Novel milliliter scale, external-loop-airlift bioreactors for cell growth studies: low-cost assembly , CFD analysis and experimental characterization

Jorge Eduardo Marroquín-Fandiño[5], **Carlos Manuel Ramírez-Acosta**[2]*, Hector Javier Luna-Wandurraga [1], Joaquín Andrés Valderrama-Rincón [1], Juan C. Cruz [3,4], Luis H. Reyes [2], and Juan D. Valderrama-Rincon [1*]

[1] Grupo GRESIA, Department of Environmental Engineering, Universidad Antonio Nariño, Bogotá, Colombia
[2] Grupo de Diseño de Productos y Procesos (GDPP), Department of Chemical and Food Engineering, Universidad de los Andes, Bogotá, Colombia
[3] School of Chemical Engineering and Advanced Materials, The University of Adelaide, South Australia, 5005, Australia
[4] Department of Biomedical Engineering, Universidad de los Andes, Cra. 1E No. 19a – 40, Bogotá, DC 111711, Colombia
[5] Department of Chemistry, Universidad Distrital, Bogotá, Colombia

***Correspondence:**
Corresponding: Juan C. Cruz: juan.cruz@adelaide.edu.au, Luis H. Reyes: lh.reyes@uniandes.edu.co

Keywords: Scale-up, airlift bioreactors, CFD, Optical sensors, Cell growth.

Abstract

Introduction

Small-scale systems are of great interest as they can help reduce consumption of expensive reagents, facilitate the handling of the by -products, and reduce economic costs related to fully instrumented laboratory equipment. R esearch laboratories are therefore becoming more interested in implementing bioreactors with volumes in the milliliter scale Consequently, there has been an increased interest in the design, manufacturing, and testing of such systems . Small-scale systems may also represent an advantage when prototyping as they help to analyze key performance parameters that later could be used in a scale-up scheme. These millibioreactor systems might find many different applications , but the research in bioprocess engineering and particularly in the study of cell growth is quite appealing. This is because the standard flasks in an incubator shaker fail to achieve proper mixing conditions compared with millibioreactors . Moreover, the ease of assembly of milibioreactors facilitates testing different geometries and configurations and in particular those related to modular air-agitated systems such as bubble columns and airlift systems . Here, we introduce a new family of millibioreactors that can be manufactured at low cost and be adjusted to the needs of the experiment with no extra capital investments. Due to its simplicity, flexibility, ease of assembling and testing and superior mixing conditions, we selected the external-loop airlift configuration for a proof-of-concept. Prior to manufacturing, we conducted a throughout *in silico* evaluation through computational fluid dynamics (CFD). With this information, we assembled the system after manufacturing its parts via additive manufacturing and molding under 100 USD cost. The system was instrumented with a home-made optical absorbance sensor (OAS) for the in-line and real-time measurement of cell biomass.

Methods

This system was evaluated *in silico* to characterize the system hydrodynamics using CFD simulations in COMSOL Multiphysics (v.5.3.1). This was critical to estimate whether a home-made monitoring device for biomass was suitable for in-line measurements in the downcomer as it requires no continuous pass of air bubbles. Moreover, it was valuable to assess the proposed operation conditions and guarantee the system's ideal mixing. The computational domain was set in a 2D geometry and focused on the solution of fluid flow dynamics and mass transfer of a two-phase fluid. Also, a convergence analysis was conducted to assure mesh quality. The bioreactor was then manufactured in polyester resin, which allows casting the pieces separately. The pieces were assembled and bonded using manufacturing techniques. The Hydraulic retention time (HRT) distribution was conducted as the reactor was operating under continuous conditions. Our results confirmed the feasibility of installing an optical absorbance sensor (OAS) as a monitoring device of biomass. The optical sensor was cased in a black PLA material printed in the laboratory so that there is no other light source interference. Then, the agitation and the mixing were tested simultaneously with the aid of BG tracer, placing the OAS in the downcomer's glass tube and measuring the absorbance at 420 nm, 630 nm, and 680 nm for 24h. Moreover, we tested four different configurations in the inlet and outlet of the system and the possibility of more than one bioreactor connected in series. Finally, as the main objective of the milibioreactor is the study of cellular growth, *Escherichia coli* MC4100 was selected as the model microorganism to test the performance of the system.

Results and discussion

The performance and behavior of the milibioreactor were, as expected, of a homogeneous ideal mixing airlift bioreactor, with the possibility of measuring in-line and real-time biomass formation as demonstrated by reliable measurements of the *E. coli* MC4100. The *in silico* design showed the mean velocity and agitation conditions of the milibioreactor with the continuous inlet of air. Also, the simulation results allowed us to confirm the system's capacity for mass transfer between phases. The simulation led us to the correct operating conditions to assure that the downcomer remained bubbleless, and the shear stress was low enough not to compromise the cellular integrity.

The assembled reactor was first calibrated and then tested with a tracer. The calibration was made such that the OAS could measure with no difference from a spectrophotometer. The results showed that the OAS presented similar behavior in slope and time response as those from the spectrophotometer but still with different values. This phenomenon may be due to the unique assembly configuration. For example, the LED position with respect to the photoresistor. Despite these possible limitations, the implementation of such a sensor instead of the spectrophotometer reduced the costs of the bioreactor considerably. The OAS system was then used in the tracer test in which two sensors were placed at the inlet and outlet of the reactor so that the HRT could be estimated. The obtained curves were similar for four different configurations. Moving the inlet and outlet and continuous operation between bioreactors led to the conclusion that regardless of the geometrical considerations, the OAS measurements are able to track low absorbance changes. Also, the results show that the operation of three millibioreactors in series improves the retention time drastically. Finally, the growth rate and maximum cell density obtained by the millibioreactor are similar to previously obtained by similar methods.

Moreover, the kinetic constants obtained for the organism agree well with those reported in a lab-scale production, regardless of the volume. In conclusion, the proposed system is an economical alternative for starting the scale-up of a bioprocess and the research experimentation of continuous cell growth. The bioreactor proposed here offers a path for proof-of-concept experiments, reducing the reagents consumption and overall operation costs. The prototype can be considered as the beginning of a small-scale research project in bioprocess engineering. It enables the possibility of measuring cell density in-line and in real-time. Some of this device's applications include changes in substrates, optimization of productivity, or even response to inhibitors, which are typical in the field.