

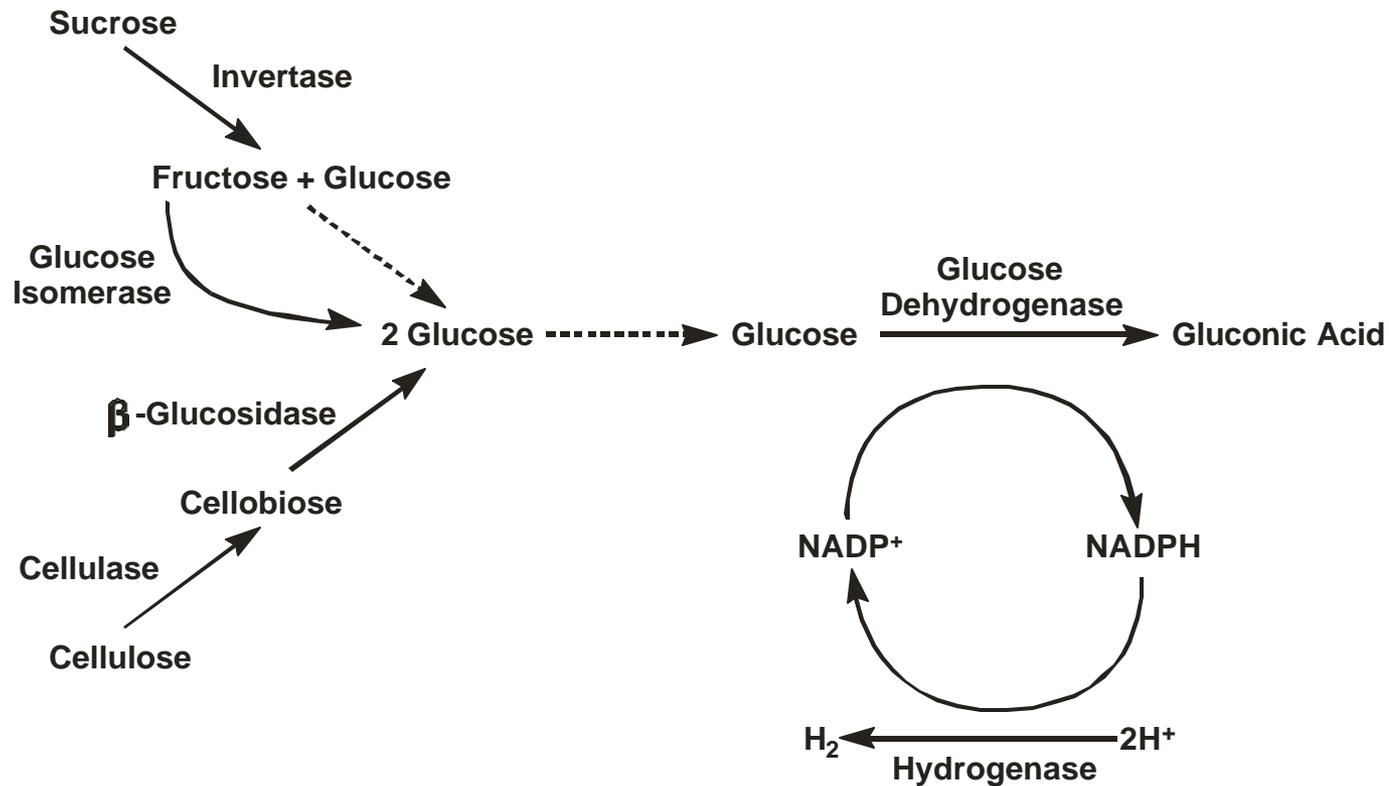
Construction of a Bio-Hydrogen Fuel Cell: Utilization of Environmental Sources of Carbohydrates

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Pathway for the Enzymatic Production of H₂ from Environmental Sources of Carbohydrates



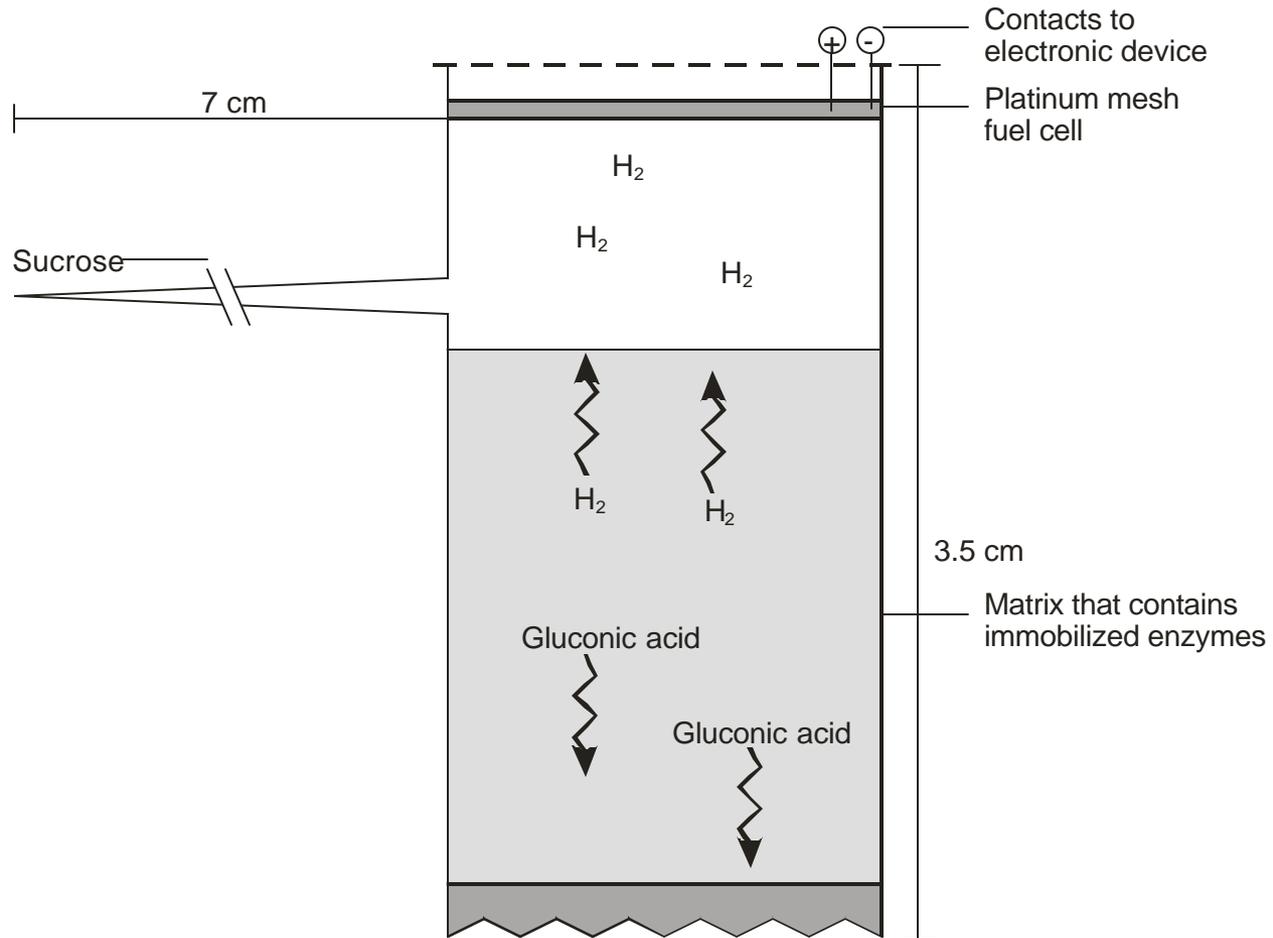
Research Challenges

1. The kinetic optimization of the enzyme/coenzyme system
2. Immobilization and stabilization of the enzymes of the pathway and the demonstration of recovery of activity and reuse.
3. Immobilization and stabilization of NADP⁺ in a manner that it can communicate effectively with the enzymes.
4. Demonstration of the operation of an electronic device powered by a fuel cell that utilizes the H₂ produced from the enzymatic oxidation of sucrose.

Research Objective

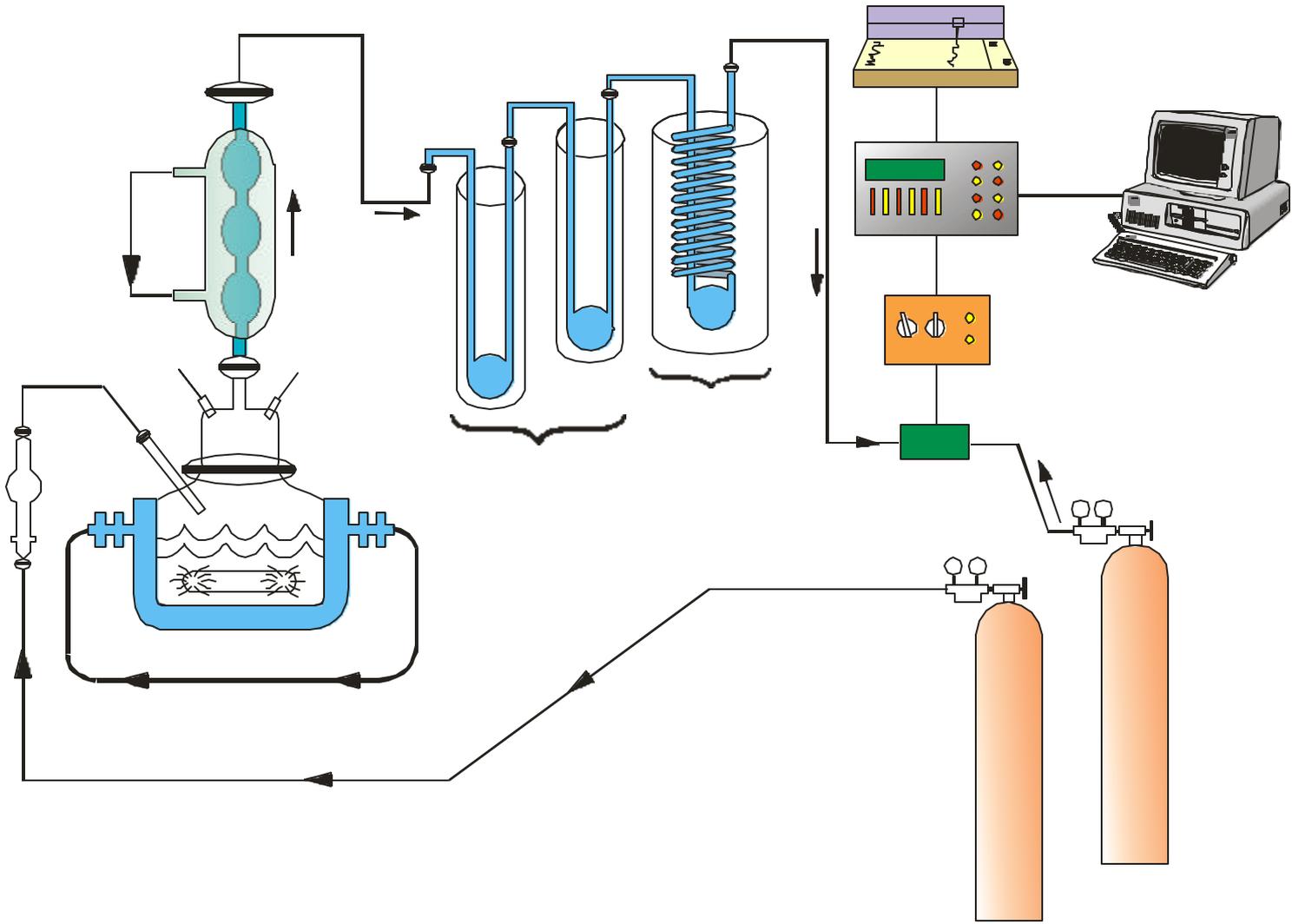
The development of biofuel cell technology employing environmental sources of energy for the operation of low power-requiring sensors.

Schematic Diagram of Fuel Cell



S

CO_2 (KOH)
apert



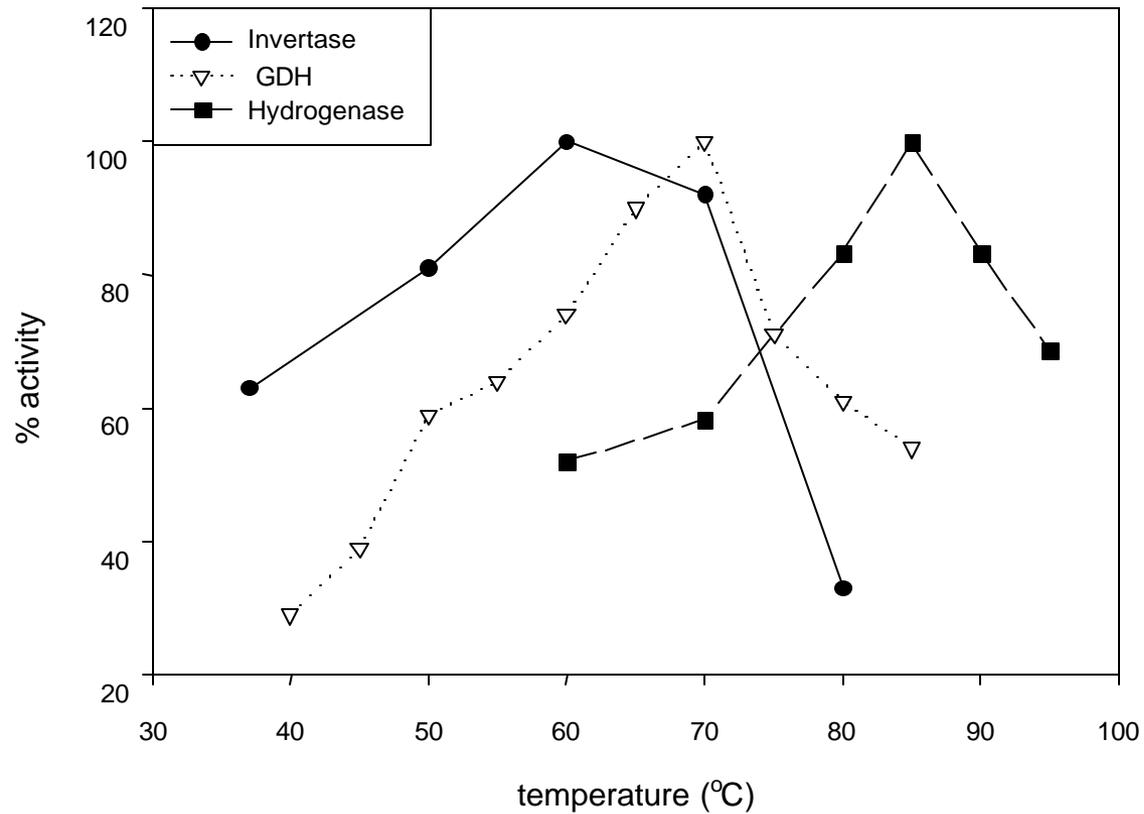
Catalytic Characteristics of the Enzymes of the Reaction Pathway

Enzyme	Optimal pH	Optimal temp. (°C)	Km (mM)	Turnover no. ² (min ⁻¹)
Hydrogenase ¹ <i>P. furiosus</i>	8.5	80 ¹	0.2 (NADPH)	1667
Glucose dehydrogenase <i>T. acidophilum</i>	6.5	70	0.11 (NADP ⁺) 10.0 (Glucose)	39,891
Invertase <i>Candida utilis</i>	5.0	60	28 (Sucrose)	73,170

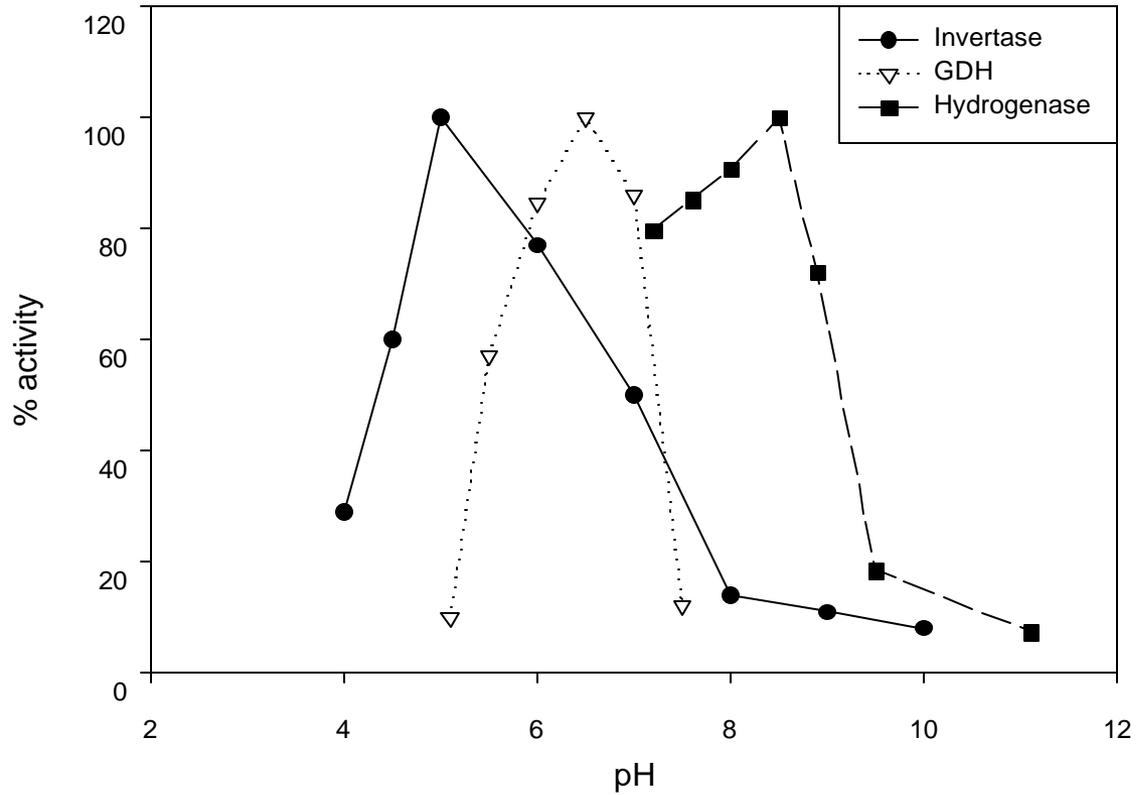
Notes: ¹ Hydrogenase has greater activity at higher temperatures but is less stable.

² Calculated at the optimal pH and temperature for activity

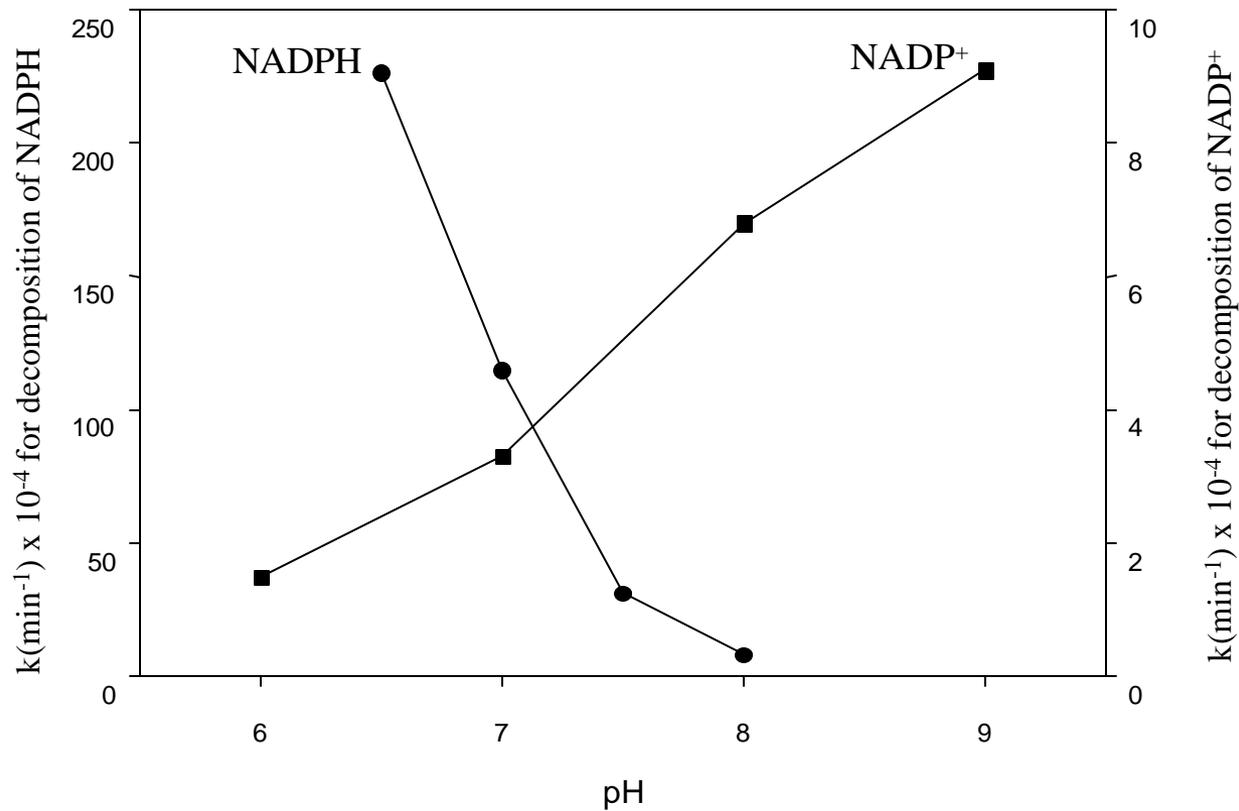
The Effect of Temperature on the Activities of Invertase, Glucose Dehydrogenase and Hydrogenase



The Effect of pH on the Activities of Invertase, Glucose Dehydrogenase and Hydrogenase



Rate Constants for the Decomposition of NADP⁺ and NADPH at Different pH Values



The Stability of NADPH Incubated at 55°C at Various pH Values

pH	k (min ⁻¹)	T _{1/2} (min)
6.5	0.02263	30.62
7.0	0.01145	60.52
7.5	0.0031	233.5
8.0	0.000785	882.8

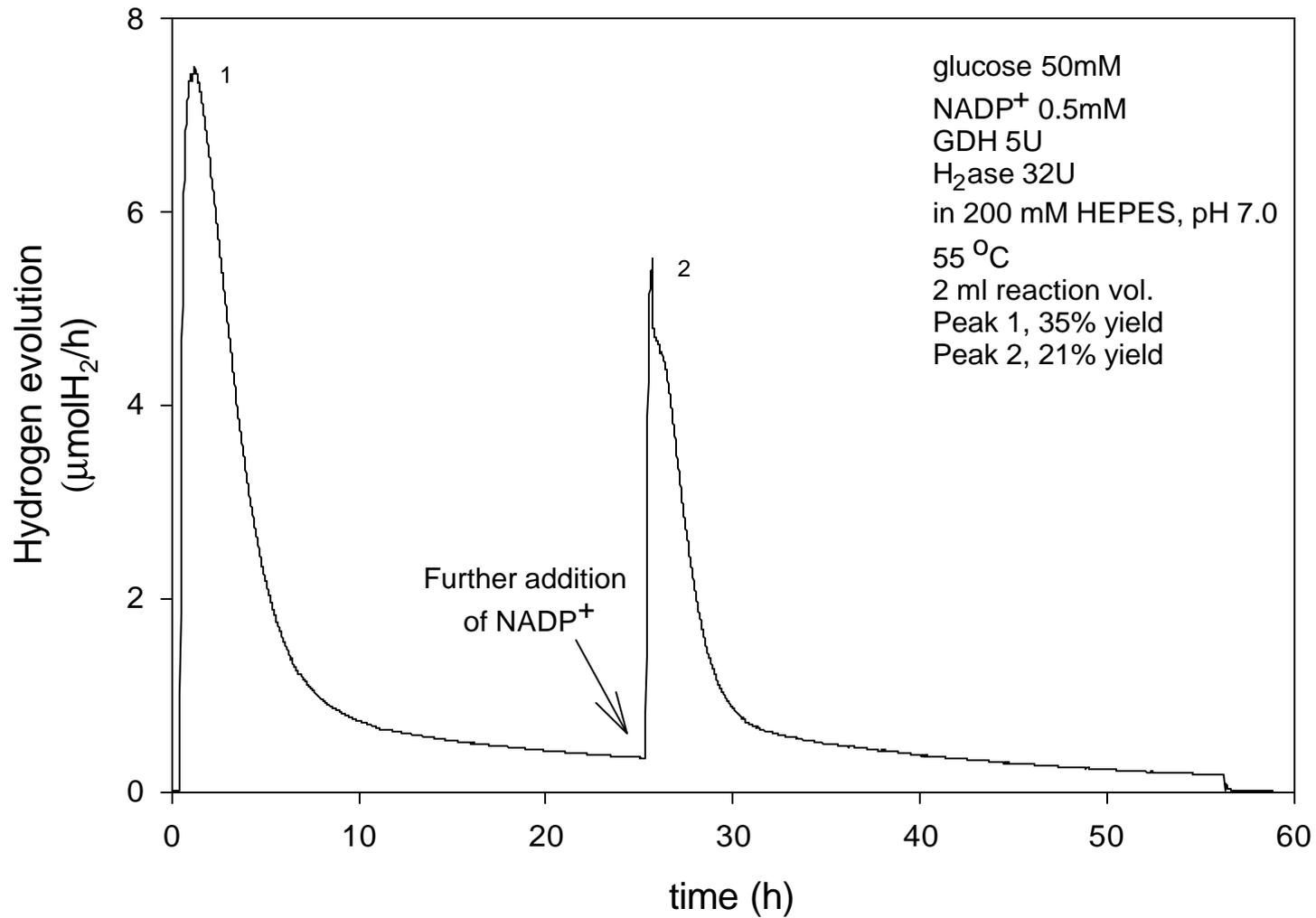
Notes: The reactions were carried out in 50mM HEPES at the indicated pH at 55°C. The initial concentration of NADPH was 150 μM and its decomposition was recorded by measurement of absorbance at 340 nm.

The Stability of NADP⁺ at 55°C Incubated at Various pH Values

pH	k (min ⁻¹)	T _{1/2} (min)
6	0.00015	4620
7	0.00033	2100
8	0.00068	1014
9	0.00091	756

Notes: The reactions at pH 6-8 were carried out in 50 mM HEPES at the indicated pH at 55 °C. The reaction at pH 9 was carried out in a 50 mM Tris buffer. The breakdown of NADP⁺ was measured indirectly by measurement of the amount of NADP⁺ that was reduced to NADPH in a glucose dehydrogenase assay. The initial concentration of NADP⁺ was 3.125 mM, 50µl samples were removed at intervals for assay.

The Effect of Additional NADP^+ on the Yield of H_2

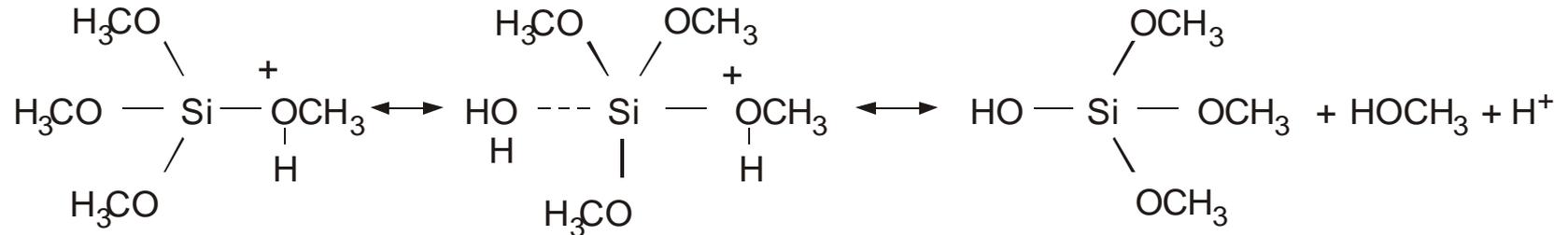


Enzyme Immobilization Studies

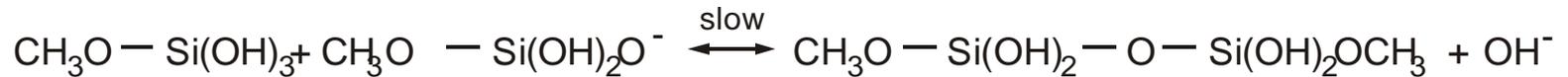
- Enzymes investigated
 - Invertase
 - β -glucosidase
 - glucose dehydrogenase
- Immobilization of invertase
 - Sol-gels
 - Propylene glycol alginate-gelatin beads

The Chemistry of the Sol-gel Process

Acid Catalyzed Hydrolysis



Base Catalyzed Condensation

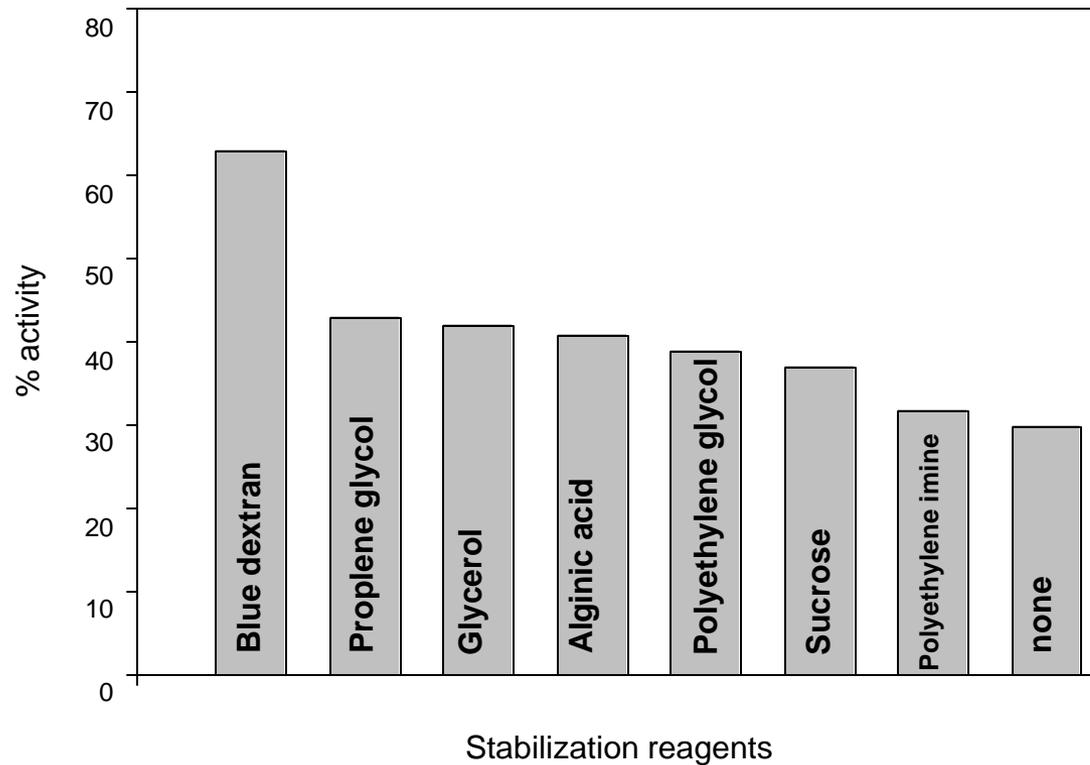


Immobilization of Enzymes in Sol-Gels

Enzyme	Initial activity*	Activity after immobilization*	Recovered activity
	(Units)	(Units)	(%)
Invertase	0.60	0.18	30.0
Glucose dehydrogenase	0.44	0.01	2.2
β -glucosidase	0.47	0.13	27.8

* per 50 μ l bead

The Effect of Various Stabilization Reagents on the Activity of Immobilized Invertase

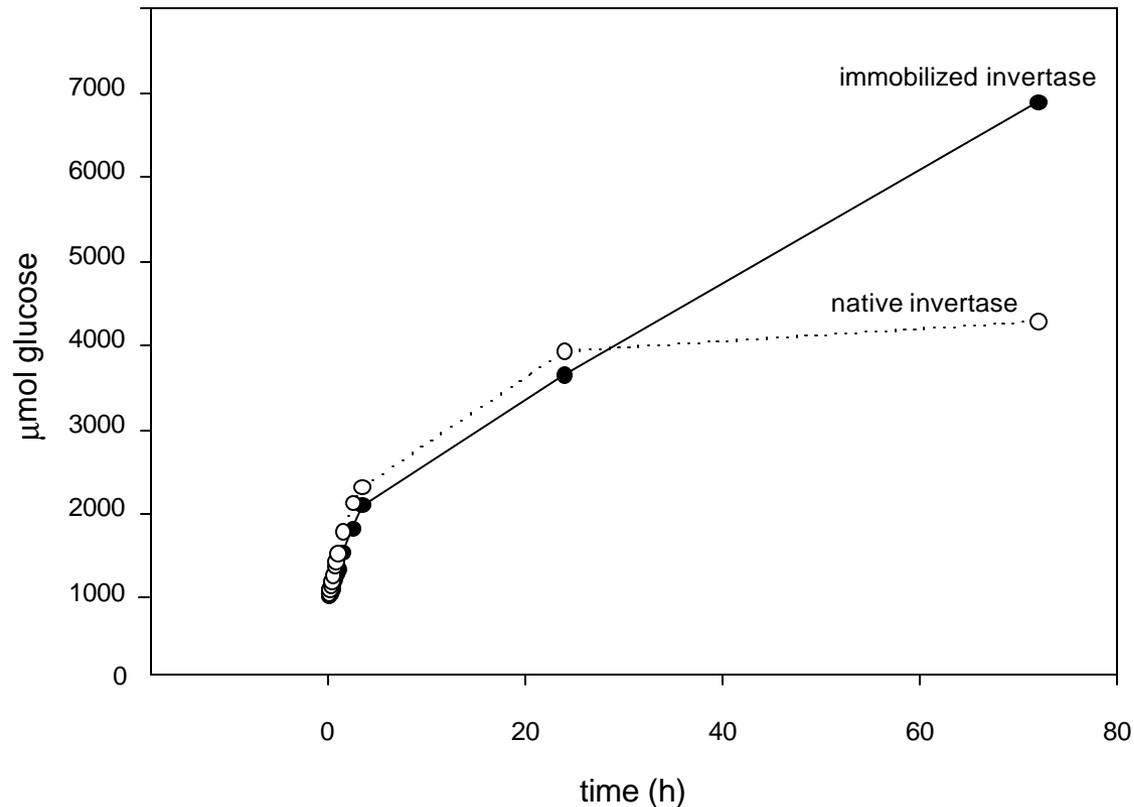


Effect of Blue Dextran on the Recovery of Enzyme Activity

Enzyme	Initial activity*	Activity after immobilization*	Recovered activity
	(Units)	(Units)	(%)
Invertase	0.60	0.38	63.0
Glucose dehydrogenase	0.44	0.01	3.0
β -glucosidase	0.47	0.24	51.5

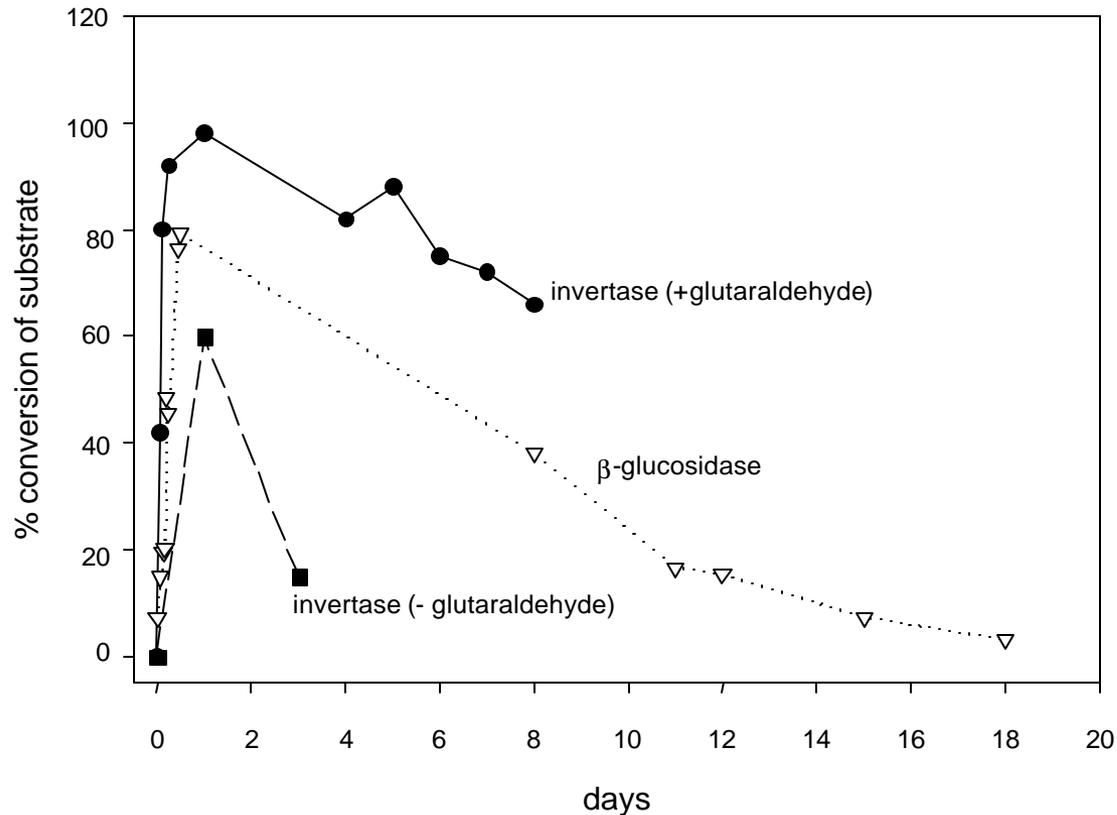
* per 50 μ l bead

The Operational Stability of Invertase Immobilized in Sol-Gels Compared to Native Invertase



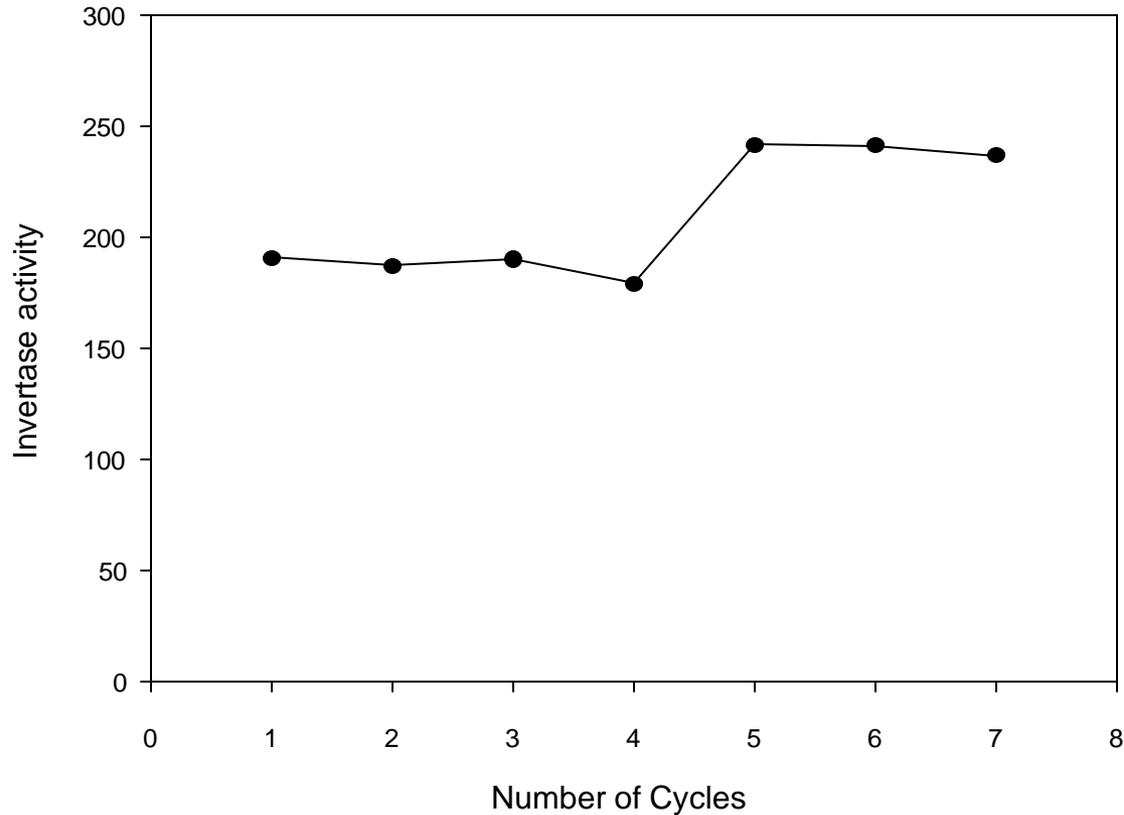
Notes: The enzymes (0.32 U activity) were incubated in 50 mM sucrose at 70°C. Samples (0.1mL) were removed at time intervals and the amount of glucose present was determined.

Sol-Gel Immobilized Enzymes in Continuous Flow Bioreactor Apparatus



Notes. Invertase (+glutaraldehyde): Sucrose (5mM) pumped at $0.1 \text{ ml} \cdot \text{min}^{-1}$ at 50°C
Invertase (-glutaraldehyde): Sucrose (10mM) pumped at $0.25 \text{ ml} \cdot \text{min}^{-1}$ at 50°C
β-glucosidase: Cellobiose (10 mM) pumped at $0.04 \text{ ml} \cdot \text{min}^{-1}$ at 72°C

The Effect of Dehydration and Rehydration on the Activity of Invertase Immobilized in Propylene Glycol Alginate-Gelatin Beads

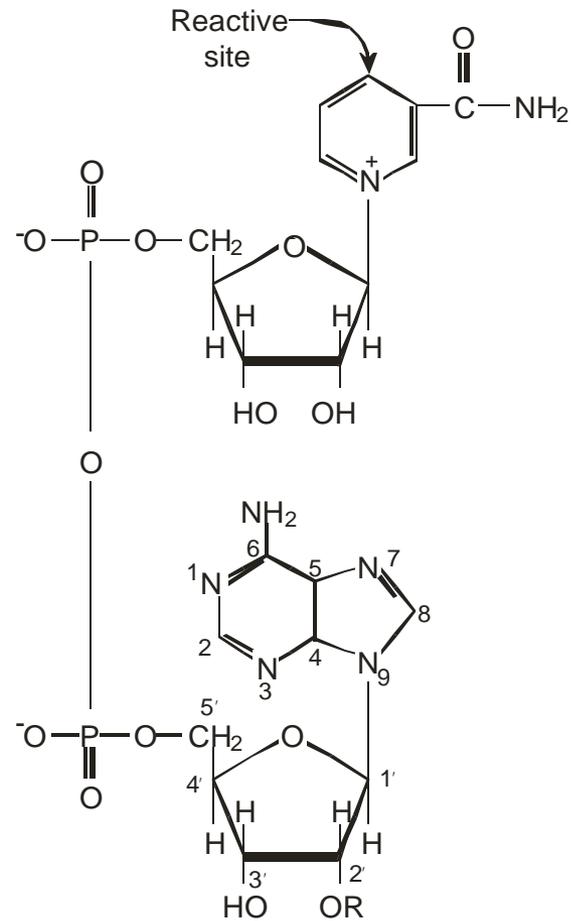


Invertase activity was expressed as nmole glucose formed $\text{ml}^{-1} \text{min}^{-1}$.

Immobilization of Glucose Dehydrogenase

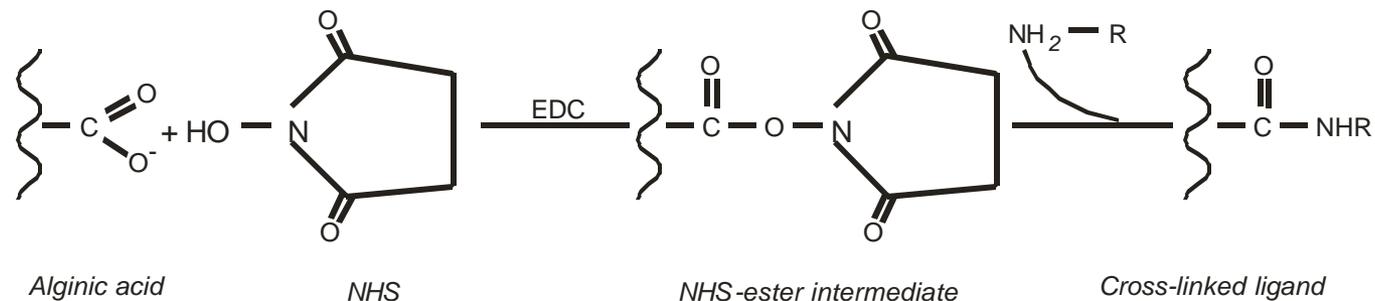
- Sol-gel
- Propylene glycol alginate-gelatin beads
- Polyacrylamide functionalized with azlactone groups
- On silicon chips using;
 - poly-L-lysine
 - gelatin (entrapment)
 - epoxysilane (covalent)

Structure of the Oxidized Forms of NAD⁺ and NADP⁺

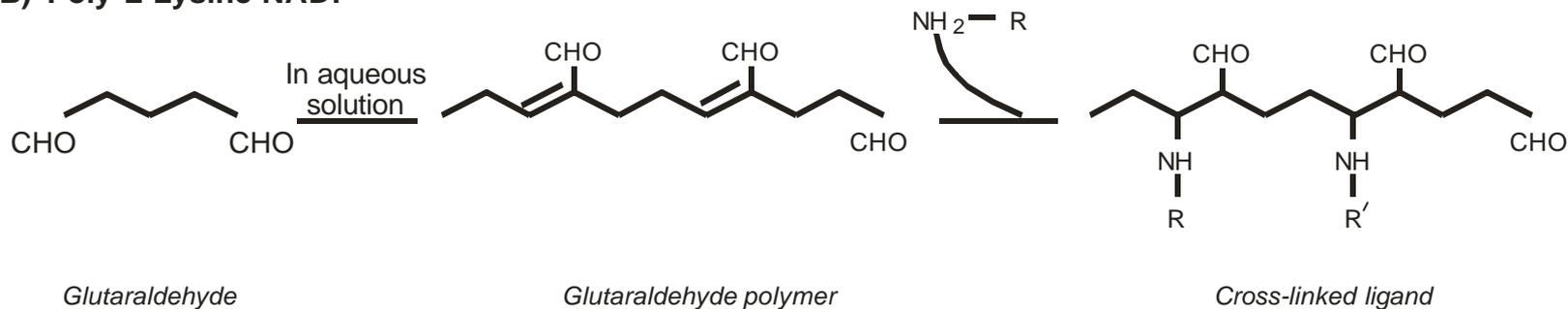


In NAD⁺, R = H; in NADP⁺, R = PO₃²⁻

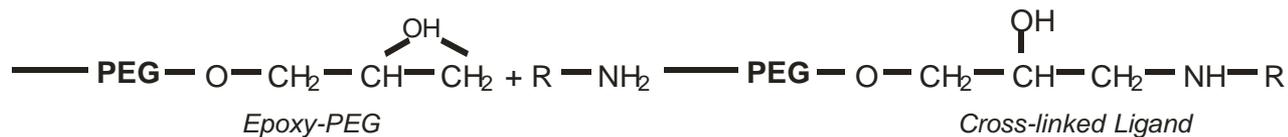
A) Alginate-NADP⁺



B) Poly-L-Lysine-NADP⁺



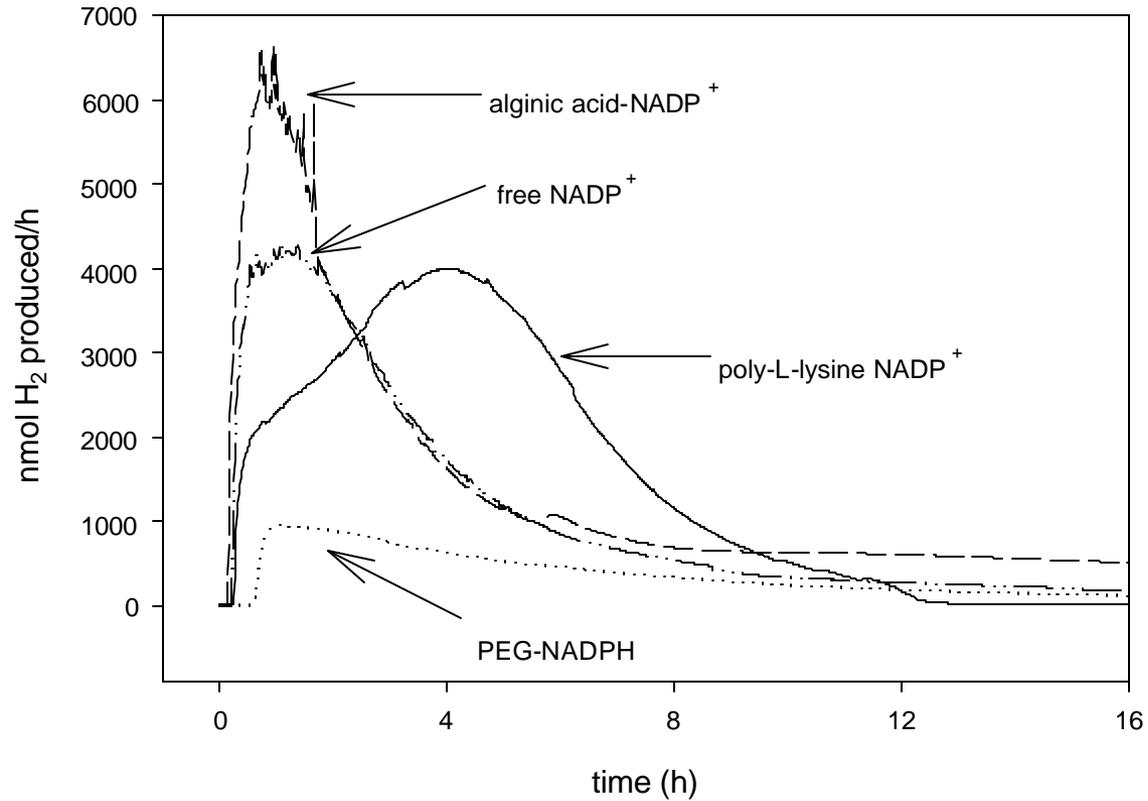
C) PEG-NADPH



The proposed reaction schemes for the coupling of NADP⁺ with various polymers.

NHS, *N*-hydroxysuccinimide; EDC, 1 ethyl-3-(3-dimethyl aminopropyl) carbodiimide; PEG, polyethylene glycol; NH₂-R, free amine at N⁶ on the adenosine portion of NADP⁺; NH₂-R', poly-L-lysine.

Hydrogen production using NADP^+ attached to different polymers



The reactions contained 0.2 mM NADP^+ , 50mM Glucose, 2 U glucose dehydrogenase, and 20 U hydrogenase in 50 mM HEPES buffer, pH 7.0

H₂ Production Using Different NADP⁺ Polymers

Cofactor polymer	Yield of H ₂ (μmol)	Maximal rate (μmol/h)	% of theoretical yield
Free NADP ⁺	20.6	4.15	20.6
PEG-NADPH	6.22	0.97	6.22
Alginic acid- NADP ⁺	23.5	6.28	23.5
PLL-NADP ⁺	23.33	3.81	23.33

Future work

- Determination of the optimum conditions for the production of H₂ from glucose using metabolic modeling software with the kinetic data that has been collected to date.
- The conditions for the immobilization of NADP⁺ on alginic acid will be optimized. Other methods for the immobilization of NADP⁺ will also be examined.
- The co-immobilization of the four enzymes of the pathway, invertase, glucose isomerase, glucose dehydrogenase and hydrogenase, in an enzyme cartridge.
- Demonstration of the fuel cell.
- A study into the stabilization of the cofactor and use of cofactor analogs will be carried out.

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