I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ** XV SHEB ** XV ENZITEC FLORIANÓPOLIS ** 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
BIOPROCESS ENGINEERING

EFFECT OF INTERNAL TUBE INCLINATION ON MIXING TIME IN AIRLIFT REACTOR WITH THREE-PHASE MIXING

Nicole Brena Marques Soares[1], Daniele Gonçalves de Oliveira[1], Vitor Teixeira Mazziero[1], Bruno Willian Picão[1], Ivan Ilich Kerbauy Veloso[1] & Marcel Otávio Cerri[1*]

[1] *São Paulo State University (UNESP), Dept. of Bioprocess Engineering and Biotechnology, School of Pharmaceutical Sciences, Araraquara, São Paulo, Brazil*

** Corresponding author's email address: marcel.cerri@unesp.br*

ABSTRACT

The operation of an airlift bioreactor involves no moving parts, reducing construction complexity and contamination risk. Internal geometry changes in this reactor can improve homogenization and mixing efficiency. Variations in inner tube geometry, including diameter, crucially control gas, liquid, and solid flow, influencing hydrodynamic parameters. Thus, in this work a study was carried out on different internal tubes, varying their diameters to obtain the shortest mixing time of a three-phase flow with a solution of 30% glycerin, air (1vvm) and 2% (v/v) PLA (polylactic acid) in relation to the liquid phase. Inner tube designs were 3D printed using Autodesk® Fusion 360 and Creality® CR10s Pro v2. The conical shape of the tube optimized mixing efficiency by increased fluid outlet velocity in both the riser and the downcomer, resulting in the total recirculation of three-phase mixing. However, excessive reduction in top diameter obstructed the riser, compromising mixing efficiency. Tubes with larger top diameters and smaller base diameters yielded favorable results but were inefficient in separating the riser and downcomer regions, leading to intermittent settlement of solid particles and two-phase system formation.

Keywords: Airlift bioreactor. Mixing time. Geometry. Three-phase. Internal tube.

1 INTRODUCTION

The airlift bioreactor works without moving parts, presenting less construction complexity and less contamination due to the lack of a mechanical seal, which is present in agitated tanks[1]. Concentric tube airlift reactors have an inner tube, placed exactly in the sprinkler region, creating an upward gas-liquid flow in the region known as the riser and a region known as the downcomer, where the liquid descends externally to the tube[2]. Due to several advantages, these bioreactors have important applications in biotechnological processes, such as the production of biomolecules, the culture of animal cells and the treatment of effluents[3-4-5].

When designing an airlift bioreactor, parameters such as mixing time must be analyzed to better understand the bioreactor's hydrodynamics. The mixing time (t_m) is the time required for a substance to mix inside the bioreactor, making the medium homogeneous again[6]. The determination of this parameter is carried out through pulse-type tests, which a certain variable change after the injection of a tracer. The variables observed to complete this test are temperature, pH, conductivity and absorbance[7]. The quality of the mixture is an important factor in the performance of bioreactors, as it provides microorganisms with an adequate environment for their growth, and which makes them produce their metabolites[8]. Geometric modifications in airlift bioreactor can promote different operational and hydrodynamic conditions[9].

Therefore, it is known that hydrodynamic parameters of the reactor can be changed according to the characteristics of the mixture. The addition of solids in gas-liquid system, for example, influences the mixing capacity and hydrodynamic performance of airlift, where factors such as mass and heat transfer, size, distribution and rising speed of bubbles and viscosity of the liquid can alter the homogenization capacity of the medium[10].

The airlift's internal geometry also influences its hydrodynamic performance. In an internal circulation airlift, for example, the inner tube is responsible for directing the flow of gaseous, liquid and solid phases, promoting a cyclic mixture within the reactor[11]. The ratio between the diameter of the internal tube and the diameter of the airlift column determines the general behavior of the reactor and the pressure losses in the system, as it divides it into two flow zones, the riser and downcomer[12]. Some studies showed geometric changes in the inner tube. Hu et al. (2020)[13] for example, investigated the use of an airlift bioreactor with internal tubes of variable diameter in several stages. The results obtained showed that the use of variable diameters can increase the liquid velocity inside the bioreactor, resulting in a significant improvement in mass transfer. In this context, the present project proposes the modification and study of different inclined internal tubes for the application of multiphase mixtures.

2 MATERIAL & METHODS

The internal-loop airlift bioreactor, constructed from transparent borosilicate glass and a stainless-steel base, features a useful volume of 1L with an internal diameter of 75 mm. The bioreactor is operated with a crosshead sprinkler having a 44 mm diameter. The experiment was conducted in triplicate with a 30% glycerin solution (viscosity of 0.0026 Pa. s and density of 890 kg/m³) as liquid phase, compressed air at 1vvm as gas phase and 2% (v/v) crushed PLA in relation to the liquid phase. The PLA was ground into 12 mesh in a knife mill with a density equal to 1240 kg/m³ as a solid phase.

The bioreactor, maintained at 20°C by a thermostatic bath via the jacket, was equipped with three temperature sensors arranged in different locations, allowing temperature monitoring throughout the reactor. These sensors were integrated through an Arduino microcontroller board to facilitate data collection. In parallel, various inner tubes, exhibiting different inner diameters, as shown in Table 1 were designed employing Autodesk® Fusion 360 and 3D printed using PLA on a Creality® CR10s Pro v2.

The determination of mixing time involved the temperature pulse method[14]. Specifically, 50 mL of the 30% glycerin solution was heated to 70°C and introduced into the bioreactor. The mixing time was then calculated based on the temperature difference between the initial pulse injection and the stabilization of temperature as indicated by the three sensors. The volume equivalent to each pulse injection was subsequently removed from the bioreactor to maintain a constant volume.

Table 1 Draft tube dimensions design for the tests in airlift bioreactor.

Draft tube	$Ø_T$ (mm)	$Ø_B$ (mm)
DT - 1	50	50
DT - 2	40	60
DT - 3	35.8	50
DT - 4	60	40
DT - 5	50	35.8

3 RESULTS & DISCUSSION

Several articles demonstrate how geometry and its changes in a bioreactor can affect hydrodynamic factors [7,15,16]. De Oliveira (2021) [17], for example, proposed the use of a conical tube together with a rounded bottom to improve the mixing time of the three-phase system with different percentages of solids, these being represented by enzymes.

To optimize the mixing time of the airlift bioreactor, most of the internal tubes in the present work have a conical trunk geometry, making it necessary to compare their mixing times in relation to those with a constant diameter. The results are shown in Table 2.

Table 2 Values found from the mixing time tests for the different tubes tested.

Draft tube	t_m(s)
DT - 1	36.33 ± 1.53
DT - 2	18.67 ± 0.58
DT - 3	25.67 ± 1.15
DT - 4	26.33 ± 3.06
DT – 5	22.00 ± 2.65

In all tubes it was possible to observe a drop-in mixing time compared to the concentric tube with constant diameter.

The tube DT-2 exhibited the shortest mixing time (18.67 ± 0.58) compared to the other tubes. The reduction in mixing time can be linked to the smaller top diameter, which increases the exit velocity of the mixture. This means that the dispersion of bubbles in the disengagement zone is reduced, allowing the liquid to rise faster through the riser and increasing the return of the mixture through the downcomer. At the same way, the increase in base diameter also contributed to improving mixing efficiency, reducing the annular space, and improving the performance of the three-phase system. Similar results were achieved in the studies conducted by De Oliveira et al. (2024)[15] while exploring various internal tubes with varying inclinations and diameters within an internal circulation reactor to enhance the triphasic mixture during an acidolysis reaction with immobilized enzymes. The authors noted that using an inner tube inclined at 5º with a top diameter of 64 mm led to a reduction in mixing time and enhanced homogeneity in three-phase mixing.

The DT-3 shares the same configuration as the DT-2, but promoted a slightly longer mixing time, this can be explained by an obstruction in the riser, where the top diameter may have been reduced too much. This obstruction was evident during the experiments, as sedimentation of the solids at the bottom of the bioreactor was observed, potentially due to a reduction in the outlet velocity of three-phase mixing in this region. As a result, the mixing efficiency of the three-phase system was compromised.

Tubes with larger top diameters and smaller base diameters, such as DT-4 and DT-5, yielded favorable mixing results compared to those with constant diameters. However, these tubes, particularly DT-5, featured base diameters smaller than the size of the sprinkler, leading to inefficient separation of the riser and the downcomer. During experiments, bubbles were observed rising through the downcomer, resulting in inefficient mixing of the three-phase system and intermittent settlement of solid particles, leading to a two-phase system. As a result, despite DT-5 exhibiting the second shortest mixing time (22.00 ± 2.65), it was deemed the least efficient in relation to homogenization.

4 CONCLUSIONS

Through experimental observations following modifications to the inner tube, it was observed that adjustments in inner tube diameter have a significant impact on the performance of the bioreactor. A tube with a reduced top diameter and an increased bottom diameter proved to optimize the process by improving the mixing time, as evidenced in scenario DT-2. However, it is essential to ensure that the top diameter is not excessively smaller, as this can cause an obstruction in the riser and hinder efficient mixing, as observed in the DT-3 case. In addition, inverted conical tubes are not ideal for the bioreactor, due to their smaller base diameter compared to the sprinkler, which results in inadequate mixing in the riser and downcomer regions.

REFERENCES

1 JUNIOR, C. G. **Optimization of dissolved oxygen control in a pressurized airlift bioreactor:** application in recombinant *Escherichia coli* cultures. Thesis (Doctorate in Chemical Engineering) - Center for Exact Sciences and Technology, Federal University of São Carlos. São Carlos, 2018.

2 KASPARY, M.R. **Production of polyglutamic acid in submerged cultivation in an airlift bioreactor from bacteria cells of the genus *Bacillus*.** Thesis (Doctorate in Environmental Sciences) - Institute of Science and Technology, São Paulo State University. Sorocaba, 2022.

3 AL-QODAH, Z. Continuous production of antibiotics in an airlift fermenter utilizing a transverse magnetic field. **Applied biochemistry and biotechnology**, vol. 87, p. 37-55, 2000.

4 COZMA, P.; GAVRILESCU, M. Airlift reactors: applications in wastewater treatment. **Environmental Engineering & Management Journal (EEMJ)**, v. 11, no. 8, 2012.

5 BADINO, C. A. et al. Pneumatic Bioreactors: Simple and Efficient. **Brazilian Journal of Chemical Engineering**, p. 24–33, 2016.

6 ZHANG, T. et al. Impacts of solid physical properties on the performances of a slurry external airlift loop reactor integrating mixing and separation. **Chinese Journal of Chemical Engineering.** V. 55, March 2023, Pages 1-12. https://doi.org/10.1016/j.cjche.2022.05.001

7 ESPERANÇA, N. M. **Influence of geometric aspects on the hydrodynamics and oxygen transfer of internal circulation airlift bioreactors**. Thesis (Master's degree in Chemical Engineering) - Center for Exact Sciences and Technology, Federal University of São Carlos. São Carlos, 2014.

8 CERRI, M. **Hydrodynamics and oxygen transfer in three geometrically similar internal circulation airlift bioreactors**. Thesis (Doctorate in Chemical Engineering) - Center for Exact Sciences and Technology, Federal University of São Carlos. São Carlos, 2009.

9 MERCHUK, J.C. Concentric-Tube Airlift Geometrical Design Reactors: Effects of on Performance. **American Institute of Chemical Engineers Journal,** no. 7, vol. 40. Jul. 1994.

10 AL-AZZI, Ali Abdul-Rahman; AL-KUFFE, Laith SS. Influence of Draft Tube Diameter on Operation Behavior of Air Lift Loop Reactors. **Al-Khwarizmi Engineering Journal**, v. 6, n. 2, p. 21-32, 2010.

11 BADINO, C. A. et al. Pneumatic Bioreactors: Simple and Efficient. **Brazilian Journal of Chemical Engineering**, p. 24–33, 2016.

12 AL-AZZI, Ali Abdul-Rahman; AL-KUFFE, Laith SS. Influence of Draft Tube Diameter on Operation Behavior of Air Lift Loop Reactors. **Al-Khwarizmi Engineering Journal**, v. 6, n. 2, p. 21-32, 2010.

13 HU, D. et al. A sustainable anti-infective pharmaceutical wastewater treatment technology: multi-stage vertical variable diameter membrane bioreactor with DO online controlling. **Bioresource Technology**. 311 (2020). https://doi.org/10.1016/j.biortech.2020.123507.

14 MAYR, B. HORVAT, P. MOSER, A. Engineering approach to mixing quantification in bioreactors. **Bioprocess Engineering**. (1992) 137–143. https://doi.org/10.1007/BF01254229.

15 DE OLIVEIRA, G. D. et al. Effect of geometrical modifications on the mixing and enzymatic conversion of structured lipids in internal-loop airlift bioreactors. **Biochemical engineering journal,** p. 109316–109316, 2024. https://doi.org/10.1016/j.bej.2024.109316.

16 TAO, J. et al. Experimental investigation of hydrodynamics and mass transfer in a slurry multistage internal airlift loop reactor. **Chemical engineering journa**l (1996. Print), v. 386, p. 122769–122769, 2020. https://doi.org/10.1016/j.cej.2019.122769.

17 DE OLIVEIRA, G. D. Adaptation of the airlift-type bioreactor for enzymatic reaction using immobilized lipase® in the synthesis of MLM-type structured lipids. 2021.

ACKNOWLEDGEMENTS

I want to express my gratitude to my research group for their valuable advice and knowledge contribution, particularly Daniele Gonçalves De Oliveira and Marcel Otávio Cerri for their support and teachings. I also extend special thanks to São Paulo State University for funding this work (ID:7720). FAPESP; CAPES (Financing Code 001); CNPq.