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Article

Enzyme Immobilization on Stimuli-Responsive Hydrogels - Part B. Impact of Carrier Responsiveness on Reactor Performance

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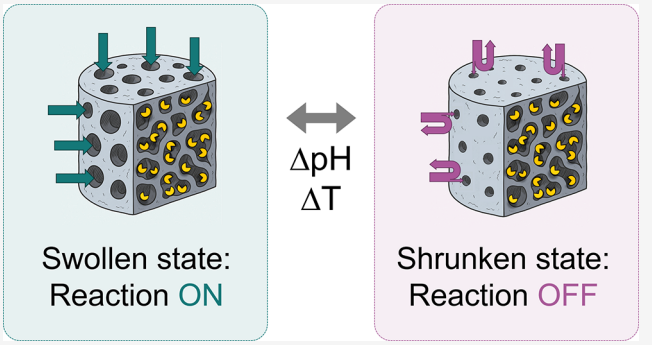
ABSTRACT: Stimuli-responsive gels, known as smart materials, undergo reversible changes through solvent uptake or expulsion. Building on prior material characterization (Paper A), this study evaluates their performance in a plug-flow reactor under changing temperature (25−40 °C) and pH (8−4) conditions using formate dehydrogenase for NADH regeneration from NAD^+. Temperature changes caused transient increases in activity at elevated temperatures, followed by partial or pronounced loss after returning to mild conditions, indicating limited effects of gel responsiveness. In contrast, under pH changes, activity decreased under acidic conditions but fully recovered for responsive hydrogels, maintaining high residual activity (93.3 ± 26.3% for pH-responsive and 91.0 ± 9.8% for dual-responsive carriers), whereas non-responsive silica carriers showed irreversible loss, retaining only 27.6 ± 10.2% of the initial yield. This behavior is attributed to gel shrinkage, which restricts mass transport and reduces enzyme exposure to the surrounding environment. These results demonstrate the potential of responsive hydrogels as adaptive enzyme carriers for biocatalysis.



INTRODUCTION

Currently, industrial processes are engineered to manufacture products from substrates of consistent quality and composition. However, this design constrains production to a particular source, impeding the utilization of raw biomass, which exhibits varying compositions based on seasonal and regional factors.[1] Future bioprocesses aiming for sustainability and economic viability require reactors that can autonomously adapt to such variability. Concerning biocatalytic processes, this will involve designing reactors with in-line reactor control.[2]

Stimuli-responsive hydrogels are characterized by their ability to undergo macroscopic changes in response to external stimuli.[3,4] These hydrogels, known as smart materials, represent a promising option for various applications due to their responsiveness to pH and temperature[5], electrical fields[6] light, and other stimuli[7]. Applications include drug delivery and wound healing[7,8] as well as sensors and actuators.[9] Moreover, they are employed in classical flow-regulating applications, such as flow control in microfluidics[10] and in recent studies as responsive packings[11,12]. Beyond these applications, stimuli-responsive materials can also serve as smart (bio)catalyst carriers,[12−14] enabling the development of autonomous bioreactor systems.

In our preliminary work (Part A: Dittmer et al.[15]), we developed and thoroughly characterized stimuli-responsive hydrogels as smart enzyme carriers for the immobilization of formate dehydrogenase (FDH) from *Candida boidinii*. The analysis encompassed detailed gel characterization, such as swelling behavior and mechanical properties, as well as the evaluation of different immobilization methods on the immobilization performance, including immobilization yield, activity and leaching. These fundamental studies established both the synthesis and functional properties of responsive hydrogels as enzyme carriers.

In Part B, we employ the smart enzyme carriers within a plug-flow reactor (PFR) operated under fluctuating temperature and pH conditions. By simulating possible variations typically encountered with raw biomass feedstocks[1] we investigate how the responsive behavior of the gels affects

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the enzymatic performance, and the overall resilience of the biocatalyst under fluctuating reactor conditions. This study aims to evaluate the feasibility of applying responsive polymer gels to protect sensitive enzymes in practical process environments. In addition, it explores autonomous reactor control as a step toward developing biocatalytic reactors capable of adjusting to fluctuating operating conditions. By promoting cleaner and more sustainable bioprocess engineering methods, the UNU Hub at TUHH contributes to the United Nations' sustainability agenda, particularly SDG 12 (Responsible Consumption and Production) and SDG 9 (Industry, Innovation and Infrastructure). To evaluate the impact of the response behavior, smart gels are compared to non-responsive carriers.

EXPERIMENTAL METHODS

This study applies the methods developed in Dittmer et al.[15] in a plug flow reactor. The first steps include the hydrogel synthesis and enzyme immobilization according to Dittmer et al.[15] This study compares three types of responsive hydrogels: poly(2-hydroxyethyl methacrylate-*co*-itaconic acid) (p(HEMA-*co*-IA)), poly(*N*-isopropylacrylamide) (p(NIPAM)), and poly(*N*-isopropylacrylamide-*co*-itaconic acid) (p(NIPAM-*co*-IA)) and their performance as enzyme carrier with respect to stability of the enzyme activity in fluctuating reactor conditions.

Materials

N-Isopropylacrylamide (NIPAM, ≥98%) and *N*-hydroxysuccinimide (NHS, 98%) were purchased from Abcr GmbH (Karlsruhe, Germany). *N*,*N*′-Methylenebis(acrylamide) (MBA, 99%) and 2-hydroxyethyl methacrylate (HEMA, 97%) were obtained from Merck Sigma-Aldrich (St. Louis, MO, USA). Sodium metabisulfite (NaDS, ≥97%) was supplied by Honeywell Fluka (Buchs, Switzerland), and gaseous nitrogen (99.99%) by Westfalen AG (Münster, Germany).

NAD^+ (≥95% purity), sodium formate (HCOONa, ≥99%), itaconic acid (IA, ≥98%), silica gel orange (2−5 mm, with indicator, pearl shaped), ammonium persulfate (APS, ≥98%), monopotassium phosphate (KH_2PO_4, ≥98%), dipotassium phosphate (K_2HPO_4, ≥98%), ortho-phosphoric acid (H_3PO_4, 85%), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC, ≥99%), and 2-morpholinoethanesulfonic acid (MES; ≥99%) were all purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany).

FDH (2 U/mg) was supplied by Megazyme Ltd. (Bray, Ireland).

Carrier Preparation

The responsive hydrogels were synthesized by radical polymerization. The copolymeric hydrogels consisted of 10 mol % itaconic acid and 90 mol % of HEMA or NIPAM, following the procedure described in Dittmer et al.[15] The hydrogels were synthesized based on the mass quantities listed in Table 1.

After the polymerization, the hydrogels were cast in 1 mL B. Braun SE (Melsungen, Germany) syringes with inner diameter of 0.5 cm. The gelation process took 24−48 h. After the completion of the gelation process, the hydrogels were removed from the syringes and cut into monoliths of 0.5 cm in length. Finally, the hydrogels were washed with deionized water for 3 days to remove the unreacted components.

The silica beads as reference material were sieved using a 4 mm mesh prior to usage. With this, particles (>4 mm) were obtained with a diameter approximately similar to the hydrogel particles. Silica beads were immobilized with FDH using the standard adsorption procedure, but prewetting based on vapor at 40 °C overnight was required to prevent fracturing upon immersion of buffer due to capillary forces in the mesoporous structure.

For the application in the PFR, the enzyme FDH was immobilized on the distinct gel monoliths using covalent binding with EDC/NHS hydrogel functionalization. As silica does not contain suitable functional groups, adsorptive immobilization was employed on these carriers. The procedure of Dittmer et al.[15] was followed.

Table 1. Hydrogel synthesis: p(HEMA-*co*-IA), p(NIPAM-*co*-IA), pNIPAM

Chemicals	pH-responsive p(HEMA-*co*-IA)	Dual-responsive p(NIPAM-*co*-IA)	Temperature-responsive pNIPAM
HEMA (mmol)	27.66	-	-
NIPAM (mmol)	-	27.66	30.94
IA (mmol)	3.08	3.08	-
MBA (mmol)	0.62	0.62	0.62
APS (mmol)	0.31	0.31	0.31
NaDS (mmol)	0.20	0.20	0.20
Water (g)	20.00	20.00	20.00

The gels were pre-equilibrated in deionized water for 24 h. Subsequently, the hydrogels were functionalized by EDC/NHS activation for 3 h at 25 °C using 52 mM EDC and 86.8 mM NHS in 100 mM MES buffer (pH 5). After activation, the hydrogels were rinsed with 10 mL potassium phosphate buffer (pH 8) to remove residual activation reagents. FDH was subsequently immobilized onto the activated hydrogels using an enzyme solution of 0.5 U/mL in 50 mM potassium phosphate buffer (pH 8) for 24 h at 4 °C.

For immobilization on silica carriers, only the final enzyme immobilization step was performed analogously. Prewetted carriers were incubated with the same FDH solution under identical conditions without prior EDC/NHS activation.

Plug-Flow Bioreactor

The plug flow reactor used in this study was designed to evaluate the catalytic performance and stability of immobilized FDH under various flow conditions catalyzing the NADH regeneration (eq 1):

$$HCOO^- + NAD^+ \rightarrow CO_2 + NADH \quad (1)$$

The reactor consisted of a vertically oriented glass column (inner diameter: 0.918 cm, height: 15 cm), which was filled with glass beads and hydrogel monoliths. Both layers contained similar masses of glass beads, resulting in a total glass bead mass of approximately 2.8 g in the PFR. To ensure consistent flow conditions and comparable residence times across all hydrogel formulations, the height of the fixed bed of carriers was kept constant at 4 cm, regardless of the hydrogel type. Therefore, the wet and dry masses varied across the formulations, resulting in differences in reaction yield.

The hydrogels were secured in place by layers of glass beads positioned above and below the carrier bed to prevent displacement during operation and to enhance mass transport properties. The reactor was operated with an Aladdin SyringeONE-1060 syringe pump (World Precision Instruments; Sarasota, FL, USA), using a substrate solution consisting of 200 mM sodium formate and 1 mM NAD^+ in 50 mM potassium phosphate buffer (pH 8 or pH 4). The acidic buffer was prepared by titration with phosphoric acid to the required pH. For the PFR operation, a residence time (τ) of 20 min was chosen (compare the result section "Impact of Flow Rate on Yield"). Flow rates were individually adjusted based on the void volume (V_{void}) of each hydrogel-packed column using eq 2, ensuring a fixed residence time in all experiments. This approach balanced maintaining sufficient yield for analysis with accurately reflecting the catalytic performance in the PFR. The void volume was determined by filling the carrier-loaded plug-flow reactor with water and calculating the required water volume gravimetrically. To prevent water uptake during the measurement, the gels were equilibrated in the buffer solution (pH 8) prior to the experiment.

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$$\dot{V} = \frac{V_{void}}{\tau} \quad (2)$$

The performance of the PFR experiments was evaluated based on the relative yield $y_{rel}(t)$ over the operation time. The yield, defined as the ratio of product (c_{NADH}) to initial substrate (c_{NAD^+}) concentration, was divided by the mass of immobilized enzyme ($m_{imm.Enzyme}$) to calculate the specific yield $y(t)$ (eq 3). From this, the relative yield was determined according to eq 4. The substrate and product concentrations were quantified according to the method described by Dittmer et al.[15] where the absorbance of NADH was measured at 340 nm using a 200 PRO microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

$$y(t) = \frac{\frac{c_{NADH}(t)}{c_{NAD^+}}}{m_{imm.Enzyme}} \quad (3)$$

$$y_{rel}(t) = \frac{y(t)}{y(t = 1h)} \quad (4)$$

$$y_{res} = \frac{y(t = t_{final})}{y(t = 1h)} \quad (5)$$

Furthermore, the residual yield (y_{res}, eq 5), of the different enzyme carriers after experiment duration (t_{final}) of 6 h including pH or temperature shift treatment, was compared.

Flow Rate Determination. To identify the optimal residence time, a preliminary experiment was conducted in which flow rates ranging from 0.25 to 2.3 mL/min were tested. This approach enabled the evaluation of how varying residence times, controlled via flow rate modulation, influence enzymatic yield and performance under dynamic conditions.

Enzyme Stability in Fluctuating Reactor Conditions. With the prepared materials, the stability and performance of FDH under fluctuating environmental conditions were subsequently evaluated in the PFR. The experiments aimed to evaluate the activity of immobilized FDH in response to variations in pH and temperature during continuous operation. Each condition was maintained for 2 h, resulting in a total sequence duration of 6 h (Figure 1). In this study, two reference systems were applied. First, responsive hydrogels were tested under constant reactor conditions (operational reference), enabling differentiation between activity loss as a result of time-dependent deactivation and the effects induced by fluctuating reactor conditions. The second reference system involved the use of non-responsive carriers (material reference). For this purpose, commercial non-responsive silica beads were tested under fluctuating reactor conditions to assess the influence of carrier responsiveness on the enzyme activity over time. Operational reference experiments were conducted for each hydrogel at pH 8 and 25 °C for 6 h, employing a residence time (τ) of 20 min. In the experiments effluent samples were collected every 30 min to monitor NADH formation using UV-Vis, analyzing absorbance at 340 nm.

The reactor was operated inside a temperature-controlled chamber (Incucenter, SalvisLab AG; Rotkreuz, Switzerland) in all experiments to maintain a constant temperature throughout the column. At the operating conditions, the oven temperature fluctuations are within ± 0.5 °C. All measurements were performed in triplicates.

RESULTS AND DISCUSSION

Hydrogels are widely studied as materials for enzyme immobilization, whereas stimuli-responsive hydrogels are primarily applied in flow control or drug delivery.[16−19] However, their application as enzyme carriers is still in its early stages.[20,21] To address this gap, this study investigates the biocatalytic performance of FDH immobilized on responsive hydrogel carriers, focusing on their performance in bioreactors under fluctuating, non-optimum operating conditions. During reactor operation, these fluctuations act as stimuli, inducing shrinkage or swelling of the hydrogels and thereby altering the mass transport properties of the carrier matrices.

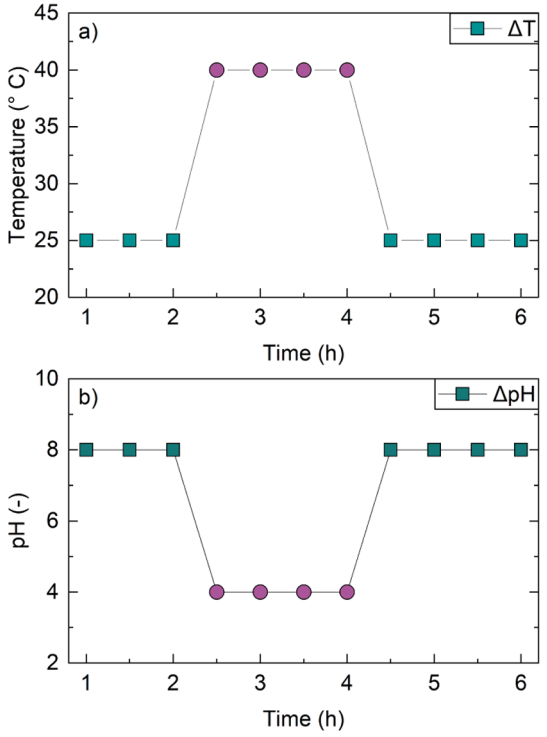

Figure 1. Reactor conditions simulating fluctuating temperature (a) and pH (b). Mild conditions (turquoise squares), harsh conditions (pink circles). Additionally, constant operating conditions (25 °C and pH 8) were investigated as an operational reference for all gel formulations.

The overall objective is to evaluate the performance and stability of enzymes immobilized on smart gel carriers in a plug-flow reactor (PFR) under dynamically changing temperature and pH conditions. In particular, the study assesses the influence of temperature- and pH-responsiveness of the carrier materials on the catalytic performance.

Impact of Flow Rate on Yield

Prior to application in fluctuating reactor systems, the product yield was analyzed as a function of the flow rate to identify the optimal flow rate for subsequent experiments. Two key effects were observed: overall yield depended on both the hydrogel formulation and the applied flow rate.

Significant differences in yield were observed among the various hydrogel formulations (Figure 2). The pNIPAM hydrogels exhibited the lowest yields, whereas substantially higher yields were obtained with p(HEMA-*co*-IA) and p(NIPAM-*co*-IA) hydrogels in the PFR. This disparity likely arises from differences in the pore structure. pNIPAM forms a dense, compact network, while p(HEMA-*co*-IA) and p-(NIPAM-*co*-IA) exhibit larger pores in lyophilized hydrogels[15] enabling improved mass transport and consequently higher yields in the PFR. In addition to structural effects, enzyme activity also depends on the compatibility between the enzyme and the carrier material. Previous analyses demonstrated that FDH exhibits significantly lower activity on pNIPAM gels compared to other carrier materials.[15] However, direct comparison between hydrogel types is limited, as equal bed heights require different amounts of hydrogel material, resulting in different residence times at identical pump rates.

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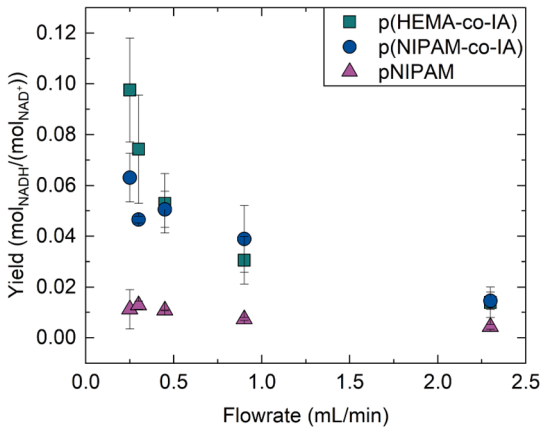

Figure 2. Enzymatic reaction yield in the PFR across various flow rates using the responsive carriers: p(HEMA-*co*-IA) (turquoise squares), p(NIPAM-*co*-IA) (blue circles), pNIPAM (pink triangles). Data represent mean ± mean deviation (n = 2). Conditions: 200 mM sodium formate and 1 mM NAD^+ in 50 mM potassium phosphate buffer (pH 8) at 25 °C.

Alongside formulation-dependent effects, a consistent decrease in yield with increasing flow rate was observed across all hydrogel types (Figure 2). This effect was most pronounced for the pH-responsive hydrogels p(HEMA-*co*-IA) and p-(NIPAM-*co*-IA), and least pronounced for pNIPAM. Although higher flow rates increase the volumetric substrate throughput, the yield decreased rather than improved. This indicates that the system is limited by the residence time rather than substrate transport under these conditions. Based on these results, a residence time of 20 min was chosen, corresponding to a flow rate of about 0.25 mL/min for the reactor operation in the following work. No further reduction of the flow rate was pursued, as this would have resulted in disproportionately long residence times relative to the overall experimental duration. In future studies, recycling the product stream into the reactor can represent a possible alternative to achieve longer residence times at higher flow rates while maintaining sufficient product yields. Alternatively, the lengths of the PFR can be increased.

Beside this, reducing the size of the gel carriers could potentially increase the activity of the immobilized enzyme by shortening diffusion path lengths within the gel and should be explored in future studies.

Temperature-Responsive Formulations

To evaluate the effect of temperature-induced response, the reactor was operated at 25 °C for the first 2 h, followed by 2 h at 40 °C, and then returned to 25 °C for another 2 h (see Figure 1). Under these conditions, the responsive carriers are expected to transition from a swollen to a shrunken state and reswollen during the final stage of the experiment, affecting both mass transport into the carrier matrix and, consequently, the observed reaction yield due to limited substrate accessibility. With this experimental setup, the effects of temperature on enzyme activity of FDH and substrate accessibility can only be investigated simultaneously.

Responsive carriers were tested under these conditions. In addition, a non-responsive, commercially available carrier material was subjected to the same treatment and used as a material reference to quantify the specific impact of the carrier's temperature responsiveness during the experiments. Both responsive and non-responsive carriers were additionally evaluated under mild conditions (25 °C and pH 8) for the full duration of the experiment (6 h) as an operational reference ("Const. 25 °C").

When comparing the temperature-responsive carriers to the non-responsive silica gel, distinct trends were observed (Figure

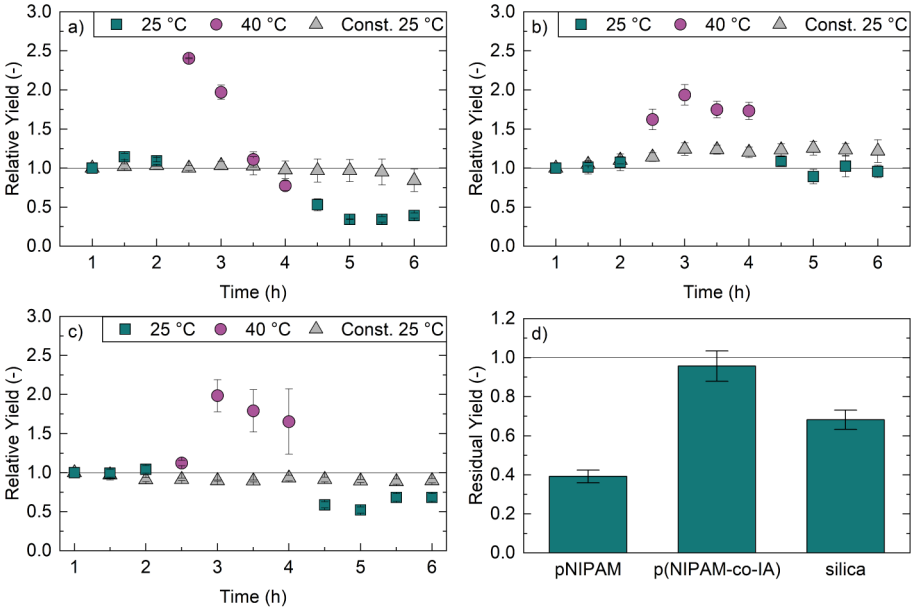

Figure 3. Enzyme activity indirectly expressed as relative yield in the PFR under fluctuating temperature conditions. The enzyme activity using the responsive carriers pNIPAM (a), p(NIPAM-*co*-IA) (b), and the non-responsive silica carriers (c). The system is operated between mild conditions at 25 °C (turquoise squares) and harsh conditions at 40 °C (pink circles), and compared to constant 25 °C as an operational reference (gray triangles). The residual yield for the carriers after heat treatment is summarized in (d). Data represent mean ± standard deviation (n = 3). Conditions: 200 mM sodium formate and 1 mM NAD^+ in 50 mM potassium phosphate buffer (pH 8) with temperature fluctuations according to Figure 1.

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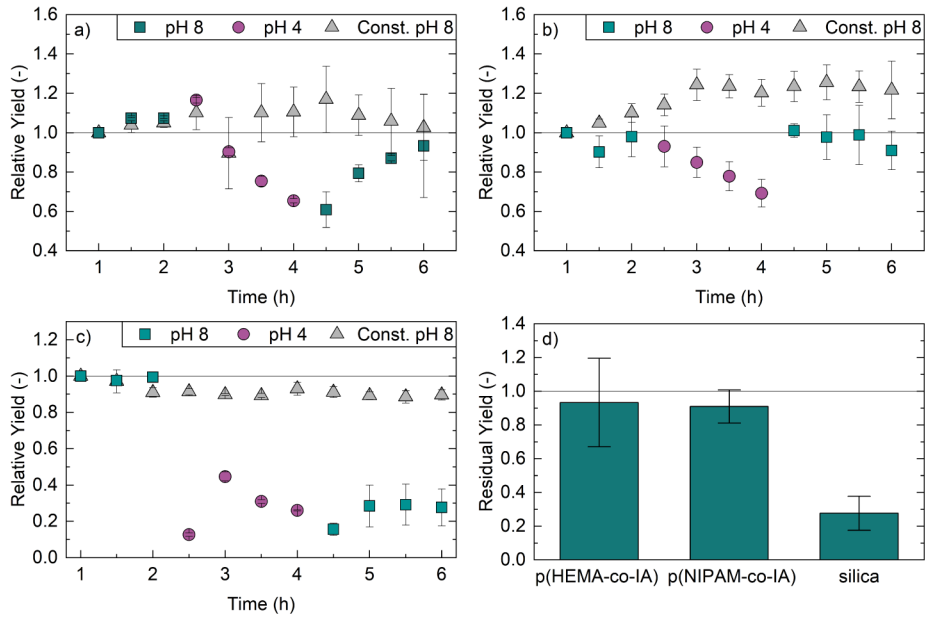

Figure 4. Enzyme activity indirectly expressed as relative yield in the PFR under fluctuating pH conditions. The enzyme activity using the responsive carriers p(HEMA-*co*-IA) (a), p(NIPAM-*co*-IA) (b), and the non-responsive silica carriers (c). The system is operated between mild conditions at pH 8 (turquoise squares) and harsh conditions at pH 4 (pink circles), and compared to constant pH 8 as an operational reference (gray triangles). The residual yield for the carriers after pH treatment is summarized in (d). Data represent mean ± standard deviation (n = 3). Conditions: 200 mM sodium formate and 1 mM NAD^+ in 50 mM potassium phosphate buffer at 25 °C with pH fluctuations according to Figure 1.

3). In all tested carriers, the initial increase in temperature led to a significant increase in yield caused by enhanced biocatalytic activity at elevated temperatures. For pNIPAM, which represents a purely temperature-responsive gel, this increase was followed by a pronounced decrease in yield during the high-temperature phase. In contrast, for p(NIPAM-*co*-IA) and the non-responsive silica gel, no decrease was observed during the elevated temperature period. Instead, the rapid decrease in yield occurred only after cooling back to 25 °C.

When using purely temperature-responsive pNIPAM gels, prolonged thermal treatment caused irreversible enzyme denaturation within the polymer matrix, as indicated by the pronounced decrease in reaction yield during the elevated temperature phase. A slight decrease was also observed with non-responsive gels, suggesting similar but less severe effects. In contrast, the dual-responsive p(NIPAM-*co*-IA) formulation applied in the PFR allowed the reaction yield to recover to pretreatment levels. These findings suggest that copolymeric formulations enhance biocatalyst resilience compared to other carrier types. However, since this recovery effect was absent in the purely temperature-responsive pNIPAM but observed in the less temperature-responsive p(NIPAM-*co*-IA) formulation, it is likely not driven by the material's responsiveness but rather by the insulating properties of the carrier matrix. This assumption is supported by the experiments with non-responsive silica gels where slower heat transfer, evidenced by a delayed increase in enzyme activity of FDH, was followed by only a minor reduction in reaction yield after thermal treatment. Given that insulating properties are influenced by the porous structure, future studies could tailor these properties, as swelling and shrinkage not only alter the macroscopic volume but also directly affect the material's pore sizes.[22] Additionally, enzyme immobilization within responsive carriers could be further characterized using NMR relaxometry, following the approach of Serial et al.[23].

pH-Responsive Formulations

The pH was alternated between 8 and 4 to induce swelling and shrinkage of the gels, respectively. Since the FDH enzyme exhibits optimal activity around pH 7.5[24] a shift to pH 4 is expected to reduce the enzyme activity directly, and indirectly by shrinking the gel and imposing mass transport limitations in the pH-responsive carriers. These diffusion limitations can therefore enable control of enzyme accessibility and, consequently, reaction control. In consequence, the following results reflect the combined effect of hydrogel shrinkage, which limits substrate accessibility, and the intrinsic pH dependence of enzyme activity.

In the experiments using pH-responsive carriers, decreasing the pH resulted in an intermittent reduction in enzymatic activity, as indicated by lower yields. Upon returning to the initial pH, the yield increased to the value observed prior to the pH change (Figure 4). The decrease in activity during the pH change can be directly attributed to the changed conditions and not to enzyme deactivation over time, based on the comparison of the yields of the operational reference at constant conditions ("Const. pH 8"). In this setup, the responsive carriers behave as actuators within the biocatalytic process, enabling autonomous reaction control by allowing the reaction to proceed in the swollen state or disrupting it in the shrunken state. For the first time, the feasibility of autonomous control of a biocatalytic reaction under fluctuating pH conditions within a PFR is demonstrated.

Responsive carriers can provide not only autonomous reaction control, but may also offer a protective environment for the biocatalysts. In non-responsive carriers, irreversible enzyme deactivation was observed (as reflected by the absence of recovery in yield following the pH shift). Conversely, the reaction yield in responsive carriers fully recovered after the pH change. This behavior can be attributed to gel shrinkage at low pH values, which reduces the pore size and thereby

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increases diffusion limitations within the carrier matrix. It was observed that the reaction progress is regulated by the swelling behavior of the responsive carriers, in contrast to the non-responsive silica particles. In the experiments conducted in this study, the response of the carriers was relatively slow, partly due to the gel's size. Shortening their response time, as already discussed in the literature[9,25−27] could significantly enhance their applicability as actuators.

Upon exposure to acidic bulk conditions, the pH-responsive carriers shrink, thereby restricting mass transport into the gel matrix. This diffusion limitation modulates the reaction rate and, moreover, may reduce the exposure of the enzyme to the harsh bulk conditions. As a result, the reaction yield observed prior to the pH treatment is reached again after mild conditions are restored.

Non-responsive silica carriers do not exhibit such adaptive diffusion behavior. In these systems, pH variations directly affect the immobilized FDH, potentially altering surface charge interactions and promoting enzyme leaching, while mass transport remains largely unchanged.

In summary, encapsulation within the responsive hydrogel matrix, creates a protective microenvironment that contributes to a resilient and adaptive enzyme carrier system, as illustrated in Figure 5. While heat transport is not significantly affected by hydrogel shrinkage and, therefore, does not provide a protective function, changes in mass transport enable enzyme protection and facilitate autonomous reaction control.

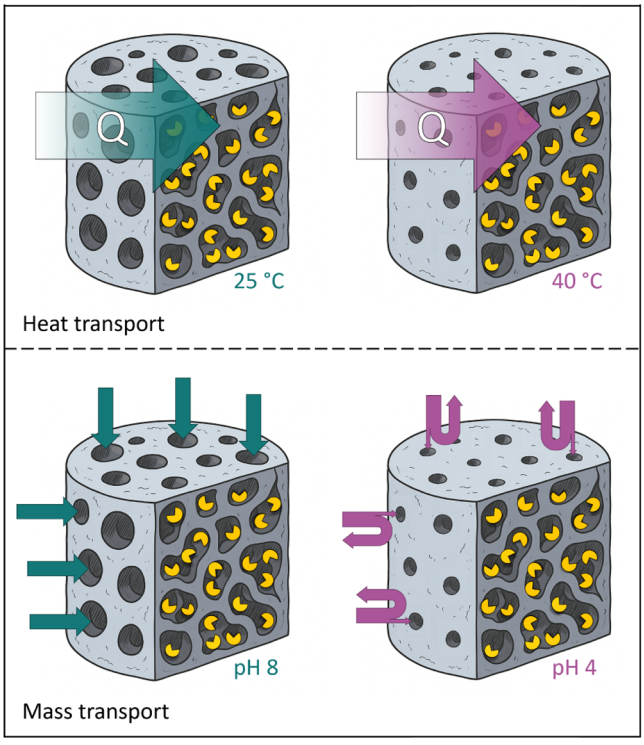

Figure 5. Schematic visualization of gel shrinkage illustrating its application as a smart carrier for enzyme protection at different temperatures and pH values.

CONCLUSIONS

This study highlights the potential of stimuli-responsive hydrogels as adaptive enzyme carriers under fluctuating process conditions in a plug-flow reactor. By evaluating temperature- and pH-responsive formulations, we demonstrated that shrinkage and swelling of the gels, triggered by temperature or pH changes, can modulate transport properties within the carrier matrix. As a result, enhanced enzyme stability can be achieved by employing responsive carrier matrices.

Under fluctuating temperature conditions, elevated temperatures caused partial enzyme deactivation of FDH in pNIPAM and non-responsive silica gels. However, p(NIPAM-*co*-IA) enabled full activity recovery upon return to mild conditions. Under fluctuating pH conditions, both pH-responsive carriers enabled full recovery of reaction yield, whereas in non-responsive systems irreversible deactivation was observed. These findings suggest that stimuli-responsive gels can be applied to achieve self-regulation of a plug-flow reactor under fluctuating pH conditions.

Overall, the results suggest the potential of responsive hydrogels as protective, resilient and adaptable enzyme carriers. Future studies could aim to optimize the balance between responsiveness and permeability and further investigate the influence of carrier geometry and porosity to enhance long-term biocatalytic performance and support the development of novel, tailored carrier matrices and reactor technologies for smart reactor control.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.iecr.6c00960.

Table S1: reaction yield for varying flow rates, comparing the different hydrogel carrier materials; Table S2: relative yield of temperature-responsive hydrogels and silica as non-responsive reference, comparing constant and fluctuating conditions; Table S3: relative yield of pH-responsive hydrogels and silica as non-responsive reference, comparing constant and fluctuating conditions (pH 8 and 4) (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Nickel, D. B.; Fornell, R.; Janssen, M.; Franzén, C. J. Multi-Scale Variability Analysis of Wheat Straw- Based Ethanol Biorefineries Identifies Bioprocess Designs Robust Against Process Input Variations. *Front. Energy Res.* **2020**, *8*, 55.

(2) Ohde, D.; Kühn, S.; Liese, A. *In Introduction to Enzyme Technology*; Jaeger, K.-E.; Liese, A.; Syldatk, C. eds., Springer International Publishing: Cham; 2024, pp. 91−121.

(3) Wang, J.; Chen, Z.; Mauk, M.; Hong, K.-S.; Li, M.; Yang, S.; Bau, H. H. Self-Actuated Thermo- Responsive Hydrogel Valves for Lab on a Chip. *Biomed. Microdev.* **2005**, *7*, 313−322.

(4) Kocak, G.; Tuncer, C.; Bütün, V. pH-Responsive polymers. *Polym. Chem.* **2017**, *8*, 144−176.

(5) Milašinović, N.; Milosavljević, N.; Filipović, J.; Knežević-Jugović, Z.; Krušić, M. K. Synthesis, characterization and application of poly(N-isopropylacrylamide-co-itaconic acid) hydrogels as supports for lipase immobilization. *React. Funct. Polym.* **2010**, *70*, 807−814.

(6) Díaz Díaz, D.; Kühbeck, D.; Koopmans, R. J. Stimuli-responsive gels as reaction vessels and reusable catalysts. *Chem. Soc. Rev.* **2011**, *40*, 427−448.

(7) Maity, S.; Mahata, K.; Meshram, B.; Banerjee, S. Smart Polymer-Derived Injectable Hydrogels: Current Status and Future Perspectives. *ACS Polym. Au* **2025**, *5*, 680−711.

(8) Shen, C.; Wang, Y.; Xiao, X.; Yang, R.; Chen, H.; Yuan, P.; Zhang, Y.; Lyu, G.; Shin, J.; Chen, G.; et al. Hydrogels for In Vivo Biomedical Applications: Recent Advances and Future Perspectives. *J. Mater. Chem. B* **2026**, *14*, 2386−2406.

(9) *Hydrogel Sensors and Actuators: engineering and Technology*; Gerlach, G.; Arndt, K.-F. eds., Springer Berlin Heidelberg: Berlin, Heidelberg; 2010, Vol. *6*.

(10) Johnson, B. D.; Beebe, D. J.; Crone, W. C. Effects of swelling on the mechanical properties of a pH-sensitive hydrogel for use in microfluidic devices. *Mater. Sci. Eng.* **2004**, *24*, 575−581.

(11) Hu, X.; Karnetzke, J.; Fassbender, M.; Drücker, S.; Bettermann, S.; Schroeter, B.; Pauer, W.; Moritz, H.-U.; Fiedler, B.; Luinstra, G.; Smirnova, I. Smart reactors - Combining stimuli-responsive hydrogels and 3D printing. *Chem. Eng. J.* **2020**, *387*, 123413.

(12) Eixenberger, D.; Kumar, A.; Klinger, S.; Scharnagl, N.; Dawood, A. W. H.; Liese, A. Polymer-Grafted 3D-Printed Material for Enzyme Immobilization−Designing a Smart Enzyme Carrier. *Catalysts* **2023**, *13* (7), 1130.

(13) González-Sáiz, J. M.; Pizarro, C. Polyacrylamide gels as support for enzyme immobilization by entrapment. Effect of polyelectrolyte carrier, pH and temperature on enzyme action and kinetics parameters. *Eur. Polym. J.* **2001**, *37*, 435−444.

(14) Cullen, S. P.; Liu, X.; Mandel, I. C.; Himpsel, F. J.; Gopalan, P. Polymeric Brushes as Functional Templates for Immobilizing Ribonuclease A: Study of Binding Kinetics and Activity. *Langmuir* **2008**, *24*, 913−920.

(15) Dittmer, K. R.; Eckert, K. M.; Setiawan, S.; Ohde, D.; Smirnova, I.; Liese, A. Enzyme Immobilization on Stimuli-Responsive Hydrogels - Part A. Influence of Carrier Properties on Immobilization Efficiency and Catalytic Performance. *Ind. Eng. Chem. Res. Appl.*

(16) Romero, M. R.; Wolfel, A.; Igarzabal, C. I. A. Smart valve: Polymer actuator to moisture soil control. *Sens. Actuators, B* **2016**, *234*, 53−62.

(17) Richter, A.; Kuckling, D.; Howitz, S.; Gehring, T.; Arndt, K. Electronically controllable microvalves based on smart hydrogels: Magnitudes and potential applications. *J. Microelectromech. Syst.* **2003**, *12*, 748−753.

(18) Chatterjee, S.; Hui, P. C.-L. Review of Applications and Future Prospects of Stimuli-Responsive Hydrogel Based on Thermo-Responsive Biopolymers in Drug Delivery Systems. *Polymers* **2021**, *13* (13), 2086.

(19) Bajpai, A. K.; Shukla, S.; Bhanu, S.; Kankane, S. Responsive Polymers in Controlled Drug Delivery. *Prog. Polym. Sci.* **2008**, *33*, 1088−1118.

(20) Chou, C.; Syu, S.; Chang, J.-H.; Aimar, P.; Chang, Y. Bioinspired Pseudozwitterionic Hydrogels with Bioactive Enzyme Immobilization via pH-Responsive Regulation. *Langmuir* **2019**, *35*, 1909−1918.

(21) Dubey, N. C.; Gaur, D.; Tripathi, B. P. Responsive microgels and microgel assemblies in biocatalytic applications. *J. Polym. Sci.* **2023**, *61*, 1730−1748.

(22) Bilican, A.; Leutzsch, M.; Schmidt, W. Evaluating Nano-Scale Shifts: Quantifying Mesopore Shrinkage in Porous Polymers via NMR Cryoporometry. *Small* **2025**, *21*, 2500343.

(23) Serial, M. R.; Schmidt, L.; Adrian, M.; Brauckmann, G.; Benders, S.; Bueschler, V.; Liese, A.; Penn, A. A novel method for quantifying enzyme immobilization in porous carriers using simple NMR relaxometry. *Biochem. Eng. J.* **2026**, *225*, 109909.

(24) Yildirim, D.; Alagöz, D.; Toprak, A.; Tükel, S.; Fernandez-Lafuente, R. Tuning dimeric formate dehydrogenases reduction/oxidation activities by immobilization. *Process Biochem.* **2019**, *85*, 97−105.

(25) Eckert, K. M.; Bonsen, J.; Hajnal, A.; Gmeiner, J.; Hasse, J.; Adrian, M.; Karsten, J.; Kißling, P. A.; Penn, A.; Fiedler, B.; Luinstra, G. A.; Smirnova, I. Enhancing swelling kinetics of pNIPAM lyogels: The role of crosslinking, copolymerization, and solvent. *Fluid Phase Equilib.* **2025**, *597*, 114462.

(26) Switacz, V. K.; Wypysek, S. K.; Degen, R.; Crassous, J. J.; Spehr, M.; Richtering, W. Influence of Size and Cross-Linking Density of Microgels on Cellular Uptake and Uptake Kinetics. *Biomacromolecules* **2020**, *21*, 4532−4544.

(27) Haq, M. A.; Su, Y.; Wang, D. Mechanical properties of PNIPAM based hydrogels: A review. *Mater. Sci. Eng.* **2017**, *70*, 842−855.

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