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ANALYSIS OF RESIDENCE TIME DISTRIBUTION PARAMETERS IN THREE REACTOR DESIGNS FOR CONTINUOUS-FLOW ENZYMATIC REACTIONS

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ABSTRACT

The study of continuous-flow enzymatic microreactors has significant industrial implications, especially in the use of enzyme-based biosensors for continuous detection of biomolecules. This work investigated the impact of the transition of peroxidase reactions from batch to continuous-flow operation in three flow distribution geometries. Employing a crude enzymatic extract containing approximately 18 U mL^{-1} of peroxidase activity and guaiacol as tracers, the main residence time distribution (RTD) parameters were quantified. Additionally, catalytic efficiency was measured in continuous-flow assays and compared to batch assays by calculating relative enzymatic activity. The RTD results revealed a potential mismatch between enzyme and substrate in the reaction volume due to diffusion phenomena. Also, the RTD curves indicated a non-ideal system with dead zones for both tracers and designs. Despite this, the continuous-flow conversion assays of guaiacol to tetraguaiacol showed that diffusion did not significantly compromise catalytic efficiency. The relative enzymatic activity in reactor design C was higher (89%) than in designs A and B. This can be attributed to the axial mixture of enzyme and guaiacol. It is essential to highlight that all three reactor designs exhibited relative activity >70%, suggesting the potential application of these microreactors for conducting enzymatic reactions in continuous flow.

Keywords: 3D-Printing. Reactor Development. Diffusivity. Contact Time. Fluid Dynamics.

1 INTRODUCTION

In enzymatic reactions, the contact time between the enzyme and the substrate is crucial for the active sites to be occupied and the products to be formed.[1] This factor favors the use of batch reactors. However, considering industrial applications, batch processes have disadvantages compared to flow systems. For example, biochemistry brings interesting applications of flow processes, such as biosensors with peroxidase that can continuously detect biomolecules of interest.[2] This enzyme is one of the most common for labeling antibodies and detecting analytes such as toxins, pathogens, and tumor markers.[3,4]

Considering the change in contact dynamics between enzyme and substrate from batch to flow operation, studies have shown that the reaction can be compromised in enzymatic microreactors operated in continuous flow.[5] The residence time distribution (RTD) of molecules in the reactor can be a determining factor in maximizing substrate conversion. Additionally, the influence of diffusion effects can play an important role in catalytic efficiency.[6] These phenomena can be assessed through RTD assays, in which the concentration of a tracer at the reactor outlet is evaluated over time. Thus, it is possible to observe the behavior of a given solute in contact with its solvent in the reaction volume and how different flow geometries affect this behavior.[7,8,9]

Through an individual analysis of distribution parameters, using peroxidase and guaiacol as tracers in three different flow designs, this study aimed to determine whether there is a mismatch between the enzyme and its substrate in the reaction volume. In addition, by conducting the enzymatic reaction in a continuous-flow regime, it was examined whether this mismatch impairs catalytic efficiency compared to the batch system.

2 MATERIAL & METHODS

The enzymatic extract was produced by *Trichoderma koningiopsis* (MK860714) supplemented with fresh *Chlorella spp.* biomass (89% humidity). After 72 hours of submerged fermentation, the Erlenmeyer content was filtered to remove the fungal and microalgal biomass, and the liquid permeate was centrifuged (2000 rpm and 4 °C for 10 min). The resulting supernatant corresponds to the enzymatic extract used in this work, containing approximately 18 U mL^{-1} of peroxidase activity and 0.1 mg mL^{-1} of total protein.

The continuous-flow devices used in this study are 3D-printed flat-plate reactors with geometries detailed in Figure 1 (restricted access to further details due to patent restrictions). The reactors were made of acrylonitrile-styrene-acrylate (ASA) and operated using a precision syringe pump (Harvard Apparatus, 11 Elite Series). RTD assays were carried out in triplicate using the enzymatic extract and guaiacol (substrate) as tracers. After setting up the system by feeding the reactor with water at a fixed volumetric flow rate, corresponding to a space time of 10 min, a single pulse of 0.5 mL of enzymatic extract was introduced. The pulse for guaiacol (1% v v^{-1}) was also 0.5 mL.

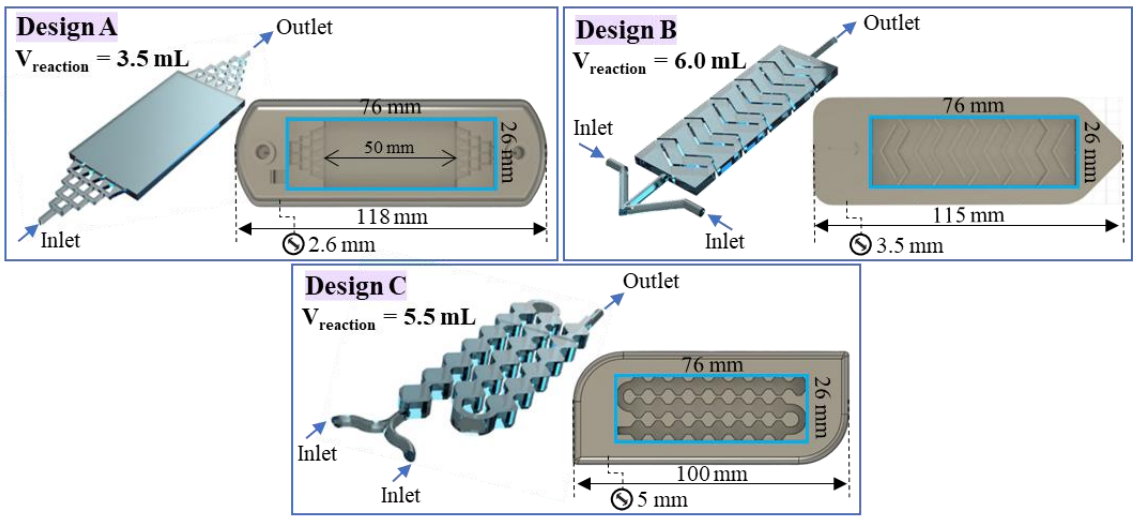

Figure 1 Details of the microstructured 3D-printed flat-plate reactors with different internal flow geometries. The area delimited by the light blue rectangle inside the reactor corresponds to the reaction volume.

After the tracer pulse at the inlet, samples were collected every 45 seconds at the reactor outlet and analyzed in terms of protein concentration using the Bradford reagent for the peroxidase RTD study. For guaiacol analysis, Ultra-fast liquid chromatography (UFLC LC-20AD, DAD detector, Shimadzu) was used. A reverse-phase C18 column (100 Å, 5 µm, 4.6×250 mm) was used, with a mobile phase of methanol and water (50:50 v v^{-1}, 1.0 mL min^{-1}). The oven temperature was maintained at 40 °C, and the injection volume was 20 µL. The concentration of guaiacol was detected at 250 nm and retention time of 5.4 min.

From the experimental data for the two tracers over time, the residence time distribution E(t) and the main RTD parameters were calculated.[8,9] Subsequently, the conversion of guaiacol to tetraguaiacol was monitored using a spectrophotometer (470 nm) in continuous-flow assays. In order to maintain the reaction medium:enzyme ratio identical to that used in batch enzymatic assays (4.5:1 v v^{-1}), the pump flow rates were adjusted to result in a combined flow at the reactor inlet. The catalytic efficiency was compared with that obtained in the batch assay by calculating the relative enzymatic activity.[10]

3 RESULTS & DISCUSSION

The residence time distribution in Fig. 2 shows that the three proposed geometries had a pronounced deviation between the space time (10 min) and the average residence time (t_m) for the enzyme; the deviation from minor to major follows the order A > B > C. For guaiacol, reactor design C had the lower deviation (4.49 min), while designs A and B had a deviation of 8.42 and 6.02 min, respectively. The E(t) curve indicates a non-ideal system with dead zones for both tracers. The RTD parameters presented in Table 1 reinforces the mixing problems for the studied tracers. As reactor design A was previously studied for dyes and no axial mixing occurred, this pronouced behavior for the enzyme can be attributed to its higher molar weight and complex molecular structure.[8,11]

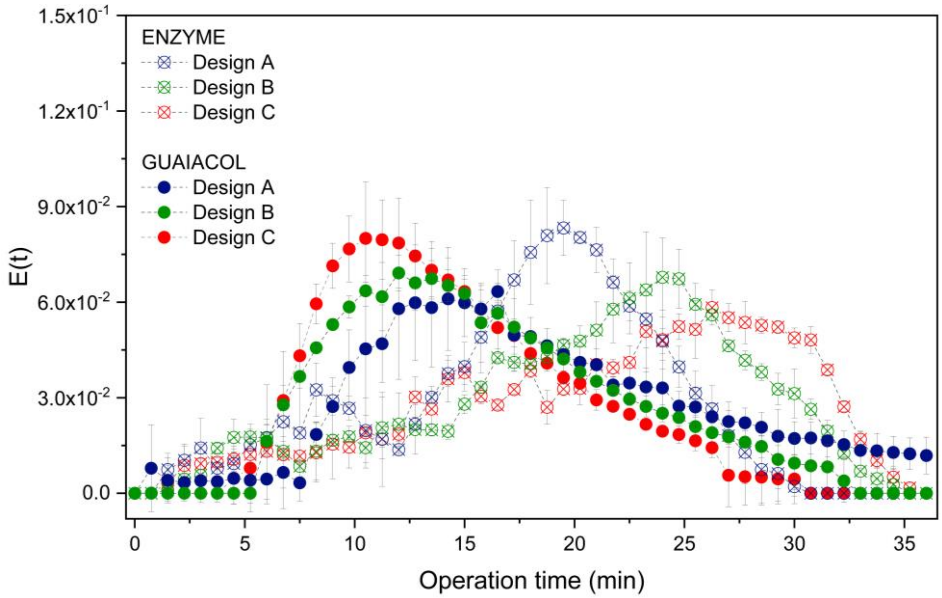

Figure 2 RTD results with a space time of 10 minutes for the tracers enzyme (unfilled symbols) and guaiacol (filled symbols).

The comparison between the t_m values for the enzyme and guaiacol for each geometry raises a concern regarding the catalytic efficiency of continuous-flow enzymatic reactions. As indicated in Fig. 1 and the results in Table 1, the diffusion of both tracers suggests a potential mismatch between enzyme and substrate in the reaction volume.[6] Despite this phenomenon, the continuous-flow conversion assays of guaiacol to tetraguaiacol showed that diffusion did not significantly compromise catalytic efficiency. For the three proposed reactor geometries the relative enzymatic activity was over 70% (Table 1).

The catalytic efficiency, from minor to major, follows the order A > B > C. Similarly, the diffusivity coefficient of guaiacol also follows this same order (Table 1). Thus, the diffusion behavior of guaiacol in reactor design C can explain the favoring of the enzymatic reaction for this flow distribution geometry. The high t_m value of peroxidase in design C reinforces the occurrence of diffusion, which can be explained by the high molar weight of the enzyme.[7] The retention of enzyme and substrate in the reaction volume favors the catalytic reaction and makes the reaction environment more similar to the batch system.

Table 1 Results of the mean residence time (t_m), standard deviation (σ), skewness (s^3), diffusivity coefficient (D), and enzymatic catalytic efficiency obtained experimentally for a fixed space time of 10 min.

Reactor Design	Enzyme				Guaiacol				Catalytic Efficiency* (%)
	t_m (min)	σ (min)	s^3 (min^3)	D (×10^{-6} m^2 s^{-1})	t_m (min)	σ (min)	s^3 (min^3)	D (×10^{-6} m^2 s^{-1})	
A	17.53 ± 0.57	6.12	-9.46	3.38	18.42 ± 1.02	7.26	7.58	198	70.68 ± 0.80
B	20.49 ± 0.01	7.14	-11.41	2.89	16.02 ± 0.69	5.82	7.62	226	72.56 ± 0.36
C	21.43 ± 0.86	7.83	-12.05	2.77	14.49 ± 0.33	5.29	7.24	245	89.27 ± 2.98

*Relative to the enzymatic activity of the batch system (100%).

4 CONCLUSION

The study of the flow distribution parameters for the three reactor designs allowed a quantitative assessment of the effects of peroxidase diffusion compared to guaiacol distribution in the reaction volume. With regard to the difference between the space time and mean residence times for the enzyme and the substrate, a smaller mismatch was observed for design A. Thus, theoretically, this reactor would be the most suitable for conducting enzymatic reactions.

However, diffusion effects were observed in all three geometries and for both tracers, promoting the retention of substrate and enzyme for a longer time in the reaction volume. In this regard, although the RTD results indicate that reactor design C had the most significant mismatch between peroxidase and guaiacol, the enzymatic reaction was favored in this flow distribution geometry due to the diffusion and the enzyme's ability to convert the substrate over a wide concentration range.

Notably, all three studied reactors exhibited relative enzymatic activity above 70%, showcasing the potential applications of these flow designs for conducting enzymatic reactions in continuous flow, despite behaving as a non-ideal system with dead zones.

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