OPTIMIZATION OF BIOHYDROGEN PRODUCTION IN A MICROREACTOR

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Acronym: MicroBioH$_2$

INTRODUCTION

Microreactors represent a promising platform for process intensification in biocatalytic applications, including enzymatic biohydrogen production (Figure 1.). Their high surface-to-volume ratio promotes efficient heat and mass transfer, enhances mixing, and reduces concentration and temperature gradients within the reaction system. In addition, their small internal volumes enable precise control of key operating parameters such as temperature, pressure, and residence time, which is particularly advantageous for processes involving sensitive biocatalysts.

This study evaluates the potential of microreactor technology for continuous biohydrogen production from NADH using recombinant [NiFe]-hydrogenase as the biocatalyst. Hydrogen production is based on the enzymatic oxidation of NADH, with the released reducing equivalents directed towards H_2 formation. The process was investigated in both batch and microreactor configurations, with particular focus on conversion, yield, and volumetric productivity. The effects of residence time and initial NADH concentration were systematically examined to determine their influence on reactor performance. Based on these results, operating conditions that promote efficient and stable biohydrogen production were identified.

Aim of the study

- ✓ to find optimal conditions for NAD^+ regeneration and biohydrogen production in a batch reactor
- ✓ to intensify the reaction of biohydrogen production in a microreactor

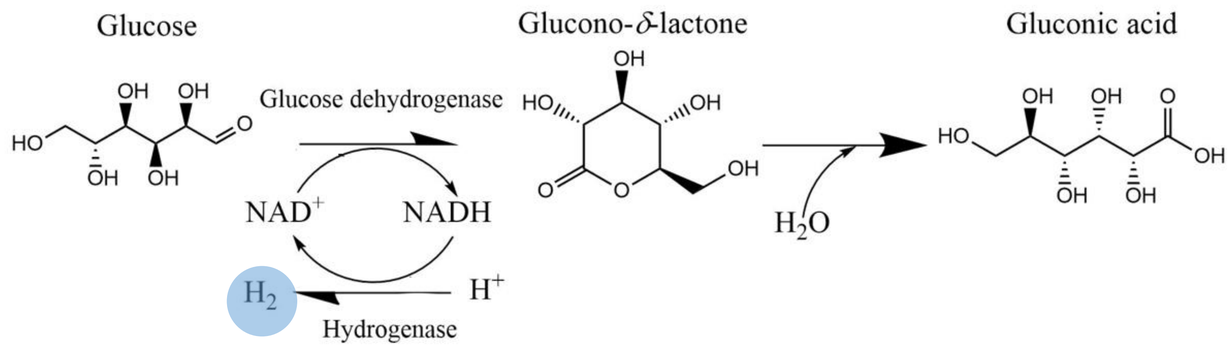
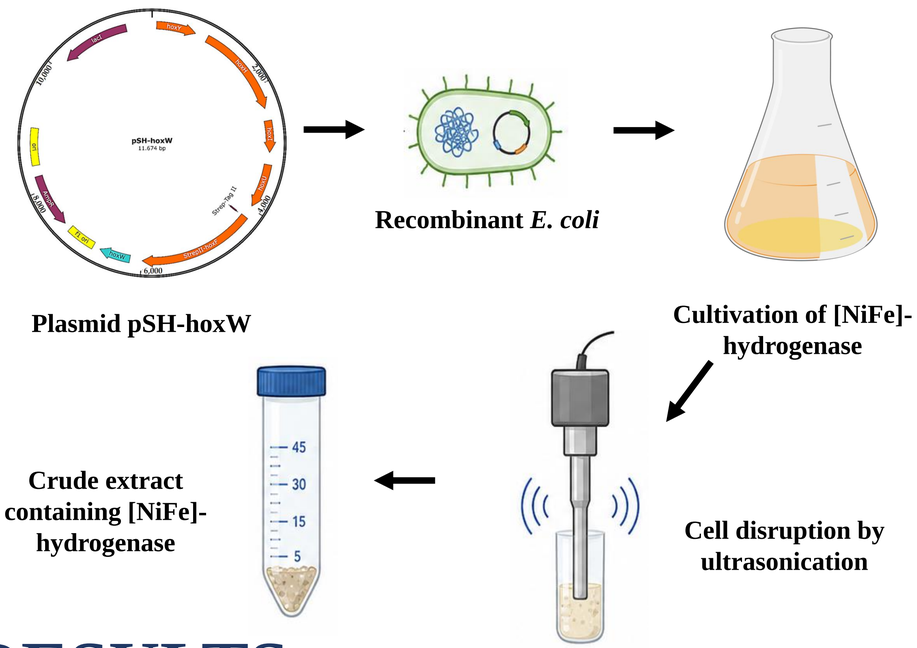


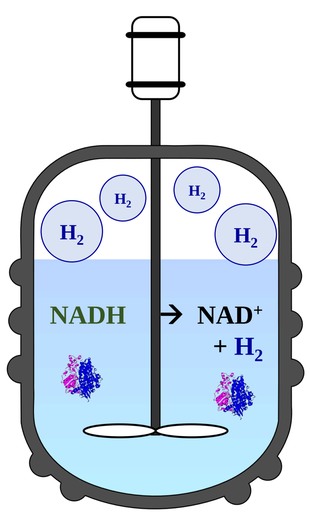
Figure 1. Glucose oxidation catalysed by the GDH from *Pseudomonas spp*. with parallel biohydrogen production catalysed by hydrogenase

MATERIALS AND METHODS

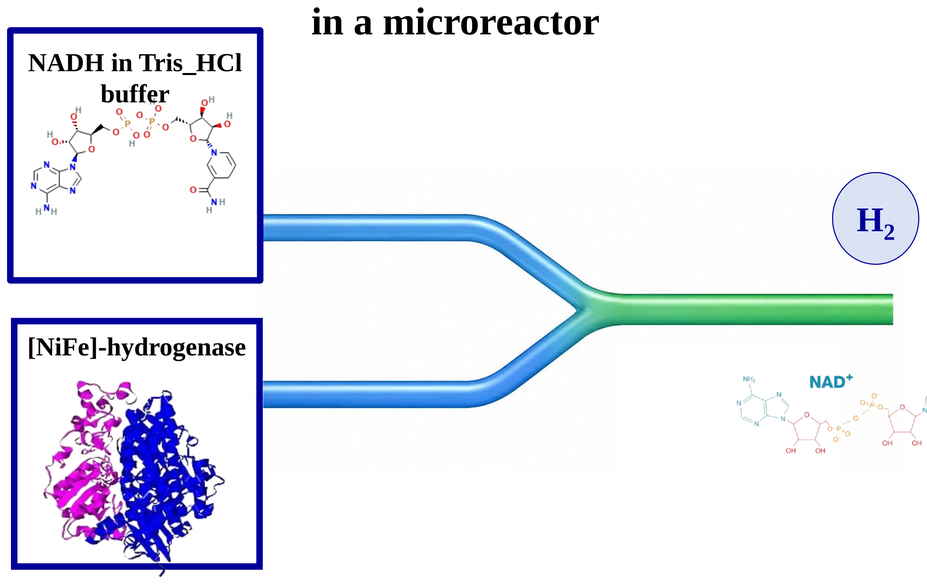
STEP 1: Recombinant [NiFe]-hydrogenase production



STEP 2: NADH oxidation in a batch reactor



STEP 3: Continuous NADH oxidation in a microreactor



RESULTS

Batch reactor

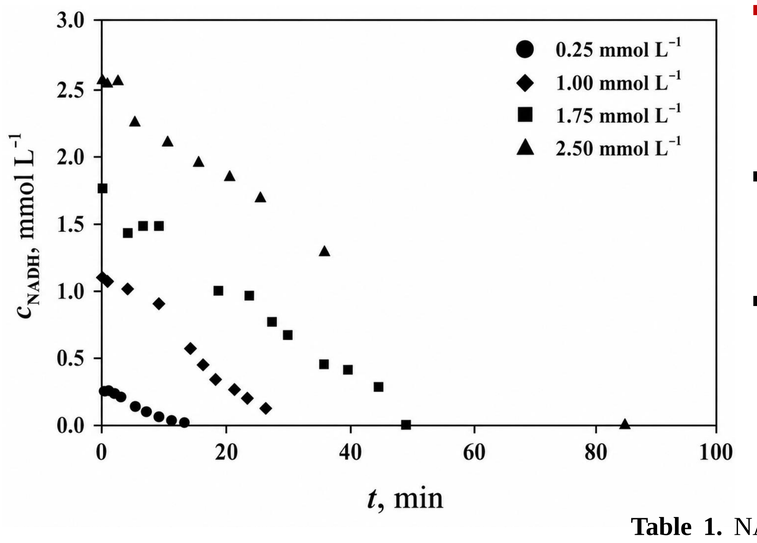


Figure 2. Dynamic changes in NADH concentration in a batch reactor at different initial NADH concentrations

- **Complete NADH conversion** (100 %) was achieved in batch experiments across all tested initial concentrations (0.25 - 2.50 mmol L^{-1}) (Figure 2.)
- Maximum NADH consumption rate: 0.0379 mmol L^{-1} min^{-1} at initial NADH concentration of 1.00 mmol L^{-1} (Table 1.)
- Increasing NADH concentration did not further increase the consumption rate → suggesting enzyme limitation and/or inhibitory effects.

Table 1. NADH consumption rate for different initial concentrations of NADH in the batch reactor

c_{NADH}, mmol L^{-1}	0.25	**1.00**	1.75	2.50
r_{NADH}, mmol L^{-1} min^{-1}	0.0179	**0.0379**	0.0365	0.0307

Microreactor

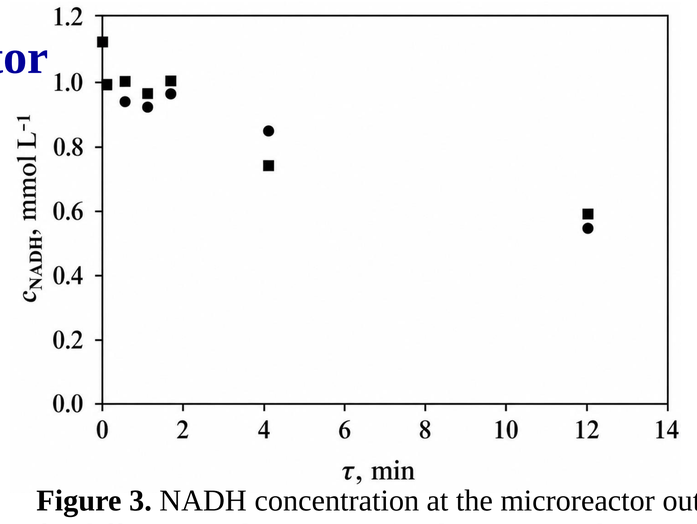


Figure 3. NADH concentration at the microreactor outlet for different residence times (■ d = 1 mm, ● d = 0.5 mm)

- **NADH conversion increased with residence time**, reaching 47.18 % and 43.07 % at 11.800 min in the 0.5 and 1.0 mm microreactor (Figure 3.)
- NADH consumption rates were similar to the batch system, indicating no significant process intensification under the investigated conditions

Table 2. NADH consumption rate for the NADH oxidation reaction carried out in microreactors of different dimensions and at different retention times.

τ, min	r_{NADH}, mmol L^{-1} min^{-1} (d = 0.5 mm)	r_{NADH}, mmol L^{-1} min^{-1} (d = 1 mm)
0.118	0.1476	0.0812
0.492	0.1310	0.0115
0.983	0.0834	0.0416
1.475	0.0277	0.0025
3.933	0.0413	0.0687
11.800	**0.0399**	**0.0365**

CONCLUSION

In the batch reactor, an initial NADH concentration of 1.00 mmol L^{-1} resulted in complete conversion and a maximum oxidation rate of 0.0379 mmol L^{-1} min^{-1}. Under comparable conditions, the microreactors achieved NADH conversions of approximately 43 – 47 % at the longest investigated residence time of 11.800 min, with NADH consumption rates similar to those obtained in the batch system. These findings indicate that microreactor operation did not provide significant process intensification under the investigated conditions, nor was any improvement observed upon decreasing the channel dimensions, suggesting that the reaction was limited primarily by enzyme activity and availability rather than by mass-transfer constraints. Further process improvement should therefore focus on optimizing residence time and enzyme and substrate concentrations, as well as exploring enzyme immobilization and integration with a continuous NADH regeneration system.