EFFECT OF ADDING SOLIDS ON THE MIXING TIME OF AN INTERNAL LOOP AIRLIFT REACTOR WITH VISCOUS LIQUID

Daniele G. Oliveira[1]*, Vítor T. Mazzieiro[1], Bruno W. Picão[1], Mateus N. Esperança[2], Ariela V. Paula[1] & Marcel O. Cerri[1]

[1] Department of Bioprocess Engineering and Biotechnology, Paulista State University (UNESP), Araraquara, Brazil.
[2] Federal Institute of Education, Science and Technology of São Paulo (IFSP), Capivari, Brazil.
** Corresponding author's email address: dg.oliveira@unesp.br*

ABSTRACT

Bioprocesses involving cells and enzymes immobilized necessitate well-designed bioreactors and optimal conditions to ensure efficient biocatalyst performance and process viability. Airlift-type pneumatic bioreactors, renowned for their mixing efficiency and low energy consumption, offer a promising solution for such processes, particularly in solid-liquid-gas systems. This study explored the influence of solids, specifically immobilized lipases, on mixing time within an internal loop airlift (1 L, working volume) reactor utilized for acidolysis grape-seed oil, aiming to produce structured lipids. The liquid phase consisted of grape-seed oil, the gas phase of comprised compressed air, and the solid phase utilized was Lipozyme RM IM® (RM IM). Mixing time was identified using the temperature pulse technique, for this utilized solid load from 0 to 3% (v/v) in relation to the liquid phase and airflow from 1 to 3 vvm. A decrease in mixing time across the various airflow rates studied was correlated with an increase in solid load, indicating improved mixing efficiency and enhanced bioreactor performance.

Keywords: Airlift bioreactor. Mixing time. Three-phase mixing. Grape-seed oil. Immobilized lipase.

1 INTRODUCTION

In the biotechnology industry, three-phase processes involving distinct gaseous, liquid, and solid phases as those with cells and immobilized enzymes are extensively utilized for producing diverse products, including antibiotics, monoclonal antibodies, vitamins, organic acids, and structured lipids[1,2]. Pneumatic airlift reactors with concentric tubes are excellent choices for these processes. These bioreactors promote mixing by introducing gas at the bottom of the reactor. They excel in mass and heat transfer, offer low shear stress and ensure good homogeneity with minimal energy consumption[3,4].

Nevertheless, the inclusion of solid particulates within the gas-liquid systems impacts the mixing efficiency and hydrodynamic performance of the airlift reactor[5]. Some authors have demonstrated both positive and negative effects on various hydrodynamic parameters, such as gas holdup, circulation velocity, mass and heat transfer, and the volumetric gas-liquid mass transfer coefficient[6,7,8,9]. Besides these parameters, another very important one when studying the performance of bioreactors is the mixing time.

Mixing time (t_m) is a hydrodynamic parameter representing the time required for homogenization of the medium within the reactor. It is essential for airlift bioreactor design as uniform substrate dispersion directly impacts process efficiency[10,11].This parameter is influenced by axial and radial mixing and is measured using tracers to monitor variations in temperature, conductivity, pH, and absorbance[10].

In this context, the objective of this study is to investigate the impact of solids (specifically Lipozyme RM IM®) on the mixing time of an airlift reactor for acidolysis involving grape-seed oil for producing structured lipids.

2 MATERIAL & METHODS

The three-phase system for enzymatic acidolysis consisted of a pneumatic system with the solid phase composed of immobilized lipase from Rhizomucor miehei (Lipozyme RM IM®, Novozymes A/S - Bagsvaerd, Denmark), a liquid phase of grape-seed oil (Vitis vinifera L.), and compressed air as the gas phase. The process took place in a 1L internal-loop airlift reactor with a diameter of 90.8 mm (Figure 1a). The reactor utilized a cross-type sparger with a diameter of 0.545 m, having 0.5 mm diameter holes spaced 2 mm apart.

To obtain the density of Lipozyme RM IM, a 10 mL volumetric flask was weighed empty and then filled with distilled water at a known temperature. The water mass was determined by the difference in weight, and the volume was calculated using the mass/density ratio of the water. After drying the flask, 0.1 g of RM IM was weighed and transferred into the flask. A small volume of water was added, and the system was kept under vacuum to remove air bubbles. The flask was then filled with water and weighed again. The water mass was determined by the difference in weight, and the volume was calculated similarly. The enzyme immobilized density was calculated by the difference in water volumes with and without the immobilized enzyme, resulting in the solid volume. The crystalline density was obtained using Equation (1), where d is the density, V is the solid volume, and m is the mass of the biocatalyst. Thus, RM IM density is 1842.9 ± 122.1 kg/m^3.

$$d = \frac{m}{v} \quad (1)$$

The dynamic viscosity and density of grape seed oil were assessed using a Stabinger Viscosimeter™ Anton Paar (SVM 3000) viscometer, resulting in values of 0.024 Pa·s and 920 kg/m³, respectively.

The mixing time was determined using the temperature pulse technique[11]. To achieve this, an Arduino microcontroller board interfaced with three DS18B20 temperature sensors, which were strategically positioned at different points within the reactor (Figure 1b). A custom data collection program, developed by the research team, operated this setup. The mixing time (t_m) was calculated as the time taken for the temperature to stabilize across all three sensors after the injection of a thermal pulse, indicating that all sensors had reached the same temperature. The oil was maintained at 45℃ throughout all experiments, with a thermal bath connected to the reactor's thermal jacket to ensure a constant temperature.

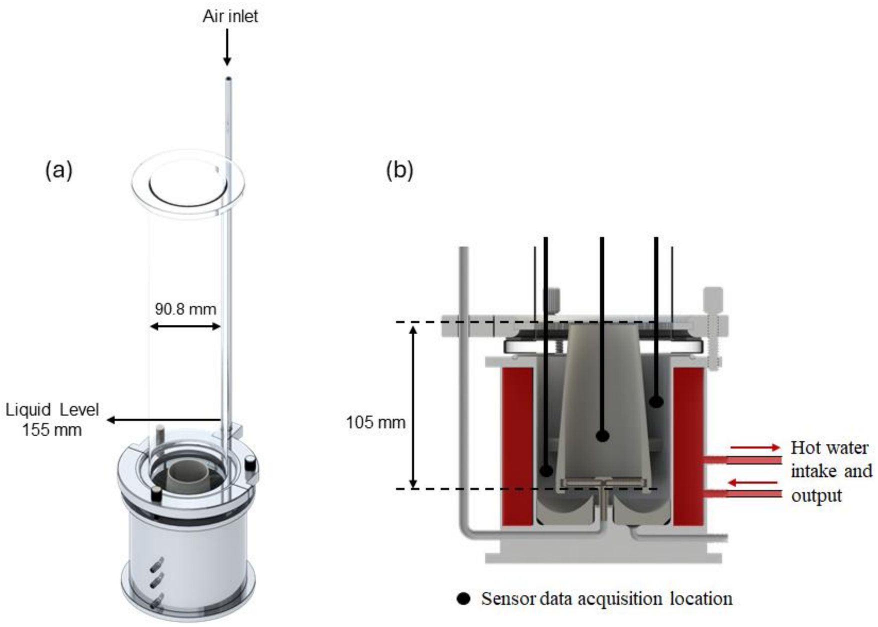

Figure 1 (a) Concentric tube internal-loop airlift reactor. **(b)** Position of the temperature sensors in the airlift reactor for conducting the mixing time test.

In this experiment, 60 mL of grape seed oil, heated to create a thermal pulse with a 50°C difference from the oil inside the reactor, was injected at the liquid surface. The tests were conducted under varying conditions: air-flow rates of 1, 2, and 3 vvm, and solid loads of 1%, 2%, and 3% (v/v) relative to the liquid volume. Tukey's test assessed the significance of differences between experiments with different solid loading and flow rate values, all conducted in triplicate.

3 RESULTS & DISCUSSION

The addition of solids in gas-liquid systems can positively or negatively the mixing efficiency[5,8]. To investigate the influence of solids (Lipozyme RM IM®) influence on mixing in a concentric tube internal-loop airlift reactor for acidolysis reactions for structured lipid production, Figure 2 presents mixing time (t_m) for different conditions of solids loading and airflow rate.

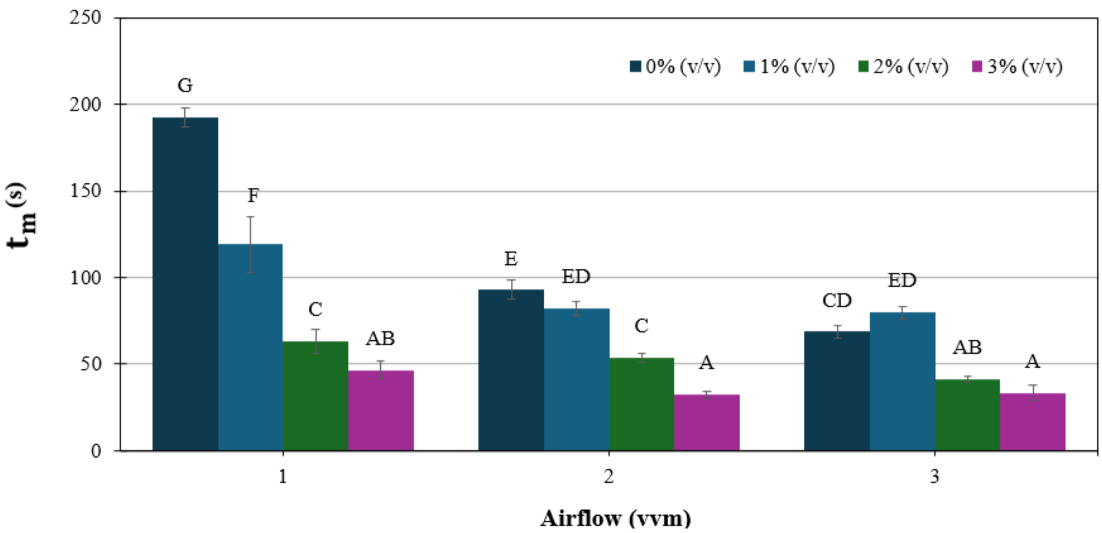

Figure 2 Mixing time obtained by varying solids loading (from 0% to 3% (v/v)) and the airflow (from 1 to 3 vvm). The bars represent the standard deviation of the triplicate and the letters represent significant differences, as assessed using Tukey's test ($p < 0.05$).

Increasing the airflow rate is known to reduce the mixing time[12], this trend was observed in this work. Another significant observation was that increasing the solid load also decreased mixing time at all studied flow rates, as seen in Figure 2. Therefore, the addition of solids to the gas-liquid systems studied positively affected the mixing capacity and bioreactor performance.

These results align with other studies that have shown similar trends, indicating a like effect across different solid-liquid-gas systems. Littejohns et al.[13], in their studies with a concentric tube airlift using water and 10% (v/v) rubber spheres and varying airflows at 0.09, 0.18, 0.27, and 0.36 vvm, observed that the addition of these particles reduced mixing time by about 33% compared to the airlift in a biphasic system. Sharp et al.[14] investigated the effect of using various concentrations of immobilized cells on the hydrodynamics of an external circulation airlift, using distilled water as the liquid phase and varying the load of alginate granules (500 to 800 μm) from 0 to 25%. They observed that, at an airflow rate of 0.5 vvm, adding 10% alginate granules reduced the mean t_m from 75 s to 38 s. Freitas et al.[15] investigated the hydrodynamics of a three-phase mixture in a concentric tube airlift bioreactor, where water was used as the liquid phase and calcium alginate granules as the solid phase. They varied the solid load from 0% to 40% (v/v), the solid density from 1016 to 1038 kg/m^3, and the air rate from 1.9 to 90.2 L/min. The mixing time reached its maximum value at a 20% (v/v) load; above this value, t_m decreased. The authors suggested that increasing the solid load increases collisions between particles, resulting in more intense vortices, thus improving mixing efficiency.

The influence of solids on the gas-liquid system mixing depends on their physicochemical characteristics, as well as the properties of the liquid phase[5]. For the percentages of solids and airflow used in the present study, a positive effect was observed. However, it is believed that at other percentages and airflow rates, these effects could be negative. For a better understanding of this phenomenon, more studies should be conducted.

4 CONCLUSION

The addition of different loads of Lipozyme RM IM® to the pneumatic system with grape-seed oil at the three air flow rates studied promoted a positive effect on the mixing of the concentric tube airlift reactor because there was a decrease in mixing time. The reduction in mixing time signifies improved homogeneity and avoidance of stagnant zones within the reactor, essential for efficient biocatalyst performance and process viability.

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