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Short communication

A hydrodynamic description of the flow behavior in shaken flasks

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ABSTRACT

Shaking flasks bioreactors have been employed extensively in biotechnology research for a long time. Despite of their wide application and importance, there is still insufficient knowledge about the hydrodynamic factors that determine the correct performance of growing cultures. The objective of this work is to provide a hydrodynamic description of the parameters which control the effective performance of such bioprocesses. The flow behavior of shaken flasks was examined using the particle image velocimetry technique (PIV) to capture the mixing dynamics for a range of operating conditions. The velocity fields and turbulent intensities were obtained. For all cases, the chaotic-like fluid motion increased with the orbital speed of the shaker. The behavior of conventional, coiled and baffled flasks was analyzed for shaking speeds ranging from 25 to 250 rpm; at high shaking rates the turbulent distribution increased for each flask configuration but the average value differed significantly. The highest turbulent intensity was found for the one-baffle arrangement, which is about 25% larger than the other configurations. We found that the highest turbulent production for all the different geometric conditions occurred at a shaking speed of about 150 rpm, which is in good agreement with the findings reported for the production of bacterial cultures at such shaking rate.

1. Introduction

Since the beginning of the century, shaking reactors have been used in many biotechnological processes [1,2]. For many years shaken flasks have been implemented in microbial fermentation, bacterial growth and in the pharmaceutical industry, among others [3–5]. The extensive use of shaken flasks relies on its simplicity, low cost and easy operation. In the biochemical industry the selection of the first step of a production process is very important prior to the scaling up to industrial scales. For this reason the characterization and determination of the appropriate operating conditions play a significant role in every process. Despite the importance of this initial step, only few investigations have attempted to understand the hydrodynamic foundations of orbital shakers. Büchs et al. [6–8], were the first authors to identify the hydrodynamic condition that restricts the operating performance in such systems. They reported a range of operation conditions. The regime called "out-of-phase" shaking, is distinguished by an augmentation of the fluid not following the orbital motion of the flask walls. In this case the specific power consumption is minimal, reducing the mass transfer rate and the mixing times. For this reason, in order to obtain a correct performance in such systems this behavior must be avoided. In shaking flask operations, multiple variables exist and have to be taken into account to describe the agitated system. Some of these parameters are the vessel geometry, size and orientation of the baffles, the liquid volume and operating conditions such as shaking radius and frequency, just to name a few. The conventional experiments performed in shaken flasks contribute to the knowledge of the operational conditions necessary to achieve the correct degree of mixing or the oxygen quantity needed in biochemical reactions. The main parameter considered in most studies is the rotational speed, which itself does not quantify other important engineering parameters as the oxygen mass transfer [9,10], the specific power consumption [11,12] or the hydromechanical stress [13–15] for which some cultures growing could be affected. For many years these parameters were inferred through large scale experiments or by the use of empirical correlations. But there are still large disagreements about the use of the physical variables that describe the hydrodynamic state in such bioreactors.

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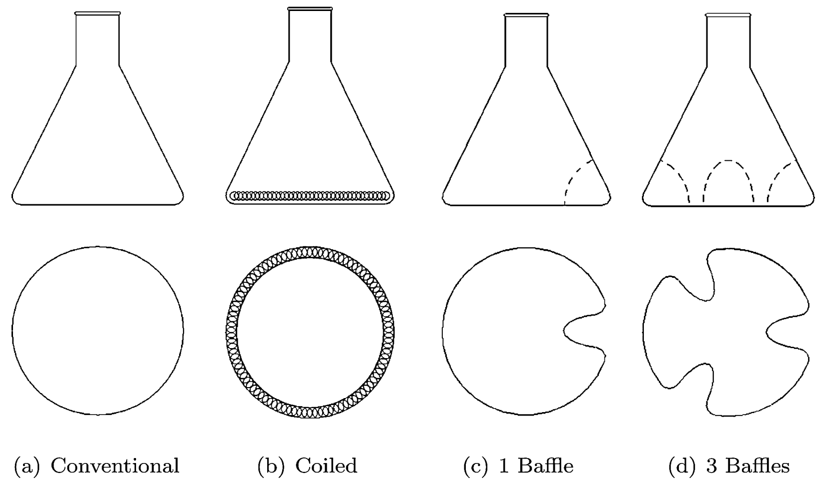

Fig. 1. Shaken flask configurations. The 2 and 4-baffles configurations are not shown.

As mentioned before, the specific power consumption has been used as key parameter for scaling up of growing culture processes [16]. Besides the power drawn, the influence of the flask geometry on the hydrodynamic performance should be also considered for scaling up purposes. Results from this study may help to select the appropriate impeller geometry by providing large turbulent intensities with the aim of enhancing mixing while shortening mixing times.

For such a purpose and in order to accomplish a better understanding of the internal process, it is necessary to analyze the flow fields and the spatial distribution of the turbulent intensity. The goal of this study is to get a deeper insight of the mixing behavior of shaken flasks as a function of their geometry with the aim of elucidating its performance from the standpoint of basic physical phenomena. Note that in this study, the effect of flow on growing cell culture was not assessed. We focused only on the hydrodynamic state of shaken flasks and how better mixing can be achieved with different configurations and shaking speeds.

2. Materials and methods

2.1. Experimental setup

Six different configurations of 250 ml Erlenmeyer flasks (Pyrex,Mexico) with 82 mm of maximum diameter were tested: conventional, baffled and coiled as shown in Fig. 1. Similar flask configurations were used by Gamboa-Suasnavart et al. [17]. The conventional flasks have a standard design which consists of a round circular section. Baffled flasks had a different number of baffles and indentations (1, 2, 3 and 4) placed at equidistant sections with 20 mm depth and 45 mm height. The coiled configuration consists of conventional flask into which a 30 cm stainless steel spring (1.3 cm diameter, 19 SW) is inserted. To have similar fluid characteristics, each flask was filled with 50 ml of Luria-Bertani's medium and a solution of 34% (w/v) sucrose was added. The liquid depth (from bottom to liquid surface) was 10 ± 2 mm depending on the flask configuration. The fluid had the following properties: $\rho = 1300\ \mathrm{kg/m^3}$, $\mu = 3.5\ \mathrm{mPa{\cdot}s}$ and $\sigma = 62\ \mathrm{mN/m}$ (surface tension). The flasks were agitated in an orbital standard shaker (VWR, model Signature DS 500), with a counterbalanced eccentric drive mechanism that travels over a 19 mm circular orbit (shaking diameter) for a speed range from 50 to 200 rpm (shaking frequency).

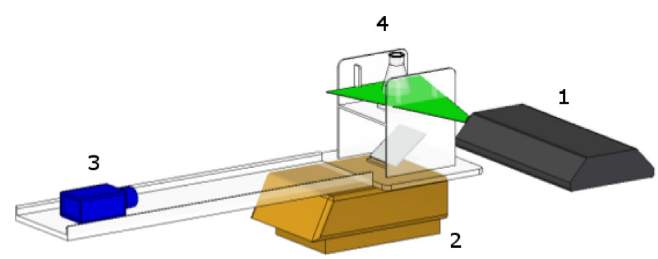

Fig. 2. Experimental setup: The laser (1), orbital shaking device (2), camera (3) and the flask array (4) are depicted.

2.2. Velocity field measurements

Fig. 2 shows the experimental setup used for determining the flow fields using the particle image velocimetry technique (PIV). It consisted of a pulsed laser light (Solo III HiSense Dynamics, wavelength of 532 nm, energy of 150 mJ), an optical array for creating a light sheet of 2 mm width and a CCD camera (Kodak Megaplus, Model ES 1.0) and a 35 mm lens, both placed in front of a mirror, inclined 45 degrees. The flask was placed into a square jacket containing water for minimizing the optical distortion due to the flask curvature. The laser sheet was aligned parallel to the base of the flask and with a height of 5 mm from the bottom; i.e. in the middle of the total depth. Note that in our measurements the camera was mounted on the same shaking plate where the flask was placed: the fluid velocity was obtained in the reference frame of the flask. For this study, an acquisition rate of 4 Hz was chosen. The Flow Manager software (Dantec Dynamics) was used to control the system and to process the images. The time between image pairs was varied from 1000 μs to 2500 μs, depending on the velocity of the orbital plate. Sets of 300 images were captured for each experiment in order to ensure a statistically robust average. The incorporation of air bubbles during the shaking caused unwanted laser reflections, which were filtered out using fluorescent particles (10 μm, Dantec Dynamics) and a 550 nm optical filter (mounted on the camera lens). The velocity fields were inferred using the standard cross correlation method used in PIV, considering interrogation areas of 32 × 32 square pixels area (about 1.6 $\mathrm{mm^2}$) with an overlap of 50 × 50%. A spatial filter (3 × 3 pixels) was applied to eliminate spurious vectors. The velocity fields were obtained from the statistical average of the images. The velocity magnitude and turbulence intensity were inferred from the time averaged velocity fields.