Chemical Engineering Research and Design xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Chemical Engineering Research and Design

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

Macro- and micro-scale mixing in a shaken bioreactor for fluids of high viscosity

Gregorio Rodriguez[a], *Martina Micheletti*[a], *Andrea Ducci*[b,*]

[a] *Biochemical Engineering Department, University College London, Torrington Place, WC1E 7JE, UK*
[b] *Mechanical Engineering Department, University College London, Torrington Place, WC1E 7JE, UK*

ARTICLE INFO

Article history:
Received 2 November 2017
Received in revised form 21 December 2017
Accepted 5 January 2018
Available online xxx

Keywords:
Mixing
Shaken bioreactor
High viscosity fluids
PLIF

ABSTRACT

Orbitally shaken bioreactors, OSRs, are commonly employed in the pharmaceutical industry at the drug development and screening stage, because they provide a high throughput solution where several cell cultures can be run in parallel. In general cell-culture media used in bioprocessing exhibit a viscosity close to water, but this hypothesis is less valid when high density cell cultures are considered, such as in continuous fermentations, where cells are retained in the bioreactor at large densities and the drug-product is continuously removed and fed to the downstream capture steps of the process. In this context the viscosity of the culture media increases with culture time and with the amount of biomass present in the reactor. In this frame of work two sets of measurements, based on DIMST and pLIF, were carried out to further study the mixing dynamics in a orbitally shaken cylindrical reactor when fluids of viscosity higher than water are considered. These data allowed to identify different flow transitions, which have not been previously observed in the PIV experiments of Ducci and Weheliye (2014). The mixing time measurements highlighted the presence of a poor mixing region for all the flow regimes considered, irrespective of the vortical structure present in the flow, and an attempt was made to quantify the diffusion process occurring at the edges of this region. A more detailed understanding of the flow and deformation dynamics occurring at small scales can be gained from the pLIF measurements. These allowed to visualise the lamellar structures induced by the flow deformation occurring over several orbital cycles. The growth rate of the material lines was estimated and compared to models commonly employed in the literature to assess mixing dynamics. These information are relevant for micro-mixing models, when the mixing process is limited by the growth of the interfacial area rather than the reaction time.

1. Introduction

Orbitally shaken reactors, OSRs, are ubiquitous in cell cultures and bioprocess development of animal or plant cells with low oxygen demand. Small scale OSRs, such as microtitre plates and/or Erlenmeyer flasks, are commonly employed at the drug development and screening stage because they provide a high throughput technology where several cell cultures can be run in parallel, and are characterised by a well defined free-surface interface and controlled shear conditions (Klöckner et al., 2014). In recent years large orbital single-use bioreactors up to 1000 L have proven successful at sustaining cell culture for shear sensitive cell lines. For example the Kühner OrbShake employs the agitation principle of shaken flasks and micro-well plates, providing a unique single-use technology for the entire upstream process, thus facilitating scaling-up and simplifying regulatory approval.

Recent works have provided a better understanding of the flow and mixing characteristics of cylindrical OSRs. At low agitation rate and for fluid of water-like viscosity the flow dynamics in OSRs are characterised by a toroidal vortex inclined below the free surface (Weheliye et al., 2013). Weheliye et al. (2013) report that the toroidal vortex expands

* *Corresponding author.*
E-mail address: a.ducci@ucl.ac.uk (A. Ducci).
https://doi.org/10.1016/j.cherd.2018.01.018
0263-8762/

Nomenclature

Abbreviations

CCW	counter-clockwise
CW	clockwise
LHS	left hand side
OSR	orbitally shaken bio-reactor
P_L	primary vortical cell, left
P_R	primary vortical cell, right
pLIF	planar laser induced fluorescence
RHS	right hand side
S_L	secondary vortical cell, left
S_R	secondary vortical cell, right
T_L	tertiary vortical cell, left
T_R	tertiary vortical cell, right

Greek symbols

μ	dynamic viscosity, Pa s
μ_w	dynamic viscosity of water, Pa s
ν	kinematic viscosity, $m^2\ s^{-1}$
ν_w	kinematic viscosity of water, $m^2\ s^{-1}$
φ	phase angle of the table, °

Roman symbols

a_o	constant of proportionality, –
a_{ow}	constant of proportionality for water, –
d_i	inner diameter of the cylinder, m
d_o	orbital diameter, m
Fr	Froude number, –
Fr_c	critical/transitional Froude number, –
g	gravitational acceleration, m/s^2
h	fluid height at rest, m
ℓ	length of material dye, m
N	shaker agitation speed, s^{-1}
Nt_m	mixing number, –
p_{vc}	perimeter of the vortical cell, m
Re	Reynold's number, –

in size towards the bottom of the vessel and increases in intensity with increasing Froude number (i.e. Froude number, $Fr = 2\pi^2 N^2 d_o/g$). When the vortex has reached the size of the tank (critical Froude, Fr_{cr}), a flow transition occurs and the fluid dynamics within the reactor are characterised by an axial vortex precessing around the vessel axis. This transition is also denoted by an increasing phase lag between the wave of the fluid free surface and the orbital movement of the reactor (i.e. out-of-phase condition). The scaling law of Weheliye et al. (2013) can predict the onset of the flow transition, and depending on the non-dimensional fluid height considered the critical Froude number can be estimated either from Eq. (1) ($h/d_i \le (d_o/d_i)^{0.5}$) or Eq. (2) ($h/d_i \ge (d_o/d_i)^{0.5}$).

$$Fr_{cr} = \frac{h(d_o/d_i)^{0.5}}{a_o d_i} \tag{1}$$

$$Fr_{cr} = \frac{1}{a_o} \tag{2}$$

where the coefficient $a_0 = 1.4$ for water. Most of the works found in the literature on OSRs concern fluids of water-like viscosity, which is in effect a close representation of cell-culture media commonly employed in bioprocessing (Büchs et al., 2000a; Maier and Büchs, 2001; Micheletti et al., 2006; Zhang et al., 2008; Rodriguez et al., 2013, 2014, 2016). This assumption is less valid for high density cell culture, where a 2–3 fold increase in viscosity has been observed with culture time and the biomass increase (Ozturk, 1996). Büchs et al. (2000b) report that an increase in viscosity promotes "out-of-phase" conditions with a significant reduction of oxygen transfer and mixing intensity, and altered cell metabolism. Büchs et al. (2000b) measured the power consumption for fluids with dynamic viscosity up to $\mu = 200$ mPa s (cf. water, $\mu = 1$ mPa s), whilst Kim and Kizito (2009) performed numerical simulations and flow visualisations for kinematic viscosity in the range $1 \times 10^{-6} \le \nu \le 1.6 \times 10^{-5}\ m^2\ s^{-1}$. Ducci and Weheliye (2014) carried out Particle Image Velocimetry (PIV) experiments to investigate the effects of fluid viscosity on the flow in a cylindrical OSR. The linear relationship between the inclination of the free surface wave and the Froude number, was confirmed also for fluid of high viscosity. The constant of proportionality, a_o, was found to decrease with increasing viscosity according to the power-law of Eq. (3):

$$a_o = a_{o_w}\left(\frac{\nu}{\nu_w}\right)^{-0.0256} \tag{3}$$

where a_{o_w} is the constant for water (i.e. 1.4), and ν_w and ν are the kinematic viscosities of water and of the viscous fluid under study, respectively. Ducci and Weheliye (2014) summarised the different flow dynamics encountered in the shaken reactor in a Fr–Re transition map. For $\nu \le 1.7 \times 10^{-6}\ m^2 s^{-1}$ ($Re >$ 10,000) they found that the flow exhibited similar dynamics to those obtained for water with toroidal and axial vortices controlling the flow for in-phase and out-of-phase conditions, respectively. For $\nu \ge 1 \times 10^{-5}\ m^2 s^{-1}$ ($Re < 5000$) the flow dynamics were significantly different: at low Fr the flow exhibited a vortex region below the lowest side of the free surface which spread over the bottom of the reactor as Fr was increased. At high Fr, the free surface was wavy and a toroidal vortex rotating in the opposite direction to that reported for water-like viscosity and in-phase conditions, was reported.

Discacciati et al. (2013) investigated numerically and experimentally the free-surface dynamics in high-viscosity fluids ($\mu = 1$ Pa s, 98% aqueous glycerol solution). They found that a two-dimensional free surface occurred at $Fr_{do} = 0.12$, while at $Fr_{do} = 0.21$ it was highly three-dimensional. Path-lines of virtual fluid parcels seeded within the reactor were reconstructed from the simulated velocity fields. Fluid parcels were tracked for 8 s and it was found that their trajectory was characterised by spiral motions for $Fr_{do} < 0.15$, while for $Fr_{do} > 0.21$, when the free surface was three-dimensional, a more uniform particle dispersion was reached over the time-frame considered.

Mixing dynamics in shaken bioreactors have been investigated in the works of Rodriguez et al. (2013, 2014) and Tissot et al. (2010), where a pH-colorimetric methodology was employed to determine macro-mixing time (Dual Indicator System for Mixing Time, DISMT). At low rotational speed two different mixing zones were identified within the reactor: a zone below the free surface, where mixing was significantly controlled by the toroidal vortex structure, and a slow mixing region close to the bottom of the reactor, where mixing occurred mainly by diffusion. The macro-mixing time was highly dependant on the feed locations, as mixing was found more effective when reagents insertion was made close to the edges of the toroidal vortex (Rodriguez et al., 2014). Tan et al. (2011) evaluated the effects of viscosity on mixing