Bacillus megaterium: A Potential Microbial Cell Factory for Biofuel & Chemicals

B. megaterium **Workshop**

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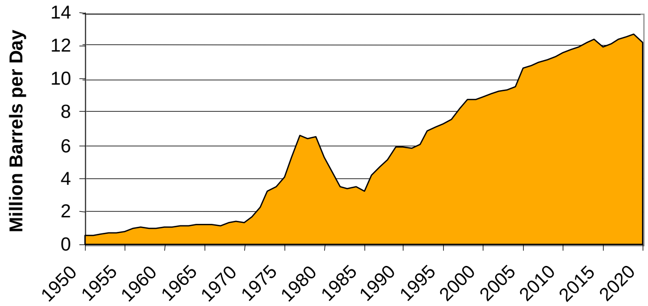
Genome Sequencing

- **Basic research (NSF)**
- **Medical related pathogenic island (NIH)**
- **Unique pathways for bioremediation of contaminated sites / regions (DOE/EPA)**
- **Unique pathways for industrial applications such as production of biofuels / biochemical compounds (DOE/USDA)**

U.S. Dependence on Crude Oil Imports



55% of the oil consumed in the U.S. is imported.

Half of the oil is used as automotive fuel (180 billion gal/year).

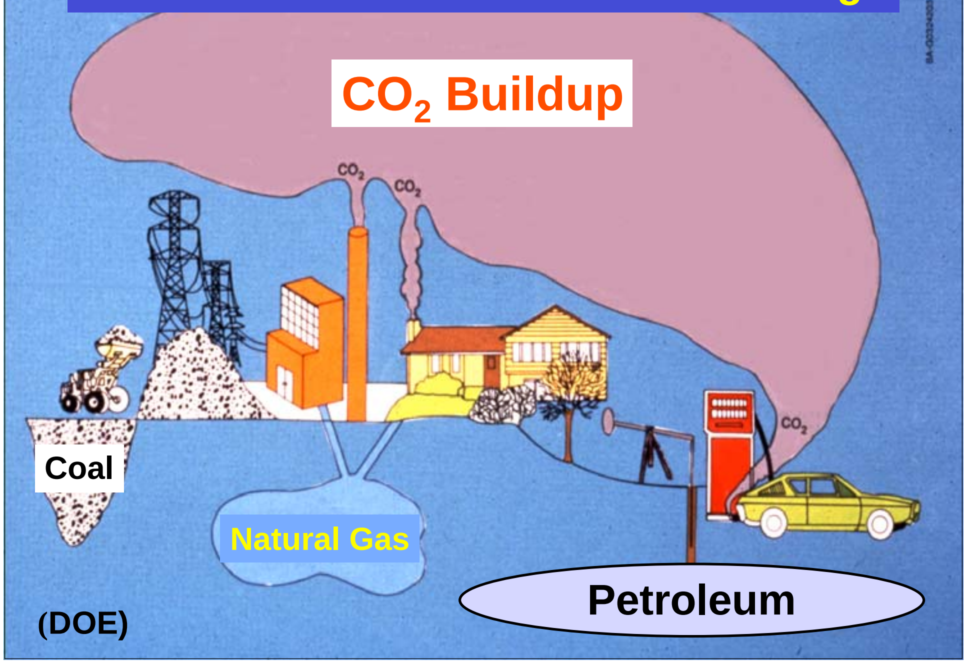
Approximately 15% is used for chemicals and plastics.

Problems created by oil dependence:

1) Sustainability and stability of supply; 2) Trade imbalance; 3) Environmental damage such as the green house effect

Modified from: U.S. Department of Energy, Energy Information Administration

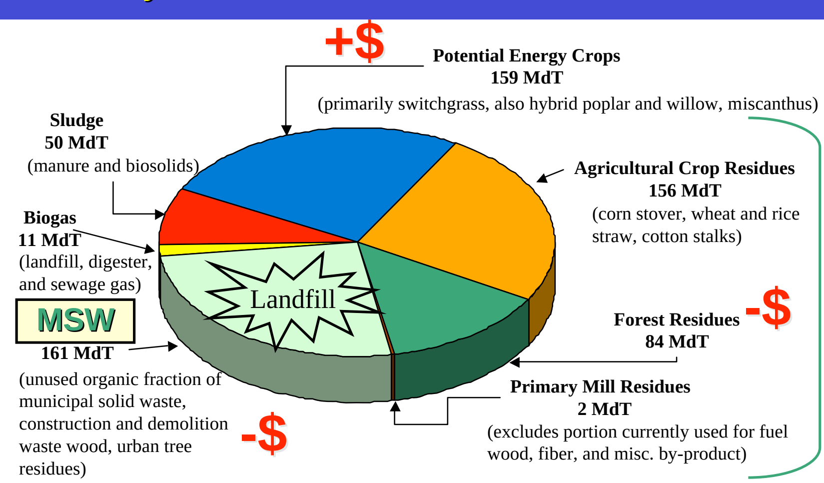
Green House Effect / Global Warming



Impacts of Climate Change

Consequences of the climate change:
1/3 of biological species are in danger…

Bio-refinery of Biomass Waste for Fuels and Chemicals

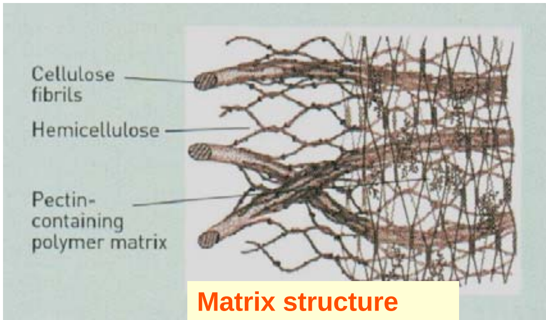


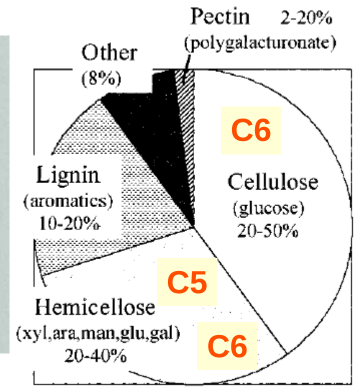
Total Feedstocks Available: 623 Million dry Tons (MdT) per year
Energy Equivalent: 16 quadrillion Btu (~1/3 of automotive fuel)

Current data: > 1.3 billion tons

DOE, Biobased Products and Bioenergy Roadmapt

Challenges to Microbial Cell Factory





Challenges: Hydrolysis of the complex to monomer sugars.
Metabolize the C6 and C5 sugars.

Desirable Traits of Microbial Cell Factory

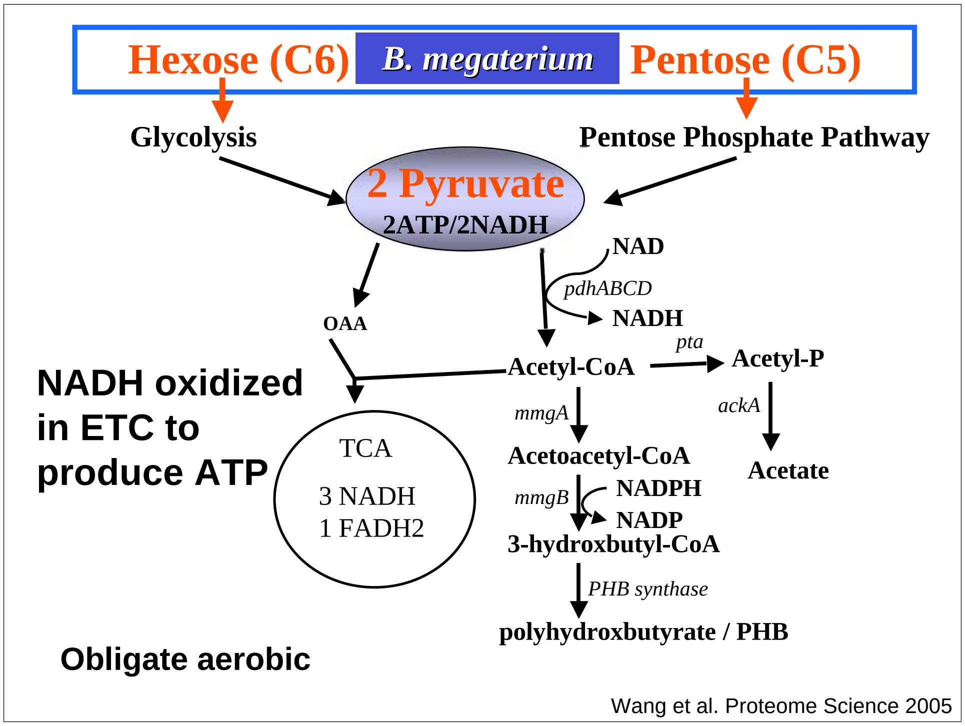
1. Use all sugars derived from biomass (C6 and C5 sugar)
2. Produce enzymes to hydrolyze hemicellulose & cellulose
3. High product yield with little by-products
4. Grow well in mineral salts media
5. Grow well in high temperature and low pH

No natural microorganisms have all these traits

B. megaterium: An Ideal Cell Factory Candidate

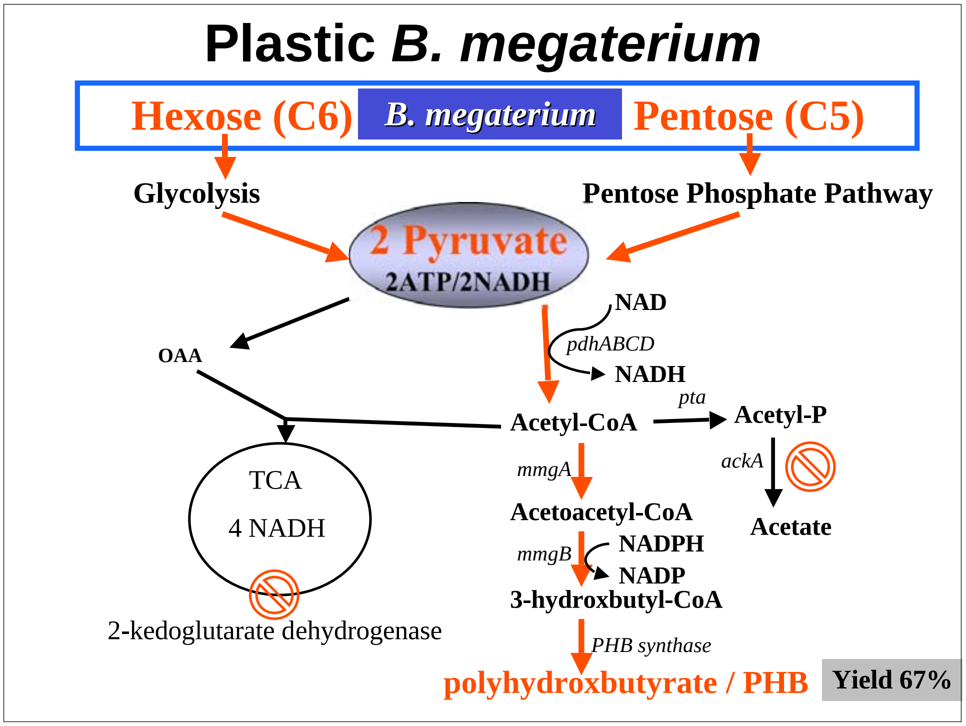
1. Has been used for producing HIV protein, antibiotics, antibody, Vitamin B12…..
2. Has no alkaline protease (good for cloning/expression)
3. Uses both C5 and C6 sugar (maybe cellobiose)
4. Grows in minimal medium and low pH (pH 4.5)
5. Produces certain enzymes such as amylase
6. Gram^{+}, easy to clone/express/secretion cellulase gene
7. Stable maintain 7 plasmids / plasmidless strain ready
8. Genome sequence is available now, ready for metabolic pathway engineering

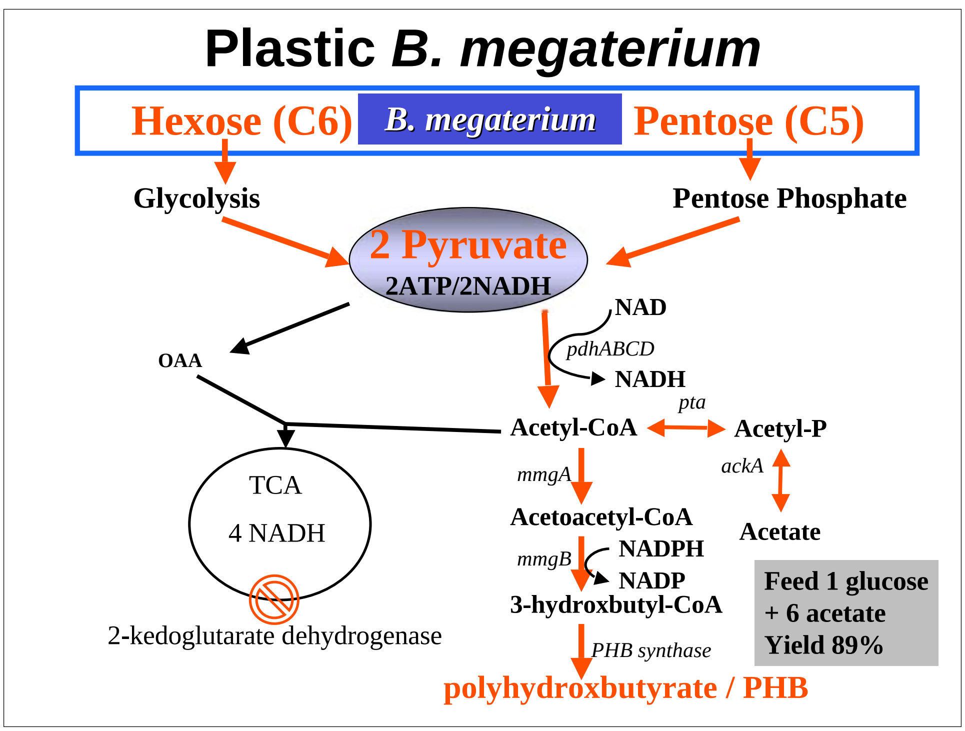
What We Know about *B. megaterium* Metabolism



Potential Projects

1. **Plastic *B. megaterium***
   (PHB biodegradable plastic)
2. Plastic *E. coli*
3. Vinegar *B. megaterium*
4. Alcoholic *B. megaterium* (ethanol and butanol)

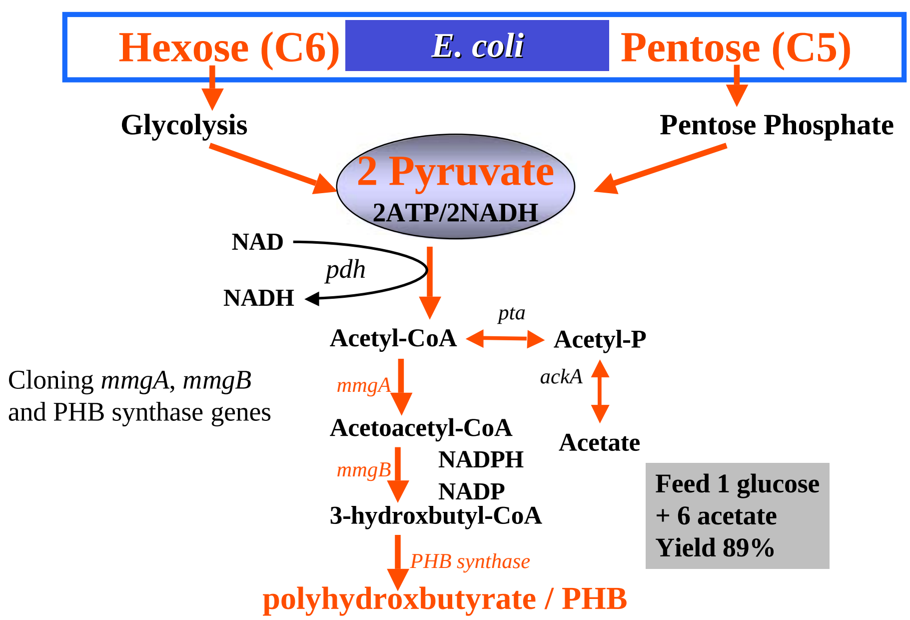

Plastic B. megaterium
Hexose (C6)
B. megaterium
Pentose (C5)
Glycolysis
Pentose Phosphate Pathway
2 Pyruvate
2ATP/2NADH
NAD
pdhABCD
NADH
OAA
Acetyl-CoA
pta
Acetyl-P
ackA
Acetate
mmgA
Acetoacetyl-CoA
mmgB
NADPH
NADP
3-hydroxbutyl-CoA
TCA
4 NADH
2-kedoglutarate dehydrogenase
PHB synthase
polyhydroxbutyrate / PHB
Yield 67%


Plastic B. megaterium
Hexose (C6)
B. megaterium
Pentose (C5)
Glycolysis
Pentose Phosphate
2 Pyruvate
2ATP/2NADH
NAD
pdhABCD
NADH
OAA
Acetyl-CoA
pta
Acetyl-P
ackA
Acetate
mmgA
Acetoacetyl-CoA
mmgB
NADPH
NADP
3-hydroxbutyl-CoA
TCA
4 NADH
2-kedoglutarate dehydrogenase
PHB synthase
polyhydroxbutyrate / PHB
Feed 1 glucose
+ 6 acetate
Yield 89%

Potential Projects

1. Plastic *B. megaterium*
   (PHB biodegradable plastic)
2. **Plastic *E. coli***
3. Vinegar *B. megaterium*
4. Alcoholic *B. megaterium* (ethanol and butanol)

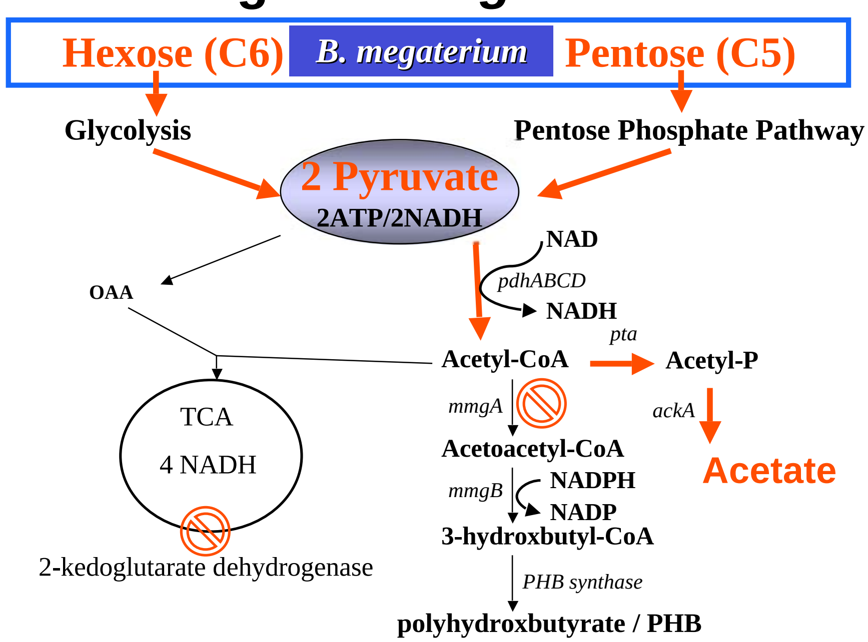
Plastic *E. coli*



Potential Projects

1. Plastic *B. megaterium* (PHB biodegradable plastic)
2. Plastic *E. coli*
3. **Vinegar *B. megaterium* (acetate, deicing)**
4. Alcoholic *B. megaterium* (ethanol and butanol)

Vinegar *B. megaterium*



Potential Projects

1. Plastic *B. megaterium*
   (PHB biodegradable plastic)
2. Plastic *E. coli*
3. Vinegar *B. megaterium*
4. **Alcoholic *B. megaterium* (ethanol and butanol).**

Problem: *B. megaterium* is obligate aerobe

1) Why obligate aerobe (Role of O_2) ?

2) Why not growing fermentatively ?

3) Can we make it grow fermentatively?

Can We Make *B. megaterium* Grow Without Oxygen ?

- **Ability to produce the building block compounds for biosynthesis (yes).**
- **Ability to accept the electron (NADH) by compounds other than oxygen.**
- **Tests: provide extra-cellular electron acceptor such as nitrate (anaerobic respiration).**

Building Blocks for G^+ Cell Growth

Protein amino acids**: 20 amino acids**

RNA nucleotides**: ATP GTP CTP and UTP**

DNA nucleotides**: dATP dGTP dCTP and dTTP**

Lipid components**:Glycerol phosphate, Serine and fatty acids**

Peptidoglycan**: UDP-N-acetylglucosamine, UDP-N-acetylmuramic acid ....**

The Requirements for Biosynthesis of the Building Blocks

1. Energy: ATP

2. Reducing power: NADPH (NADH)

3. Carbon skeleton: Precursor metabolites

Precursor Metabolites

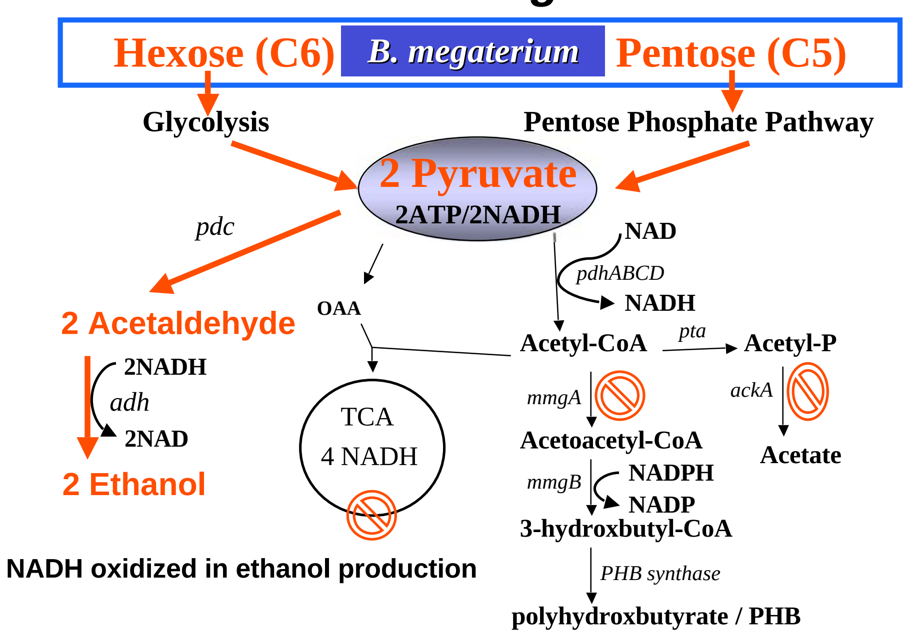
Glucose-6-phosphate	Fructose-6-phosphate
Ribose-5-phosphate	Erythrose-4-phosphate
Triose-phosphate	3-phosphoglycerate
Phosphoenolpyruvate	Pyruvate
Acetyl-CoA	2-ketoglutarate
Succinyl CoA	Oxaloacetate

Provide carbon skeleton for biosynthesis

Can We Make *B. megaterium* Grow Without Oxygen ?

- Ability to produce the building block compounds for biosynthesis (yes).
- **Ability to accept the electron (NADH) by compounds other than oxygen.**
- **Tests: provide extra-cellular electron acceptor such as nitrate (anaerobic respiration)**
- **Make pyruvate/its derivative as the electron acceptor (fermentation).**

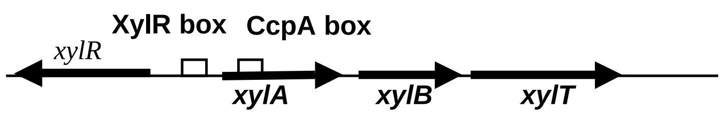
Alcoholic *B. megaterium*



Potential Challenges of Alcoholic *B. megaterium*

- **Anaerobic expression of *pdh* operon (some expression but not too much).**
- **Efficient expression of *pdc/adh* genes (use strong promoter and high copy number plasmid).**
- **The catabolite repression of xylose (deletion of *xylR* and the *ccpA* gene).**

Xylose Uptake Operon in *B. megaterium*



XylR: repressor; CcpA catabolite repressor

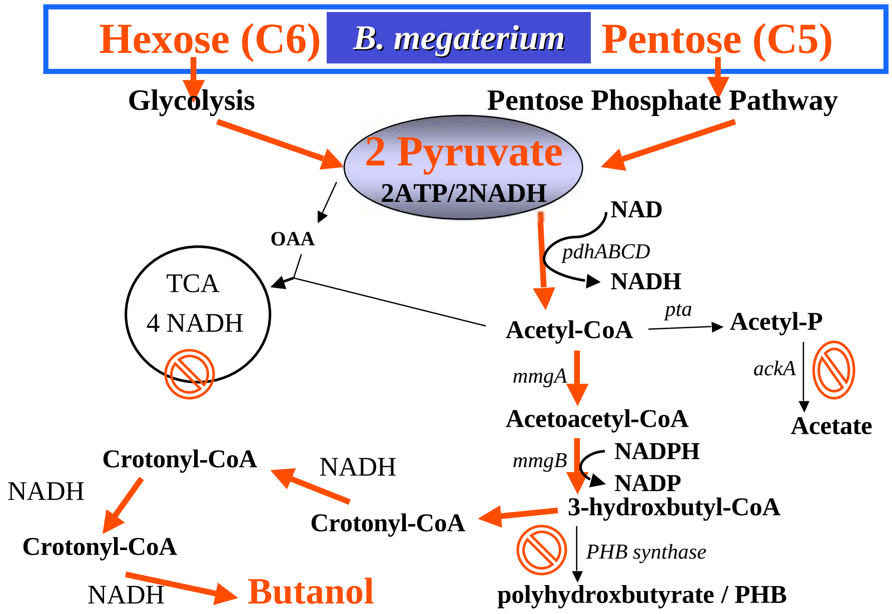
XylA: xylose isomerase

XylB: xylulose kinase

XylT: H^+-dependent symporter

Hillen et al. Molecular Microbiology 1997, 23:1053-1062

Alcoholic *B. megaterium*



Suggestions and Comments

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Thank you