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Mixing time and kinetic energy measurements in a shaken cylindrical bioreactor

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ABSTRACT

The mixing dynamics in a cylindrical shaken bioreactor are investigated by means of velocity and kinetic energy and mixing time measurements obtained with phase-resolved PIV and a dual pH indicator system, respectively. The objective of the work is to correlate the kinetic energy of the flow and the mixing number measured under different operating conditions. The results provide evidence that the onset of a laminar-turbulent flow transition occurs when the previously reported transition to out-of-phase flow takes place, and that the mixing number is highly dependent on the position of the feeding pipe. Insertion close to the vessel walls and thus outside the vortical structures present near the centre of the reactor can enhance mixing.

Keywords: Shaken bioreactor; Mixing time; Dual indicator method; PIV

1. Introduction

The pharmaceutical industry is at the forefront of the production of viruses, viral vectors and antibodies using mammalian cell-based cultures. Mammalian cells are usually preferred to other host cells, such as yeast, bacteria, insects and plants, because of their favourable folding characteristics as well as assembly and post-translational abilities, which define the efficacy and functioning of an antibody (Wurm, 2004). At laboratory scale, cells are usually grown in low shear devices, with shaken bioreactors being largely employed in the early stages of bioprocess development because they offer a low power consumption solution to screen several conditions in parallel. This low energy requirement, combined with the well-defined liquid-gas interface occurring in shaken bioreactors, provides a promising environment for mammalian cell cultivation in terms of oxygen transfer and cell metabolism requirements. Once the process is optimised at small scale, it is then adapted to stirred tank reactors (STRs), which is the type of bioreactor most commonly used at production level. STRs consist of an impeller for culture medium agitation, spargers for gas aeration, pH and temperature control systems and have been thoroughly characterized in the literature (see for example the works on STR mixing and fluid dynamics of Doulgerakis et al., 2009, 2011; Escudie and Line, 2003; Kresta and Wood, 1993; Yianneskis et al., 1987).

It should be emphasised however that cell growth and environmental conditions vary significantly between shaken cultures and those encountered in large STRs. This often becomes a bottleneck in bioprocess development as cells respond differently to the different shapes and mixing mechanisms taking place in the two scales and types of bioreactors. Such differences, together with other drawbacks of STRs, such as excessive shear levels, have prompted the industry to look at production scale Orbitally Shaken Bioreactors, OSRs (see for example Anderlei et al., 2009; Liu and Hong, 2001). OSRs employ the agitation principle of shaken flasks and microwell plates, thus facilitating scaling-up and simplifying regulatory approval. The bioprocess industry has been investigating implementation of OSRs to provide a single piece of equipment for different cell culture processes at multiple scales, because of the simplified scaling-up procedures based on a single agitation mechanism, of the possibility to implement more efficiently single-use manufacturing processes and of

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Nomenclature

2D	two-dimensional
3D	three-dimensional
CFD	computational fluid dynamics
PIV	particle image velocimetry

Greek symbols units

φ	phase angle of the table (°)
σ	standard deviation of the normalised green channel across the field at time t (–)

Roman symbols units

a_o	constant (–)
d_i	inner diameter of the cylinder (m)
d_o	orbital diameter (m)
g	gravitational acceleration (m s^{-2})
$G^*_{i,j}$	normalised intensity of the green channel in the ith jth pixel (–)
h	fluid height (m)
Δh	free surface height (m)
I_a	free surface interfacial area (m^2)
k_{ij}	kinetic energy of the periodic motion estimated from the ith and jth velocity components (m^2 s^{-2})
k^*_{ij}	space average of the kinetic energy of the periodic motion (m^2 s^{-2})
k'_{ij}	kinetic energy due to the random velocity fluctuations in the ith and jth directions (m^2 s^{-2})
k'^*_{ij}	space average of the kinetic energy due to the random velocity fluctuations (m^2 s^{-2})
N	shaker table rotational speed (s^{-1})
M	degree of mixing (–)
Δt	time between two consecutive images (s)
t_m	mixing time (s)
$t_{m_{ij}}$	mixing time of the ith and jth pixel (s)
u_i	velocity component in the ith direction (m s^{-1})
$\langle u_i \rangle$	phase average of the velocity component in the ith direction (m s^{-1})
u'_i	random velocity fluctuation in the ith direction (m s^{-1})

the flexibility and adaptability of this technology. Despite their extended use at research and lab scales, only a few publications can be found in the literature which address engineering aspects of shaken bioreactors (Büchs, 2001).

The study of Gardner and Tatterson (1992) is one of the first reported in the published literature that investigated the flow and mixing dynamics in a shaken, partially filled, cylindrical vessel. Dye visualization techniques with different degree of mixture of water and corn syrup were employed to assess the variation of the homogenization time with increasing Reynolds number, $Re = NT^2/\nu$, where the characteristic length scale T is obtained as the cubic root of the filling volume $V^{1/3}$, while N and ν are the shaker table rotational speed and fluid viscosity, respectively. The authors identified three mixing regimes: (i) a laminar regime, $Re = 0.8$–1000, controlled by a toroidal vortex and characterised by the homogenization time being inversely proportional to Re; (ii) a transitional regime, for $Re \sim 1000$, characterised by a vertical axial vortex inducing unmixed dye regions at the top and at the bottom of the filling volume; (iii) a turbulent regime at higher Re where the flow is dominated by severe splashing and the homogenization time is nearly constant. It should be noted that, in the experiments of Gardner and Tatterson (1992), the addition of the dye occurred prior to the shaker motion; hence diffusion would start before the flow structures would have been established.

Kim and Kizito (2009) performed numerical analysis to study the flow present in a shaken system with emphasis on the deforming free surface. They found that the flow was quasi-steady when a coordinate system rotating with the shaker table was considered. More specifically, the highest free surface height occurred on the vertical plane identified by the directions of the centrifugal and gravitational accelerations. This plane changes its orientation in space and rotates around the bioreactor axis as the shaker table proceeds along its circular orbit. Their numerical simulations showed that the velocity field of the deforming free surface is 90° out of phase with the centrifugal body force. The time-averaged flow fields showed the presence of two strong vortical flow structures, with their axis in the tangential direction, in proximity of the cylindrical vessel walls. As the fluid viscosity was increased the two vortices were pushed towards the centre of the bioreactor, and the thickness of the boundary layers next to the cylinder walls grew in size. Kim and Kizito (2009) assessed their numerical results against flow visualizations obtained in a cylindrical vessel of 4.35 cm internal diameter, for a shaking diameter $d_o = 2$ cm and a shaking frequency $N = 60$ rpm. Water–ethylene glycol mixtures were used as the working fluid, while a laser was employed to illuminate the cross-section of the cylinder. Experimental results confirmed the presence of large vortices on the side walls which created a strong upstream flow in the core region of the vessel. The sizes of the vortical structures depended on fluid height and viscosity. Kim and Kizito (2009) concluded that mixing processes were driven by these side vortices when the aspect ratio $h/d_i \sim 1$, where h and d_i are the fluid height and cylindrical vessel diameter, respectively. At higher aspect ratios mixing was found to be primarily caused by the free surface travelling wave. Apart from the work of Kim and Kizito (2009), other numerical simulation studies are those carried out by Zhang et al. (2005) and Zhang et al. (2008) on 250 mL Erleymeyer flask and 24- and 96-microwell bioreactors, respectively. These analyses aimed at estimating macro-parameters, such as power consumption, from the integral of the viscous dissipation rate of kinetic energy, interfacial area and volumetric mass transfer coefficients for different types of bioreactor, but do not provide insight into the flow and mixing dynamics occurring inside the bioreactor.

Moreover it is worth mentioning the work of Mehmood et al. (2010) who found a correlation between numerical estimates of the power dissipation and the production of pristinamycins by Streptomyces pristinaespiralis in a shaken bioreactor of conical shape. From this study they identified an optimal production for a power consumption range of 5.5-8.5 kW m^{-3}.

Büchs et al. (2000) estimated the power consumption in Erlenmeyer flasks by measuring the torque on the drive acting upon the shaker table and taking into account friction and other system losses. Several operating conditions were investigated by varying the size of the container, filling volume, shaking diameter, shaking frequency and liquid viscosity. The results showed that at certain agitation regimes an 'out-of-phase' condition occurs, and the liquid in the shaken flask does not move in synchronization with the shaker table. The variation of a modified Newton number Ne' (i.e. power