CARREFOURS
DE L'INNOVATION AGRONOMIQUE

Bioénergies durables

Innovations dans les territoires et les filières

Mardi 22 novembre 2016

INRA
SCIENCE & IMPACT

Biofuels from lignocellulosic resources by yeast and bacteria :

current status and prospects

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Agricultural residues
Forest residues
Dedicated biomass
Industrial wastes

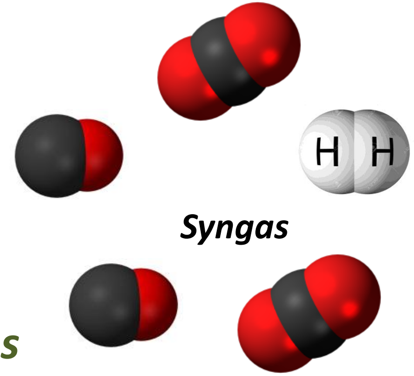
Lignocellulosic biomass

Energetic molecules

Challenges
Reduce environmental impact
Increase energy independence
Meet the increasing energy demand

Two routes

Thermal process



Syngas

Fermentation

Down stream process

Lignocellulosic biomass

Energetic molecules

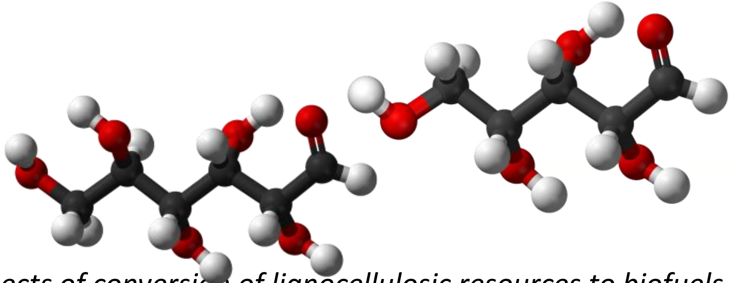
Pre- treatment

Hydrolysis

Fermentation

Downs tream process

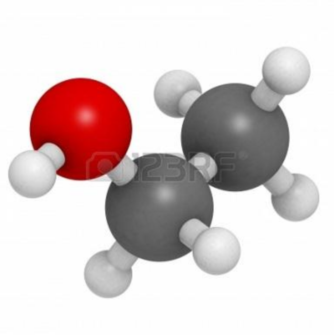
Liquid hydrolysates

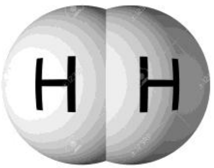


S. Alfenore, C. Molina-Jouve, Current status and future prospects of conversion of lignocellulosic resources to biofuels using yeasts and bacteria, Process Biochem (2016), http://dx.doi.org/10.1016/j.procbio.2016.07.028

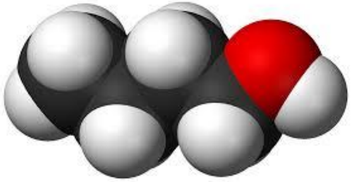
Main Biofuels

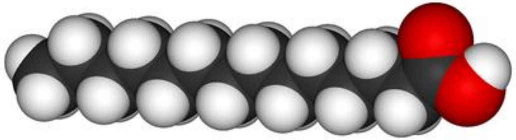
Alcohols (ethanol, acetone–butanol–ethanol (ABE)…), lipids as biofuel precursors, hydrogen





120,5 MJ/kg





Ethanol 28,9 MJ/kg

Gasoline : 47,3 MJ/kg
Gazole : 44,8 MJ/kg

Isobutanol 32,959 MJ/kg

Few studies have focused on the biofuel production **from real lignocellulosic substrates**

Physico-chemical-biological route

Pre- treatment
Hydrolysis
Fermentation
Downs tream process

Objectives

Efficient deconstruction of biomass according to criteria as
- lignin, hemicellulose, cellulose extraction
- cellulose crystallinity and polymerization degree
- by product production

Numerous pretreatment processes

Physico-chemical-biological route

Pre- treatment | Hydrolysis | Fermentation | Downs tream process

Challenges

High yield in carbohydrates recovery, efficient lignin recovery

Reduce energy consumption

Reduce/avoid production of toxic molecules that inhibit bioconversion processes

Under progress

Combined pretreatment processes

Screw extrusion with steam explosion or enzymatic and alkali deconstruction
twin-screw extrusion – with and without the filtration step – with alkali addition,
milling in an oxalic acid medium with microwave-induced hydrothermal treatment
fungal pretreatment to chemical steps such as organosolv or alkali and oxidative pretreatments

Further investigate lignocellulose deconstruction mechanisms

Physico-chemical-biological route

Pre- treatment

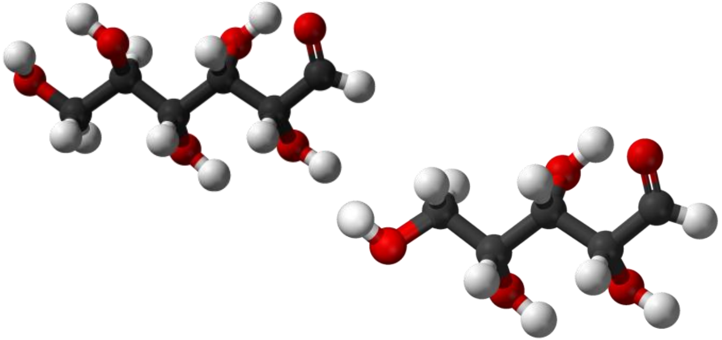
Hydrolysis

Fermentation

Downs tream process

Objectives

Lignocellulose degradation
Depolymerization into monomeric C6 and C5 sugars



Chemical hydrolysis

Enzymatic hydrolysis

(corrosion, environmental and utilities costs…

Physico-chemical-biological route

Pre- treatment

Hydrolysis

Fermentation

Downs tream process

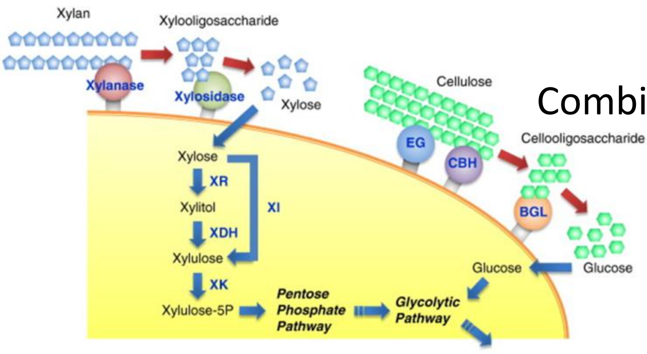
Challenges

Enzymatic hydrolysis

High-performance and robust enzymes
enzyme stability vs pH, T, inhibitors…
High sugar concentrations

Under progress

Better understanding of enzyme binding to cellulose and hemicellulose
Design new and high performance engineered enzymes and strains (consolidated bioprocessing)
Design cellulosomes/hemicellulosomes,
Explore biodiversity
Combine pretreatments and hydrolysis to improve yields at high solids loadings
Develop cleanup systems to remove inhibitors



Physico-chemical-biological route

Pre- treatment

Hydrolysis

Fermentation

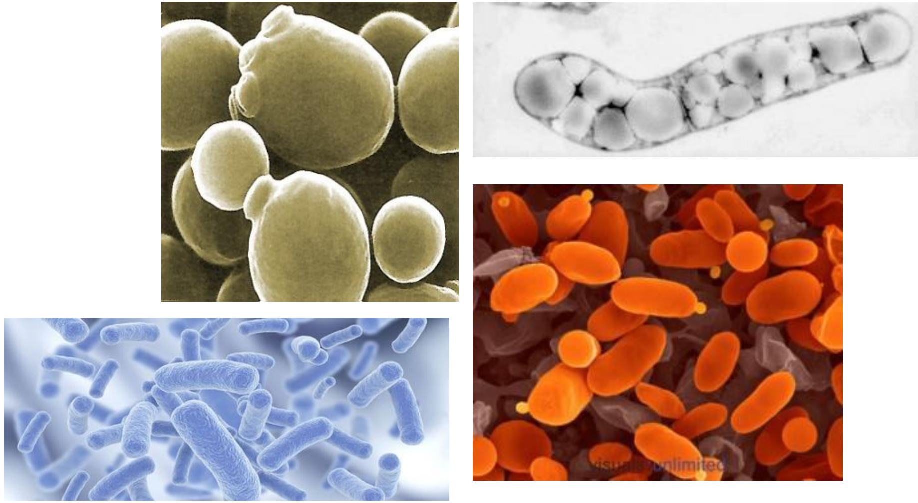
Downs tream process

Objectives

Hexose and pentose microbial conversion

Mature technology

Scale up



Physico-chemical-biological route


Pre- treatment

Hydrolysis

Fermentation

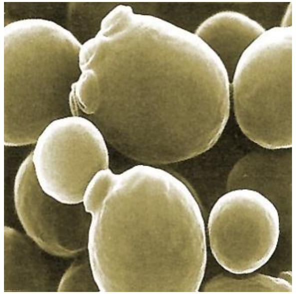
Downs tream process

Challenges

yield and titer and productivity
High-performance cell, robustness and inhibitor tolerance
reduce nutrient supplementation of the hydrolysates

Ethanol *depending on the pretreatment*

Khoo HH. Renewable and Sustainable Energy Reviews 2015;46:100-119.



175–270 kg ethanol / t corn stover
190–370 kg ethanol / t bagasse
144 kg ethanol / t switchgrass
200 kg ethanol / t rice straw

Physico-chemical-biological route

Pre- treatment

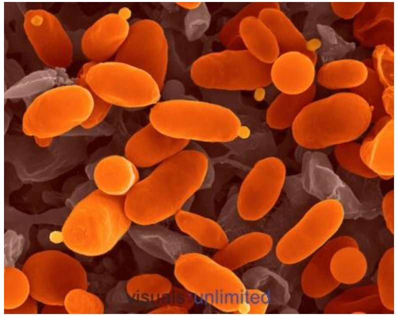
Hydrolysis

Fermentation

Downs tream process

Challenges

increase yield, titer and productivity
cell robustness and inhibitor tolerance
Ethanol and…



ABE : from pretreated *Miscanthus giganteus* by *Clostridium beijerinckii* NCIMB 8052
Zhang et al. (2014)

Lipids : on various lignocellulosic biomass hydrolysates (wheat straw, *Miscanthus*, corncob…)

Number of relevant studies still very low.

on nondetoxified corncob hydrolysates, *R. glutinis*,
biomass concentration of 70.8 g/L with a lipid content of 47.2%
Liu YT, Wang YP, Liu HJ, Zhang JA. Bioresource Technology 2015;180:32-39.

Physico-chemical-biological route

Pre- treatment

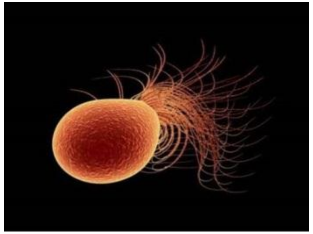
Hydrolysis

Fermentation

Downs tream process

Hydrogen

Clostridium, Pyrococcus, Thermotoga…



from pretreated *Miscanthus* biomass by *Thermotoga elfii*
82.2 and 42.4 mmol/L of hydrogen and acetate, respectively hydrogen yield : 2.7 moles per mole of total consumed sugars *(theoretical value for glucose alone : 4 moles of hydrogen per mole of glucose)*
De Vrije T et col. Journal of Hydrogen Energy 2002;27:1381-1390.

Under progress

Development of overproducing engineered strains
with increased inhibitor resistance and/or excrete intracellular metabolites
Combined hydrolysis, detoxification and fermentation processes

Thermal route

Thermal process

Fermentation

Down stream process

Syngas *: carbon monoxide (CO), hydrogen (H_2), nitrogen (N_2), carbon dioxide (CO_2), minor amounts of methane (CH_4), and a variety of trace gases.*

Objectives

Microbial conversion of syngas into energetic molecules

Challenges

better understand carbon fixation, product formation, genetic targets

high-performing strains

solubility of CO and H_2

Thermal route

Thermal process

Fermentation

Down stream process

a membrane bioreactor (polypropylene (PP) hollow-fiber membranes) : highest value of volumetric mass transfer coefficient of 1096.2 h^{-1}

Yasin M et al Bioresource Technology 2015;177:361-374.

ethanol from CO/H2: acetogenic bacteria as *C. ljungdahlii, Clostridium autoethanogenum, Acetobacterium woodii, Clostridium carboxidivorans, Butyribacterium methylotrophicum.* with *C. ljungdahlii* : ethanol 48 g/L after 560 h

Klasson KT et al.. Fuel 1993;72:1673-1678.

H2 from CO : Hydrogenogenic bacteria including heterotrophic anaerobes, heterotrophic facultative anaerobes, heterotrophic strict anaerobes, and photosynthetic bacteria

maximum rates of hydrogen with *Thermococcus onnurineus* 102.6 mmol/L/h

Kim YK et al. Bioresource Technology 2014;159:446-450.

Performances are very low for biofuel applications

Numerous works under progress on metabolic engineering

Conclusions and perspectives

Thermal process

Fermentation

Down stream process

Lignocellulosic biomass

Energetic molecules

Pre- treatment

Hydrolysis

Fermentation

Downs tream process

Enhance carbohydrate release with combinations of pretreatments

Combine pretreatments and hydrolysis to improve yields at high solids loadings and to achieve economic feasibility

Conclusions and perspectives

Thermal process

Fermentation

Down stream process

Lignocellulosic biomass

Energetic molecules

Pre- treatment

Hydrolysis

Fermentation

Downs tream process

Maximize and accelerate the conversion of sugar monomers into the final products

Improve enzymes and microorganisms

Develop a cleanup system to remove inhibitors present in the substrate (liquid or gas)

Conclusions and perspectives

Thermal process

Fermentation

Down stream process

Lignocellulosic biomass

Energetic molecules

Pre- treatment

Hydrolysis

Fermentation

Downs tream process

Improve fermentation performances (nutritional complementation of both liquid and gaseous media, mass transfer)
Design new bioreactor configurations
Develop overproducing engineered strains with increased inhibitor resistance

Improve performances, green solvant, low energy

Conclusions and perspectives

Biorefinery

combines high-added-value chemical and energetic applications by optimizing by-product valorization and recycling.

Thermal process

Fermentation

Down stream process

Lignocellulosic biomass

Energetic molecules

€€

And **high-added-value products**

Pre- treatment

Hydrolysis

Fermentation

Downs tream process

with recycling

Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés

UMR CNRS/INSA N°5504
INSIS, INSB, INEE

UMR INRA/INSA N° 792
MICA, CEPIA

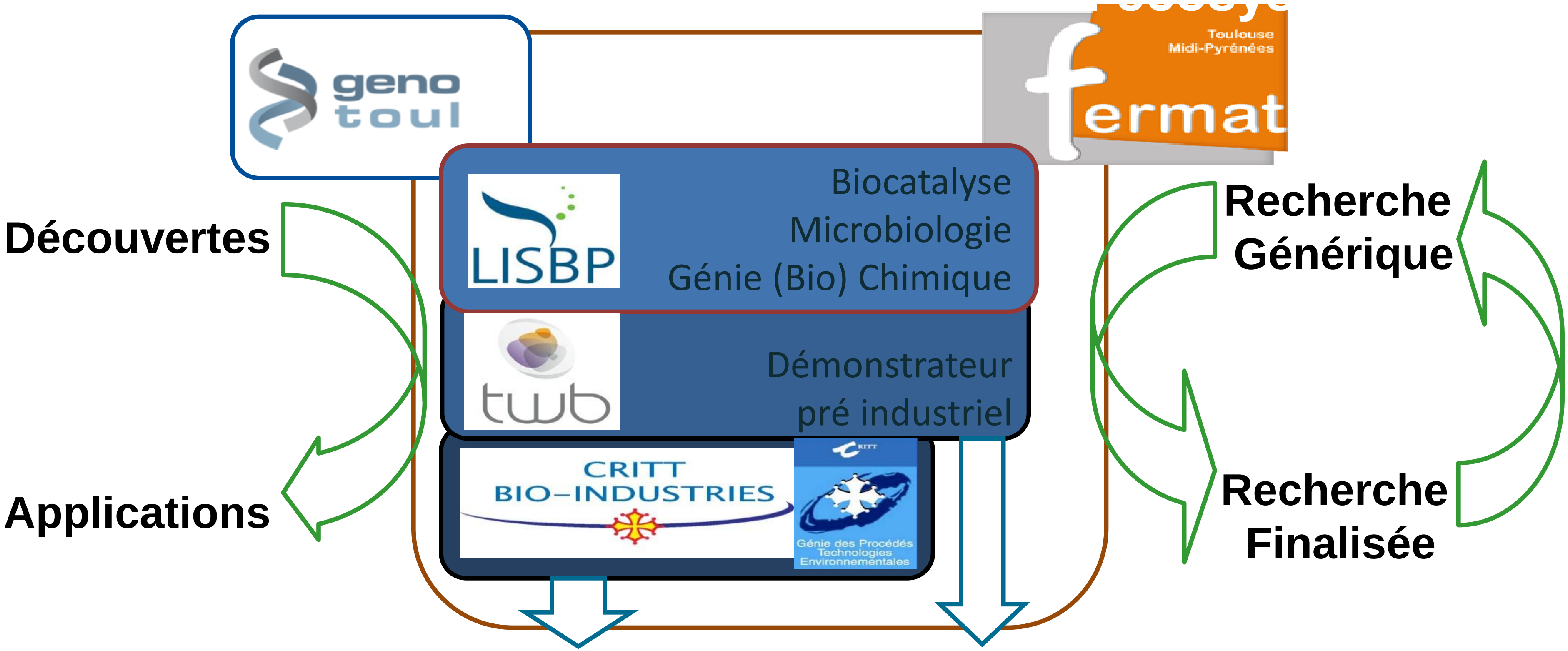

2019

- **Enzyme and Metabolic Engineering:** rational knowledge-driven improvement of a system's performance based upon in depth understanding of the underlying scientific mechanisms.

- **Systems Biology:** biological systems respond in a complex and dynamic manner to their ambient environment. Understanding these interactions and the basic regulatory networks involved is a key objective for modern industrial biotechnology.

- **(Bio)Chemical Engineering:** Understanding the fundamental interactions between physico-chemical-biological phenomena involved in (bio)processes to identify bottlenecks, deduce innovation and allow realistic integrated process development (scale up).

Basic research input for an integrated approach to modern industrial biotechnology with wide applications in BIOENERGY, synthons, biomaterials, pharmaceuticals, food and feed, water resources, etc



Biocatalyse
Microbiologie
Génie (Bio) Chimique

Démonstrateur
pré industriel

Découvertes

Applications

Recherche Générique

Recherche Finalisée

Transfert des concepts validés vers l'industrie

LISBP

Laboratoire d’Ingénierie des Systèmes Biologiques et des Procédés

Un réseau national de collaborations de recherche en bioénergie

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Grands projets fédérateurs
Soutiens publics et privés

Et des collaborations internationale
USA, Mexique, Viet Nam, Tunisie…

En vous remerciant de votre attention

J' ai refait tous les calculs, ils confirment l'opinion des spécialistes. Notre idée est irréalisable.
Il ne reste qu'une seule chose à faire : réaliser.

P.G. Latécoère (1990)