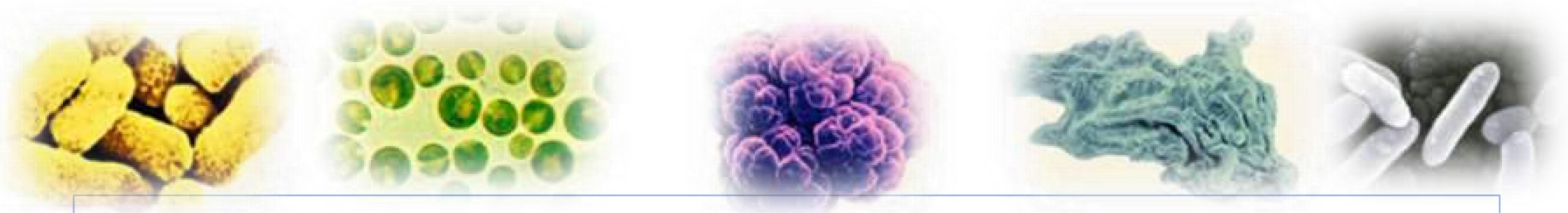
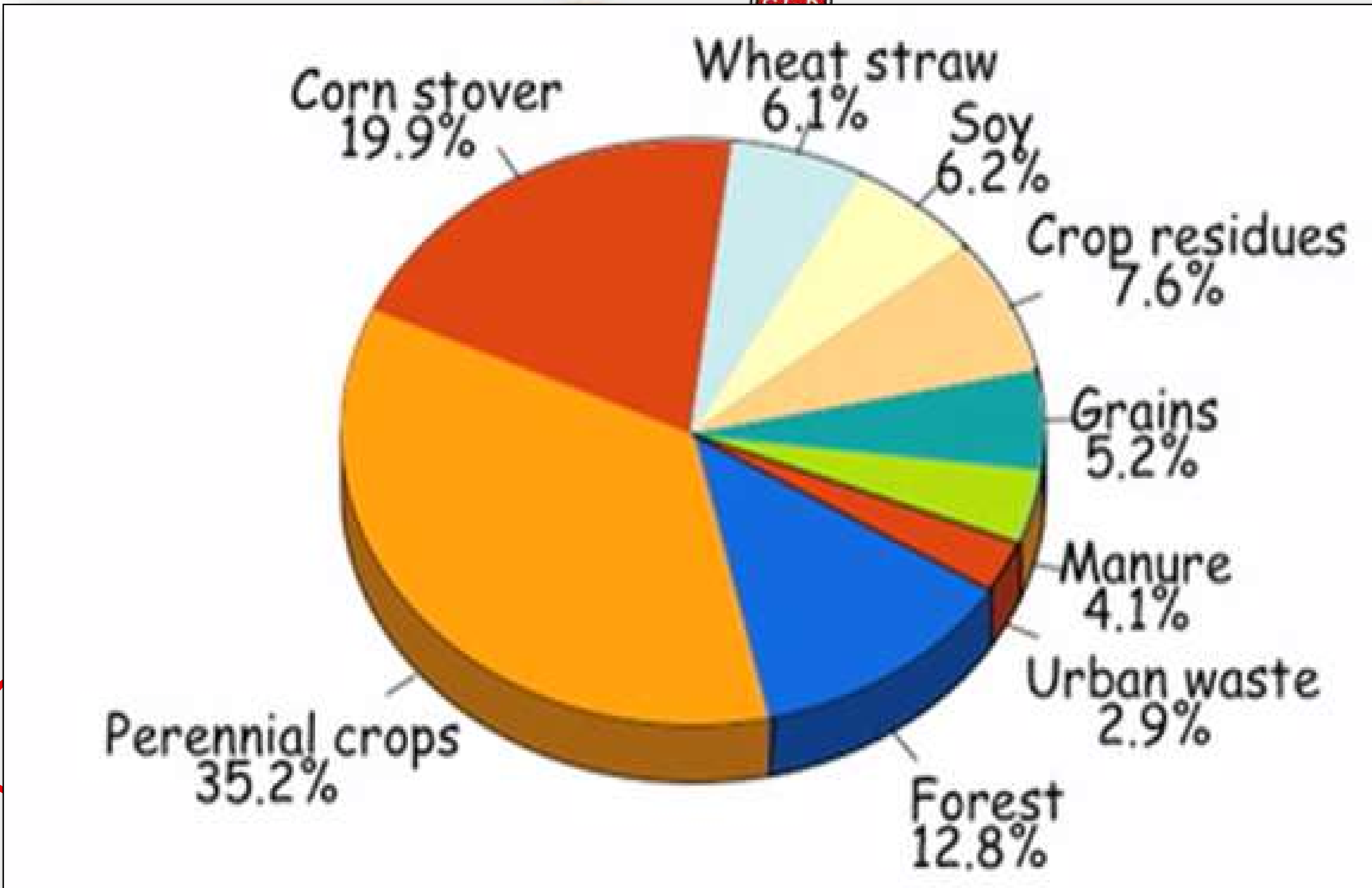
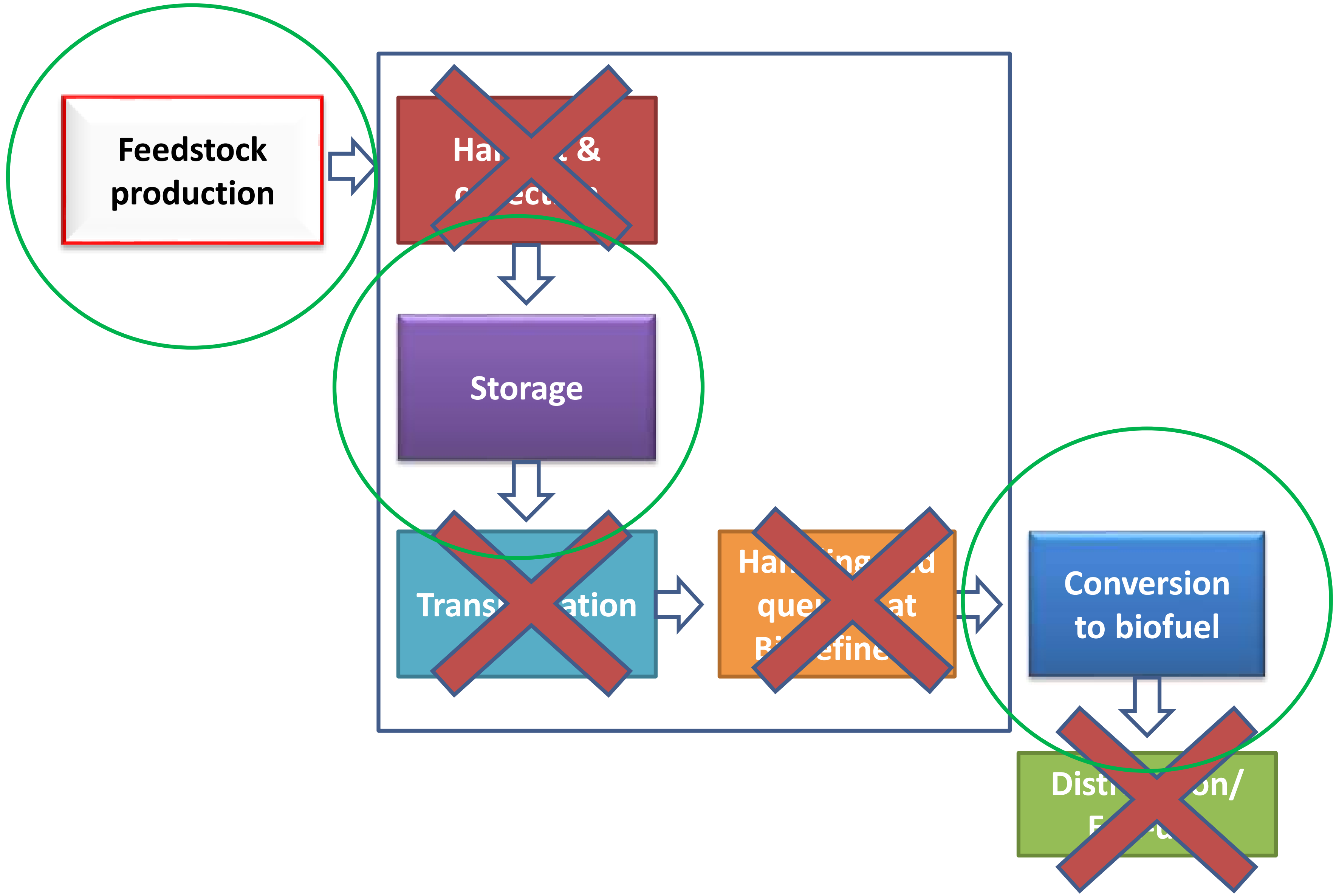


Microorganisms in Biofuel Production.

Bioenergy resources



Biomass-BIOFUEL system



Role of microbes

- Bioprocessors
- Source of biocatalyst (enzymes)
  - Preprocessing of feedstock for fuel production
  - Cell wall modification
  - Feedstock preservation
  - Pretreatment
  - Hydrolysis
  - Fuel production (An array of conversion options)

- **Other [energy saving & environmental roles**
- **Carbon recycling**
- **Micro-carbon sinks**
- **Carbon extension???**
- **Waste treatment/Bioremediation**

Biofuel energy options

- [Trans]esterification
- Combustion
- Combine Heat and Power (CHP)
- Pyrolysis
- Gasification
- Hydroprocessing/treatment
- Fermentation
- Anaerobic digestion
- Microbial Fuel cells
  - Electrosynthesis (liquid fuels)
  - Electromethanogenesis/Electrohydrogenesis (gaseous fuels)
  - Electricity

Chemical route

Thermal route

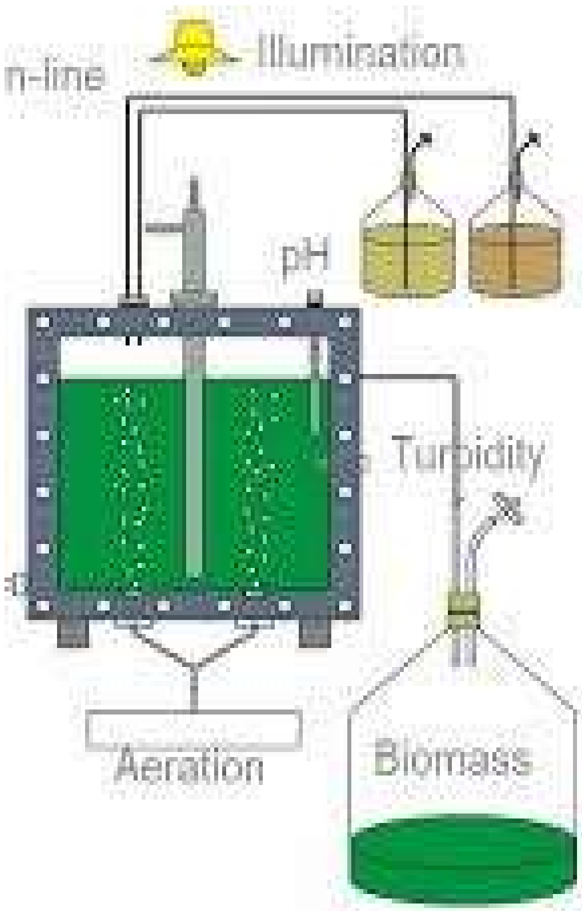
Thermochemical route

Biochemical route

Require biocatalyst (enzymes or microbes)

Bio-electrochemical route

Microbial Communities



Conversion

Microbial Communities

Conversion to biofuel

What are will looking for ?

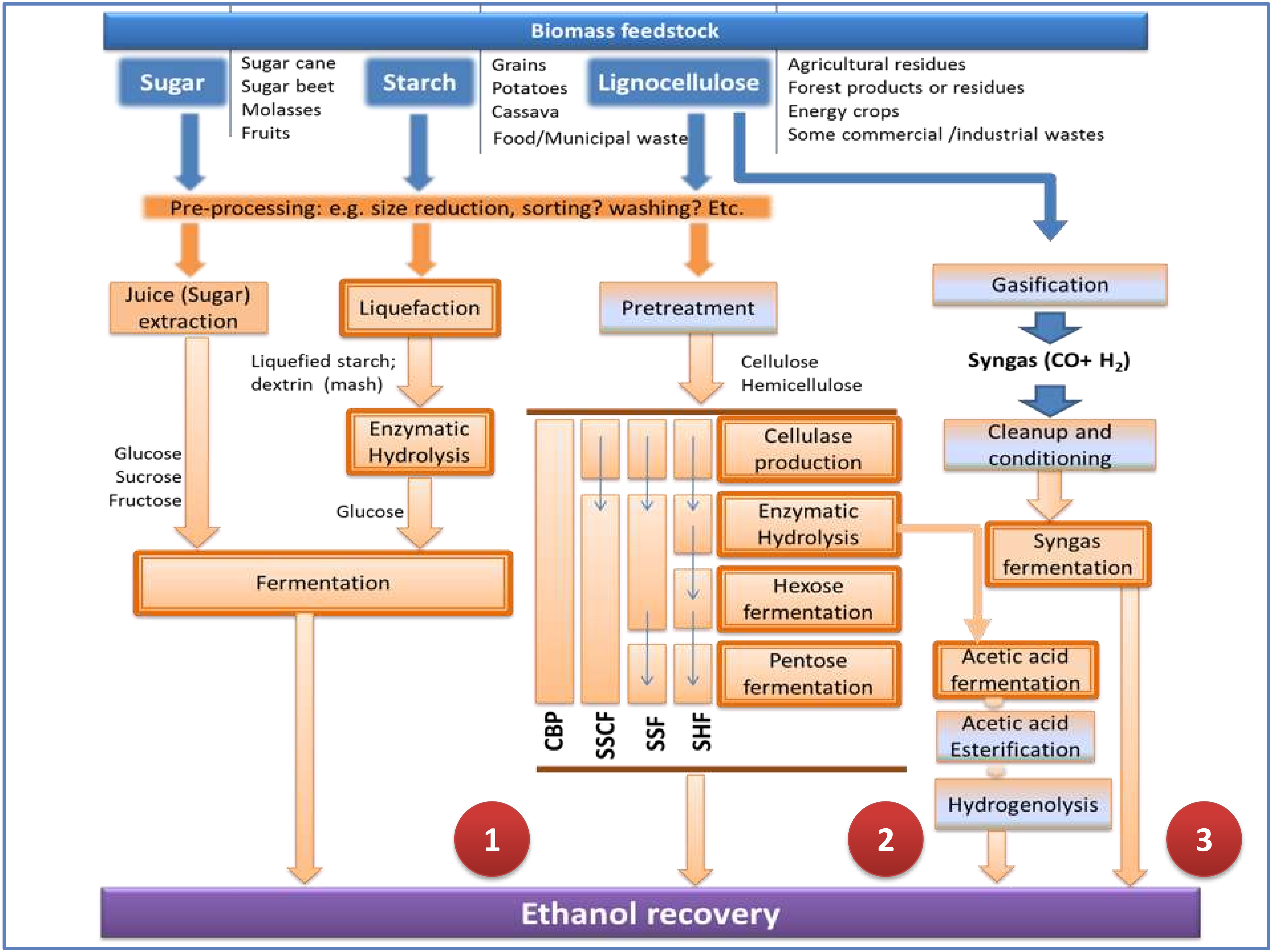
- Growth in high substrate concentration
- Tolerance to stress
- Substrate flexibility
- Product specificity
- High productivity
- High yield
- Fast doubling time???

Type of Biofuel

- **Media composition**
- **Operational conditions**
- **Biological parameters**

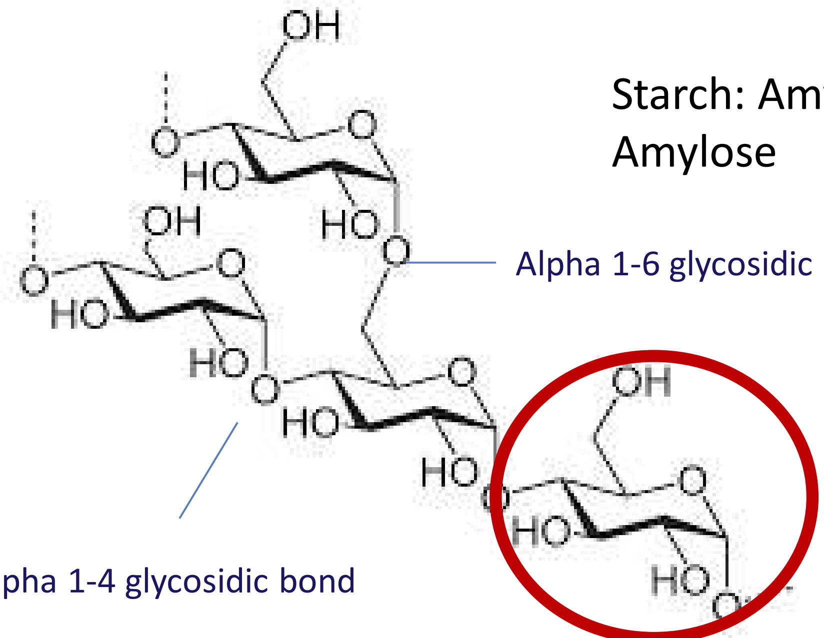
Reactor design

Ethanol production


Biomass feedstock
Sugar
Sugar cane
Sugar beet
Molasses
Fruits
Starch
Grains
Potatoes
Cassava
Food/Municipal waste
Lignocellulose
Agricultural residues
Forest products or residues
Energy crops
Some commercial /industrial wastes
Pre-processing: e.g. size reduction, sorting? washing? Etc.
Juice (Sugar) extraction
Liquefaction
Pretreatment
Gasification
Syngas (CO+ H2)
Liquefied starch; dextrin (mash)
Cellulose
Hemicellulose
Enzymatic Hydrolysis
Cleanup and conditioning
Glucose
Sucrose
Fructose
Glucose
Cellulase production
Enzymatic Hydrolysis
Hexose fermentation
Pentose fermentation
Syngas fermentation
Fermentation
Acetic acid fermentation
Acetic acid Esterification
Hydrogenolysis
CBP
SSCF
SSF
SHF
1
2
3
Ethanol recovery

Hydrolysis: carbohydrate

Starch: Amylopectin
Amylose

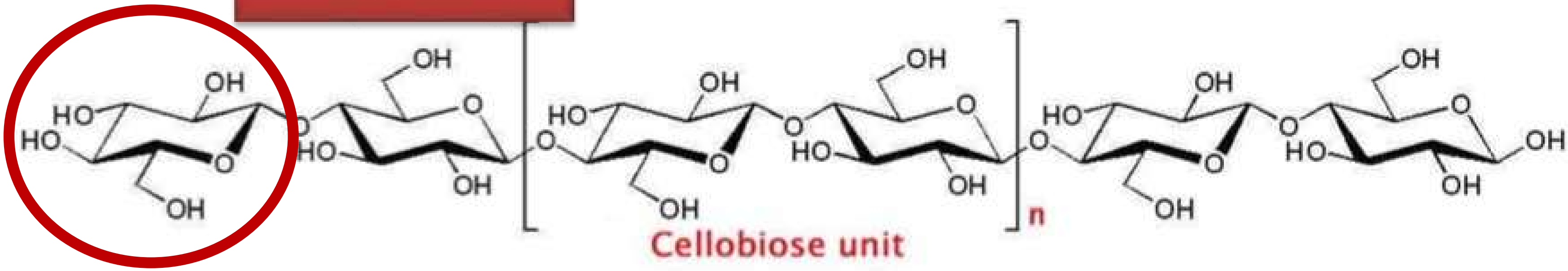


Alpha 1-6 glycosidic bond

Alpha 1-4 glycosidic bond

Glucose Units

Cellulose



Microbial Communities

Conversion to Ethanol

Saccharolytic microbes

Fermentative microbes

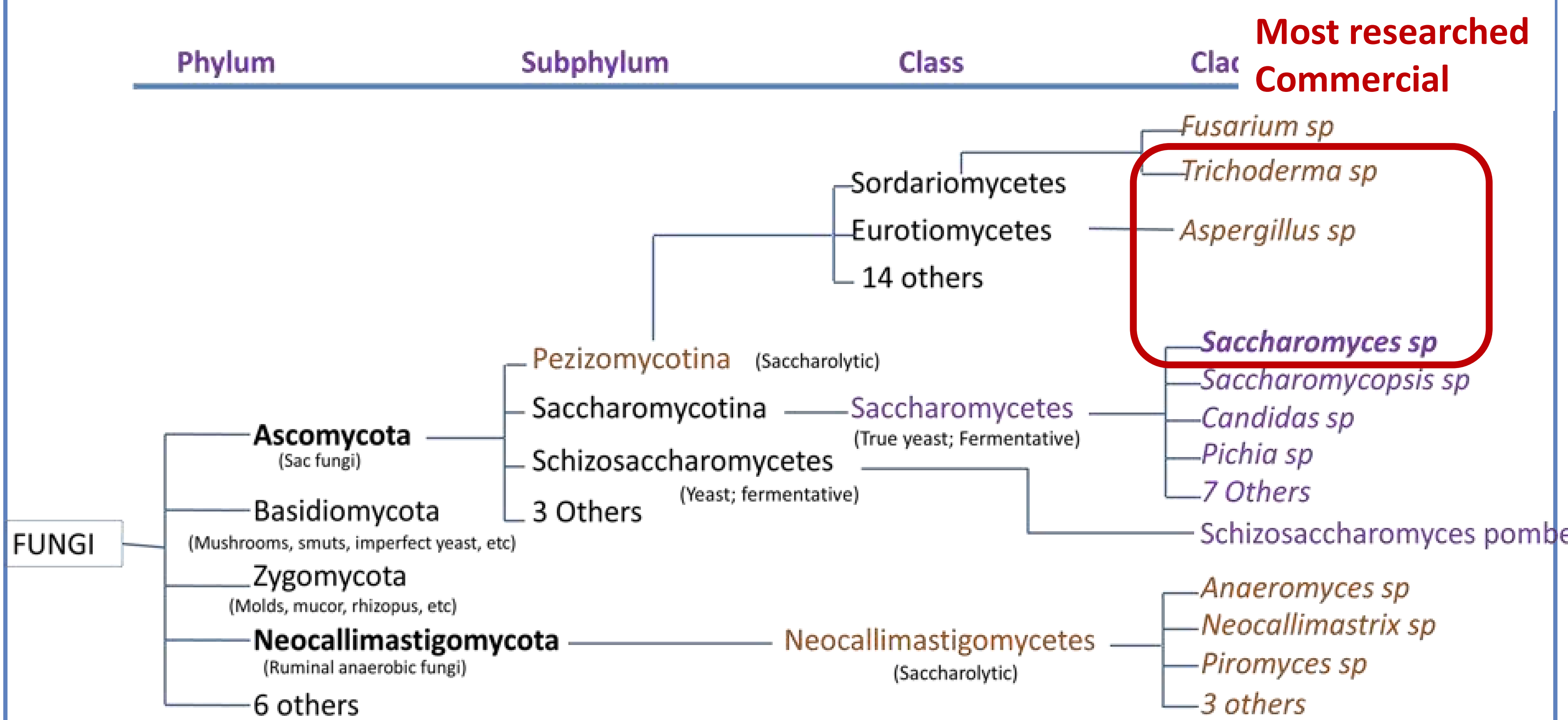
Hydrolytic enzymes

Single species

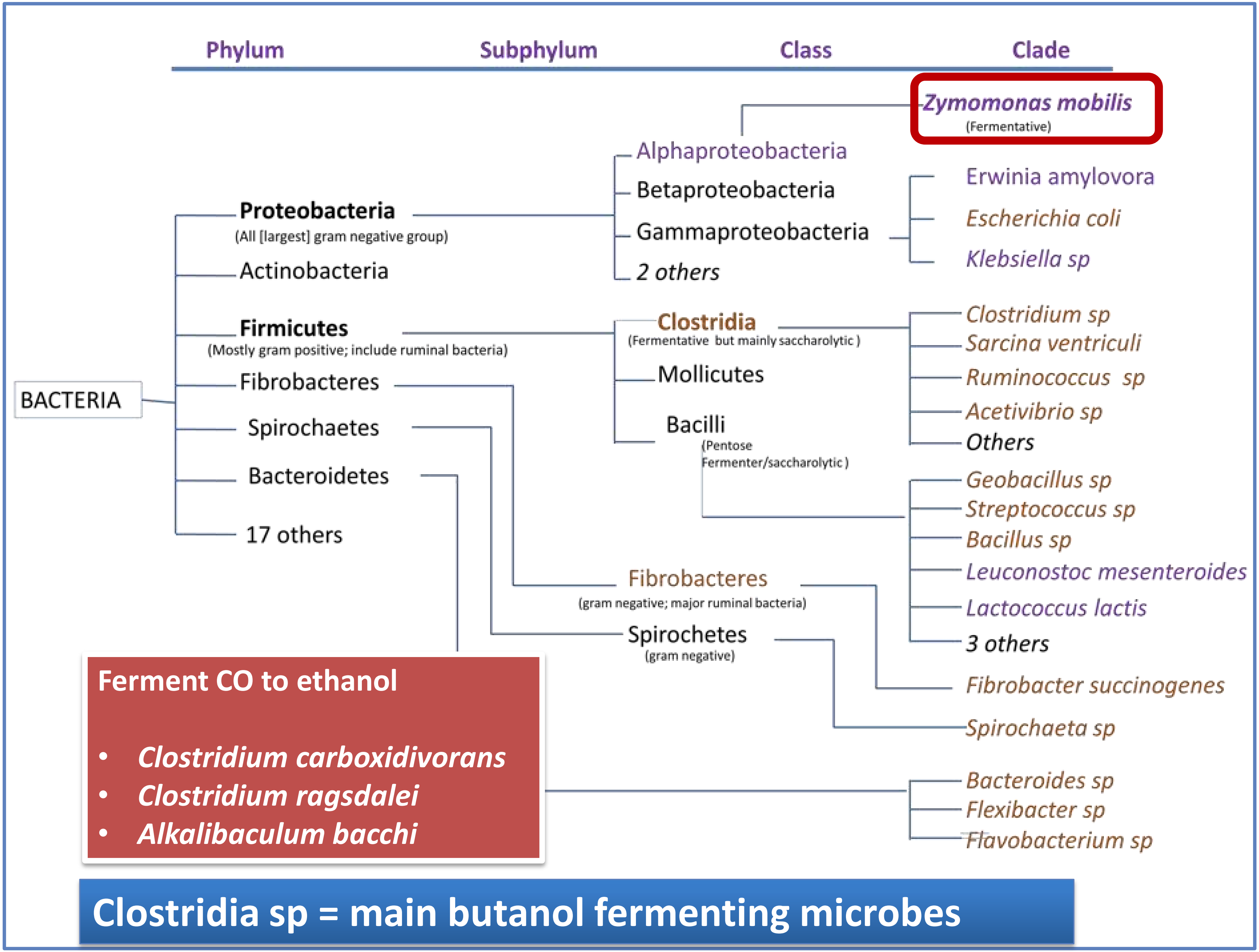
Fungi

Complex of multiple enzymes (**Cellulosome**)

Bacteria



Most researched
Commercial
Phylum
Subphylum
Class
Clac
FUNGI
Ascomycota
(Sac fungi)
Basidiomycota
(Mushrooms, smuts, imperfect yeast, etc)
Zygomycota
(Molds, mucor, rhizopus, etc)
Neocallimastigomycota
(Ruminal anaerobic fungi)
6 others
Pezizomycotina
(Saccharolytic)
Saccharomycotina
Schizosaccharomycetes
(Yeast; fermentative)
3 Others
Sordariomycetes
Eurotiomycetes
14 others
Saccharomycetes
(True yeast; Fermentative)
Neocallimastigomycetes
(Saccharolytic)
Fusarium sp
Trichoderma sp
Aspergillus sp
Saccharomyces sp
Saccharomycopsis sp
Candidas sp
Pichia sp
7 Others
Schizosaccharomyces pombe
Anaeromyces sp
Neocallimastrix sp
Piromyces sp
3 others


Phylum
Subphylum
Class
Clade
Zymomonas mobilis
(Fermentative)
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
2 others
Erwinia amylovora
Escherichia coli
Klebsiella sp
Proteobacteria
(All [largest] gram negative group)
Actinobacteria
Firmicutes
(Mostly gram positive; include ruminal bacteria)
Fibrobacteres
Spirochaetes
Bacteroidetes
17 others
BACTERIA
Clostridia
(Fermentative but mainly saccharolytic)
Mollicutes
Bacilli
(Pentose Fermenter/saccharolytic)
Clostridium sp
Sarcina ventriculi
Ruminococcus sp
Acetivibrio sp
Others
Geobacillus sp
Streptococcus sp
Bacillus sp
Leuconostoc mesenteroides
Lactococcus lactis
3 others
Fibrobacteres
(gram negative; major ruminal bacteria)
Spirochetes
(gram negative)
Fibrobacter succinogenes
Spirochaeta sp
Bacteroides sp
Flexibacter sp
Flavobacterium sp
Ferment CO to ethanol
• Clostridium carboxidivorans
• Clostridium ragsdalei
• Alkalibaculum bacchi
Clostridia sp = main butanol fermenting microbes

Ethanol production

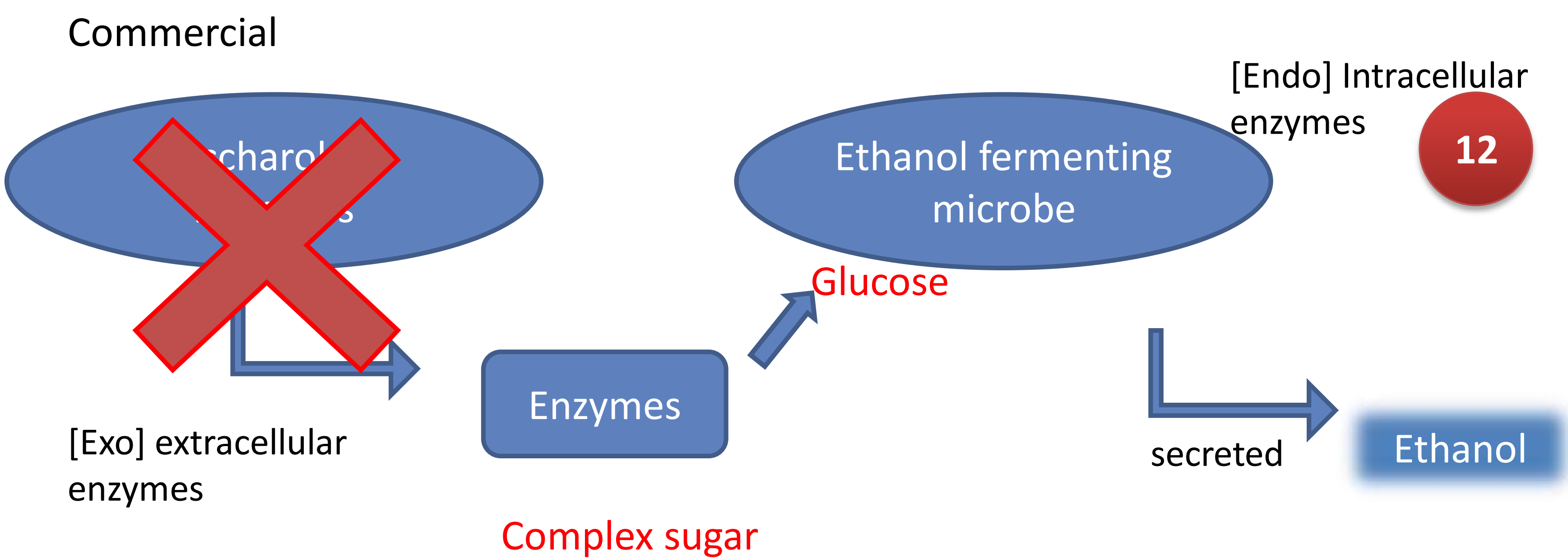
Carbohydrates: **Cellulose** **Starch** **Glucose/sucrose**

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

1 mole glucose = 2 moles ethanol + 2 moles carbon-dioxide

$$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$$

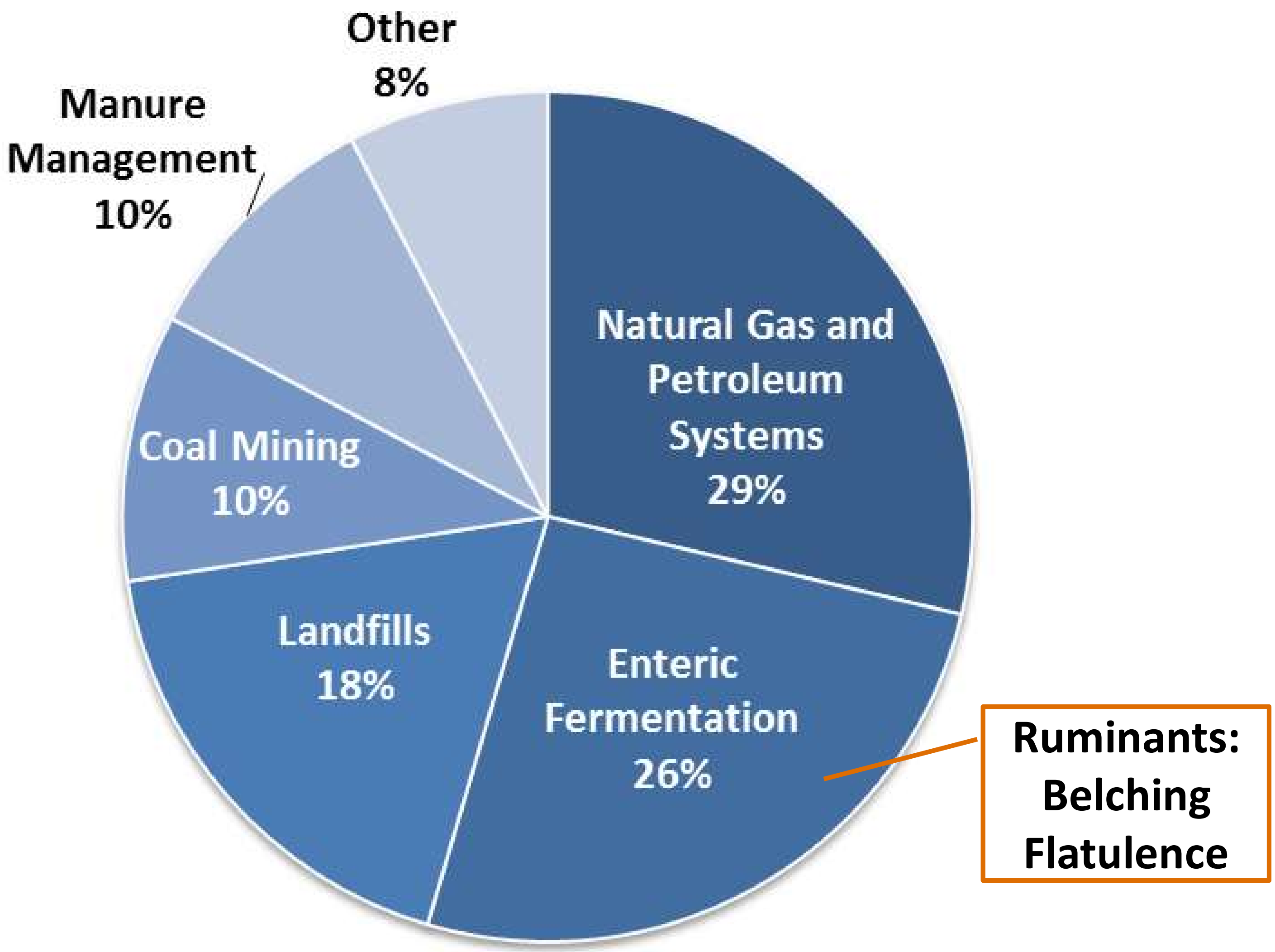
3 moles xylose = 5 moles ethanol + 5 moles carbon-dioxide



Alcohol fermentors (Lab-scale reactors)

Anaerobic Digestion

U.S. Methane Emissions, By Source



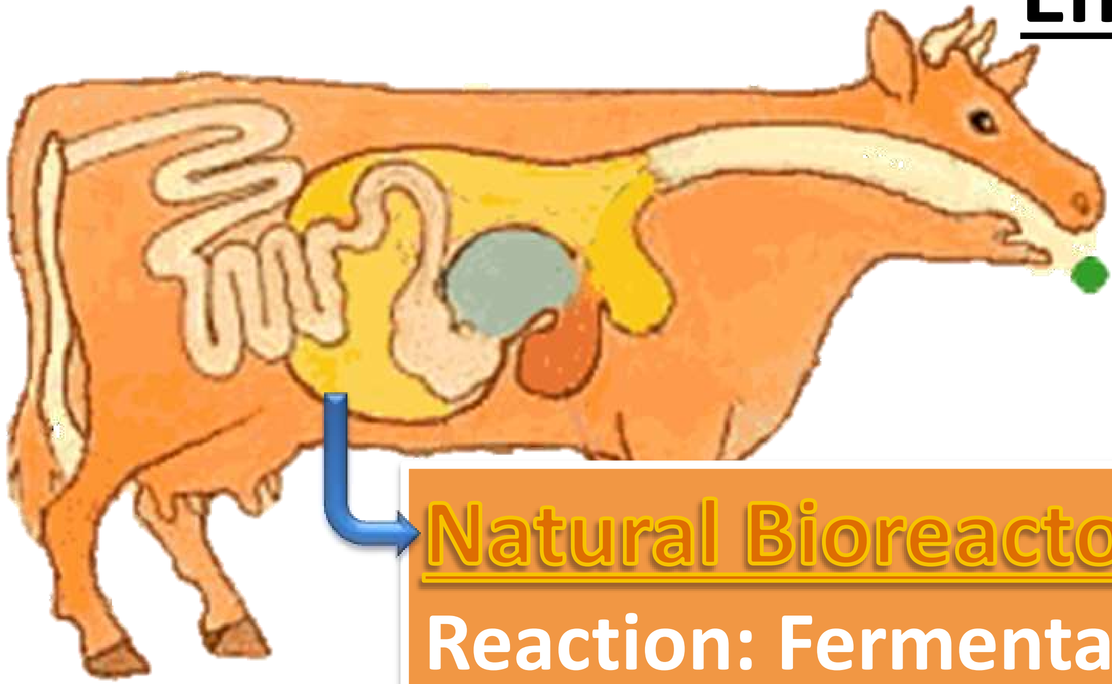
Note: All emission estimates from the *Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2013*.

Enteric Fermentation

Gas composition	Human Fart	Ruminant Burp
• Nitrogen	59%	7%
• **Hydrogen**	**21%**	0.2%
• Carbon-dioxide	9%	65%
• **Methane**	**7%**	**27%**
• Oxygen	4%	0.5%
• Hydrogen sulfide/Mercaptans	1%	

95% of enteric emissions from belching rather than farting

Enteric Fermentation



Biogas digesters

Natural Bioreactor

Reaction: Fermentation

Capacity: 40 to 60 gallons of material

Microbes: 150 billion/teaspoon

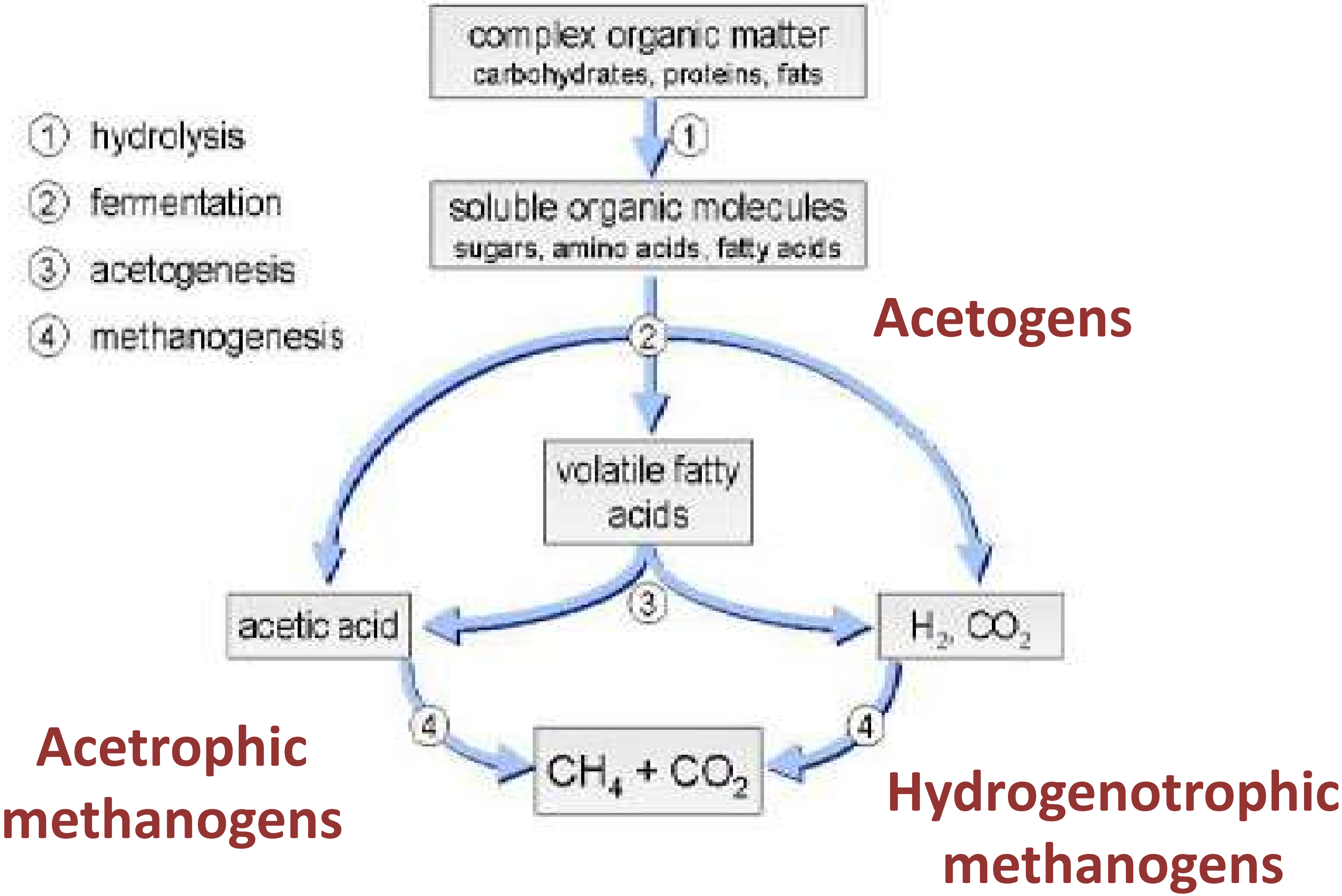
> 400 different species

Output: Energy/nutrients

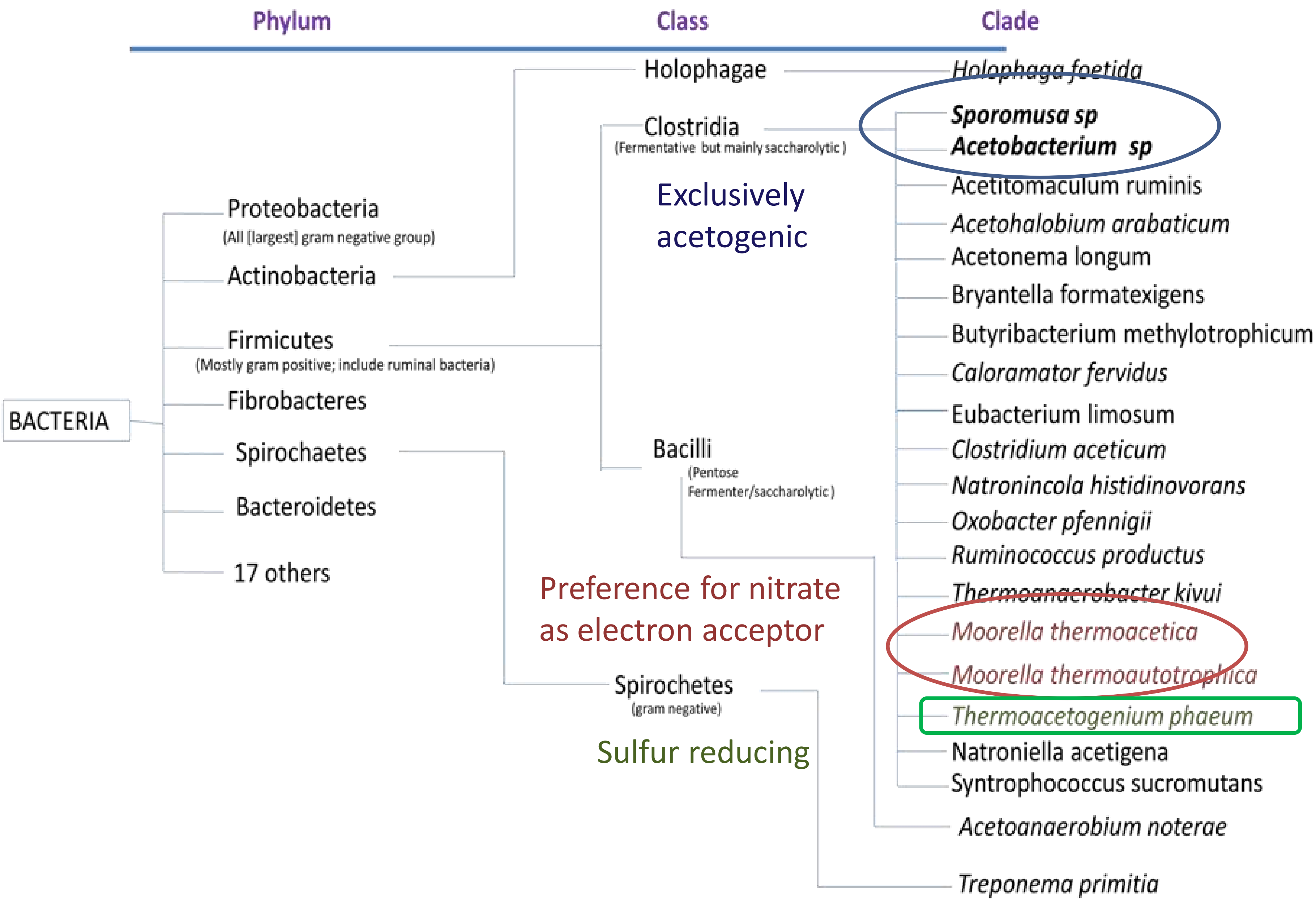
132 - 264 gal of ruminal gas belched/day

30 – 50 gallons methane/cow/day

Anaerobic digestion



Acetogens



Phylum
Class
Clade
BACTERIA
Proteobacteria
(All [largest] gram negative group)
Actinobacteria
Firmicutes
(Mostly gram positive; include ruminal bacteria)
Fibrobacteres
Spirochaetes
Bacteroidetes
17 others
Holophagae
Clostridia
(Fermentative but mainly saccharolytic)
Exclusively acetogenic
Bacilli
(Pentose Fermenter/saccharolytic)
Preference for nitrate as electron acceptor
Spirochetes
(gram negative)
Sulfur reducing
Holophaga foetida
Sporomusa sp
Acetobacterium sp
Acetitomaculum ruminis
Acetohalobium arabaticum
Acetonema longum
Bryantella formatexigens
Butyribacterium methylotrophicum
Caloramator fervidus
Eubacterium limosum
Clostridium aceticum
Natronincola histidinovorans
Oxobacter pfennigii
Ruminococcus productus
Thermoanaerobacter kivui
Moorella thermoacetica
Moorella thermoautotrophica
Thermoacetogenium phaeum
Natroniella acetigena
Syntrophococcus sucromutans
Acetoanaerobium noterae
Treponema primitia

Methanogens

Dominant methanogenic groups (archaea) :

Manure
1) *Methanoculleus thermophilicus (*hydrogenotrophic)
2) *Methanosarcina thermophila* (acetotrophic)

Fruits and vegetables
1) *Methanosphaera stadtmanii* (hydrogenotrophic)
2) *Methanobrevibacter wolinii* (hydrogenotrophic)

Municipal wastes and sewage sludge
1) *Methanosaeta concilii (*acetotrophic)
2) *Methanosarcina sp* (acetotrophic)

Bioenergy to the rescue

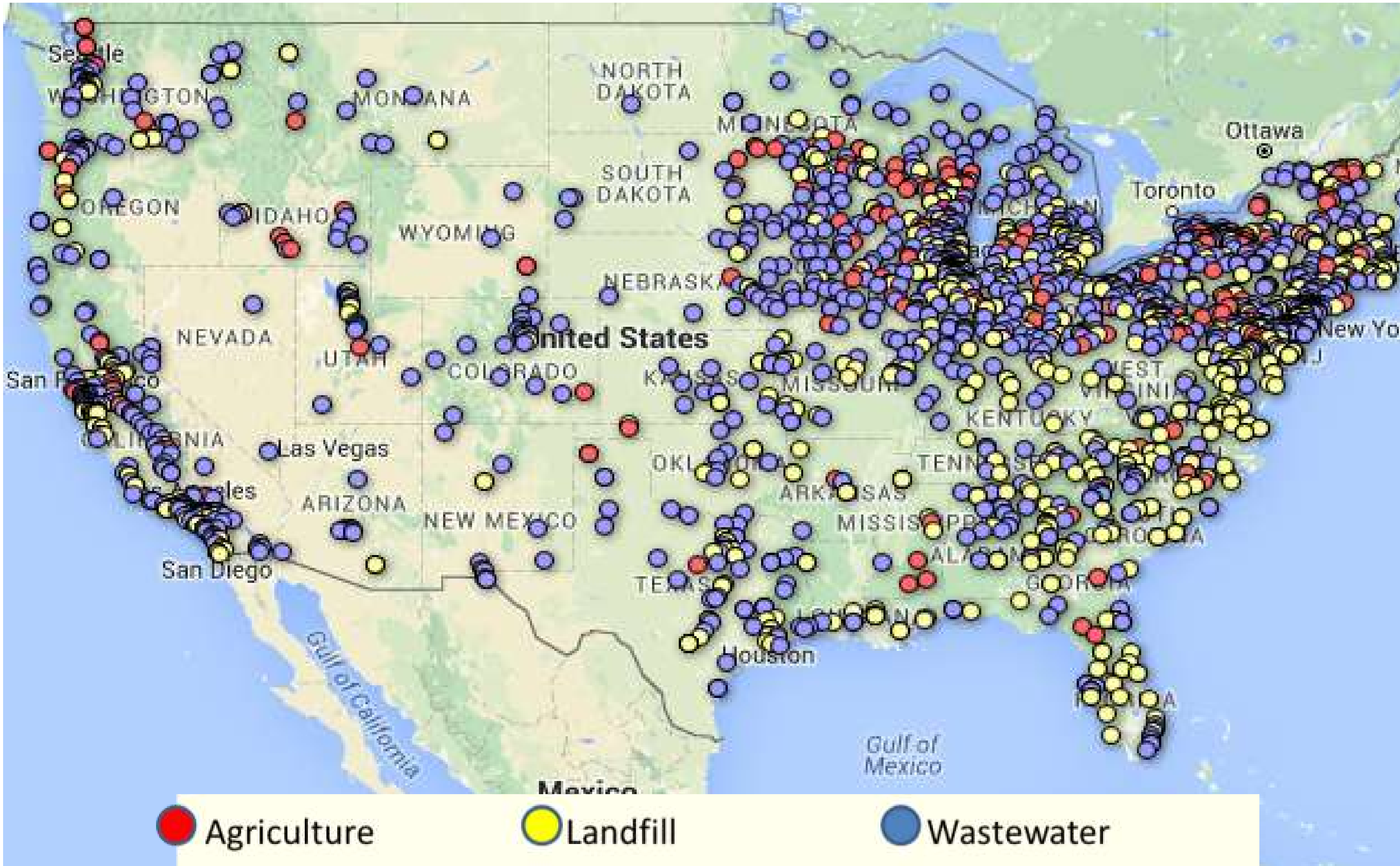
Missouri
Hog production facility
~ 2 Million hogs
80 manure Lagoons

	Emissions per day		
	Volume (m3)	Mass (kg)	Mass (ton)
Biogas	720,000.00		
Methane	432,000.00	283,392.00	312.39
CO2	288,000.00	570,240.00	628.58

Bioenergy to the rescue

- Energy production
- Pathogen reduction
- Odor reduction
- Nutrient recovery
- Mitigate Global warming

Operational Biogas Systems in the U.S.

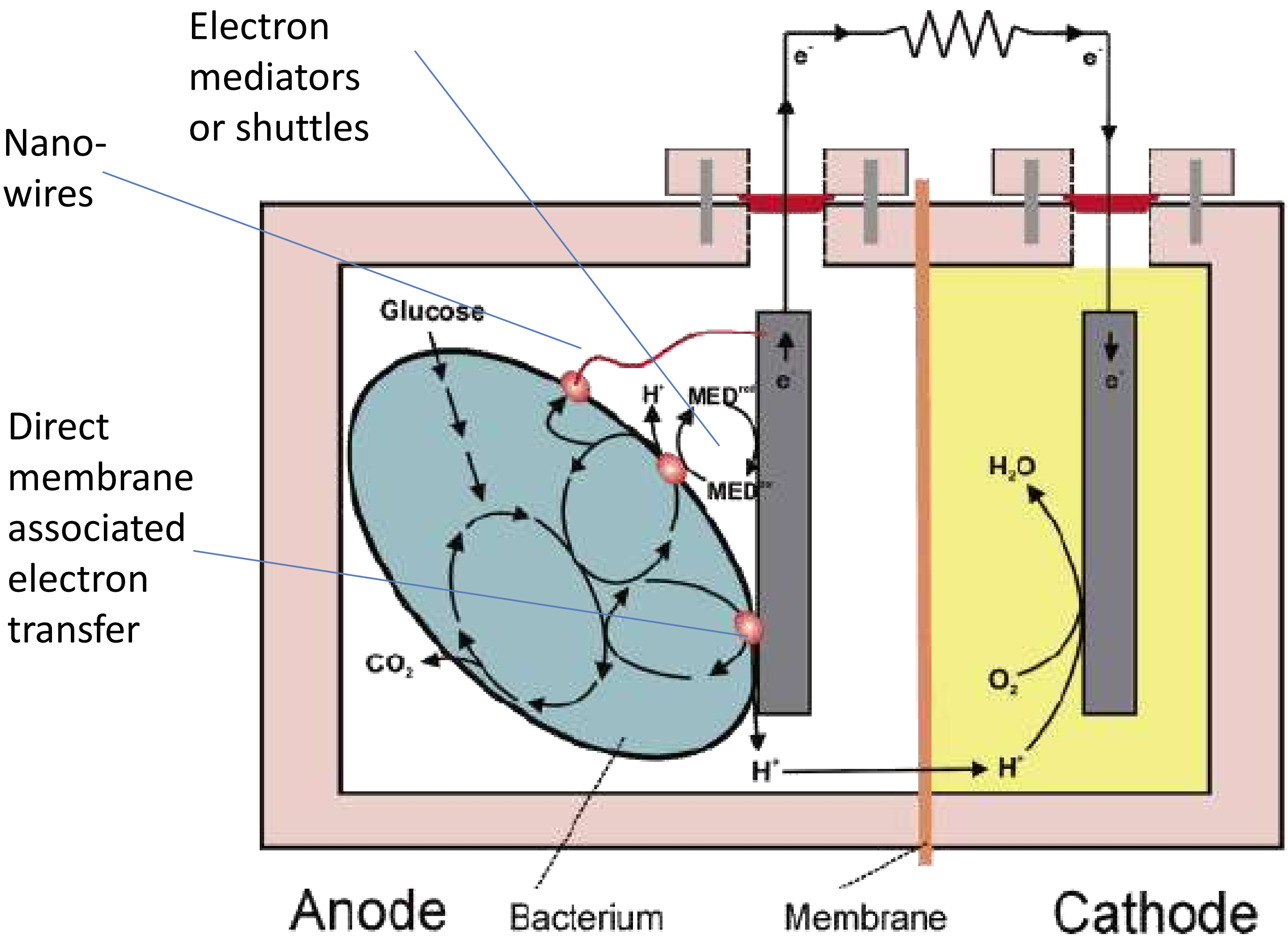


Source: American Biogas Council

Anaerobic digesters

Microbial fuel cells (MFC)

Microbial fuel cells (mfc)



Rabaey and Verstaraete, 2005. Microbial fuel cells: novel biotechnology for energy generation.
http://www.microbialfuelcell.org/Publications/Rabaey%20and%20Verstraete%20Trends%20in%20Biotechnology.pdf

Microbial Fuel Cells (Mfc)

Some MFC bacteria (electricigens)

1
- *Geobacter species*
  - *Geobacter metallireducens*
  - *Geobacter sulfurreducens*
  - *Geobacter psychrophilus*
- *Desulfuromonas acetoxidans*
- *Geopsychrobacter*

(direct membrane electron transfer mechanism - exoelectrogens)

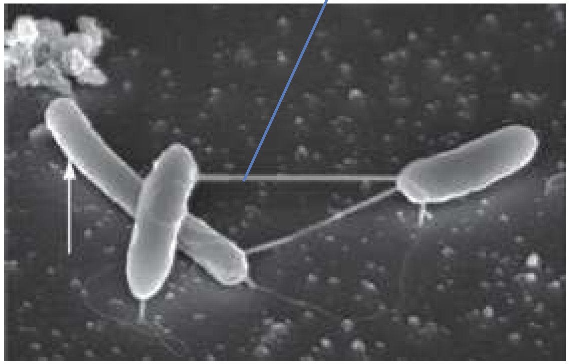
2
- *Shewanella putrefacians*
- *Pseudomonas species*
- *Geothrix ferementans*

(Produce their own chemical – redox mediators)

3
- *Shewanella oneidenensis*
- *Pseudomonas aeruginosa*

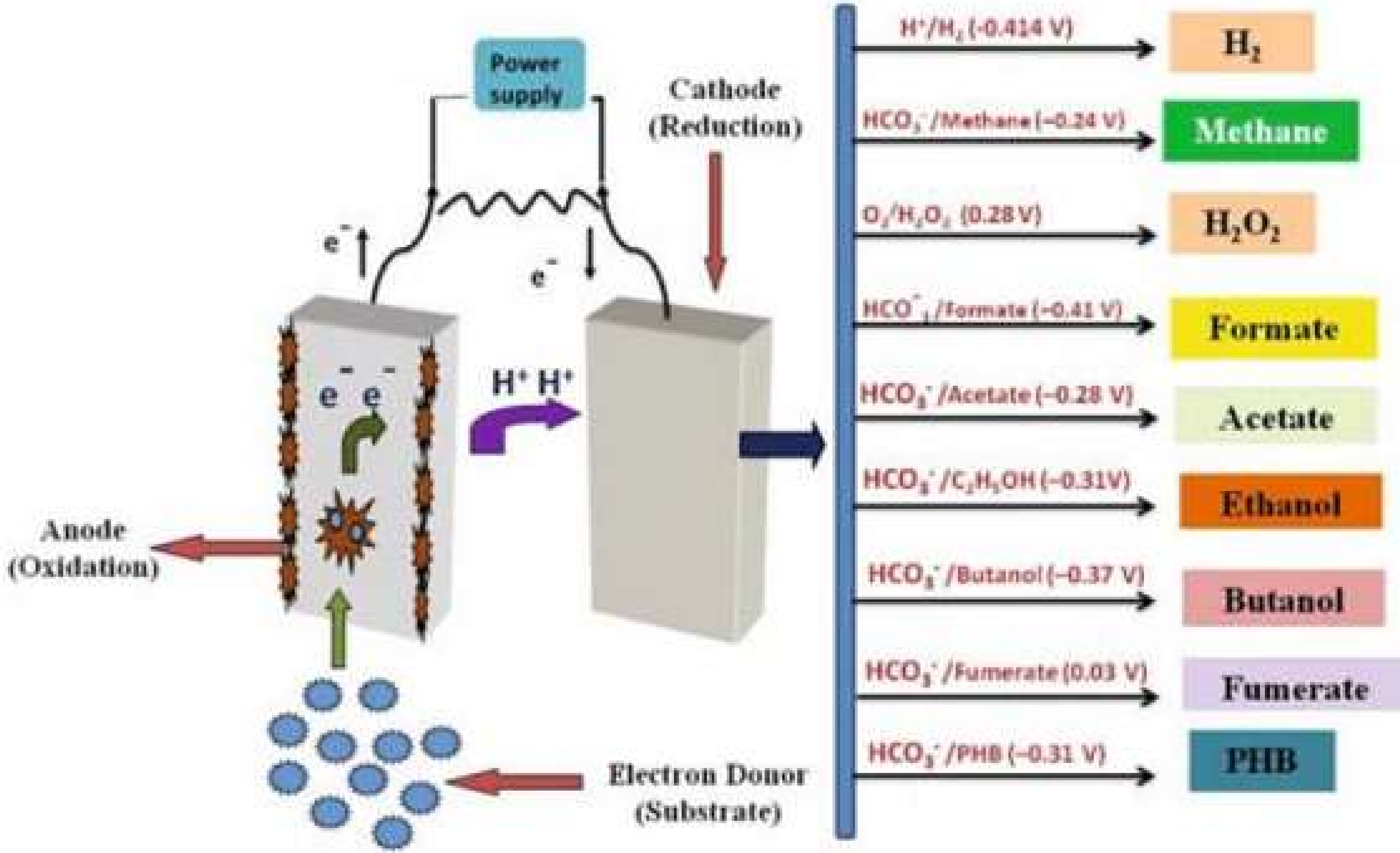
Use nanowires for electron transfer

Nanowires



http://www.nanowerk.com/spotlight/spotid=1197.php

MFC – variants



MFC – other variants

Hydrogen-producing MFC

TWO MODES

- BioElectrochemically-Assisted Microbial Reactor (BEAMR)
- Biocatalyzed electrolysis cells (BECs)
- Microbial electrolysis cells (MECs)

1

MEC reaction at cathode:

~~Oxygen~~ + H^+ + e^- = Hydrogen

Acetic acid

2

$$C_6H_{12}O_6 + 2\,H_2O \rightarrow 4\,H_2 + 2\,CO_2 + 2\,C_2H_4O_2$$

$$C_2H_4O_2 + 2\,H_2O + \text{electricity} \rightarrow 2\,CO_2 + 4\,H_2$$

Theoretically: 0.41 Volts to make H_2 from acetate,

Bacteria produce: ~0.2 to 0.3 V

Supplementary voltage: 0.2 V

Connect the positive pole of a programmable power supply to the anode and the negative pole to the cathode

MFC vs Conventional Fuel Cell (CFC)

Utilization of biomass or organic substrate

MFC

Biodegradable substrate
Acetate, Ethanol, Glucose, Waste water, etc

↓

Appropriate bacteria — Mixed Consortium preferred

↓

Electrons produced (bacterial metabolism) — Bacteria as catalyst

↓

Protons and electrons → Anode (electron acceptor) — Anoxic condition

CFC

(mainly hydrogen fuel cell)

Hydrogen rich fuels
Natural gas, Glucose, Ethanol, Liquid propane, Gasoline, etc

↓

Chemically reformed or hydrogen fermentation

↓

Hydrogen

↓

Anode (Catalytic electrode - Hydrogen dissociation) → Protons and electrons

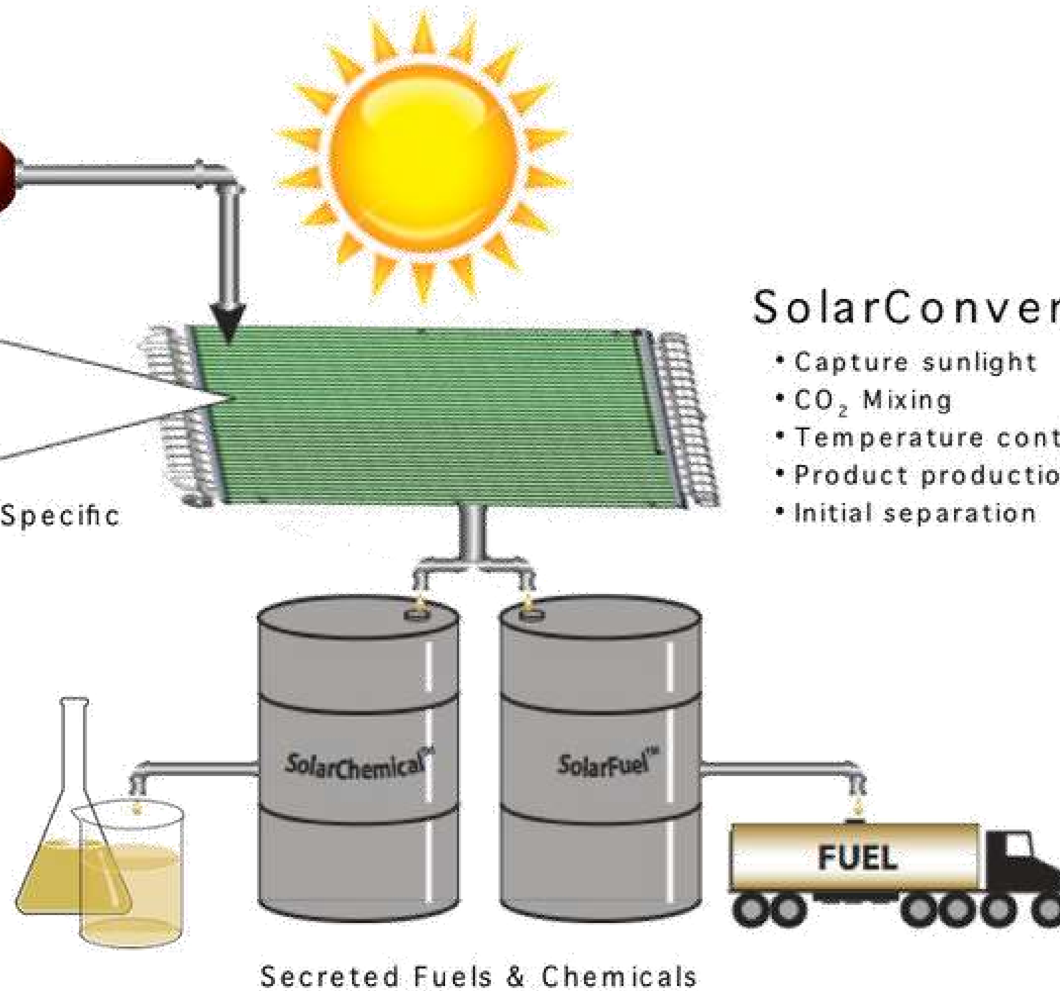
Microbial Fuel Cells (Mfc)

Single chamber

Double chamber

Emerging technology: **Oleaginous microorganisms**

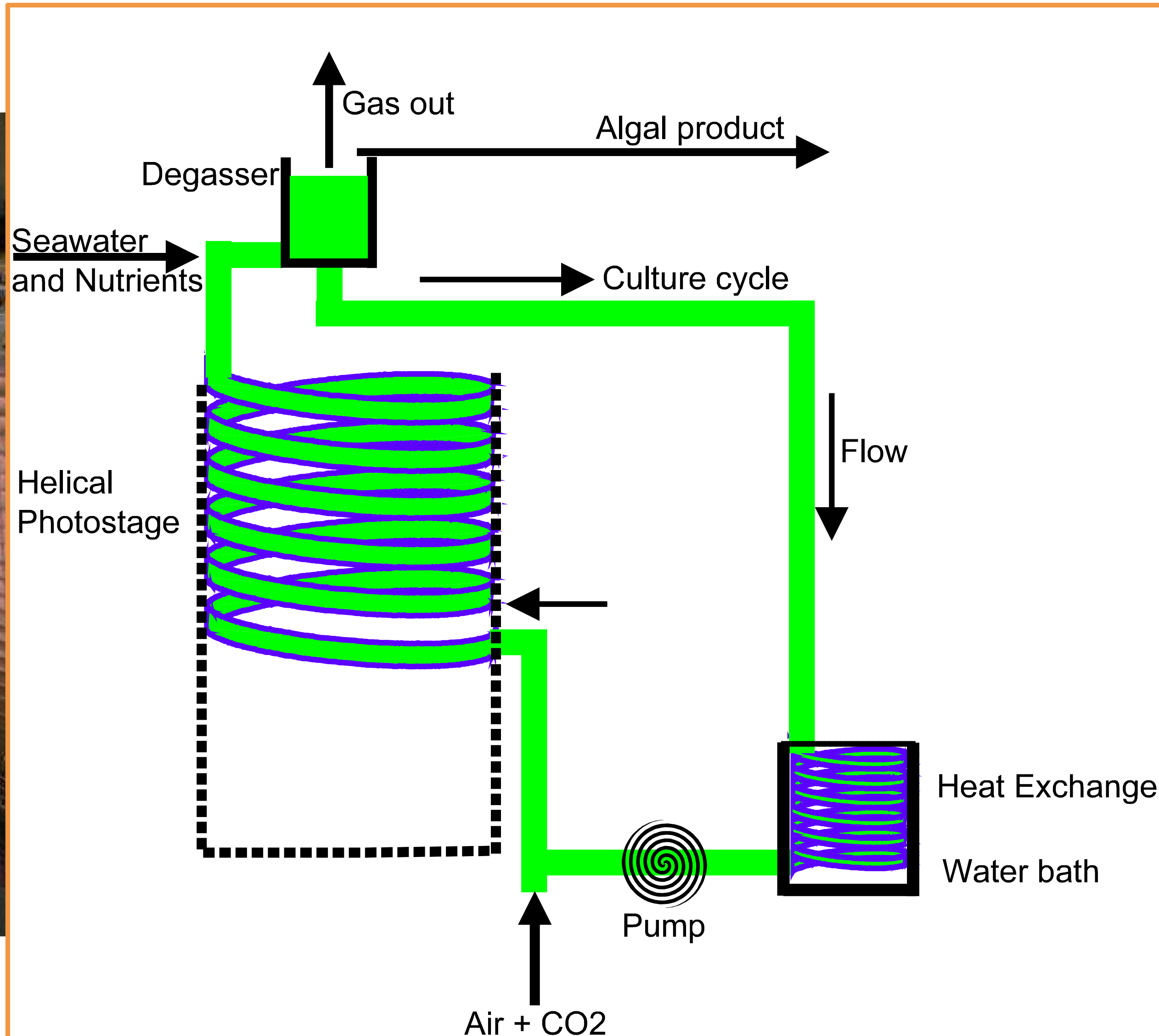
- **Microbial-based diesel and microdiesel**

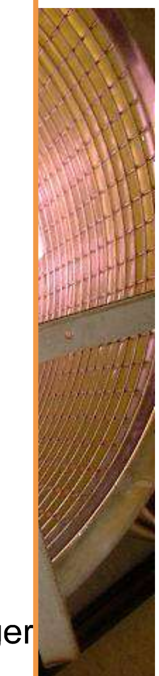


Non-destructive

- Solar Ethanol
- Solar Gasoline
- Solar Diesel
- Solar Fuels

Photobioreactor





Emerging Technologies

Microbial-based Fatty acids for biodiesel

Cyanobacteria

- Modified thioesterases
  - Clip the bonds associating the fatty acids with more complex molecules
- Modified S and peptidoglycan layers
  - allow fatty acids to more easily escape outside the cell

Emerging Technologies

Microdiesel

Genetically Modified *Escherichia coli*

- ➕ Ethanol producing genes from *Zymomonas mobilis*
- ➕ another genes from *Acinetobacter baylyi* to produce enzymatic catalyst for the reaction

- Uses oils/fatty acids and sugars
- Plant waste, food waste

Microbial Communities

Bunker silo (Horizontal)

Wrapped silage

Silage bag

Storage

Some endogenic silage microorganisms

MICROBE	SUBSTRATE	OUTCOME
Lactic acid Bacteria (LAB)		
Homolactic	Simple sugars	Lactic acid
Lactobacillus plantarum, Pediococcus acidilactici, Enterococcus faecium		
Heterolactic	Simple sugars	Lactic acid, [acetic, isobutyric, ethanol, mannitol, propandiol], CO_2
Lactobacillus buchneri, Leuconostoc pseudomesenteroides, Weissella cibaria		
Propionic acid bacteria	Simple sugars	Propionic, acetic , CO_2, H_2O
Propionibacterium shermanii, Propionibacterium jensenii		
Enterobacteria	Simple sugars	Acetic acid, ethanol, CO_2, H_2
Erwinia persicinia, Escherichia coli, Pontoea agglomerans		
Bacilli *Bacillus mageterium*	Simple sugars (Cellulolytic activity)	Lactic and acetic acids [butyric acid]
Clostridia *Clostridium butyricum, Clostridium sporogenes, Clostridium perfringens*	Simple sugars, lactic acid	Butyric acid, CO_2
Yeast (air infiltation) *Candida intermedia, Pichia fermentans, Saccharomyces martiniae*	Simple sugars, organic acids	Ethanol and CO_2
Molds (air infiltration) *Aspergillus fumigatus, Penicillium roqueforti, Mucor circinelloides*	Simple sugars (Cellulolytic activity)	CO_2 and H_2O

Most desirable | OK | aerobic stabilty | NOT desirable

Common organic acids in anaerobic solid state fermentation

Inhibitory capacity decreases ↓

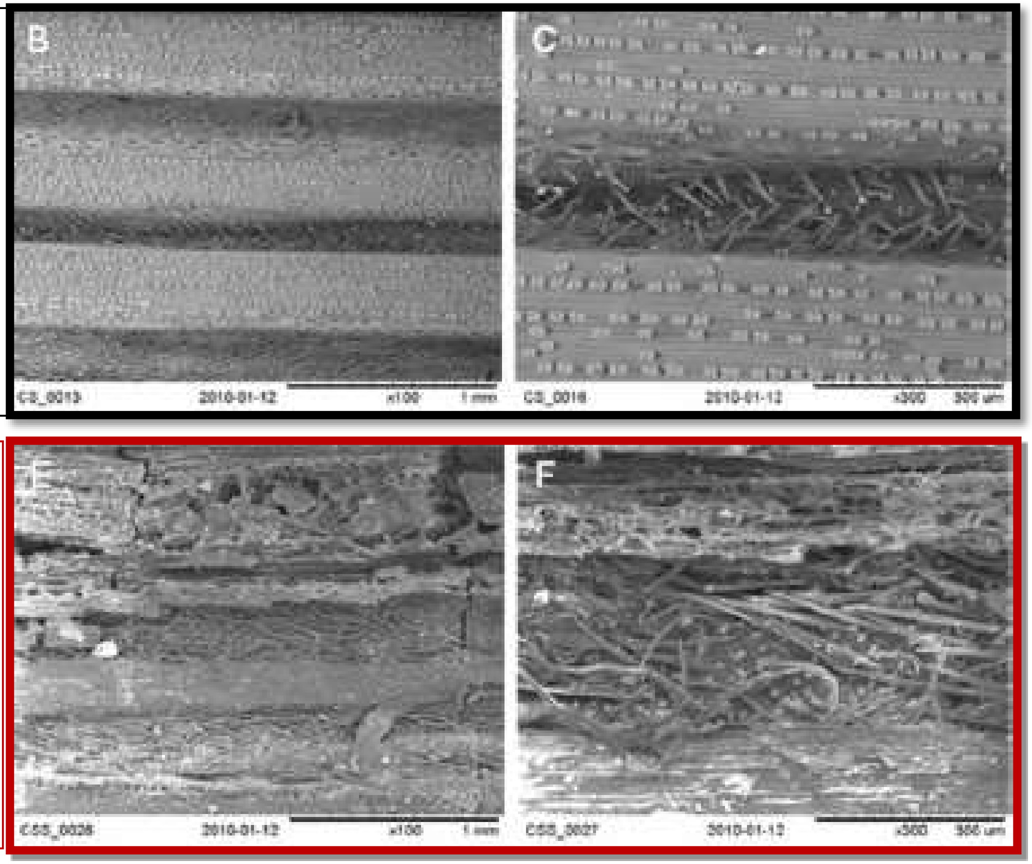
	pKa	% Dissociated at pH 4.5	Post storage advantage
Propionic	4.88	29.42	Aerobic stability
Butyric	4.82	32.37	None
Iso-butyric	4.86	30.39	Ethanol stimulant (if less than 4g/L)
Acetic	4.76	35.46	Ethanol stimulant (if less than 6 g/L)*
Lactic	3.85	81.71	Ethanol stimulant (if less than 8g/L)*

* Higher concentrations can be present singularly (up to 10 g/L and 20 g/L for acetic and lactic respectively) without any inhibitory effect on ethanol

Effect of organic acids on feedstock structure

X 100 X 300

Unensiled corn stover

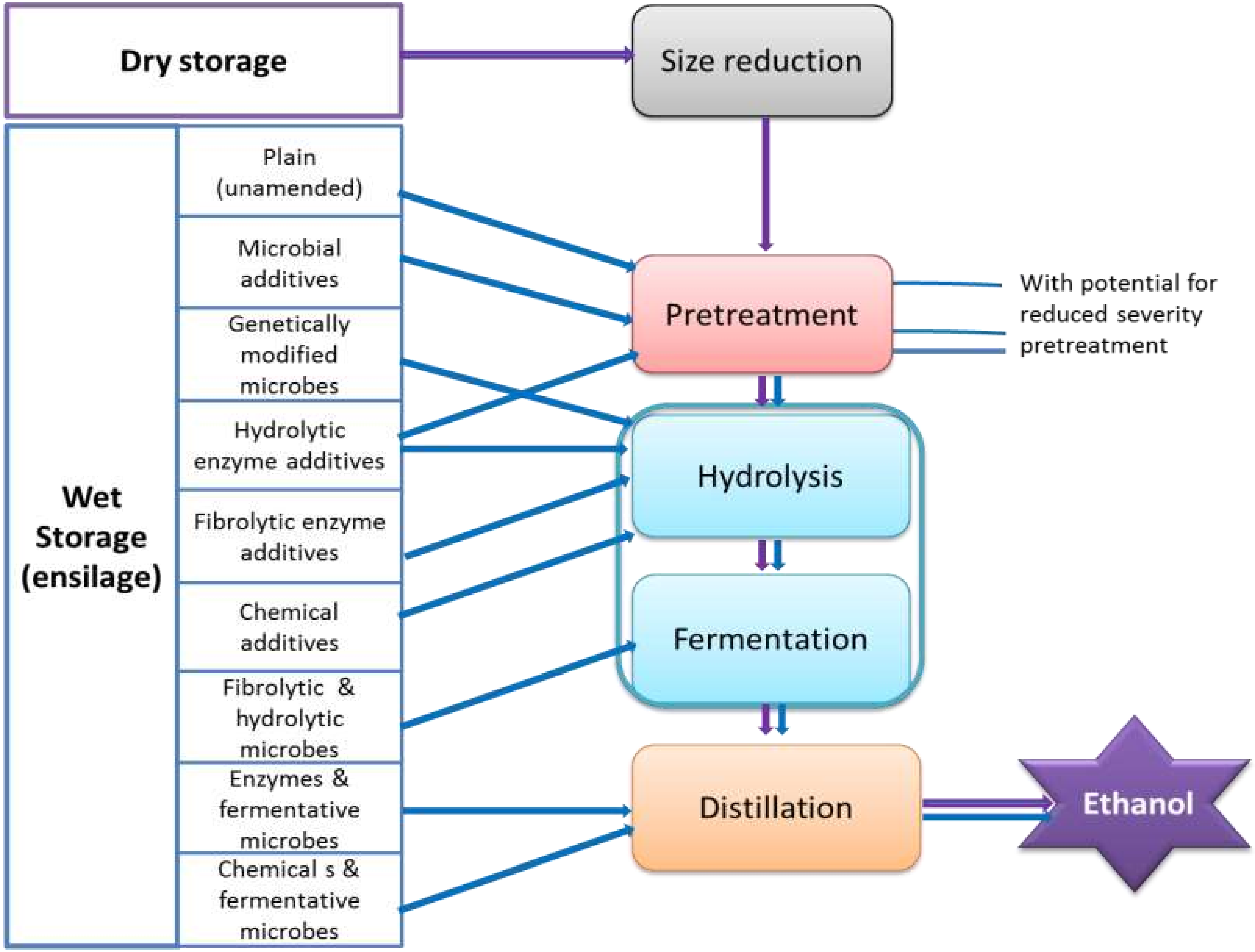


Ensiled corn stover (with microbial additive)

Additive: Biomax Si (CHR Hansen, Denmark) containing lactic acid bacteria
Storage moisture: 23 – 30%

Microscopic images comparing unensiled and ensiled corn stover (Adapted from Oleskowicz-Popiel et al. 2010)

Ultimate goal and expectations?



Microbial Communities

Feedstock production

Microbes in feedstock production

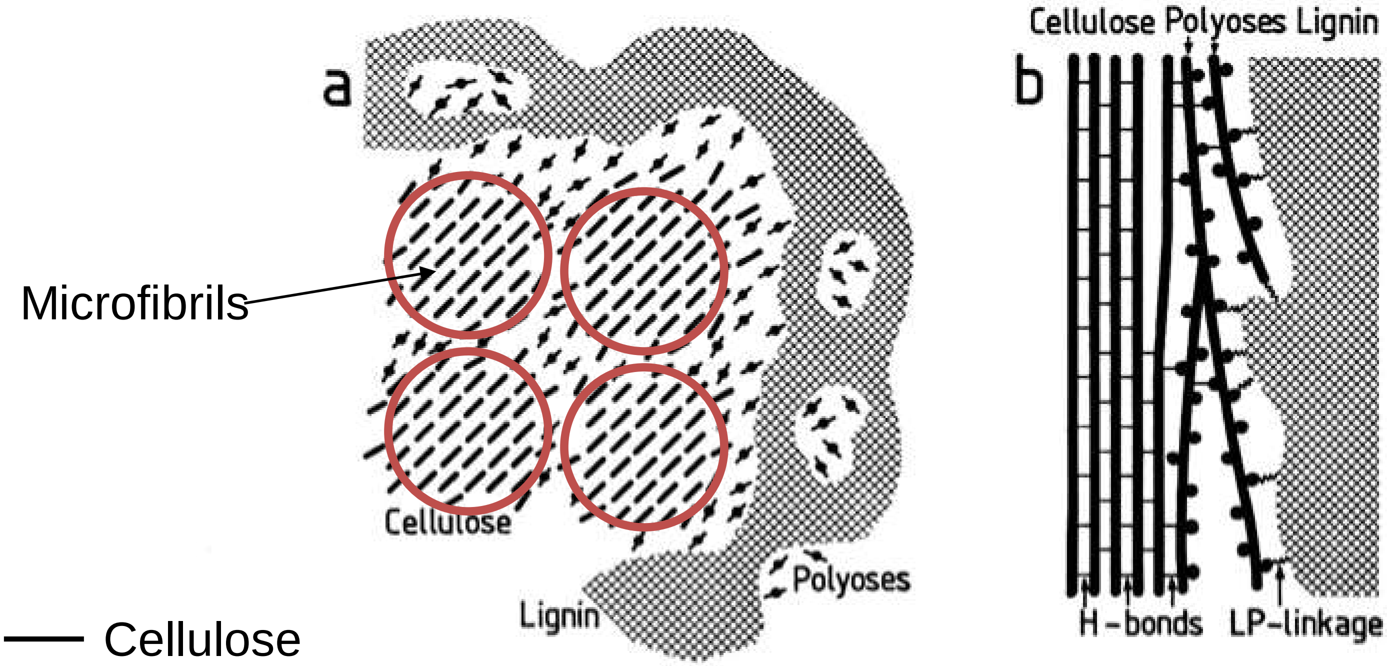
Strategy focus mainly on:

- Decreasing lignin content
- Altering/modifying lignin content
- incorporate enzymatic biocatalyst
- or microbial bioprocessers

- Down regulation of lignin synthesis enzymes e.g. shikimate hydroxycinnamoyl transferase
- Overexpression of some gene
- Replace lignin-lignin bonds with bond

- Gradually act on plant cell walls during growth
- Activated at crop maturity or at harvest or during feedstock processing to increase cell wall digestibility and make sugars more accessible

Two Models

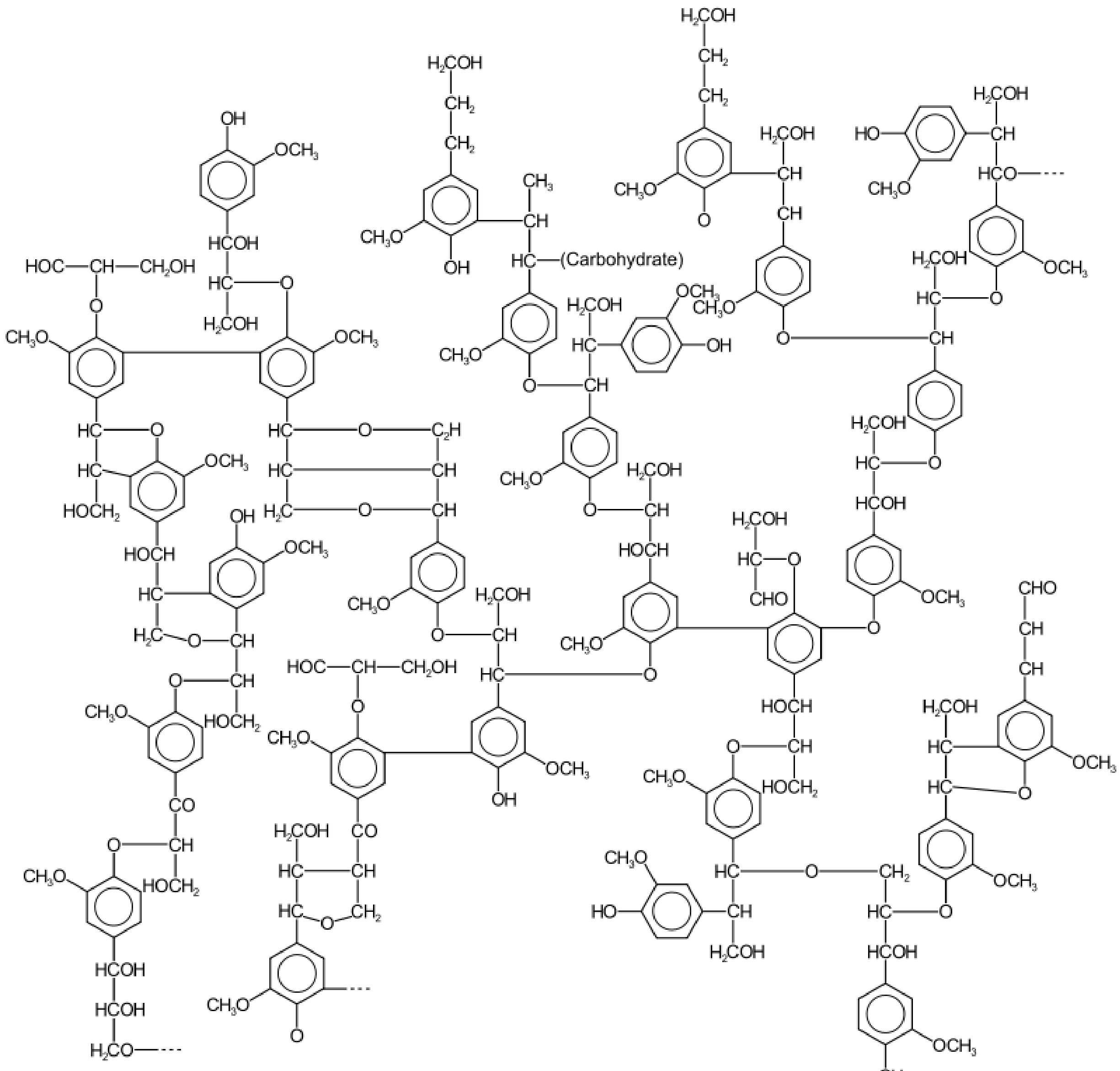


Cellulose

Polyoses (Hemicellulose)

Lignin

Lignin



Lignin
Non-carbohydrate

Biomass-degrading enzymes used

Enzymes from bacteria (e.g. ***Pseudomonas fluorescens***)

4-hydroxycinnamoyl-CoA hydratase/lyase

Lower degree of polymerization/molecular weight
Cleavage of some side chains

Enzyme	Reference
Endoglucanase	Dai *et al.* (2000)
Cellobiohydrolase	Dai *et al.* (1999)
Xylanase	Kimura *et al.* (2003)
Lignin peroxidase	Bhat and Bhat (1997)
Manganese peroxidase	Chen *et al.* (2012) and Saha (2003)
Ferulic acid hydrolase	Buanafina *et al.* (2010)
Multifunctional hydrolases	Fan and Yuan (2010)
Cocktails (endoglucanase, exoglucanase, pectate lyase, cutinase, swollenin, xylanase, acetyl xylan esterase, beta-glucosidase and lipase)	Verma *et al.* (2010)

Microbe-derived hydrolytic enzymes

Bioprospecting

Mostly based on natural analogues

Microbial Communities

Hmm…
My poop has more
value than I do

Lignocellulose Degradation

Methane (Biogas) production

Questions

?