Biotechnological Applications of Elementary Modes Analysis

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April 29, 2022

Outline

Some examples of applications of Elementary Modes Analysis:

- Pathway redesign for improving yield
- Modes of operation of the Woods–Ljungdhal pathway
- Channelling metabolism into desired routes

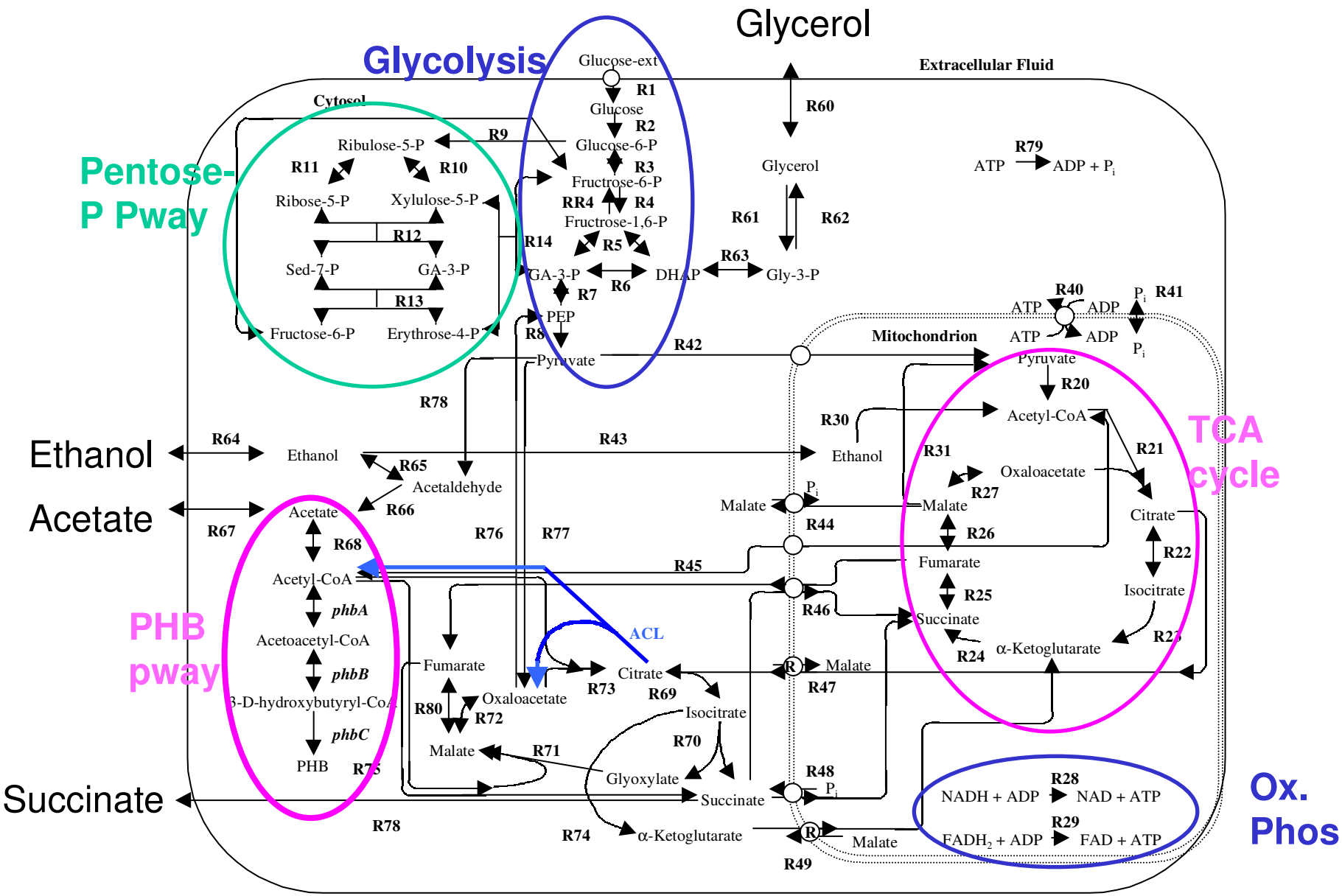
Pathway Design for Improving Yield

Biodegradable Plastics

Polyhydroxybutyrate or polyhydroxyalkanoates

Red

Polyhydroxybutyrate Synthesis in Yeast



Optimal yields of PHB synthesis

Based on highest–yielding elementary modes of the network:

Wild-type yeast + PHB pathway

1. 2 Acetate + EtOH $\rightarrow$ PHB + 2 CO_2 0.67
2. 65 Ac. + 31 EtOH $\rightarrow$ 30 PHB + 72 CO_2 0.63

(Number following each mode is the fractional carbon conversion.)

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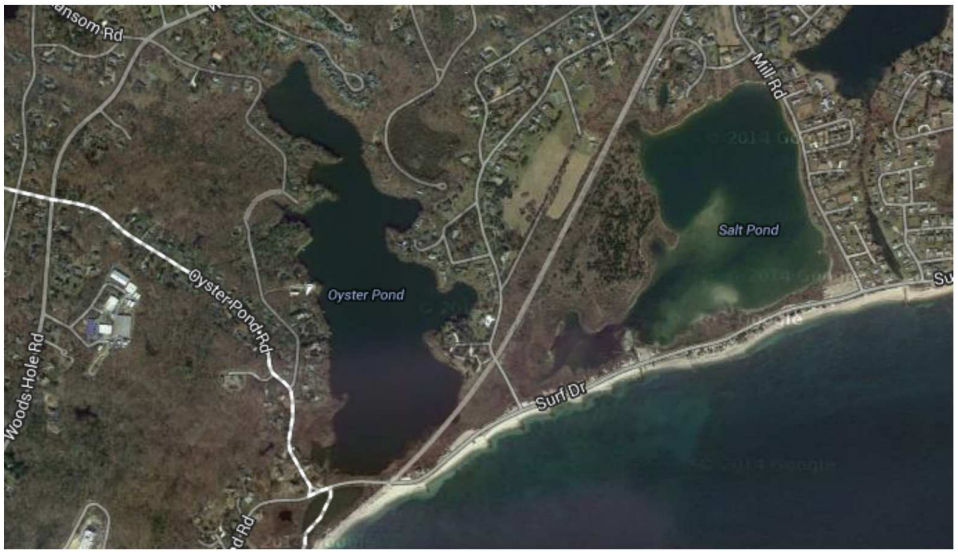
Wild-type yeast + ATP–citrate lyase + PHB pathway

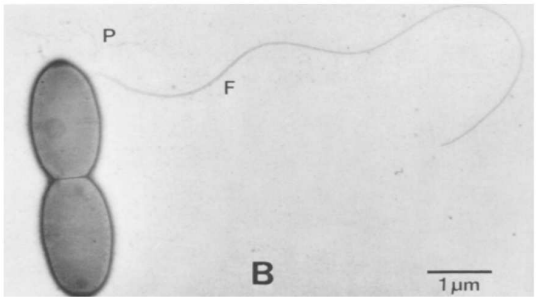
3. 12 EtOH $\rightarrow$ 5 PHB + 4 CO_2 0.83
4. 77 EtOH + 31 Glycerol $\rightarrow$
   48 PHB + 4 Ac. + 47 CO_2 0.78

(Number following each mode is the fractional carbon conversion.)

Acetobacterium woodii and the Woods-Ljungdahl Pathway

Acetobacterium Woodii





Isolated from high salinity lake ~ 32ppt

Non-pathogenic, highly motile, non spore forming and gram postive.

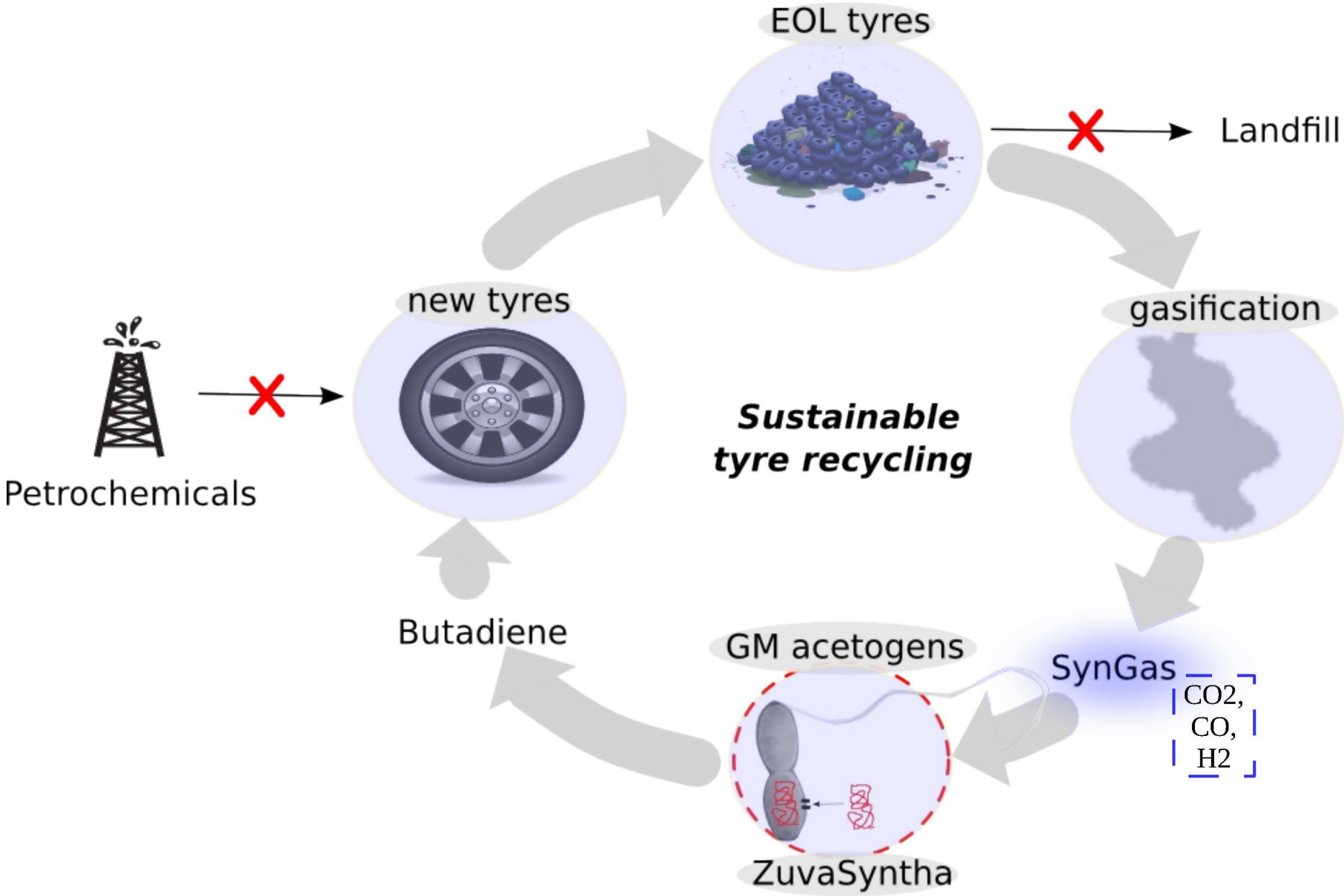
Within group of acetogens – 22 genera in soils, sediments, intestinal tracts.

Acetogens use the acetyl-CoA or Wood-Ljungdahl Pathway to grow autotrophically on inorganic substrates (H2-CO2, CO) but also metabolise organic substrates – hexoses, pentoses, alcohols, methyl groups.

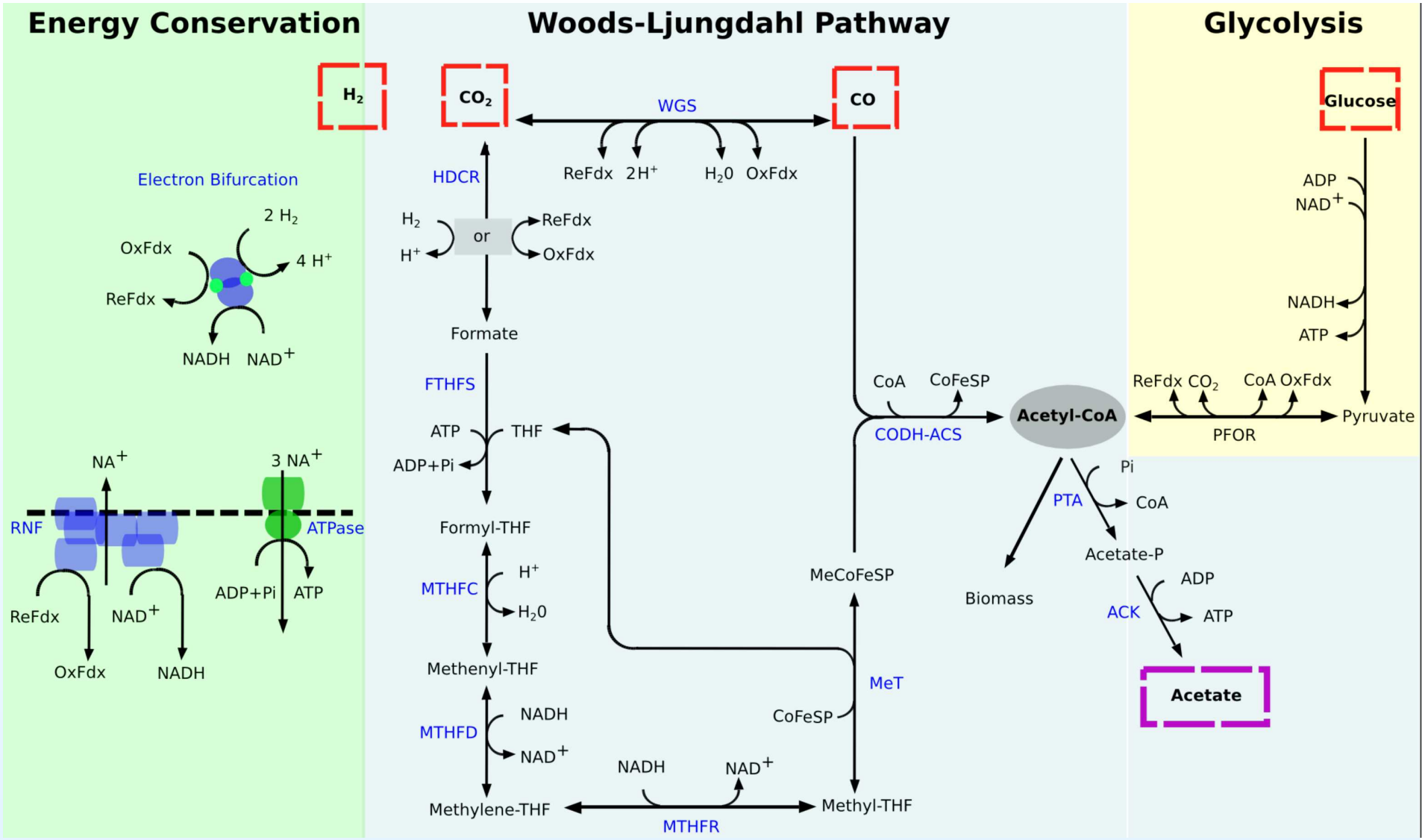
Model acetogens – Moorella thermoacetica, Acetobacterium Woodii and Clostridum Ljungdahlii

Currently categorised as a RNF Na-dependant acetogen
Genome - 4,044,777 bp, 3,473 proteins

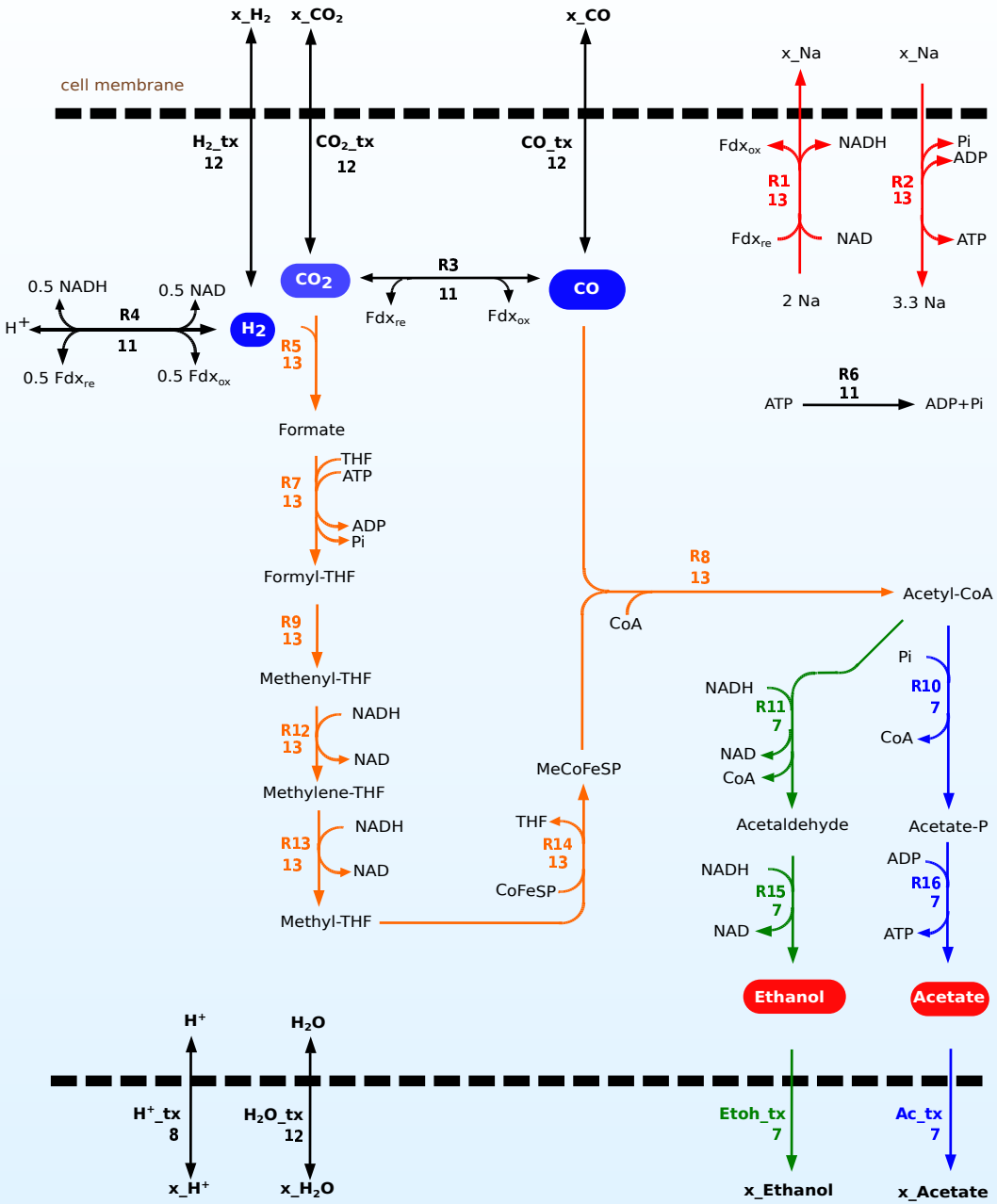
Industrial Application



Catabolism Overview



The WLP Reaction Scheme



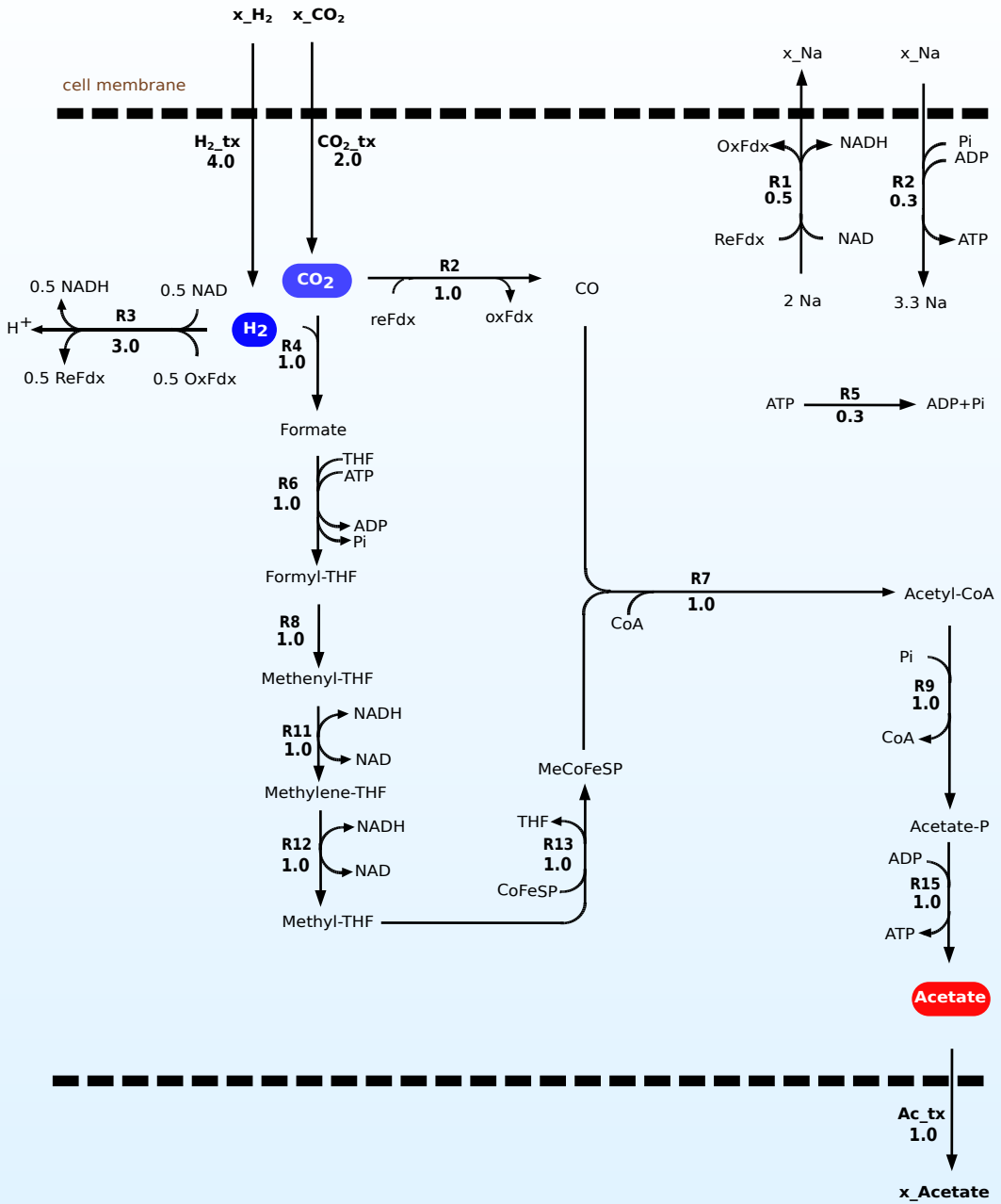
Key to reaction scheme

Metabolites with blue backgrounds have exchange transporters and those with red backgrounds have export transporters.
Dashed lines represent the cell membrane and so metabolites inside these are internal metabolites. The colour of non-black arrows represents membership to one of the four reaction subsets.
Numbers under reaction labels indicate the number of elementary modes that reaction is involved in.
R1:Rnf complex, **R2**:ATP synthase, **R3**:carbon monoxide dehydrogenase (CODH), **R4**:bifurcating hydrogenase (EBHyd), **R5**:hydrogen-dependent formate dehydrogenase (HDFD), **R6**:ATP hydrolase, **R7**:formate-THF ligase, **R8**:acetyl-CoA synthase, **R9**:methenyl-THF cyclohydrolase, **R10**:phosphate acetyltransferase, **R11**:acetaldehyde dehydrogenase, **R12**:methylene-THF dehydrogenase, **R13**:methyl-THF reductase, **R14**:methyl-THF:corrinoid/iron-sulfur methyltransferase, **R15**:alcohol dehydrogenase, **R16**:acetate kinase, **Ac_tx**:acetate transporter, **Etoh_tx**:ethanol transporter.

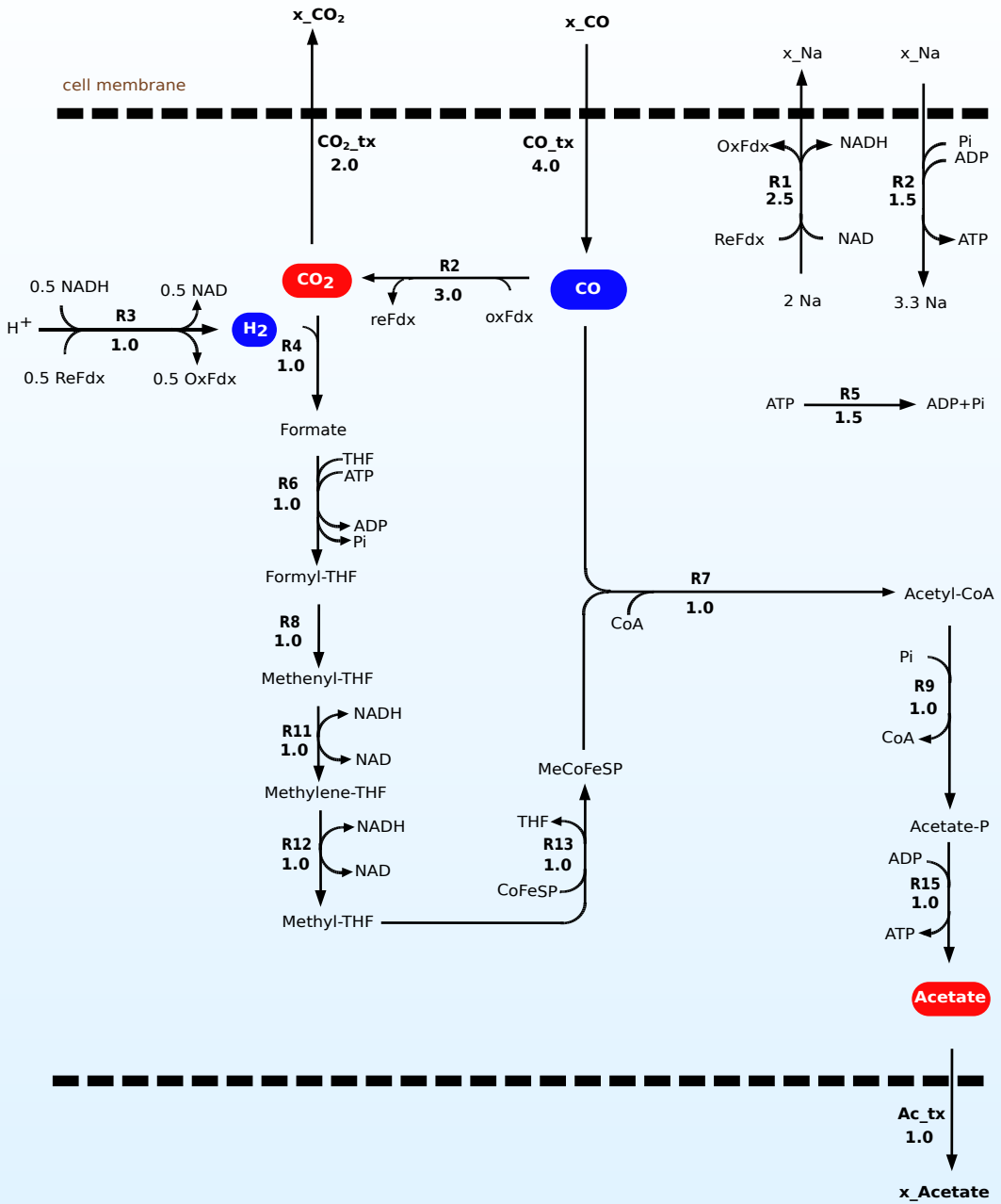
Elementary modes of the WLP

Mode	Stoichiometry	Reactions	Y^{C}_{ATP}
1	2 CO_2, 4 H_2 $\rightarrow$ Ac, 2 H_2O, H^+	19	0.15
2	26 CO_2,72 H_2 $\rightarrow$ 3 Ac, 10 EtOH, 36 H_2O, 3 H^+	19	0.00
3	3 CO_2, 5 H_2 $\rightarrow$ Ac, CO, 3 H_2O, H^+	15	0.00
4	4 CO, 2 H_2O $\rightarrow$ Ac, 2 CO_2, H^+	18	0.38
5	CO, H_2O $\rightarrow$ H_2, CO_2	9	0.30
6	6 CO, 3 H_2O $\rightarrow$ EtOH, 4 CO_2	18	0.29
7	CO_2, CO, 3 H_2 $\rightarrow$ Ac, H_2O, H^+	19	0.30
8	CO_2, CO, 5 H_2 $\rightarrow$ EtOH, 2 H_2O	18	0.11
9	17 CO_2, 3 CO, 57 H_2 $\rightarrow$ 10 EtOH, 27 H_2O	18	0.00
10	2 CO, 2 H_2 $\rightarrow$ Ac, H^+	18	0.45
11	3 CO, H_2, H_2O $\rightarrow$ Ac, CO_2, H^+	19	0.40
12	5 CO, H_2, 2 H_2O $\rightarrow$ EtOH, 3 CO_2	18	0.29
13	3 CO, 3 H_2 $\rightarrow$ EtOH, CO_2	18	0.27
14	2 CO, 4 H_2 $\rightarrow$ EtOH, H_2O	18	0.26

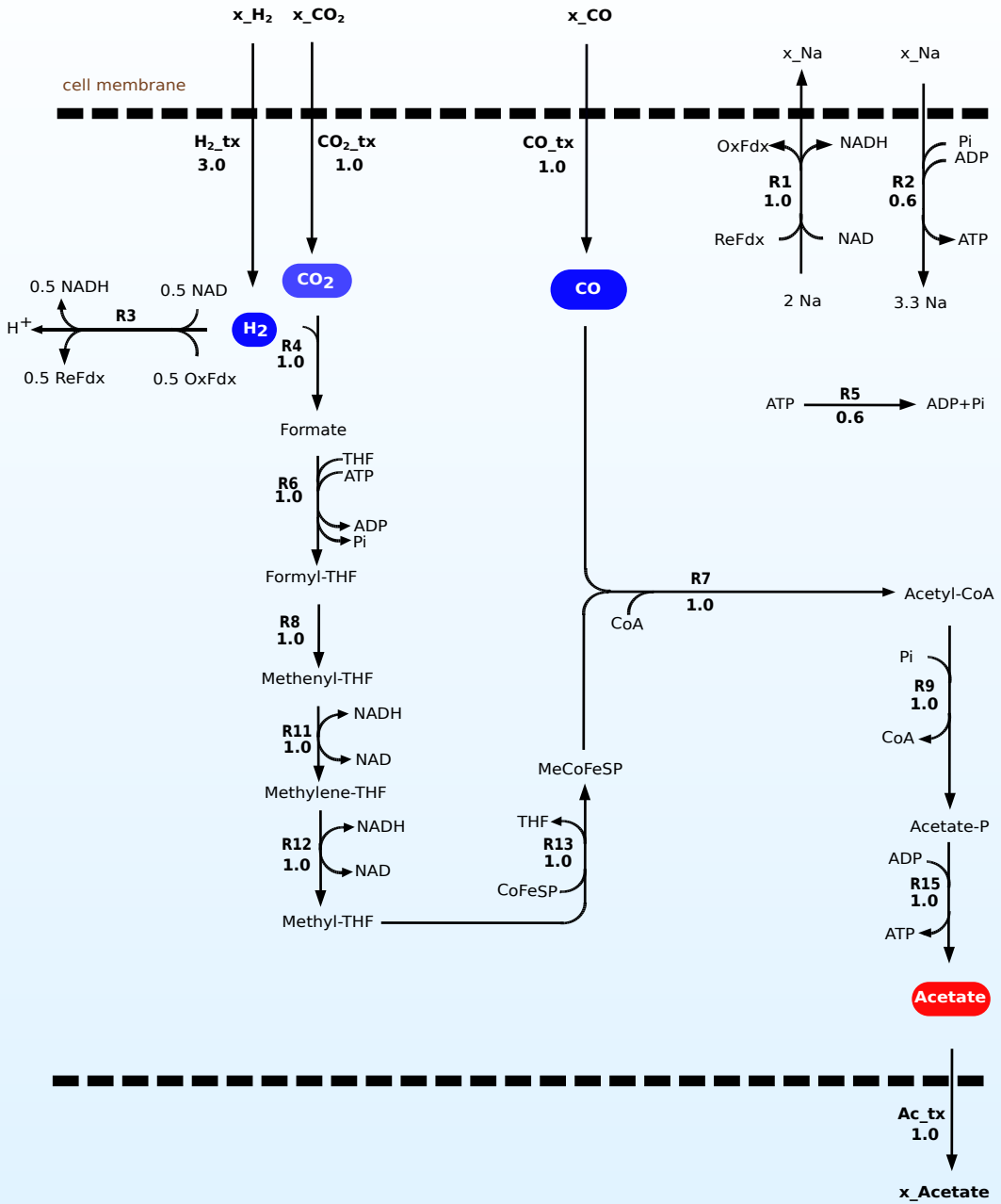
Mode 1: $CO_2 + H_2$



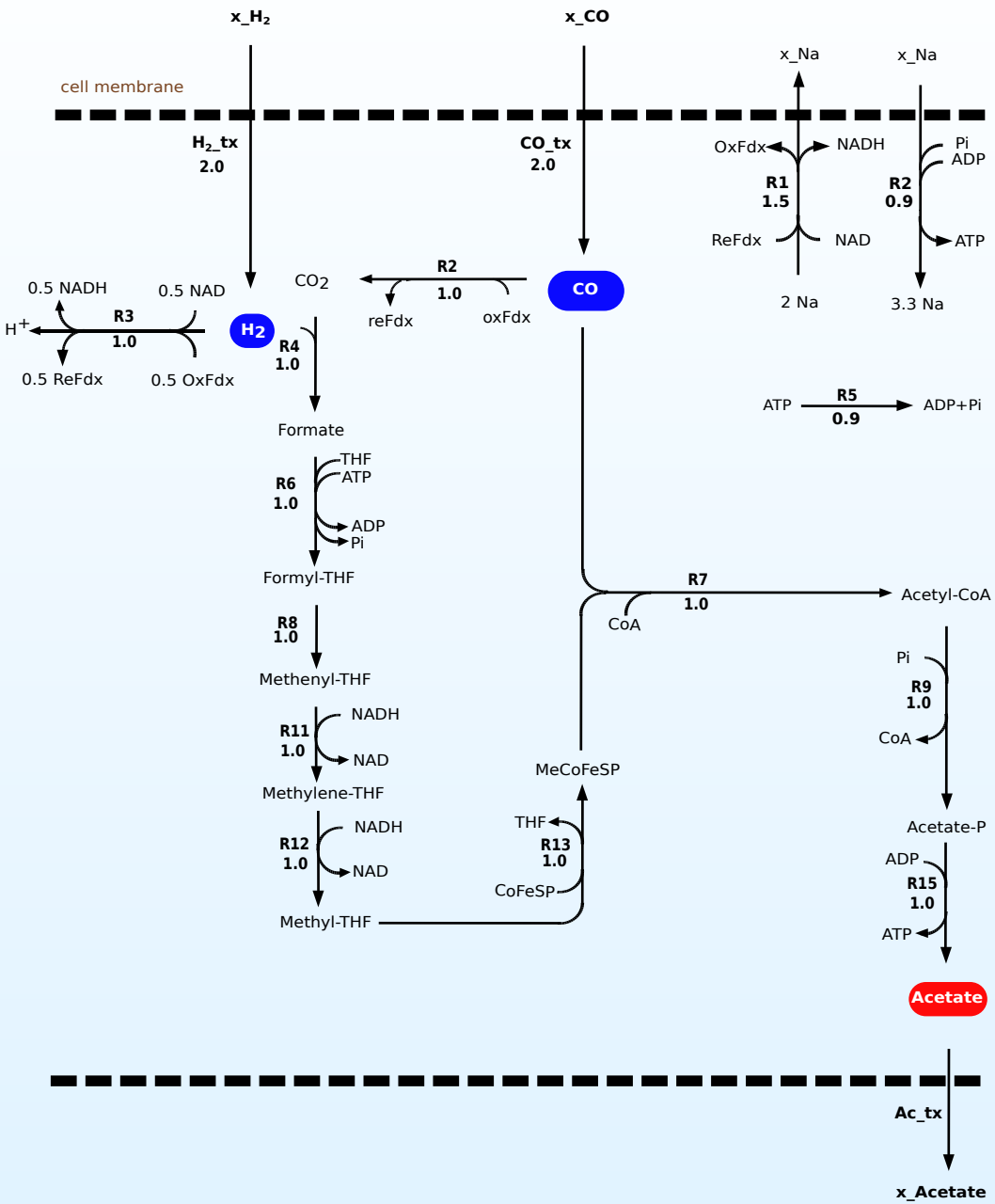
Mode 4: CO + H_2O



Mode 7: CO_2+ CO + H_2



Mode 10: CO + H_2



Channelling Metabolism into Desired Routes

Ethanol from Plant Waste

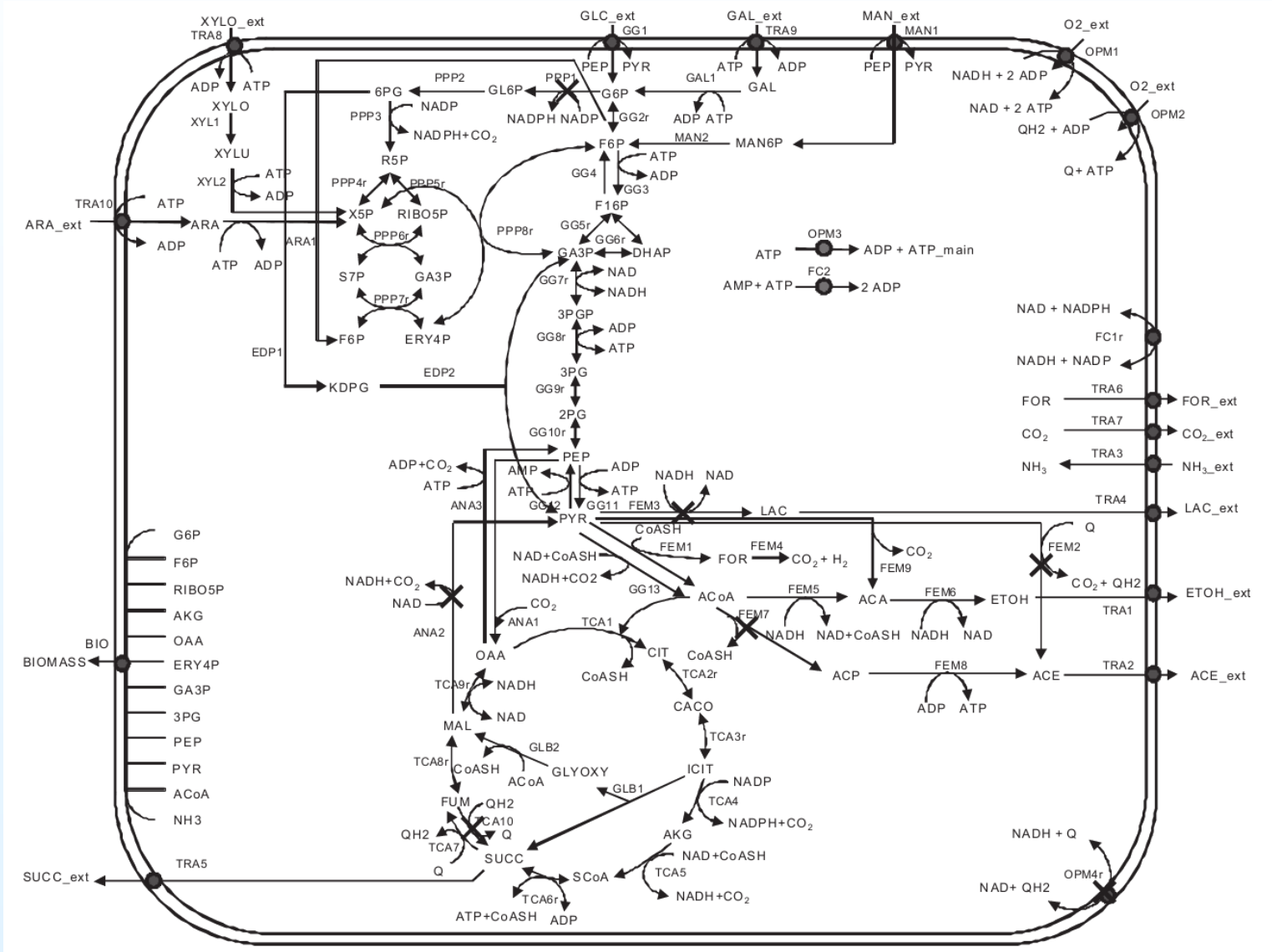
Some of the issues:

- Plant wastes (e.g. straw) contain cellulose and hemicellulose which can be hydrolysed to glucose and pentose sugars.
- Yeasts convert glucose to ethanol, but don't readily use the pentoses.
- *Escherischia coli* can use pentoses as well as glucose, but ethanol is not its preferred product.
- E. coli is easy to engineer, but can it be modified to make ethanol from pentoses in such a way that it cannot mutate back to its original state?

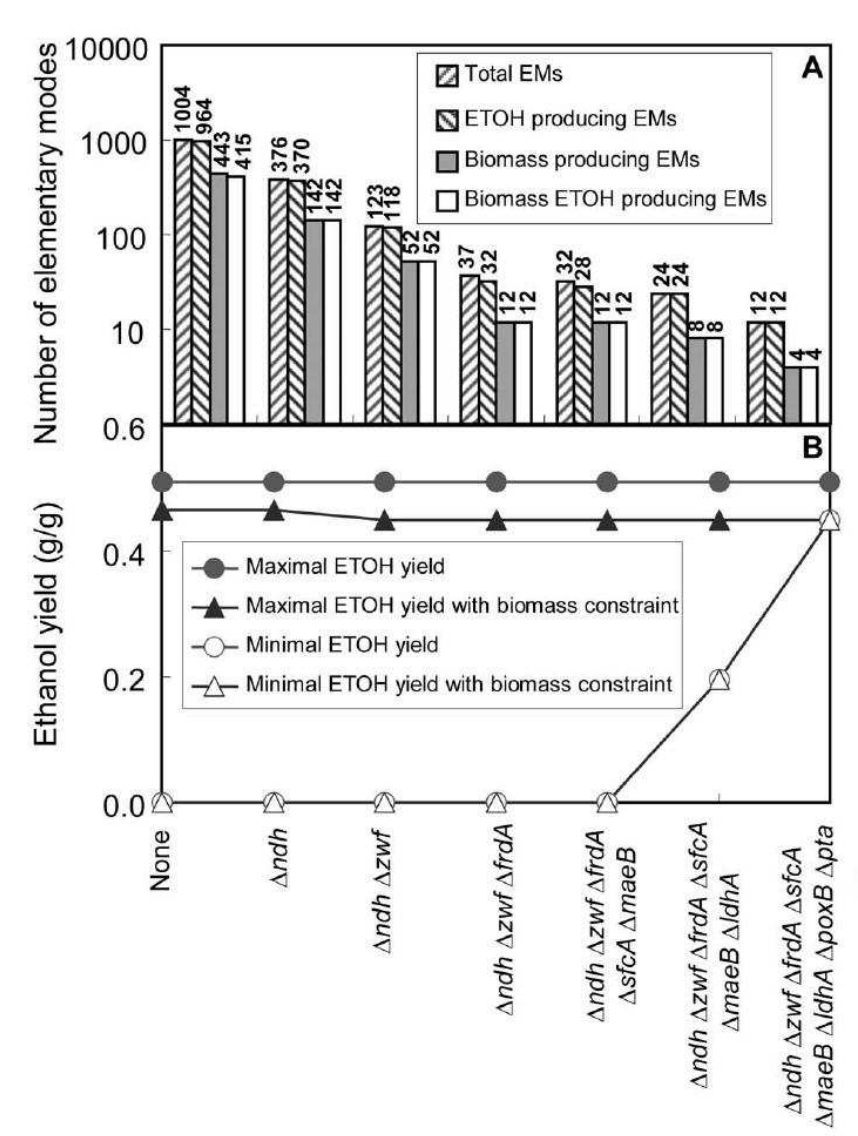
A Demonstration Solution

- Friedrich Srienc's group (Trinh et al, Appl. Env. Microbiol, 74, 3634-3643, 2008)built a medium-sized structural model of E coli central carbon metabolism.
- They computed the elementary modes leading from glucose and pentoses to products including ethanol and biomass.
- They searched for reactions that were *needed* for modes leading to other products but which were *not needed* for *some* of the routes to biomass and ethanol.
- They found a set of *eight* reactions that between them disabled all the modes except those leading to either ethanol alone or biomass and ethanol.
- They made a the deletion mutants and obtained close to the theoretically-predicted yields of ethanol.

The Model



The Analysis



Conclusion

- Elementary modes analysis can be used to design metabolic network modifications to obtain improved yields.
- Strategies can include both addition of heterologous enzymes to provide new routes, or deletion of native enzymes to block unproductive routes.

Acknowledgements

Friedrich Srienc, Ross Carlson, University of Minnesota

Brian Rudd, Michelle Gradley, BioSyntha/ZuvaSyntha

Sheila Ingemann Jensen, Alex Toftgaard Nielsen, Technical University of Denmark

Funding:

iCASE studentship Grant no. BB/L015684/1.