**BIO-HYDROGEN**

**Thermophilic enzymes**

- **Properties of thermophilic enzyme**
  - [Fig. 6. Thermal stability of the enzyme](Image)
  - Enzyme precipitation at 75°C.
  - The purified protein was incubated in 100 mM of HEPES buffer (pH 7.5).

**Pathways of the enzymatic cascade reactions**

- **Target enzymes for cascade reactions**
  - [Fig. 12 Target enzyme reaction for demonstration of cascade reaction by TIM, ALD, FBP, and PGI](Image)

**Simple low cost purification of thermo-enzymes**

- **Co-immobilization of the four enzymes in hybrid microcapsule**
  - [Fig. 18. SDS-PAGE analysis of the purified recombinant enzyme for co-immobilization, S, cell; yaeT; P, purified enzymes](Image)

**Immobilization of enzymes**

- **One-step purification & immobilization of enzyme**
  - [Fig. 8. One-step protein purification and immobilization of a CBM-tagged Clostridium Thermostoma phosphoglucomutase (PGI, EC 5.3.1.9) on a cellulose filter](Image)
  - Enzyme precipitation at 75°C.

**Thermal stability of the immobilized enzyme**

- [Fig. 11. Thermal stability (half-life time, t1/2) of free PGI (a) and immobilized CBM-PI (b) at different concentrations of enzyme in 100 mM of HEPES buffer (pH 7.5) containing 100 mM NaCl and 10 mM MgCl2 at 80 °C](Image)

**Summary**

- [Fig. 13. Scheme of enzymatic pathway containing enzyme-limited reactions and substrate-limited reactions](Image)
  - Ln (residual activity %)
  - Time (h)
  - 60

**References**


**Challenges and possible solutions in vitro SyPAb**

- [Fig. 2. The non-SyPAb for high yield hydrogen generation from starch or cellulose materials](Image)
  - Y.-P. Zhang, Microbes 4 (2009), S60.
  - CHO2+CHO2+ = THF (I) + 12% (g) + EC0 (g)

**THERMOPHILIC ENZYMES**

- **Fig. 5. Optimal thermostability of the purified FBP (EC 5.1.1.17) from hyperthermophilic bacteria Thermotoga maritima at pH 7.5**

**ENZYMATIC CASCADE REACTIONS**

- **Fig. 10. Leakage of the CBM-PGI**
  - [Different concentrations of enzyme in 100 mM of HEPES buffer (pH 7.5) containing 100 mM NaCl and 10 mM MgCl2 at 80 °C](Image)

**PURIFICATION OF ENZYMES**

- **Fig. 7. Single step low cost purification of a thermo-enzyme**
  - Thermostable Enzyme (E), FBPase (15 U/mg), EC 4.1.2.13) by heat precipitation and enzyme solubilization. Final precipitation is approximately 90% of the initial enzyme.

**IMMobilization of enzymes**

- **Fig. 9. A family 2 cellulase binding module (CBM) from the Clostridium thermostoma phosphoglucomutase (PGI, EC 5.3.1.9) on a cellulose filter**

**Fig. 14. Simulation results based the kinetic of the enzymes (substrate: 5 mM gal)**

**Fig. 15. Leakage of the CBM-PGI**

**Fig. 16. Scheme of co-immobilization in hybrid microcapsules as a biocatalytic module**

**Fig. 17. Scheme of immobilization in hybrid microcapsules as a biocatalytic module**

**Fig. 3. Two different electricity generation systems based on carbohydrates. (a) High power electricity generator. (b) Low power battery. **Y.-P. Zhang, International Journal of Hydrogen Energy. 30 (2010) 1034–1042**

**Fig. 1. Scheme of the hydrogen economy based on renewable carbohydrate**

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**Abstract**

The production of hydrogen from low-cost renewable biomass is appealing because biomass is an enriched, collectable renewable energy source. The use of carbohydrates as high-density hydrogen carriers and energy sources for hydrogen production is possible due to cell-free synthetic biology technology. Synthetic pathway biotransformation (SyPB) is the implementation of complex biochemical reactions in the vitro assembly of a large number of enzymes and coenzymes. Hydrogen can be produced from carbohydrate with an overall reaction of CH2O + 3H2O → 2H2 + 2CO2 + 4H2O. As a result, nearly 12 miles of hydrogen are produced per gallon equivalent of polyacrylamides and water by this non-natural synthetic pathway which contains 13-14 enzymes. For facilitating cascade reactions among three enzymes: Thermus thermophilus triose phosphate isomerase (TtTIM), Thermotoga maritima fructose bisphophate aldolase (TmALD), Thermotoga maritima fructose 6,6-bisphosphatase (TmFBP), and Clostridium cellulose binding module phosphoglucomutase (CmolPGI), we need find optimal conditions and proper enzyme ratios for co-immobilized enzymes. In this poster, we will present our recent progresses in properties of thermophilic enzymes, their purification and immobilization, and enzymatic cascade reactions.