Anaerobic Digestion (AD): not only methane

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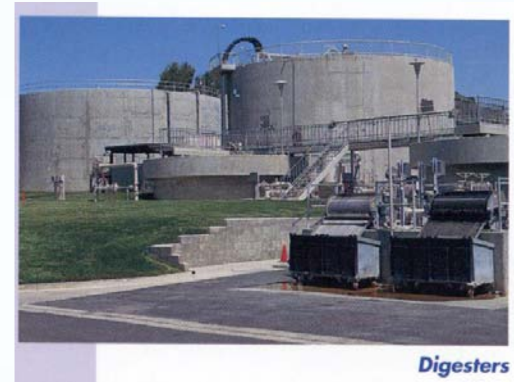
July 31, 2013

"Anaerobic digestion is a collection process by which microorganisms break down biodegradable material in the absence of oxygen. The process is used for industrial or domestic purposes to manage waste and/or to produce fuels" - methane. (Wikipedia)

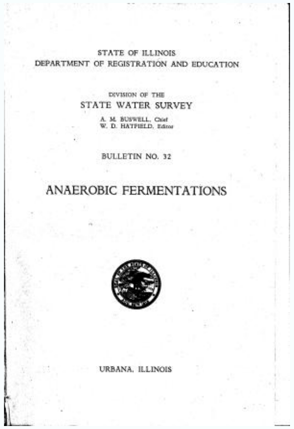


- AD and methane have throughout history been closely associated
- Robert Boyle in the 1600's (combustible gas) followed by Volta in the 1700's associated with sediments of streams and lakes decomposition of organic material
- The first anaerobic digester was built by a leper colony in Bombay, India, in 1859.
- In 1895, the technology was developed in Exeter, England, where a septic tank was used to generate gas for the sewer gas destructor lamp, a type of gas lighting.
- Some research on methanogenesis by Hoppe-Seyler and Omelianski in the late 1800's
- But it wasn't until the 1930's that research on AD began in earnest.

Arthur M. Buswell Chief
1920 - 1955

Want to make special note of four Bulletins by the State Water Survey for the State of Illinois generally referred to as the Buswell reports. (1930-35)

What did they learn –



STATE OF ILLINOIS
DEPARTMENT OF REGISTRATION AND EDUCATION

DIVISION OF THE
STATE WATER SURVEY
A. M. BUSWELL, Chief
W. D. HATFIELD, Editor

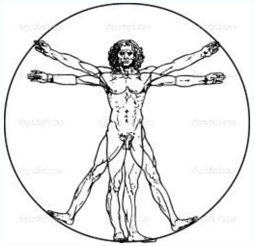
BULLETIN NO. 32

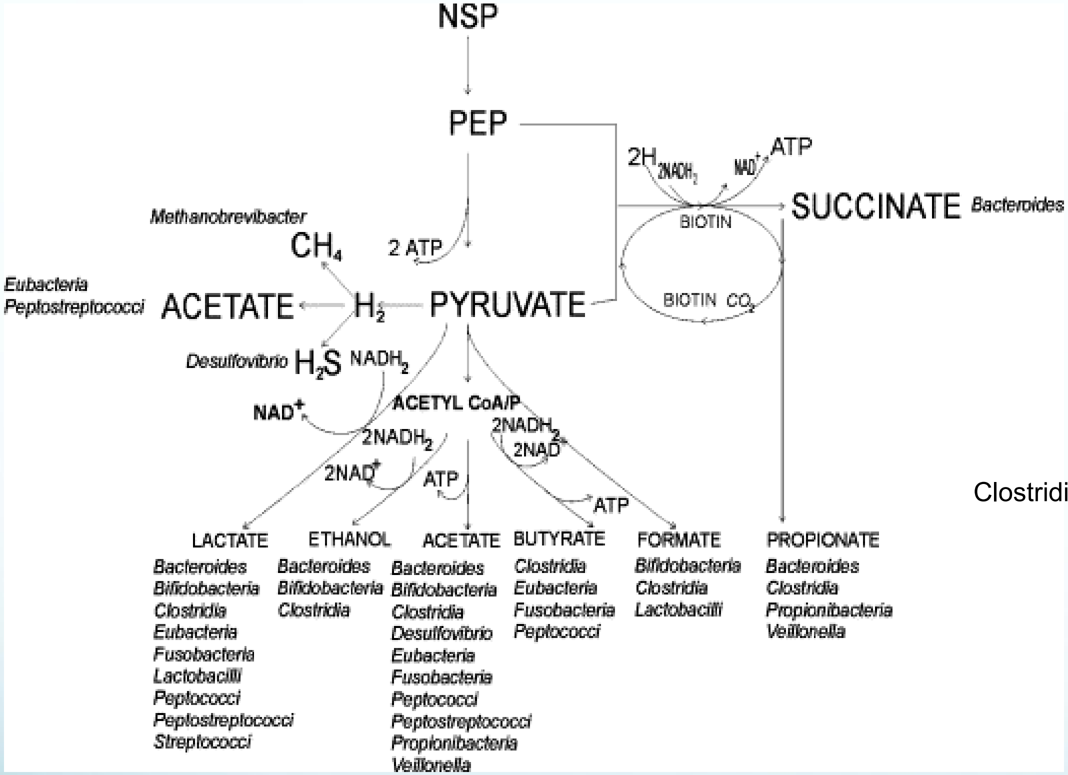
ANAEROBIC FERMENTATIONS

URBANA, ILLINOIS

- Pure and complex compounds such as Acetic Acid, Acetone, Arabinose, Benzoic acid, Butyric acid, Cellobiose, Dextrose, Dulcitol, Dextrin, Formic acid, Ethyl alcohol, Glycerol, Galactose, Inulin, Isobutyric acid, Lactose, Lactic acid, Levulose, Maltose, Mannitol, Oxalic acid, Propionic acid, Raffinose, Rhamnose, Saccharose, Starch, Stearic acid, Sucrose, Triethylene glycerol, Valeric acid, Xylose, Cellulose, Citrus Pulp, Cornstalks (green and dried), Cow/Horse/Hog Manures, Cracked Corn, Distillery Wastes, Milk Waste, Sugar Beet Wastes, and Wheat Straw are all fermentable and many were found as intermediates in the AD liquor.
- Gas yields are depended on – water activity, homogeneity of substrate, loading rate, inoculum, availability of nitrogen, temperature, residence time, substrate C/N/P, oxygen, hydraulic retention time, and mixing.

Types of anaerobic digestion



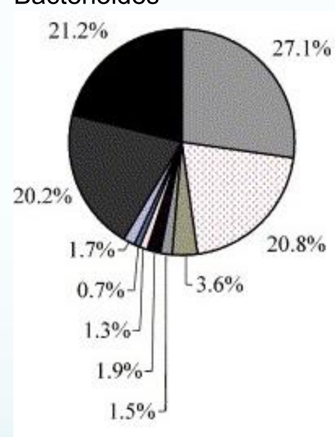


Bacterioides

Bacillus-Lactobacillus
-Streptococcus

Clostridium

Enterics



Types of anaerobic digestion intermediates

Cecum

metabolites
isoleucine
leucine
valine
ethanol
lactate
alanine
arginine
lysine
acetate
threonine
proline
glutamate
methionine
glutamine
aspartic acid
asparagine
creatine
ethanolamine
choline
phosphorylcholine
GPC
taurine
glycine
glutathione
lipids (triglycerides and fatty acids)
trimethylamine-*N*-oxide (TMAO)
tyrosine
phenylalanine
oxaloacetate
myo-inositol
scyllo-inositol
β-glucose
α-glucose
formate
uracil
fumarate
cytosine
inosine
propionate
unknown
N-acetyl-glutamate
serine
cysteine
phosphoryl-ethanolamine
GPE
acetoacetate
betaine
glycerol
tryptophan
cystamine

Francois-Pierre Martin et. al. 2007

Rumen

Metabolite[1]
AA metabolism
3-Hydroxyphenylacetate
3-Phenylpropionate
Alanine
Aspartate
Benzoate
Cadaverine
Glutamate
Glycine
Histidine
Isobutyrate (m*M*)
Isoleucine
Isovalerate (m*M*)
Leucine
Lysine
Phenylacetate
Phenylacetylglycine
Proline
Tyrosine
Valine
Butanoate metabolism
3-Hydroxybutyrate
4-Hydroxybutyrate
Acetoacetate
Butyrate (m*M*)
Fumarate
Glycolysis/gluconeogenesis
1,3-Dihydroxyacetone
Acetate (m*M*)
Ethanol
Glucose (m*M*)
Glycerol
Lactate
Maltose
Phospholipid metabolism
Choline
Ethanolamine
Methane metabolism
Dimethylamine
Methylamine
N-Nitrosodimethylamine
Formate
Nucleotide metabolism
Hypoxanthine
Ribose
Uracil
Xanthine
TCA cycle
Fumarate
Nicotinate
Propionate (m*M*)
Succinate
Phenylpropanoid synthesis
Ferulate

F. Saleem et al. 2012

AD

Fatty Acids
- **Isovaleric acid**
- **Butyrate**
- **Propionate**
- **Acetate**
- **Formate**
- **Isobutyrate**

?

?

Alcohols
- **Ethanol**
- **Butanol**

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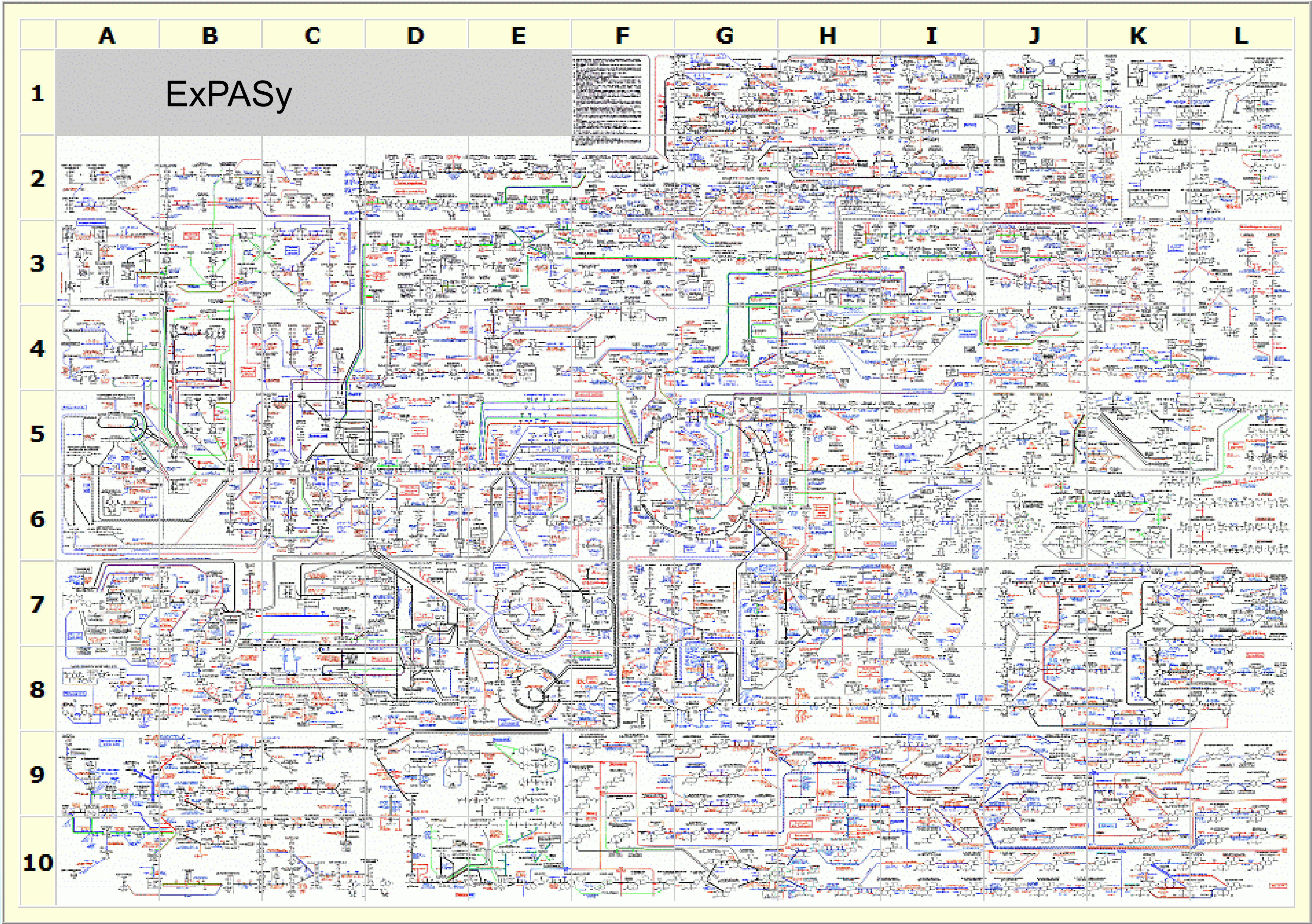
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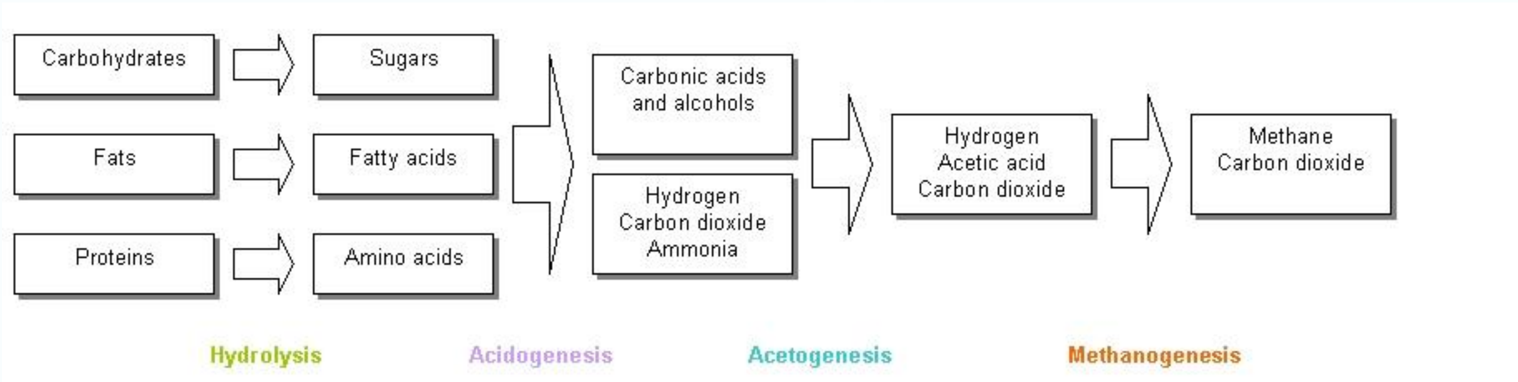
ExPASy

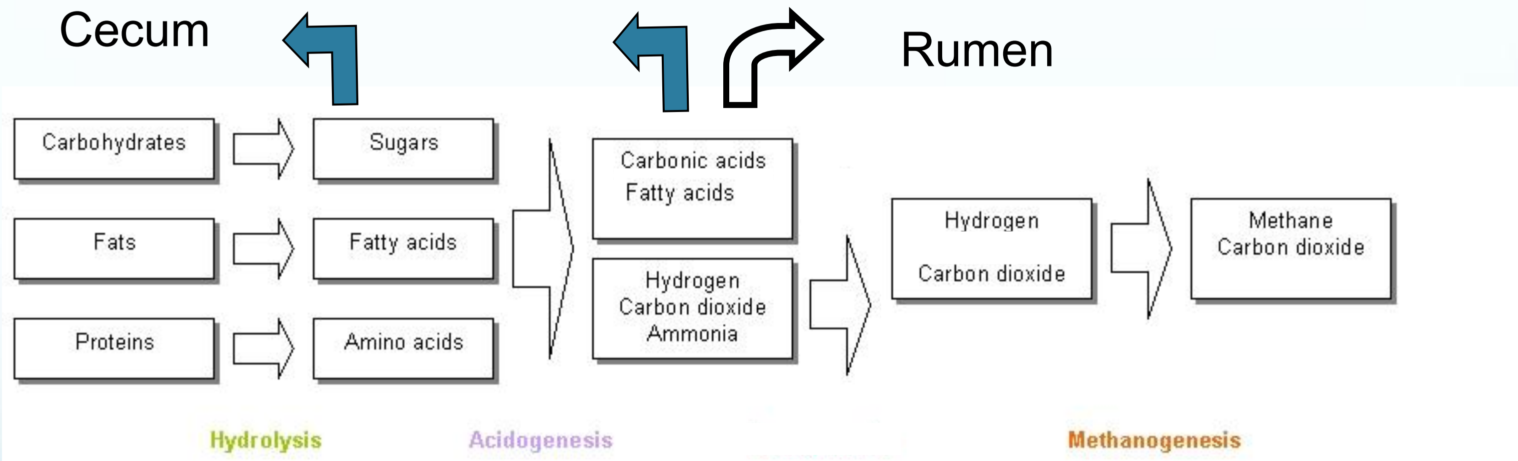
Types of anaerobic digestion carbon flow

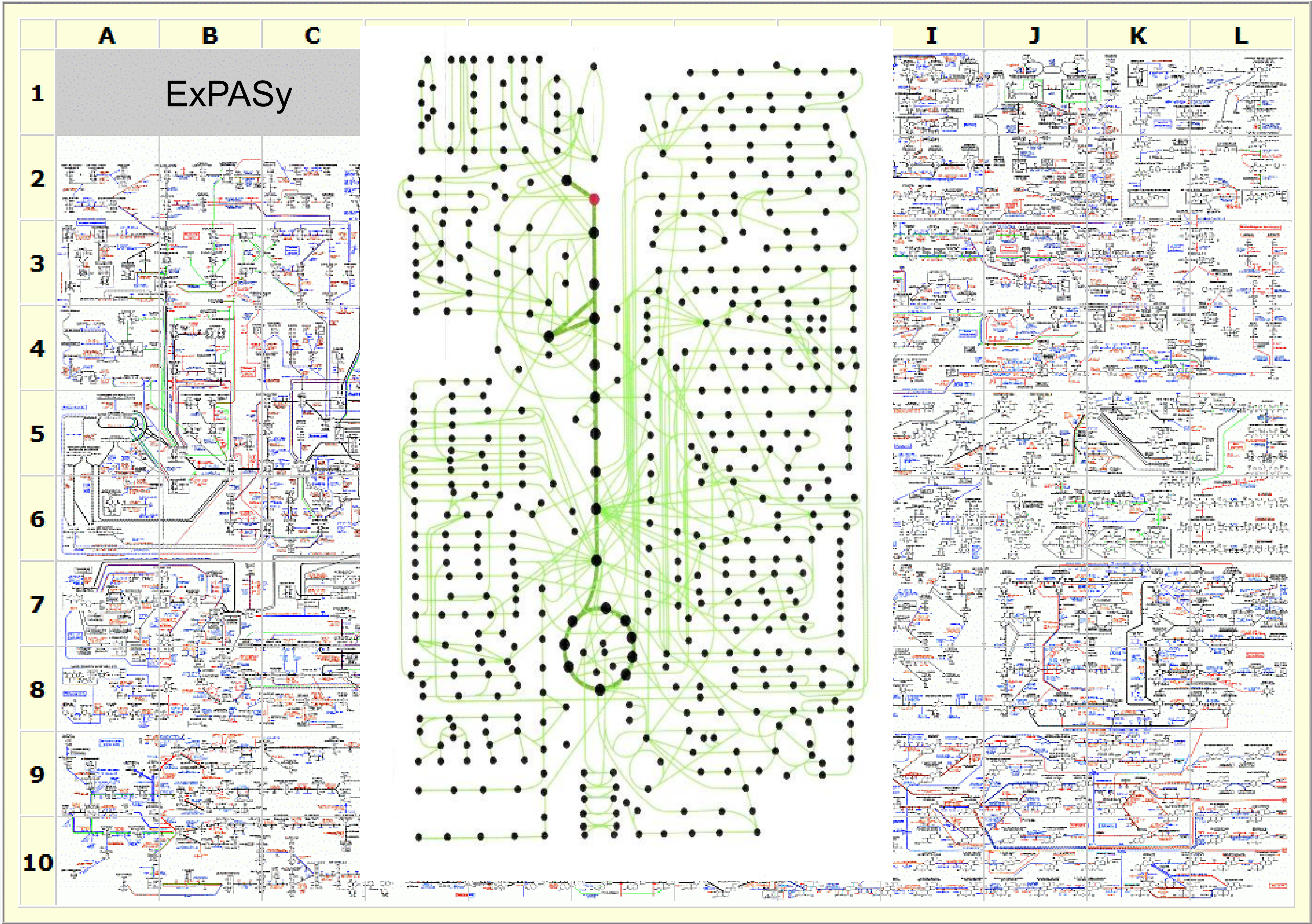
AD



Cecum

Rumen

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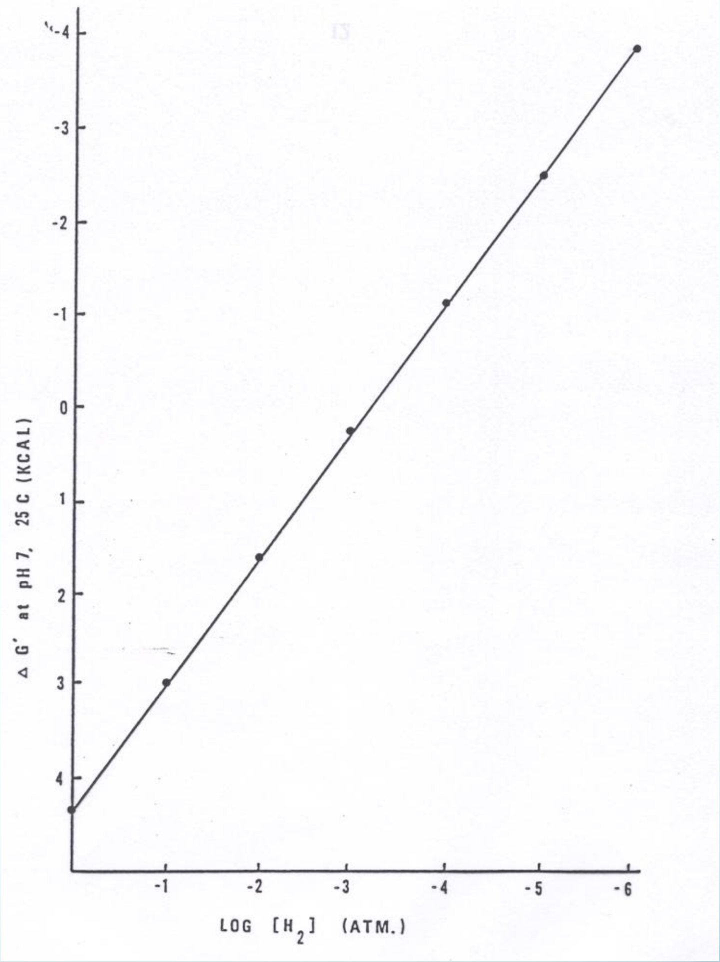
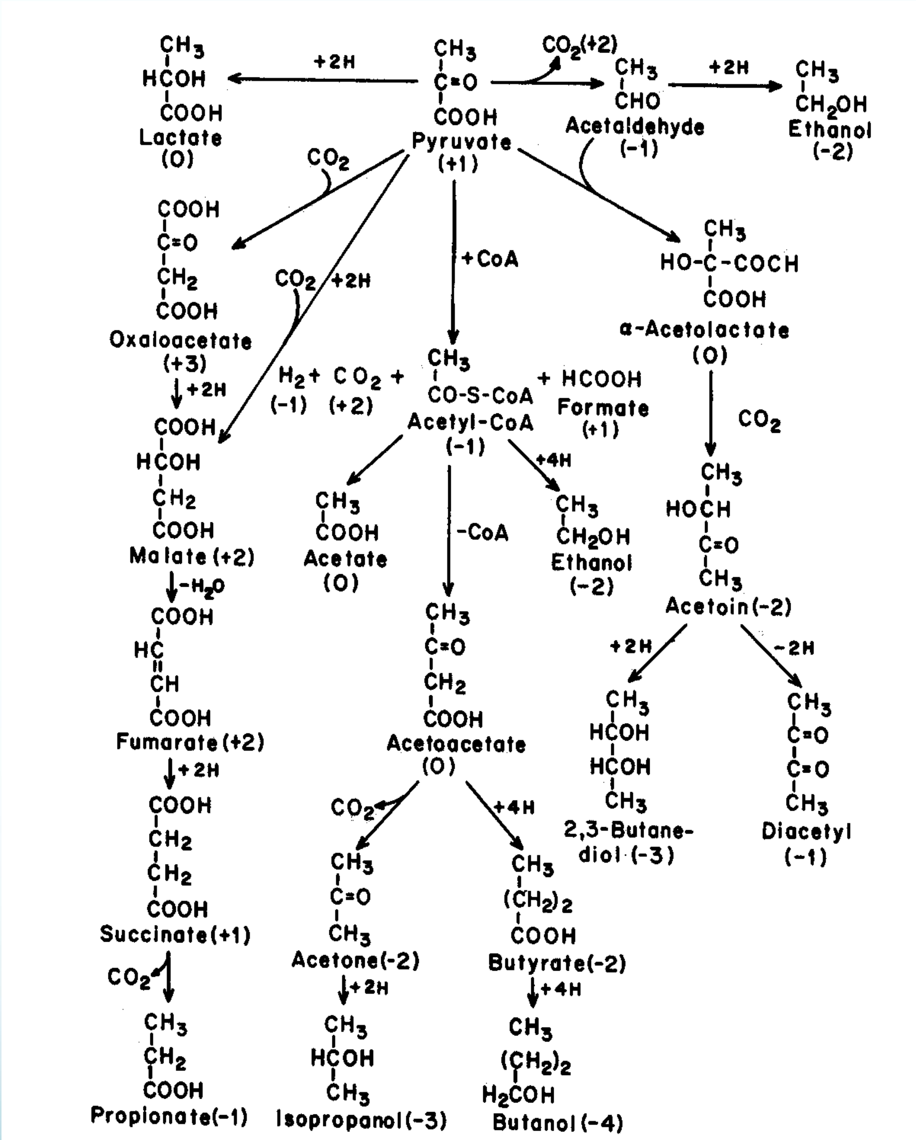
ExPASy

Interspecies Hydrogen Transfer

"Interspecies hydrogen transfer" is described as the transfer of hydrogen between non-methanogenic and methane producing bacteria. An important characteristic of this is that the hydrogen concentration in the environment is extremely low. As the partial pressure of hydrogen increases the fermentative process becomes increasing thermodynamically unfavorable. Should be expanded to hydrogen (in liquid) and electron transport (on particles) between organisms.



$$NADH + H^{+} \text{ ---> } NAD^{+} + H_2$$

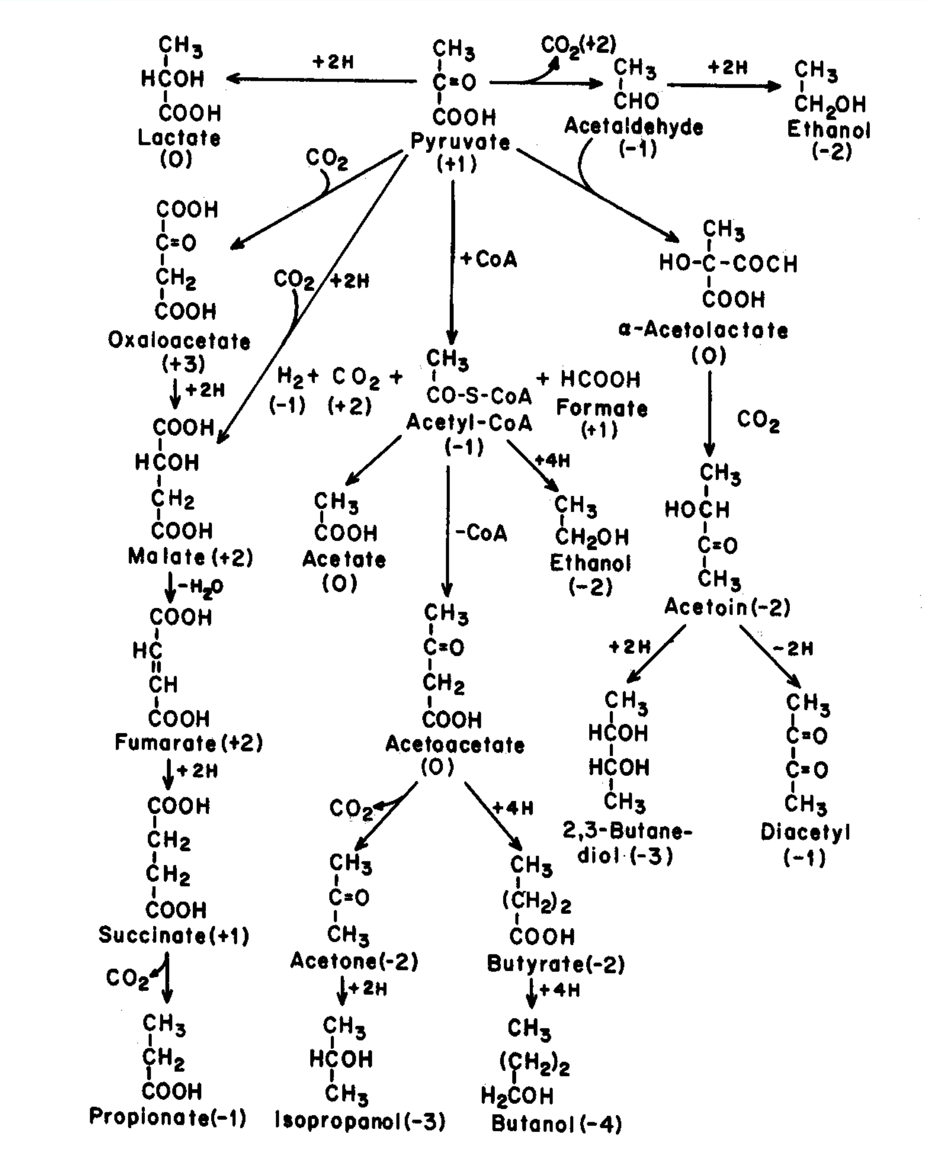


If you could eliminate hydrogen transfer the flow of hydrogen into alcohols from acids could/would occur?

Any evidence for this?

Regulation of Product Formation in Bacteroides by Interspecies Electron Transfer.

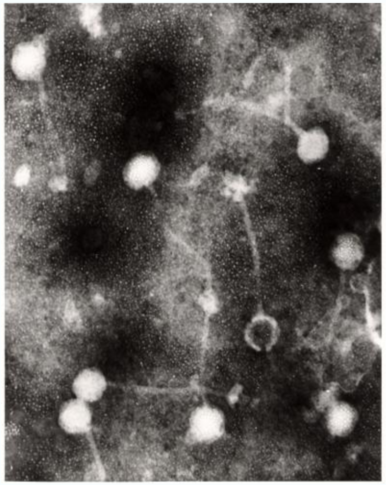
In a mixed culture of *Bacteroides xylanolyticus* and *Methanospirillum hungatei*, acetate, CO_2, and CH_4 were produced. In the pure culture *Bacteroides xylanolyticus* produced, ethanol, acetate, CO_2, and hydrogen. Biesterveld S, Zehnder AJ, Stams AJ



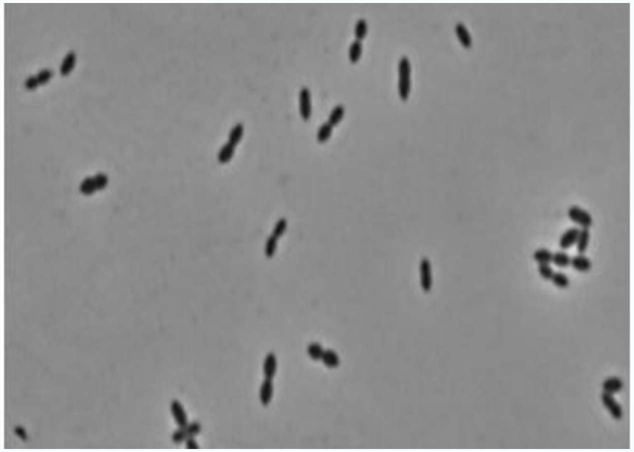
If you could eliminate hydrogen transfer the flow of hydrogen into alcohols from acids could/would occur?

So how would you do this?

Eliminate methanogens but – How?



Phage therapy ☺

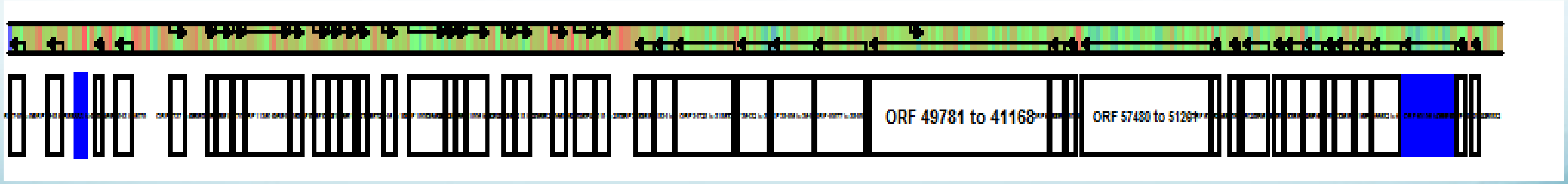


Phage G

Methanobrevibacter strain G

Methanobrevibacter strain G - Isolated from bovine rumen Serves as the only host of phage PG colonizes the human intestinal tract and digesters.

Phage G – lytic phage – burst size ~40-100 virons/cell - ds DNA. Genome was recently sequenced (by J. Craig Venter Institute). 28 genes have been annotated (homologies to known genes) 40 genes are of unknown function If the endolysin is found then controlled cell lysis or infection with a endolysin mutant would render cells metabolically inactive.



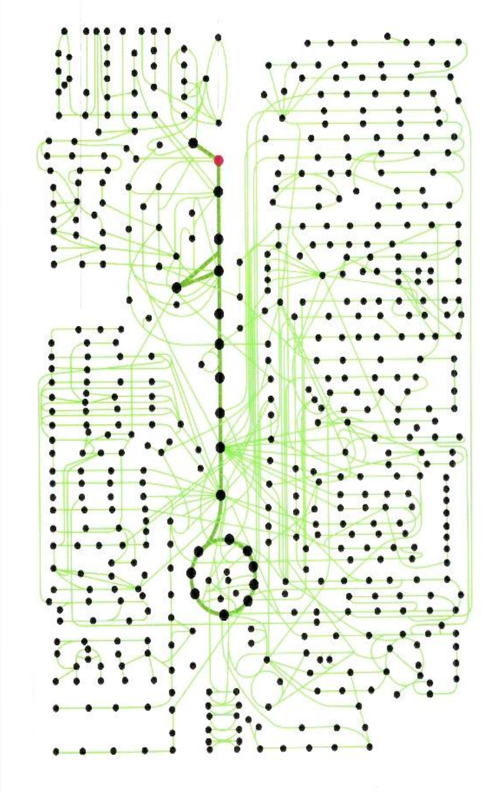
Is there another way to change the carbon flow in an AD? - Butanol

Known –

Microbial intervention or probiotics ("live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance“). There is evidence that the addition of lactic acid bacteria, bifidobacteria, yeasts, and possibly bacilli as active cultures in fermented foods changes the microbial flora and the flow of carbon in the cecum.

Speculative –

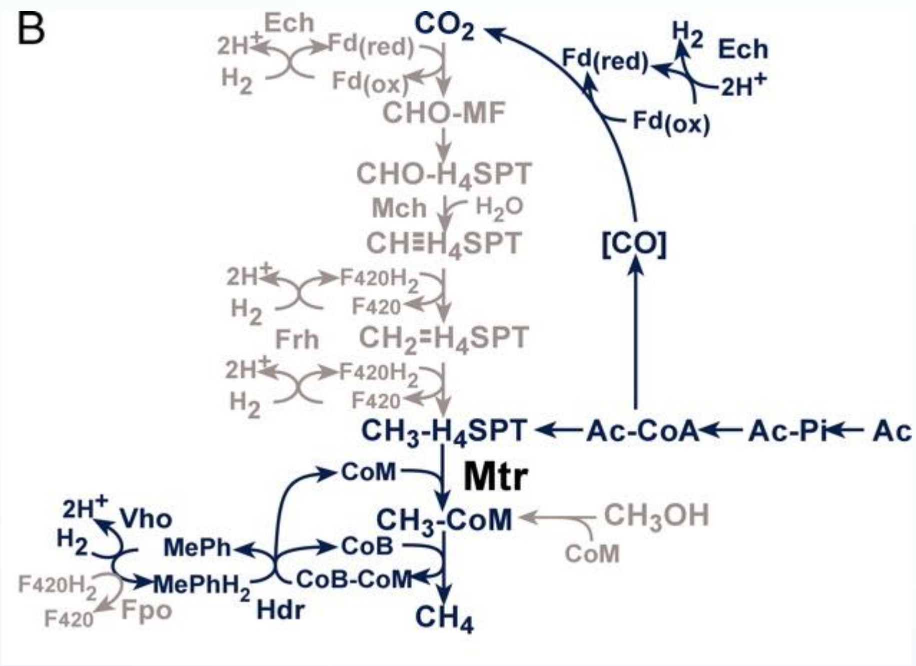
Microbial intervention in the AD by either enriching for or supplementing the AD with clostridium. This could be done for any desired end product.



Microbial Augmentation

What if we want more methane?

Methanogen Metabolic Engineering

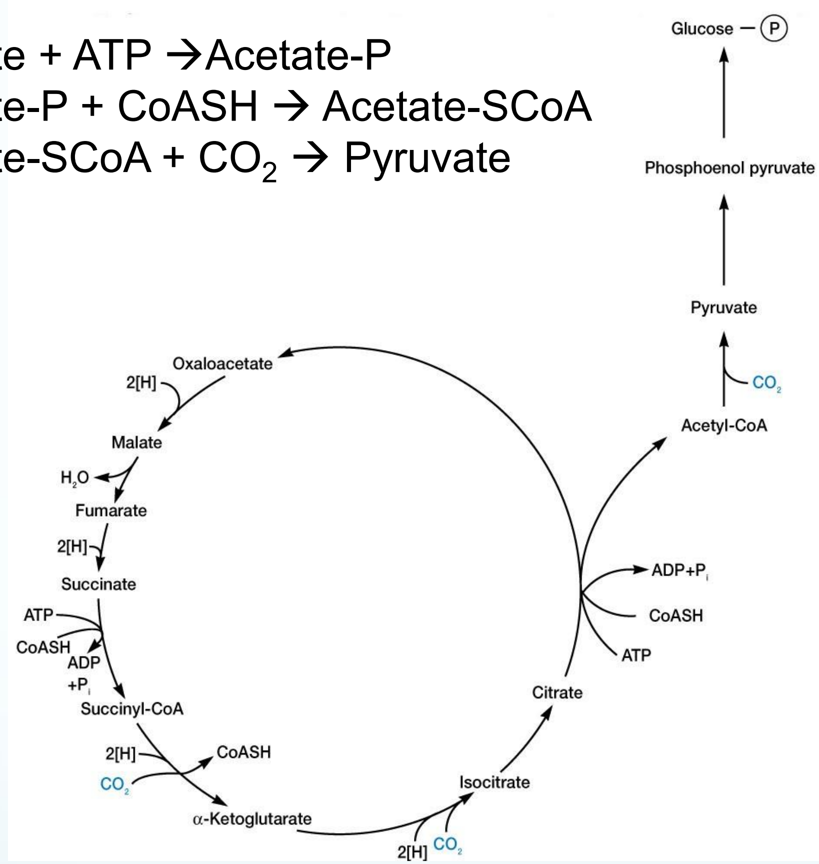


Paula V. Welander and William W. Metcalf. 2005

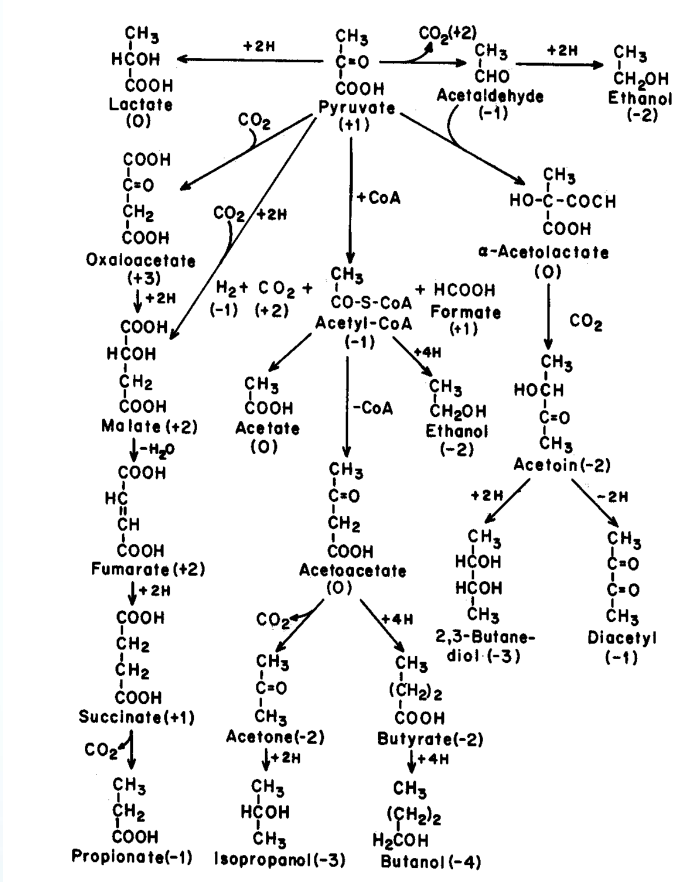
Micro array work on methanogens indicate that methanogenesis is highly regulated. So to speed up these reactions one can - add more cells or engineer the organism to be constitutive. Couple of ways to make constitutive – disable controls or genes w/o accompanying controls. Working on moving [CO] gene and or complex from *M. acetivorans* into *Methanobrevibacter* strain G a non-acetate utilizing methanogen.

What if we want the methanogen to produce ???

Acetate + ATP → Acetate-P
Acetate-P + CoASH → Acetate-SCoA
Acetate-SCoA + CO_2 → Pyruvate



Reductive TCA cycle



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Isoleucine
Isovalerate (m*M*)
Leucine
Lysine
Phenylacetate
Phenylacetylglycine
Proline
Tyrosine
Valine
Butanoate metabolism
3-Hydroxybutyrate
4-Hydroxybutyrate
Acetoacetate
Butyrate (m*M*)
Fumarate
[illegible]
Glycolysis/gluconeogenesis
1,3-Dihydroxyacetone
Acetate (m*M*)
Ethanol
Glucose (m*M*)
Glycerol
Lactate
Maltose
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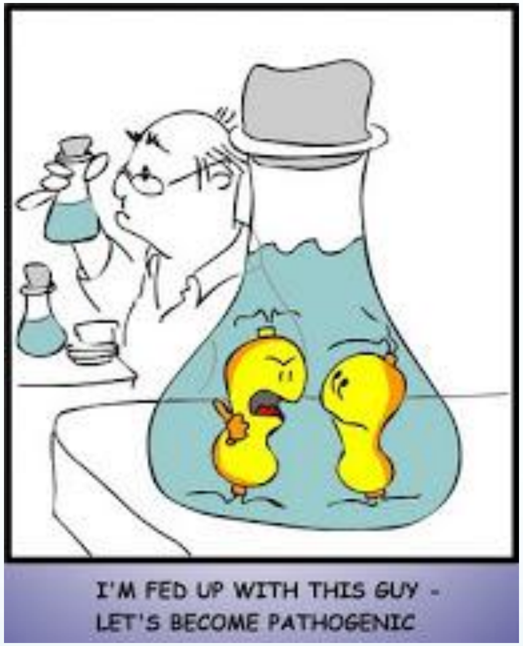
Ribose
Uracil
Xanthine
TCA cycle
Fumarate
Nicotinate
Propionate (m*M*)
Succinate
Phenylpropanoid synthesis
Ferulate

Using anaerobic fermentations (digestion) we already produce

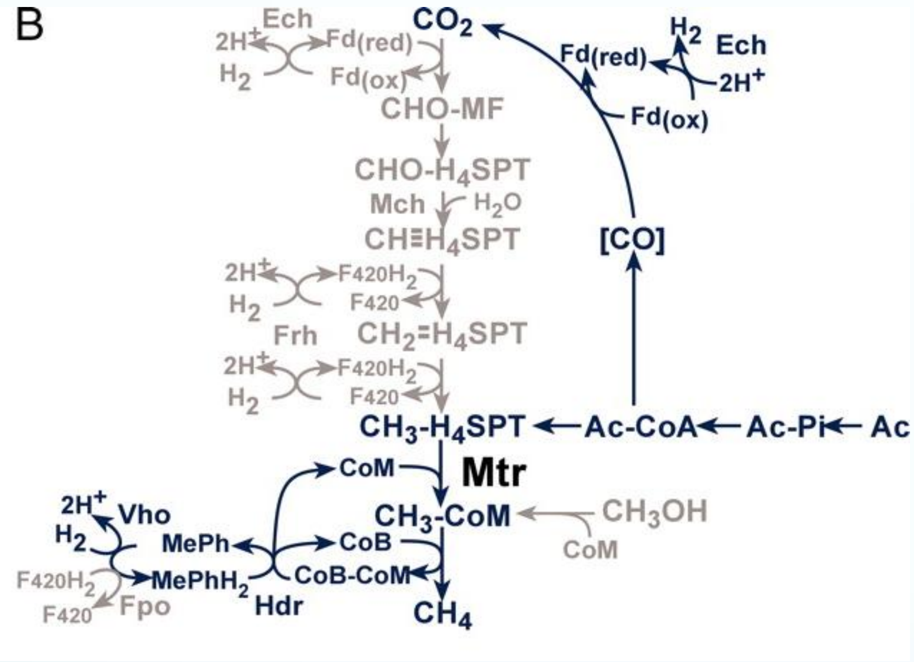
- Amino acids and sugars from proteins
- Sugars from carbohydrates
- Higher fatty acids and alcohols from lipids

So why not from an Anaerobic Digester?

The end or just the beginning!

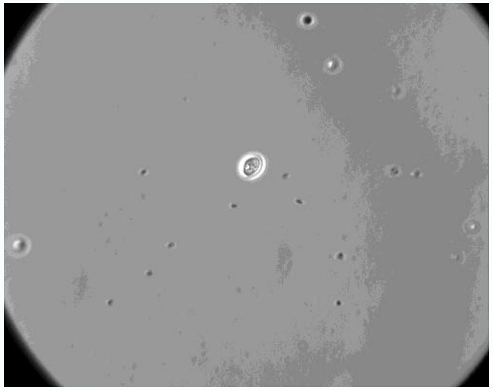


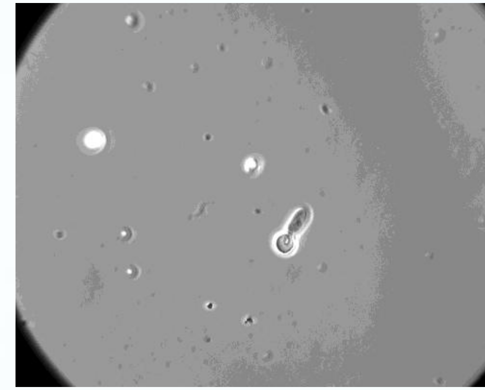
Methanogen Metabolic Engineering



Paula V. Welander and William W. Metcalf. 2005

Take then methanol complex from *M. acetivorans* and insert it into *Methanobrevibacter* strain G a non-methanol utilizing methanogen.





Or Use methanogens to Detoxify (acetate)

	No Hydrolysate	5% Hydrolysate	CH_4	Ethanol
M. acetivorans	100% (0.04 hr^{-1})	45% inhibition	+	_
S. cerevisiae	100% (0.4 hr^{-1})	10% inhibition	_	+
M. acetivorans + *S. cerevisiae*		45% inhibition Not measured	+	+

M. acetivorans - minimal with 0.5% acetate and or 0.5% methanol. Growth measured by production of methane.

S. cerevisiae - glucose, yeast extract media. Growth measured by OD and microscopic observation.