A grayscale microscopic image of various bacterial cells, including rod-shaped and spherical forms, some with flagella, set against a dark background. The image is used as a background for the title text.

# Fermentative Hydrogen Production:

## Strain Selection and Reactor Operation

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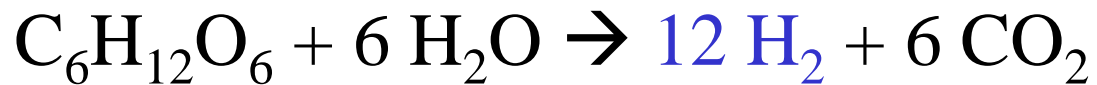
Penn State University

PENNSSTATE

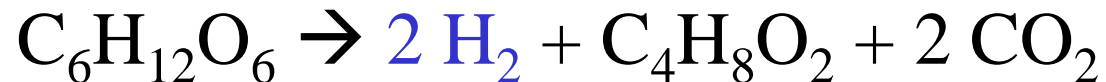


# Fermentative production of H<sub>2</sub> from sugars

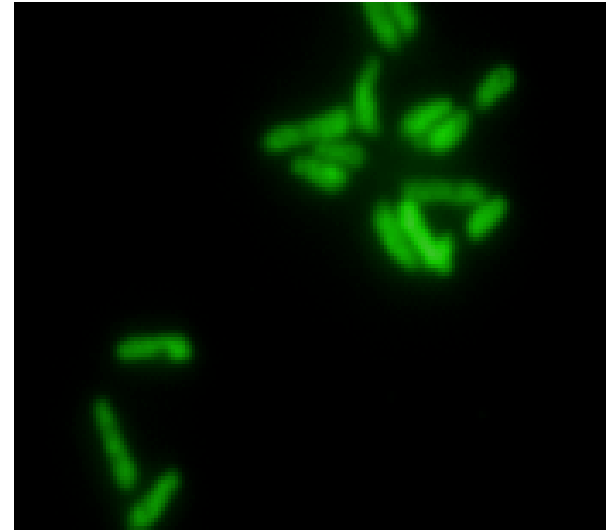
- Full oxidation of glucose to H<sub>2</sub>:



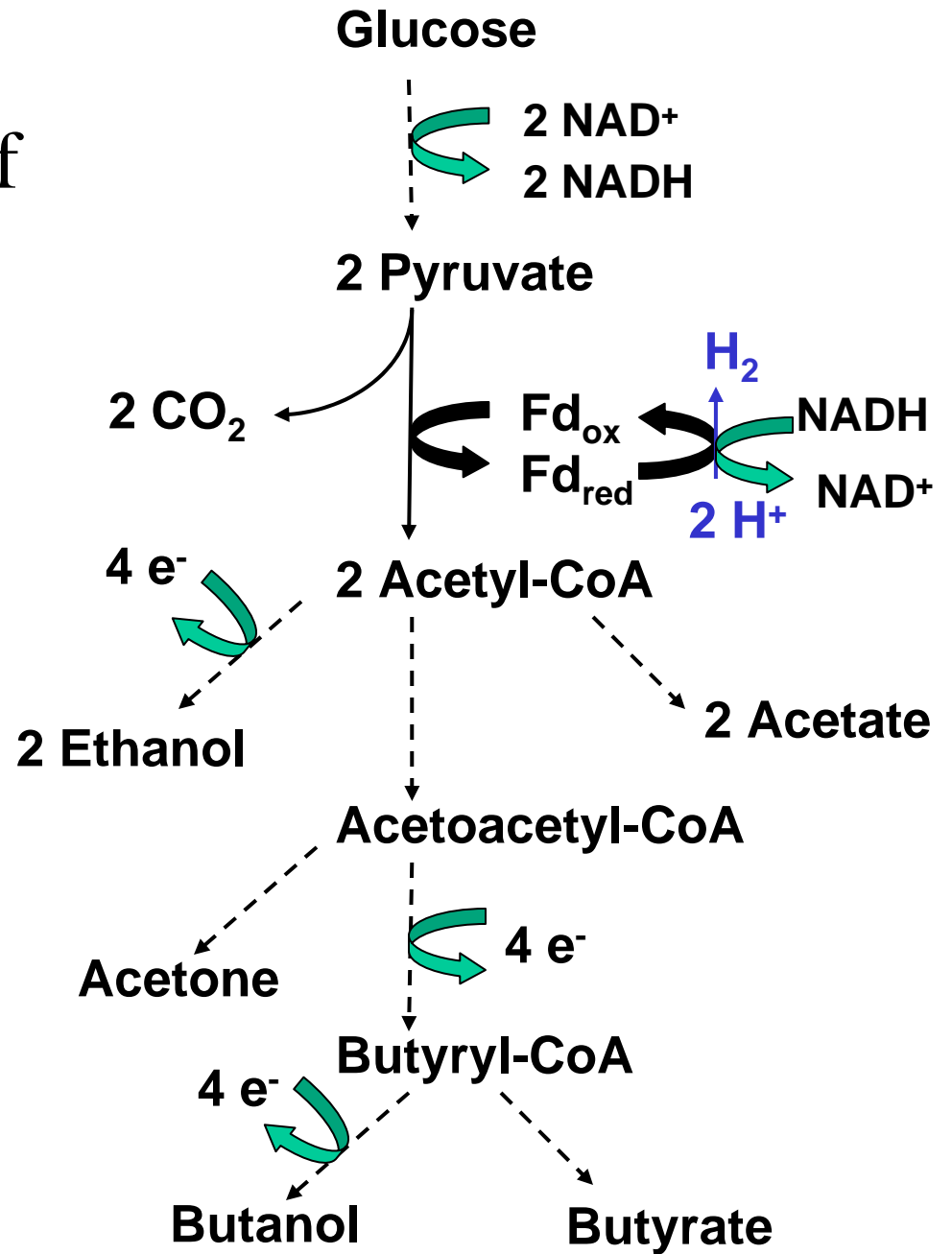
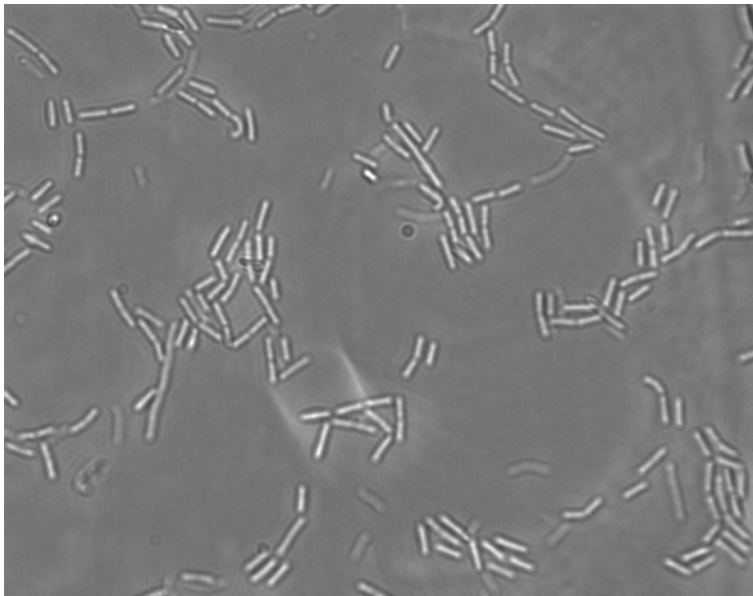
- Known pathways:



- Typical yield < 2 mol H<sub>2</sub>/mol glucose



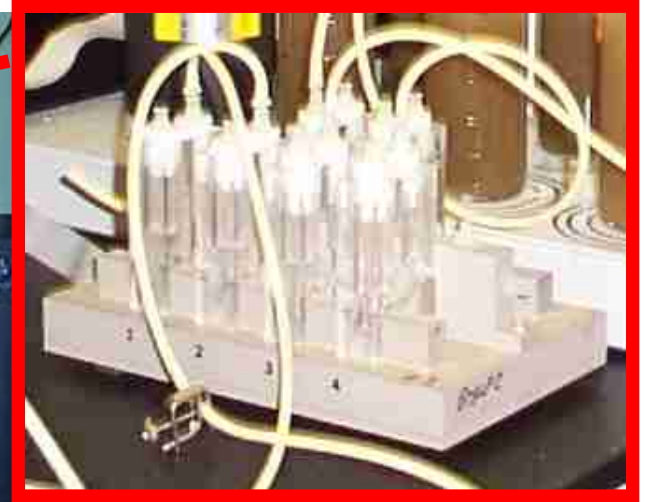
# Central Metabolism of *Clostridium acetobutylicum* 824



# Strategies to Increase Fermentative H<sub>2</sub> Yields

- Reactor configurations
  - Continuous gas release in batch systems
  - Chemostat reactors
- Uncouple hydrogen consumption (methanogenesis/homoacetogenesis)
- Strain selection

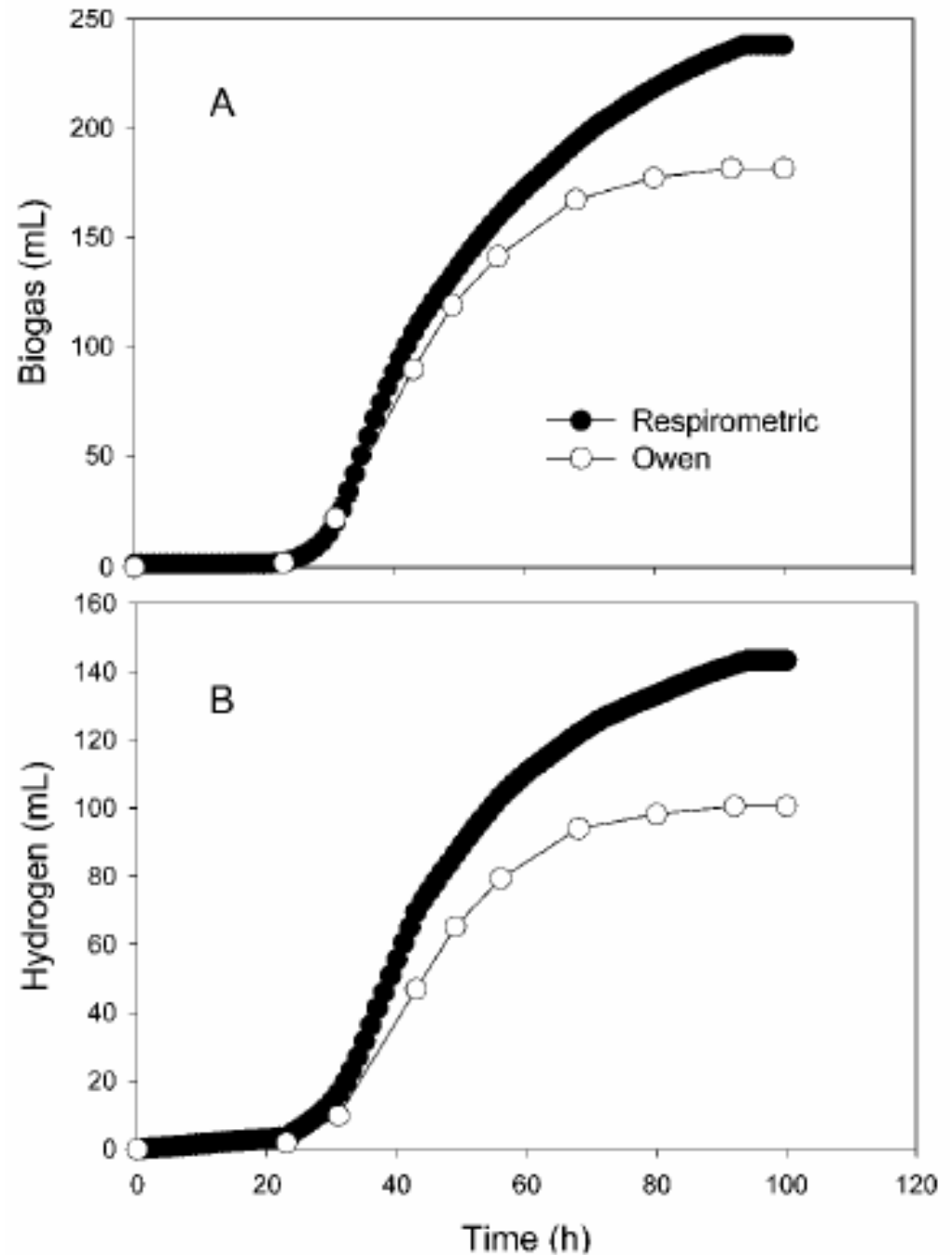
# Continuous gas release system (respirometer)



# Continuous gas release in batch cultures

43% increased H<sub>2</sub> production

Logan et al. (2002) *Environ. Sci. Tech.*, 36, 2530.



# H<sub>2</sub> production is higher in CSTR than in batch



Batch Tests: Overall conversion efficiency of 26% (8.7%)



CSTR Tests: Overall conversion efficiency of 44% (15%)

Reasons: H<sub>2</sub> loss due to acetogenesis, shifts in community structure

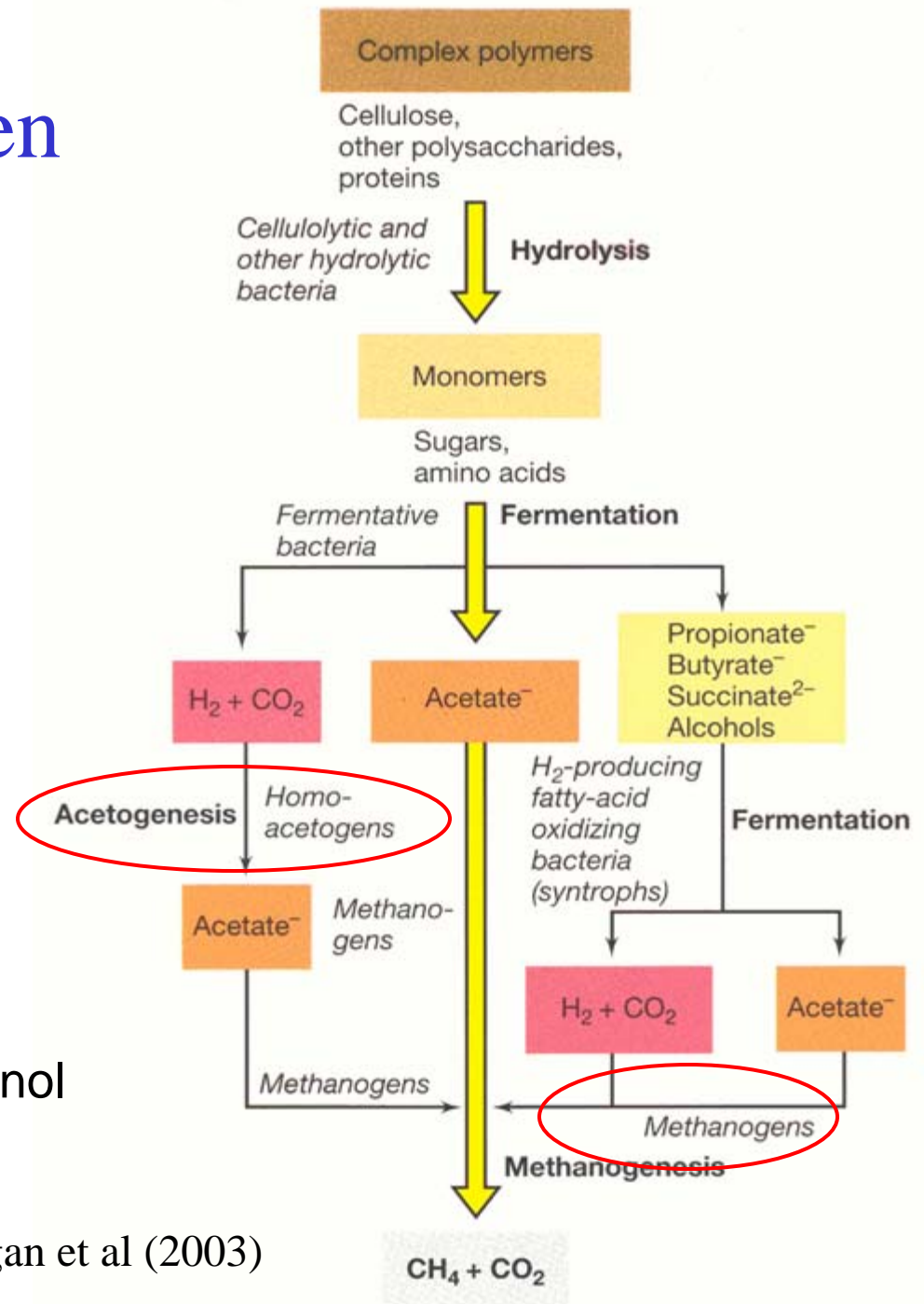
# Strategies to Increase Fermentative H<sub>2</sub> Yields

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# Uncoupling Hydrogen Consumption

- Anaerobic food chain involves interspecies  $H_2$  transfer
- $H_2$  production requires preventing:
  - Hydrogenotrophic methanogenesis
  - Homoacetogenesis
  - $H_2$ -oxidizing propionate/ethanol production



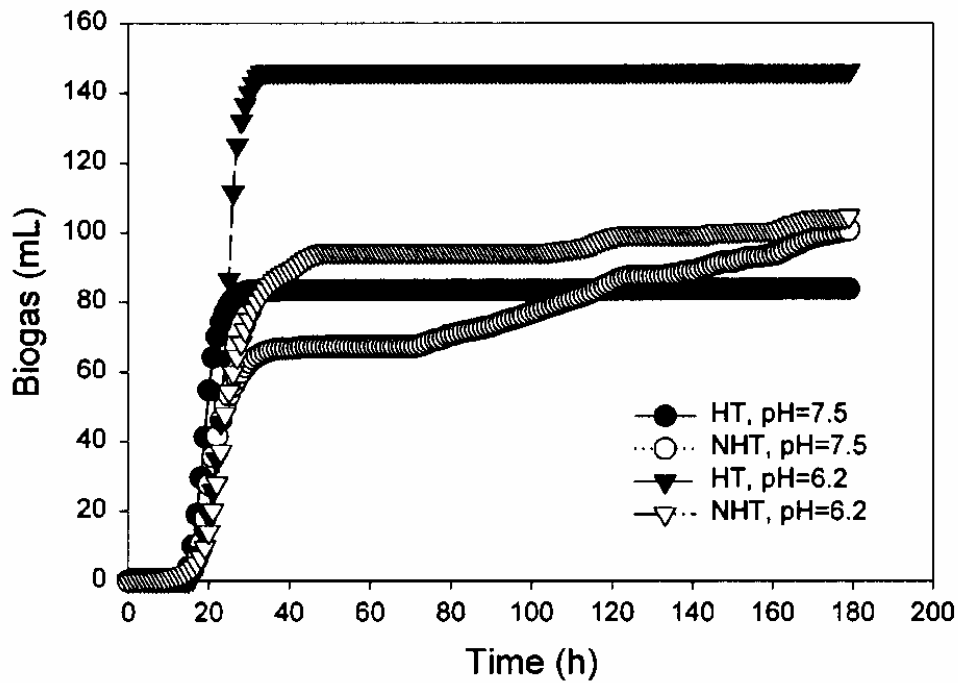
Madigan et al (2003)

# Limiting Methanogenic H<sub>2</sub> Consumption

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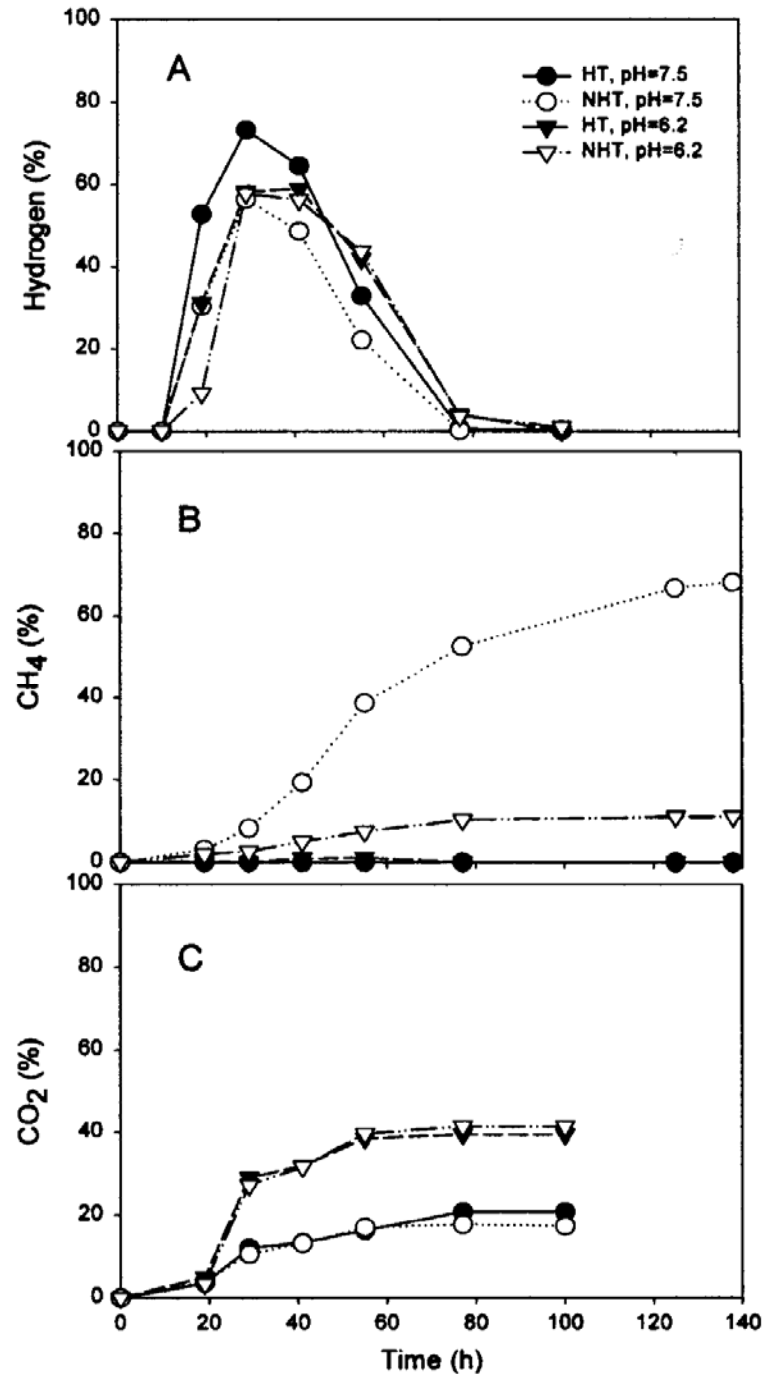
- Heat treatment of inoculum: kills non-spore forming bacteria such as methanogens that are hydrogen consumers
- Low pH (~6): limits methanogen growth
- Short SRT: methanogens grow too slowly
- Alkaline pretreatment [Cai et al (2004) Environ. Sci Technol.]

H<sub>2</sub> production maximized with heat treatment to kill non spore formers and using a low pH



HT = heat treated  
NHT = non heat treated

Oh et al. (2003) *Environ. Sci. Technol*



# Limiting Homoacetogenic H<sub>2</sub> Consumption

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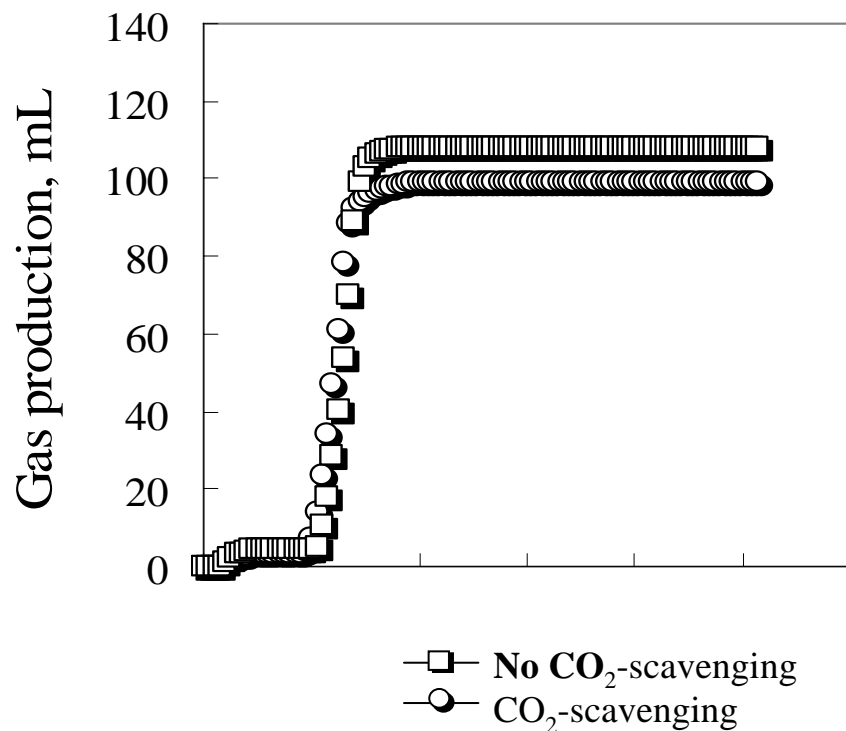
- Homoacetogenesis:



Solution:

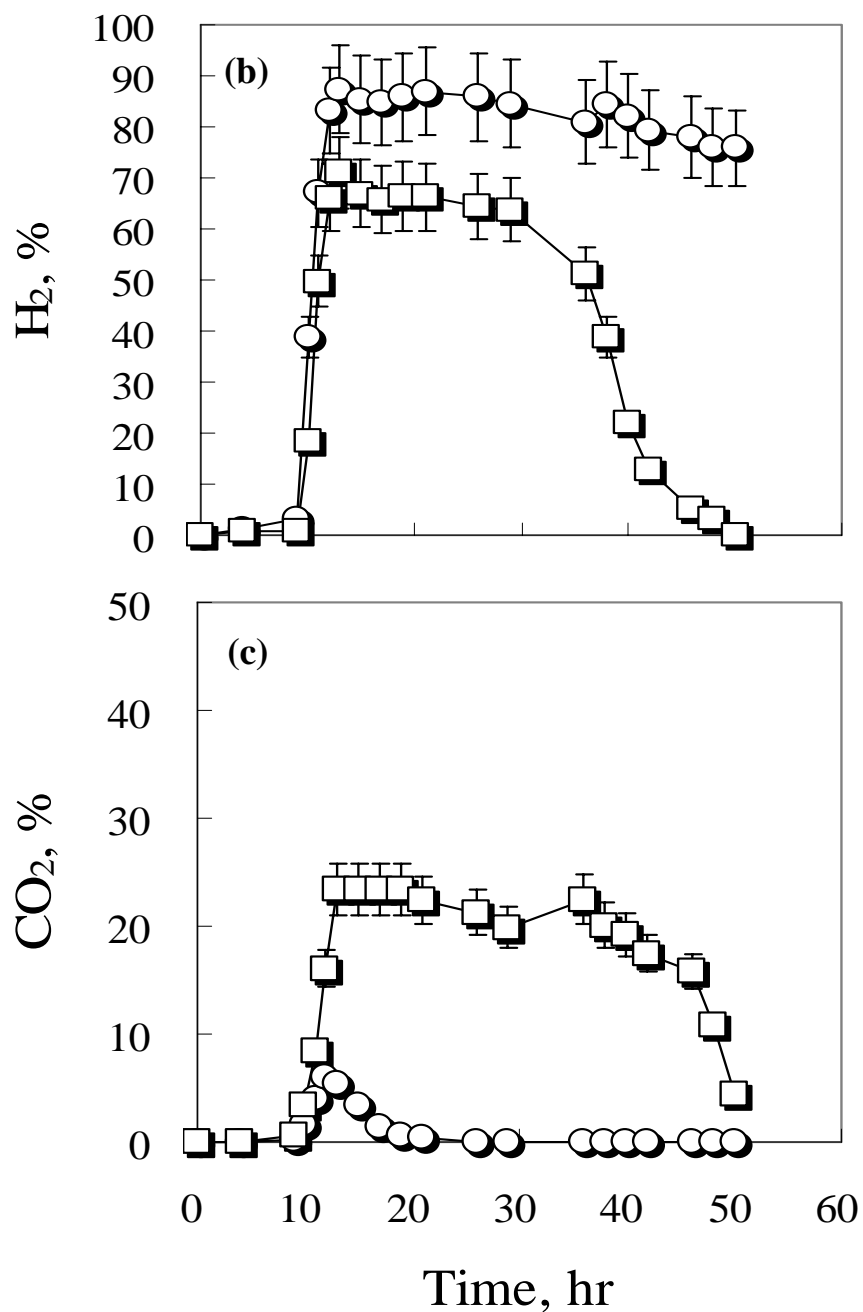
- low  $P_{\text{H}_2}$  with continuous gas release
- $\text{CO}_2$  scavenging with KOH

# Gas production



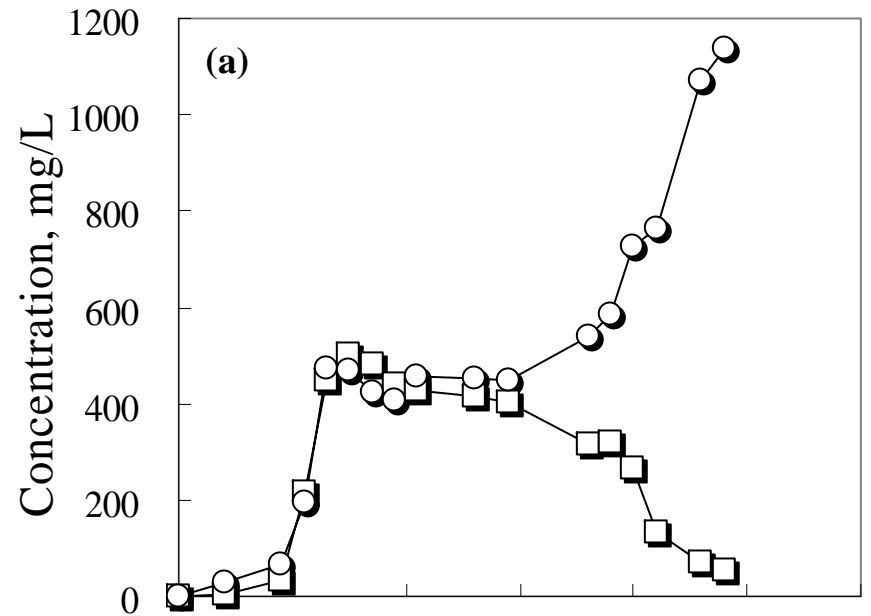
H<sub>2</sub> Yield (mol/mol):

- 2.0 w/ scavenging
- 1.4 w/o

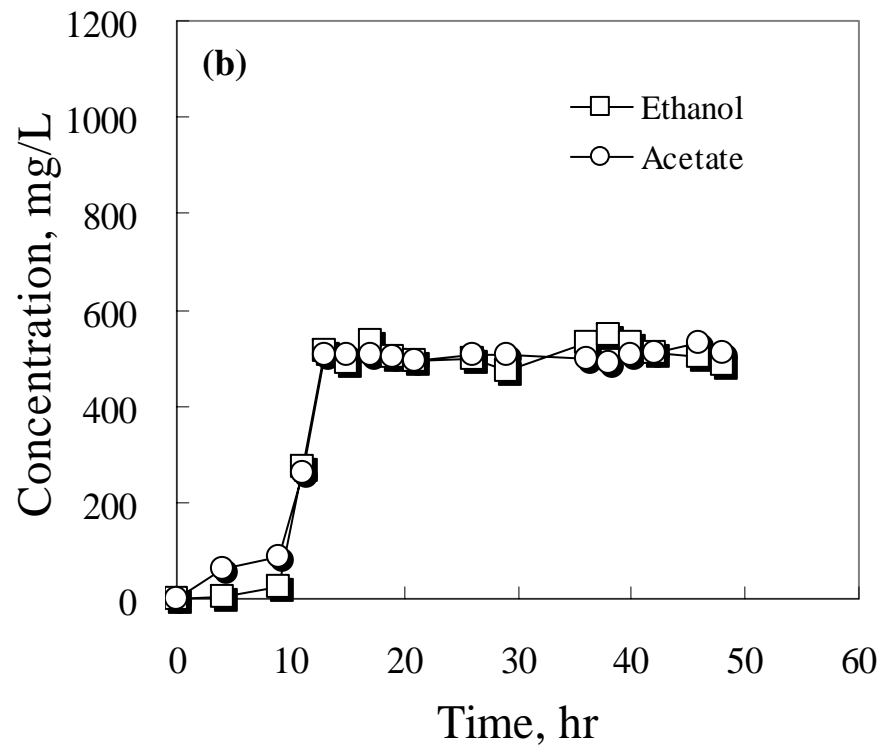


# Fermentation products

No CO<sub>2</sub> scavenging



With CO<sub>2</sub> scavenging



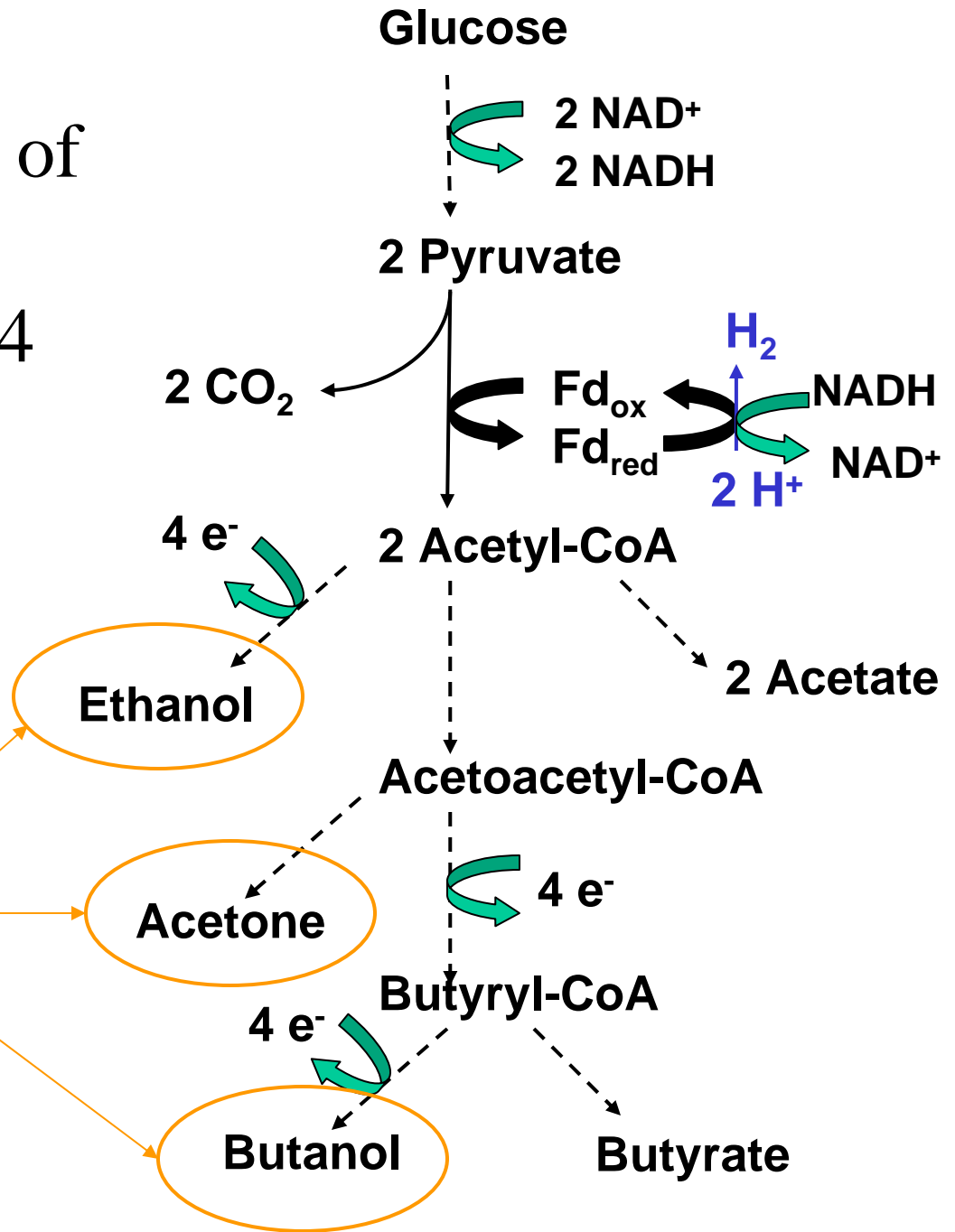
# Strategies to Increase Fermentative H<sub>2</sub> Yields

- Reactor configurations
  - Continuous gas release in batch systems
  - Chemostat reactors
- Uncouple hydrogen consumption (methanogenesis/homoacetogenesis)
- **Strain selection**

# Central Metabolism of *Clostridium acetobutylicum* 824

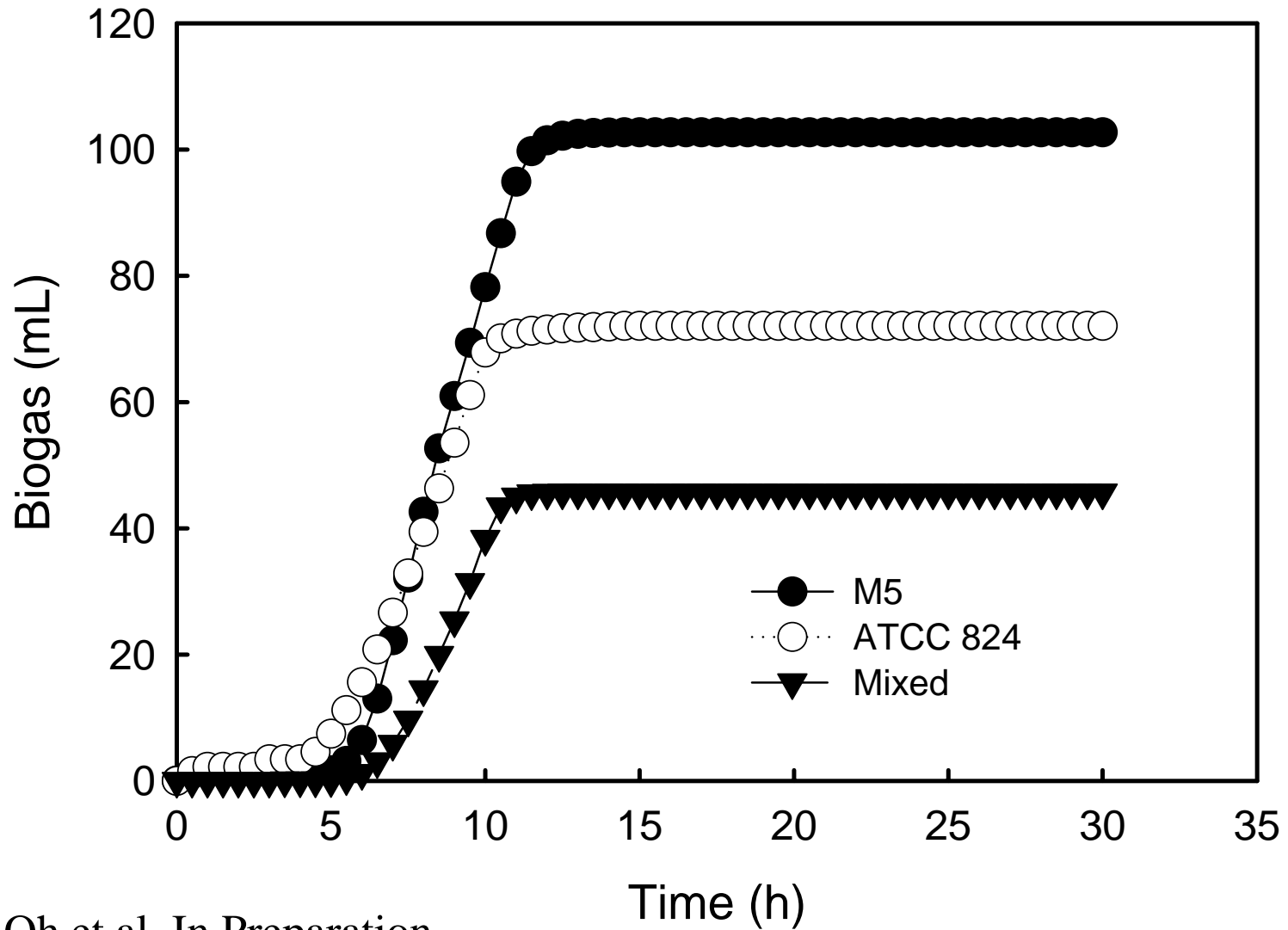
H<sub>2</sub> yield from megaplasmid-deficient strain M5?

Production decreased in megaplasmid-deficient strains



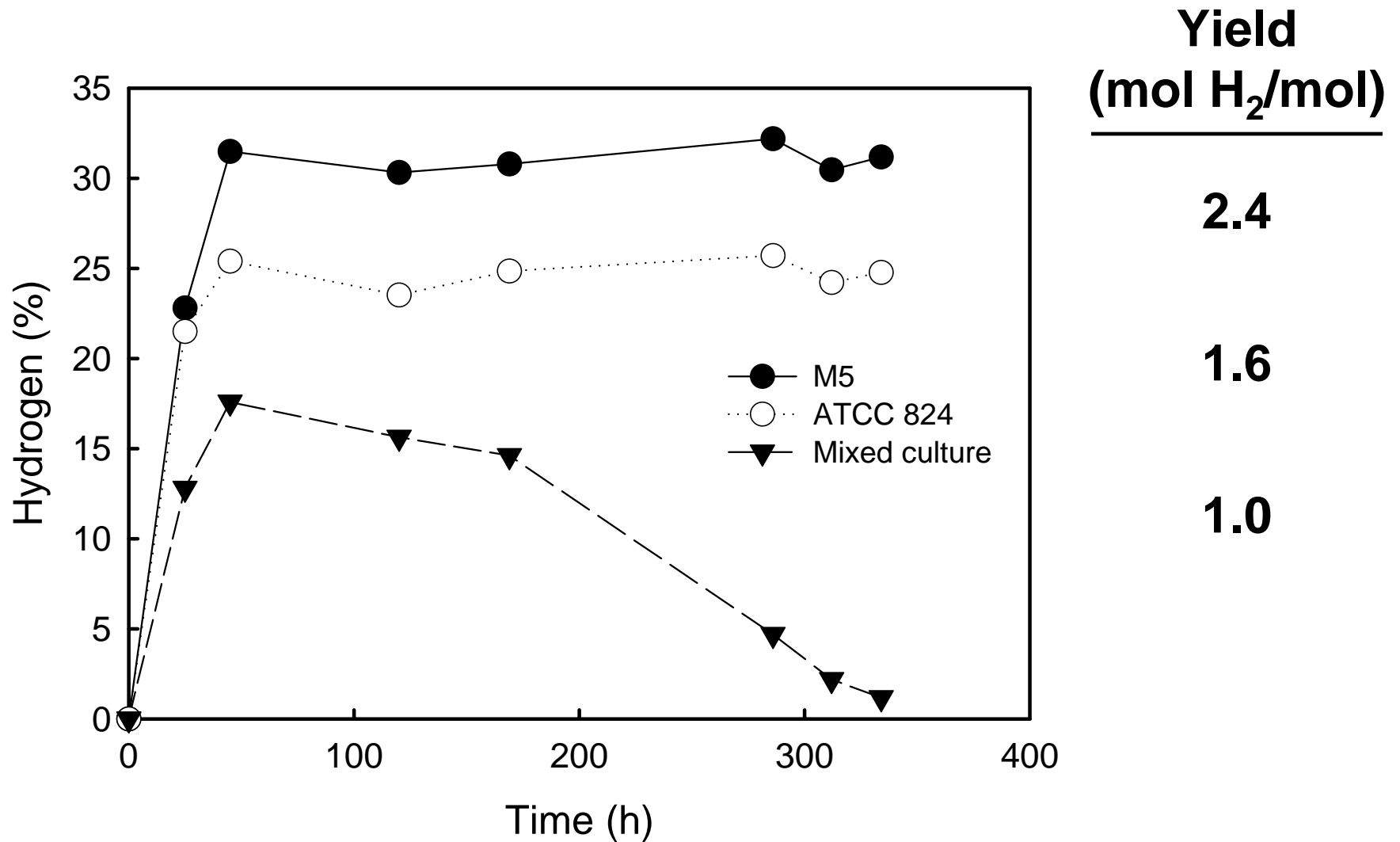


# Respirometric Results

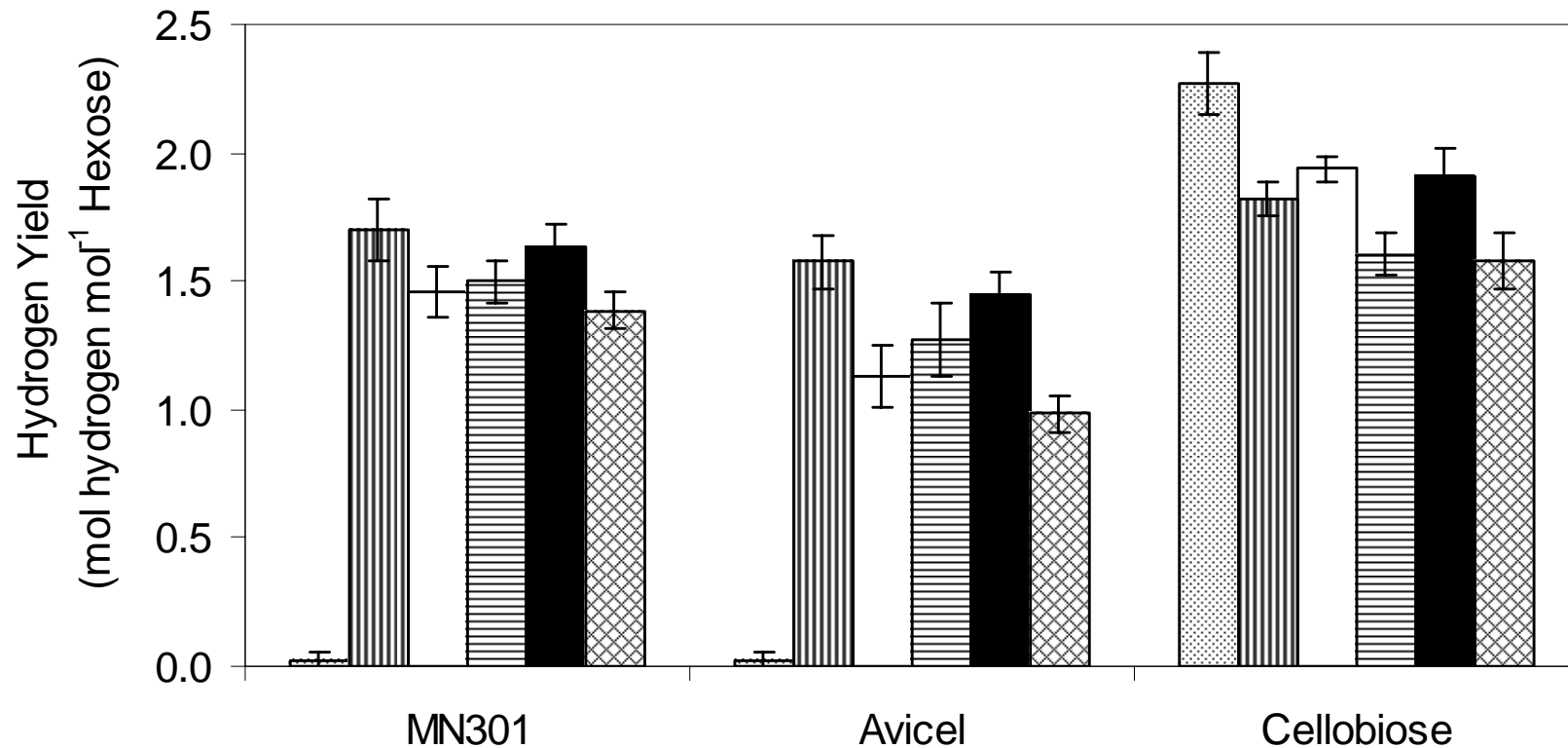


Oh et al, In Preparation

# Hydrogen Yield Results



# Comparison of H<sub>2</sub> yield for *Clostridium* spp.



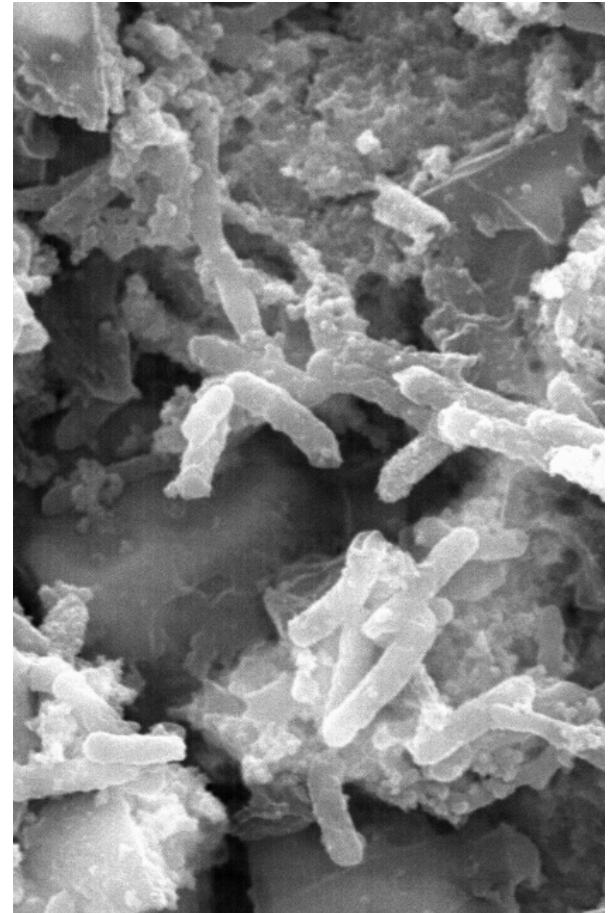
*Clostridium* species:  *acetobutylicum*,  *cellulolyticum*,  *cellobioparum*  
 *celerecrescens*,  *populeti*,  *phytofermentans*

Ren, Ward, Logan, and Regan (2007) *J. Applied Microbiol.*

# Summary

Fermentative hydrogen yields can be increased by:

- Continuous release of  $H_2$
- Continuous flow system
- Reducing  $H_2$  consumption by methanogens and acetogens
- Using strains that divert more electrons to proton reduction than other products



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Mark Gultinan, Horticulture

Mary Ann Bruns, Crop and Soil Science

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Steven Van Ginkel, Thomas Ward, Yi Zuo

## Funding:

USDA/DOE 2003 Biomass Research and Development Initiative

