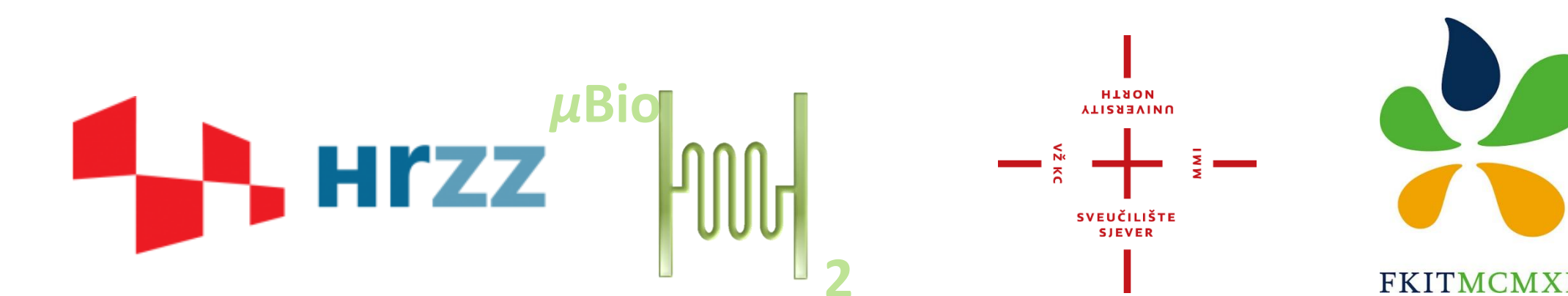




OPTIMIZATION OF GLUCOSE DEHYDROGENASE IMMOBILIZATION IN A 3D-PRINTED MILLIREACTOR



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Project: IP-2022-10-2175

Source of funding: The Croatian Science Foundation

Project leader: Prof. Bruno Zelić, PhD

Project: Integrated micro-system for enzymatic biohydrogen production

Acronym: MicroBioH₂

INTRODUCTION

Biocatalytic reactions based on enzymes are commonly used as part of main or auxiliary processes in the pharmaceutical, food, or biofuel industries. Unfortunately, enzymes lose their stability and activity when taken out of their natural environment, which is one of the main problems when using enzymes as industrial catalysts. **Fixation or entrapment of enzymes in solid supports is a promising immobilization technique to improve their stability and activity.** Immobilization also helps in the application of enzymes in continuous flow systems such as millireactors. **The dimensions of millireactor channels allow for better control of reaction conditions,** resulting in higher efficiency and product consistency. Glucose dehydrogenase (GDH) is an important enzyme with numerous applications in biotechnology, characterized by its ability to catalyse the oxidation of glucose to glucono- δ -lactone with simultaneous reduction of the coenzyme nicotinamide adenine dinucleotide (NAD⁺ to NADH). Like many other enzymes, GDH can also denature and lose activity over time. **The combination of millireactors and immobilization offers a promising solution** to these challenges as it provides a controlled environment that can improve the stability and activity of the enzyme.

The aim of this study was to investigate the effects of different enzyme immobilization strategies on the performance of GDH from *Pseudomonas* sp. in a 3D-printed millireactor.

EXPERIMENTAL

The millireactor (Figure 1) was designed using Autodesk Fusion CAD software v2.0.20256. The layer height, which corresponds to the z-axis resolution, was set to 50 μ m.

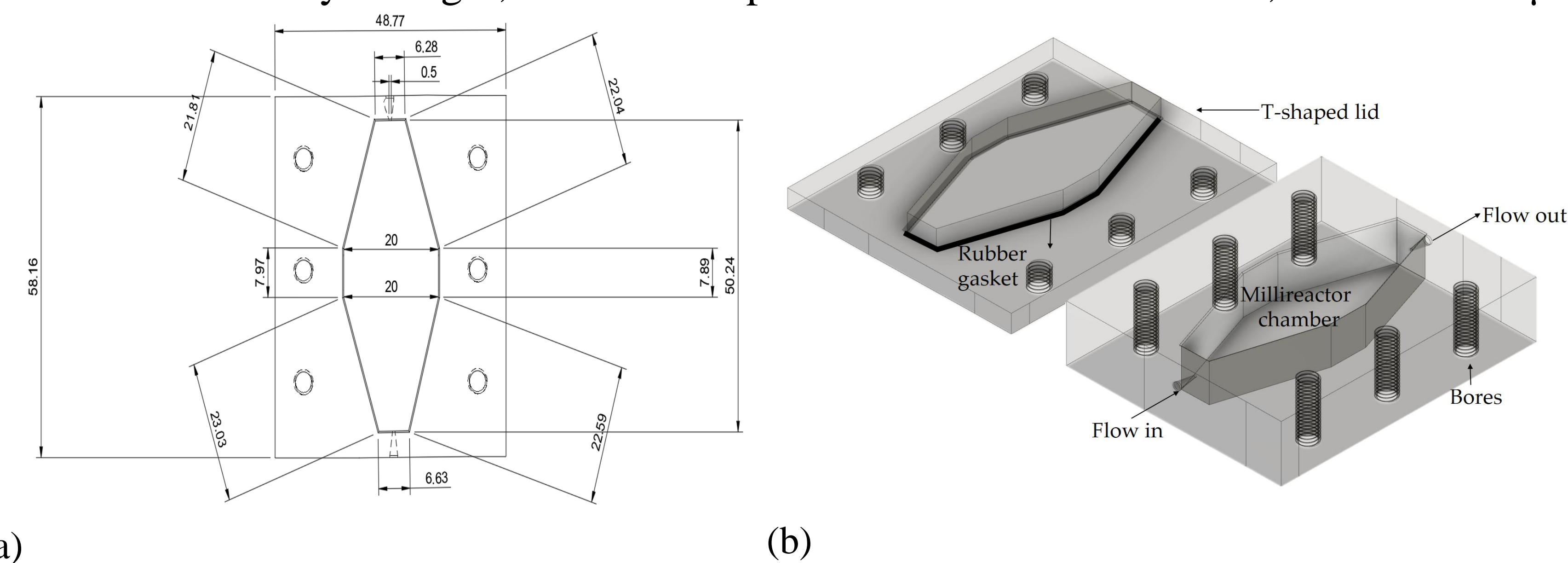


Figure 1. Schematic representation of a) design specifications (in mm) with height = 5 mm and average hydraulic diameter = 6.69 ± 0.98 mm and b) key millireactor elements.

Before performing the glucose oxidation reaction with GDH from *Pseudomonas* sp., **three strategies were used to immobilize the enzyme:** (a) in alginate beads, (b) in the form of an alginate hydrogel directly on the bottom surface of the millireactor and (c) in the form of an alginate hydrogel on both the bottom and top surfaces of the millireactor (Figure 2). For each strategy, the effect of immobilization on enzyme activity was investigated, focusing on the available surface area for enzyme–substrate interactions.

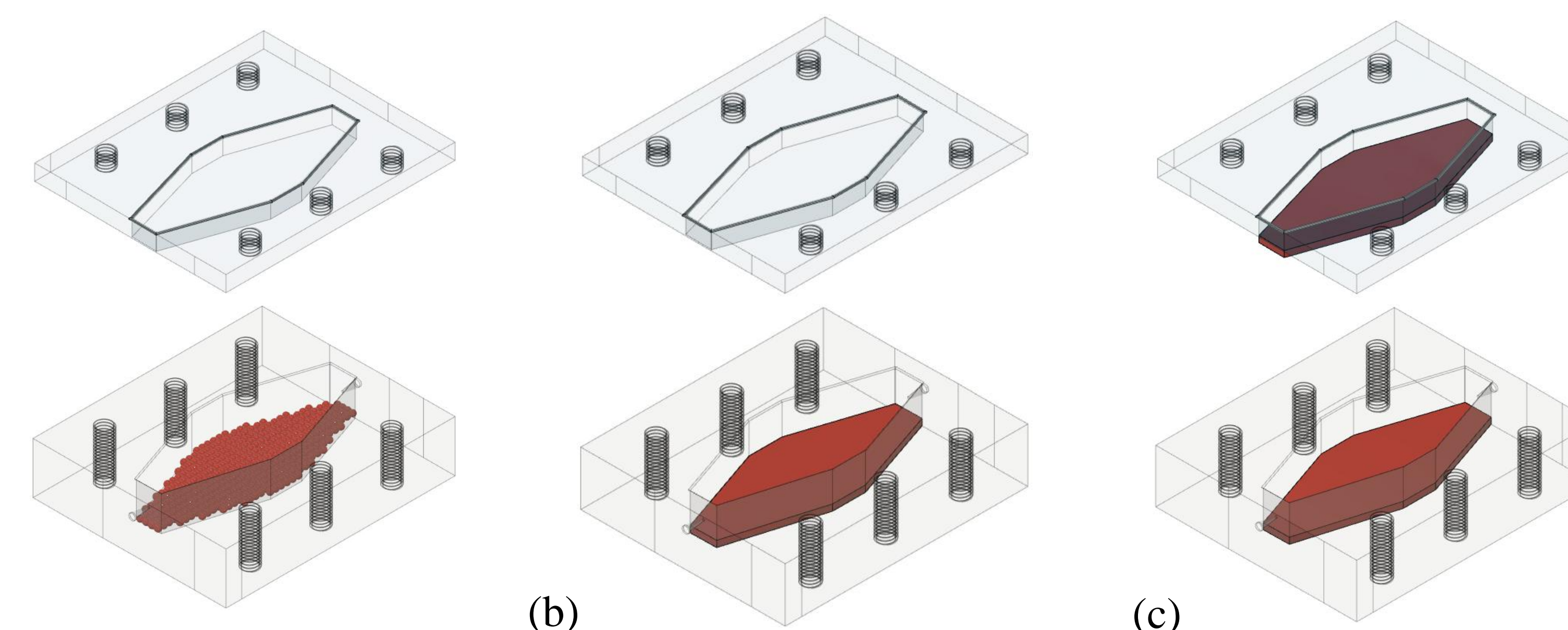


Figure 2. Different GDH immobilization strategies (a) as beads, (b) on the bottom surface of the millireactor, and (c) on both the bottom and the top surface of the millireactor.

To perform glucose oxidation in a millireactor (Figure 3), a 5 mmol/L equimolar solution of glucose and NAD⁺ was prepared in 20 mmol/L TRIS-HCl buffer at pH 7. The millireactor was submerged in a water bath with a heat regulation system. In all experiments, the flow rate was varied from 25 to 400 μ L/min to investigate influence of the residence time on glucose oxidation.

The output stream was collected in a vial placed on ice to stop the reaction. Glucose and NADH concentrations were measured in all collected samples.

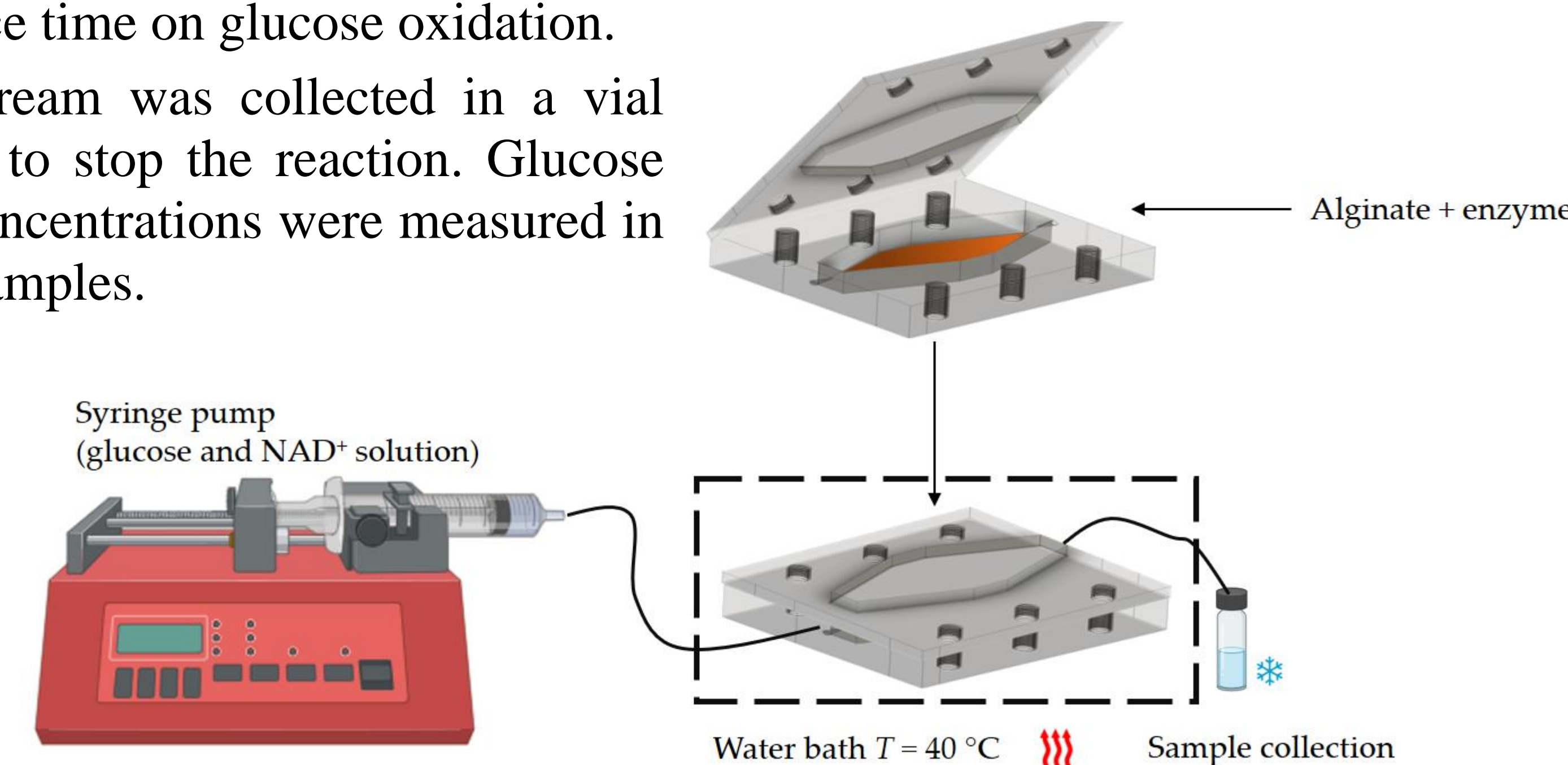


Figure 3. Experimental setup used for glucose oxidation.

RESULTS

The results showed that **the configuration in which the enzyme was immobilized on both the top and bottom surfaces of the millireactor significantly increased enzyme performance** (Figure 4). The efficiency was twice as high as immobilization with beads and four times as high as immobilization on the bottom surface.

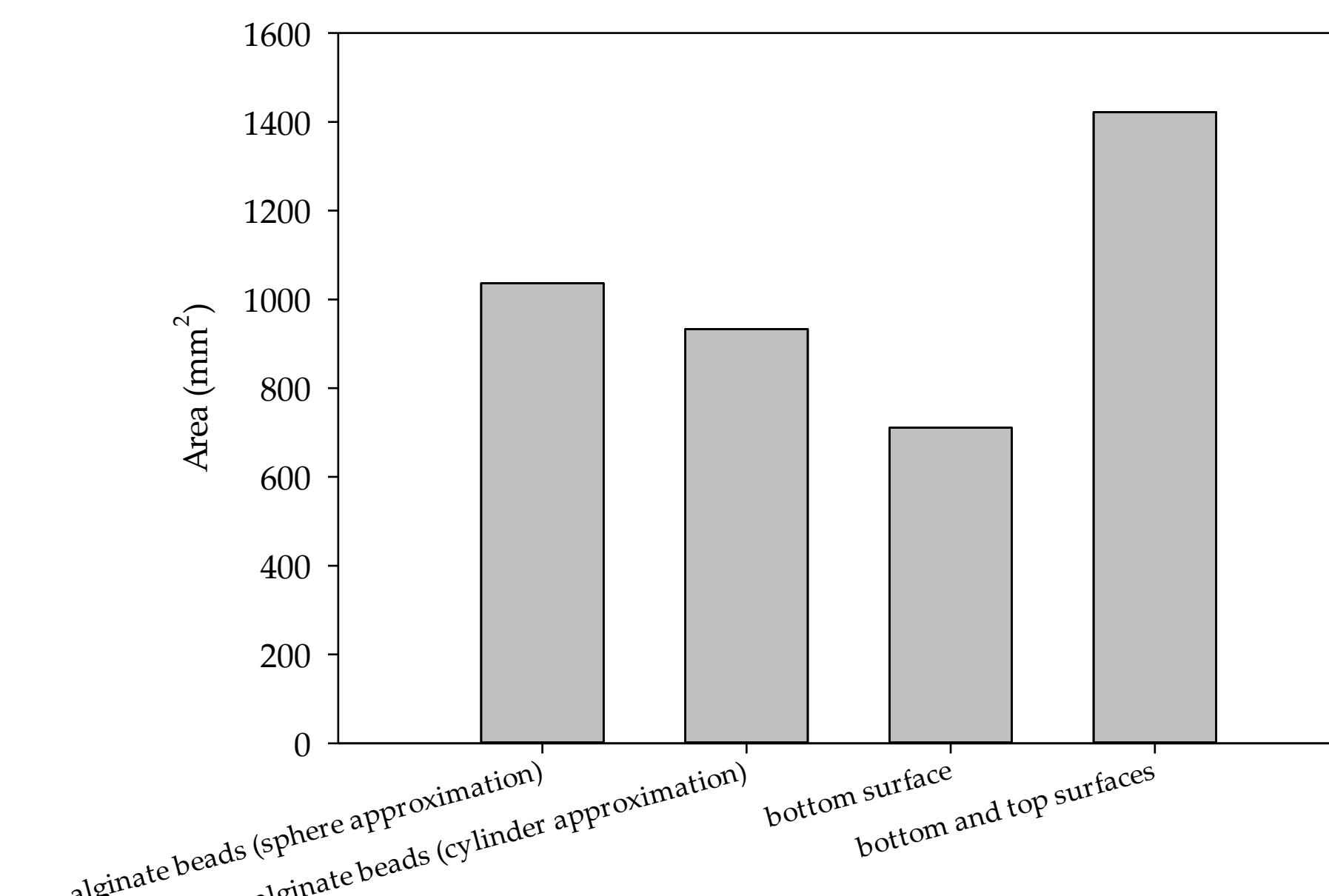


Figure 4. Comparison of the available surface area depending on the immobilization strategy.

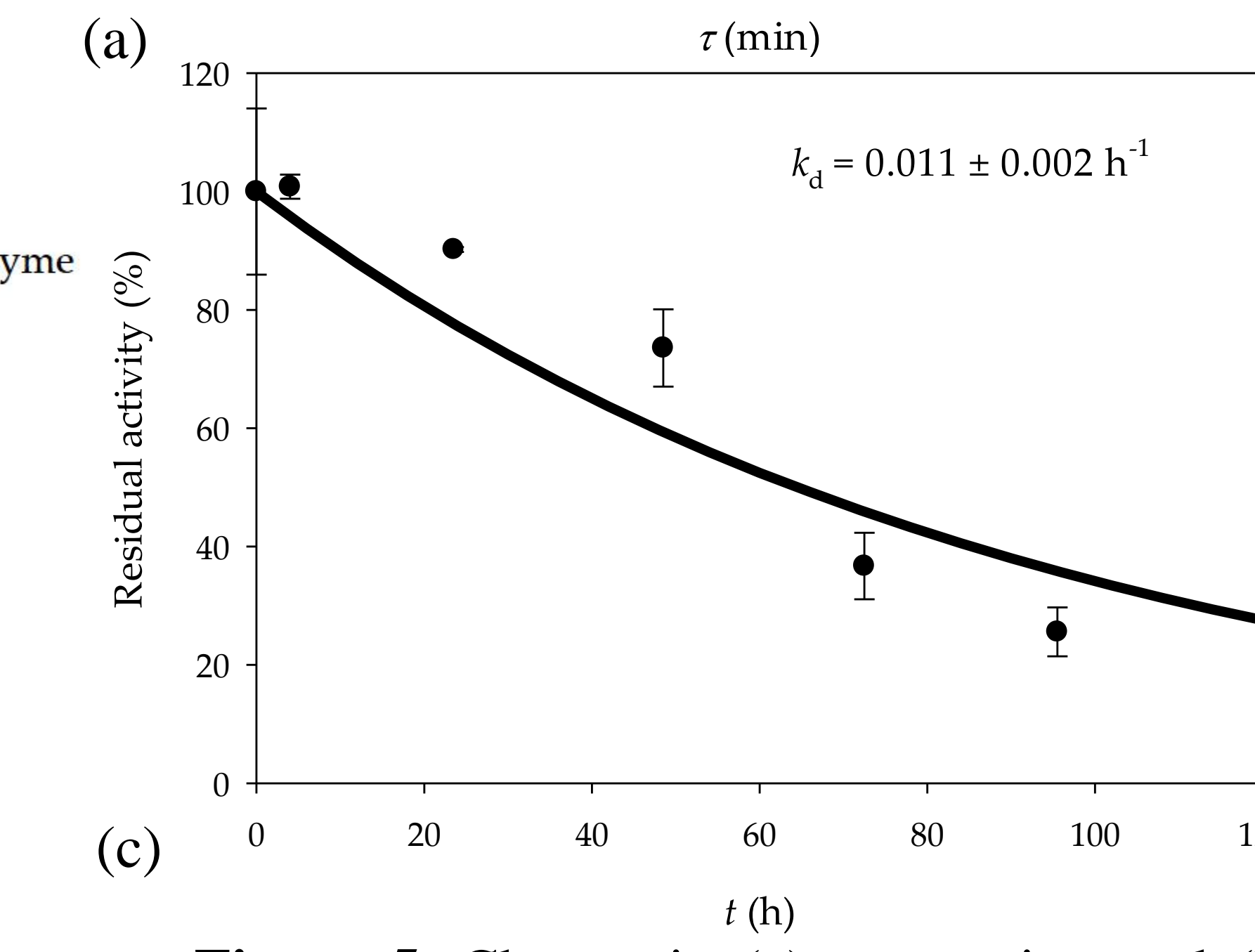
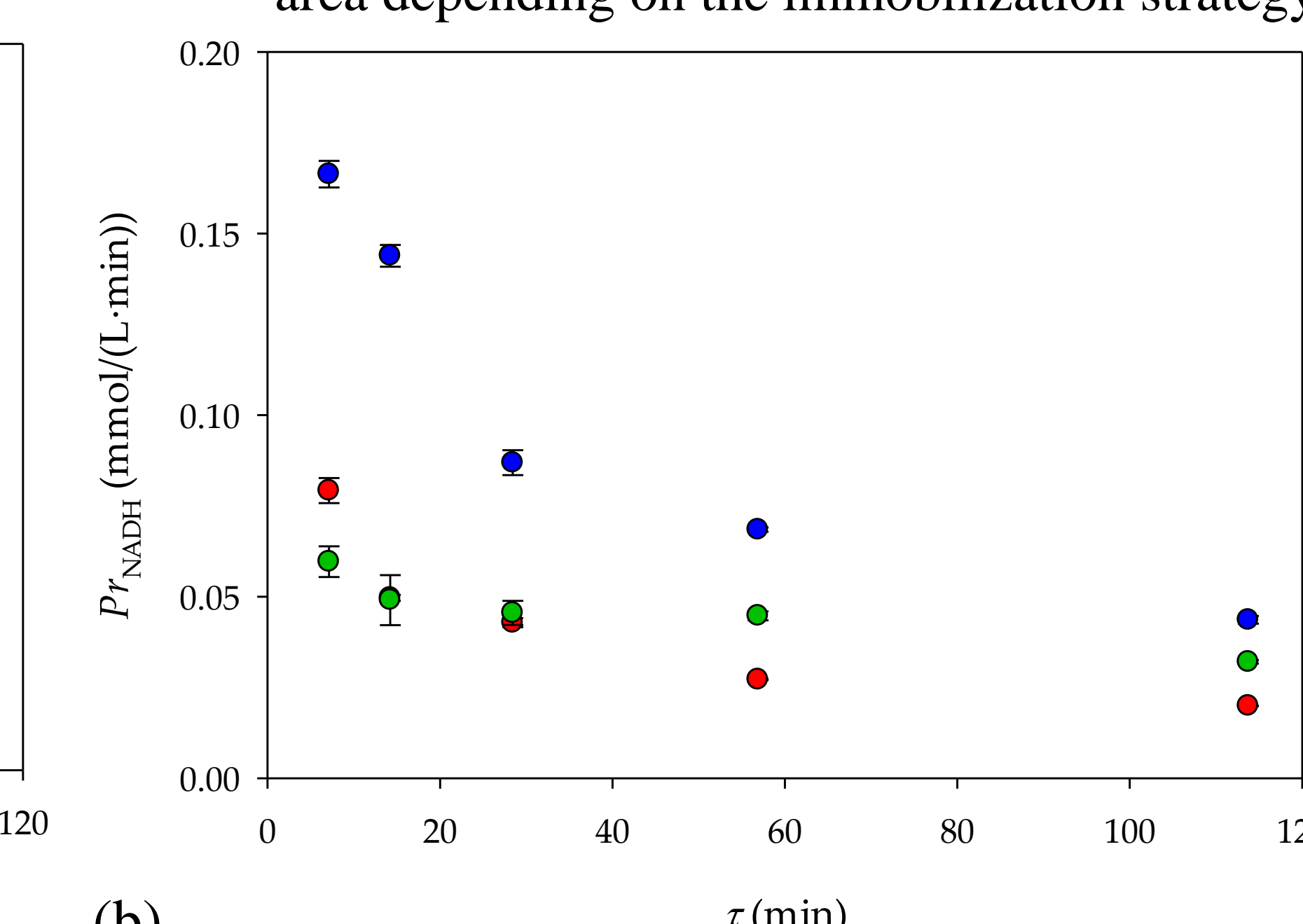
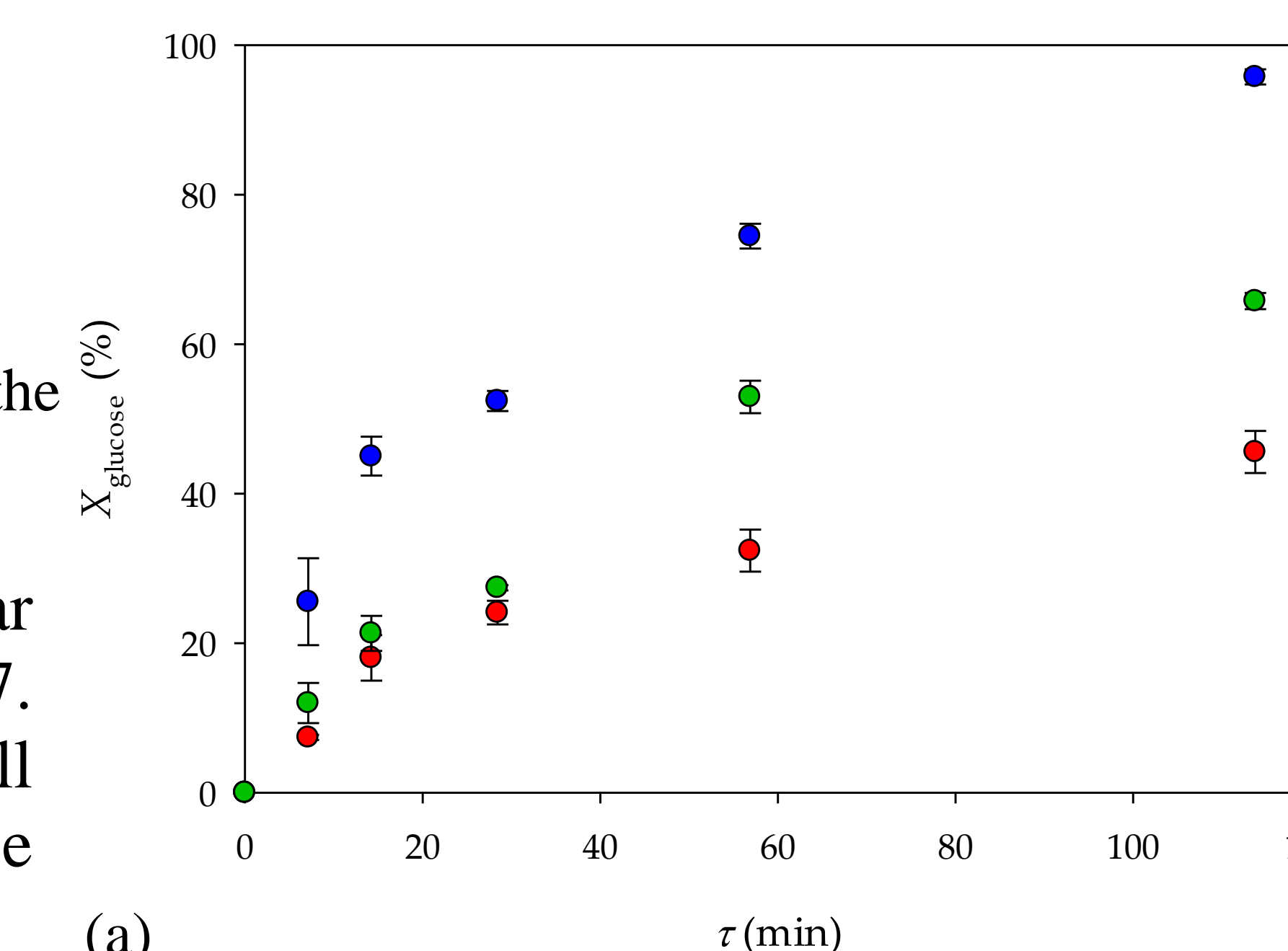


Figure 5. Change in (a) conversion and (b) productivity upon immobilization of the enzyme in alginate beads (•) and alginate hydrogen, bottom surface (•) and bottom and top surface of the millireactor (•) and (c) enzyme deactivation.

The influence of the immobilization strategy is perhaps most evident when comparing the conversion and productivity of the three strategies (Figure 5). **Conversion and productivity were highest in the millireactor in which the enzyme was immobilized in two layers** (both on the bottom and on the top surface).

CONCLUSION

In summary, this study presents an **innovative and versatile 3D-printed millireactor design** that not only addresses the main limitations of conventional rectangular millireactors, but also decouples the reactor design from the chosen enzyme immobilization method. **This flexibility significantly expands the applicability of the reactor and makes it a universal platform suitable for various biocatalytic processes.**