Valorization of biomass by fermentation

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Fermentation in biorefinery

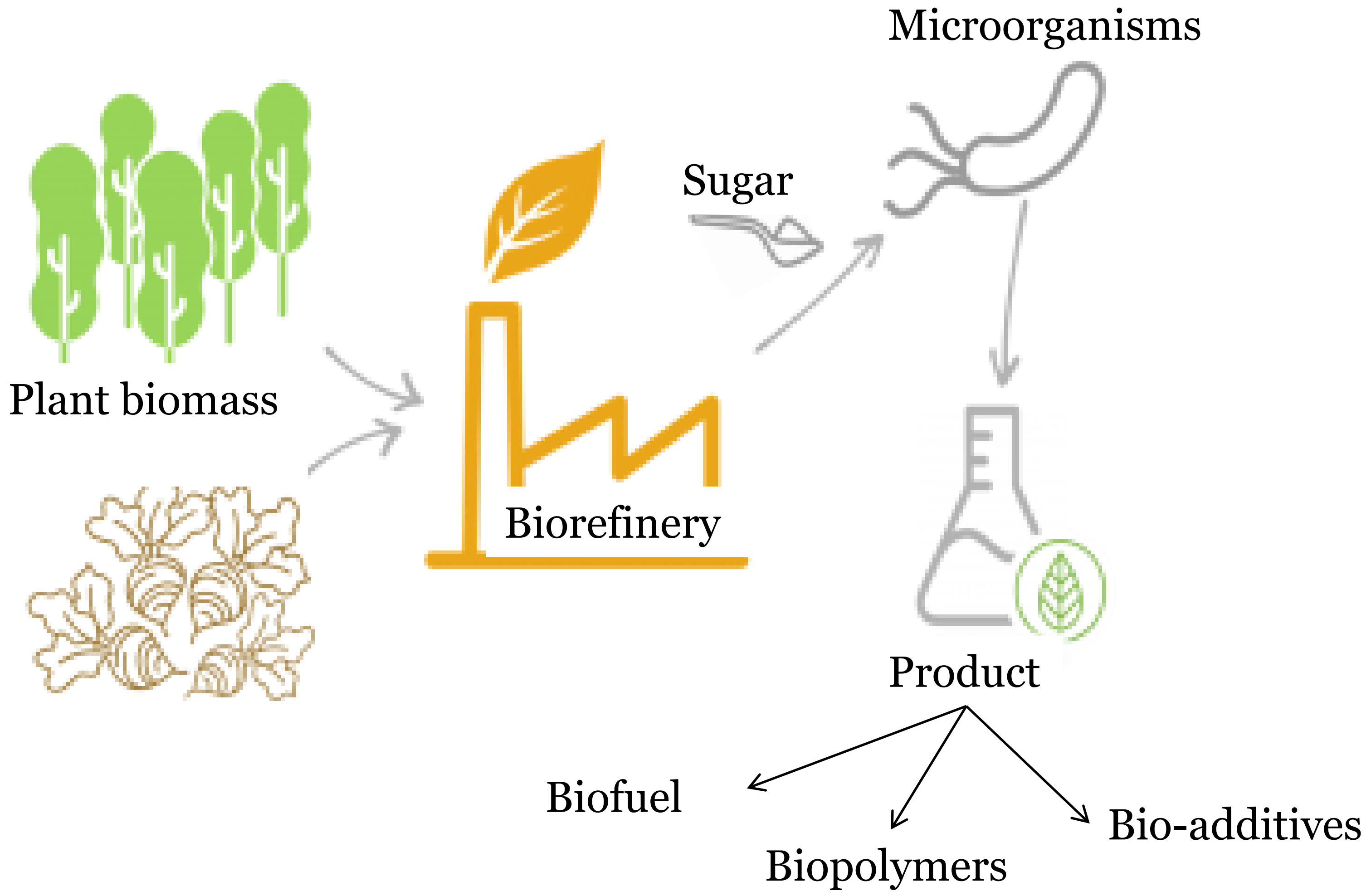
- What is the role of fermentation in the biorefinery based industry ?
  - What is fermentation
  - What is the role of fermentation
  - How does it work
  - How can fermentation be usefull to valorise biomass
  - Microalgae as a source of fermentable substrate

Fermentation in everyday life






Fermentation in biorefinery

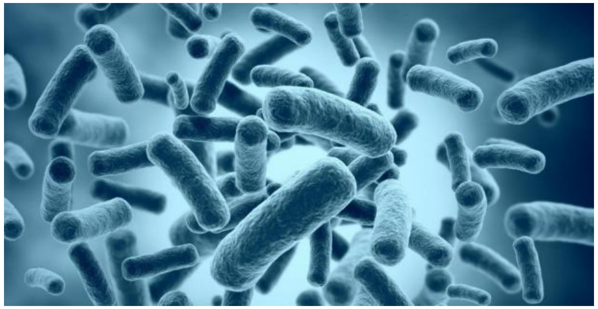


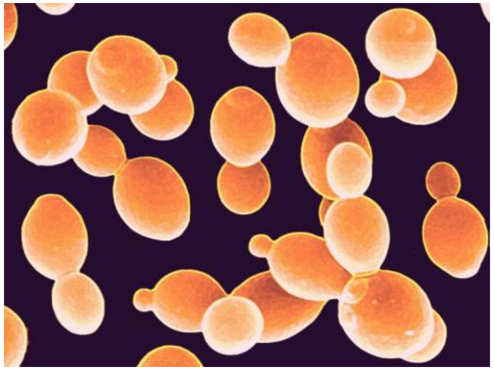
Fermentation and microbiology

→ Pasteur : the result of life without air

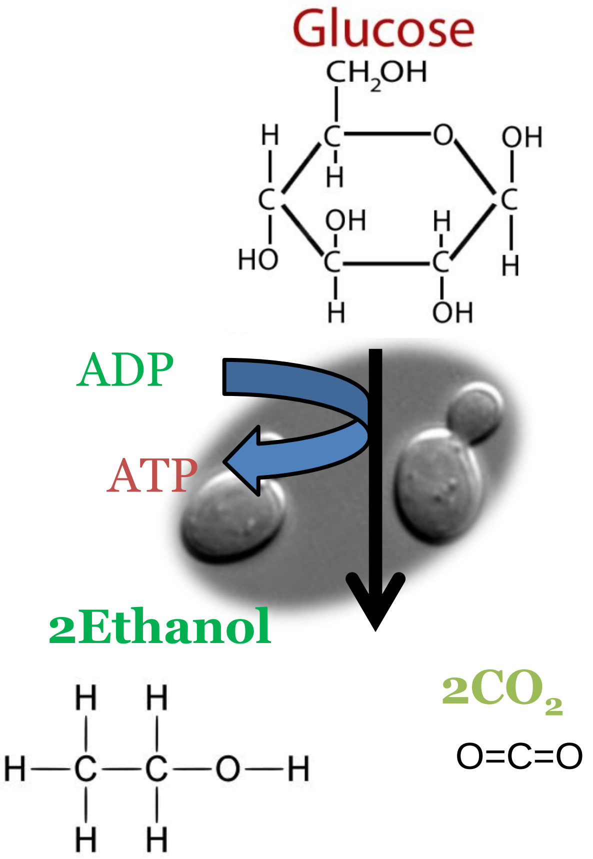
→ Energy-yielding anaerobic metabolic process in which organisms convert nutrients (typically carbohydrates) into alcohol and acids (such as lactic acid and acetic acid).

→ Modern definition : the breakdown of complex molecules in organic compounds, caused by the influence of a ferment





Fermentation biotechnologies
→ Microorganisms, natural factories



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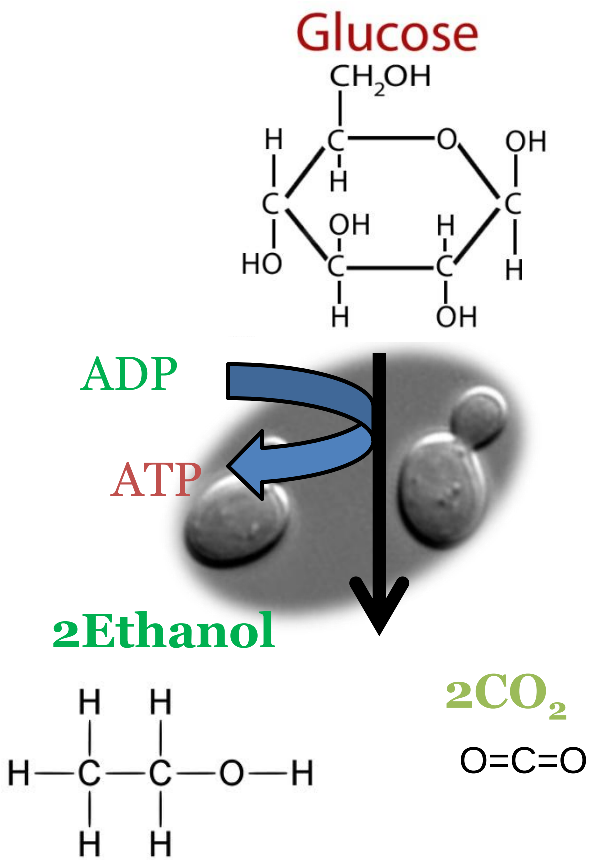

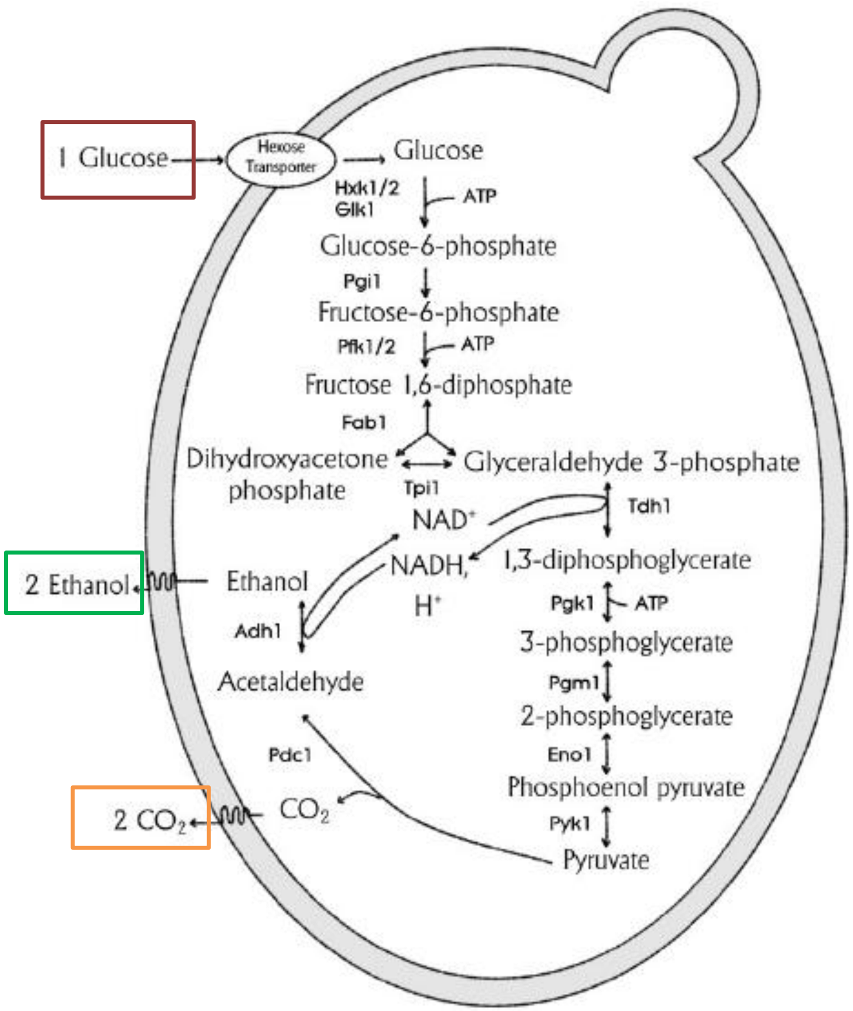

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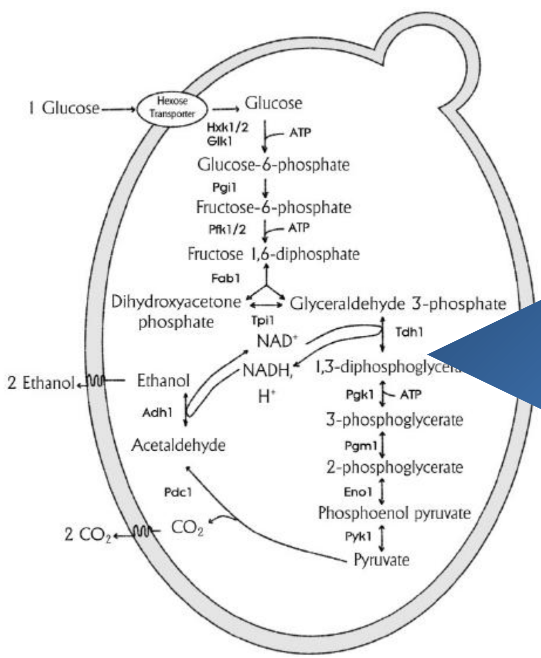
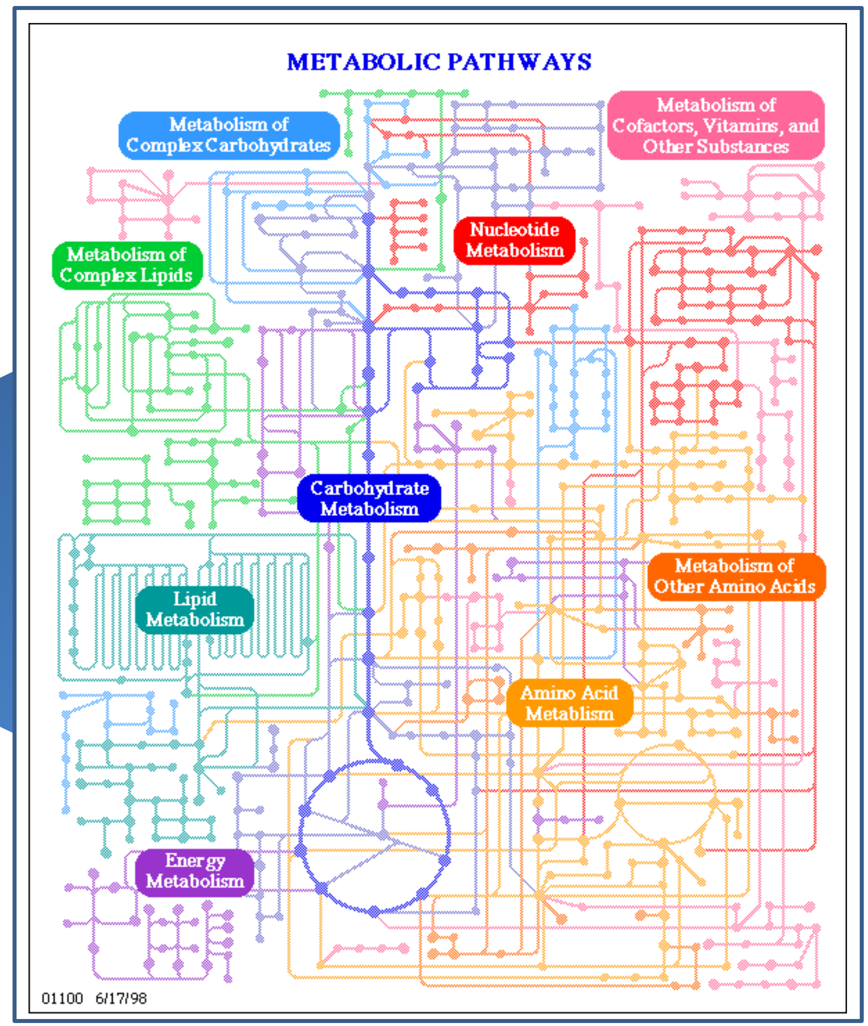
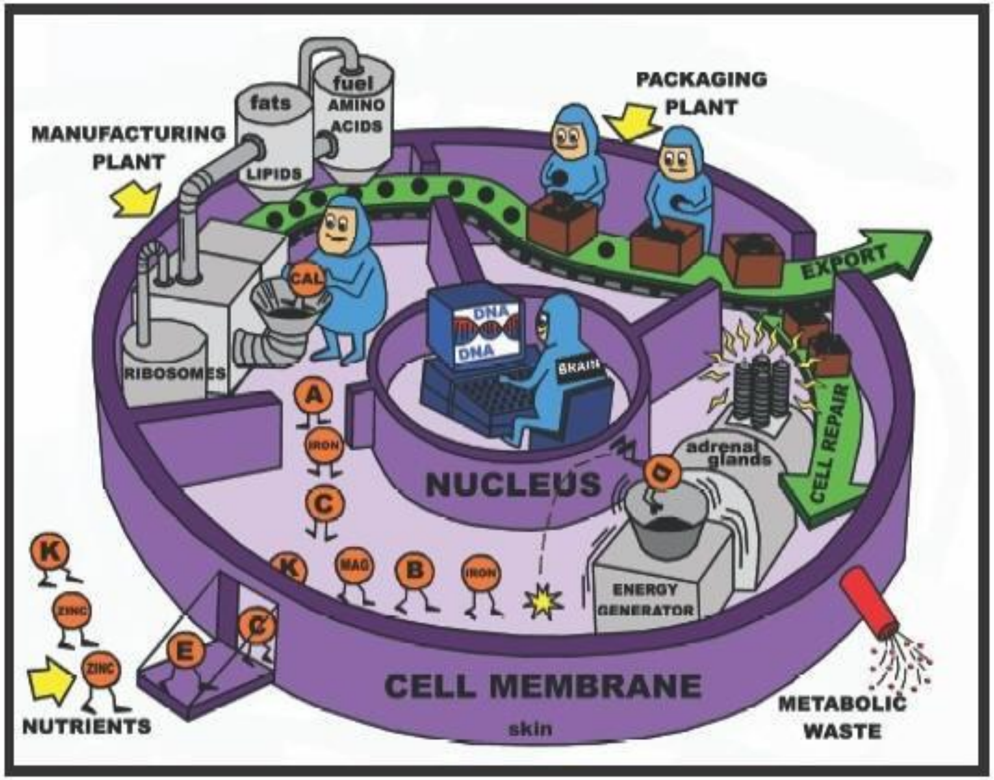




Fermentation biotechnologies → Microorganisms, natural factories

→ Use of biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use
(United Nations Convention on Biological Diversity)



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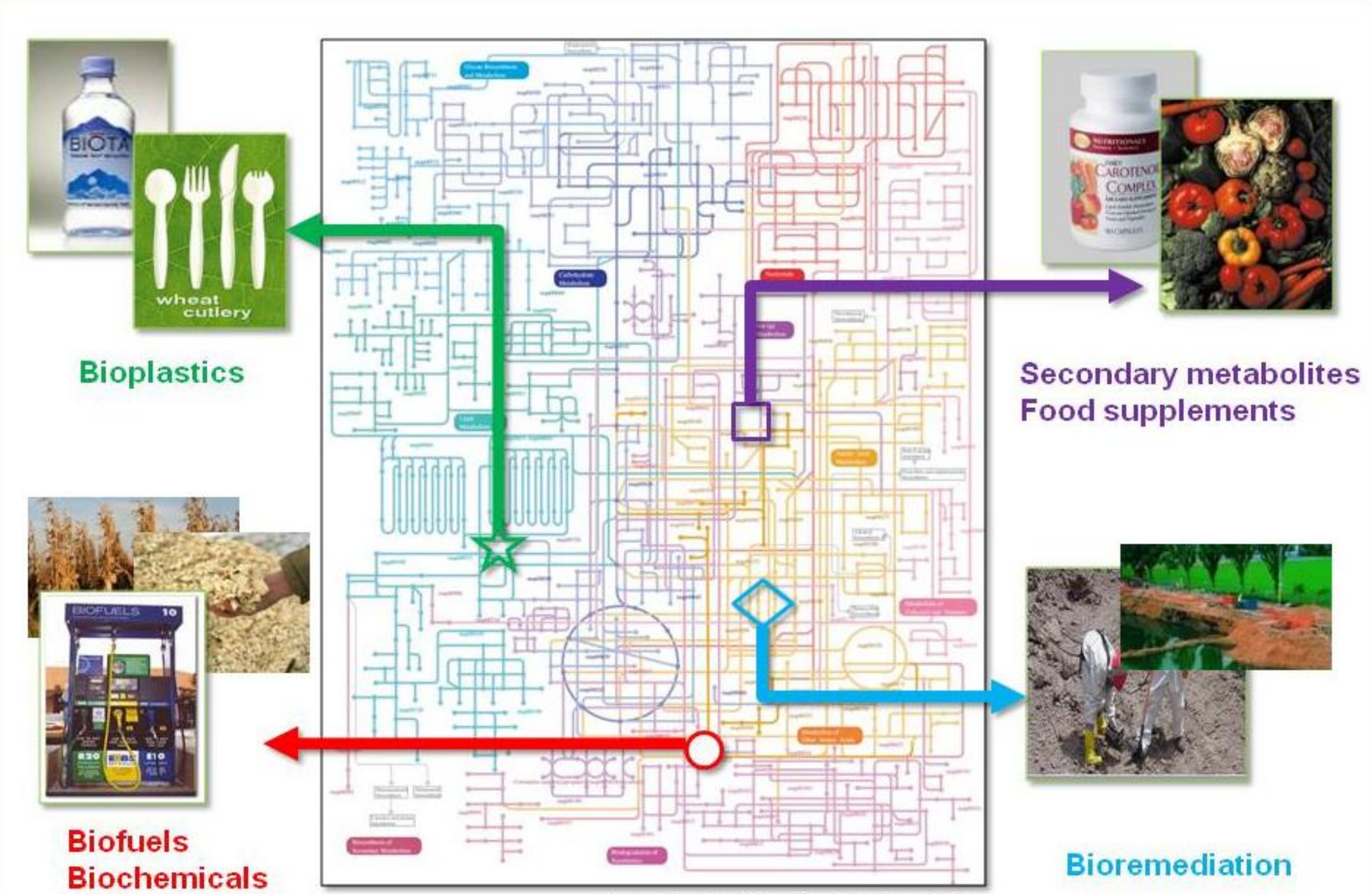


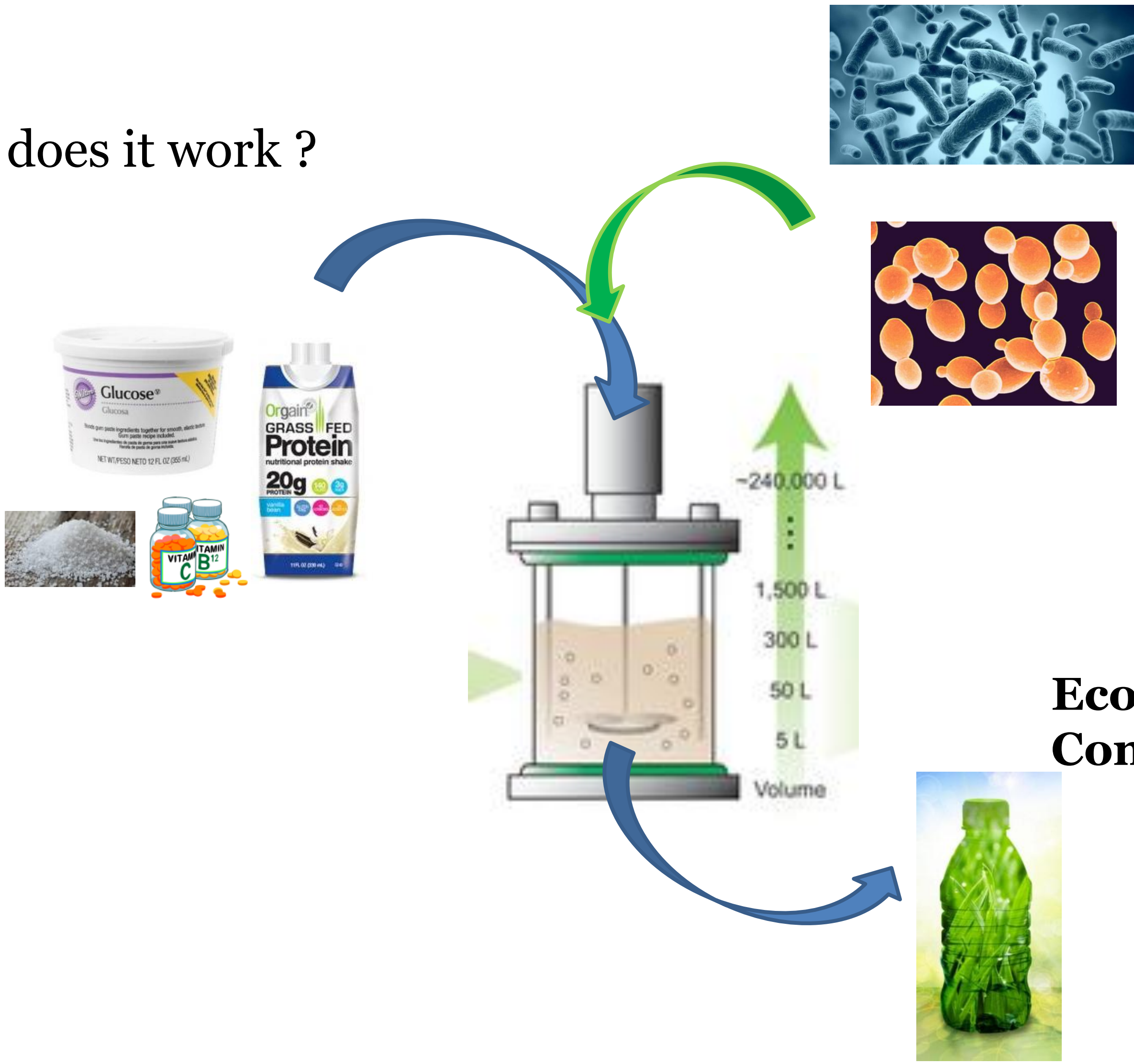
Kanehisa et al, Nucleic Acids Res, 2008.

Fermentation and the bioeconomy

- How does it work ?

Fermentation and the bioeconomy

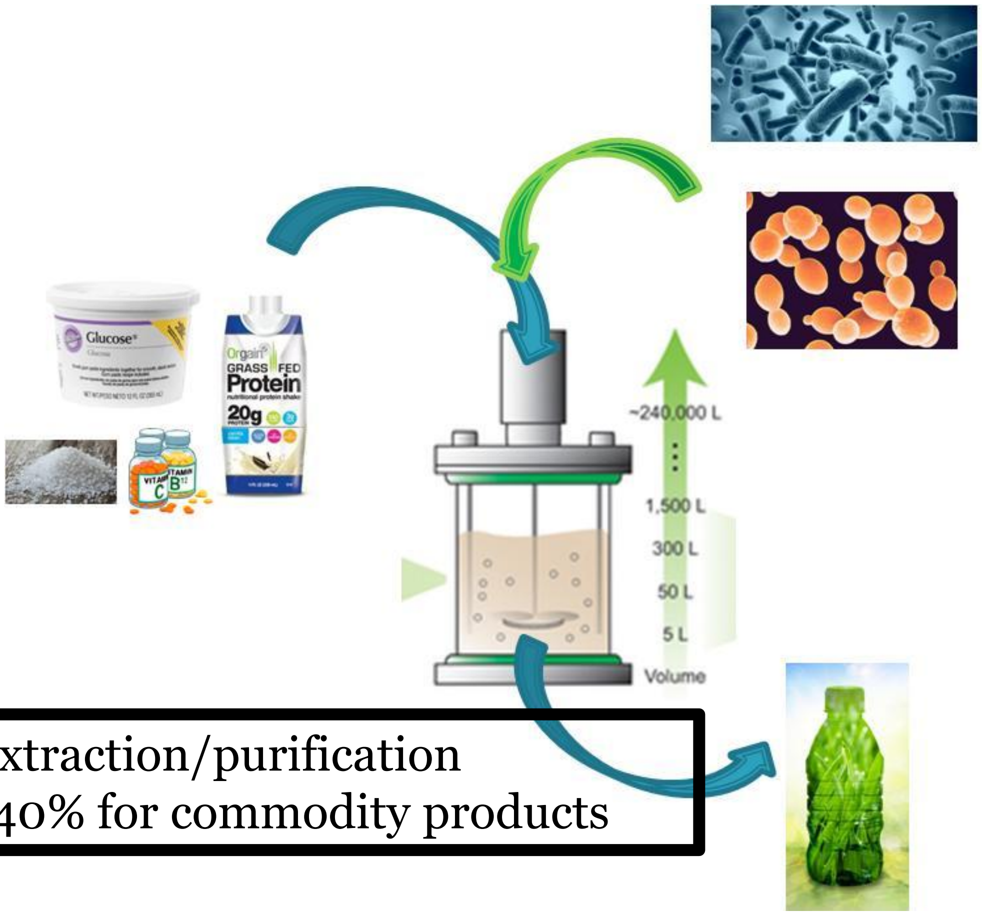
- How does it work ?



Economic Competitivness ?

Fermentation and the bioeconomy

- How to make it competitive

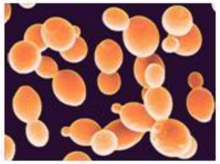


DSP: extraction/purification
→ 20-40% for commodity products

Fermentation and the bioeconomy

- How to make it competitive

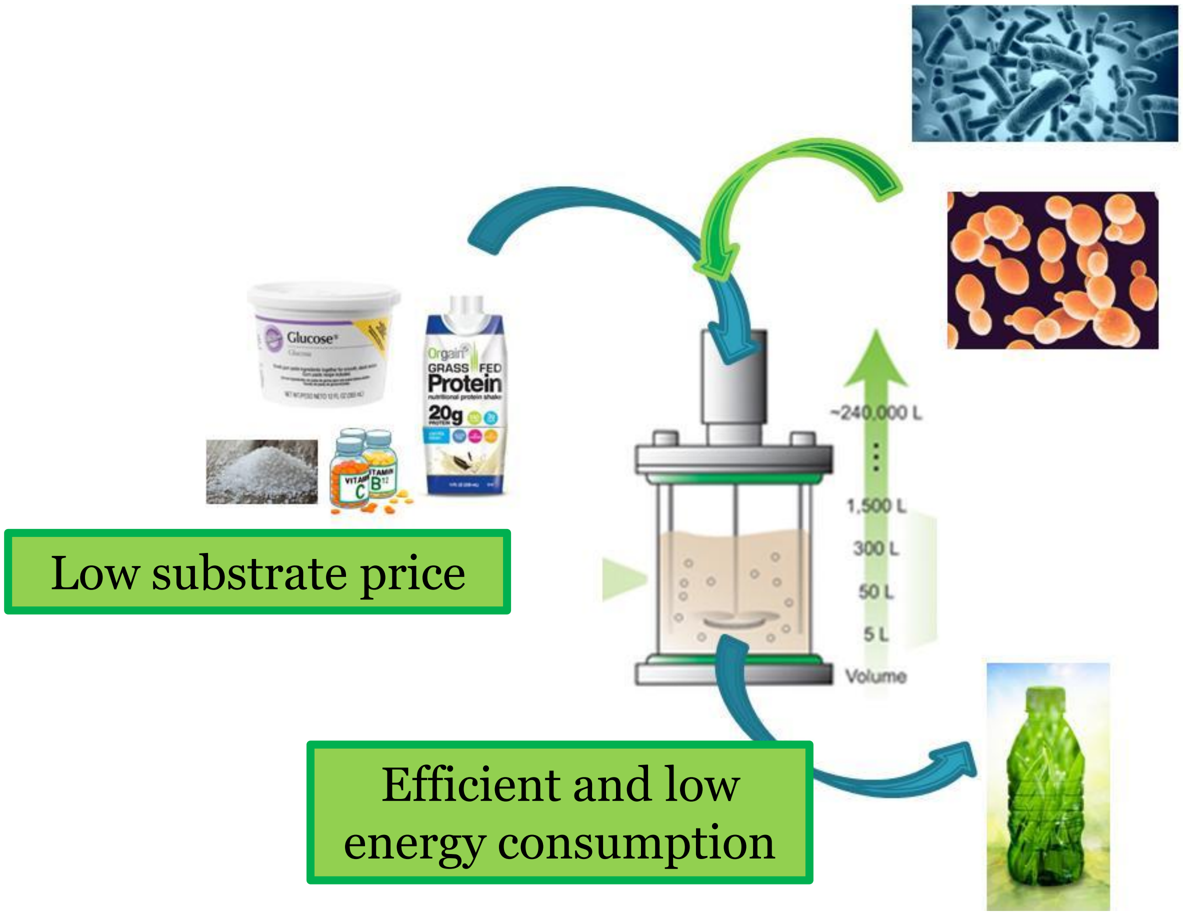
Feedstock
→ Up to 75% of
total process cost



DSP: extraction/purification
→ 20-40% for bulk products

Fermentation and the bioeconomy

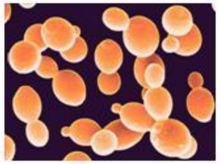
- How to make it competitive

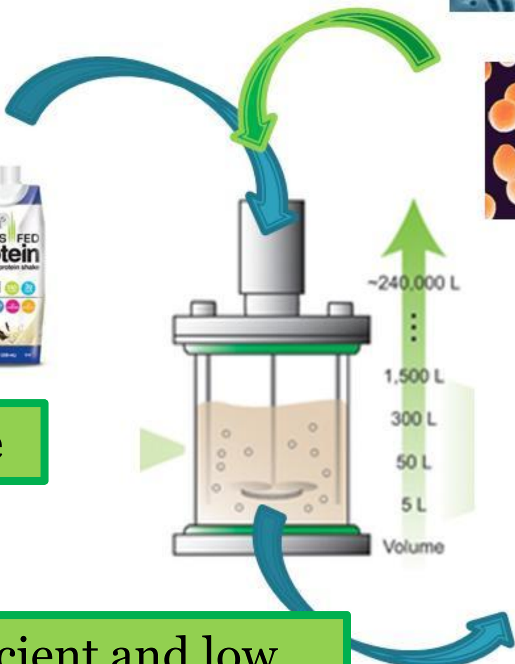


Fermentation and the bioeconomy

- How to make it competitive

→ High product yield (g product/g substrate)
→ High productivity (g/L/h)
→ High product titre (g/L)





Low substrate price

Efficient and low energy consumption

Fermentation and the bioeconomy

- How to make it competitive

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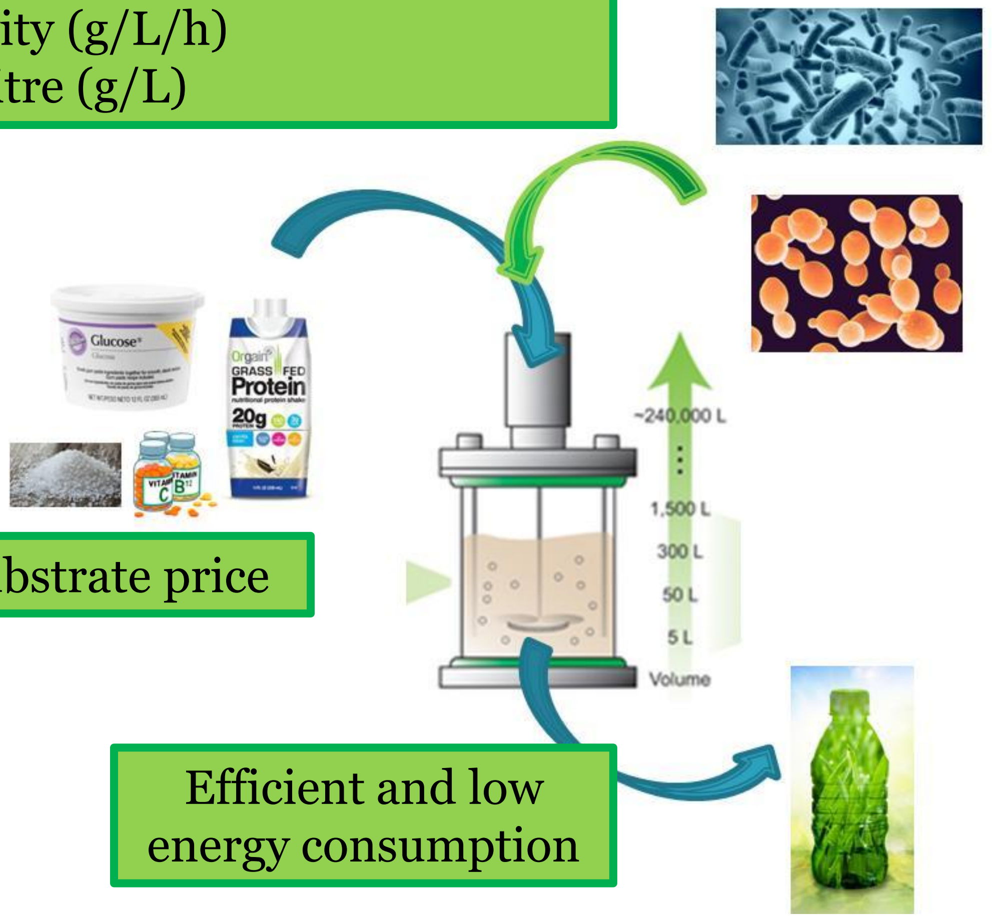


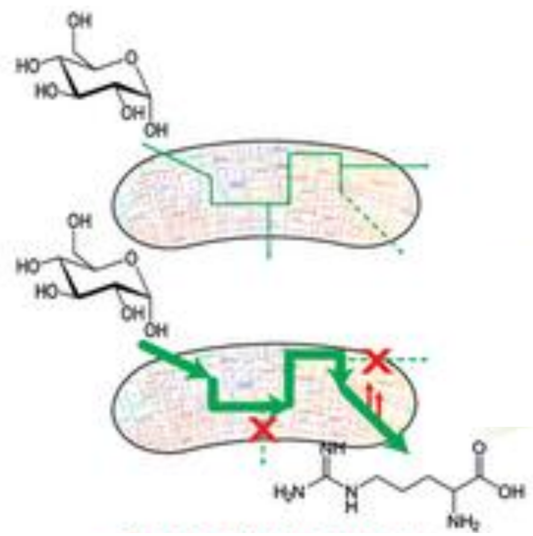
Strain development

Low substrate price

Efficient and low energy consumption

Fermentation biotechnologies → Systems metabolic engineering and synthetic biology

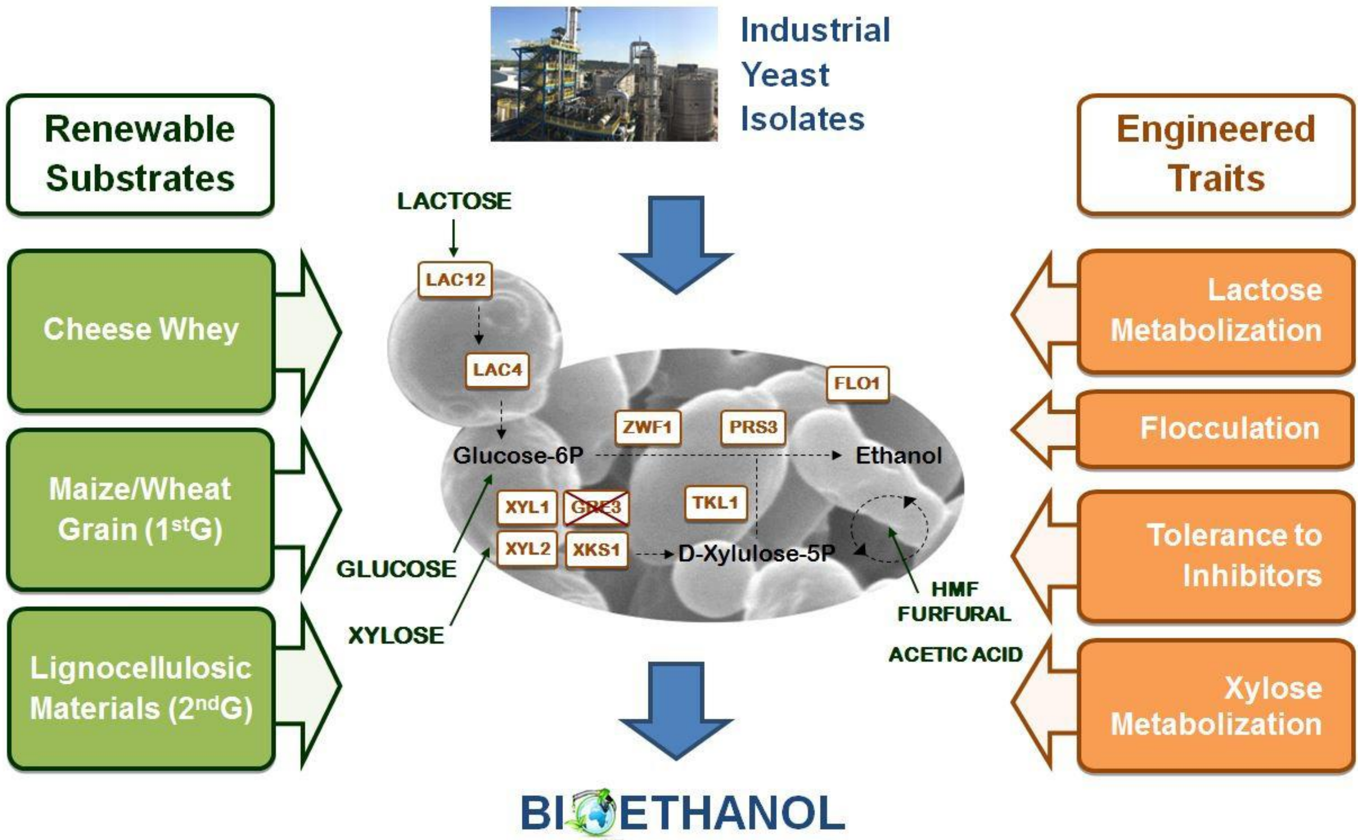
Strain development



50-300 person-years → 10 person years

Design of new metabolic pathways

Fermentation biotechnologies
→ Synthetic biology

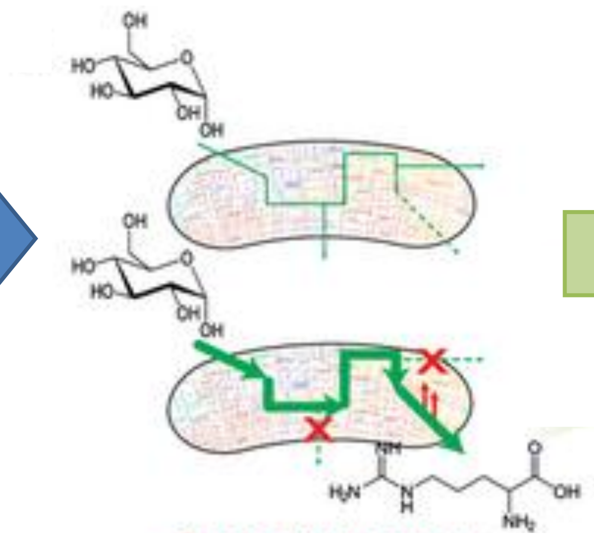


L. Domingues – *Centre of biological engineering*

Fermentation biotechnologies → Systems metabolic engineering and synthetic biology

Strain development

50-300 person-years → 10 person years



Design of new metabolic pathways

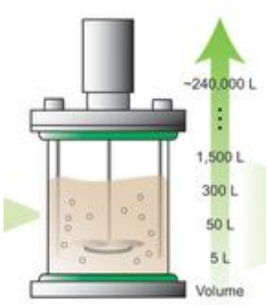
Biobased process

Sensitivity to biomass derived inhibitors

Societal acceptance of GMO

Fermentation and the bioeconomy

- Advantages and drawbacks

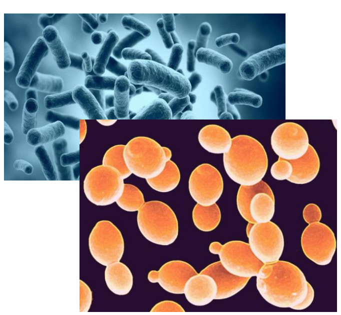


Mild conditions
Low temperature
Neutral to slightly acidic pH

High water content
DSP

Renewable substrate

Substrate cost



Biobased process

Strain development
Sensitivity to biomass derived inhibitors

Increasing product titer
Water recycling
Evolution of extraction technics
New sources of fermentable products
Synthetic biology and system biology development

Fermentation of plant biomass

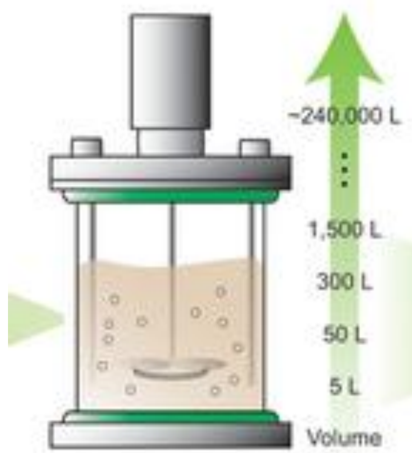
Feedstock

Low cost sustainable substrate for existing fermentation processes

Biorefinery
Adding value to agro-industrial waste

Agro-industrial waste
→ Cheese whey
→ Sugarbeet molasses
→ Wheat maltose
→ Starch

Competition with food and feed



Agro-industrial waste
→ Straw
→ Sawdust
→ Wood pulping products

Recalcitrance
Inhibitors

Fermentation at the industrial level

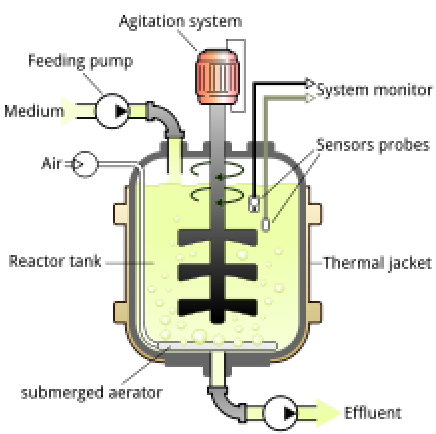
Product	Production organism	Status	Feed stock	Companies	Reference
Chemicals					
Acetone	*Clostridium acetobutylicum*	Commercialized	Corn	Green Biologics	www.greenbiologics.com
Citric acid	*Aspergillus niger*	Commercialized			
Succinic acid	*E. coli*	Commercialized	Corn sugars	BioAmber	www.bio-amber.com
	E. coli	Commercialized	Sucrose	Myriant	www.myriant.com
	S. cerevisiae	Commercialized	Starch, sugars	Reverdia	www.reverdia.com
	B. succiniproducens	Commercialized	Glycerol, sugars	Succinity	www.succinity.com
Lactic acid		Commercialized	Corn sugars + more	NatureWorks	www.natureworksllc.com
Itaconic acid	*Aspergillus terreus*	Commercialized	Biochemistry	Qingdao Kehai	www.kehai.info/en
1,3-PDO	*E. coli*	Commercialized	Corn sugars	DuPont Tate & Lyle	www.duponttateandlyle.com
1,3-BDO		Demonstration		Genomatica and Versalis	www.genomatica.com
1,4-BDO	*E. coli*	Commercialized	Sugar	Genomatica and DuPont Tate & Lyle	www.genomatica.com
1,5-PDA		Commercialized	Sugar	Cathay Industrial Biotech	www.cathaybiotech.com
3-HP		Commercialized		Metabolix	www.metabolix.com
		Demonstration		Novozymes and Cargill	www.novozymes.com
Isoprene	*S. cerevisiae*	Preparing	Sugar, cellulose	Amyris, Braskem, Michelin	www.amyris.com
		Preparing		DuPont, Goodyear	www.biosciences.dupont.com
Isobutene	*E. coli*	Demonstration	Glucose, sucrose	Global Bioenergies	www.global-bioenergies.com
Adipic acid	*Candida* sp.	Demonstration	Plant oils	Verdezyne	www.verdezyne.com
Sebacic acid	*Candida* sp.	Demonstration	Plant oils	Verdezyne	www.verdezyne.com
DDDA	*Candida* sp.	Under commercialization	Plant oils	Verdezyne	www.verdezyne.com
Squalene	*S. cerevisiae*	Commercialized	Sugarcane	Amyris	www.amyris.com
PHA	*E. coli*	Commercialized		Metabolix	www.metabolix.com
Fuels					
Ethanol	*S. cerevisiae, Zymomonas mobilis, Kluyveromyces marxianus*	Commercialized	Sugarcane, corn sugar, lignocellulose	Many	
	Clostridium autoethanogenum	Demonstration	Flue gas	Lanzatech	www.lanzatech.com
Farnesene	*S. cerevisiae*	Commercialized		Amyris	www.amyris.com
Butanol	*Clostridium acetobutylicum*	Commercialized	Corn	Green Biologics	www.greenbiologics.com
Isobutanol	Yeast	Commercialized	Sugars	Gevo	www.gevo.com

1,3-PDO, 1,3-propanediol; 1,3-BDO, 1,3-butanediol; 1,4-BDO, 1,4-butanediol; 1,5-PDA, 1,5-pentanediamine; 3-HP, 3-hydroxypropionic acid; DDDA, dodecanedioic acid; PHA, polyhydroxyalkanoates.

Gustavsson & Lee. *Microbial biotechnology*- **2016**

How to make industrial fermentation successfull ?

Microorganism
→Selection
→ Engineering



Pretreatment

Down stream processing

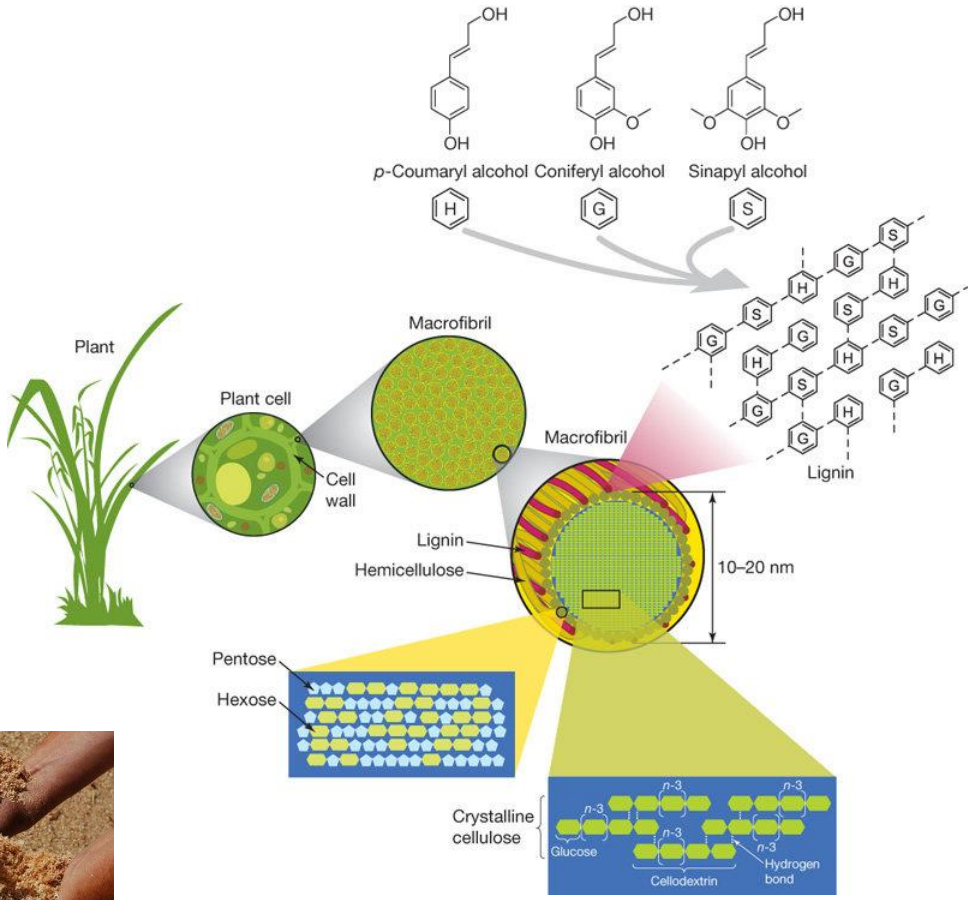
Fermentation as a way to valorize plant biomass

- Most abundant biomass on earth

→ Lignin : 30%

→ Cellulose : 40% (Polymer of glucose β 1-4)

→ Hemicellulose : 26% (Polymer of C6 and C5 carbohydrates : Xylose, Arabinose, Mannose)



Fermentation as a way of agro-industrial waste valorisation

- Accessing valuable parts

✓ High recalcitrance of the structure

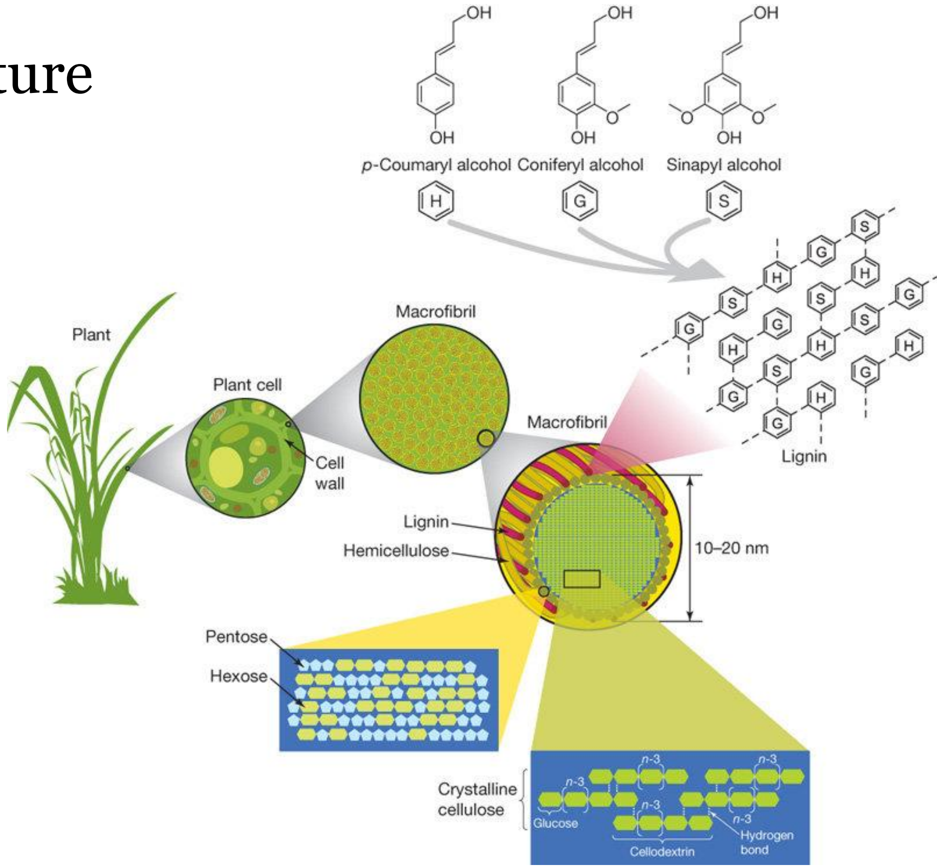
✓ Pretreatment needed

→ Deconstruction of polymers to access free sugars

→ Avoide sugar degradation

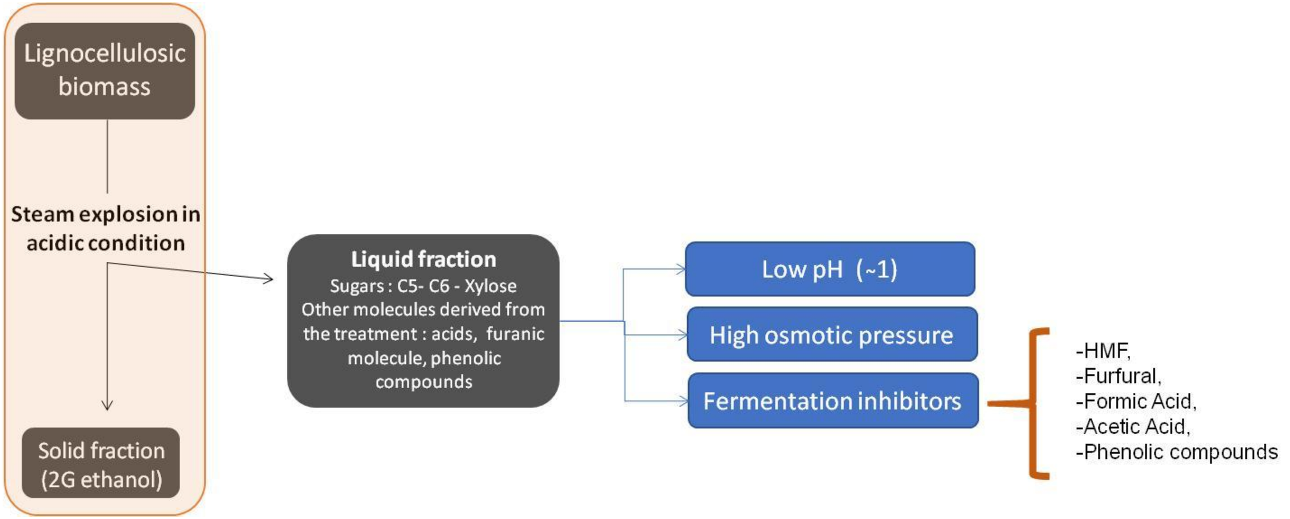
→ Low energy consumption

→ Low cost

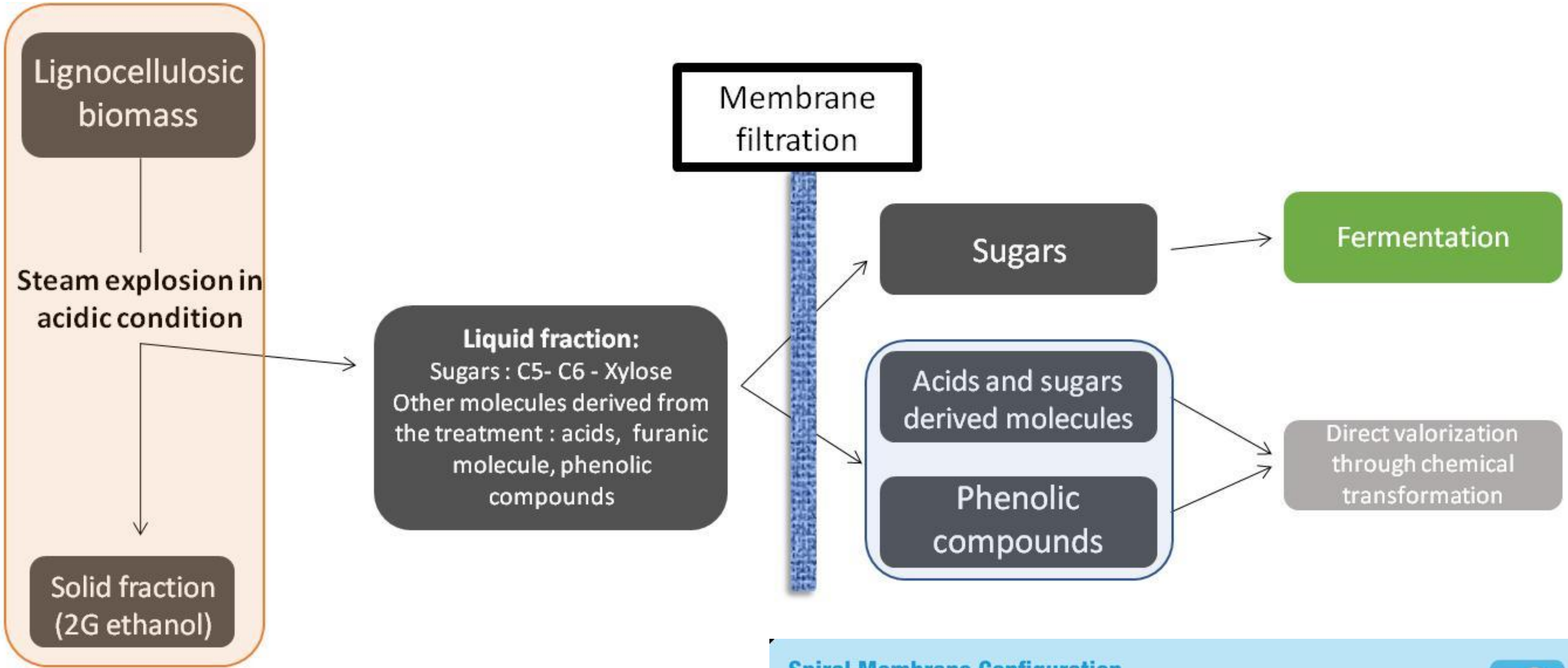


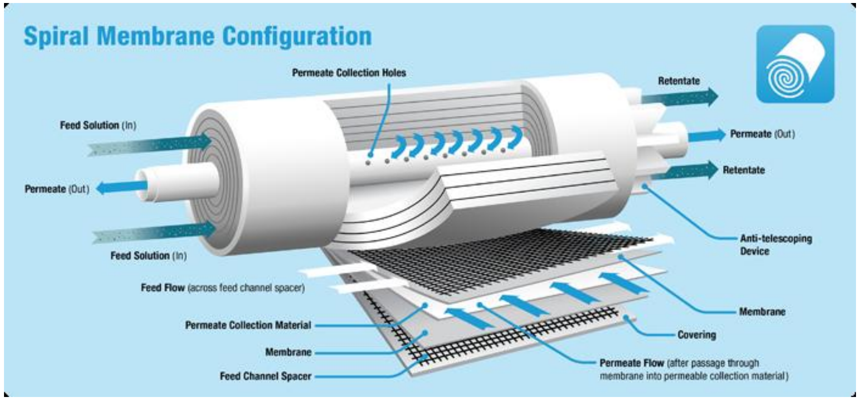
Pretreatments of biomass
→Steam explosion in acidic condition

- Short time vapor-phase cooking (~ 200°C), 2-3% H_2SO_4
- Removing the hemicellulosic barrier to make cellulose accessible to enzymatic saccharification
- Hydrolysis of hemicellulose
- Lignin → combustible, reserve of energy
- Liquid fraction → Sugars and breakdown products



Pretreatments of biomass
→ Separation of molecules by nanofiltration





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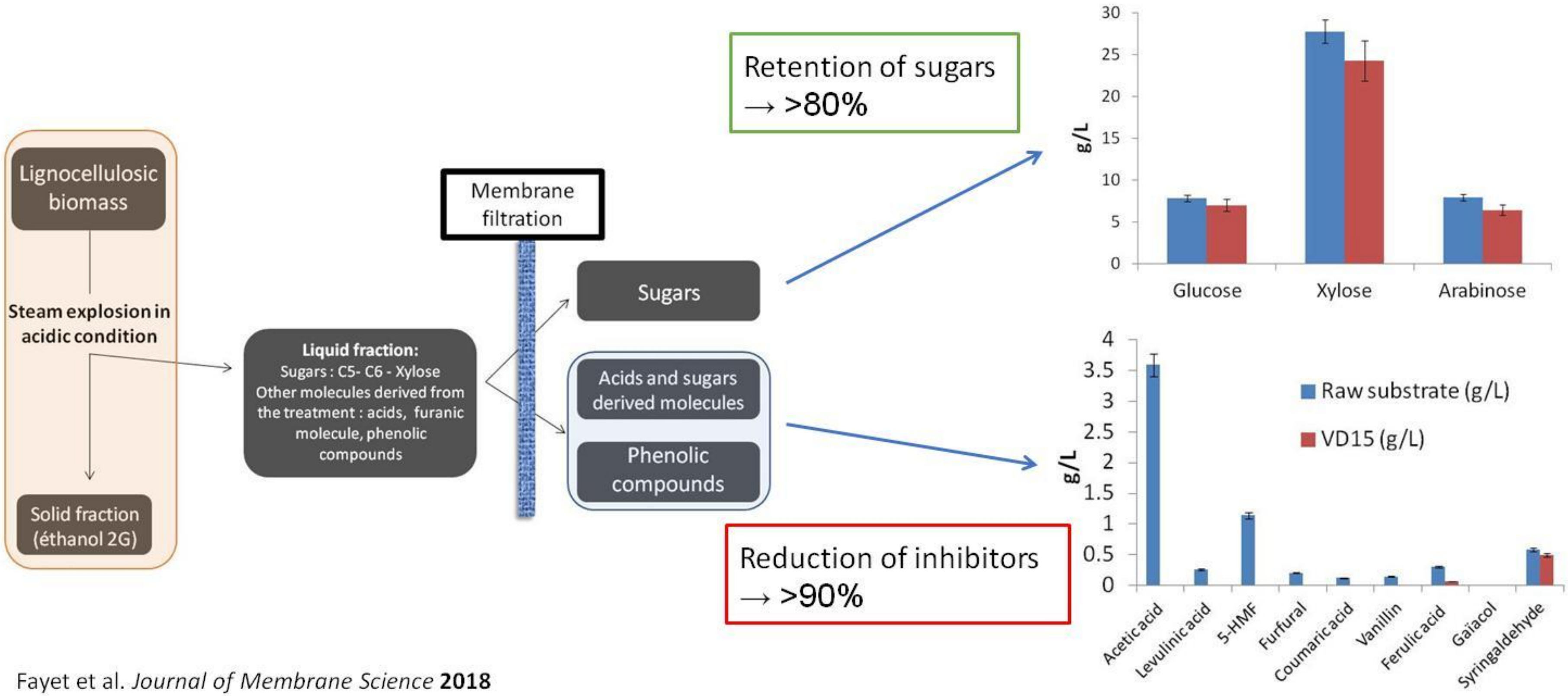


Fayet et al. *Journal of Membrane Science* **2018**

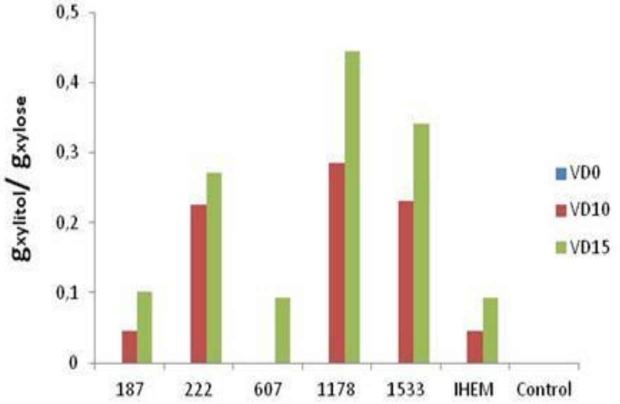
Pretreatments of biomass →Possible applications

Bacillus subtilis
-Model microorganism
-Usual chassis

No growth on detoxified medium

Xylose fermenting yeasts
-Resistance to osmotic pressure
-Large pH range
-Resistance to phenolic acids

Added value of medium detoxification concerning xylitol production



Pretreatments of biomass

Diversity of ligno-cellulosic biomass
→ Diversity of treatments

Physical	Chemical	Biological
Milling	Thermohydrolysis	Enzymatic deconstruction
Thermolysis	Steam explosion -In acidic condition -In alcalin medium	
	Organosolv	
	Chemical oxydation (O_3)	

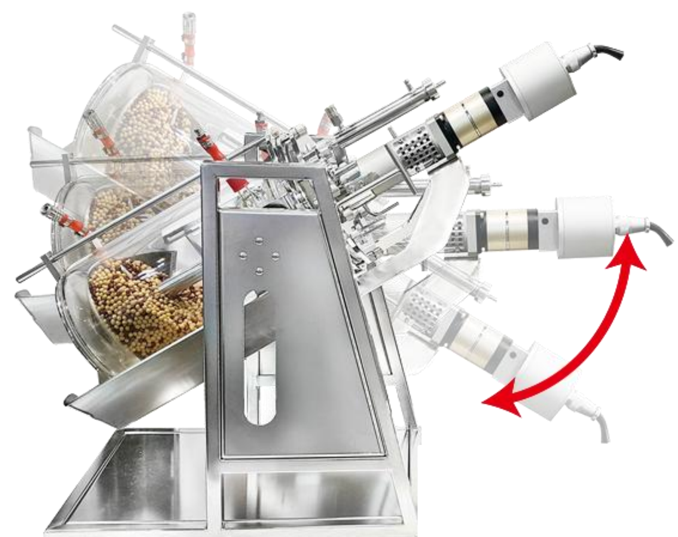
Biomass as a substrate for solid state fermentation

- ➢ Enzymatique deconstruction using microorganisms on a solid matrix
- ➢Wide range of possible substrate
  - → Agro-industrial waste : environmental benefits
  - → Decontamination, remediation

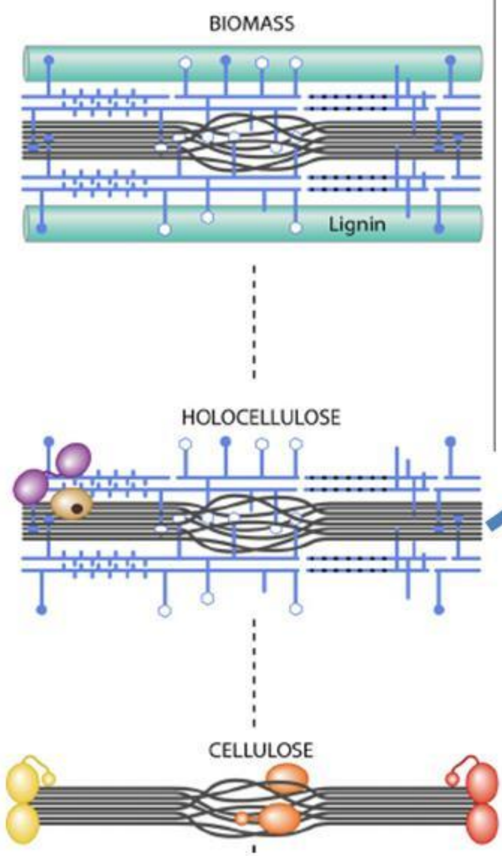
- ➢ Industrial applications: enzymes, organic acids

Low water consumption
Light substrate pretreatment
Close to natural habitat of microorganisms
Low energy demand for sterilization
High enzymatic productivity…

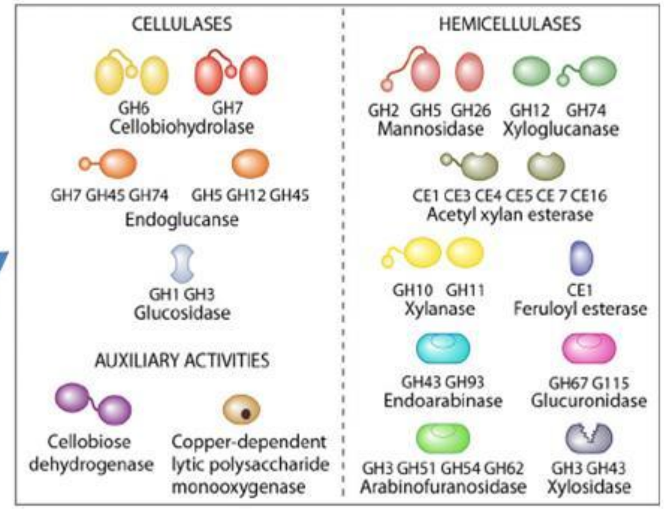
Instrumentation and control
→ Difficulty to scale up
DSP : separation of biomass after fermentation



SSF with Aspergilli
→ Biopulping to access fermentable sugars



Segato et al. *Microbiology and molecular biology review* - **2014**

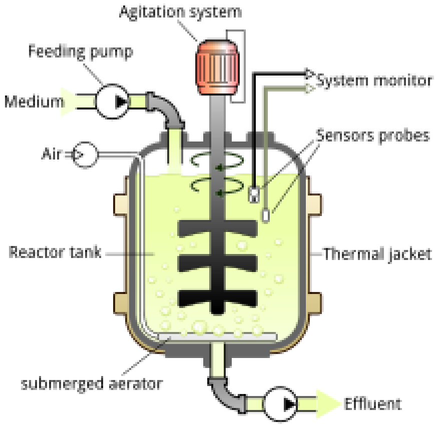


Glucose
Xylose
Arabinose

Galactose
Mannose
Fucose
Rhamnose

Fermentation

What about microalgae ?



Pretreatment

Down stream processing

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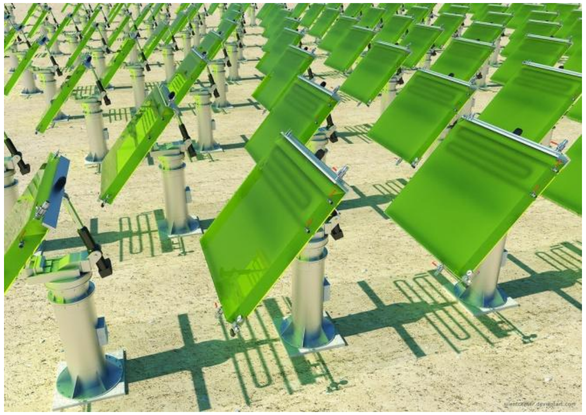
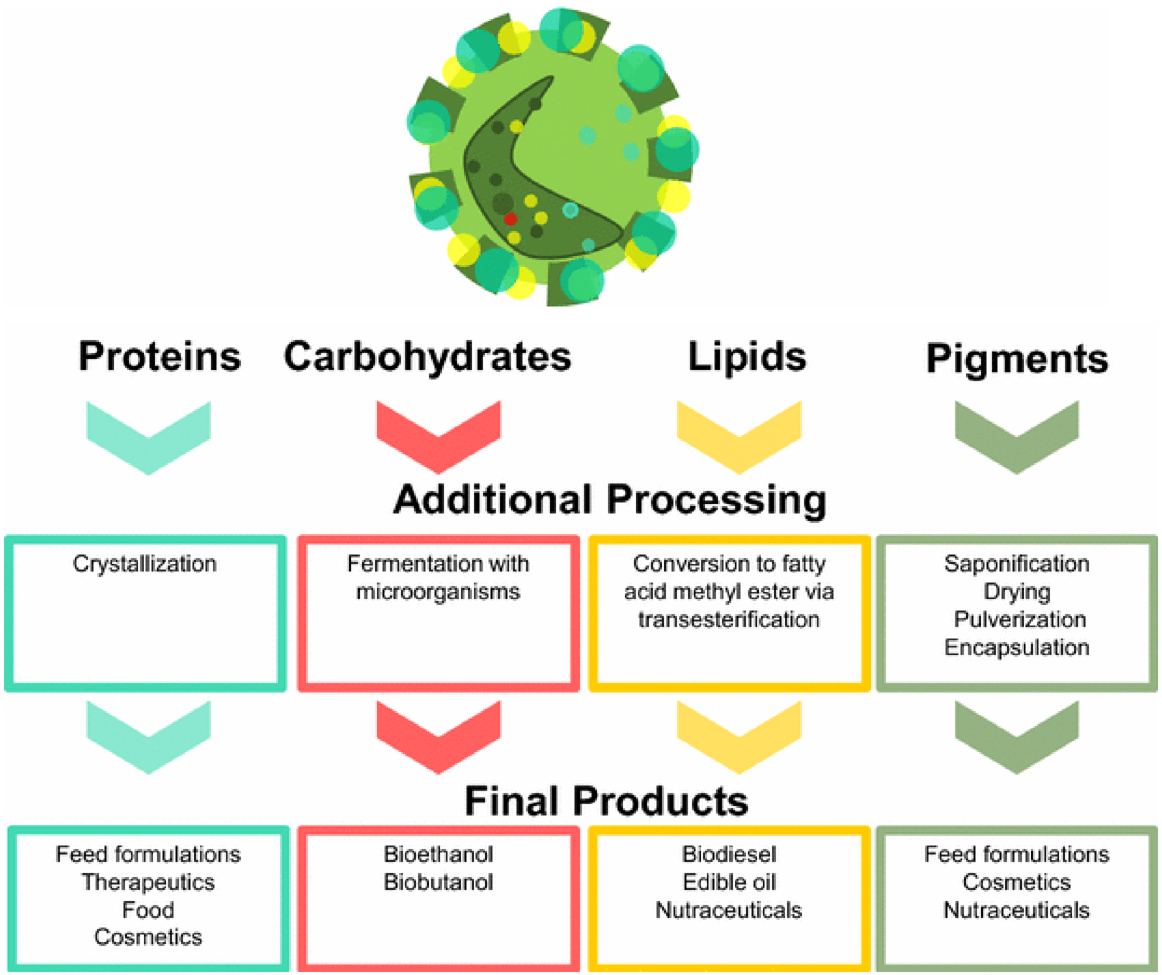


- CO_2 as carbon source
- Sunlight or low energy consuming LED

- Higher growth rate compared to plants
- Sustainable cultivation in non-arable land
- Low nutrient demand

What about microalgae ?



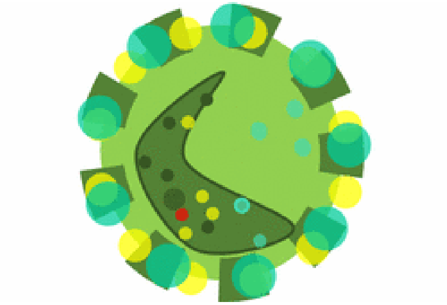
Dixon & Wilken *Bioressources and bioprocessing*- **2018**

- ➢Alternative and sustainable source of biofuels and bioproducts
- ➢Necessity of high value product to make it advantageous on the environmental and economic levels
- ➢Diversity of derived products and applications → Adapted to biorefinery

Microalgae and fermentation →Microalgal carbohydrates

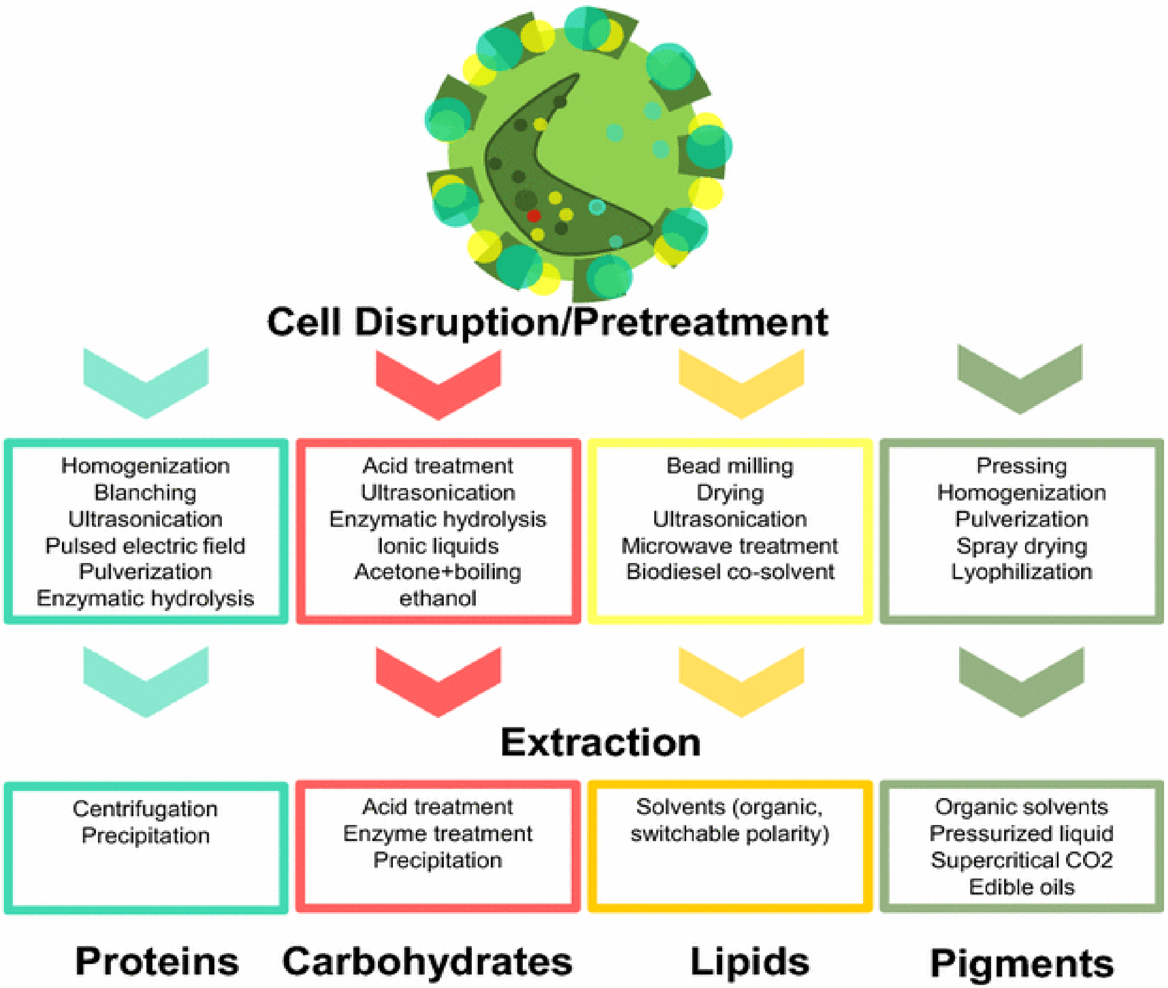
- High variability in carbohydrate content
- Depends on species and cultivation conditions
- *Scenedesmus ovalternus*, *Porphyridium purpureum*, *Tetraselmis suecica*: up to 80% DW (mainly glucose)

Microalgae and fermentation
→Microalgal carbohydrates



- Component of the microalgal cell wall
- Mainly polymeric form : Cellulose, Hemicelullose (Xyloglucan, mannan, glucuronan, (1→3)-β-glucan), ulvan
- Conversion to monomers : Rhamnose, arabinose, fucose, xylose, mannose, galactose and glucose

Microalgae and fermentation →Microalgal carbohydrates



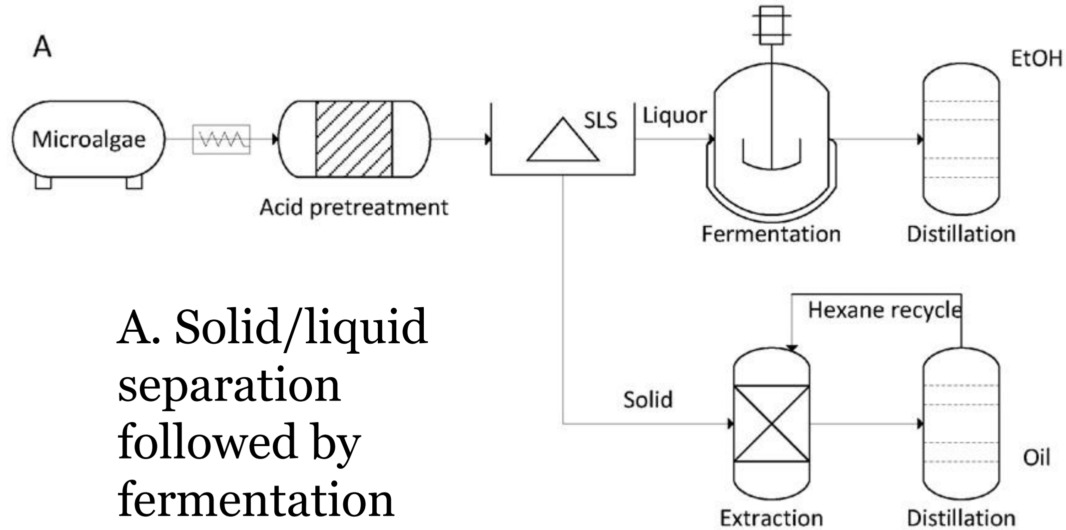
Dixon & Wilken *Bioressources and bioprocessing*- **2018**

- No lignin
- High diversity of extracellular matrices
- Problematic of cell disruption
- High water content
- Need for robust, energy-efficient process maintaining product quality (proteins, pigments etc...)

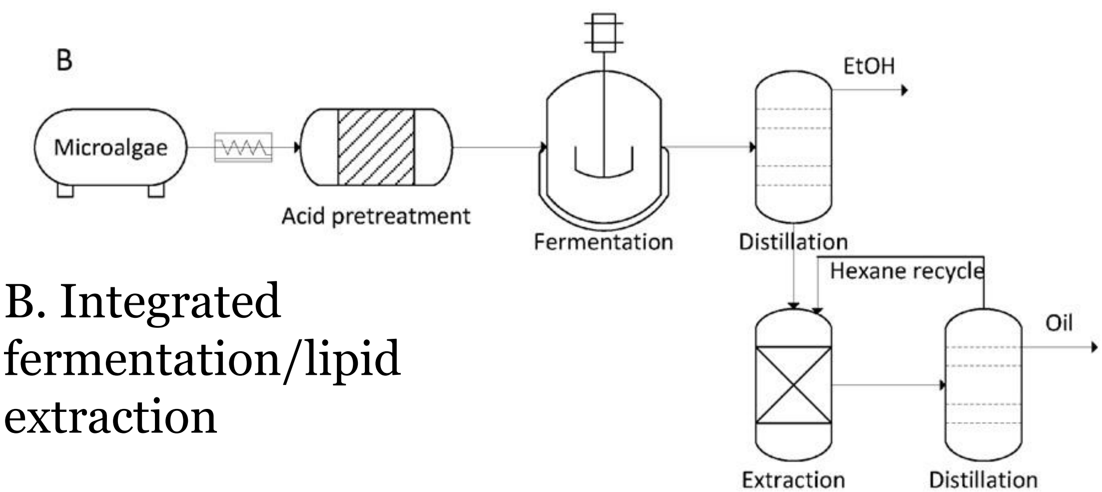
What about microalgae ?

- Combined sugar fermentation and lipid extraction from microalgae biomass

- Reduction of microalgal fuel cost by 9%



A. Solid/liquid separation followed by fermentation



B. Integrated fermentation/lipid extraction

Dong et al. Algal Research- **2016**

What about microalgae ?

- Substrate for solid state fermentation

→ *Anaerobic solid-state fermentation of bio-hydrogen from microalgal Chlorella sp. Biomass* – Phanduang et al. 2016

→ *Highly efficient methane generation from untreated microalgae biomass* – Klassen et al. 2017

- ✓ Advantages of low nitrogen content of microalgal biomass for its use in a methanisation process

Fermentation of biomass towards a biobased industry

- Fermentation technology is a dynamic and fast growing area of biotechnology that has great potential for tackling the societal challenge to go towards more renewable energy

- The rise of synthetic biology is a great opportunity for the development of bioproducts

- Many hurdles are still to overcome in order to establish microbial biorefineries and produce fuels and chemicals that can compete with oil-based chemicals, especially in the field of pretreatment

Fermentation of biomass towards a biobased industry

- The success of microbial biorefinery will depend on the development of biomass pretreatment technologies and DSP

- In this context microalgae are a promising source of fermentable substrate, although improvement of extraction techniques is still necessary in order for microalgal product to compete with market price

Thank you for your attention!

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