell metabolism can cause microbially induced corrosion [5,11]. Precipitation of minerals can occur, for example the deposition of calcium carbonate by algal biofilms. When compared to their planktonic forms, biofilm bacteria exhibit increased resistance to antimicrobials [12]. The reasons for this are not completely understood, and the mechanisms hypothesized include: direct interaction between EPS and antimicrobials, affecting diffusion and availability; an altered microenvironment within the biofilm, leading to areas of reduced or no growth; the development of biofilm/attachment-specific phenotypes; and the possibility of programmed cell death of damaged bacterial cells and persistent cells [13–16]. Biofilm elimination is difficult because of the evolution of resistant phenotypes, to the extent that control strategies that rely mainly on

* Corresponding author. Fax: +44 1223 334796.
E-mail address: diw11@cam.ac.uk (D.I. Wilson).

http://dx.doi.org/10.1016/j.bej.2015.11.006

Nomenclature

Roman

a	Arc length (m)
C_d	Discharge coefficient (–)
C_f	Friction factor (–)
d	Inner diameter of dynamic gauging tube (m)
d_t	Nozzle throat diameter (m)
D	RCR cylinder diameter
h	Clearance between nozzle and gauging surface (m)
h_0	Clearance between nozzle and gauging surface (m)
$\dot{m}$	Tube discharge mass flow rate, (kg s^{-1})
n	Normal vector of the relevant plane (–)
N_e	Number of mesh elements (–)
P_i	Pressure (Pa)
ΔP	Pressure drop (Pa)
r	Radial co-ordinate (m)
Re_a	Reynolds number of agitation (–)
Re_t	Reynolds number at the throat of the nozzle, $Re_t = 4m\text{-}dot/\pi$
s	Lip width (m)
μ_m	Mean velocity (m s^{-1})
v	Velocity vector (m s^{-1})
v	Velocity (m s^{-1})
v_{max}	Centreline velocity (m s^{-1})
v_y	Velocity in y direction (m s^{-1})
v_z	Velocity in z direction (m s^{-1})
y	Coordinate along cylinder surface (m)
z	Coordinate in direction normal to cylinder surface (m)

Greek

α	Internal divergent angle (°)
δ	Thickness of measured layer (m)
λ	Nozzle entry length (m)
θ	Nozzle angle (–)
κ	Curvature of cylinder (m^{-1})
μ	Fluid viscosity (Pa s)
ρ	Fluid density (kg m^{-3})
τ	Wall shear stress (Pa)
τ_{zy}	Wall shear stress on z-plane in the y-direction (Pa)

Acronyms

CDC	Centre for disease control and prevention
CFD	Computational fluid dynamics
CLSM	Confocal laser scanning microscopy
CNM	Concentrated nutrient medium
czFDG	Cylindrical zero-discharge fluid dynamic gauging
DNM	Diluted nutrient medium
EPS	Extracellular polymeric substance
FDG	Fluid dynamic gauging
FEM	Finite element method
HDPE	High density polyethylene
PB	Phosphate buffer
PMMA	Poly(methyl methacrylate)
RCR	Rotating cylinder reactor
SEM	Scanning electron microscopy
SS	Stainless steel

biocide action often fail against biofilms, as total inactivation of the microbial cells is rarely achieved. Moreover, when the biomass is not completely removed from the surface, there is a greater dispersion of persister cells, causing rapid growth of recidivist biofilms [22,23].

A wide range of biofilm reactors are employed to study biofilms. Mature biofilms can be formed in systems like the rotating cylinder reactor, the Centre for Disease Control (CDC) reactor, the rotating annular reactor, the rotating disc reactor, the Propella® system, the constant depth film fermenter or the flow-cell [17,19–23]. There are also many techniques for sampling and analysis that may be performed directly on test coupons, including thickness measuring or microscopy observations assisted by epifluorescence microscopy, confocal laser scanning microscopy (CLSM) [24], and scanning electron microscopy (SEM) [25]. However, these methods generally require the removal of the substrate from the bulk fluid and some treatments, to fix the cells or stain the structures that involve removing part of the water content from the sample. These dehydrating treatments compromise significantly the biofilms structure as their composition is essentially water (at least 90%) [17]. Methods to study biofilms *in situ* that minimize the manipulation of the samples are required, and particularly ones which allow the biofilm's response to biocides and other agents to be monitored. This paper reports the development of a variant of the fluid dynamic gauging (FDG) technique for measuring the thickness – and change of thickness in response to biocide application – of biofilms prepared on cylindrical surfaces. The biofilms studied are formed using the rotating cylinder reactor (RCR) developed by the LEPABE group at Porto [17,18,26,27]. The RCR mimics industrial conditions, with rotation speed manipulated to promote growth conditions with low to moderate wall shear stress. It has been used previously to form steady-state biofilms, aiming to assess their behaviour and mechanical stability, including the synergic effects of mechanical and chemical stresses in either single as well as multi-species biofilm. In this work, biofilms are prepared using the bacterium *Pseudomonas fluorescens,* a species often found in industrial environments due to its short generation time and resistance to heat treatment [28]. The biofilms are grown on cylinders of high-density polyethylene (HDPE), a polymer regularly used for drinking water pipes [29,30], and 316 stainless steel.

FDG is a non-contact technique developed for measuring the thickness of soft deposits *in situ* and in real time. Since its introduction by Tuladhar et al. [31] its functionality has been extended to study the strength of soft solid layers using computational fluid dynamics (CFD) to evaluate the stresses that the gauging fluid imposes on the surface being studied [32]. FDG has been used previously to study biofilms, including algal (*Chlorella*, [33]), cyanobacterial (*Synechococcus* sp. WH 5701, [34]), and bacterial (*Escherichia coli* and *Pseudomonas aeruginosa:* [35]) forms, all prepared on flat plates. The device presented here employs a curved surface and also employs the zero discharge mode introduced by Yang et al. [36], in which alternate ejection and suction stages mean that the total liquid volume does not change over the course of a test. This has particular advantages for aseptic operation or when liquid consumption is to be minimized. This paper details the construction, operation and proof-of-concept demonstrations of the cylindrical (geometry) zero (net discharge) fluid dynamic gauging (czFDG) system which has developed for making measurements on the Porto RCR test cells. The technique can be applied to other cylindrical geometries and does not require the gauging liquid to be transparent.

2. Materials and methods

2.1. Surfaces

The samples were cylinders of high-density polyethylene (HDPE) or 316 stainless steel (SS) with a useful surface area of 39.3 cm^2 (diameter = 2.5 cm, length = 5.0 cm). A thin vertical strip of aluminum foil was attached to the HDPE cylinders for checking