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Multiscale study of hydrodynamics, mixing and gas-liquid mass transfer in a stirred-tank bioreactor

Dissertation submitted in partial fulfillment of the requirements for the degree of *Doctor of Philosophy* in Engineering Sciences

by Anne de Lamotte
under the supervision of Pr. Dominique Toye

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"You should never, never doubt something that no one is sure of."
– Willy Wonka

Roald Dahl, *Charlie and the Chocolate Factory* (1964)

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Summary

The present PhD thesis has been funded by the National Funds for Scientific Research of Belgium (FRS-FNRS) by means of a PDR FNRS PhD grant (PDR n° T.0250.13). It has been carried out within the "PEPs - Products, Environment and Processes" group, a part of the department of *Chemical Engineering* of the University of Liège, under the supervision of Pr. Dominique Toye.

This research work is part of an FNRS-PDR project in collaboration with Gembloux Agro-Biotech, aiming at better mastering phenomena linked to gas-liquid transfer, that rule the performance of biochemical processes. It focuses on the influence of mixing and circulation imposed by hydrodynamics, within a baffled stirred-tank reactor, on the gas-liquid transfer through the liquid free-surface and on the spatiotemporal distribution of the dissolved gas concentration in the liquid phase.

The selection of agitation configuration and operating conditions has to meet two objectives. On the one hand, the influence of physical parameters on microbial physiology and on bioprocess performance has to be properly assessed. On the other hand, hydrodynamics and mass transport phenomena, as well as their coupling, need to be properly understood at and across all scales for adequate bioreactor scale-up. In this context, a characterization methodology has been developed and validated to understand, quantify and model these multiscale transport and transfer phenomena during bioreactor implementation. Moreover, even if the guidelines of this work are inspired by the biotechnology industry, the strategy adopted during this thesis is chosen to obtain outcomes that serve as large a framework as possible.

The first part of this PhD dissertation presents the state of the art relative to the culture of micro-organisms inside stirred-tank reactors, and introduces the crux of the theoretical aspects used for the present work.

Chapter 1 presents the bases of bioprocess development and of their implementation in a bioreactor, in order to highlight the necessity of a better mastery of all the parameters conditioning their performance.

Chapter 2 reviews the fundamental concepts relative to hydrodynamics, bulk fluid mixing and gas-liquid mass transfer through the free-surface inside stirred-tank bioreactors within the framework of scale-up/down, in order to evidence the need for a better characterization of them.

Chapter 3 describes the strategy chosen for this PhD thesis, with emphasis on the methodology and tools developed for the understanding, the quantification and the simulation of these three key dynamic phenomena and of their coupling. The body of the thesis manuscript is divided into three parts to underline the achievement of these thesis objectives: experimental study, computational study and application.

"Part 1 - Experimental Study" is dedicated to the understanding and the quantification of hydrodynamics, mixing and gas-liquid transfer through the free-surface inside a stirred-tank bioreactor, using simple and more advanced data acquisition and processing techniques.

Chapter 4 consists of global and local descriptions of these phenomena within a reference pilot-scale stirred-tank configuration by means of statistical research. Based on global parameter values, the most appropriate rotating speed is selected to further study local hydrodynamic quantities, as well as mixing and mass transfer dynamics, *i.e.* local concentrations of an inert tracer and of dissolved oxygen. Measurements are performed using two advanced experimental methods: Particle Image Velocimetry (PIV) and Planar Laser-Induced Fluorescence (PLIF). The averages of the obtained detailed data are compared to the global ones. Statistical analysis of their spatial distributions gives a further insight into the effects of hydrodynamics and mixing on mass transfer through the free-surface in stirred-tank bioreactors.

Chapter 5 introduces the application of modal decomposition methods to flow fields in order to extract spatial structures and their time characteristics. Two data-decomposition techniques, Proper

Orthogonal Decomposition (POD) and Dynamic Mode Decomposition (DMD), are selected because of their simplicity of application and of their potential complementarity to get a better understanding of unsteady hydrodynamic data and the underlying physical phenomena. Both analyses are applied to PIV measurements of instantaneous velocity fields in a turbulent flow generated in the reference stirred-tank configuration at two rotating speeds. The results show that the flow field is characterized by a wide range of spatial and time scales, but distinct spatial patterns and some dominant frequencies are clearly evidenced.

Chapter 6 presents the analysis of scalar fields by means of the POD and DMD methods, in order to get and compare spatial and time information on mixing and mass transfer phenomena and to relate them to flow field characteristics. PLIF measurements of instantaneous concentration fields of inert tracer and dissolved oxygen performed in the reference stirred-tank configuration, are processed using the two data-decomposition techniques. These complementary mathematical tools give information on mixing as well as on gas-liquid transfer, that is otherwise hard to highlight due to the interactions of physical mechanisms. They isolate spatial structures along with their spectral properties. Moreover, POD and DMD are proved effective in providing a low-dimensional representation of mixing and mass transfer dynamics.

"Part 2 - Computational Study" is dedicated to the modeling of hydrodynamics, mixing and gas-liquid transfer through the free-surface inside a stirred-tank bioreactor while using numerical tools.

Chapter 7 demonstrates the ability of Computational Fluid Dynamics (CFD) to simulate these three key physical phenomena in the reference baffled stirred-tank configuration. Transient simulations are carried out using a homogeneous Euler-Euler multiphase flow model coupled with a Reynolds-Averaged Navier-Stokes (RANS) turbulence closure model. Mixing and gas-liquid transfer through the free-surface are both computed, which makes it possible to follow the spatio-temporal evolution of local concentrations of inert tracer and dissolved oxygen. The numerical results are first globally and locally characterized by means of statistical analyses. These outputs are then reduced to the most relevant dynamics by applying the POD and DMD techniques. All simulations are discussed based on the comparison with PIV and PLIF experimental data.

Chapter 8 investigates the validation of a Euler-Lagrange modeling approach coupling a CFD-based compartment model (CFD/CM) and a stochastic model based on a Continuous-Time Markov Chain (CTMC/SM). This association of numerical models is much less demanding in computational time than CFD and offers the possibility to render (bio)chemical kinetics at much lower computational cost. The evolution of mixing of an inert tracer and oxygen transfer through the free-surface in the reference baffled stirred-tank configuration is reproduced using the CFD/CM and compared with PIV and PLIF experimental results. The CTMC/SM is then added to the CFD/CM to simulate the displacement of virtual particles, in order to determine residence and circulation time distributions in different zones of the vessel. Such information thus enables the mapping of the exposure time and frequency of particles under potential sensitive conditions according to basic bioreactor considerations, for example fluctuating dissolved oxygen concentrations experienced by the cells due to free-surface aeration.

"Part 3 - Application" is dedicated to the illustration of the main inputs of the developed characterization methodology by modeling the impacts of a modification in the agitation configuration on physical mechanisms ruling mixing and gas-liquid transfer through the free-surface.

Chapter 9 focuses on the comparison of hydrodynamics, mixing and gas-liquid transfer through the free surface within two standard pilot-scale stirred-tank configurations with almost the same working volume but very different flow patterns. The rotating speed of both vessels is chosen to reach similar global mixing time based on experimental tracer measurements, in order to study the effects of homogenization and surface renewal on oxygen transfer through the free-surface. Characterization of the three physical phenomena is performed using the Euler-Lagrange modeling approach coupling the CFD/CM and the CTMC/SM. The results are discussed on the basis of spatial heterogeneities induced

by the turbulent flow and fluid particles trajectories within the two tanks.

The last part of this PhD dissertation summarizes all acquired information and suggests diverse perspectives.

Chapter 10 consists of the description of the main contributions and potential development of the work carried out. Besides providing further understanding and quantification of hydrodynamics, mixing and gas-liquid transfer through the free-surface within baffled stirred-tank bioreactors, the presented methodology, in particular the numerical models, can serve as design tools to come up with rational choices about the agitation configuration and operating conditions as parts of scaling prospects.

Chapter 11 emphasizes that many experimental and computational challenges still need to be addressed in order to better assess the coupling of flow dynamics with mixing and gas-liquid transfer processes, to conduct more realistic computational models and, ultimately, to have an outright contribution of the developed characterization approach to bioreactor optimization.

Contents

Abstract The growth of industrial biotechnology has created a pull for advancing bioreactor design. The requirements of the culture system have led to a variety of technical issues that generally involve transfer of mass and energy. Predicting bioreactor performance has proved to be complex as it requires not only a deep knowledge of all the biological aspects, but also a proper characterization of transport and transfer phenomena within the bioreactor which are equipment design and scale dependent. In stirred-tank bioreactors, hydrodynamics governs bulk fluid mixing and gas-liquid mass transfer. The understanding and quantification of these three physical key aspects and of their interactions are required within the framework of scale-up or scale-down. Due to their simplicity, traditional scaling criteria based on global quantities are obviously not able to account for the intricacy of the local hydrodynamics, mixing and mass transfer properties.
This dissertation is part of a project aiming at a better mastering of phenomena linked to gas-liquid transfer that govern the performance of biochemical processes. It studies the influence of mixing and circulation imposed by hydrodynamics, within a baffled stirred-tank reactor, on the gas-liquid transfer through the liquid free surface and on the spatiotemporal distribution of the dissolved gas concentration. The major thrust of this work is to improve the description of fluid dynamics, mixing and gas-liquid mass transfer in stirred-tank bioreactors. The main input is the development and validation of a characterization experimental and computational approach that allows understanding, quantifying and modeling these multiscale transport and transfer phenomena during bioreactor implementation, in particular the selection of agitation configuration and operating conditions

Résumé La croissance de la biotechnologie industrielle a révélé l'importance d'améliorer la conception des bioréacteurs. Les exigences afférentes aux micro-organismes ont conduit à une variété de problèmes techniques, impliquant généralement un transfert de matière et d'énergie. La prédiction des performances d'un bioréacteur s'avère complexe car elle nécessite non seulement une connaissance approfondie de tous les aspects biologiques, mais aussi une caractérisation appropriée des phénomènes de transport et de transfert dans le bioréacteur, dépendants de la conception et du dimensionnement de l'équipement. Dans les bioréacteurs à cuve agitée, l'hydrodynamique régit le mélange du milieu réactionnel et le transfert gaz-liquide. La compréhension et la quantification de ces trois aspects physiques fondamentaux et de leurs interactions sont nécessaires dans le cadre d'une montée ou d'une descente en échelle. En raison de leur simplicité, les critères d'extrapolation traditionnels basés sur des paramètres globaux ne permettent pas de rendre compte de la complexité des propriétés locales propre à l'hydrodynamique, au mélange et au transfert de matière.
Cette thèse de doctorat fait partie d'un projet visant à mieux maîtriser les phénomènes liés au transfert gaz-liquide qui contrôlent le rendement des bioprocédés. Plus particulièrement, ce travail étudie l'influence du mélange et de la circulation imposées par l'hydrodynamique au sein d'un réacteur à cuve agitée, sur le transfert gaz-liquide à travers la surface libre et sur la distribution spatio-temporelle de la concentration en gaz dissous. L'objet de cette étude est ainsi d'améliorer la description de la dynamique des fluides, du mélange et du transfert de matière gaz-liquide dans les bioréacteurs à cuve agitée. Sa contribution principale est le développement et la validation d'une approche de caractérisation expérimentale et numérique permettant de comprendre, de quantifier et de modéliser ces phénomènes de transport et de transfert multi-échelles lors de la mise en oeuvre du bioréacteur, notamment la sélection du dispositif d'agitation et des conditions opératoires.

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