Conversion of biomass to hydrogen through a synthetic enzyme pathway

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Why Hydrogen?

- Hydrogen is an important industrial chemical
- ~75 million metric tons produced annually worldwide ($135 billion) from **fossil fuels**[1]
  - Ammonia synthesis (50%), hydrocracking (37%), and methanol synthesis (8%)
- 1 mole CO_2:4 mole H_2
  - 413 million metric tons of CO_2
  - 1.3% of CO_2 emissions annually[2]

Ammonia Synthesis Plant

http://www.kbr.com/Images/Content/Full/Ammonia-Synthesis-Plant.jpg

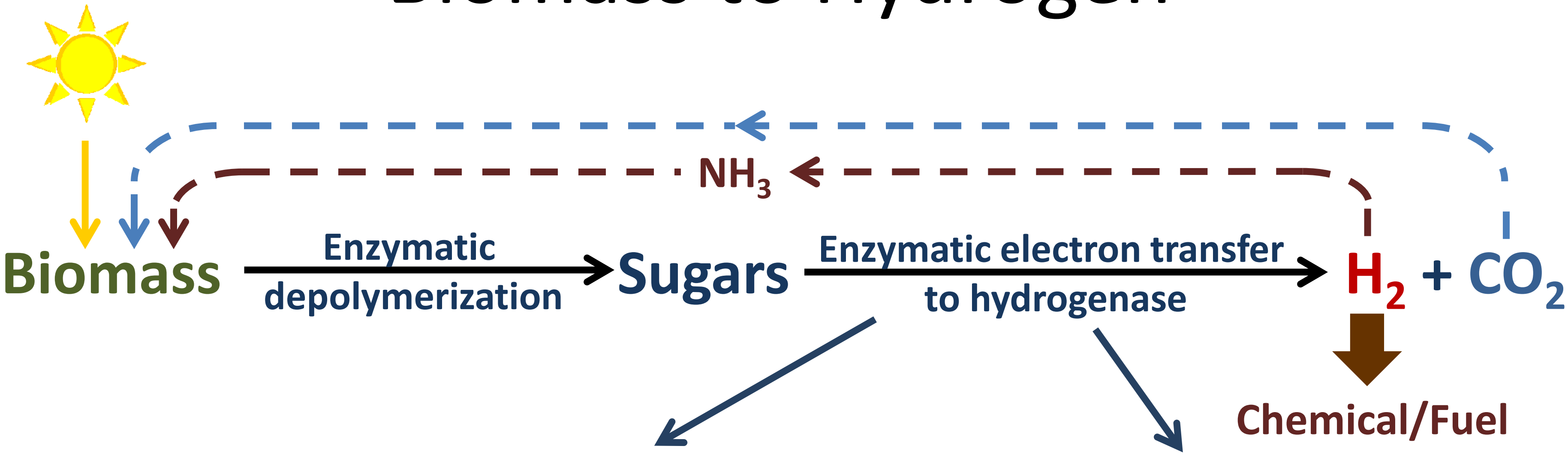
[1]Balat, 2010, Int. J. Hyd. Energy, http://en.wikipedia.org/wiki/Hydrogen_economy
[2]CO_2 calculations with Kunal Mehta, www.google.com/publicdata

Sunlight to Hydrogen

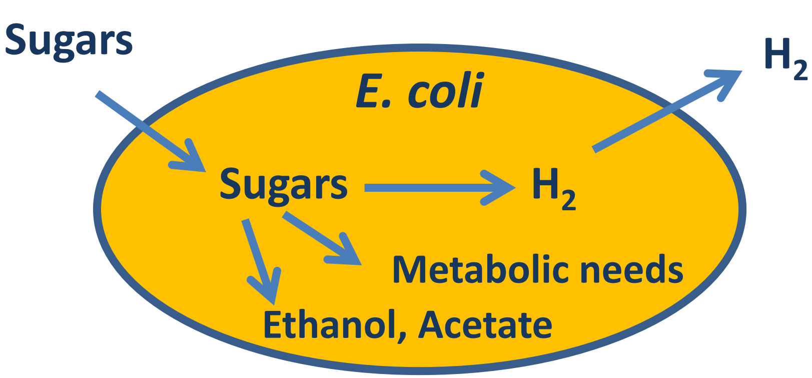
Biomass → (Millions of Years) → Fossil Fuels → (Steam Reforming) → $H_2 + CO_2$

Biomass → (Enzymatic depolymerization) → Sugars → (Enzymatic electron transfer to hydrogenase) → $H_2 + CO_2$

Biomass to Hydrogen

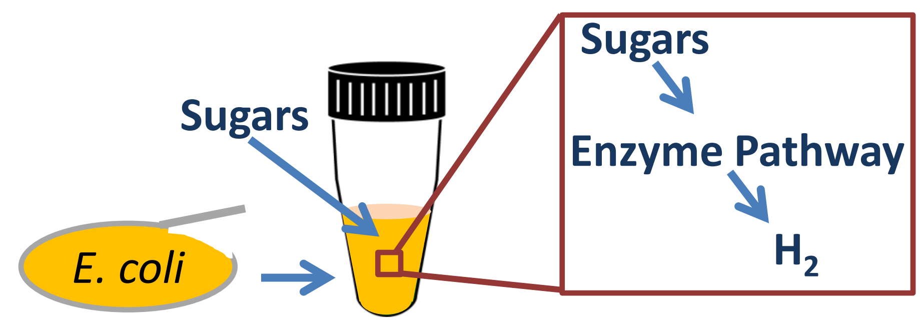


Fermentative (*in vivo*)



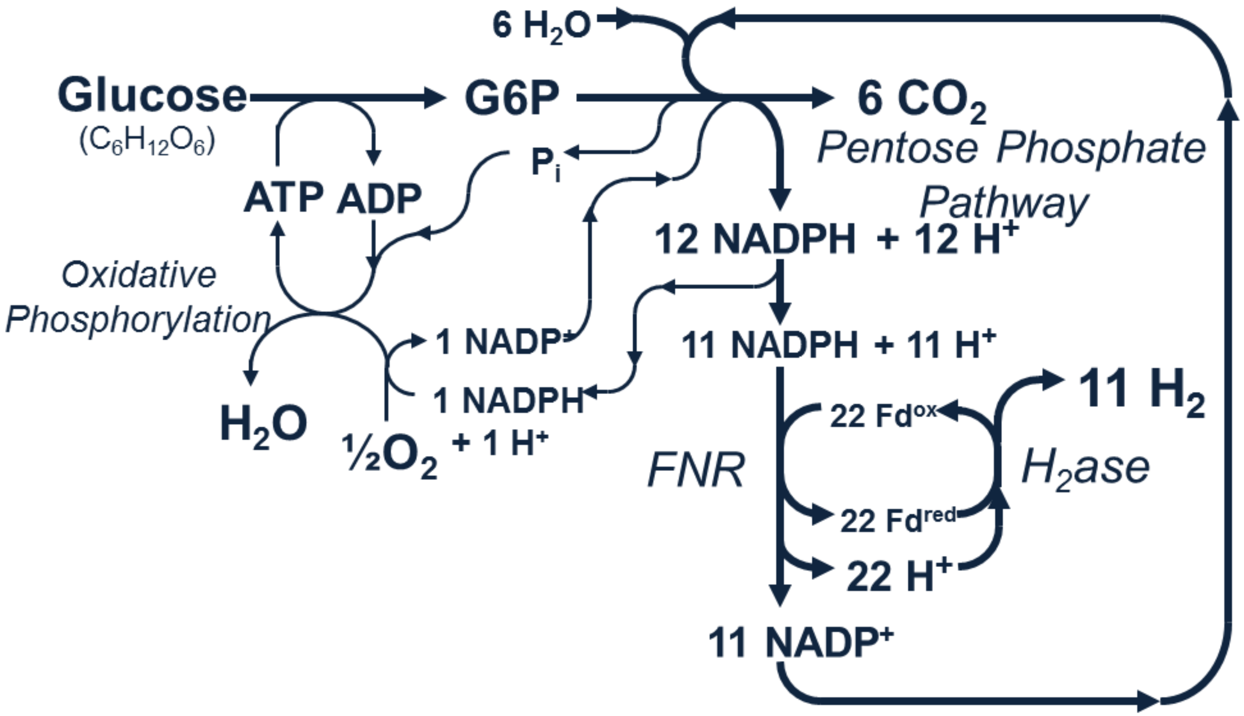
- Yield (2-4 H_2 per 1 glucose)
- Biomass hydrolysate toxicity
- Volumetric productivity

Cell-free (*in vitro*)



- Directly accessible
- High Yield (11 H_2 per 1 glucose)
- No toxicity
- Volumetric Productivity

Synthetic Enzyme Pathway



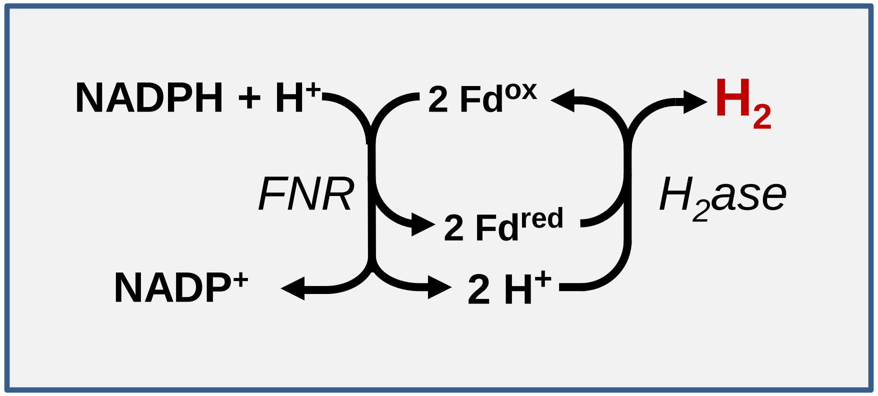
Ferredoxin-NADPH-reductase (FNR)

Ferredoxin (Fd)

[FeFe] Hydrogenase (H_2ase)

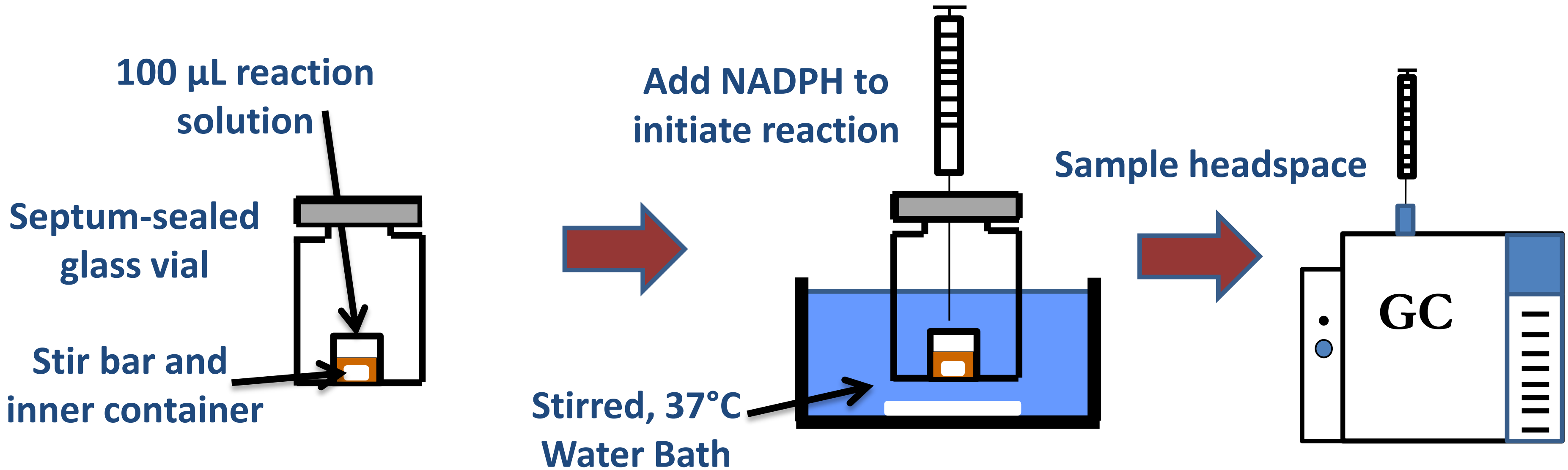
Glucose to H_2 Conversion:
$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2$
Target yield of 11 H_2 per glucose
(Maximum theoretical yield is 12)

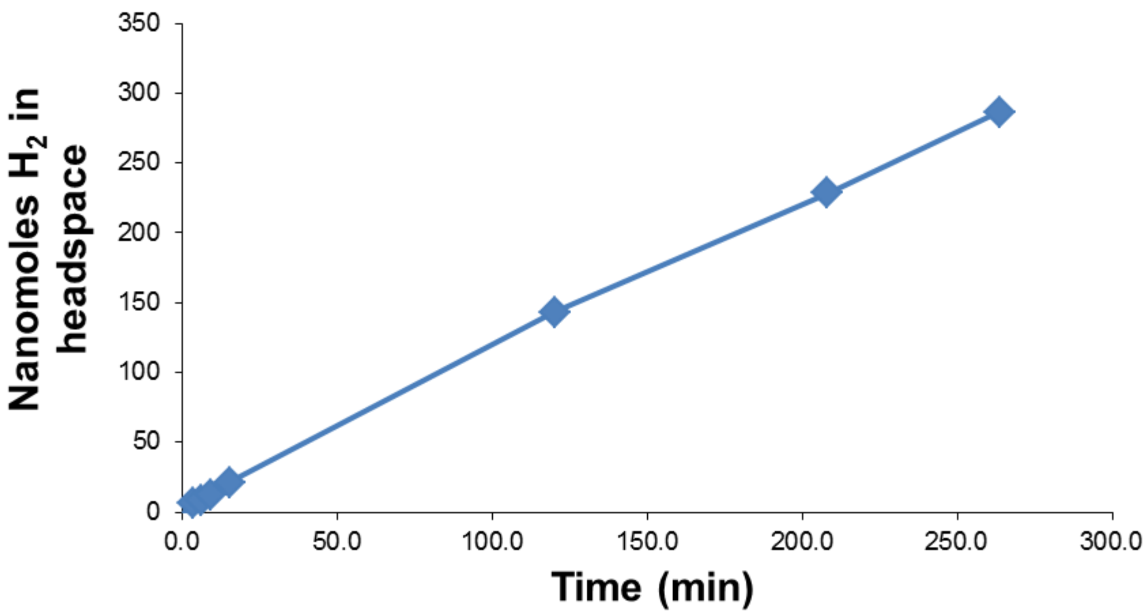
NADPH to Hydrogen: Proof of Principle



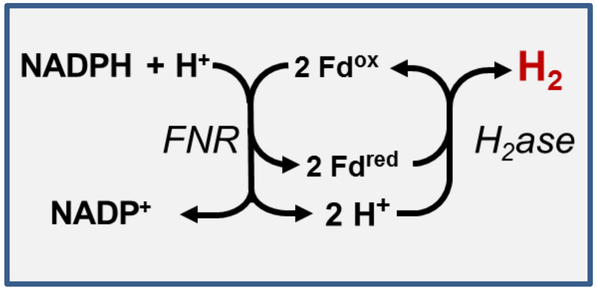
- Will these enzymes (from different microorganisms) work together at a practical rate?
- Can this sub-pathway interface with the larger pathway?
- How does this compare to other biofuel technologies?

Data Collection

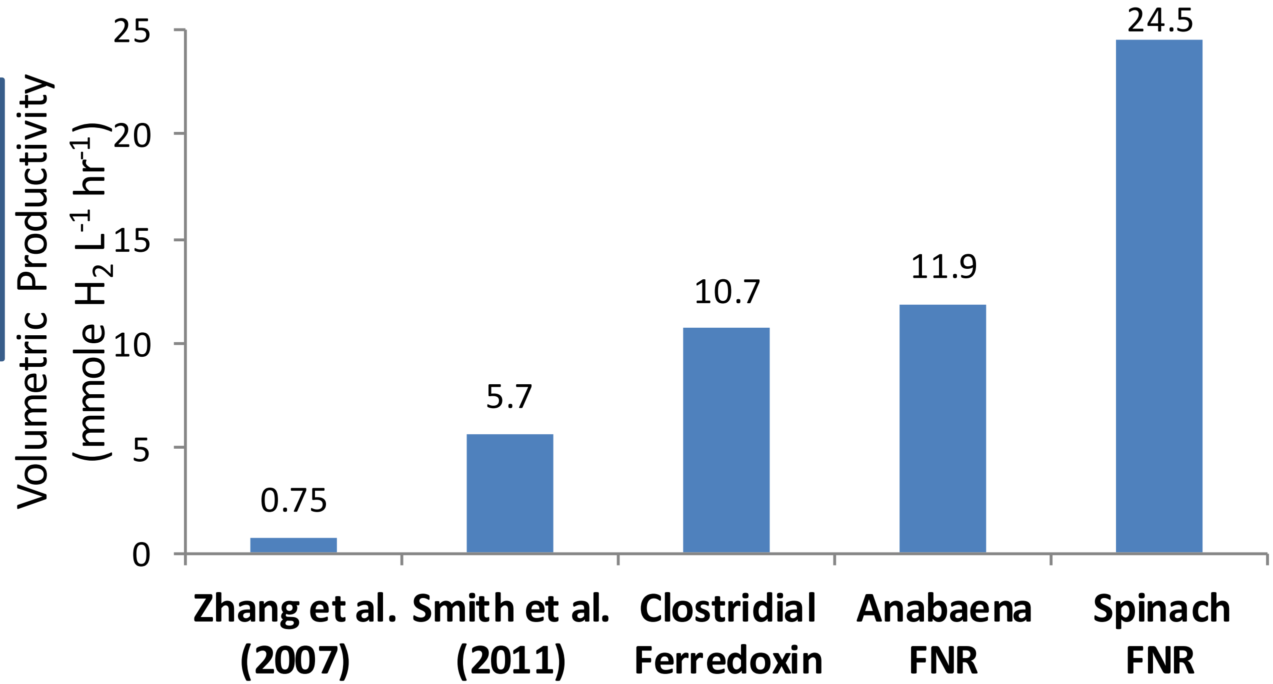




Proof of Principle: H_2 From NADPH



H_2ase = [FeFe] hydrogenase from *Clostridium pasteurianum*

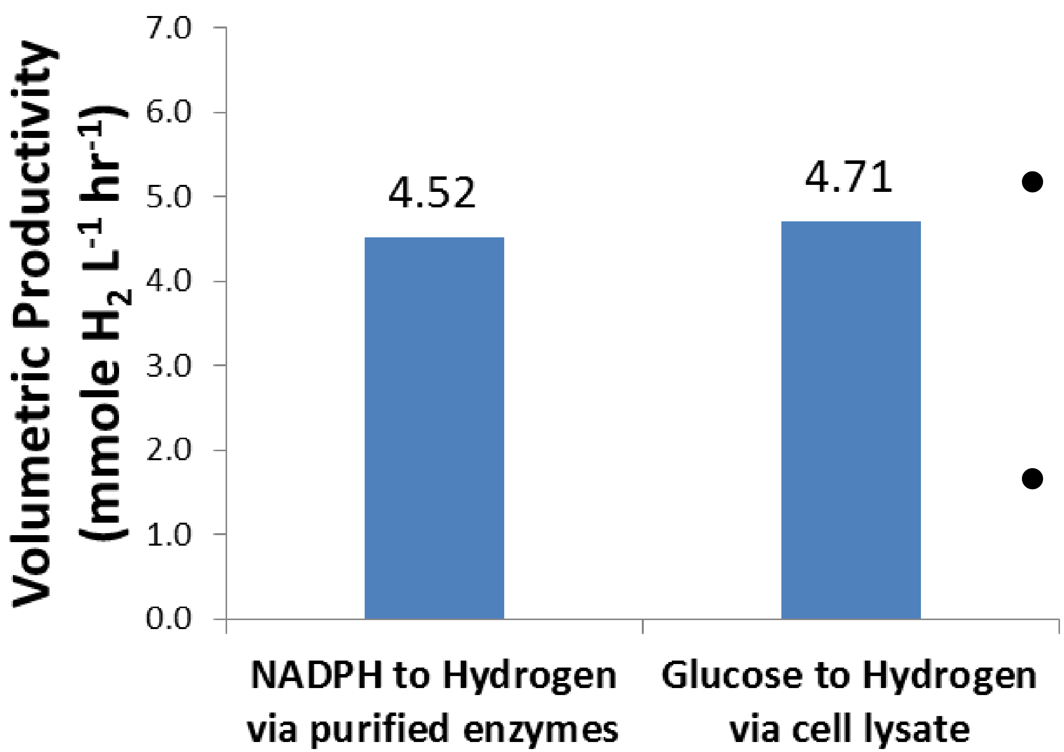


		Zhang et al. (2007)	Smith et al. (2011)	Clostridial Ferredoxin	Anabaena FNR	Spinach FNR
Ferredoxin		N/A	*Synechocystis*	*Clostridium*	*Clostridium*	*Synechocystis*
FNR		N/A	*E. coli*	*E. coli*	*Anabaena*	Spinach
FNR TON (sec^{-1})	(Max = 20-200)	N/A	0.01	0.04	0.04	0.2
Hydrogenase TON (sec^{-1})	(Max = 3000)	0.005	1.5	3.1	3.3	3.4

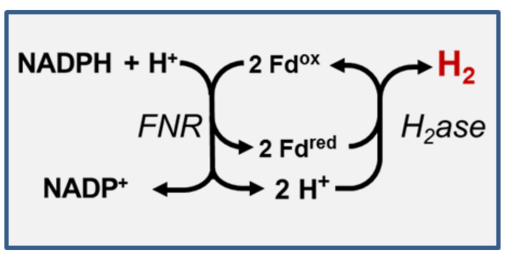
TON = turnover number, i.e., how many times an enzyme catalyzes a reaction per second

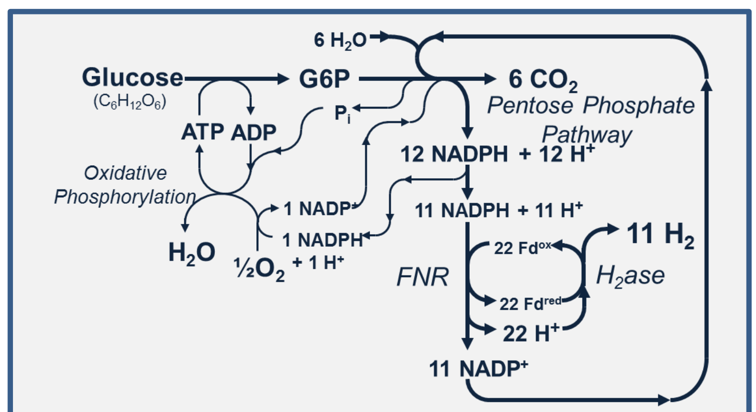
Smith, Bingham, Swartz (2011). Int. J. Hyd. Energy, in Press

Proof of Principle: Glucose to H_2

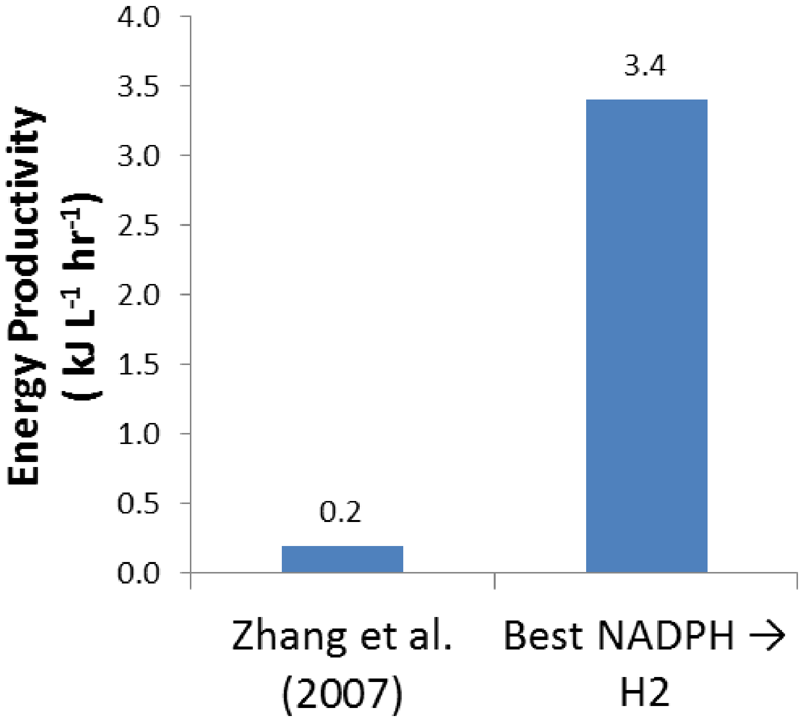


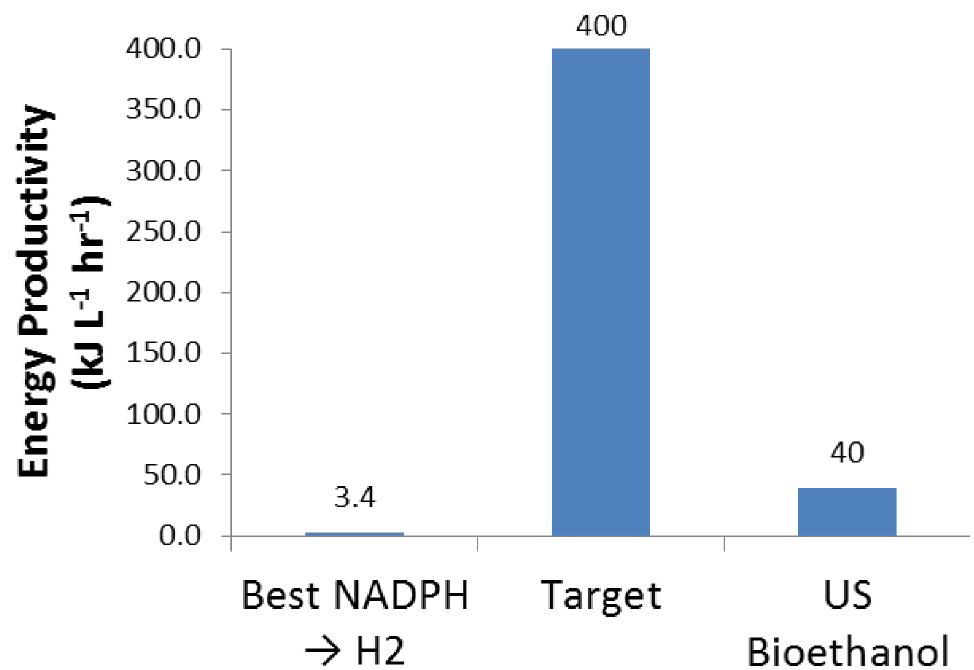
- Glucose is powering H_2 production
- The *FNR/Fd/H_2ase* pathway is limiting



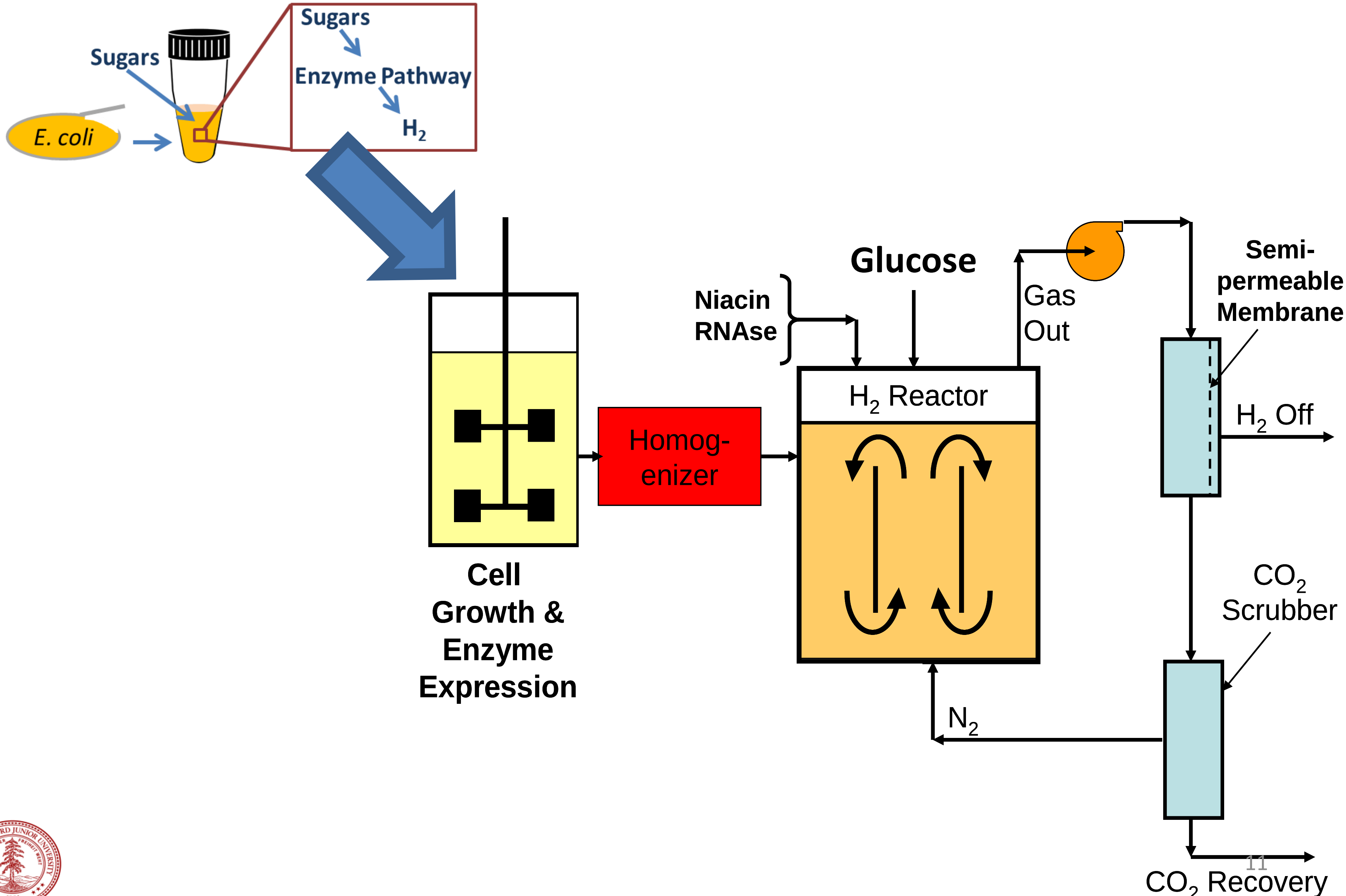


Energy Productivity





Envisioned Industrial Process



Analysis

- Limited electron transfer from *FNR* to *Fd* as shown by low turnover numbers
  - Sub-optimal binding of *FNR* and *Fd*?
  - Reduced ferredoxin regulating *FNR*?
  - Redox potential/electron transfer?

Future Work

- Create and test a kinetic model (Kunal Mehta)
- Engineer/evolve enzymes with improved binding and electron transfer

Conclusions

- Renewable, carbon-neutral production of hydrogen from biomass could significantly decrease CO_2 emissions
- We have designed a synthetic enzyme pathway to convert glucose to hydrogen and demonstrated proof of principle
- We believe that the FNR-Fd interaction is limiting and are pursuing a solution

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