# Biotechnological Applications of Elementary Modes Analysis

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# Outline

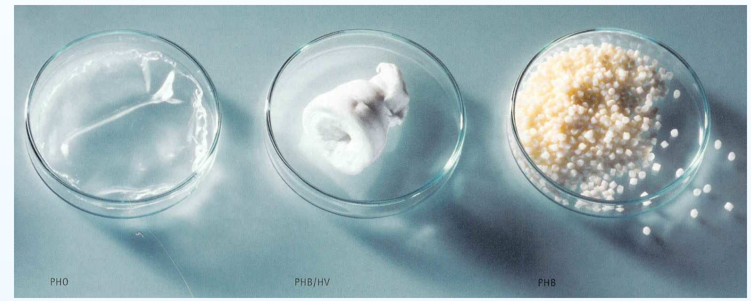
Some examples of applications of Elementary Modes Analysis:

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

# Pathway Design for Improving Yield

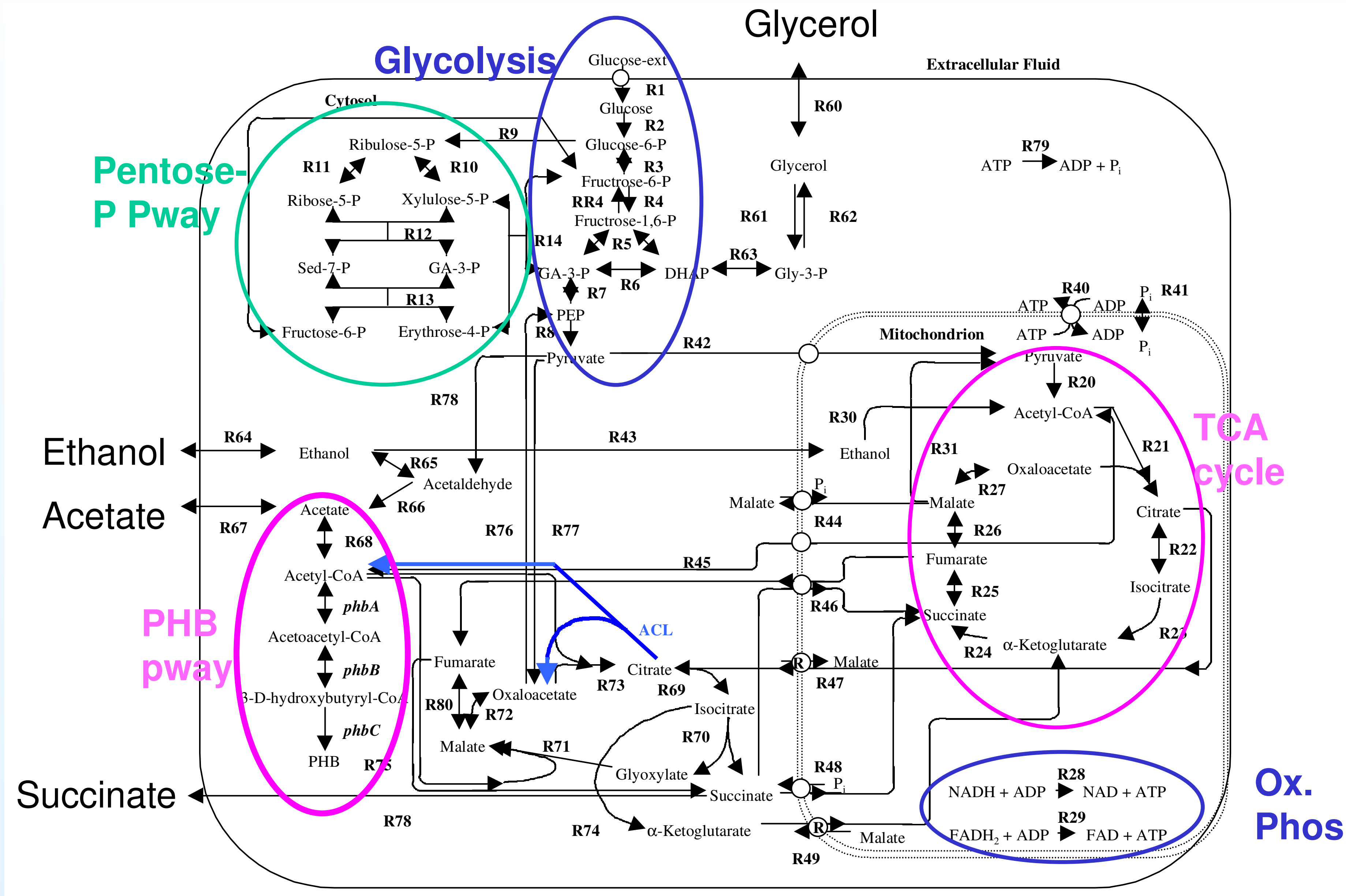
# Biodegradable Plastics

Polyhydroxybutyrate (PHA) or polyhydroxyalkanoates PHB


PHB is produced naturally as a storage compound by some bacteria, and notably by the $H_2$–utilising, $CO_2$—fixing *Cupriavidus necator* (aka *Ralstonia eutropha*).

# 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.)

Carlson et al, *Biotechnol. Bioeng.* **79**, 121–134, 2002.

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

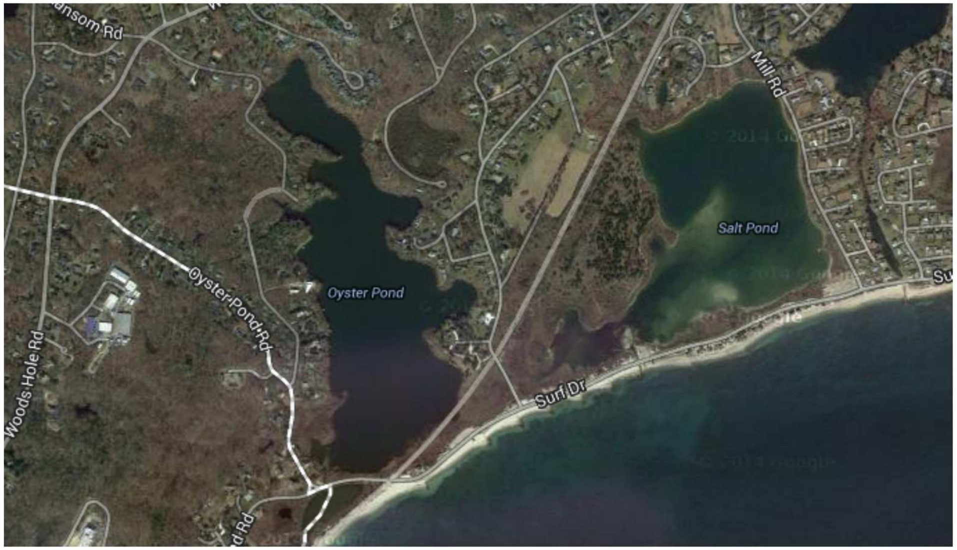
3. 12 EtOH $\rightarrow$ 5 PHB + 4 $CO_2$ 0.83
4. 77 EtOH + 31 Glycerol $\rightarrow$
   48 PHB + 4 Ac. + 47 $CO_2$ 0.78

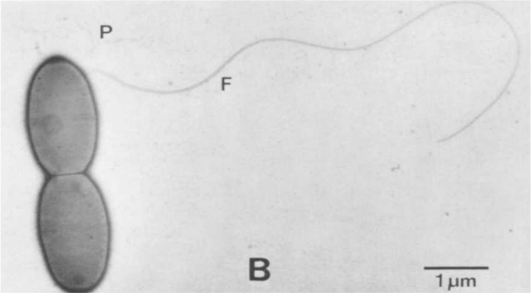
(Number following each mode is the fractional carbon conversion.)

Carlson et al, *Biotechnol. Bioeng.* **79**, 121–134, 2002.

# *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.**

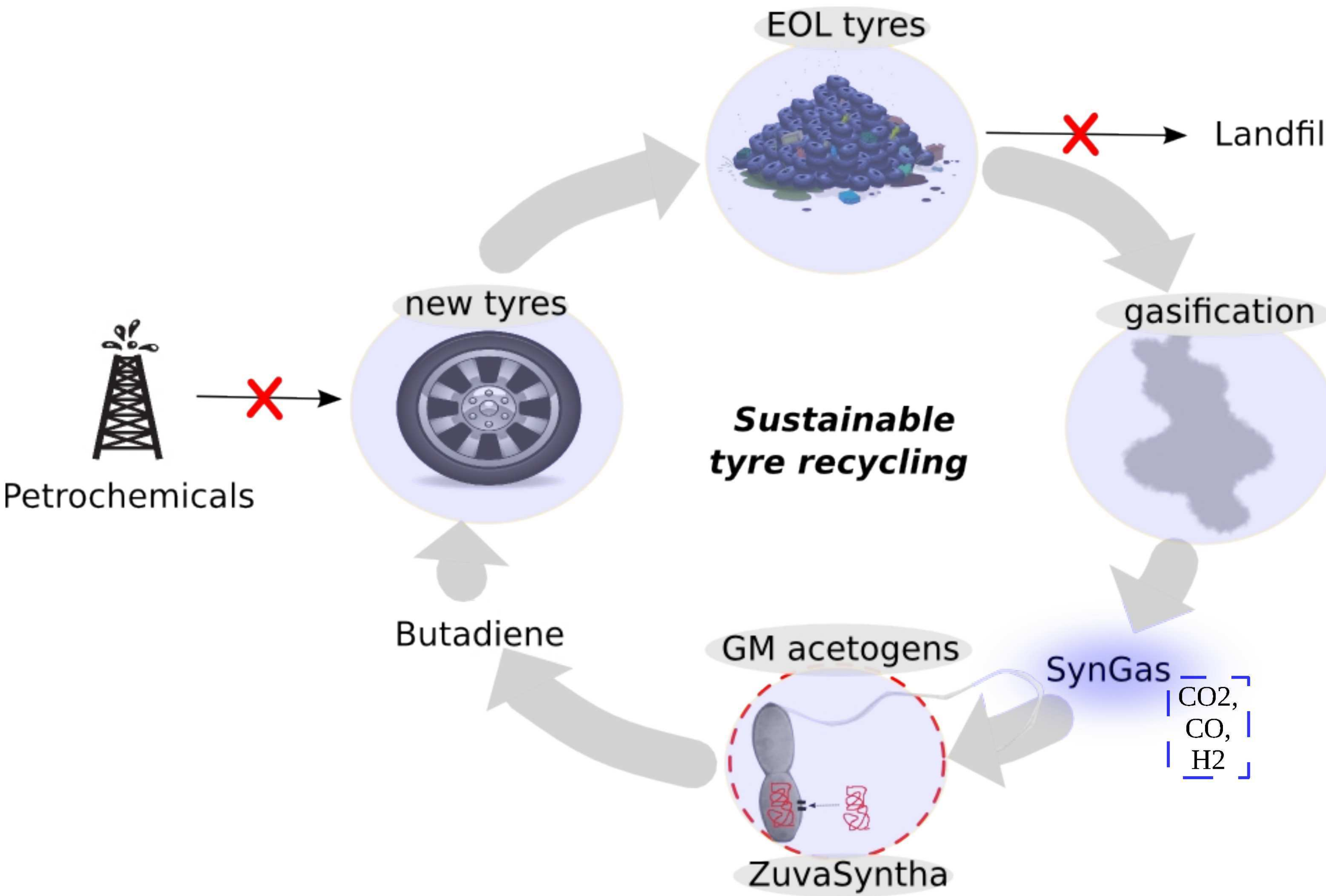
**Within group of acetogens – 22 genera in soils, sediments, intestinal tracts.**

**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.**

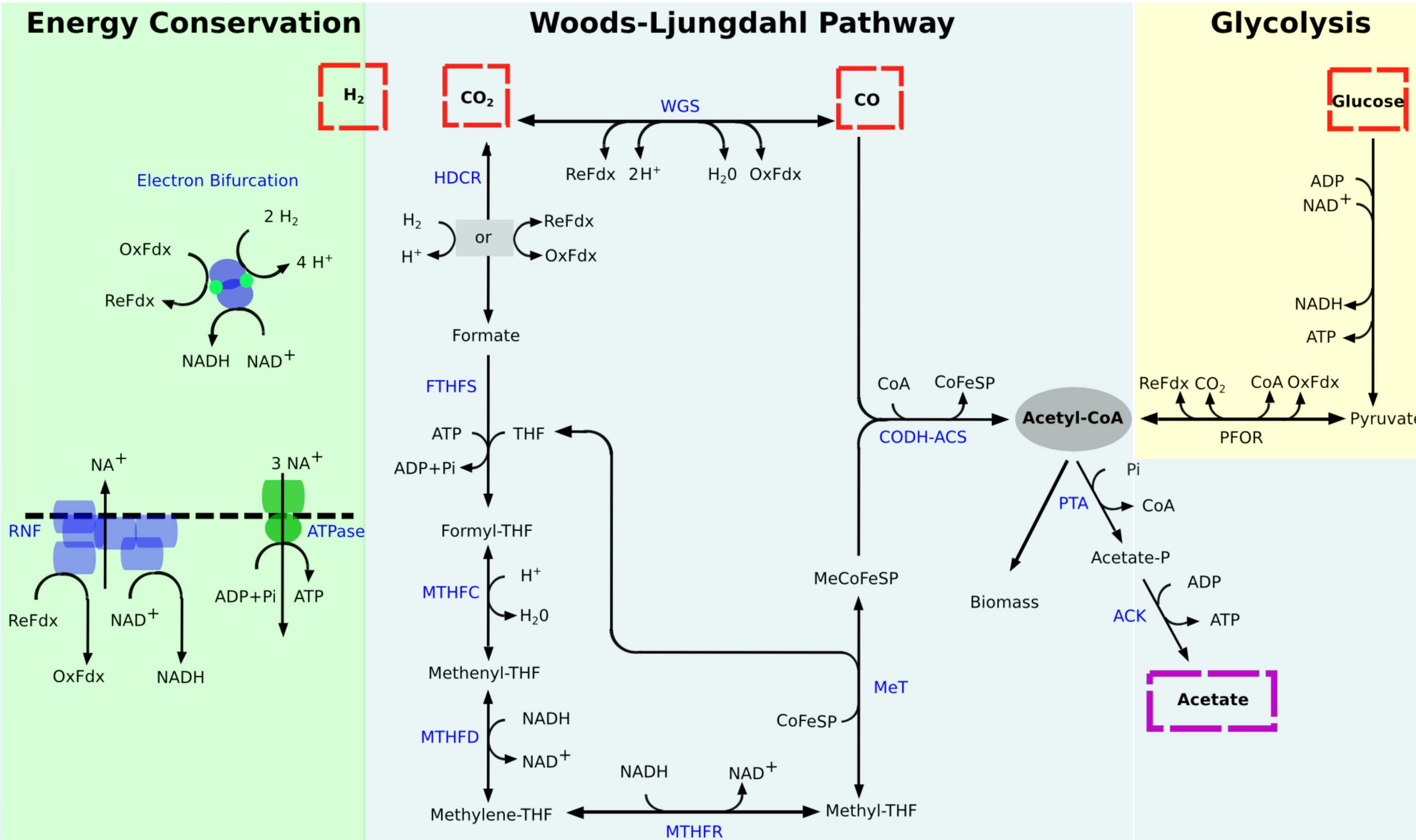
**Model acetogens – Moorella thermoacetica, Acetobacterium Woodii and Clostridum Ljungdahlii**

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

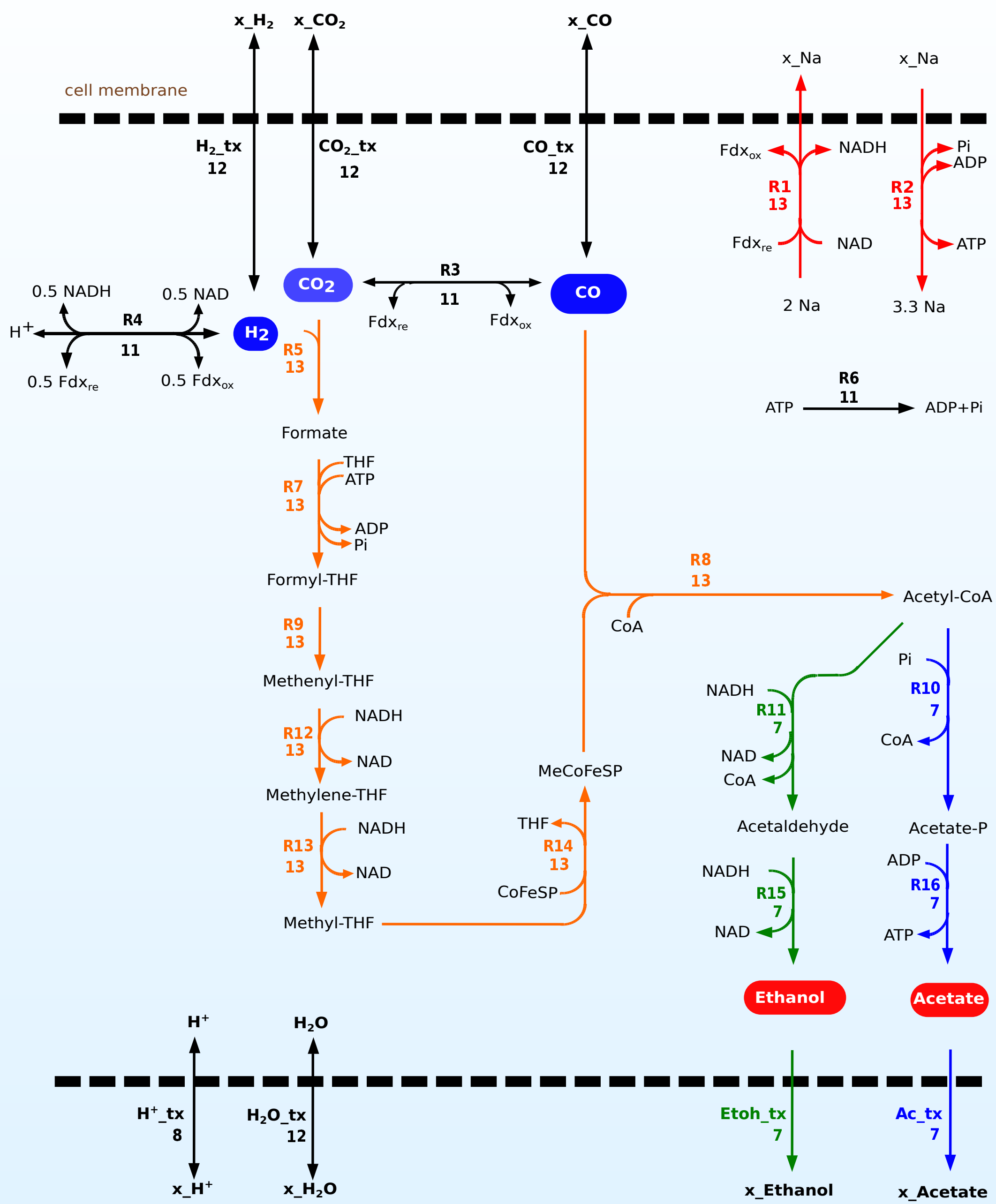
# Industrial Application



# 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.

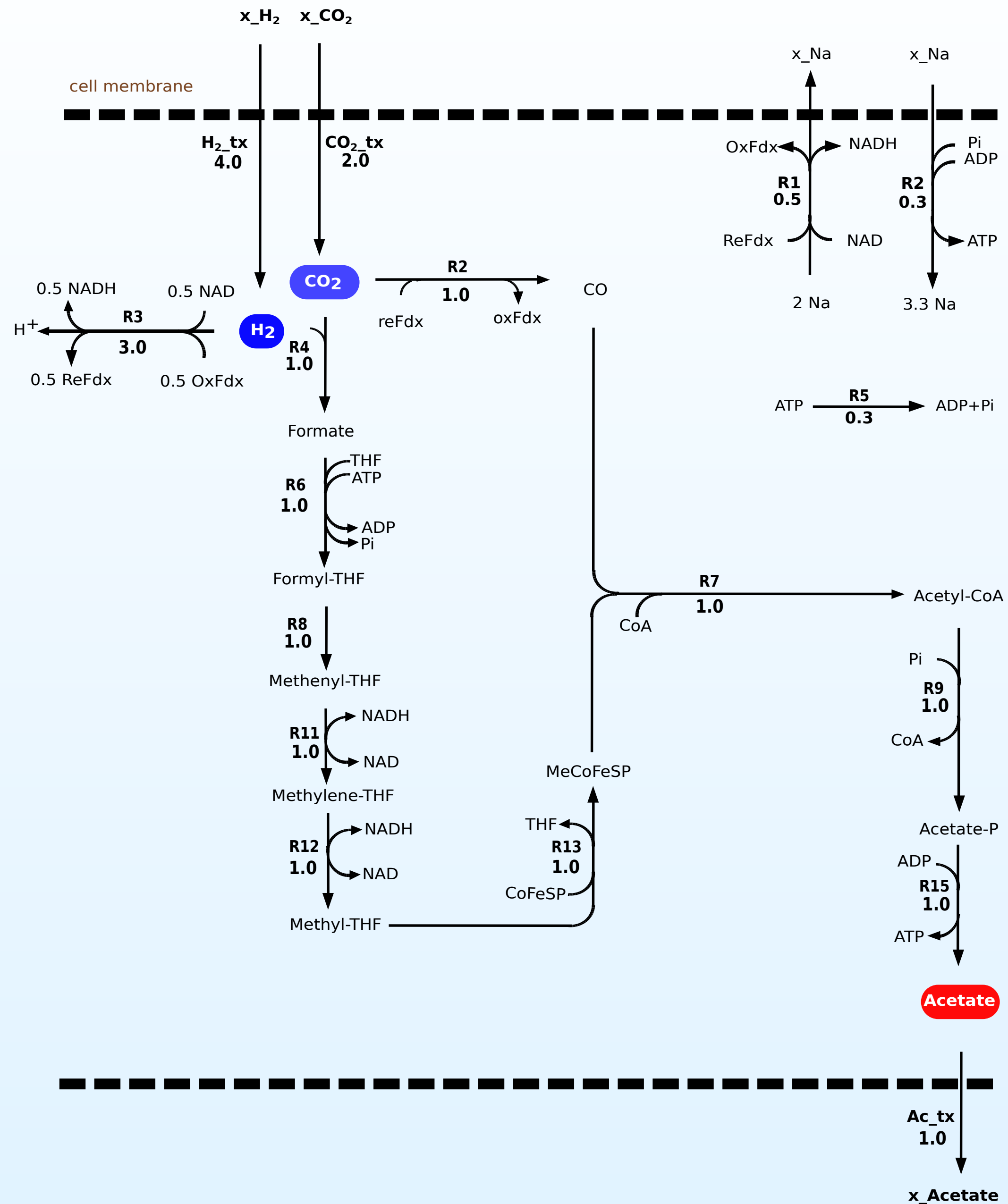
Numbers under reaction labels indicate the number of elementary modes that reaction is involved in.

**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.

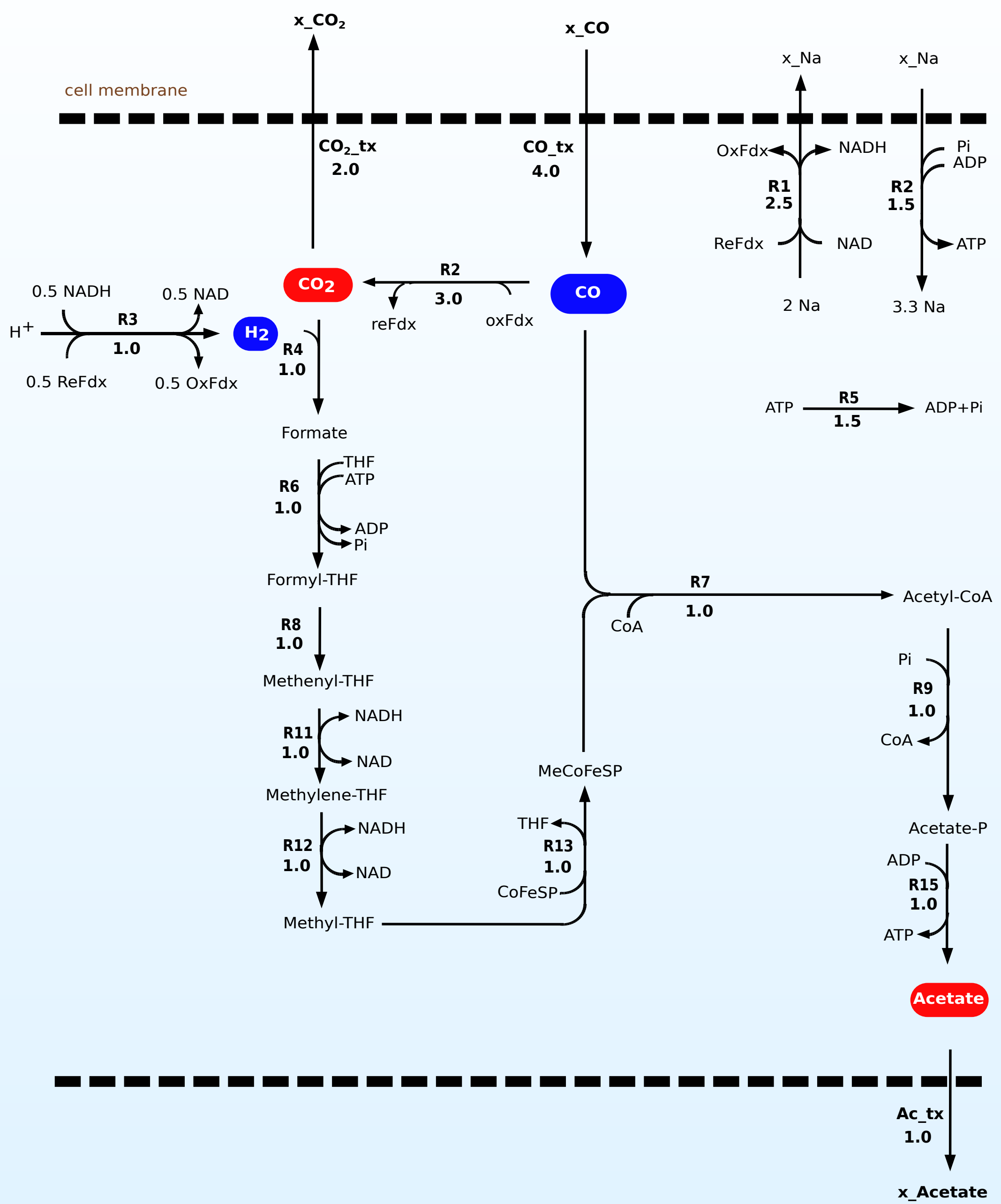
## Elementary modes of the WLP

| 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 |

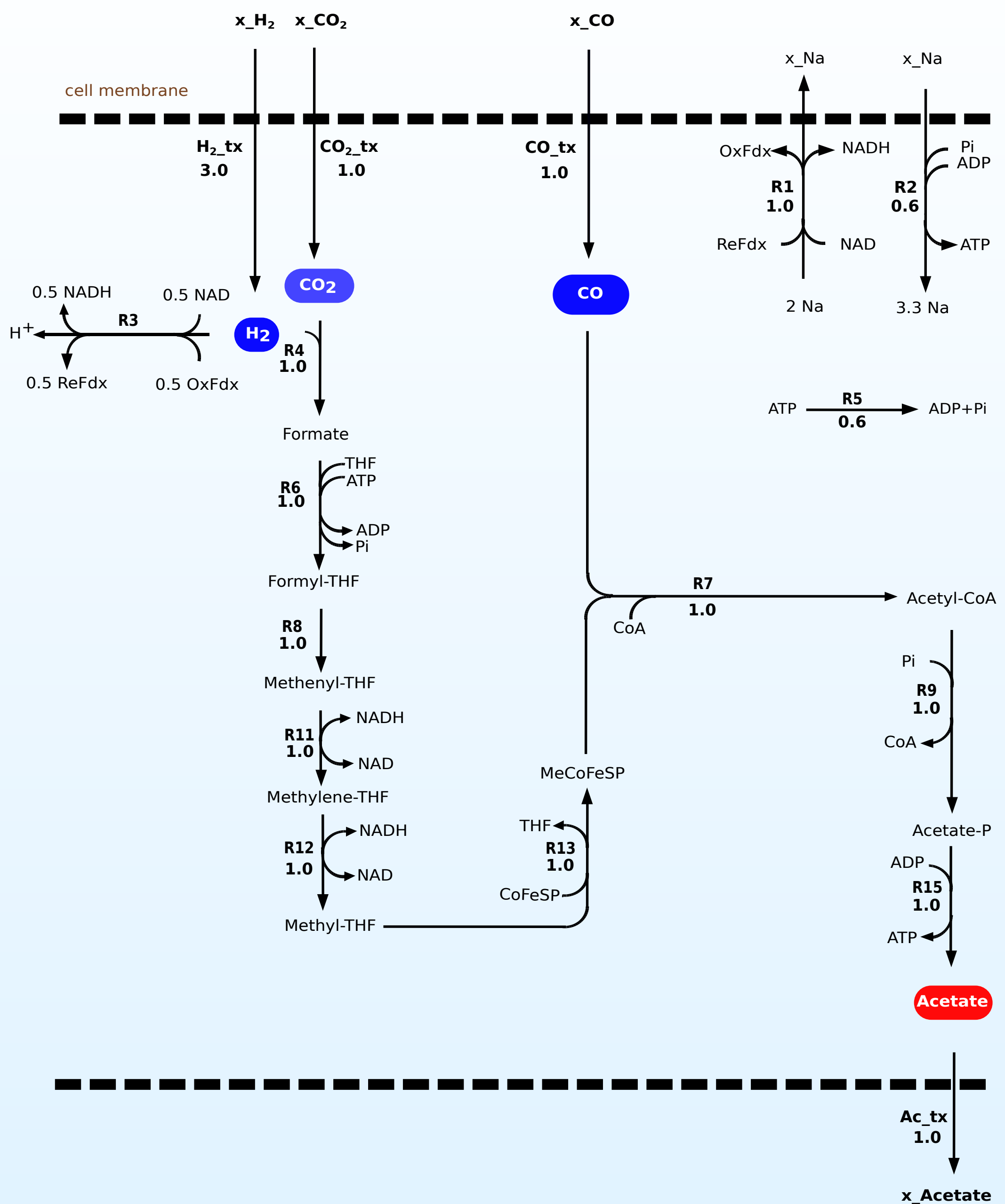
# Mode 1: $CO_2 + H_2$


x_H2
x_CO2
cell membrane
H2_tx
4.0
CO2_tx
2.0
x_Na
x_Na
OxFdx
NADH
R1
0.5
ReFdx
NAD
2 Na
Pi
ADP
R2
0.3
ATP
3.3 Na
CO2
R2
1.0
CO
reFdx
oxFdx
0.5 NADH
0.5 NAD
H+
R3
3.0
0.5 ReFdx
0.5 OxFdx
H2
R4
1.0
Formate
ATP
R5
0.3
ADP+Pi
THF
ATP
R6
1.0
ADP
Pi
Formyl-THF
R8
1.0
Methenyl-THF
R11
1.0
NADH
NAD
Methylene-THF
R12
1.0
NADH
NAD
Methyl-THF
R7
1.0
CoA
Acetyl-CoA
Pi
R9
1.0
CoA
MeCoFeSP
THF
R13
1.0
CoFeSP
Acetate-P
ADP
R15
1.0
ATP
Acetate
Ac_tx
1.0
x_Acetate

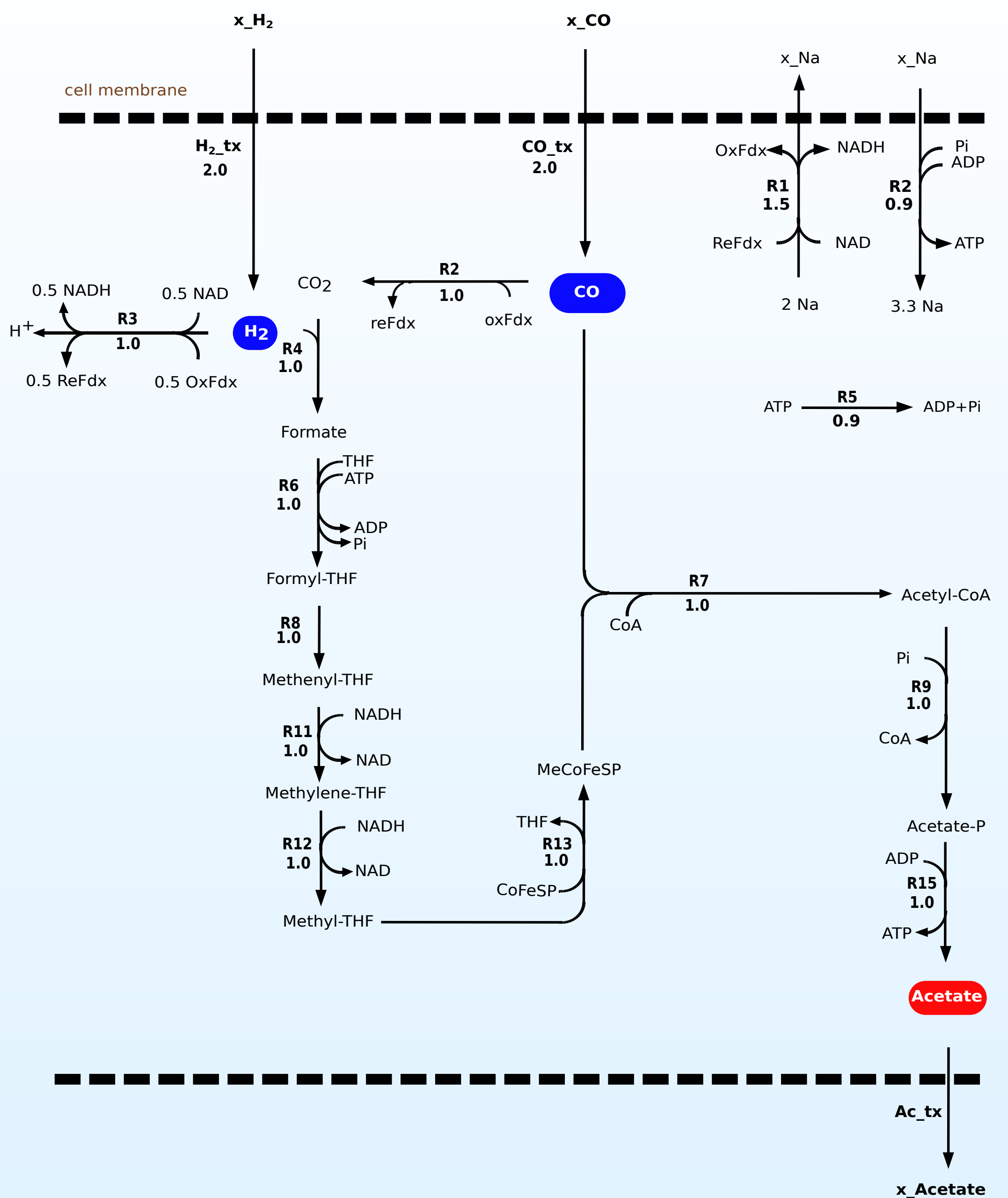
# Mode 4: CO + $H_2O$


x_CO₂
x_CO
x_Na
x_Na
cell membrane
CO₂_tx
2.0
CO_tx
4.0
OxFdx
NADH
R1
2.5
ReFdx
NAD
Pi
ADP
R2
1.5
ATP
2 Na
3.3 Na
CO₂
R2
3.0
reFdx
oxFdx
CO
0.5 NADH
0.5 NAD
H+
R3
1.0
H₂
0.5 ReFdx
0.5 OxFdx
R4
1.0
Formate
ATP
R5
1.5
ADP+Pi
THF
ATP
R6
1.0
ADP
Pi
Formyl-THF
R7
1.0
Acetyl-CoA
CoA
R8
1.0
Methenyl-THF
Pi
R9
1.0
CoA
NADH
R11
1.0
NAD
MeCoFeSP
Methylene-THF
NADH
R12
1.0
NAD
THF
R13
1.0
CoFeSP
Methyl-THF
Acetate-P
ADP
R15
1.0
ATP
Acetate
Ac_tx
1.0
x_Acetate

# Mode 7: $CO_2$+ CO + $H_2$



# Mode 10: $CO + H_2$



x_H₂
x_CO
x_Na
x_Na
cell membrane
H₂_tx
2.0
CO_tx
2.0
OxFdx
NADH
R1
1.5
ReFdx
NAD
2 Na
Pi
ADP
R2
0.9
ATP
3.3 Na
CO₂
R2
1.0
reFdx
oxFdx
CO
0.5 NADH
0.5 NAD
H⁺
R3
1.0
0.5 ReFdx
0.5 OxFdx
H₂
R4
1.0
Formate
ATP
R5
0.9
ADP+Pi
THF
ATP
R6
1.0
ADP
Pi
Formyl-THF
R7
1.0
Acetyl-CoA
CoA
R8
1.0
Methenyl-THF
Pi
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1.0
CoA
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R11
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MeCoFeSP
NADH
R12
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NAD
THF
R13
1.0
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Acetate-P
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R15
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Acetate
Ac_tx
x_Acetate

# An aside — *C. autoethanogenum*



- *Clostridium ethanogenum* uses the WL pathway to capture $CO_2$ and CO to make ethanol as well as acetic acid.
- A New Zealand start-up company **LanzaTech** developed a process with a strain that makes mainly ethanol using the off-gases from steel mills.
- It uses a specially designed fermentation vessel and several working–scale versions have been installed in steel mills and the like.
- The ethanol can be converted to various chemical precursors, and there is also a chemical process to convert it to 'sustainable' aviation fuel.

# LanzaTech

LanzaTech

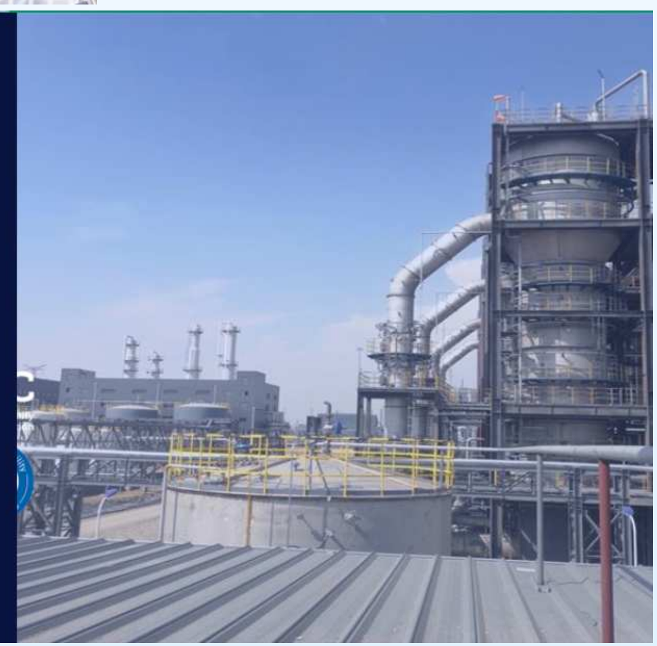
ABOUT TECHNOLOGY ▾ INVESTOR RELATIONS
NEWS ▾ RE:CARBON BLOG CAREERS CONTACT

**ABOUT**

## Carbon Recycling Technology for Today and the Future

LanzaTech's carbon recycling technology is like retrofitting a brewery onto an emission source like a steel mill or a landfill site, but instead of using sugars and yeast to make beer, pollution is converted by bacteria to fuels and chemicals! Imagine a day when your plane is powered by recycled GHG emissions, when your shampoo bottle started life as emissions from a steel mill. This future is possible today using LanzaTech technology.

| | |
|---|---|
| Startup Year | **2022** |
| Annual Ethanol Production Volume | **60,000 Metric Tons** |
| Annual $CO_2$ Abatement Volume | **˜120,000 Metric Tons** |
| Carbon Source | **Ferroalloy Mill Emissions** |



# 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 Demonstrated Approach

- 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.

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- They found a set of *seven* reactions (eight genes) that between them disabled all the modes except those leading to either ethanol alone or biomass and ethanol.

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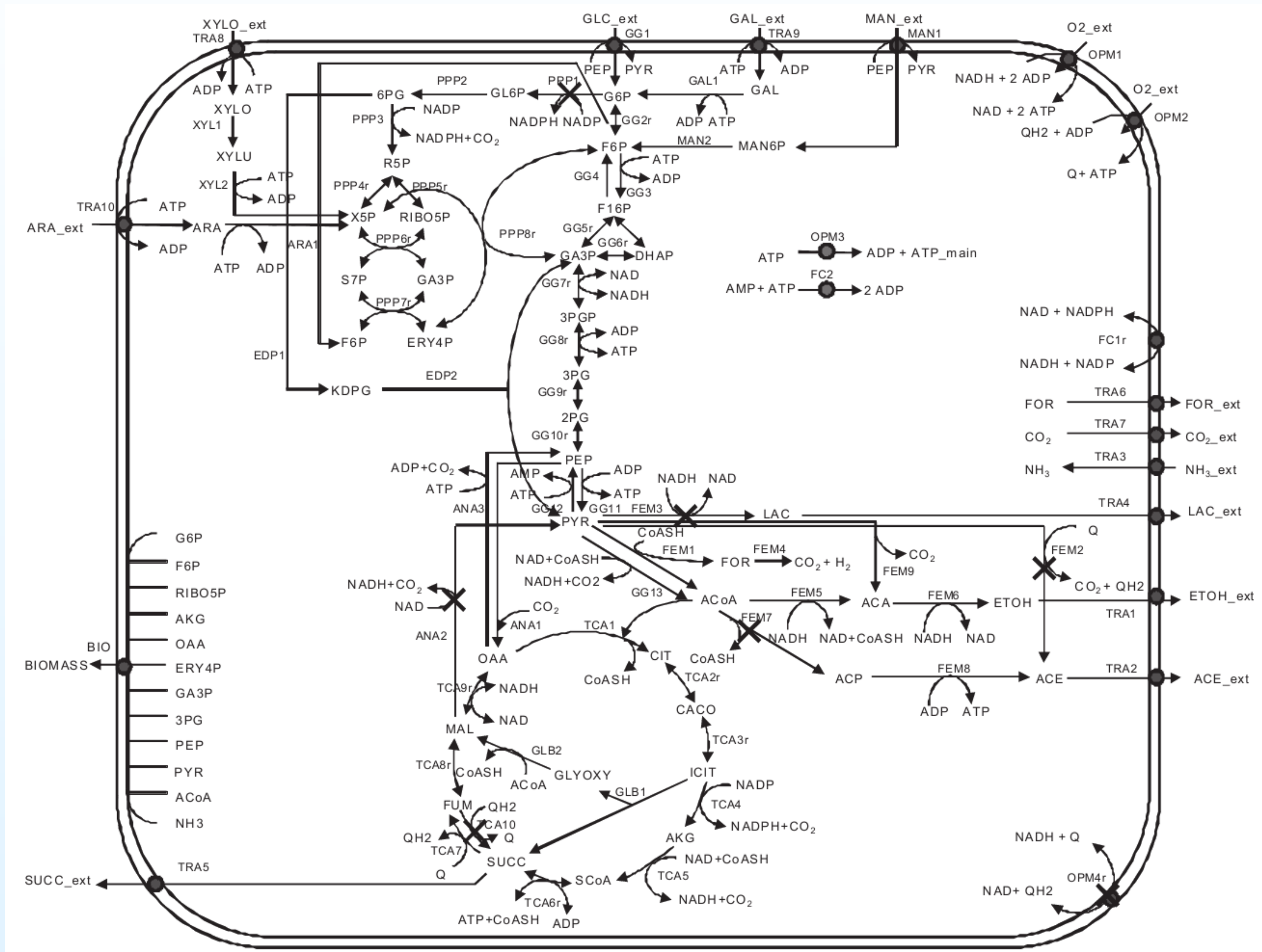
- They found a set of *seven* reactions (eight genes) that between them disabled all the modes except those leading to either ethanol alone or biomass and ethanol.
- They successively made the set of deletions where growth can only occur with ethanol production and obtained close to the theoretically-predicted yields of ethanol on glucose and xylose.

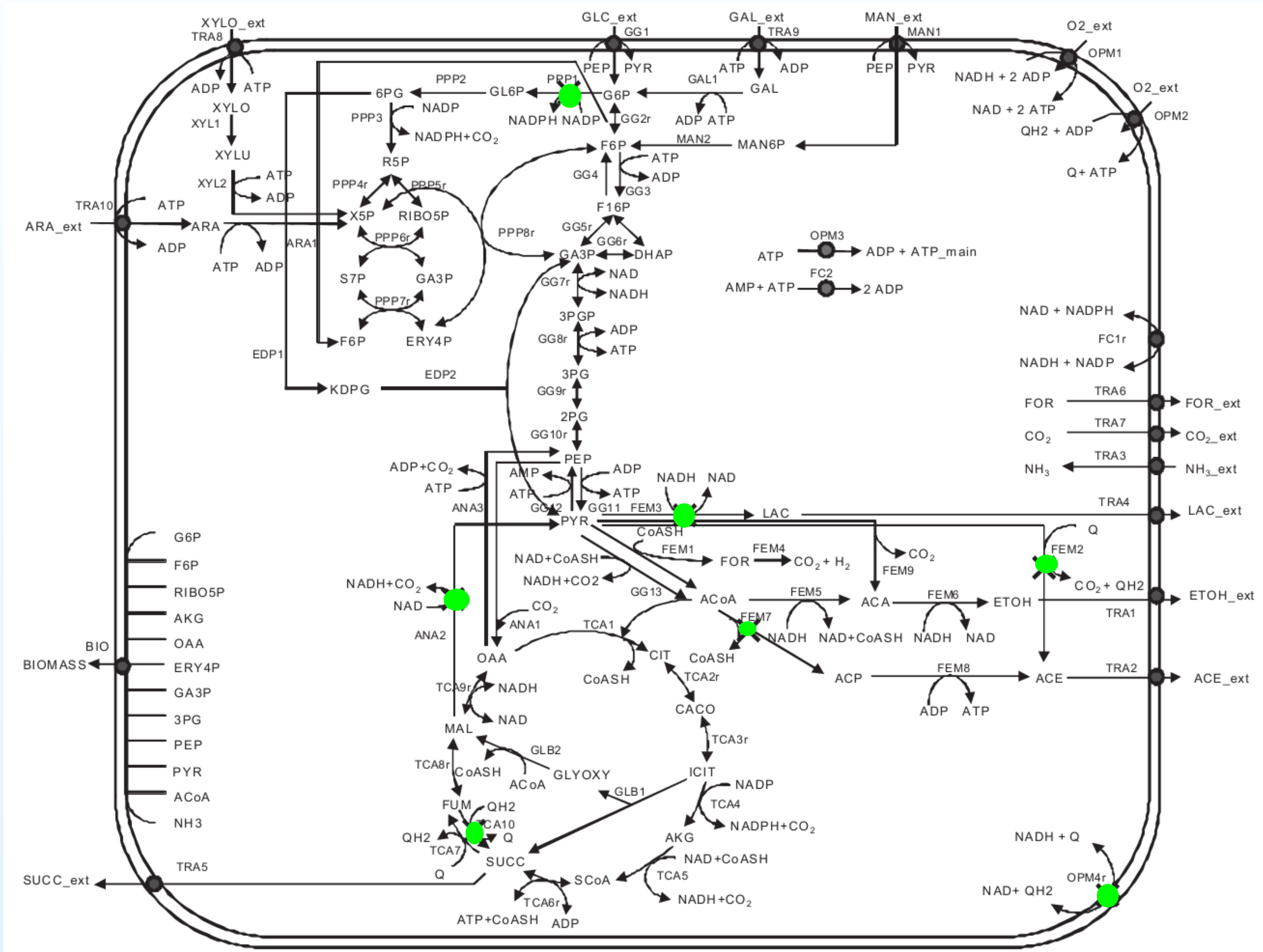
# The Metabolic Model



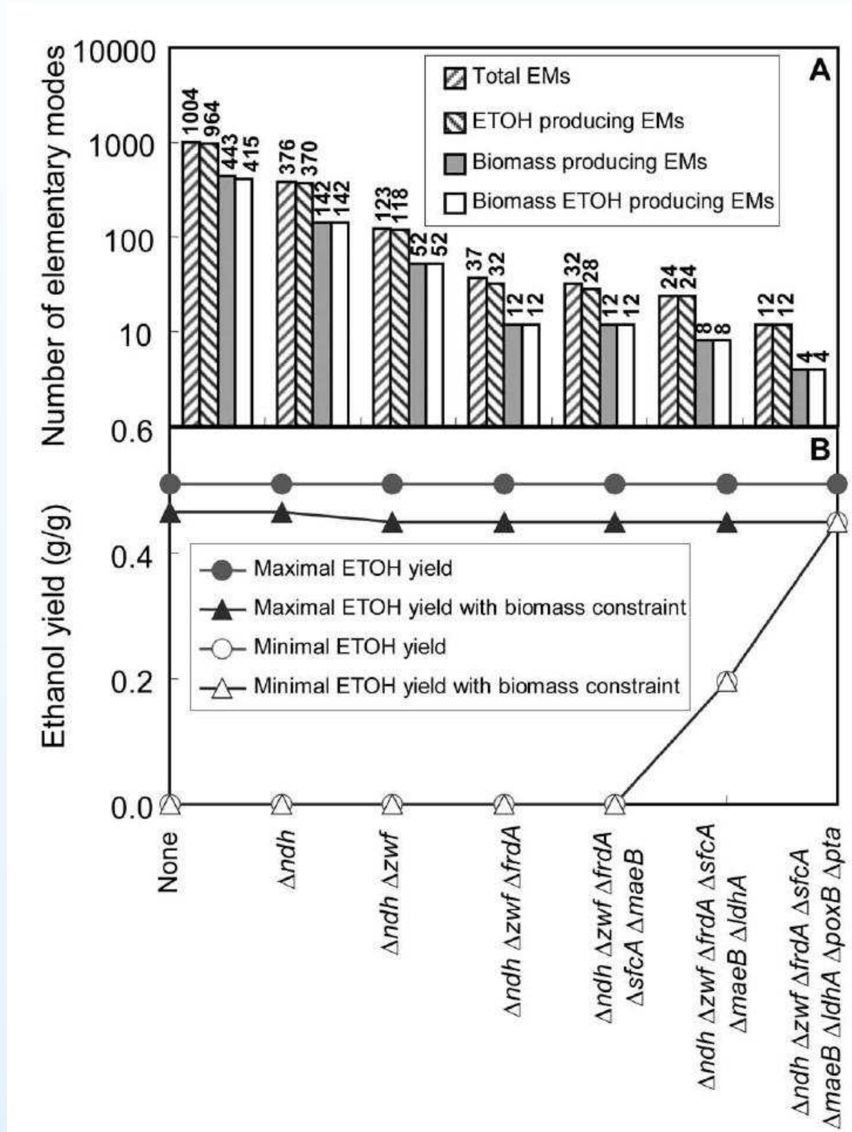
## The Targets

| Enzyme reaction | Gene(s) |
|---|---|
| Glucose–6–P DH | *zwf* |
| NADH DH II | *ndh* |
| NAD/NADP malic enzyme | *sfcA, maeB* |
| D-lactate DH | *ldhA* |
| fumarate reductase | *frdA* |
| pyruvate oxidase | *poxB* |
| Pi acetyl transferase | *pta* |

# The Targets on the Map



# The Analysis



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# Conclusion

- Elementary modes analysis can assist biotechnology projects to design metabolic network modifications for new products or 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

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