Biofuels and Bioproducts from Biomass - Generated Synthesis Gas

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Biobased Products and Energy Center
http://bioenergycenter.okstate.edu/

Oklahoma State University

The Biobased Products and Energy Team:

A multidisciplinary team dedicated to conduct research and provide educational programs in biobased product and bioenergy leading to the establishment of sustainable bioenergy and biorefinery industries in Oklahoma

Research Areas:

- **Feedstock development and feedstock production**
- **Biomass harvesting, handling and storage**
- **Conversion technologies**

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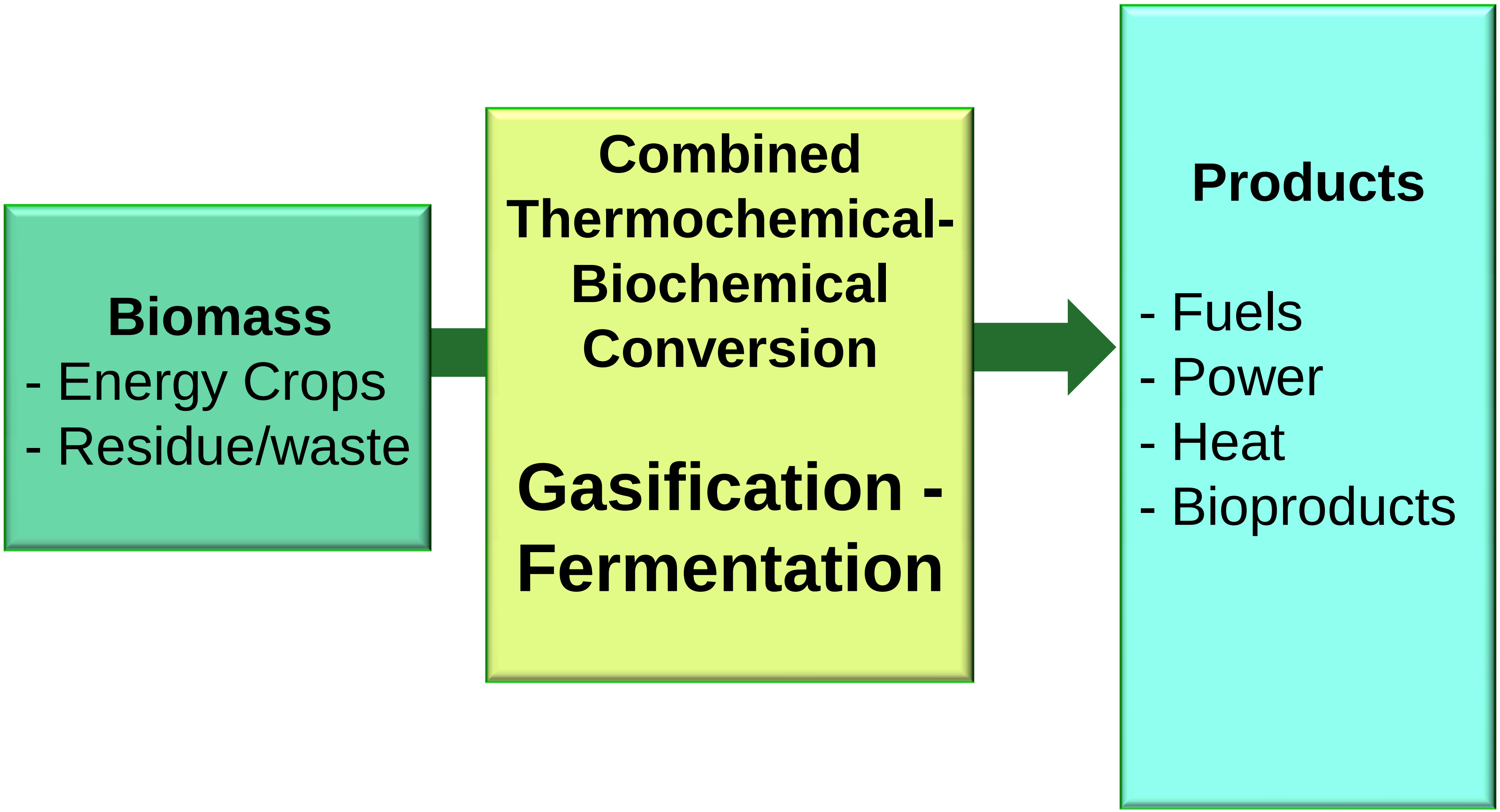
National Renewable Fuel Standard program (RFS) 2007 requires the production of 36 Billion gallons of biofuels from renewable sources by 2022, of which 16 Billion gallons are cellulosic biofuels.

Pathways for Cellulosic Biofuels



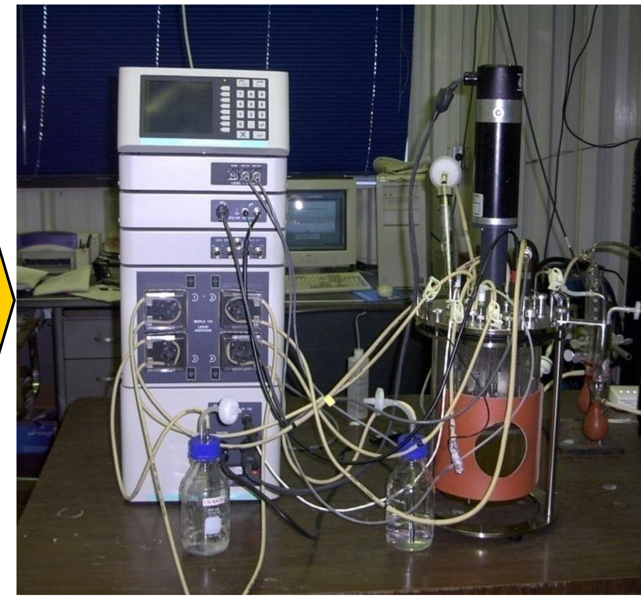
OSU GRASSohol Strategy: Using Gasification-Fermentation to Convert Biomass to Fuel-Grade Ethanol

Biomass

Grow, harvest, and transport biomass

Gasifier

Convert biomass to producer gas (CO, CO_2, H_2)



Bioreactor

Ferment producer gas to ethanol (and other useful products)

Gasification-Fermentation

Advantages

- Feedstock independent of chemical composition of biomass
- Unlike chemical catalysts, microorganisms are specific to ethanol production (i.e., specific products are produced)
- No pretreatment or enzymes required as in biochemical platform.
- Complete conversion of biomass to syngas including lignin. This reduces environmental impact of waste disposal.

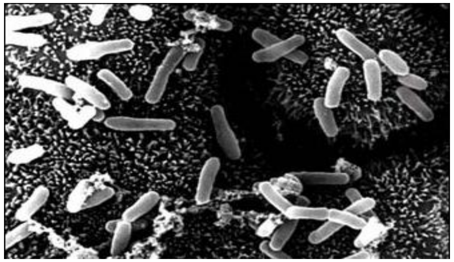
Gasification-Fermentation

Disadvantages

- Gas-liquid mass transfer limitations
- Low ethanol productivity related to low cell density
- Biomass-generated syngas impurities
- Sensitivity of microorganisms to environmental conditions (pH, O_2 concentration, redox potential).

Microorganisms Used for Ethanol Production from Syngas

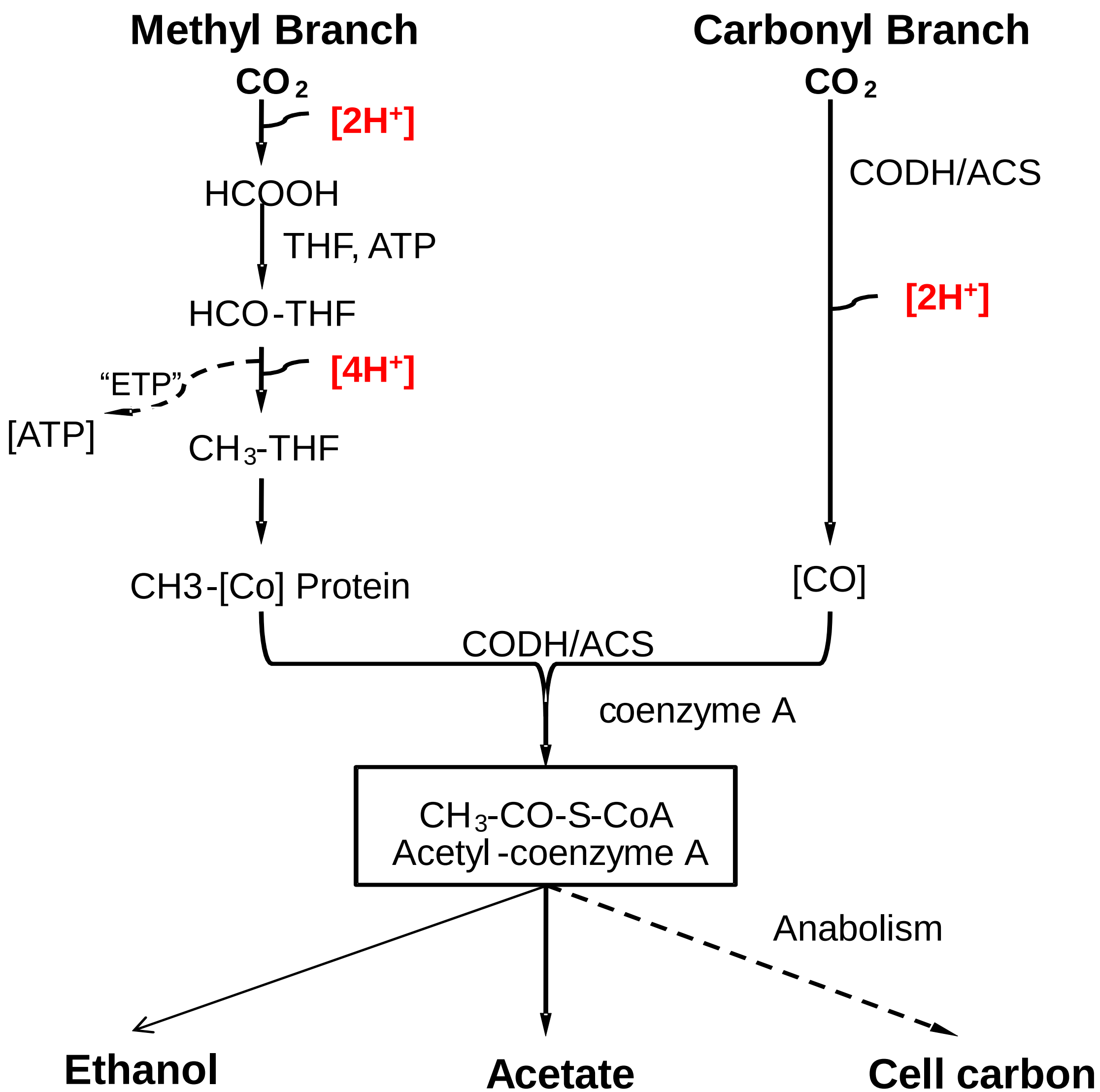
- *Clostridium ljungdahlii*
- *Acetobacterium woodii*
- *Clostridium thermoaceticum*
- *Clostridium carboxidivorans* (OSU-OU-BYU)
- ***Clostridium* strain P11 (OSU-OU-BYU)**
- **New strain CP11 (OU-OSU)**



Clostridium species

These microorganisms are acetogens that can produce ethanol, acetic acid and other products from CO and H_2 in the presence of CO_2 .

Acetyl-CoA Pathway

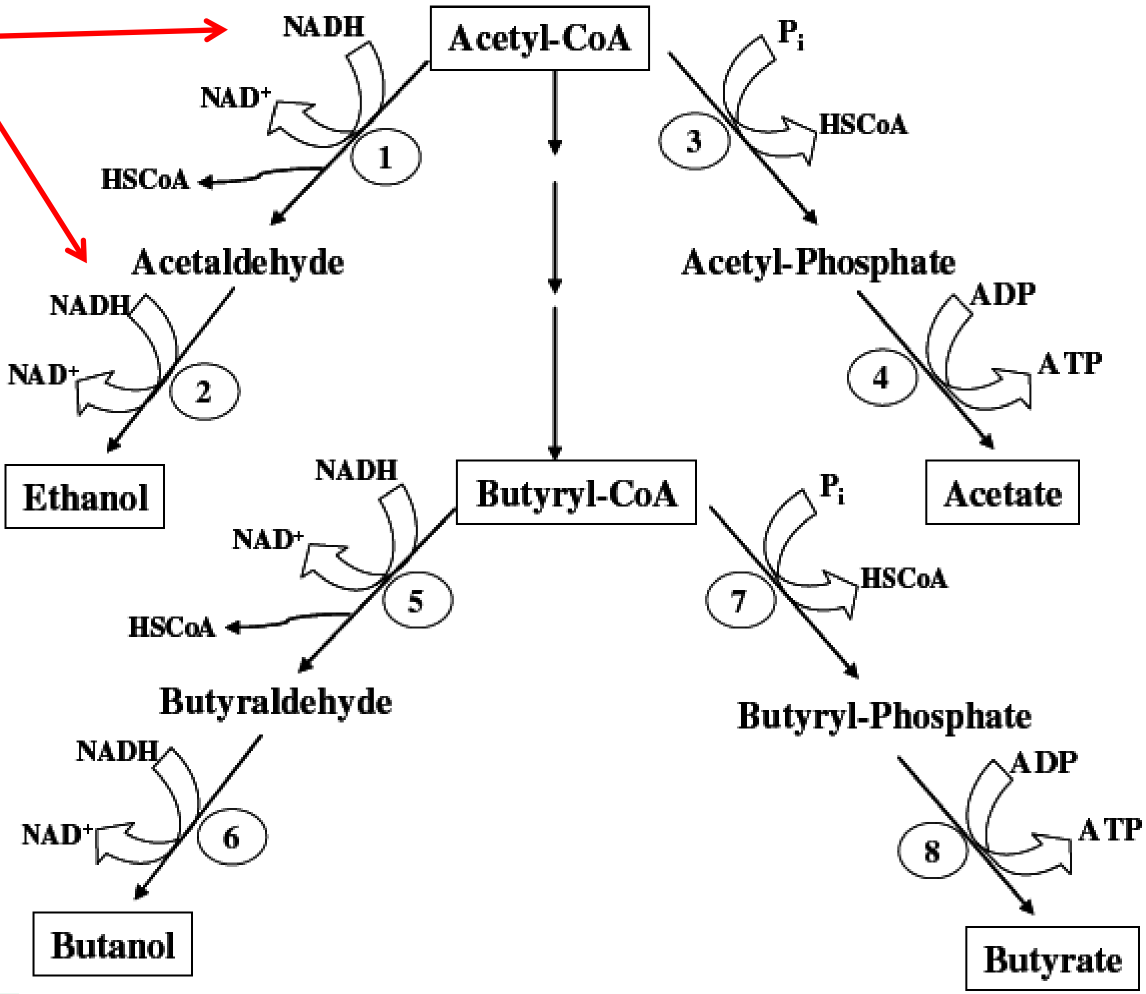


Adapted from Drake 1994

In the Acetyl-CoA Pathway

NADH is an energy rich electron donor required for the reduction of Acetyl-CoA to acetaldehyde and then reduction of acetaldehyde to ethanol.

The regeneration of NADH from NAD^+ could improve ethanol production, which can be done by the addition of a reducing agents.



Adapted from Ahmed, 2006

The effect of DTT on ethanol and acetic acid production in syngas fermentation using strain P11.

Research Gap

- Reducing agents are known to increase ethanol production in fermentation processes.

- Reducing agents act as artificial electron carriers and help regenerate NADH from NAD^+ which is directly involved in the production of acetaldehyde from acetyl-CoA and then ethanol from acetaldehyde.

$$NAD^+ + 2e^- + H^+ \underset{\text{Oxidation}}{\overset{\text{Reduction}}{\rightleftharpoons}} NADH$$

Reducing Agents Used in Fermentation Processes

- Reducing agents used in fermentation processes include:

  - methyl viologen
  - sodium sulfide
  - neutral red
  - ascorbic acid
  - cysteine sulfide
  - **dithiothreitol (DTT)**

- Dithiothreitol (DTT, $C_4H_{10}O_2S_2$), also known as Cleland's reagent, is a strong reducing agent with a redox potential of -330 mV in Standard Hydrogen Electrode (SHE) at pH 7.

- The **redox potential**, also known oxidation/reduction potential (ORP), is a measure of the affinity of a chemical specie for electrons compared with hydrogen.

- The more positive the reduction potential of a species, the greater the species' affinity to electrons and tendency to be reduced.

- DTT can enhance fermentation by reducing the redox potential and protect enzymes involved in the fermentation process.

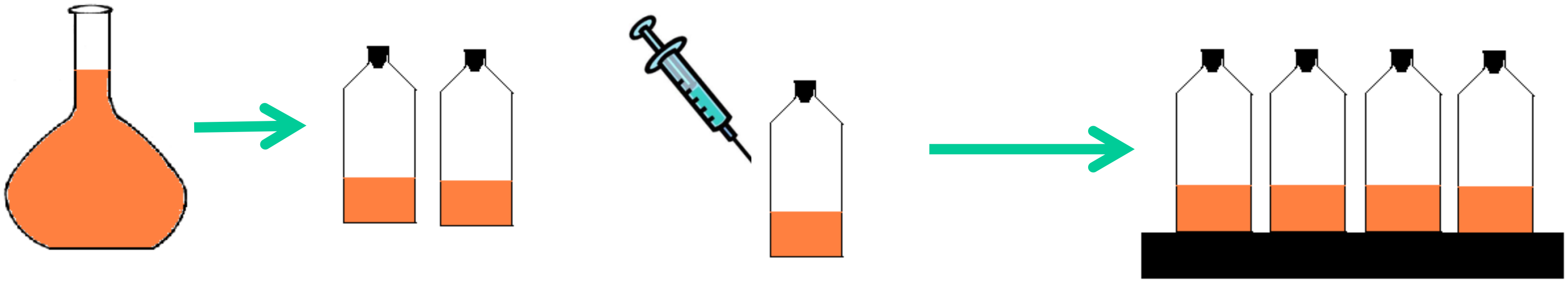
Motivation

The effect of DTT on ethanol and acetic acid production in syngas fermentation using strain P11 has not been studied.

Objective

To study the effect of reducing agent dithiothreitol (DTT) on ethanol and acetic acid production by *Clostridium* strain P11 using simulated biomass based syngas and producer gas in yeast extract (YE) and corn steep liquor (CSL) media.

Materials and Methods



100 ml medium in each 250 ml serum bottles strict anoxic conditions

Inoculate with *Clostridium* P11

Fermentation, 15 days
150 rpm & 37^{o}C

Media composition (per L)

Minerals	30 mL
Trace metals	10 mL
Vitamins	10 mL
MES buffer	10 g
Resazurin (0.1 %)	1 mL
Cysteine sulfide (4%)	10 mL
and either	
Yeast extract	1 g
or	
Corn steep liquor	10 g

DTT conc.: 0.0, 2.5, 5.0, 7.5 and 10 g/L

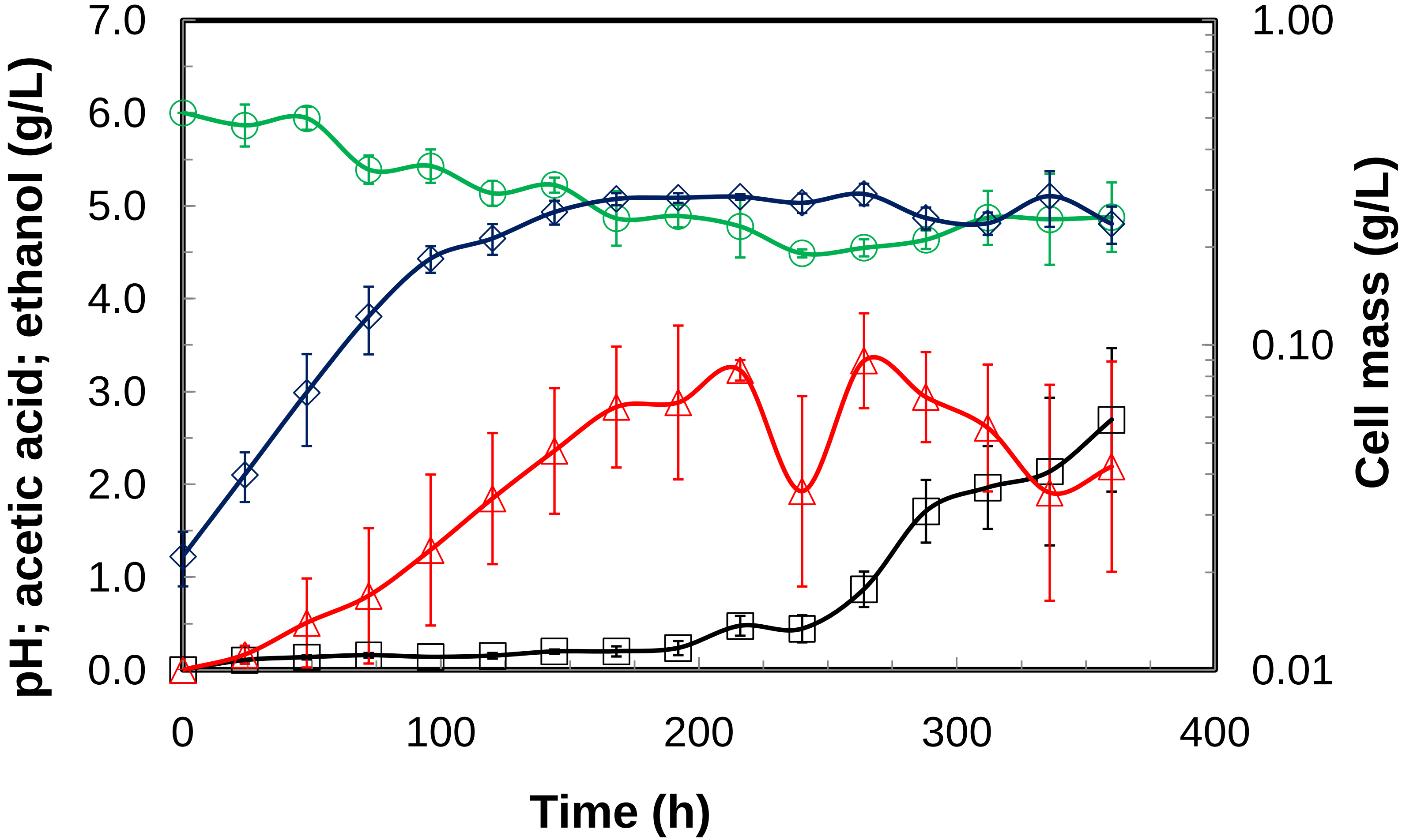
Syngas composition (mole%):

H_2=5% ; CO=20% ; CO_2=15% ; N_2=60%

Analysis

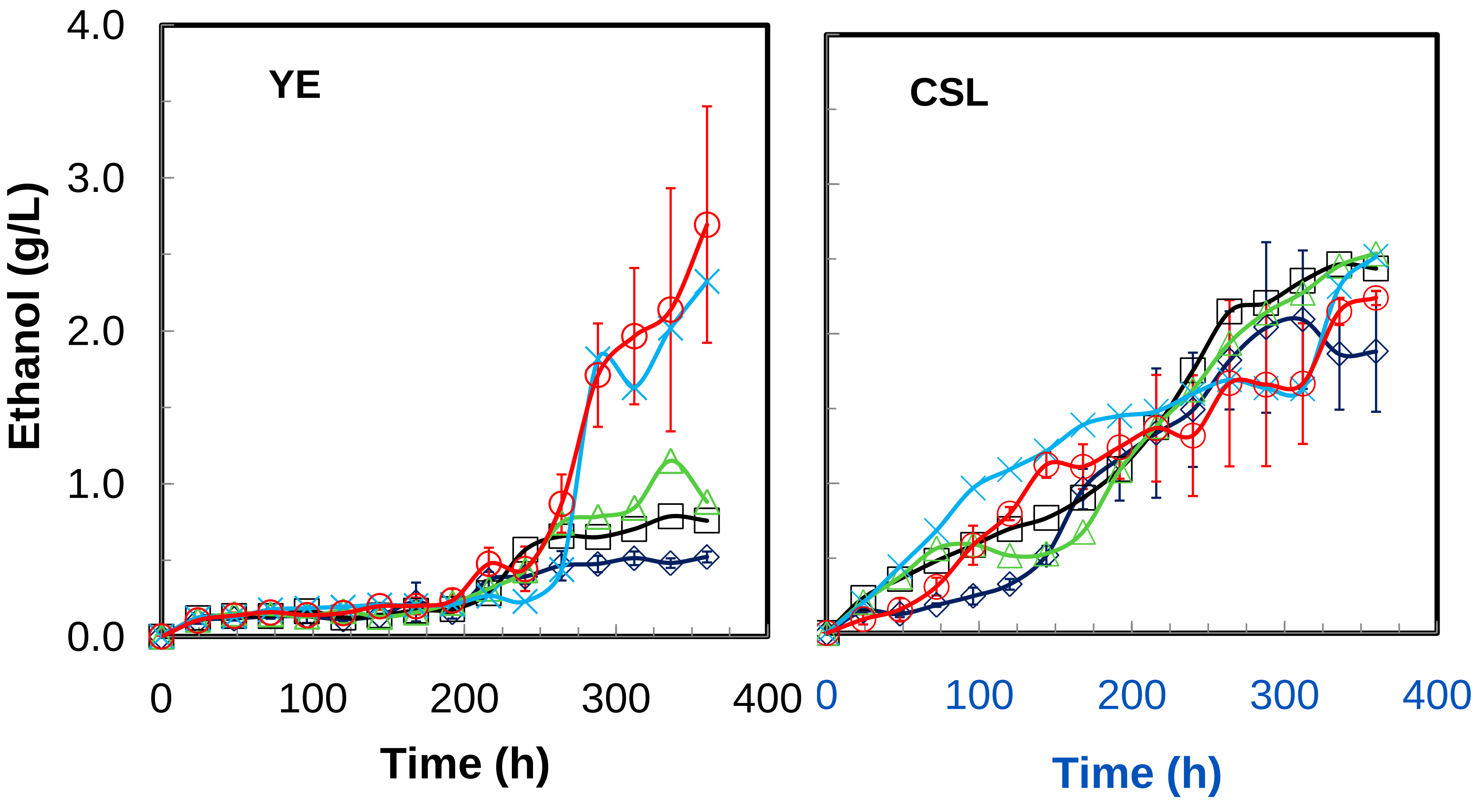
- **pH**
- **Cell mass** – **OD @ 660 nm**
- **Solvents** – **GC-YSI**

Product Profile in DTT 10 g/L (Yeast Extract Medium)



(◇) cell mass; (□) ethanol; (Δ) acetic acid; (O) pH

Ethanol Profile



Dithiothreitol (g/L): (◇) 0.0; (□) 2.5; (Δ) 5.0; (X) 7.5; (O) 10.0

Specific Growth Rate and Ethanol Yield

DTT concentration (g/L)	Specific growth rate (h^{-1})		Ethanol yield (g ethanol / g cell)	
	YE[a]	CSL[b]	YE[a]	CSL[b]
0.0	0.035	0.042	3.48	5.31
2.5	0.033	0.078	2.98	8.33
5.0	0.028	0.074	3.60	9.16
7.5	0.022	0.077	7.60	15.08
10.0	0.022	0.043	11.42	16.24

[a] YE, yeast extract medium
[b] CSL, corn steep liquor

Conclusions

- The addition of the reducing agent, DTT, substantially enhanced ethanol production.

- Over fourfold increase in ethanol production was observed in YE medium that contained at least 7.5 g/L of DTT after 360 h of fermentation compared medium without DTT.

- There was a 35% increase in ethanol production in the presence of DTT at concentrations lower than 7.5 g/L in CSL media compared to the medium without DTT.

Growth and product kinetics of *Clostridium* strain P11 using corn steep liquor and yeast extract media

Research Objectives

- To evaluate corn steep liquor (CSL) as a low cost alternative nutrient to standard yeast extract medium for syngas fermentation using *Clostridium* strain P11.
- To compare cell growth, syngas conversion efficiency and product formation during syngas fermentation.

What is Corn Steep Liquor?

- Corn steep liquor is by-product from wet corn milling plant (rich in vitamins, trace metals and minerals).
- Corn steep liquor has been used for production of many bioproducts such as:
  - Ethanol
  - Butanol
  - Mannitol
  - Enzymes
  - Antibiotics

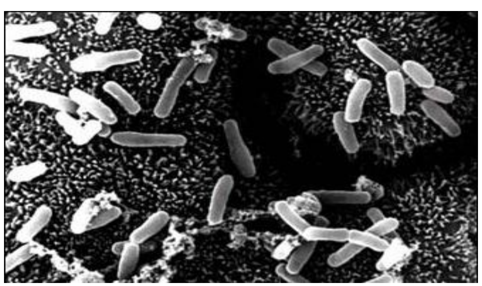
Syngas Fermentation in 7.5 L CSTR

Experimental conditions

Working volume: 3L
Agitation speed: 150 RPM
Temperature: 37°C
CSL medium or Yeast extract medium

Syngas composition (mole%):
H_2=5%; CO=20%; CO_2=15; N_2=60%

Microbial catalyst: *Clostridium* strain P11

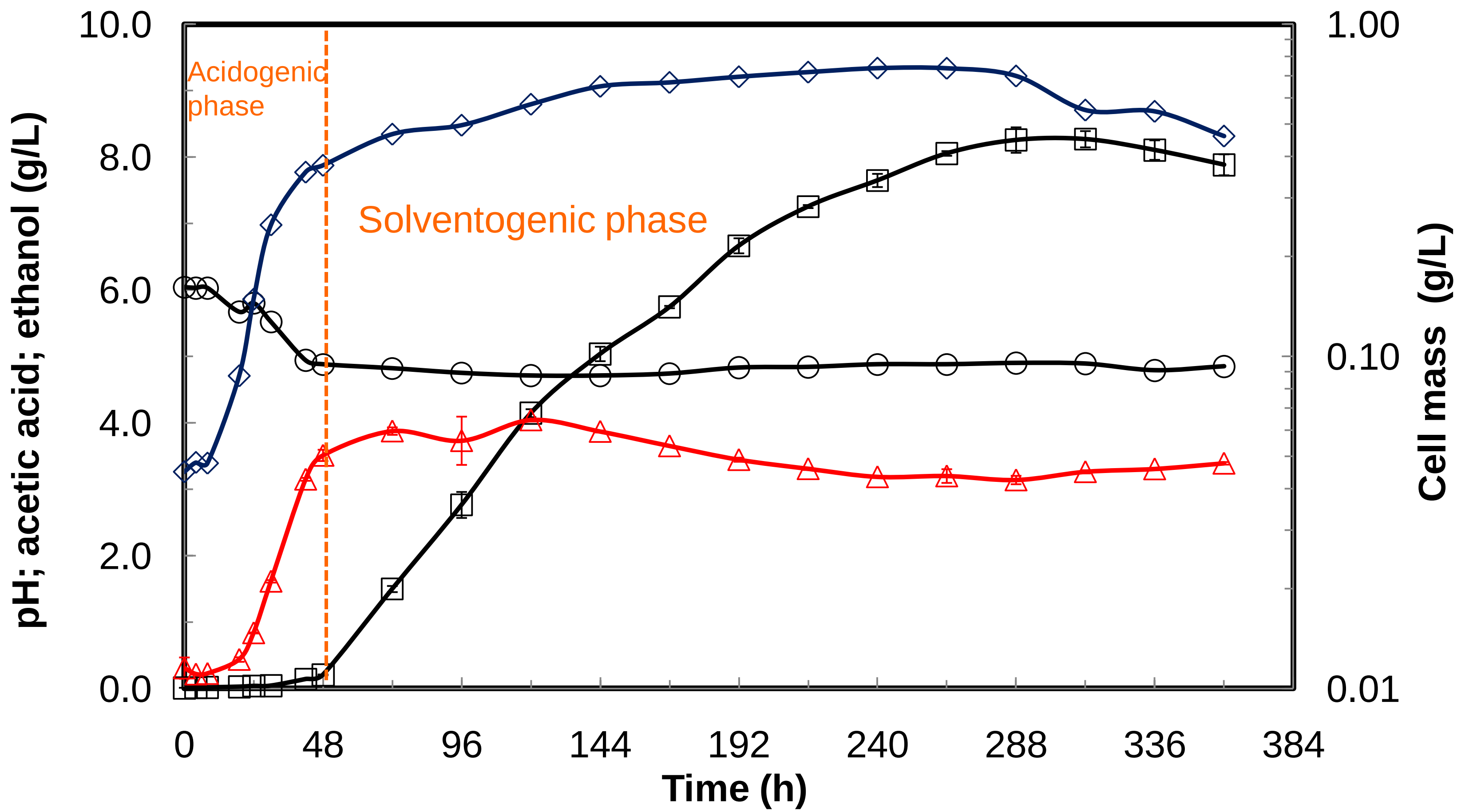


Clostridium species

Analytical Procedures:

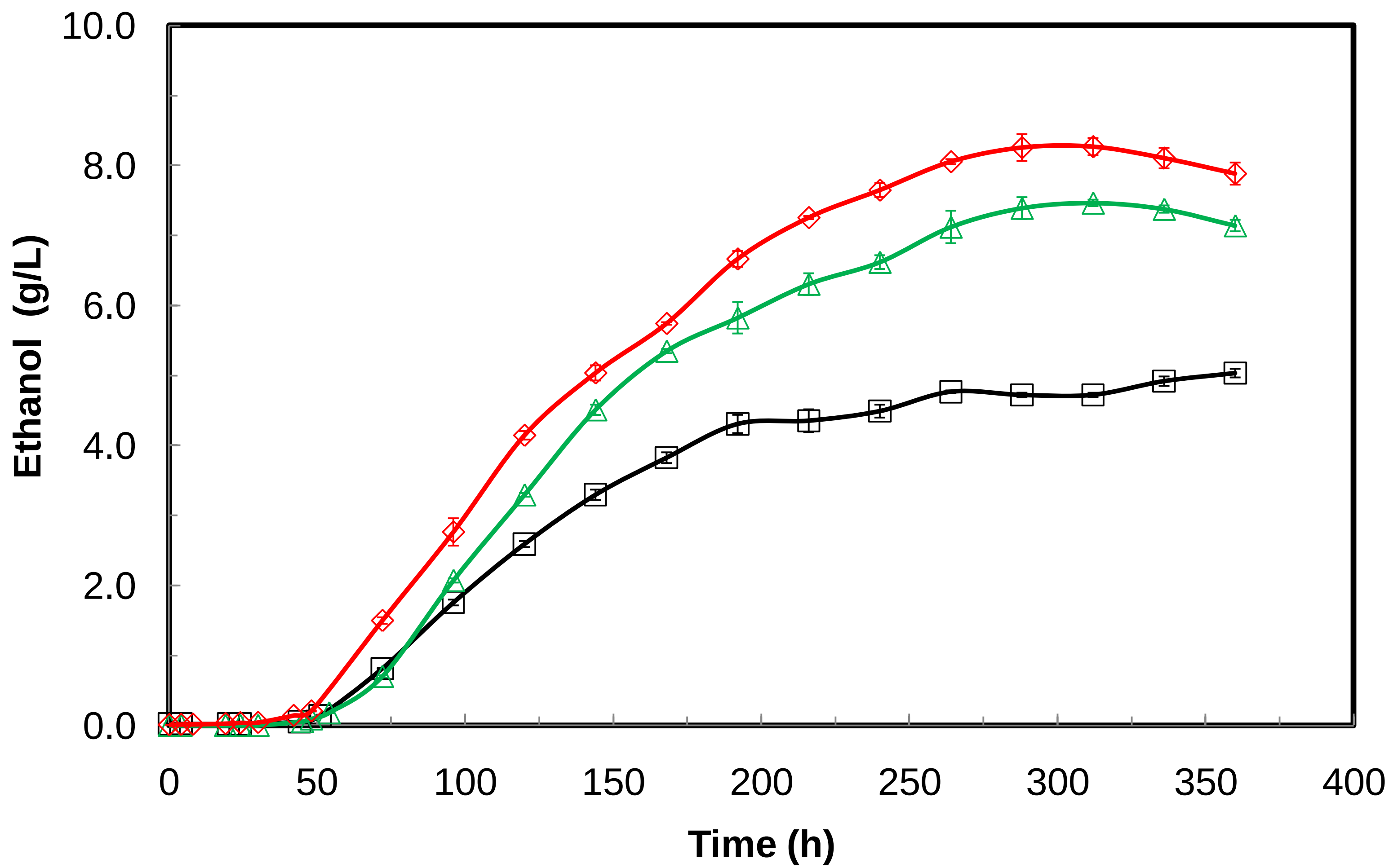
Cells concentration: spectrophotometer
Ethanol, acetic acid and butanol: GC-FID
Gas composition: GC-TCD

Growth and Product Profile in 20 g/L CSL Medium in 7.5 L Fermentor



(◇) cell mass; (□) ethanol; (Δ) acetic acid; (O) pH

Ethanol Production with P11



(□) 1 g/L Yeast extract; (Δ) 10 g/L CSL; (◇) 20 g/L CSL

Conclusions

- About, 57% and 13% more ethanol is produced in 20 g/L CSL medium compared to 1 g/L yeast extract and 10 g/L CSL media, respectively, in the 7.5 L fermentor.

- Ethanol productivity in 20 g/L CSL medium is about two times higher than in yeast extract medium.

Acknowledgments

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