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Hydrodynamic characterization of a new small-scale reactor mixed by a magnetic bar

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ABSTRACT

The purpose of this study was to evaluate the mixing characteristics of a new milliliter-scale (mL-scale) reactor developed for studying enzymatic activity or physiological cell response. The mL-scale reactor was designed to enable the integration of several sensors to carry out dynamic measurements in a controlled environment. Rapid homogeneity of the entire system is essential to ensure reproducible and reliable results, consequently the reactor was stirred to optimize both mass and heat transfers. A comparative study using three different techniques was undertaken to study mixing performances in the system. Firstly, mixing time (t_m) was estimated in the reactor using both experimental methods, including instrumental method and image analysis, and CFD. As hydrodynamics is not occurring in the fully-turbulent regime, turbulent numerical simulations using the SST transitional and the standard k-ϵ turbulence models were conducted and compared to laminar approach. Results showed good agreement between the two experimental methods and no significant differences were observed between the simulation methods. Moreover, according to results, a minimum agitation rate of 350 rpm seemed to be appropriated to obtain a quick homogenization in the system. Finally, the second part of the study seemed to indicate that probes had no significant impact in the studied reactor.

1. Introduction

The high cost and toxicity of some reagents, production costs and low availability of biocatalysts, as well as the analytical procedures, are technical limitations that often result in scientists using small volumes to study enzyme activity and physiological cell response. In small volumes (cuvettes or microplates), used with spectrophotometers, spectrofluorometers or microplate readers, environmental parameters such as pH, temperature or dissolved gases are not generally monitored. However, the physico-chemical environment may have a crucial impact on the results and better monitoring of these parameters could be the solution to ensure reproducible, reliable and comparable results. Finally, online measurement using sensors such as ionic electrodes (Mn^{2+}, Fe^{3+}, tetraphenylphosphonium, salicylate) would be of interest in many studies [1–5]. Consequently, a reactor with a small reaction volume that offers the necessary instrumentation for measuring and monitoring the physico-chemical environment would be a valuable tool for research teams. Indeed, even if commercial minibioreactor, such as ambrTM system, was proved to be powerful culture systems for small-scale culture, till now, it was dedicated to cell culture only [6].

To meet this demand, a milliliter-scale (mL-scale) reactor was especially developed to study biochemical reactions [7]. This reactor, made in stainless steel, consists of a cylindrical chamber with a working volume between 5 and 15 mL, agitated by a rotating magnetic bar in the bottom of the chamber. This system can be considered as intermediate technology between micro and conventional bioreactors (Liter-scale reactors). Several miniaturized sensors can be adapted to the mL-scale reactor allowing online measurements and monitoring of various operating parameters. Indeed, the use of a cylindrical chamber allows However, even on a small scale, and especially in the case of experiments using several sensors, the mixing quality and a quickly homogenization are fundamental parameters to ensure reproducible and reliable results.

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About it, reactor technology has led to several studies to characterize conventional bioreactors and optimize system performances in terms of mixing.

Generally, the efficiency of mixing depends on numerous factors such as vessel design, stirrer type, the occurrence of baffles, the agitation rate or liquid properties (density, viscosity). Among the various criteria available to characterize the mixing quality in mechanically stirred vessels, the mixing time is the most commonly used [8–11]. The mixing time, or t_m, can be defined as the necessary time to obtain a given degree of homogeneity of the liquid within the vessel. The different methods used to measure t_m during the mixing process can be divided into three categories.

The first category groups intrusive experimental methods based on the use of dedicated probes which measure a flow property that can be related to mixing quality. In this case, t_m corresponds to the time required to reach a given degree of homogeneity in a specific location. Among these methods, an approach based on conductivity measurements is commonly used. This method consists in recording the conductivity profiles after injection of a saline solution during the mixing process in a stirred vessel. Riedlberger et al., used this method to compare t_m obtained with two types of small bioreactor [12]. The acid pH tracer response technique is an alternative method which consists in measuring pH variation using a standard pH probe after addition of an acid tracer [9]. Another technique was proposed by Ng et al., who developed a novel spectroscopic method to measure mixing times using two in-situ fiber optic probes immersed in the vessel [13]. t_m was established according to the absorbance signal after injection of a colored tracer in the stirred vessel. The results obtained with the spectroscopic technique in 3–20 L stirred vessels were compared with those obtained using the conductivity technique; a good correlation was found between the two methods.

The second category of experimental approaches includes non-invasive techniques based on the visualization of discoloration [14] or the color change of the mixed liquid. Contrary to the previous described methods, they are non-intrusive and enable the entire stirred system to be measured until the end of mixing. Poorly mixed zones in the reactor are advantageously identified with this method. Melton et al., developed a dual indicator system for mixing time (DISMT) method based on color modification arising from the reaction between a red acid solution and a blue basic solution [15]. The change in pH which occurs during the mixing of the two solutions results in the color changing to yellow; this indicates the end of mixing. Compared to a single-indicator method, DISMT provides an interesting and global insight into the dynamics of the mixing process and allows zones that are mixed rapidly and zones that are mixed slowly to be distinguished. For example, Delaplace et al., used DISMT and were able to accurately analyze the evolution of the mixing process and determine the mixing time for highly viscous fluids [16]. Moreover, to circumvent a certain degree of subjectivity, which is often reproached to DISMT methods, authors coupled image analysis to their work. Cabaret et al., also demonstrated the reproducibility and robustness of using image analysis to describe macro-mixing in different types of stirred tank reactors [17]. Among the non-invasive methods, it is worth mentioning the study by Wabo et al. who used 3D resistance tomography [18], and more recently, Hu et al., who applied planar laser-induced fluorescence (PLIF) to characterize the spatio-temporal mixing process in a stirred tank [19].

The third category of techniques concerns numerical approaches, such as computational fluid dynamics (CFD). Indeed, CFD has become a valuable approach for predicting the mixing performance of a reactor and, more generally, for designing and scaling-up bioreactors; it is now routinely used in the literature [20–22]. CFD offers interesting advantages compared with experimental methods such as no need of specific material or reactants. Moreover, operating parameters such as liquid volume, agitation rate or bioreactor design can be easily modified for a more rapid comparison of the results. In this way, Hekmat et al., compared the mixing efficiency according to the design of an air-lift reactor and determined the optimum position of the draft tube in the system [23]. As this approach can be used whatever the size or the design of the reactor, Riedelberg et al., successfully used CFD to compare the efficiency of two impeller designs for enzymatic biomass hydrolysis in a mL-scale bioreactor [12]. Furthermore, numerical simulation can be used to evaluate other process characteristics such as power dissipation, velocity and turbulent dissipation rate, which are useful for accurately characterizing stirring systems [12]. Concerning the study of bioreactor hydrodynamics and the determination of t_m, the previous studies revealed good agreement between the numerical and the experimental results [12,24]. Nevertheless, the majority of studies focused on liter-scale reactors and few studies are available for mL-scale reactors [11,12].

The purpose of the present work was to characterize the mixing performance of an instrumented mL-scale system dedicated to study enzymatic reactions and physiological cell responses. Three different methods associating experimental and numerical approaches were compared to analyze the mixing process. Indeed, in the manner of Ng et al. [13], a novel spectroscopic method using a specific fiber optic was developed to determine t_m in a mL-scale prototype reactor, made in glass. Secondly, an image analysis was developed to estimate t_m in the same prototype. The third approach consisted in using CFD numerical simulations to characterize liquid flows in the vessel and to determine t_m. Considering the intermediate values of Reynolds numbers expected in the stirred system, three numerical approaches were used and compared in our study. For the first one, no turbulence model was used, thus supposing laminar flow. For the two other approaches, turbulent flow was supposed through the use of k-ϵ and transition SST turbulence models. Then, the impact on mixing times of the two measurement probes insertion in the mL-reactor was numerically simulated and discussed.

2. Material and methods

2.1. Experimental system

2.1.1. mL-scale reactor design

The experiments were carried out in a glass prototype (Fig. 1A) which presents the same dimensions as the cylindrical reaction chamber of a stainless steel mL-scale reactor dedicated to bioconversion. The use of a cylindrical chamber for the reactor is more convenient as several electrodes must be inserted in the system. Indeed, with a cylindrical chamber with a diameter of 1.8 cm, the working volume remains small and 6 miniaturized electrodes (diameter of 6.0 mm) may be inserted together. This is an advantage as one expectation of this system will be to allow various simultaneous measurements. Liquid contained in the prototype was agitated by a prism-shaped magnetic bar (Dutscher, Brumath, France), controlled by a magnetic stirrer and placed in the bottom of the prototype (Fig. 1C). This agitation system was chosen for different reasons. Firstly, for its low cost and for convenience: it can be easily inserted or removed from the reactor for cleaning or replacement. Moreover, with this prism-shaped bar, a plane face is in contact with the bottom of the prototype. Consequently it is more stable than other magnetic bars (egg-shape, disk). Then, with its two inclined faces in contact with the liquid, it may be compared with more conventional stirrer systems such as pitched-blade turbine. Finally, a protection cross-grid was placed above the magnetic bar to avoid any potential shocks between the magnetic bar and the sensors immersed in the reactor (Fig. 1A and 1B). Regarding