Metabolic pathways and metabolic engineering

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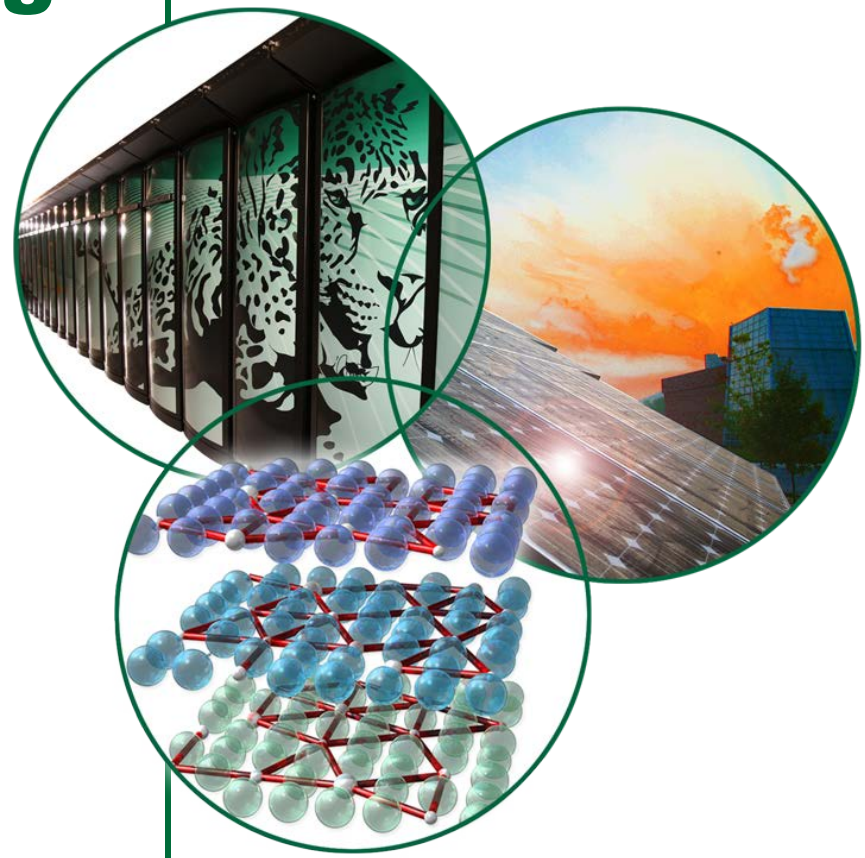

U.S. DEPARTMENT OF ENERGY

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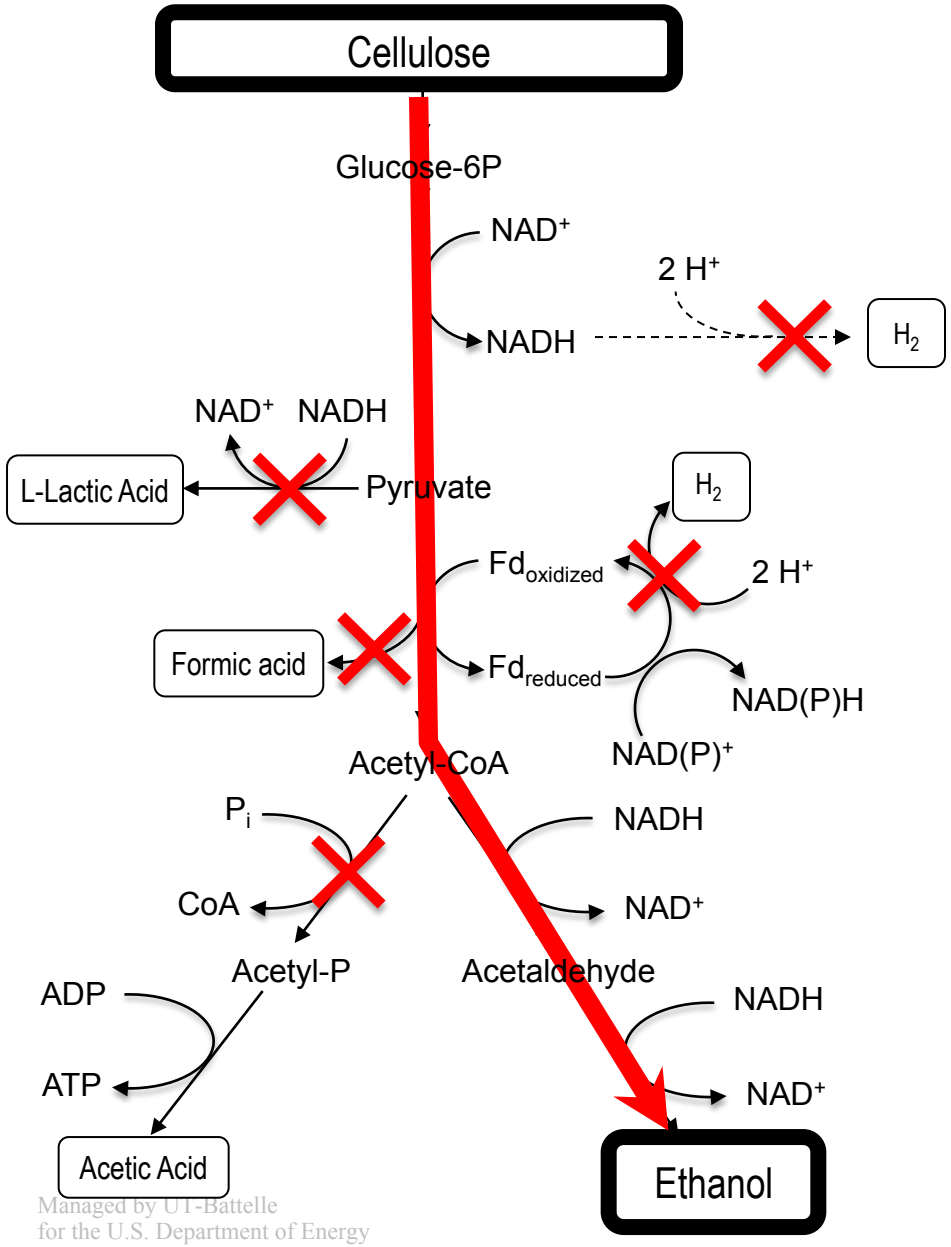
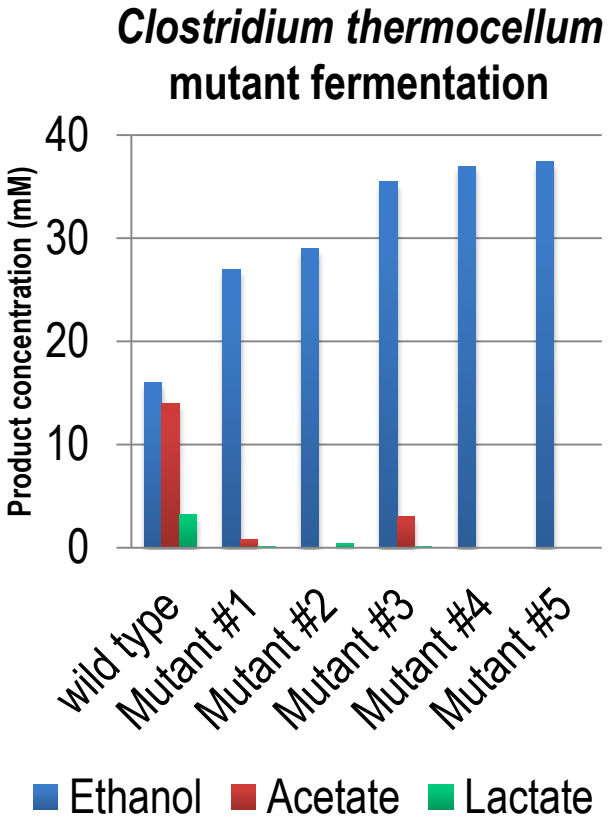
Metabolic engineering of *Clostridium thermocellum* for cellulosic ethanol production





By understanding and then modifying carbon and electron flux, we have increased ethanol yield in *C. thermocellum*

Major issues in applying metabolic engineering

- Choice of platform organism
  - Every host presents challenges
- Start with easily modified, industrial organism
  - Usually yeast or *E. coli*
  - Engineer for desired function → requires deep understanding of pathways to heterologously express
  - Control expression, mitigate toxicity of intermediates and products
- Start with organism that has unique desired capabilities
  - Develop genetic systems
  - Build understanding of metabolism, gene regulation, etc.
  - Engineer it to make only the compound of interest and be more robust

Current status of technology in metabolic engineering

- Developing new genetic systems is difficult but feasible
- Synthetic biology is allowing rapid progress on all fronts
  - DNA synthesis is relatively inexpensive, and cost is decreasing
  - Shifts the focus from tools to ideas, even in non-model organisms
- Metabolic models and other computational tools are becoming more advanced and could inform future strategies
- ^{13}C labeling and other “fluxomics” could be broadly enabling, but are typically under-utilized
  - May be less useful for H_2 production because it can not directly follow electron flux, but still important

Barriers and challenges in metabolic engineering

- Knowledge
  - Often incomplete understanding of enzymatic pathways
  - Electron flux often less well understood than carbon flux
- Complexity
  - Regulatory pathways, including mechanisms of dynamic regulation
  - Allostery; either a hindrance or a tool
  - Multiple isozymes of key enzymes
- Models
  - Limited by the information you put into them
- Vision
  - Need clear idea of how to get where you want

Key needs in metabolic engineering

Near term

- Put existing pieces together as proof-of-principle
- Target applied metrics

Medium term

- Explore the basics of native pathways to increase foundational understanding
- Explore completely new approaches