Louis Pasteur, pionnier de la Biotechnologie Industrielle… et la contribution de celle-ci à la bioéconomie naissante.

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Toulouse, FRANCE

Toulouse Biotechnology Institute
Bio & Chemical Engineering

White Biotechnology
center of excellence

Journée Bicentenaire naissance de L.Pasteur
Acad. Agric. France, 14 dec. 2022

ANR · Investissements d'avenir · INRA Science & Impact · INSA Institut National des Sciences Appliquées Toulouse · CNRS

OUTLINE

Introduction: Biotechnology & BioEconomy

Part 1 - (R)Evolution of IB over centuries and major contribution by Louis Pasteur

Part 2 - Challenges to WB in the 21st Century

Part 3 - An example: bioproduction of methionine

Part 4 - Factors that point to a bright future for WB to BioEco

Part 5 - Future trends and awareness

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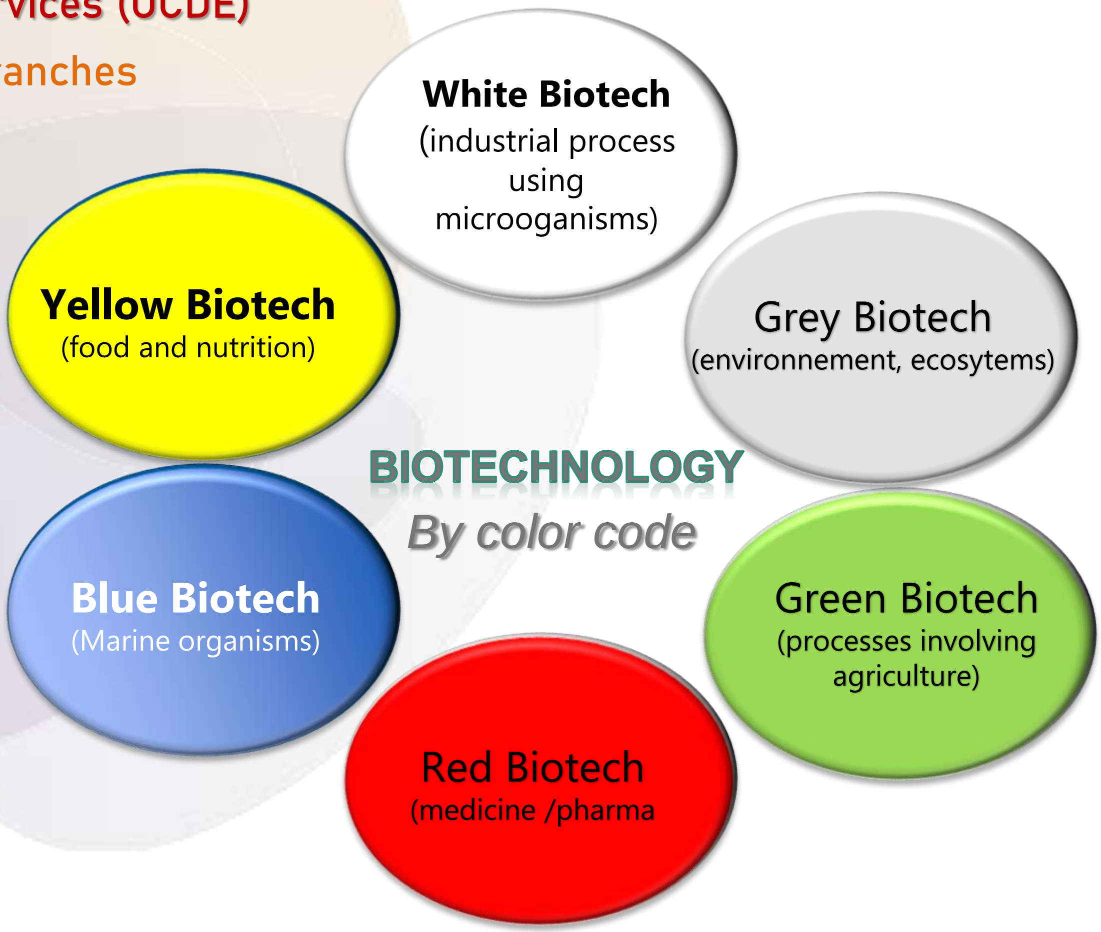
INTRODUCTION: BIOTECHNOLOGY & BIOECONOMY

Biotechnology (*words originally coined by Hungarian agronomist Karoly Ereky 1919*):

-> application of science and technology to living organisms and their parts, products or models, to modify living or non-living materials for the production of knowledge, goods and services (OCDE)

-> 10 branches

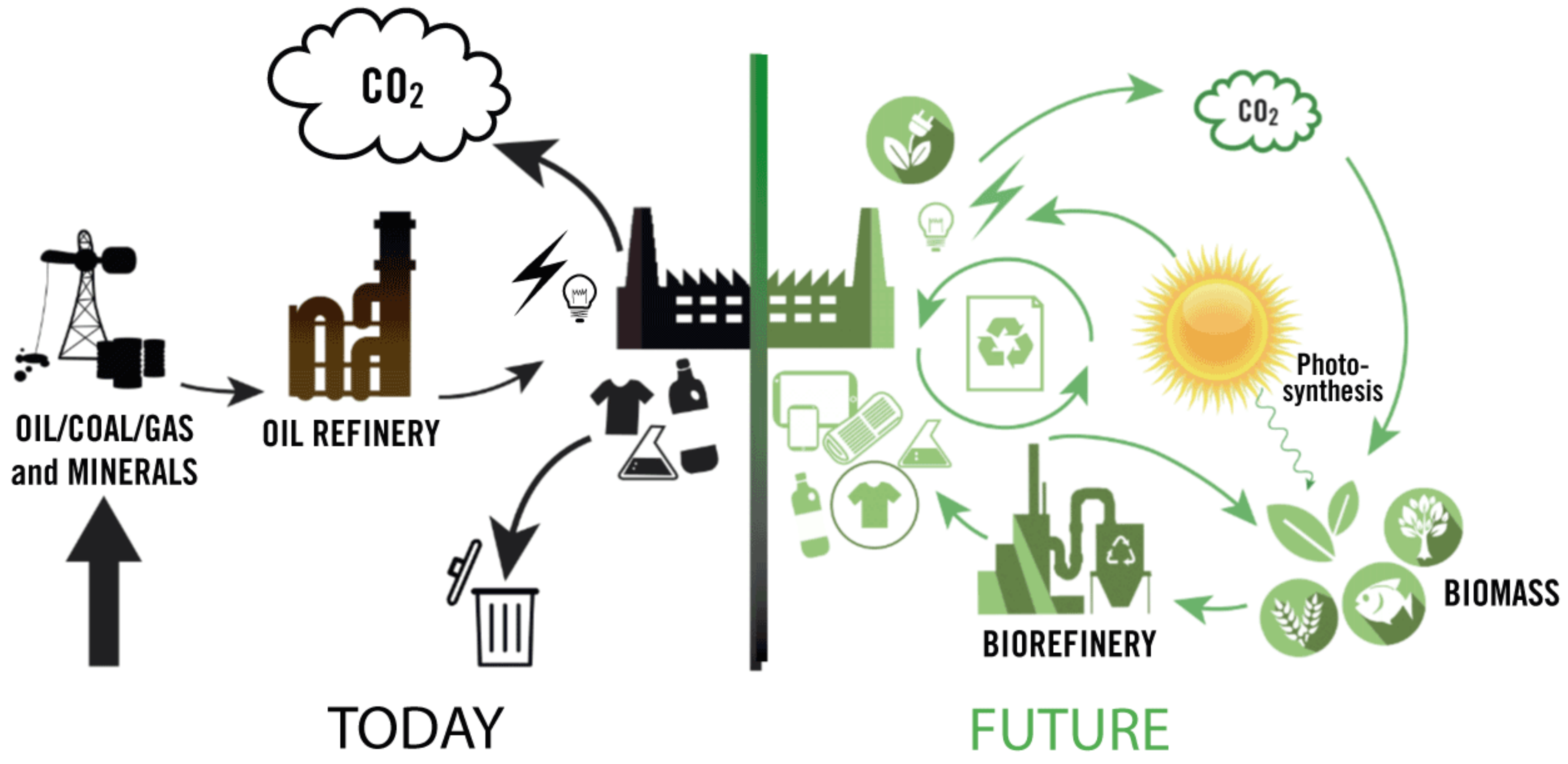


and

- **Brown** biotech
(dry, saline, desert area)
- **Gold** biotech
(bioinfo, computational)
- **purple** biotech
(ethics, philosophy)
- **black** biotech
(bioterrorism, biological weapons)

INTRODUCTION: BIOTECHNOLOGY & BIOECONOMY

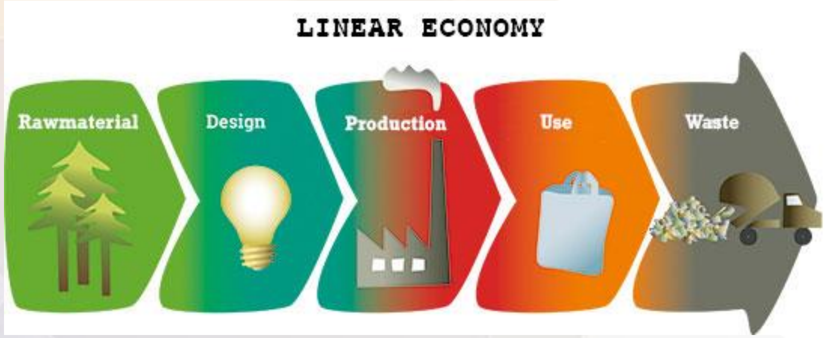
THE BIOECONOMY is economic and societal development based on RENEWABLE RESOURCES. (land, forest, sea and fresh water).



https://v-a.se/english-portal/projects/bloom/what-is-bioeconomy/

INTRODUCTION: BIOTECHNOLOGY & BIOECONOMY

BIOECONOMY is part of the CIRCULAR ECONOMY

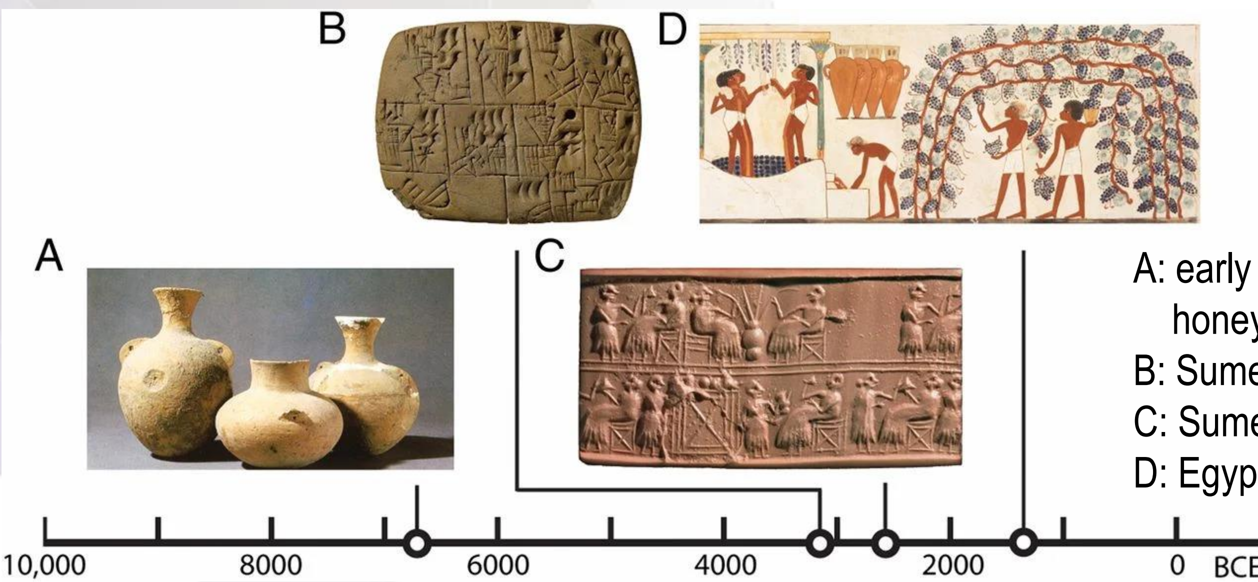


https://www.naturskyddsforeningen.se/faktablad/cirkular-ekonomi/

PART 1 - (R)EVOLUTION OF INDUSTRIAL BIOTECHNOLOGY OVER CENTURIES AND MAJOR CONTRIBUTION BY PASTEUR

❑ Primitive Biotechnology v0.0 (~ 7000 BC (Neolitic) to 19th century)

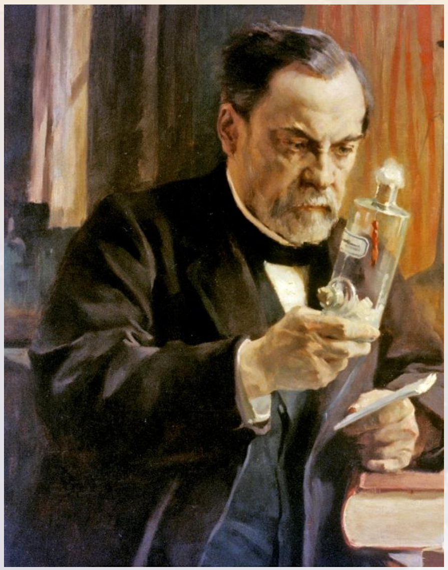
- ➢ Transformation of foods into fermented foods, wine, beverages
- Divine intervention
    - Osiris for Egyptian
    - Bacchus for Greeks
    - Small Shrine bowed to daily in Japanese factories

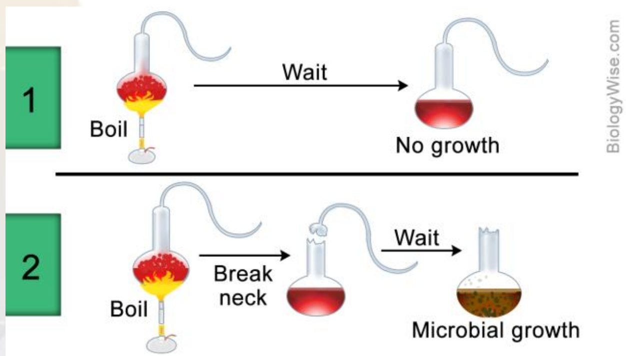


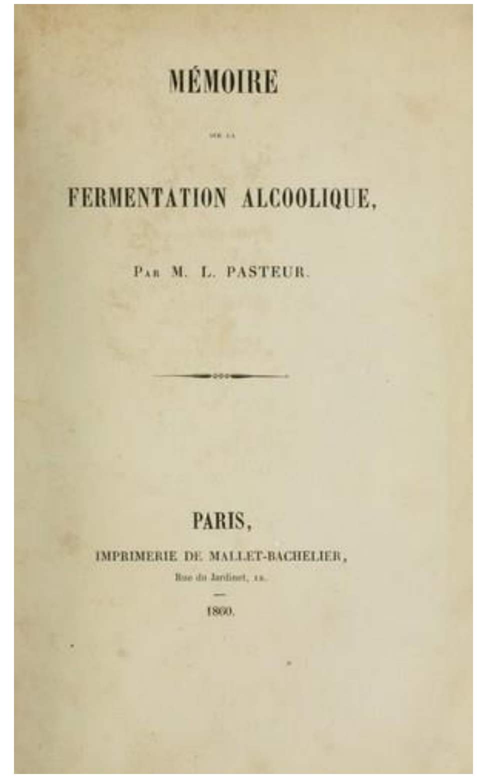
A: early Neolithic jars for fermented mix of rice, honey and fruit (Henan, China)
B: Sumerian tablets illustrating beer
C: Sumerian cylinder seal showing beer process
D: Egyptian viticulture and wine production

PART 1 - (R)EVOLUTION OF INDUSTRIAL BIOTECHNOLOGY OVER CENTURIES AND MAJOR CONTRIBUTION BY PASTEUR

❑ Industrial Biotechnology v1.0 (19th century and promoted by L. Pasteur)






MÉMOIRE

FERMENTATION ALCOOLIQUE,

PAR M. L. PASTEUR.

PARIS,
IMPRIMERIE DE MALLET-BACHELIER,
1860.

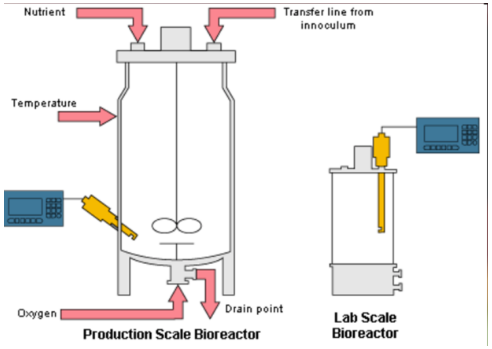
- Discredit the notion of spontaneous generation
- Identify aerobic vs anaerobic fermentation
- characterize alcoholic fermentation
- methods to isolate and cultivate microorganisms

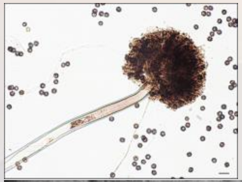
Precept of Industrial microbiology/ Biotechnology

PART 1 - (R)EVOLUTION OF INDUSTRIAL BIOTECHNOLOGY OVER CENTURIES AND MAJOR CONTRIBUTION BY PASTEUR

❑ Industrial Biotechnology v1.0 (19th century till 1970)

Large scale fermentation -bioprocess-
strong technological development early 20th century





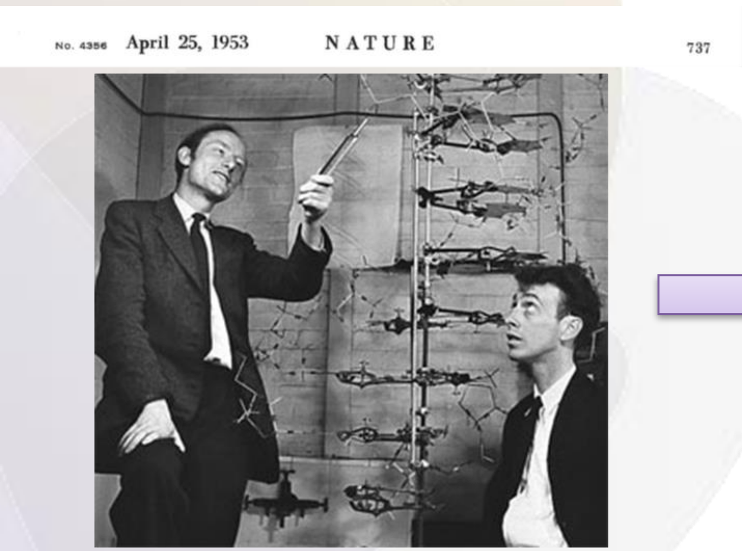
Citric acid fermentation ranks in Pfizer's Brooklyn facility, circa 1920s.
https://www.acs.org/content/acs/en/education/whatischemistry/landmarks/penicillin.html

Penicillin production by *Penicillium chrysogenum* fermentation during World ward II by Pfizer.
https://www.eurekalert.org/news-releases/491868

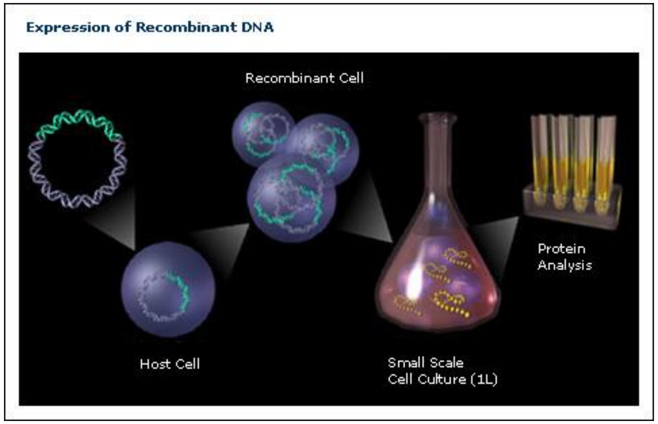
PART 1 - (R)EVOLUTION OF INDUSTRIAL BIOTECHNOLOGY OVER CENTURIES AND MAJOR CONTRIBUTION BY PASTEUR

❑ Industrial Biotechnology v2.0 (70ies to 90ies)

From DNA structure and coding information to genetic engineering



Watson & Crick, 1954



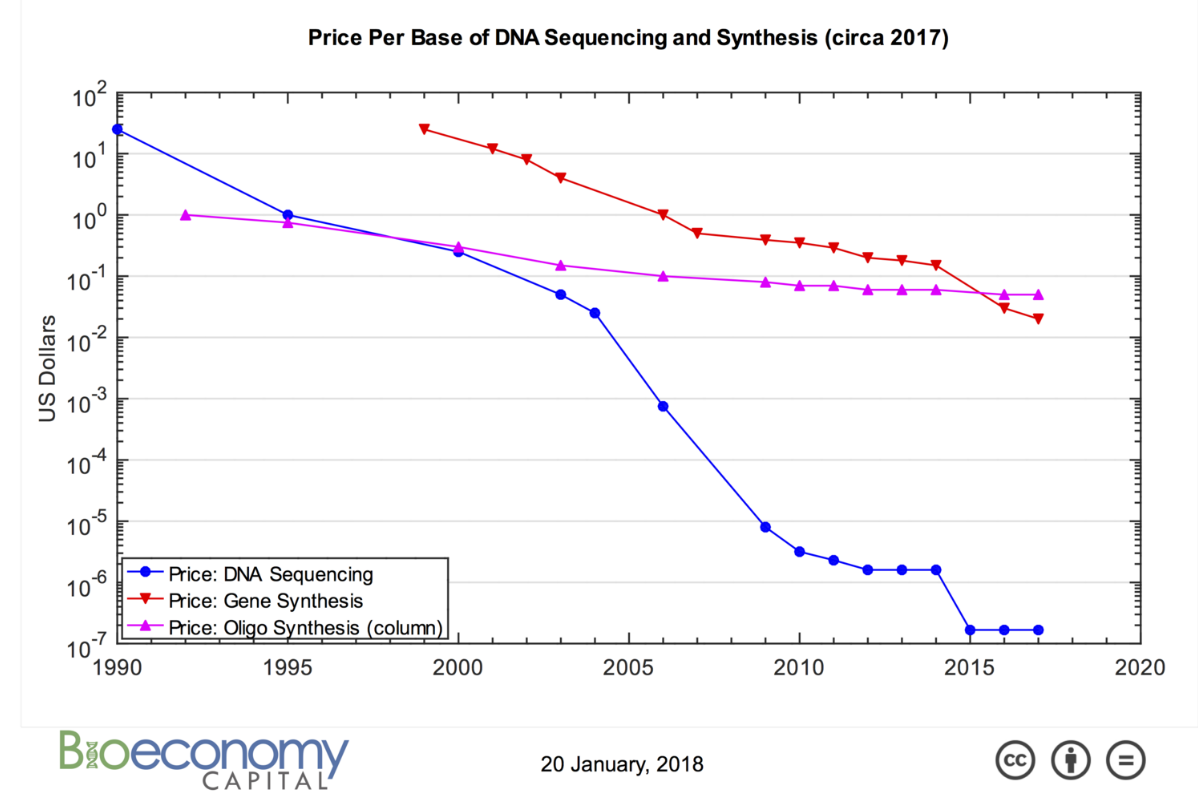
+

Creation many BIO Pharma (5000 from 70ies to 80ies in US)

❑ Industrial Biotechnology V3.0 (from early 21th …)

Emergence **Nano-Bio-Info**

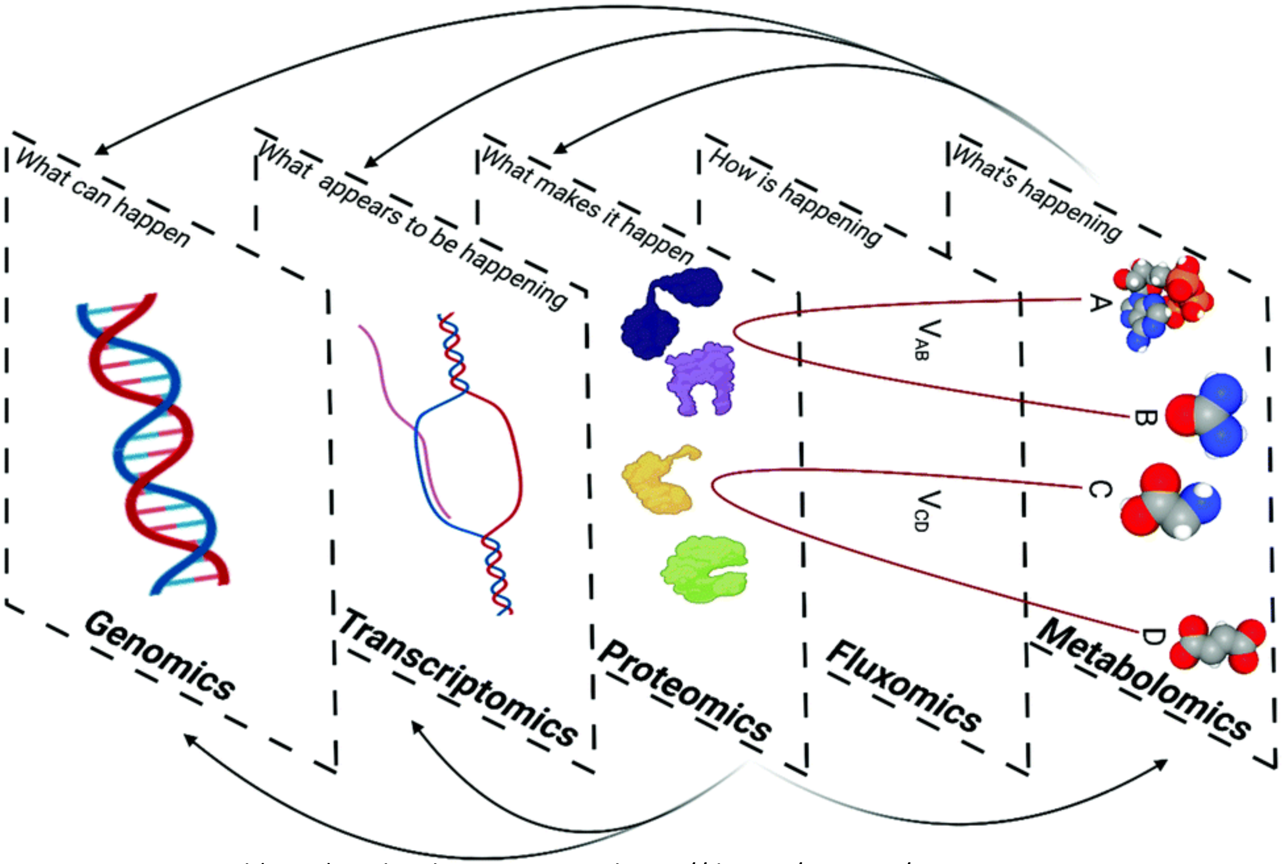
-> **new generation sequencing (NGS)**



❑ Industrial Biotechnology V3.0 (from early 21th …)

Emergence **Nano-Bio-Info**

-> **omics** technologies : access to all **cellular components at once**



From Basri et al (2022) Molecular Omics, 2, 7; https://doi.org/10.1039/D2MO00060A

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- Biotechnology v3.0

(~ 2000)

Systems Biology or SysBio

The focus is the 'whole natural' living system using -omics, mathematical and modeling tools.

It aims at recapture the whole systems from its part in order to have complete understanding of its functioning

Synthetic Biology or SynBio

The focus is to 'build or rebuild' (artificial) biological system endowed with a specific/focused function

This is fundamentally 'an engineering' application of biological science rather an attempt to do more science

A 'dream'?

Resources

fossile
Petrol
gas
charcoal

Petro-based products

Non-fossile
biomass
ex: lignocellulose
($2x10^{11}$ Mtons/year)

Bio-based products

Renewable Carbon sources (raw material at 100 - 300 €/Ton)

Bioproducts

Low volume/ high price

products/ C-source : high margin

- ***T****iter (g/L) can be low*
- ***R****ate (g/L/h) can be low*
- ***Y****ield $Y_{P/C}$ (g/g) can be low*

Ex: therapeutic; cosmetic; Recombinant proteins; etc.

-> highly competitive with petro-chemical derived products

Many (recent) success stories

- Veget burger by Impossible foods
- Hyalin by Zymergen
- Sitgaliptin by Merck
- Artemisinin (Sanofi)

high volume/ low price

products/ C-source: low margin

T*iter (g/L) must be high*
R*ate (g/L/h) must be high*
Y*ield $Y_{P/C}$ (g/g) must be high*

Ex: commodity chemicals; detergents; biopolymers, etc

-> hardly competitive versus petro-chemical derived products

Few success stories

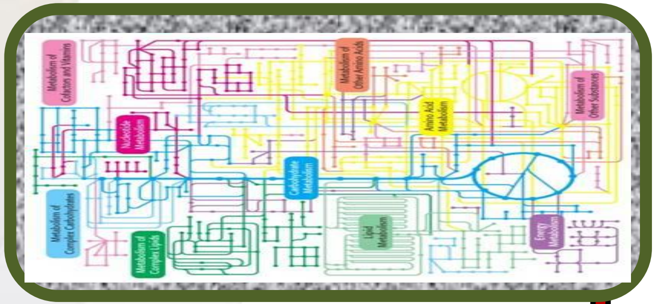
- 1,4 butanediol (Genomatica/BASF)
- 1,3 propanediol (Dupont-Genencor)

PART 2 - CHALLENGES TO WHITE BIOTECHNOLOGY IN THE 21ST CENTURY

❖ **1st challenge : *cell' vs biotech' objective***

Cell's objective

C-source →



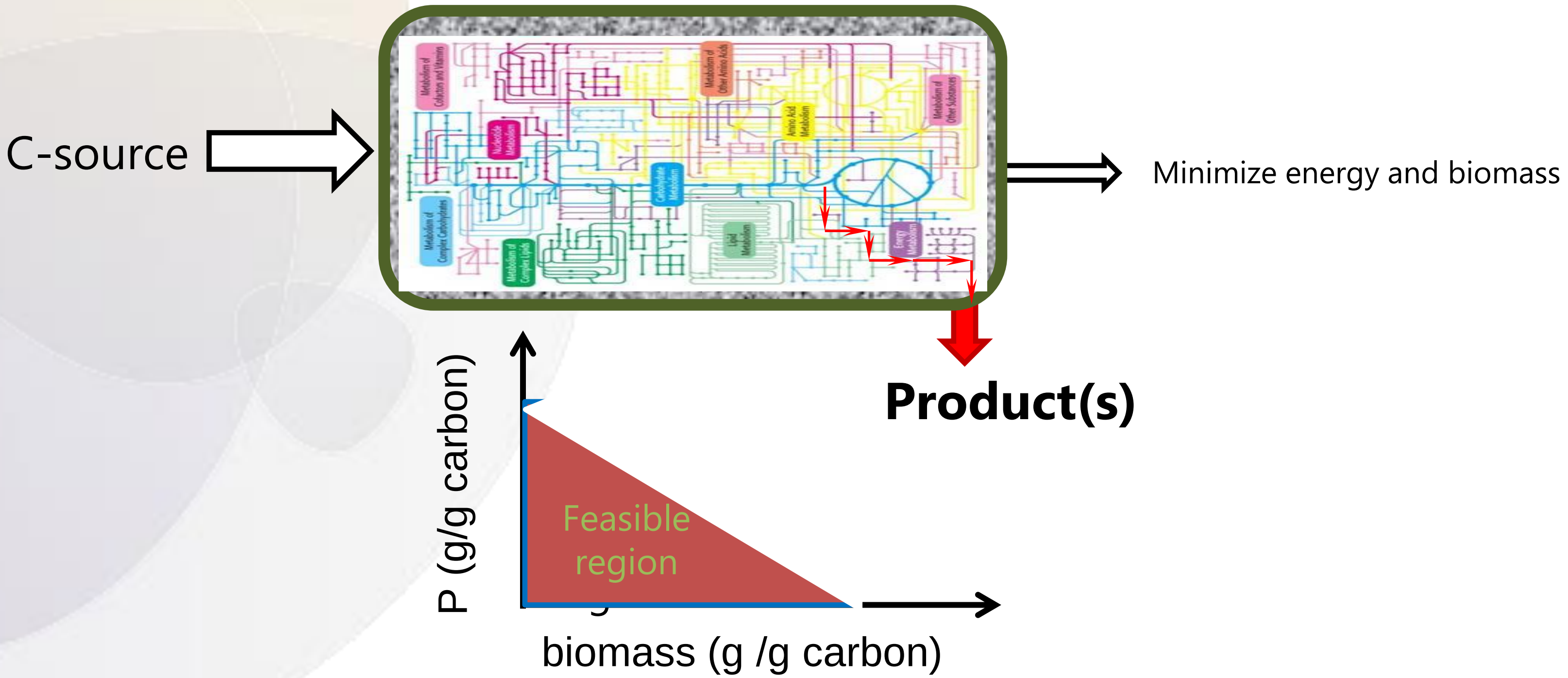
→ **Maximize energy/ biomass**

↓ **Byproducts**

(solving redox/ carbon balance)

❖ 1st challenge : *cell vs biotech objective*

(Bio)technological objective



-> Are natural pathways truly optimal for product formation from (bio)chemical point of view ?

❖ 2nd challenge: carbon conservation

- **Electron balance of substrate and product – Degree of reduction (γ) concept**

-> Thermodynamic yield $Y_{th} = \gamma_S / \gamma_P$

- **The cellular metabolism**

-> Stoichiometric yield Y_{st}

- **Optimization criterion**

-> Pathway efficiency: $E = Y_{st} / Y_{th}$

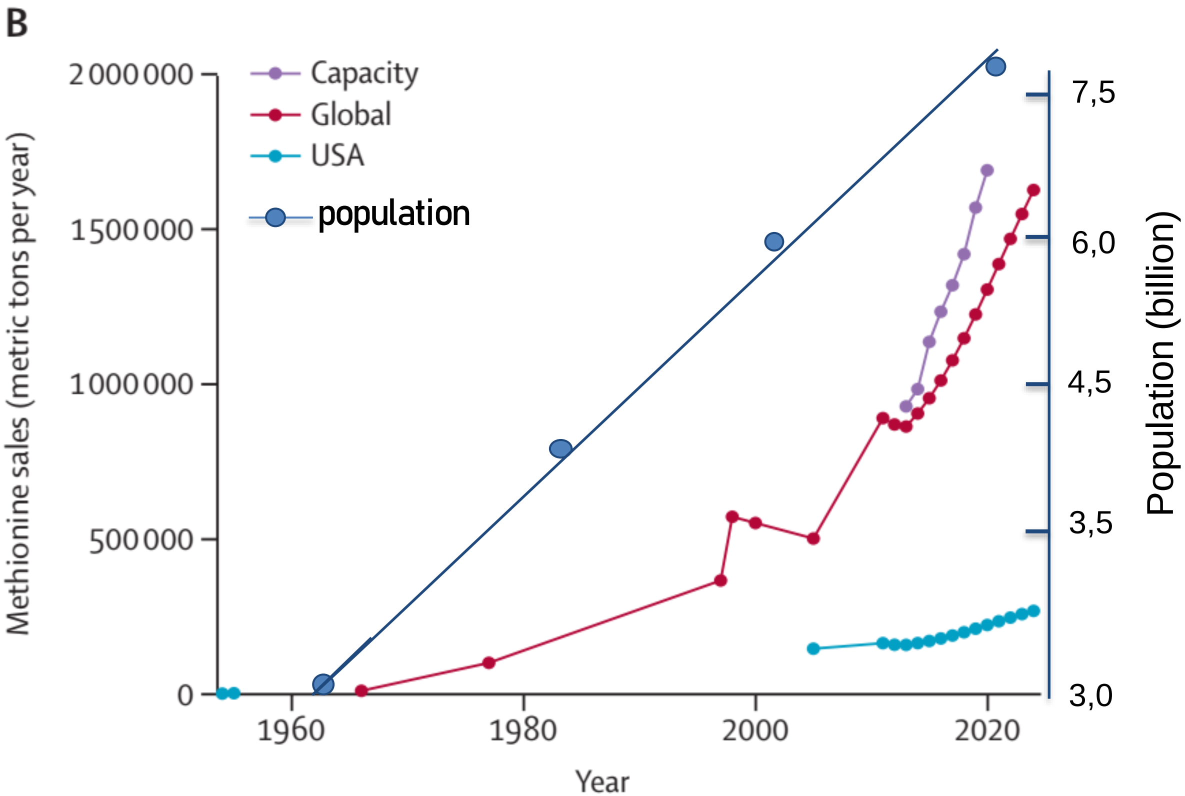
(source : Dugar & Stephanopoulos, Nat. Biotech 2011; Cueto-Rojas et al., Trends Biotech, 2014)

Substrate	Product	Degree of reduction		Stoichiometric reaction	Theor. yield [mol/mol]		Pathway eff. [%]
		γ_S	γ_P		Y_{th}	Y_{st}	Y_{st}/Y_{th}
Glucose	Ethanol	24	12	$C_6H_{12}O_6$ -> 2 C_2H_6O + 2 CO_2	2	2	100
Glucose	Acetate	24	8	$C_6H_{12}O_6$ + 2O_2 -> 2 $C_2H_4O_2$+ 2 CO_2 + 2H_20	3	2	75
glucose	Glycolic acid	24	6	$C_6H_{12}O_6$ + 3 O_2 -> 2 $C_2H_4O_3$+ 2 CO_2 + 2H_20	4	2	50

➡ The natural pathway is not always the most efficient one!

PART 3 - EXAMPLE: BIOPRODUCTION OF METHIONINE: **HOW** AND *WHY*

Methionine : mass commodity in global economy



(*Source: Neubauer & Landecker, Lancet Planet Health, 2021, 5 e60-69)*

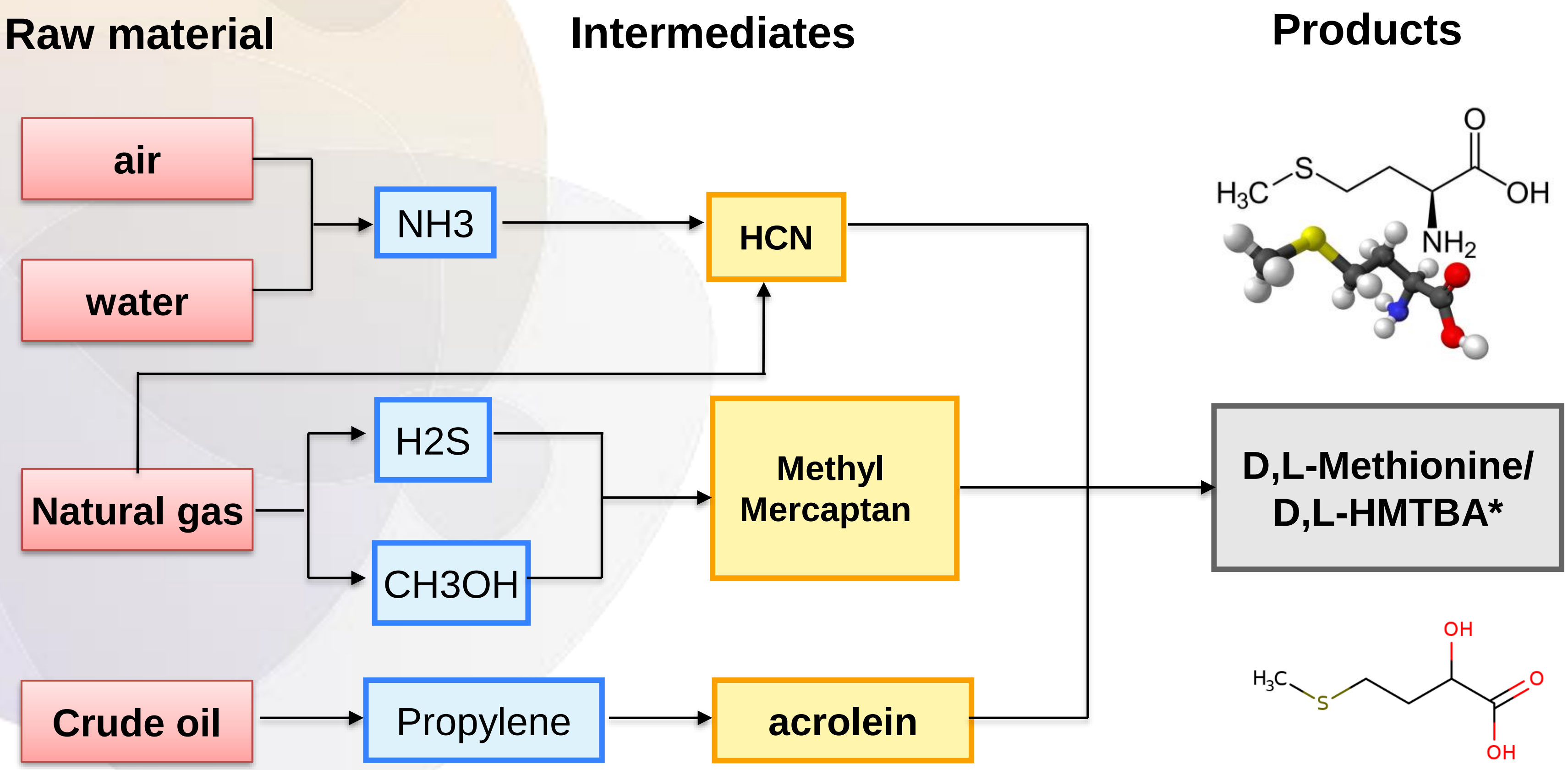
Market of Methionine:

- 1,2 to 2,0 €/kg
- > 90 % for poultry industry
- CAGR ~ 6%
- Increase with rise of population

New plant of 150 KT

every 30 month

Methionine : > 95% by chemical process



* 2-hydroxyl 4-methyl-thio-butanoic acid

Main technical and economical challenges

KEY TECHNOLOGICAL PERFORMANCES FOR an economically viable Biotechnological process of 'bulky' or commodity compounds

- Key indices or TRY

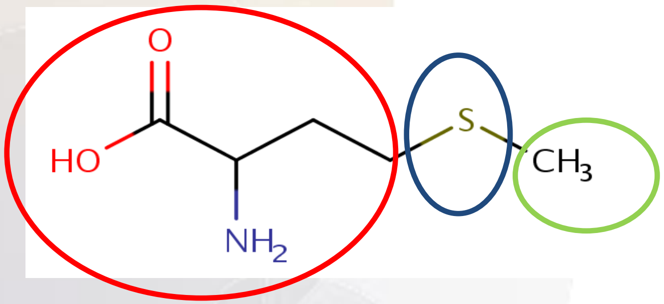
- Product Titers > 100 g/l
- Production Rate > 2 g/h/l
- Product Yield > 50 % (g/g carbon source)

=> This is the case for many amino acids (glutamate, threonine, lysine..)
(through metabolic engineering of the natural pathway)

BUT NOT METHIONINE .... Why?
(as well as for many bio-based molecules !)

Biosourced production of methionine: challenges

1) Methionine synthesis involved 3 interconnected pathways (3 intermediates)



- C4-carbon skeleton (*aspartate -homoserine path*)
- Sulfur group (*sulfate ions*)
- Methyl group (*serine to glycine through CH3-THF and/or glycine cleavage system*)

2) Metabolic pathway highly costly in ATP and reduced cofactor

+ Metabolic pathway highly complex and regulated

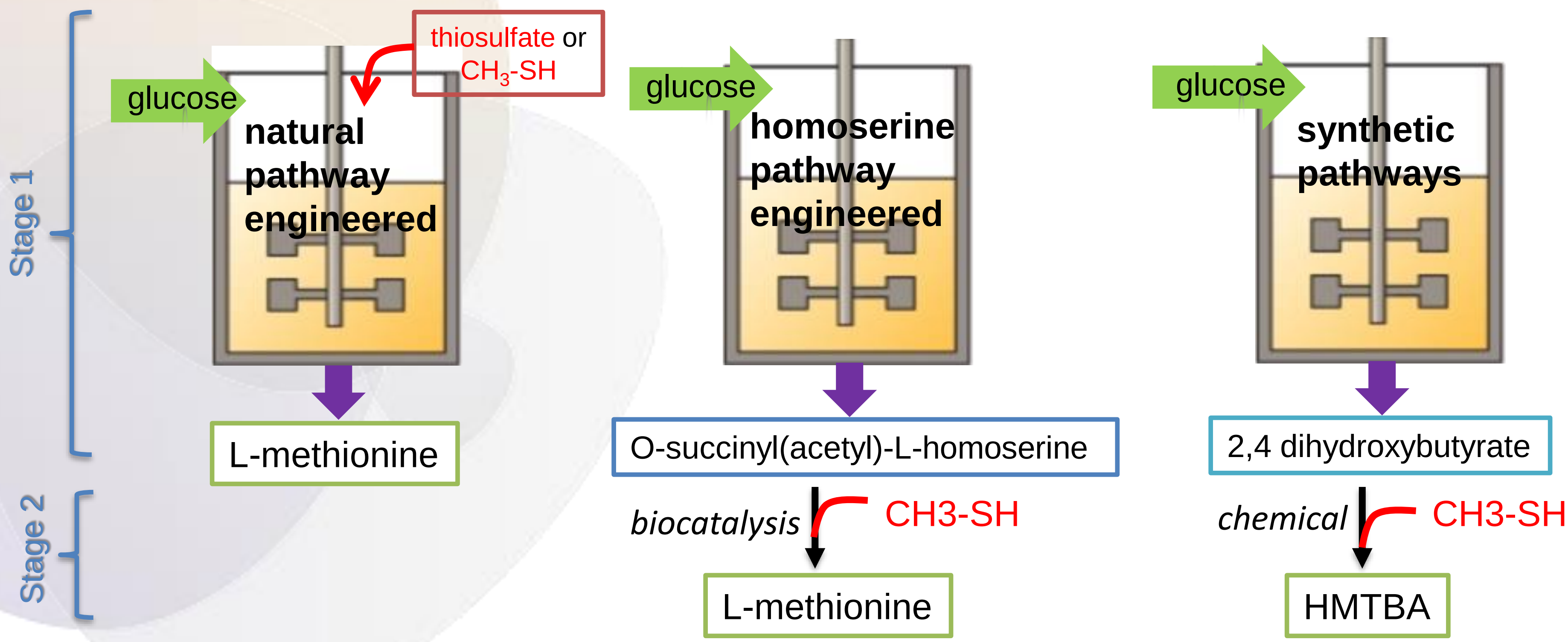
-> Maximal yield from natural pathway < 0.5 g/g glucose

4) Low solubility of methionine in water (< 55 g/l at 25°C)

PART 3 - EXAMPLE: BIOPRODUCTION OF METHIONINE: **HOW** AND *WHY*

Biosourced production of methionine: current process in development

a) Fermentation | b) Hybrid | c) Mixed



By Evonik/ MeteX
Y^{max}: 0.85 g/g using CH_3-SH

By CJ CheilJedang
Y^{max}: 0.8 g/g

By Adisseo
Y^{max}: 1.31 g/g

Methionine production by mixed process

Chemical process

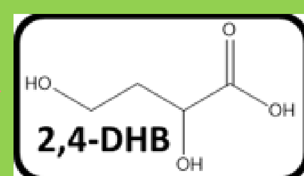
fossil ressource — **Intermediates** — **Final product**

Natural gas → H_2S + CH_3OH → **Methyl Mercaptan** → **Hydroxy analog Methionine**

Fermentation process for maximizing carbon conservation

Renewable C-sources

Synthetic pathway →

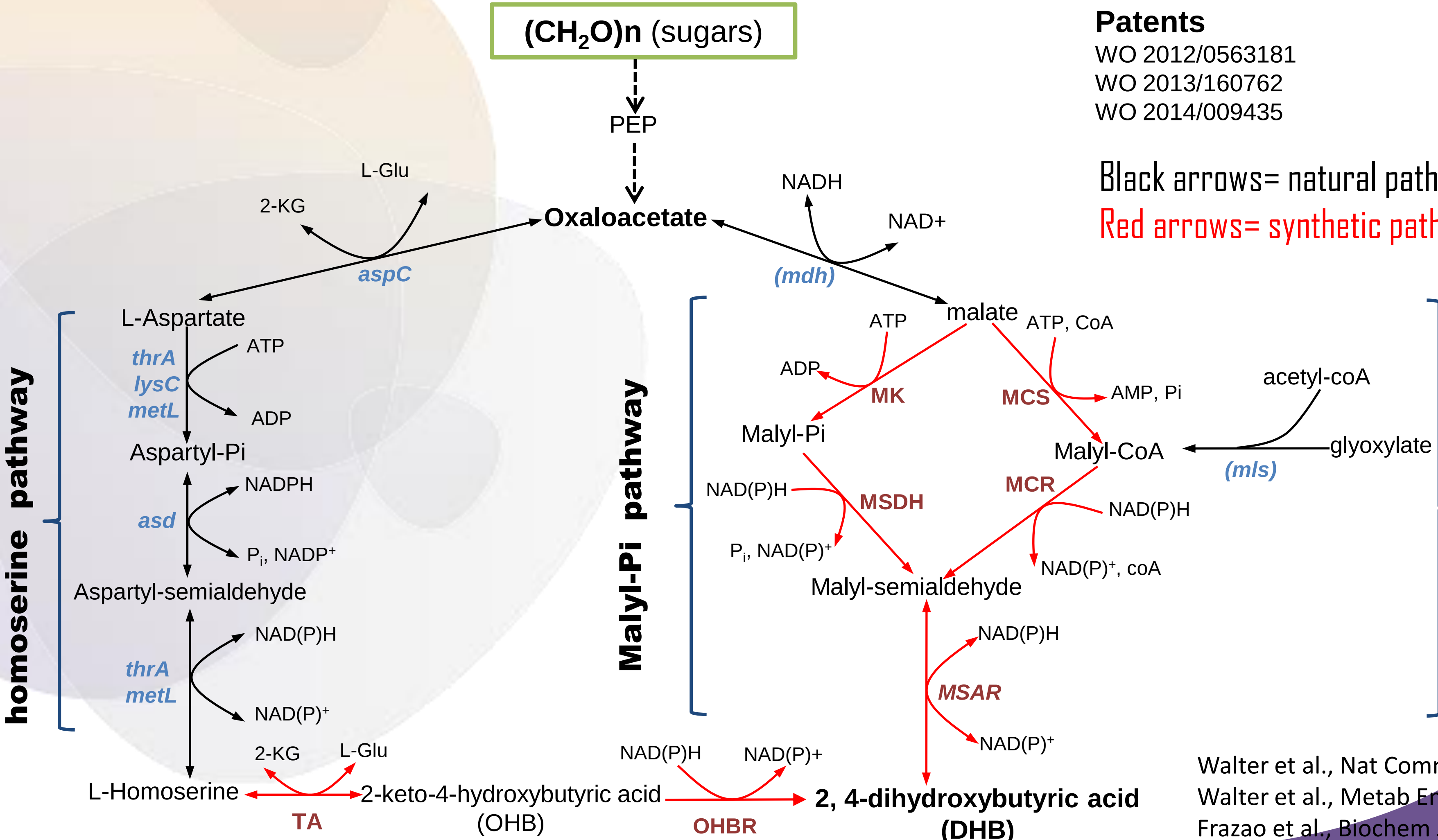


(non-natural)

DHB : successful access through three synthetic pathways

Patents
WO 2012/0563181
WO 2013/160762
WO 2014/009435

Black arrows= natural pathway
Red arrows= synthetic pathway



Walter et al., Nat Comm., 2017
Walter et al., Metab Eng. 2017
Frazao et al., Biochem J. , 2028

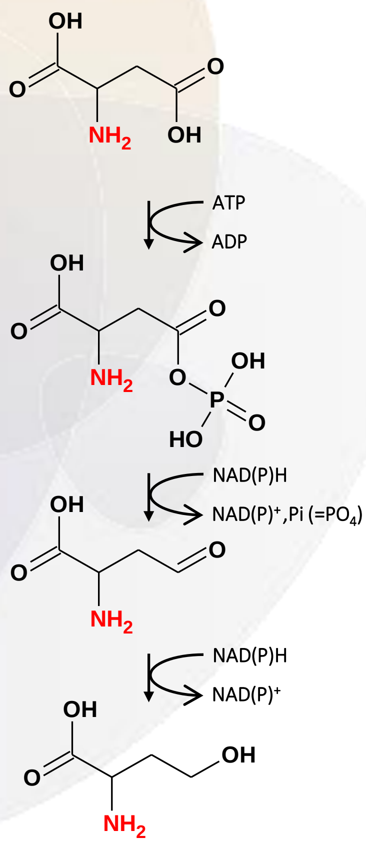
A: Enzyme engineering for Malyl-Pi pathway

Homoserine natural pathway

Aspartate kinase
AK

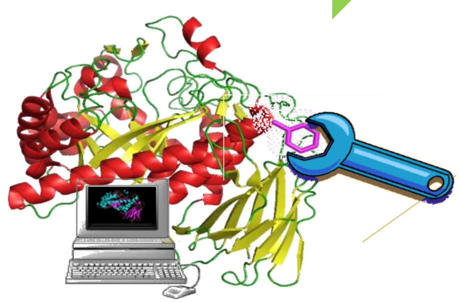
Aspartate semialdehyde dehydrogenase
ASDH

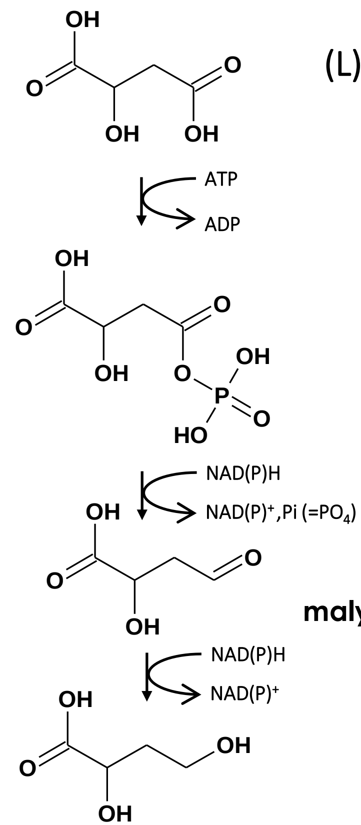
Homoserine dehydrogenase
HomDH



(L)-homoserine

We created 3 new enzymes





(L)-malate

Malate Kinase
MK

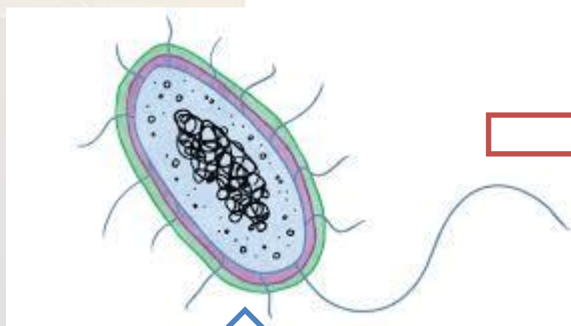
Malate semialdehyde dehydrogenase
MSA

malyl-4-semialdehyde

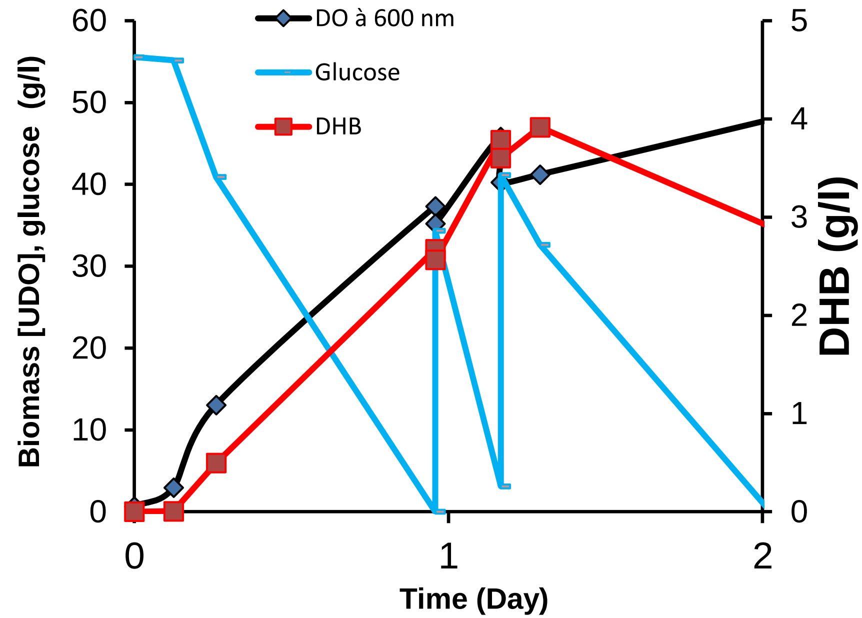
Malate semialdehyde Reductase
MSR

(L)-2,4-dihydroxybutyric acid

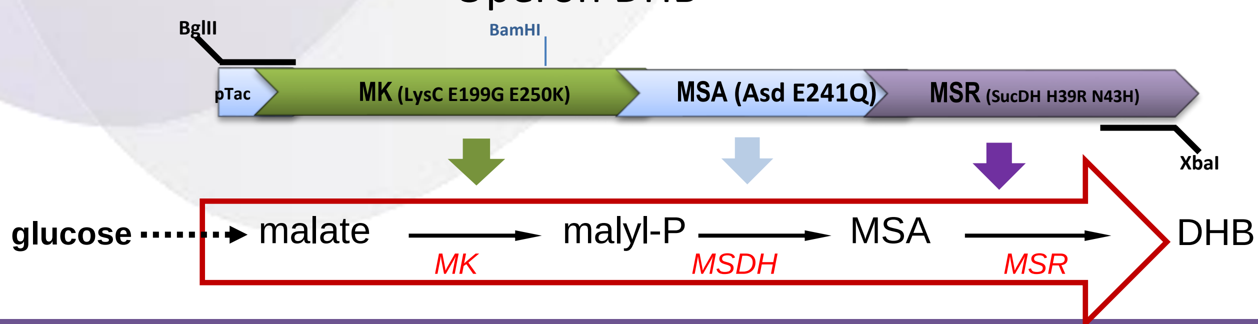
B: Strains engineering and performance



Fed-batch culture

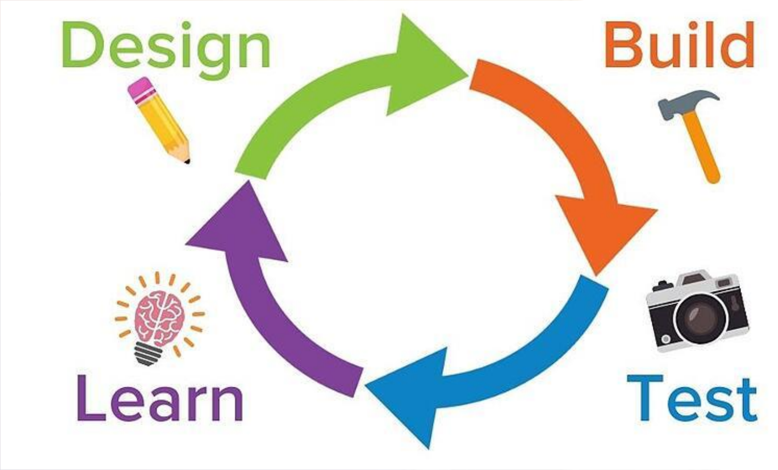


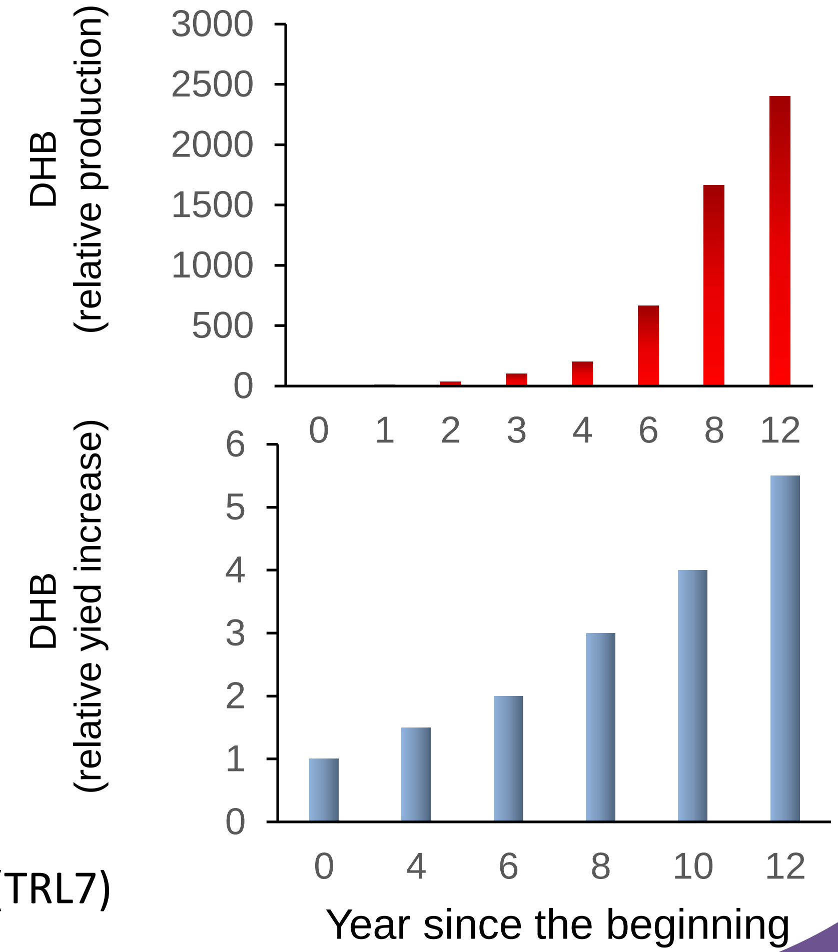
Operon DHB



B: Strains engineering and performance

- **Strains performance (*DBTL cycle*)**

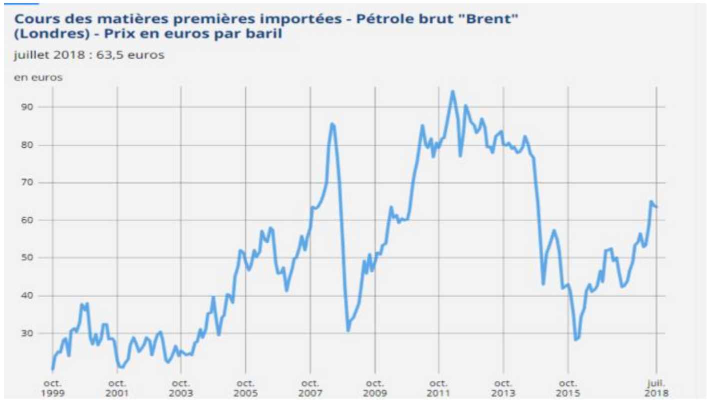




⇒ Reach today: ‘prototype/demonstrator’ (TRL7)

Factors not favouring bioproduction of methionine

1- The cost of fossil resource is still 'low'



2- D or L-methionine or D or L- hydroxyl methionine are all nutritionally valuable in poultry

-> chemical attractiveness

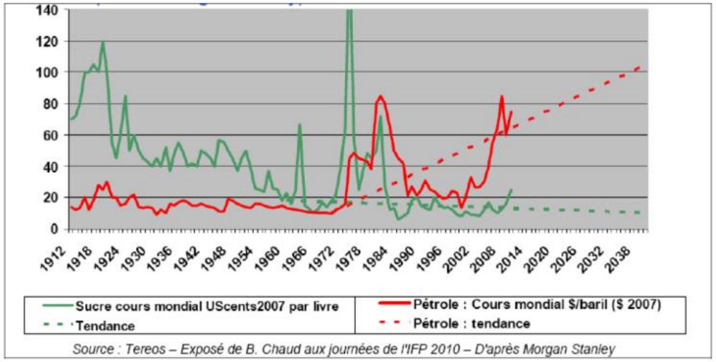
3- Pure petro-chemical process is highly mature

-> TEA highly competitive

-> LCA competitive to biotechnological production on actual amino acids (lysine, glutamate)

Factors favouring production of biosourced methionine

1- fossil resource is steadily decreasing and
thus price shall raise



2- Petro chemical process is highly hazardous
-> Seveso High
-> use HCN, acrolein, solvents, high acidic conditions

3- plant biotech is 'smaller' and "smatter"
-> build "locally' taking into account environmental and societal factors
- local carbon resource (straw, molasses, sugar canes, wood)
- local human activities

=> reduce transport (reduced greenhouse effect)

PART 4 - FACTORS FAVORING BRIGHT FUTURE OF WB

1st: Instruments to de-risk and innovate in IB

'Technology Readiness Level' ou TRL

Cost **risk**

10 -15 years

1. Idea or basic principles
2. Technology concept formulated
3. Experimental proof of concept (*-> Patent*)
4. Technology validated in lab
5. Technology validated in a relevant environment
6. Technology demonstrated in an industrial context
7. System prototype demonstration
8. System complete et qualified
9. Actual system proven operational

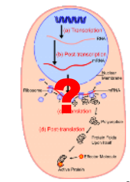
Cost	risk
5	85
10	10
85	5

Recherche

Industrialisation

Development

Idea



Lab-scale

Demo plant

Industrial plant

1st: Instruments to de-risk and innovate in IB

Technology Readiness Level ou TRL

10-15 years

1. Idea or basic principles
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3. Experimental proof of concept *(-> Patent)*
4. Technology validated in lab ← **Valley of Death!**
5. Technology validated in a relevant environment
6. Technology demonstrated in an industrial context
7. System prototype demonstration
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PART 4 - FACTORS FAVORING BRIGHT FUTURE OF WB

1st: Instruments to de-risk and innovate in IB

how to get out of the valley of death?

❖ Start-ups in Biotech

- concept of innovation and de-risking
- Several means for fund raising

❖ Transfer Technology Center

ex: Toulouse White Biotechnology (TWB)

3 missions

- accelerate the development of industrial biotech
- facilitate the interface between public research and industry
- promote the development of sustainable production methods

➢ Require strong Public financial support for de-risking

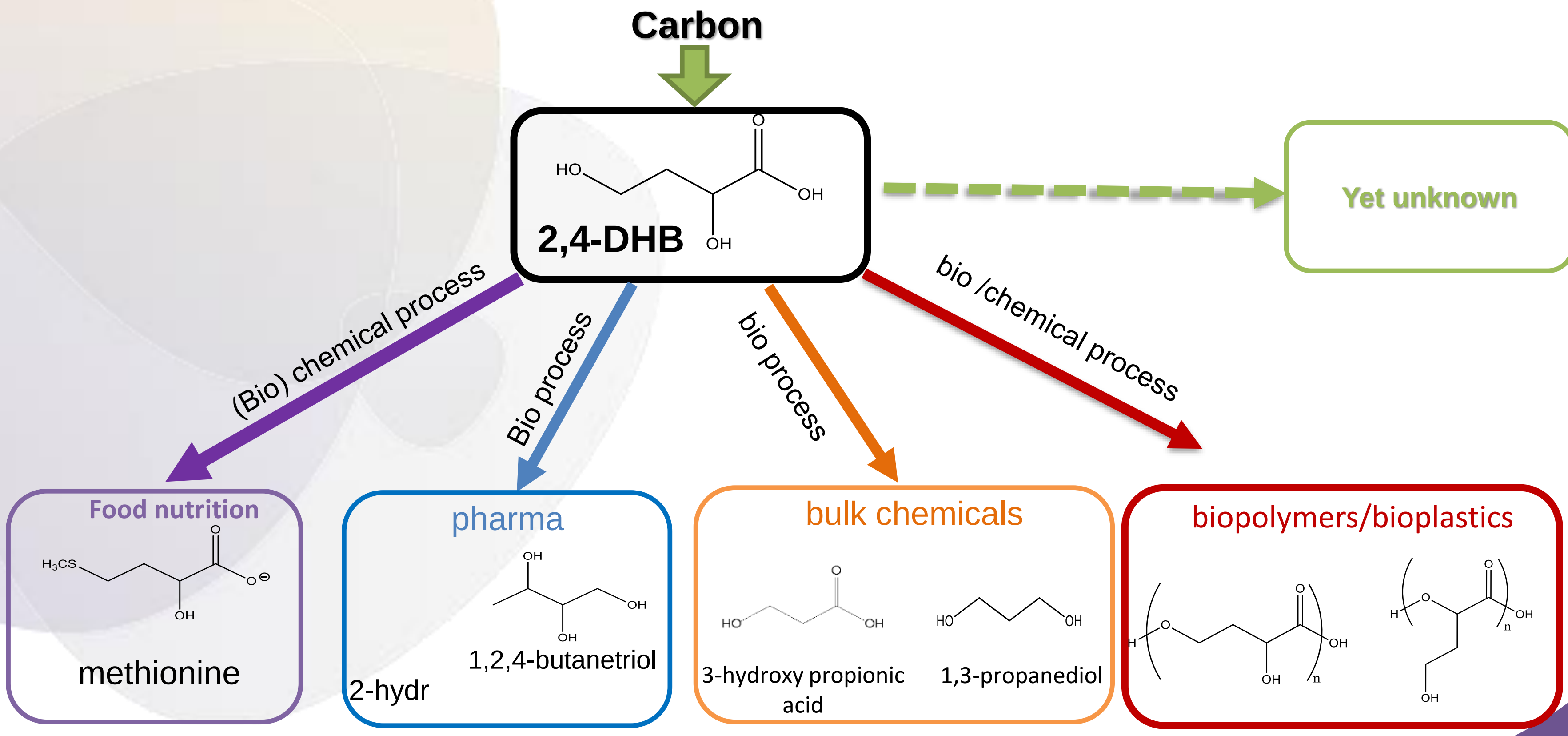
- ANR (TRL2-5)
- PIA-BPI/ADEME (TRL 5-7)
- France Biotech 2030 (TRL 5-8)

2nd: Techno-scientific (*business model*): the bio-based product is a platform molecule

additionally

should be easier to produce biologically than chemically and

should reduces significantly GWP and CO_2 emission (Life cycle assessment)



PART 4 - FACTORS FAVORING BRIGHT FUTURE OF WB

3th: socio- economic: Size reduction and Sharing

BIG chemical Industries that do all in all → Smaller and 'local' Biotech plant

- Can be installed where there are "resources"
- Will reduce global pollution
- Will reduce GWP and CO2 (transportation)
- Will promote local 'reindustrialization'
- Will increase diversification / new jobs

PART 5 - FUTURE TRENDS & AWARENESS

- ❖ New microbial chasses must be developed to use all type of carbon sources
- ❖ CO_2, plastic waste as alternative carbon source
- ❖ Fermentation process must be realized with less water
- ❖ Acceleration of innovation and development by integration of IA into Biology

- ! Need 'long-tail public funding over many years to de-risk the field
- ! Public acceptance of 'Synthetic Biology Organism' (SBO)
- ! Increase consumer awareness of global sustainability from bio-based solutions, even if they will be less 'economically' attractive

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Collaborators

Technological Platforms

Financial support

Institutional support