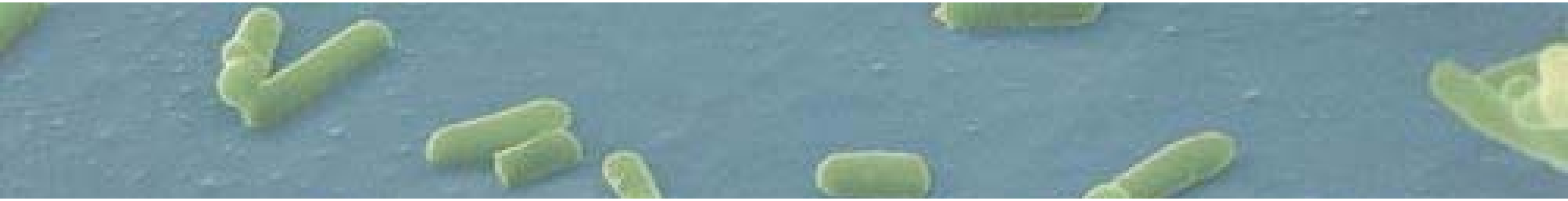
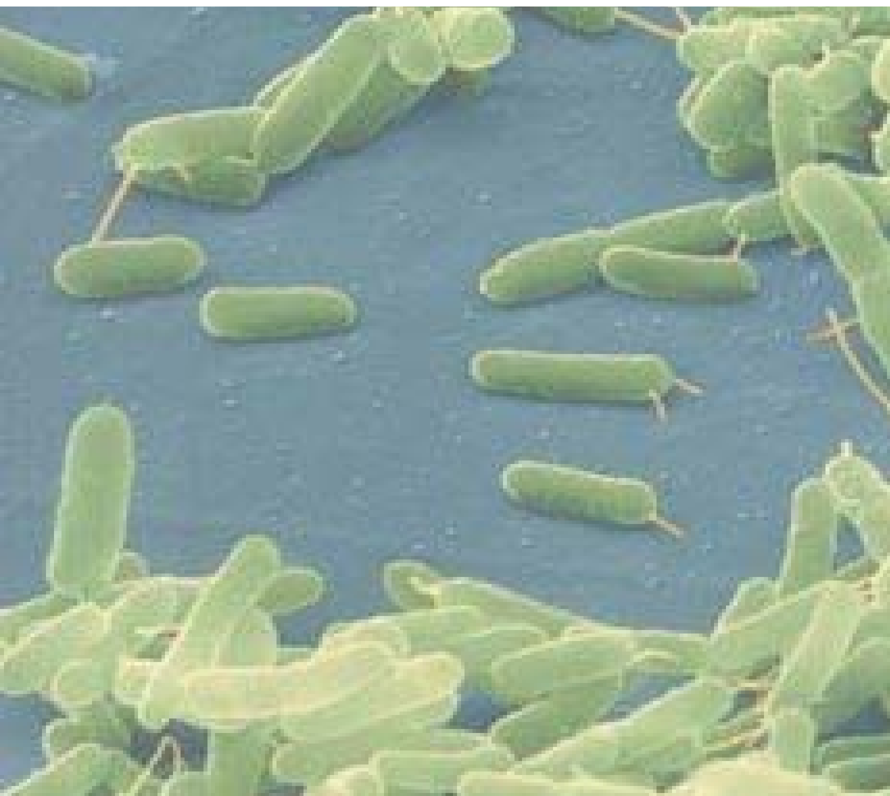


Metabolically engineered microbial systems and the conversion of agricultural biomass into simple sugars for the production of biofuels and value-added products



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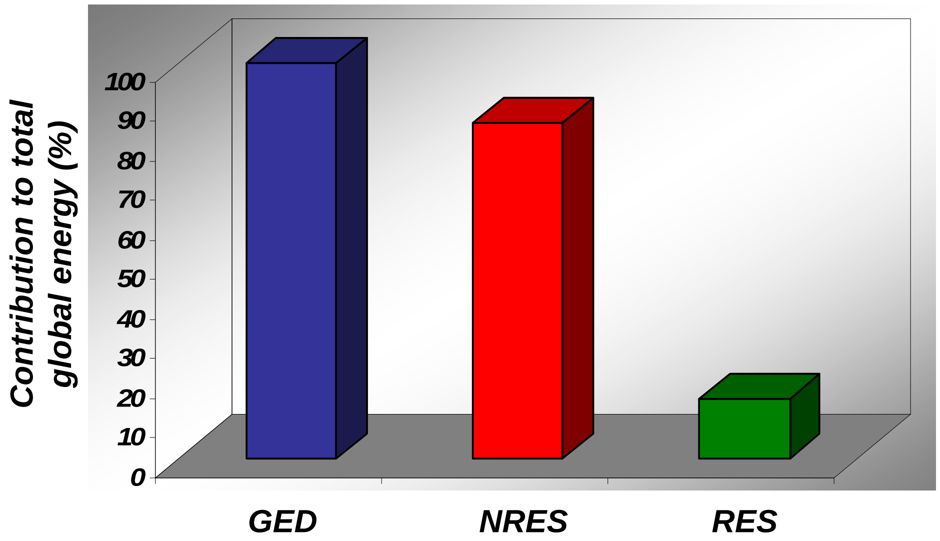
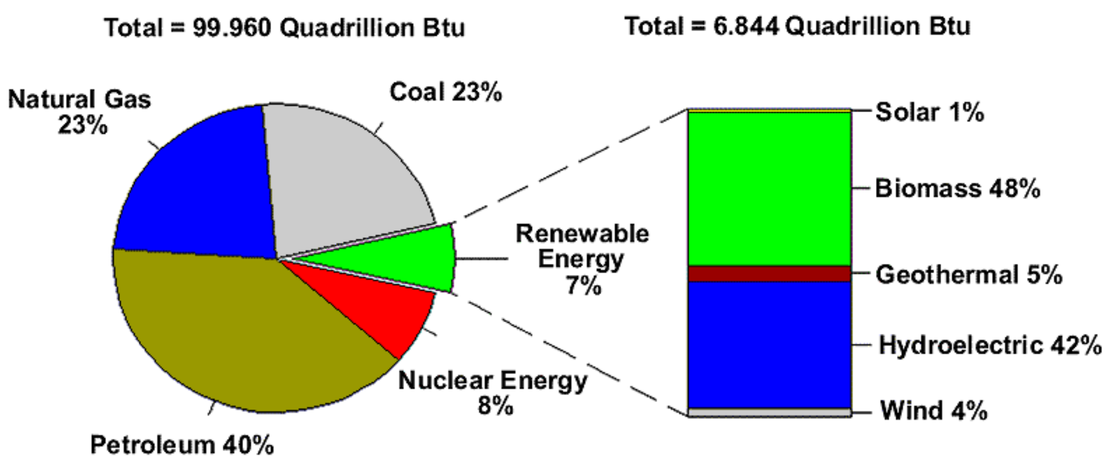
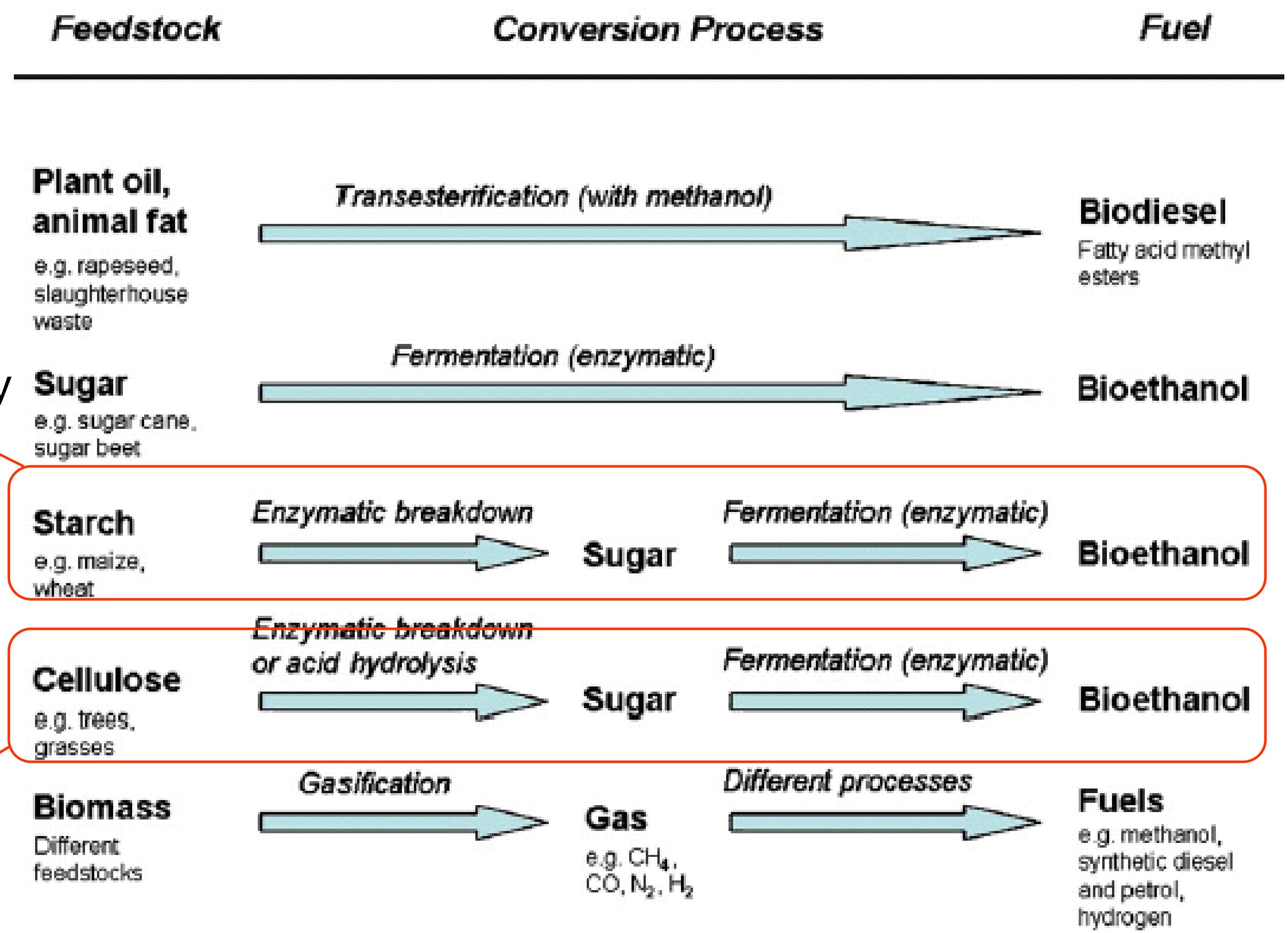
Global energy demand (GED)





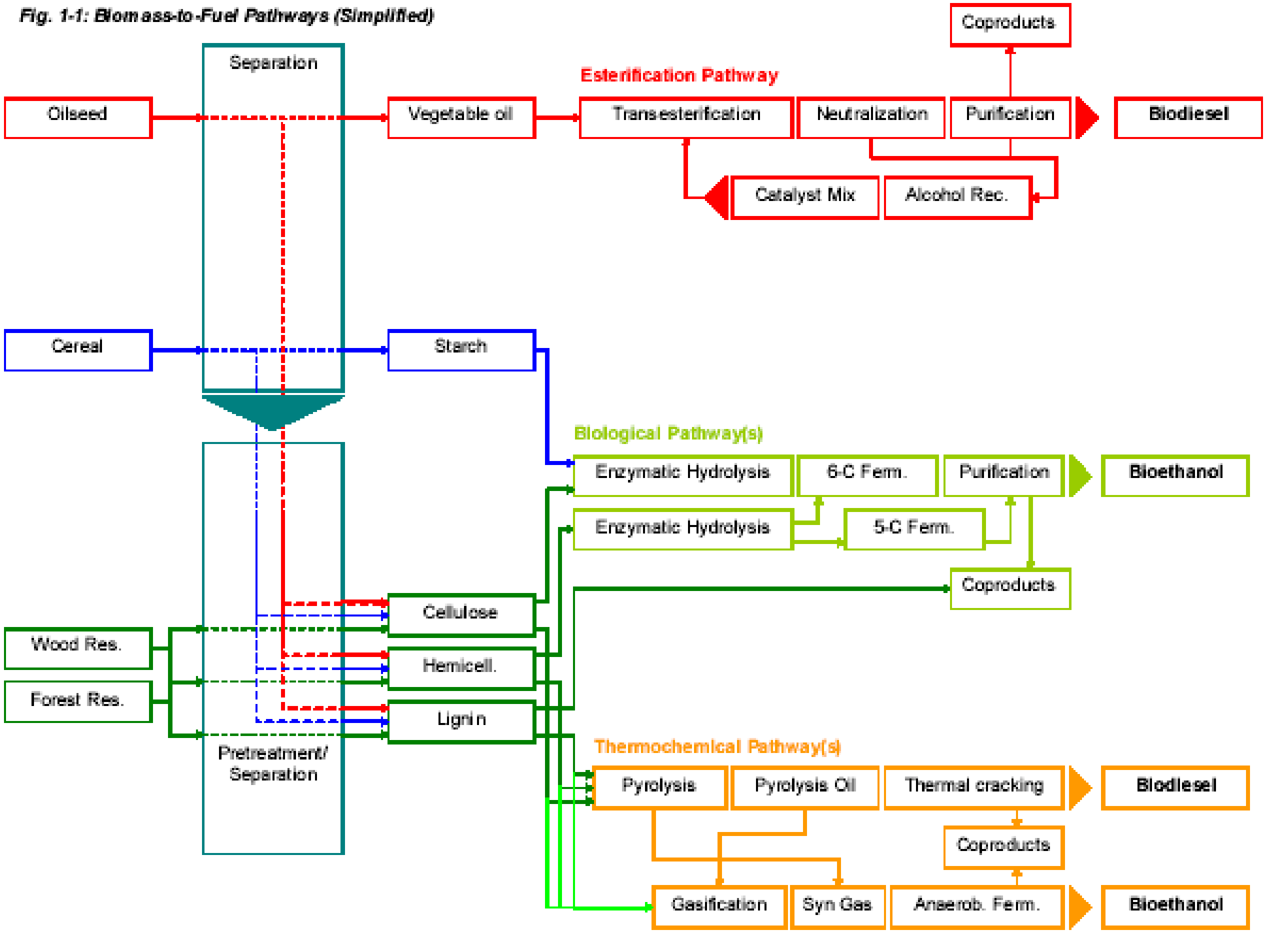
Biomass-derived energy



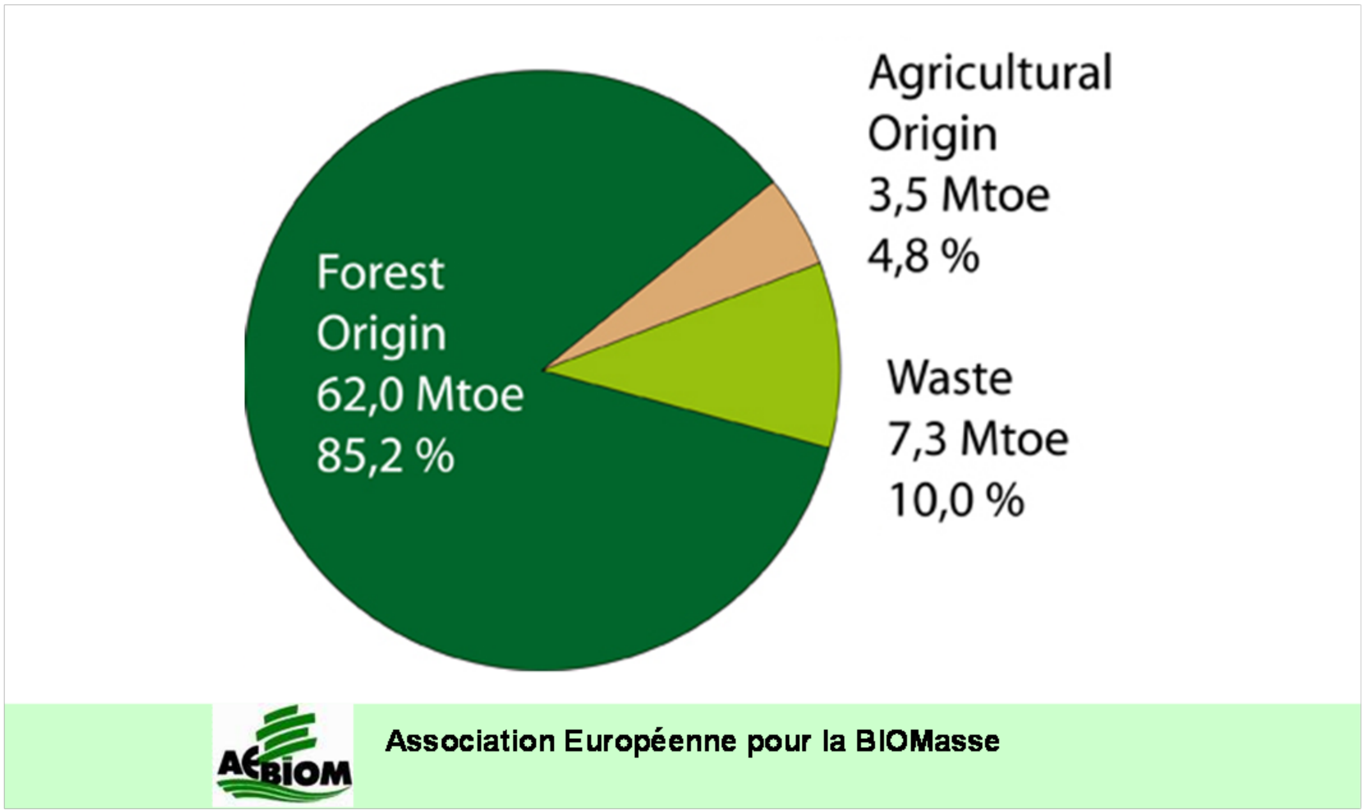
Energy costly

Lack of available enzyme

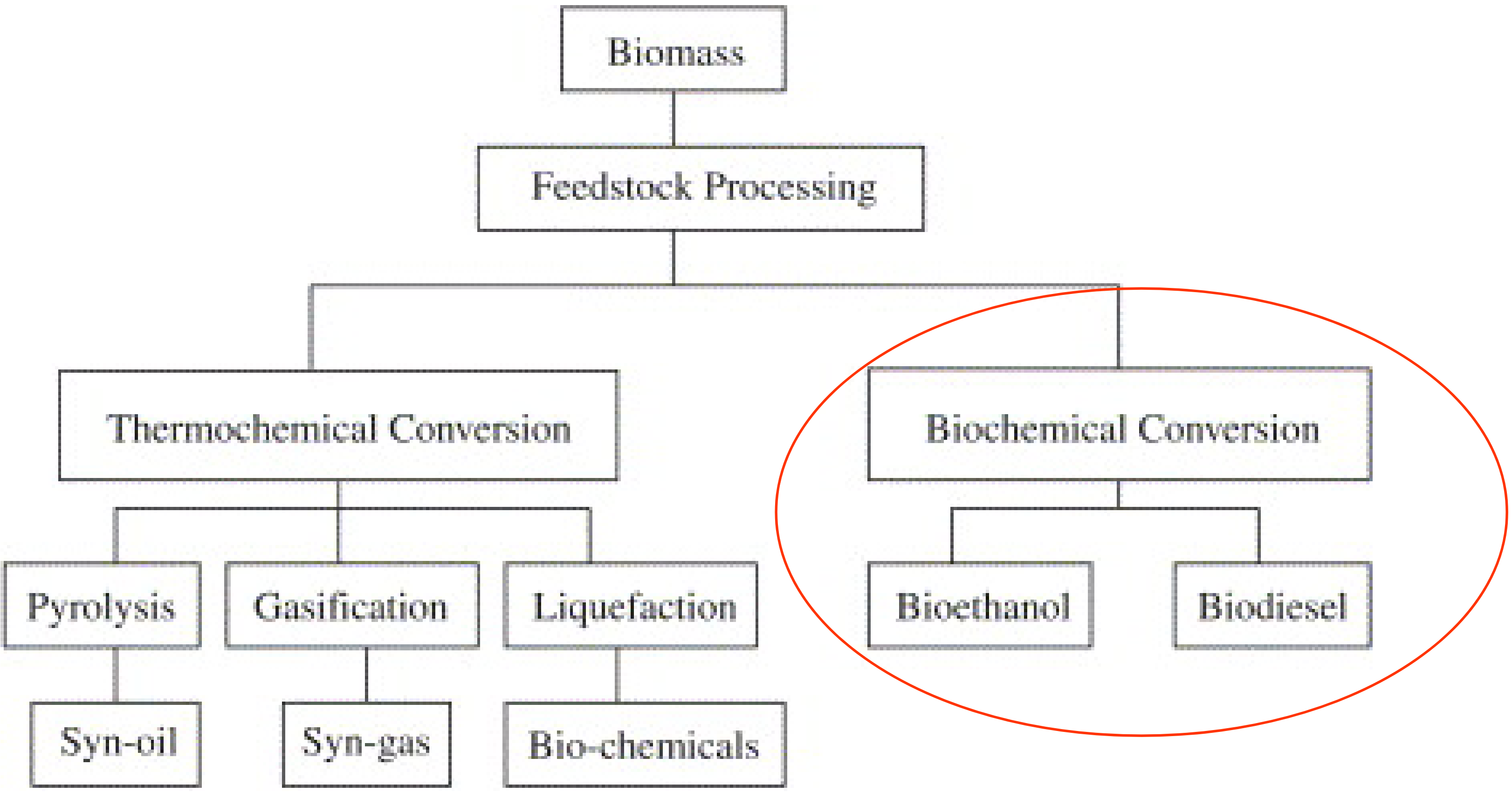
Biofuel production pathways



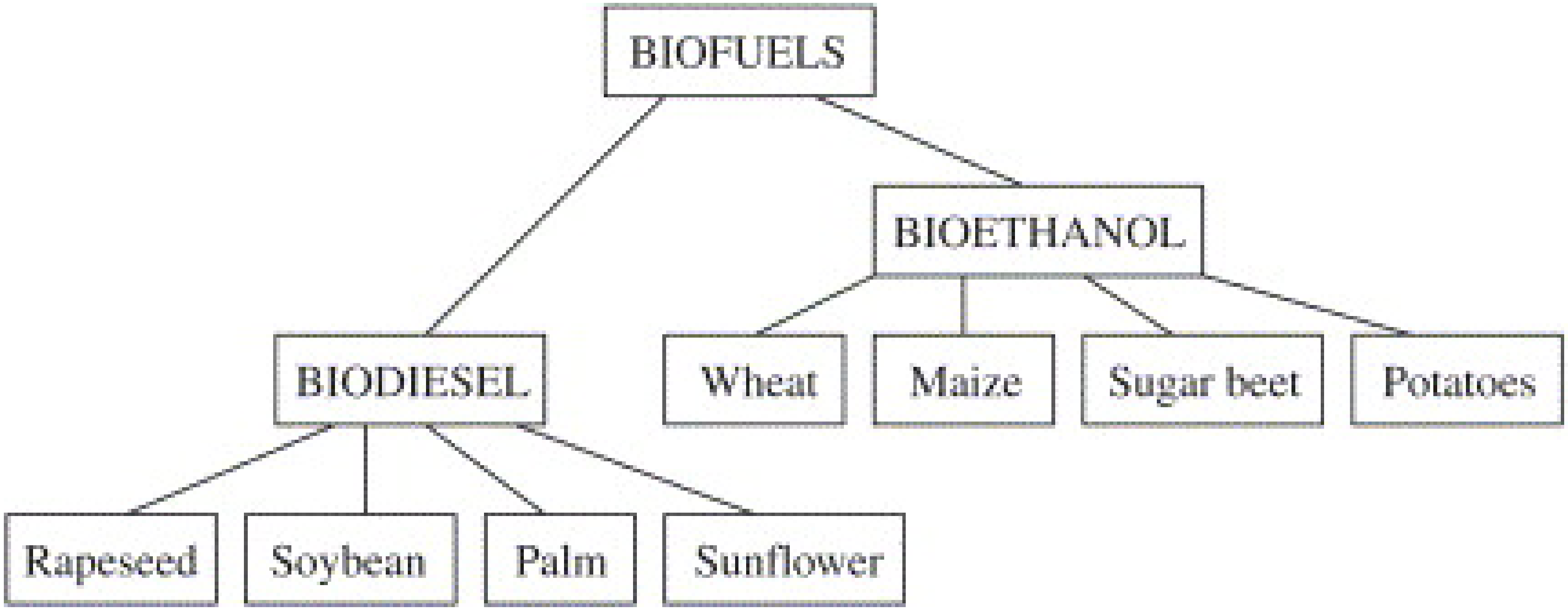
Composition of biomass as energy carrier



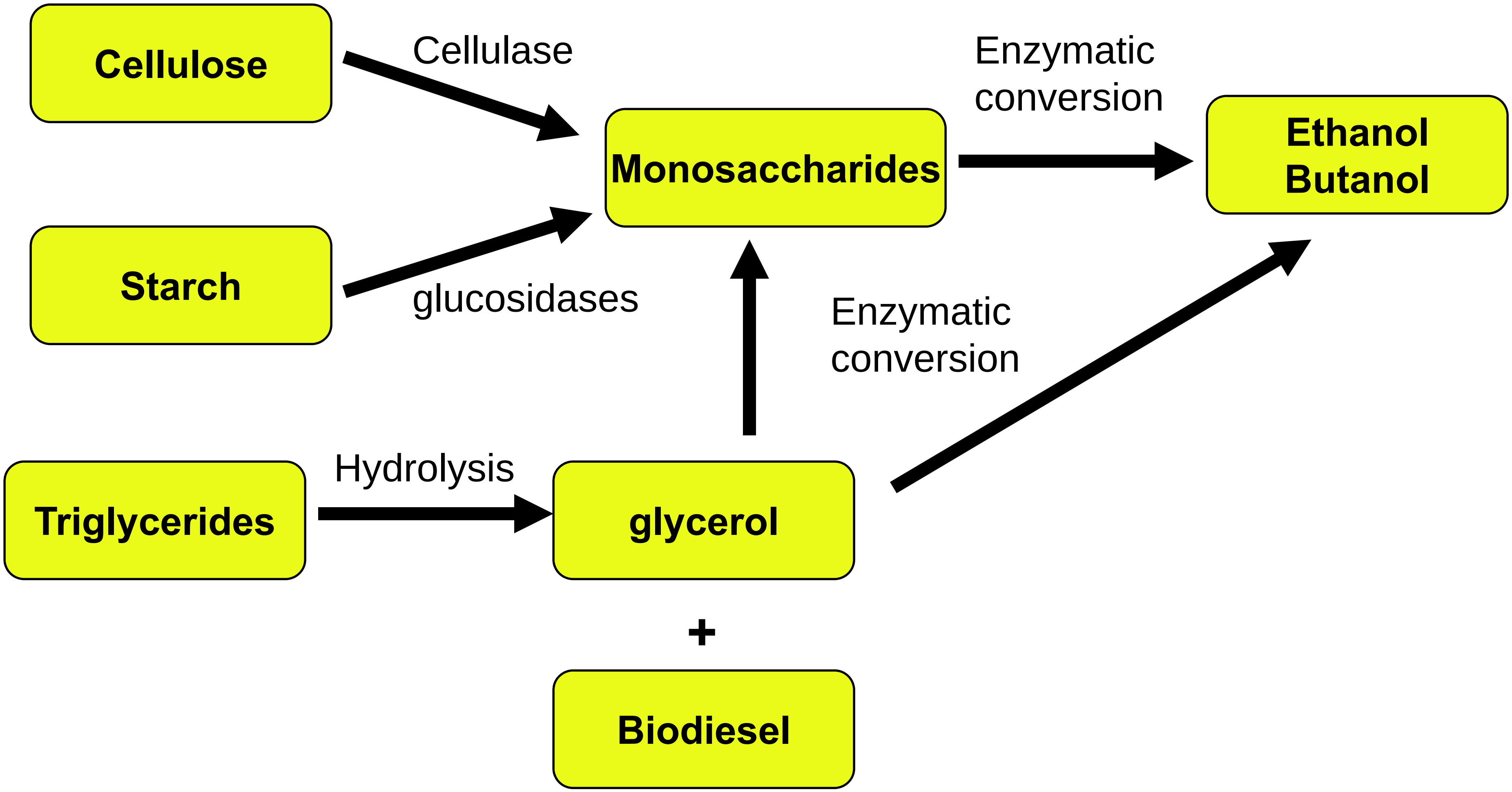
Biofuel Production



Biofuel production



The biochemistry of biofuel production



Potential raw materials for bioconversion

Potential raw materials for bioconversion to chemicals, solvents, and animal feed

Sugar containing	Starch containing	Lignocellulosic
Molasses	Cereal grains	Agricultural residues
Whey	corn	Forest residues
Fruit juices	sorghum	Wood sulfite waste
Sweet sorghum	barley	Fruit/vegetable waste
Sugarbeet	Wheat bran	Waste paper
Sugarcane	Root tubers	Municipal solid waste

Must remove lignin

The saccharide composition of different forest residues

Chemical composition of some forest residues

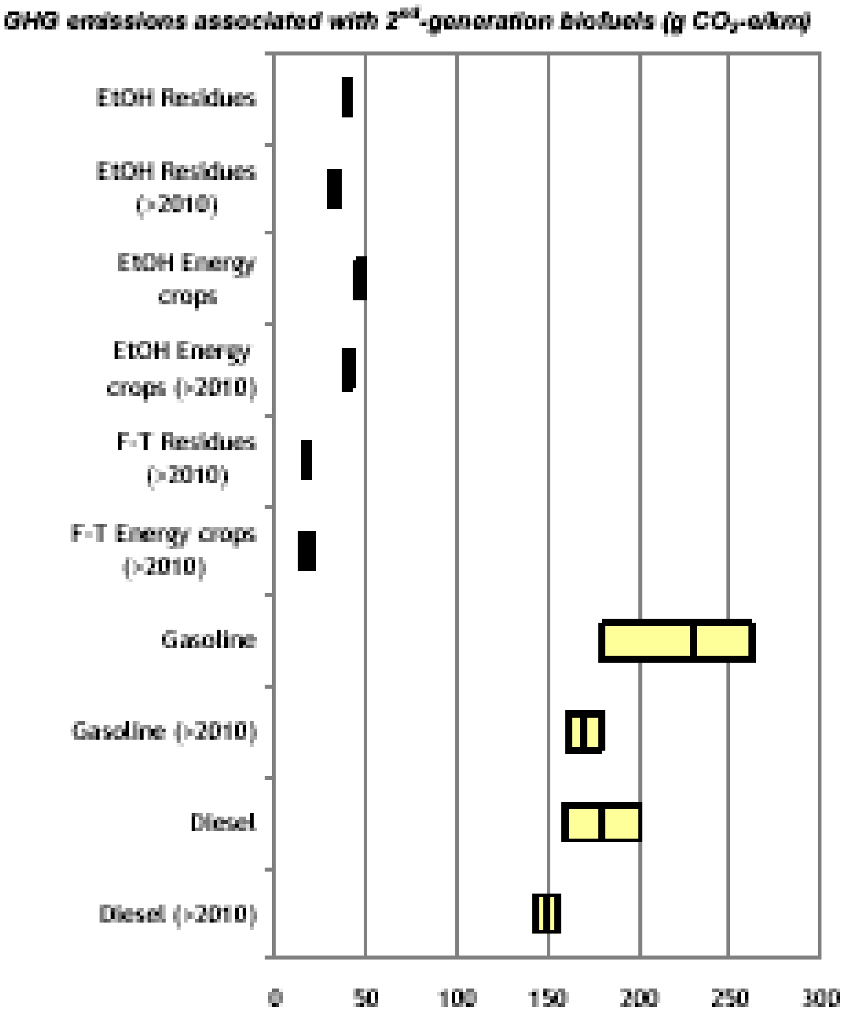
Residues	% Dry weight			
	Hexosans	Pentosans	Lignin	Ash
Hardwoods				
Aspen	50	28	15	0.3
American beech	47	20	23	0.2
Paper birch	41	26	25	1.0
Yellow birch	40	33	21	0.8
Cottonwood	46	19	24	0.6
Sugar maple	42	21	23	0.2
Silver maple	47	18	21	0.2
Red maple	39	33	23	1.0
Poplar	45	19	20	0.1
Black cherry	45	20	21	0.1
White oak	48	18	28	0.4
Sweet gum	40	24	19	1.0
Softwoods				
Balsam fir	42	11	29	0.5
Douglas fir	57	8	24	0.4
White fir	56	12	24	0.7
Eastern hemlock	43	10	32	0.4
Jack pine	41	10	27	0.1
White pine	44	11	28	0.1
Red pine	46	12	24	0.2
Black spruce	44	11	27	0.3
Red spruce	43	12	27	0.2
White spruce	44	10	27	0.3

Saccharide composition of different agricultural residues

Chemical composition of some agricultural lignocellulosic residues

Residues	% Dry weight			
	Hexosans	Pentosans	Lignin	Ash
Bagasse	33	30	29	4
Barley straw	40	20	15	11
Corn stover	42	39	14	2
Corn stalks	35	15	19	5
Cotton stalks	42	12	15	6
Groundnut shells	38	36	16	5
Oat straw	41	16	11	12
Rice straw	32	24	13	18
Rice husk	36	15	19	20
Sorghum straw	33	18	15	10
Wheat straw	30	24	18	10
Rye straw	37	30	19	4
Flax shives	35	24	22	3
Soybean stalks	34	25	20	2

Economic value of biomass-derived fuels



Fuel	***Energy Yield (Btu)**	**Net Energy (loss) or gain**
Gasoline	0.74	(26 percent)
Diesel	0.83	(17 percent)
Ethanol	1.34	34 percent (corn ethanol)
Biodiesel	3.20	220 percent

Bioconversion of agricultural/forest waste streams into biofuels

Agricultural and forest biomass (straw, stover, lignincellulose, hemicellulose, and biodiesel waste)

↓

Microbial metabolism and conversion

↓

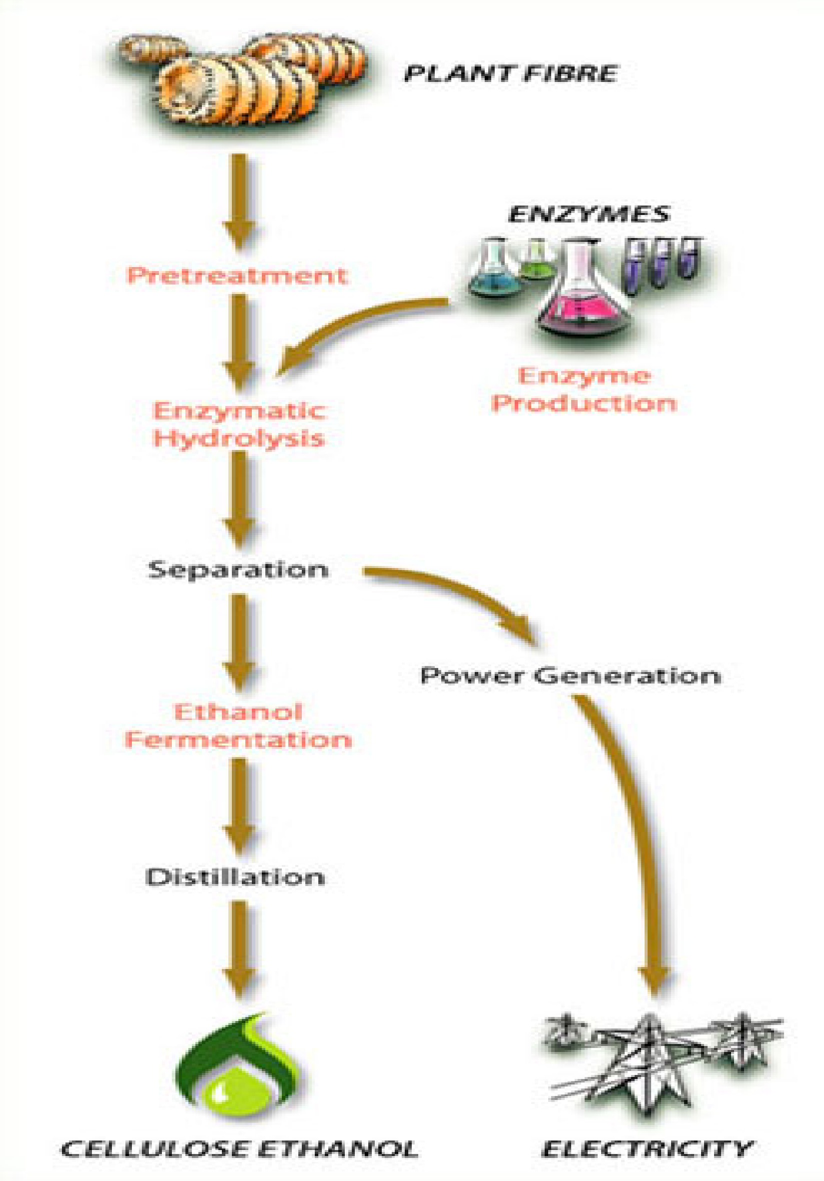
Simple sugars (glucose, fructose, disaccharides)

↓

Value-added products

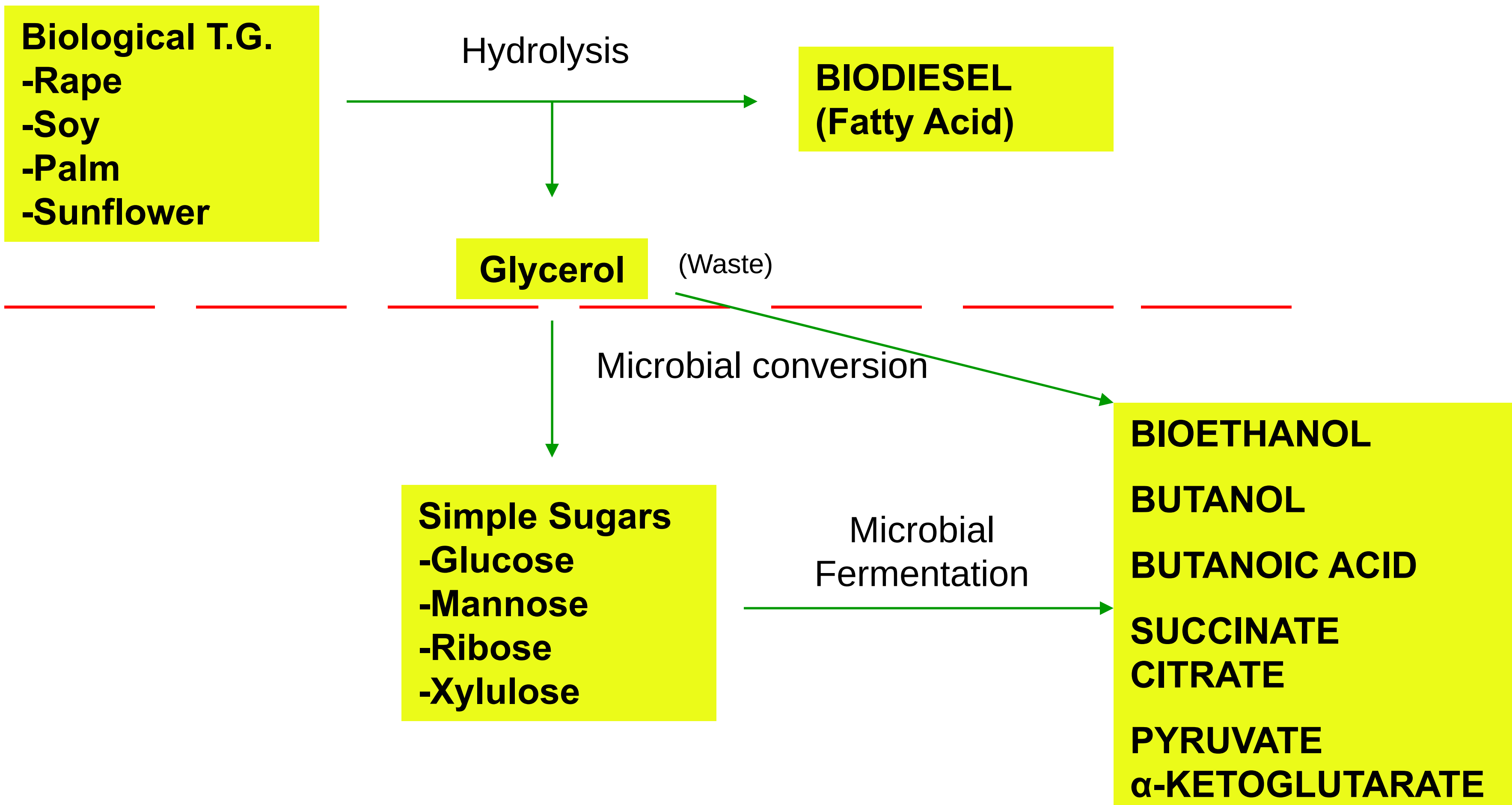
pyruvate citrate α-ketoglutarate succinate butanol ethanol butanoate

Conversion of cellulose into ethanol



IOGEN® CORPORATION

Glycerol waste can be used for biofuel production



Commercial value of products generated from glycerol

Glycerol	$7.60/100g
Succinic Acid	$21.80/100g
Citric Acid	$23.00/100g
Ethanol	$ 0.2/liter
Butanol	$ 1.2/liter
DHA	$32.20/100g
G3PDH	$38.60/100u
GDH	$124.00/100u
ADH	$379.50/100u

Our system

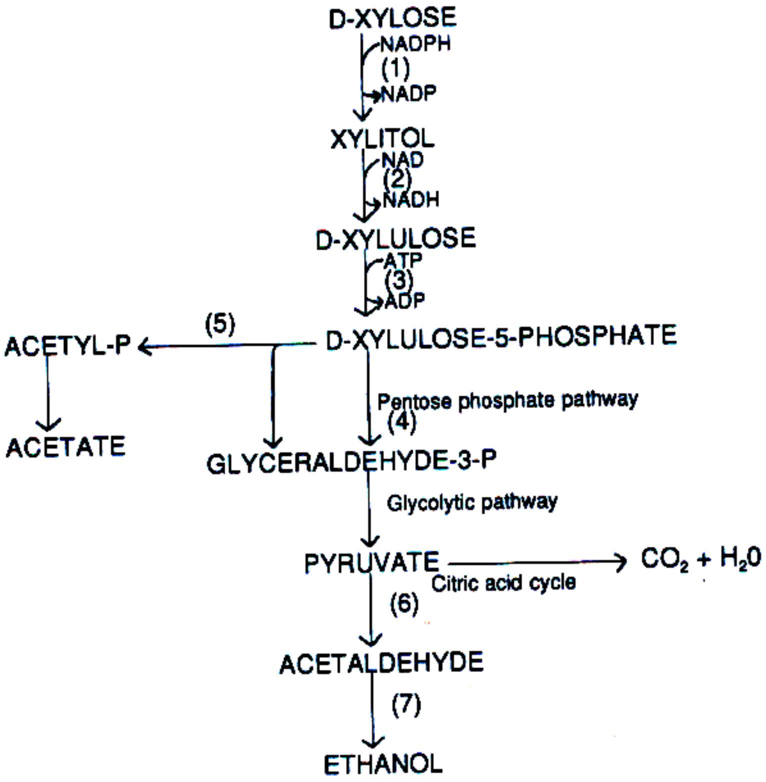
- Does not employ GMO
- Uses selective biological pressure directed evolution
- Low cost
- Low energy demand
- Minimizes toxic wastes and unusable materials (no sulfur containing compounds produced)
- No pretreatment of the biomass materials
- Highly specific process (tailored to our desire) with bioconversion into value-added products

TRANSLATES INTO GREEN TECHNOLOGY

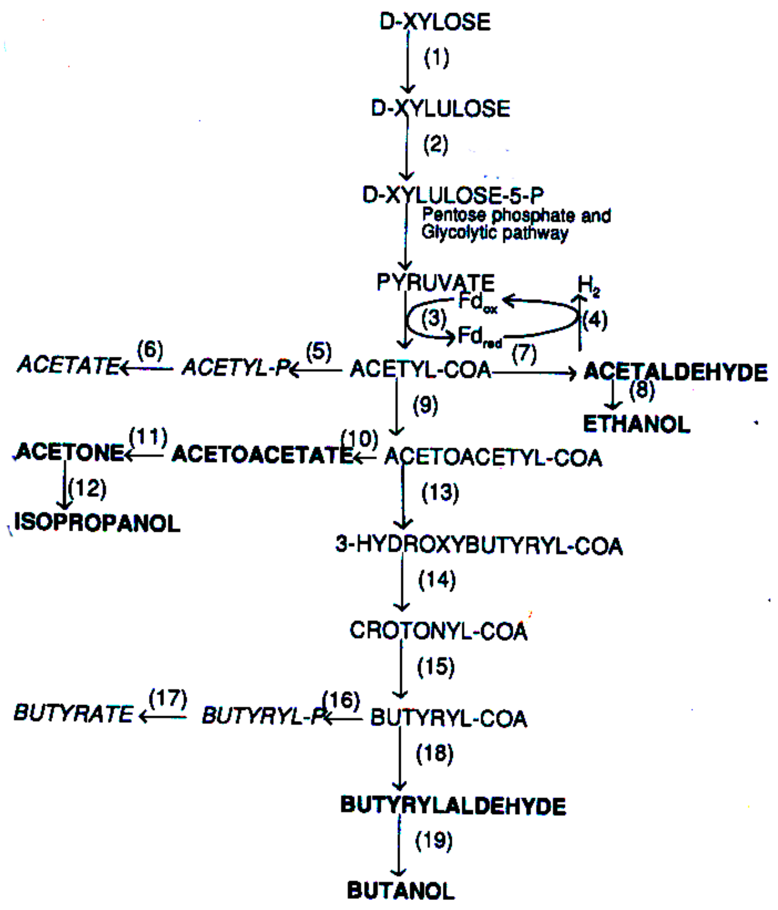
Xylose and Arabinose….content in hemicellulose

Residue	% of total hemicellulose sugars	
	Xylose	Arabinose
Agricultural		
Corn cobs	65	10
Corn stalks	71	9
Corn husk	54	13
Wheat straw	58	9
Soybean stalks	60	7
Soybean hull	27	13
Sunflower	61	2
Flax straw	65	13
Peanut hull	46	5
Sugarcane bagasse	60	15
Wood		
Maple	33	1
Alder	20	1
Birch	39	3
Beech	28	2
Poplar	24	3
English oak	26	1
Pine	9	2
Tamarack	7	2
Spruce	7	2
Balsam fir	5	1

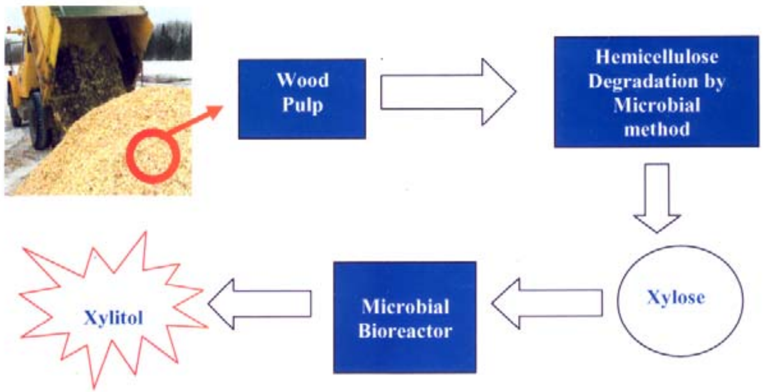
Ethanol production from xylose



Butanol production from xylose

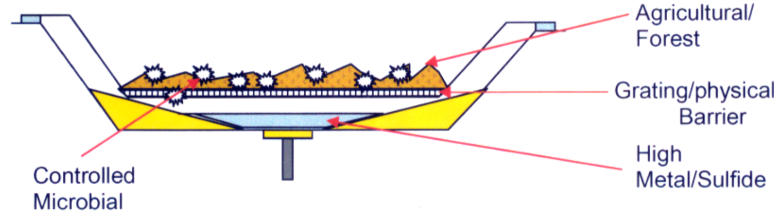


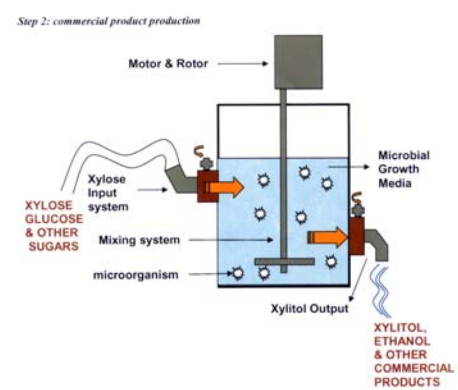
Wood bioreactors: conversion of xylose into commercial products



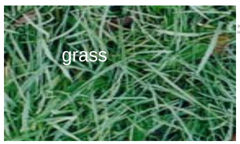
Microbial Bioreactor:

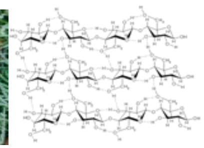
Step 1: degradation of wood products





Grass reactors and the production of simple sugars

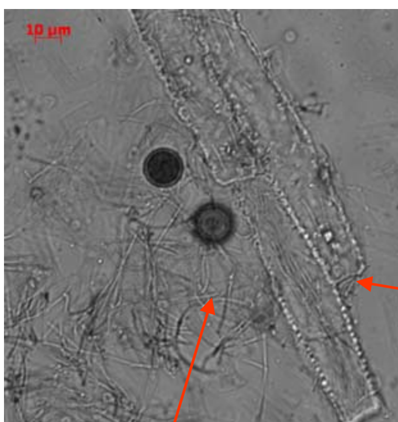




Enzymatic breakdown of cellulose and hemicellulose

D-glucose, cellobiose, cellotriose, cellotetraose

Enzymatic conversion into value-added products



Grass cells

microbes

Stat culture 1 day growth

Stat culture 7 days growth

Picture of Dan’s xylose culture

Consordium which gained confluency in 3 days in 20mM xylose

Cellulose and hemicellulose enriched media

↓ Enzymatic breakdown by acclimatized organisms

Remove liquor (contains xylose, arabinose, glucose, cellobiose,etc)

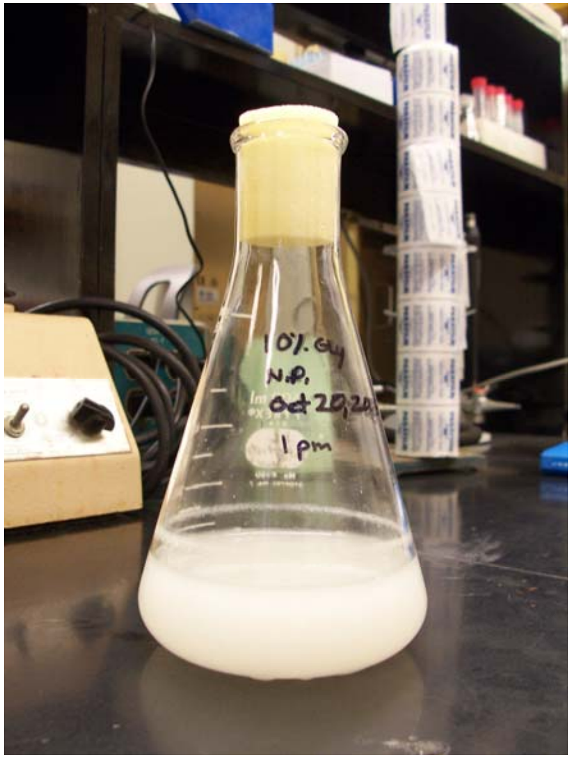
↓ Enzymatic break down by organisms acclimatized to xylose

Biofuels, bioethanol, butanol

Generation of value-added products and biofuels from agricultural/forestry waste streams

	Products	Value-added products
Grass	Simple carbohydrates (total conversion of **35%**)	Butanol, ethanol, commercial acids
Straw	Simple carbohydrates (total conversion of **37%**)	Butanol, ethanol, commercial acids
Peanut shell	Proteins (total conversion of **40%**)	Protein –enriched feed
Xylose	Simple carbohydrates (total conversion of **60%**)	Butanol, ethanol, commercial acids
Wood chips	Simple carbohydrates (total conversion of **20%**)	Butanol, ethanol, commercial acids

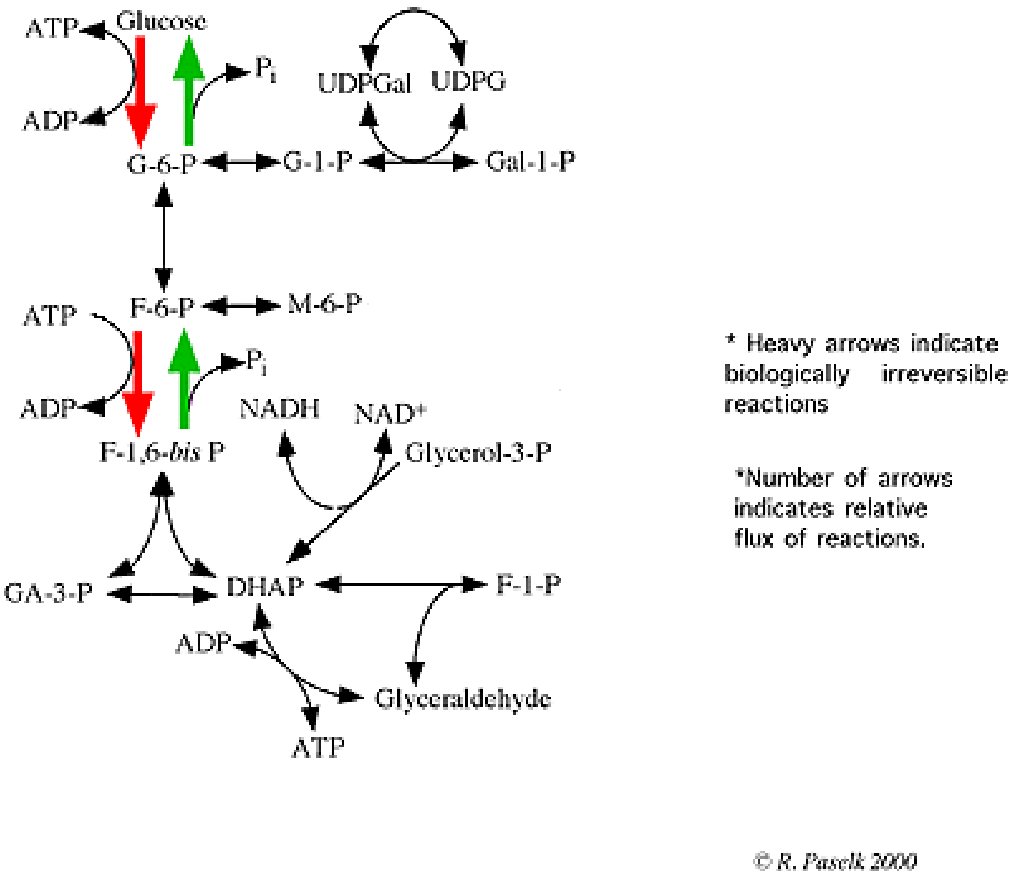
Glycerol reactors produce biofuels and value added products



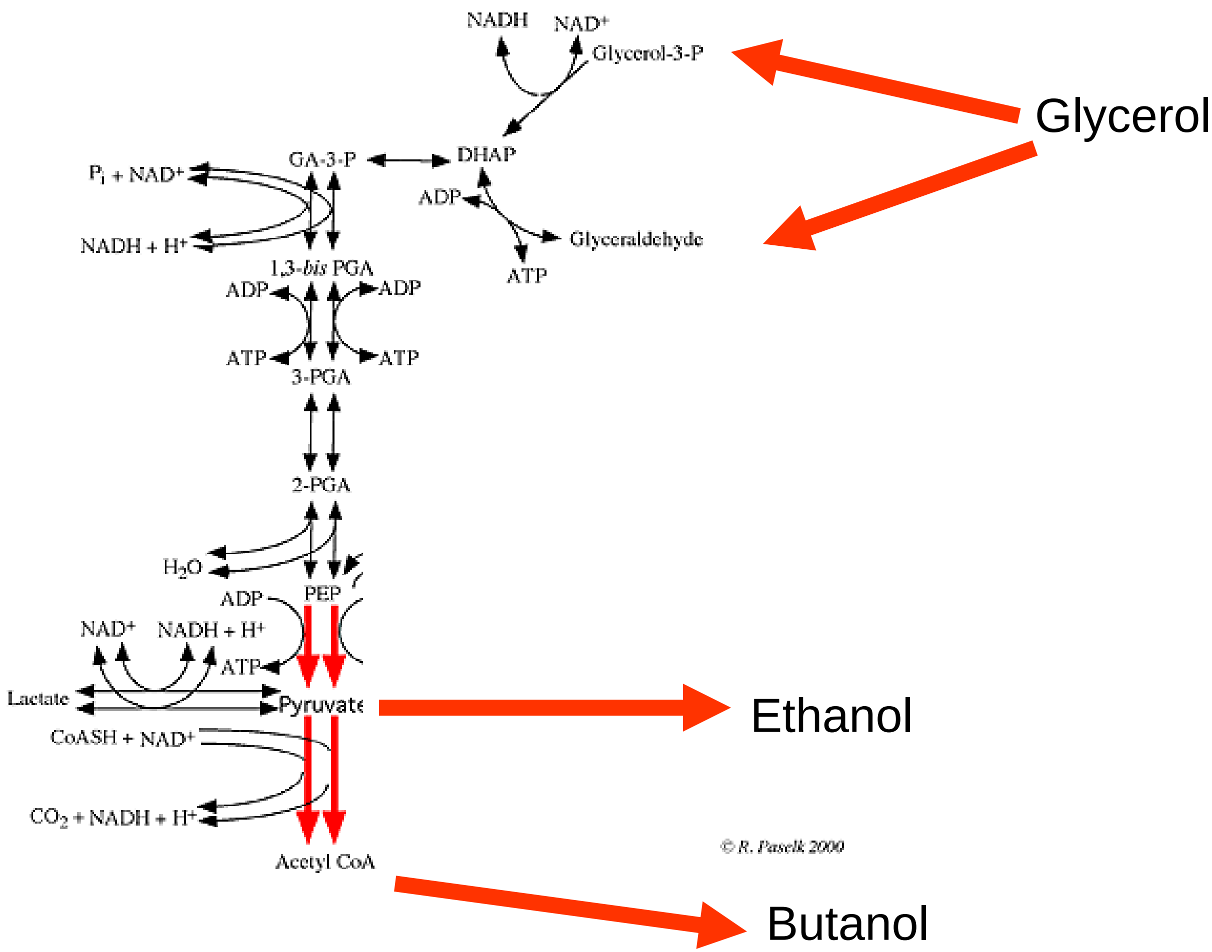
Grow in abnormally high concentration of glycerol
Produce both fermentable sugars and value added products
Use a nutrient stress system

Glycerol metabolism and biofuel production

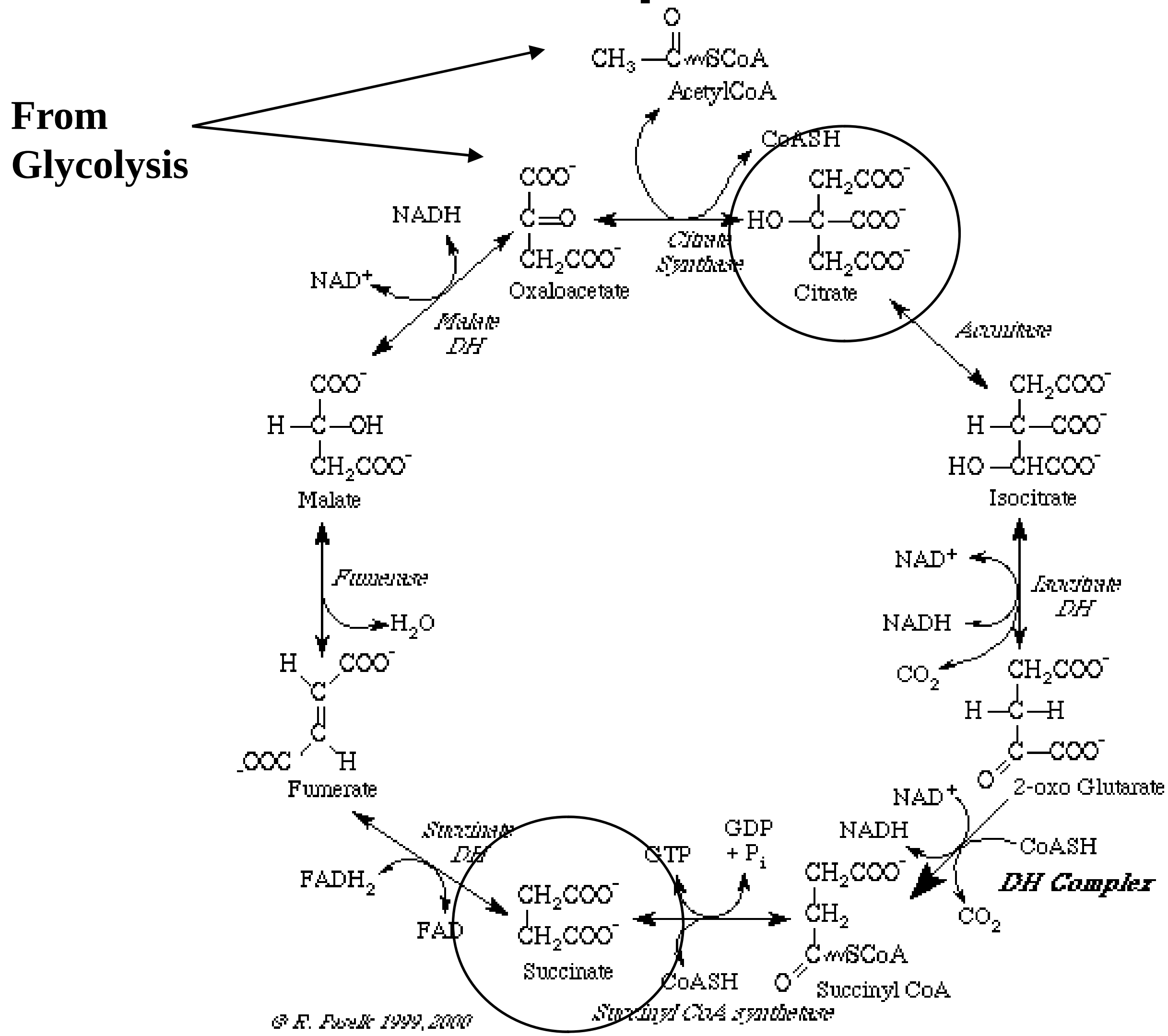
Glycerol →



Glycerol as a source of ethanol and butanol



The TCA cycle can provide value-added products



Glycerol conversion into biofuels

	Total carbohydrates (g/L)	Total Proteins (g/L)
control	4.9	2.7
Nutrient stress	7.7	2.8

Amount of carbohydrates and protein in the media were measured following a 70h growth period.
Total carbohydrates measured by Dubois assay
Total protein measured by Bradford assay

Proliferating medium. This has not been tested in the nonproliferating
But I have the samples frozen. Can still test for carbs before you leave.

Conversion of glycerol into keto acids

	Vol Glyc (ml)	Grams Glyc used by system	Grams Pyruvate produced	Total yield (glyc/pyr x 100%)
10% glycerol	20	17.6	3	17%
5% glycerol	10	7.2	2.6	36%

Density of glycerol = 1.261g/ml
200ml cultures were used
Glyc = Glycerol
Pyr = Pyruvate

NOTE: 10% culture max pyr production at 72h.
5% culture max pyr production at 24h
Cultures are nutrient stress non-proliferating medium

1% Nonproliferating converts glycerol to KG

	Grams of glycerol consumed	Grams of α-ketoglutarate produced	% conversion of glycerol to α-ketoglutarate (glycerol/KG)
Control (8h)	5.4	3.4	63%
Nutrient stress (8h)	5.6	5.1	91%

This is in non-proliferating media containing 1% v/v glycerol. **No pyruvate was detected.**

Max KG detected at 8h incubation.

Microbial Systems For The Production of Biofuels and Value-Added Products