IMMOBILIZATION OF GLUCOSE DEHYDROGENASE IN HYDROGELS

21 Ružičkini dani
DANAS ZNANOST – SUTRA INDUSTRIJA
9. – 11. rujna 2026. • Vukovar • Hrvatska

Nera Bebek[1,*], Renata Vičević Šimac[1], Anita Šalić[1], Bruno Zelić[1,2]
[1]University of Zagreb Faculty of Chemical Engineering and Technology, Trg Marka Marulića 19, HR-10000 Zagreb, Croatia
[2]University North, Department of Packaging, Recycling and Environmental Protection, Trg dr. Žarka Dolinara 1, HR-48000 Koprivnica, Croatia
*email: nbebek@fkit.unizg.hr

Introduction

The growing demand for sustainable alternatives to fossil fuels has increased interest in biohydrogen production. Enzymatic biohydrogen production using multienzyme systems with cofactor regeneration represents a promising approach; however, the practical application of enzymes is often limited by their insufficient stability and by difficulties in recovery and reuse. Therefore, enzyme immobilization is an important strategy for improving enzyme stability, enabling enzyme reuse, and facilitating enzyme retention within reactors. Hydrogels are particularly suitable for enzyme immobilization due to their hydrated polymer network, which can retain enzymes while allowing smaller molecules, such as substrates and products, to diffuse through the matrix. In this study, glucose dehydrogenase (GDH) was immobilized in a hydrogel matrix as part of a multienzyme system for enzymatic biohydrogen production. Four different hydrogel systems were investigated as immobilization matrices: alginate, chitosan-alginate polyelectrolyte complex, gelatin, and agar-agar.

Materials and methods

The reaction scheme for enzymatic biohydrogen production catalyzed by GDH and hydrogenase is presented in Figure 1. As the first step in the development of this system, GDH immobilization was investigated using four different hydrogel matrices: alginate, chitosan-alginate polyelectrolyte complex, gelatin, and agar-agar. The selected hydrogels were compared to determine their suitability as supports for GDH immobilization and to identify the most appropriate matrix for further application in the biohydrogen production system. GDH was immobilized in micro- and millireactors (Figure 2.) using the selected hydrogel matrices.

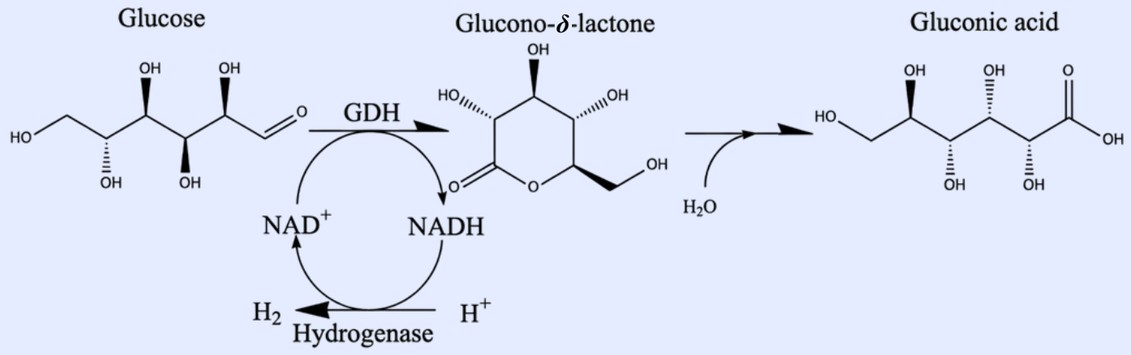

Figure 1. Glucose oxidation catalyzed by GDH with simultaneous biohydrogen production catalyzed by hydrogenase.

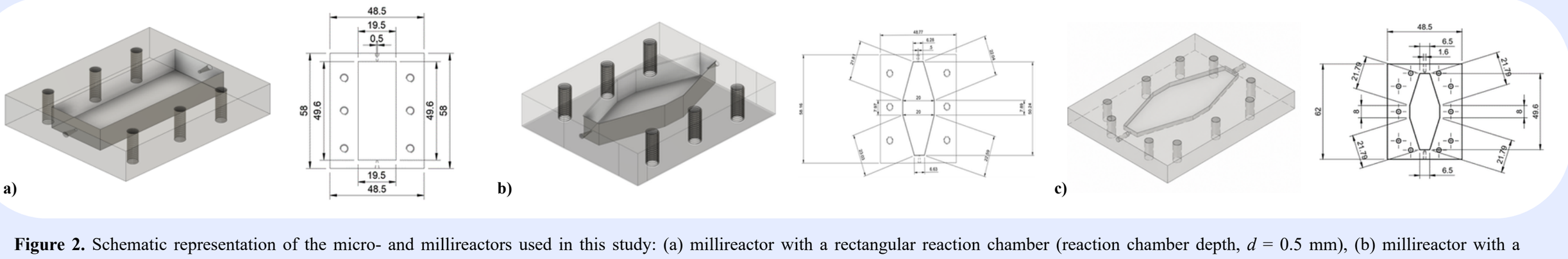

Figure 2. Schematic representation of the micro- and millireactors used in this study: (a) millireactor with a rectangular reaction chamber (reaction chamber depth, d = 0.5 mm), (b) millireactor with a modified reaction chamber with beveled corners (d = 0.5 mm), and (c) microreactor with a modified reaction chamber with beveled corners (d = 0.1 mm).

Results and Discussion

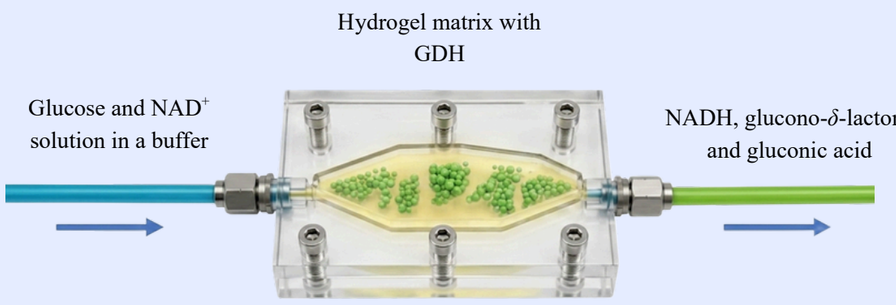

Figure 3. Schematic representation of the continuous-flow microreactor system.

The operating principle of the microreactor with immobilized GDH is illustrated in Figure 3. The substrate solution containing glucose and NAD^+ flows through the reactor, where GDH catalyzes the oxidation of glucose to glucono-δ-lactone, accompanied by the reduction of NAD^+ to NADH. Glucono-δ-lactone is subsequently hydrolyzed to gluconic acid.

Glucose conversion as a function of residence time was investigated for GDH immobilized in an alginate hydrogel using three different configurations (Figure 4.): hydrogel placed at the bottom of the reaction chamber (●), hydrogel distributed at the bottom and on the lid of the reaction chamber (▼), and hydrogel beads (○) in modified and rectangular millireactors.

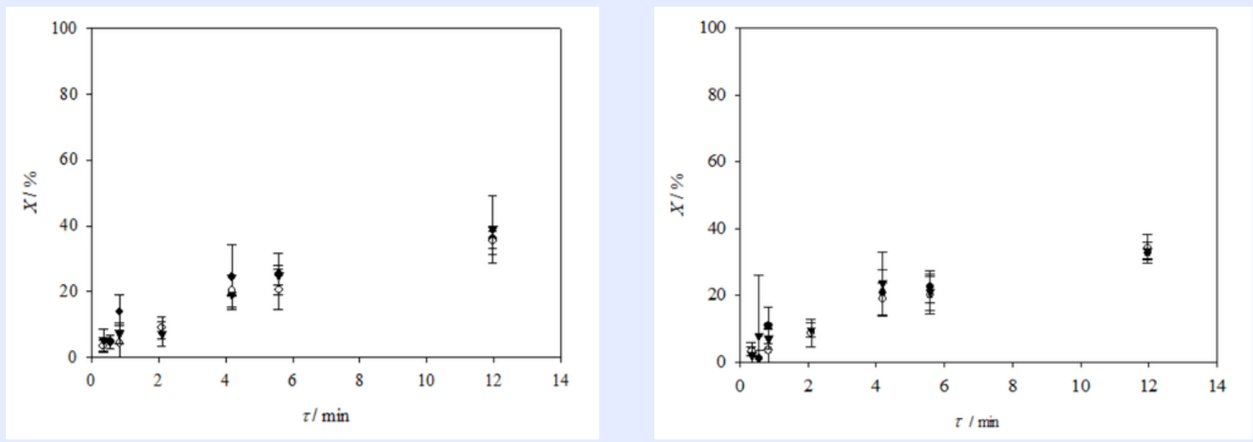

Figure 4. Dependence of glucose conversion on residence time in (a) the millireactor with a modified reaction chamber with beveled corners and (b) the rectangular millireactor.

The configuration with alginate hydrogel placed at the bottom and on the lid of the reaction chamber showed the highest glucose conversion and was therefore further evaluated in the microreactor (Figure 5.), followed by evaluation of the other hydrogel matrices (Figure 6.)

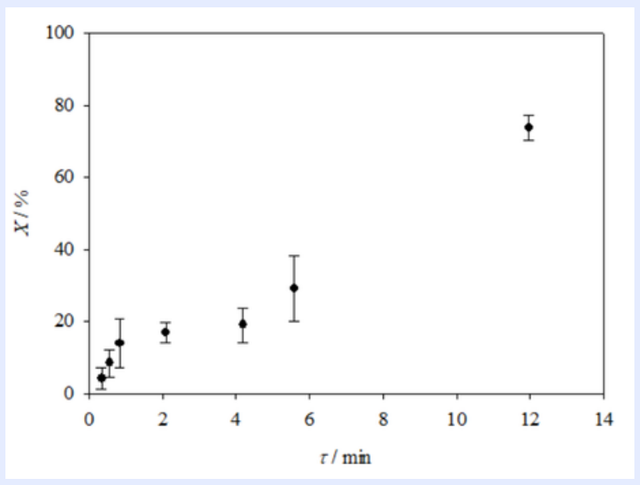

Figure 5. Glucose conversion as a function of residence time in the modified microreactor.

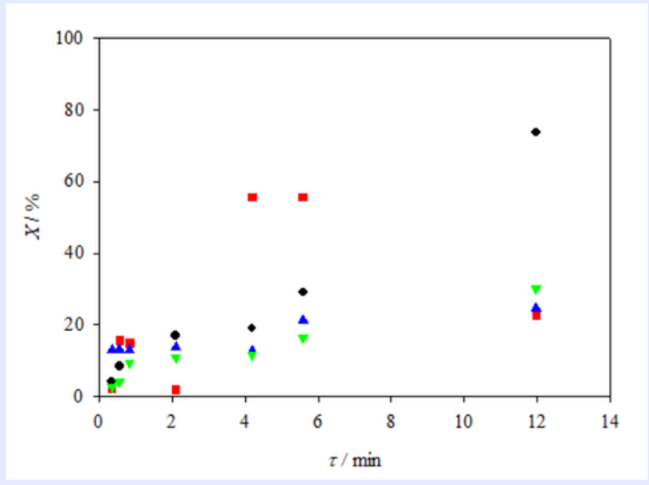

Figure 6. Glucose conversion as a function of residence time for GDH immobilized in four different hydrogels: sodium alginate (●), gelatin (■), chitosan-alginate polyelectrolyte complex (▼), and agar-agar (▲).

Conclusion

Immobilization of GDH in hydrogel matrices enabled continuous glucose oxidation in micro- and millireactors. Among the investigated reactor configurations, alginate hydrogel placed at the bottom and on the lid of the reaction chamber showed the best performance. In the modified microreactor, the highest glucose conversion of 73.72 +/- 3.42% was achieved at a residence time of 11.97 min. Among the four investigated hydrogel matrices, alginate provided the highest conversion at the longest residence time. These results identify alginate as the most suitable matrix for further development of a continuous GDH–hydrogenase system for enzymatic biohydrogen production process.

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: MicroBioH$_2$