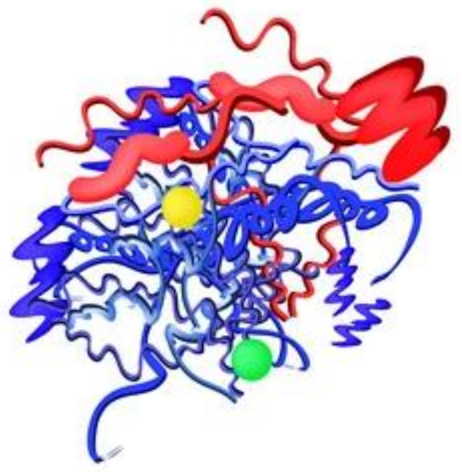
October 2023

How enzymes can bring stability and efficiency for cosmetic applications

Givaudan
Human by nature

Why enzymes for cosmetic application ?



1. Naturality

2. Selectivity

3. Efficiency

4. less by products emission

5. Less energy needed

6. Traceability & sustainability

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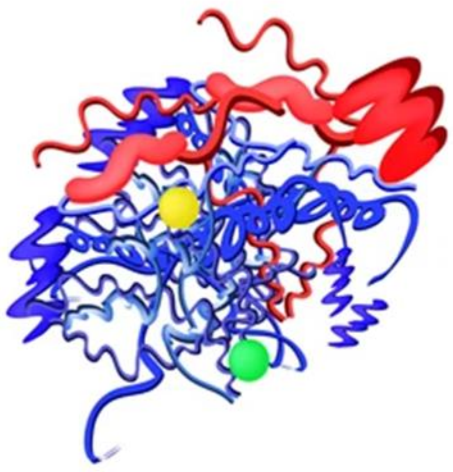
Cosmetic used it for what purpose ?

1. Actives



Peeling with Papain, Bromelain, ficin etc…

Hair coloration with laccase…

Reparation / protection with antiox such as SOD, Catalase, peroxidase…

2. Additives to stabilise or to deliver

Anti microbial applications with Glucose oxidase, oxydo-reductase

Nutrient for skin and hare care with Proteolytic enzyme or lipase to produce oligo-peptides, Pseudo céramides, fatty acid

3. Enzyme to express as a target

for whitening, slimming etc.. Action on Tyrosinase, lipases, Proteinases

4.Processing aids => Biocatalysis and fermentation

For self tanning, GDH (DHA), moisturizing, Hyaluronanes (HA), anti ageing /whitening, C vitamin 2 Glucosides (AA2G)

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Givaudan put a focus on processing aids using fermentation

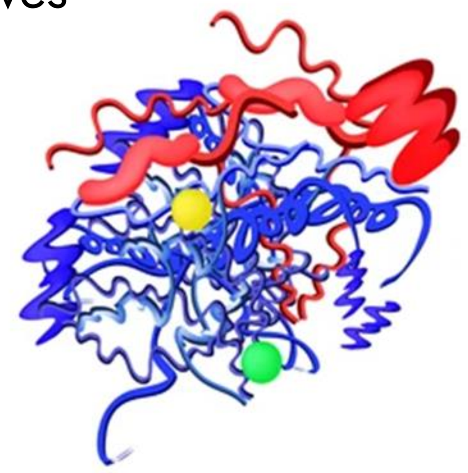
=> To Produce enzyme by fermentation for bio-catalysis and then to obtain efficient actives

Leuconostoc for Glycosylation and Phosphorylation

=> To use or to introduce enzyme in microorganisms as processing aids

GDH with Gluconobacter suboxydans for DHA (self tanning agent)

Hyaluronanes synthase or / and Hyaluronidases with Saccharomyces cerevisiae for HA



The key challenge is to develop all theses technolgies into **cost-effective industrial scale** that deliver **products** at market **prices**

Bio-catalysis

1°) Applied Biocatalysis in Cosmetics

Glucosylation | Phosphorylation

Increases solubility in water

- Glucosylation: **Hesperedin** (Hayashibara, 1989); **Rutin** (Hayashibara, 1990)
- Phosphorylation: **Ascorbyl-P and Alkyl-Ascorbyl-P**; **Tocopheryl-P** (use: LVMH, US 5387579, filed July 31st 1992)

Increases chemical stability

- Glucosylation: **Hesperedin**; **AA2G** (Hayashibara, 1990); **α-Arbutin** (Ezaki Glico, 2002)
- Phosphorylation: **Polyphenols : gallotannins, resveratrol** (Omnichem-Ajinomoto, US 2008/0076833 A1) (Estee Lauder; precursors; US 2008/0095866 A1)

Is a reversible modification

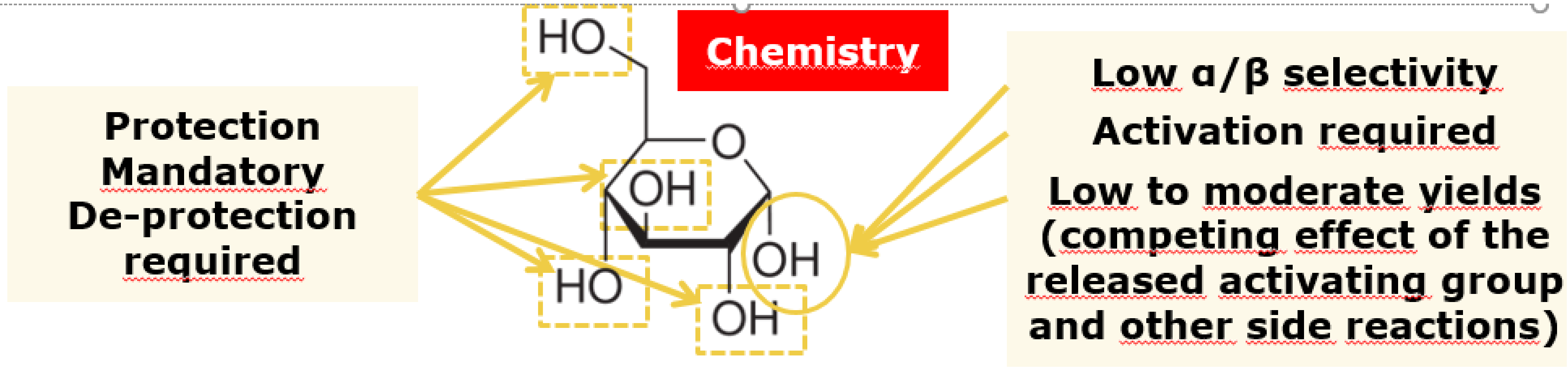
- Glucosylation: **Hesperedin**; **δ-tocopherol** (PFDC, depigm., US 2012/0093749 A1, 2012); **Polyphenols** (Chanel / Rhodia, bioprecursor, WO/2006/134282)
- Phosphorylation: **Polyphenols : gallotannins, resveratrol** (Omnichem-Ajinomoto, US 2008/0076833 A1) (Estee Lauder; precursors; US 2008/0095866 A1)

Gives NEW active molecules

- Glucosylation: **Prebiotics** (Lactosucrose, Hayashibara, 1990)(Malto/isomaltoOS, GlcOS, GalOS, FOS, XylOS, …); **Melibiose** (Galactoside)

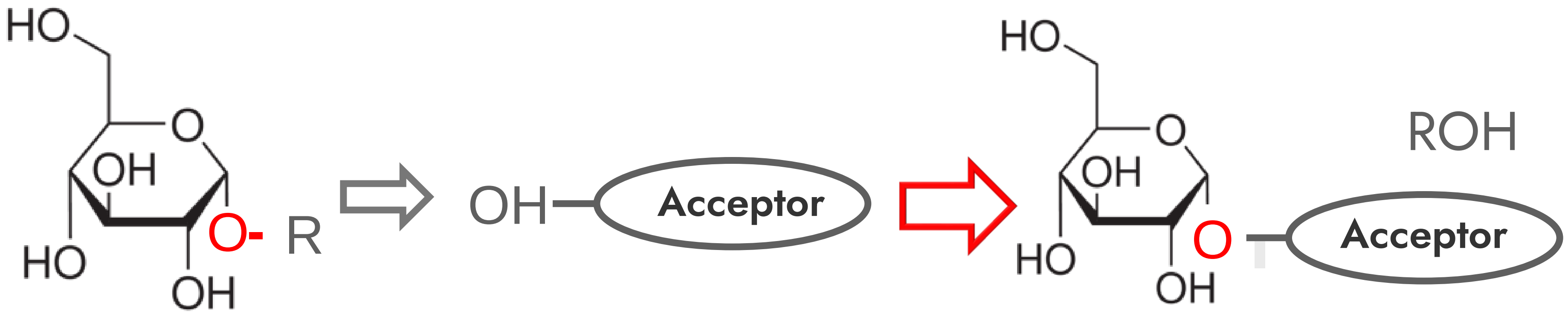
Glucosylation

- Glycosides play various roles: structural molecules, energy reserve, labels that allow the cells to communicate
- Hydroxyls group are very reactives but chemistery is not enough selective and need protection and deprotection to be grafted



Glucosylation with enzyme

- Sucrose is a glucose donor cheaper than cyclodextrins
- Dextransucrases are described to add only one Glucose residue
- Dextransucrases are not commercial enzymes: to be produced but IP is possible
- **Targeting poorly water soluble actives substances to deliver water soluble forms: the most critical work will be to find conditions delivering sufficiently high product concentrations (sugars are only soluble in water or polar solvents)**



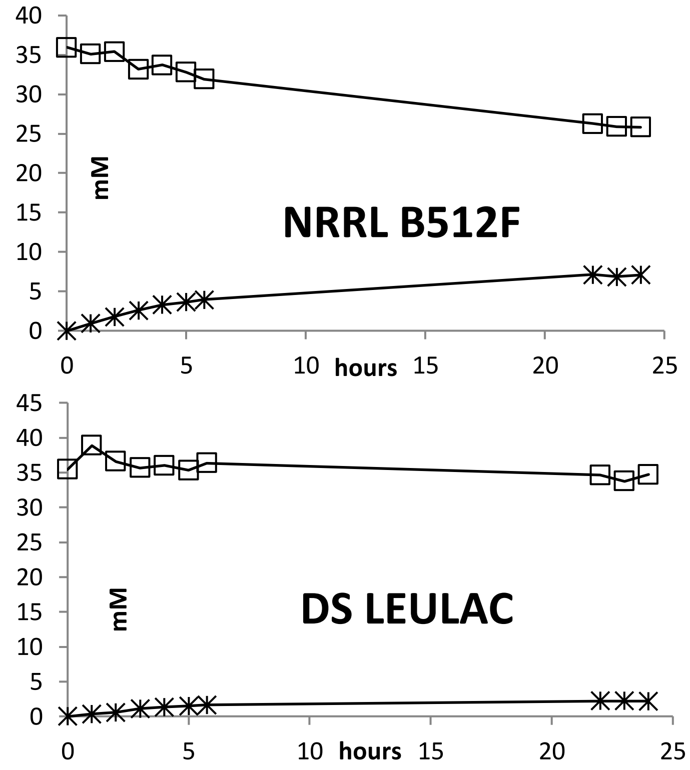
Glucosylation selection @ Givaudan

First step was to select the best strain

Second step to harvest it

Last step to stabilize it

Code	Origin
NRRL B 512 F	*Leuconostoc mesenteroides*
ATCC 11449	*Leuconostoc mesenteroides subsp. mesenteroides*
ATCC 8086	*Leuconostoc mesenteroides subsp. dextranicum*
DSM 14295	*Weissella cibaria*
DSM 20188	*Leuconostoc amelibiosum Schillinger*
DSM 46216	*Leuconostoc mesenteroides subsp. dextranicum*
DS LEULAC	*Leuconostoc lactis* (recombinant)



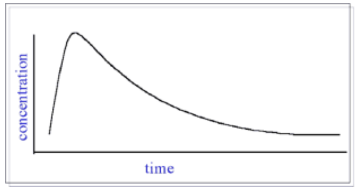
Glucosylation @ Givaudan

➡ **Modulation of the physico-chemical properties through glucosylation**

Water Solubility

Chemical Stability (oxidation)

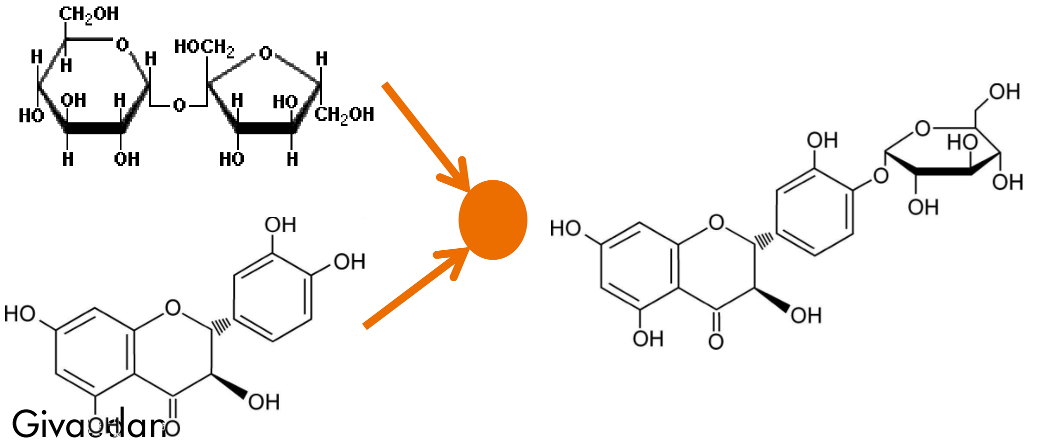
Structure well characterized



Bioavailability

➡ **Dextransucrase produced by *Leuconostoc mesenteoides* NRRL B512F : a breach in the acceptor recognition specificity**

We developed our technology with
Caffeïc acid
Polyphénols
Taxifolin
Resviratrol



Glucosylation of small molecules @ Givaudan

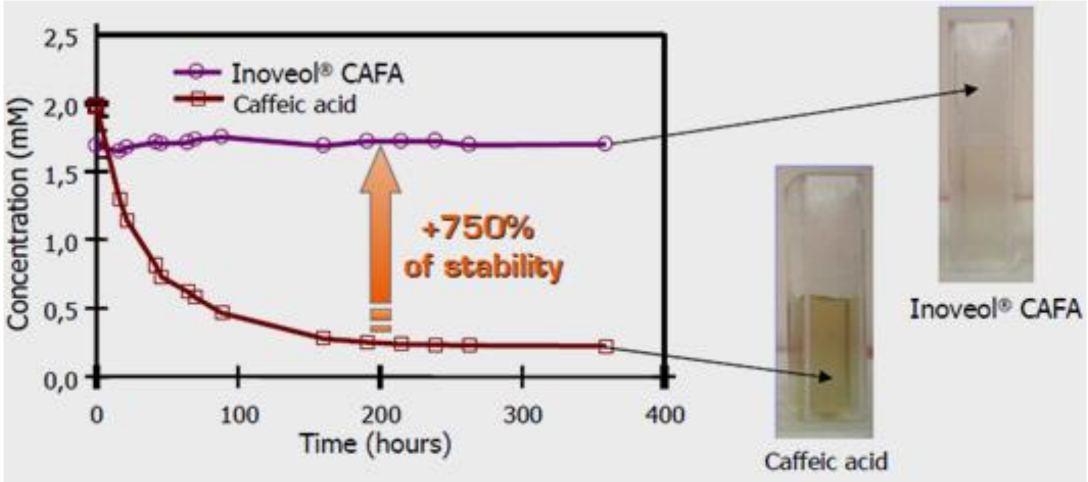
➡ **Route for the production of α-D-Glucosides**

1. Enzyme Production	2. Conversion	3. Purification	4. Performance
Culture of the GRAS bacteria Enzyme isolation / storage	Free Enzyme: 30°C, pH 5.2, 24 – 36 hours 5 – 25 g/L/d	Sugars and Enzyme removal α-D-Glucoside recovery	$Y_{Gde/Ac}$: 80% Purity: > 96% Sev. > 500 kg/Y

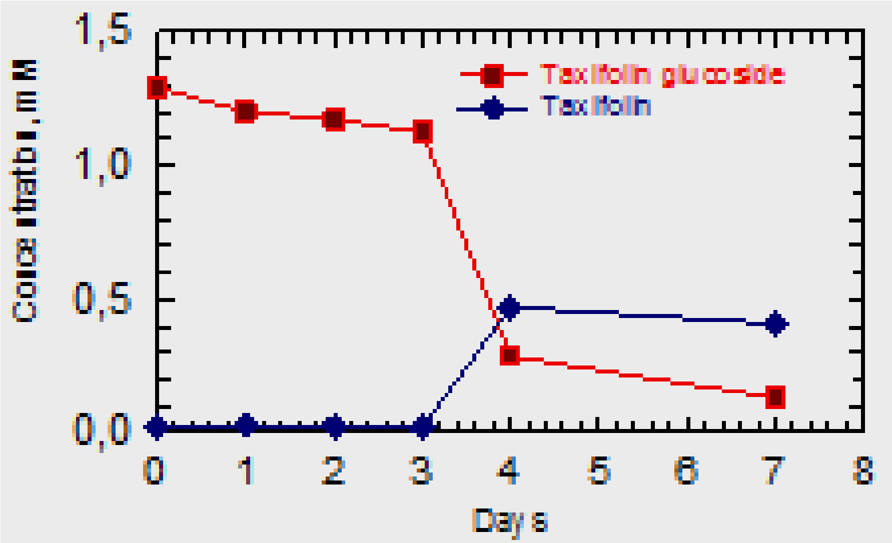
Key point to fix and to purify the glycosyl

Glucosylation stabilize and make it efficient

- **Adjusted properties through glucosylation**
  - Solubility in water, pH 5.2: > 100 g/L
  - Caffeic acid Glucoside : stable at pH 7.1,



- **Taxifolin can be enzymatically released from Taxifolin glucoside on the skin**



Conclusion

- Biocatalyse is used since years for cosmetic application

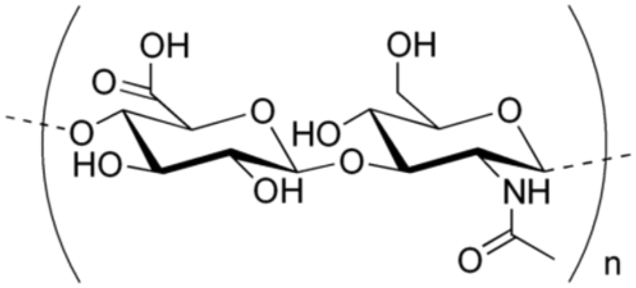
- Givaudan developed processes to produce specific enzymes (dextrane saccharase, Phosphatase) and used it to stabilise reinforces or to make it soluble and more effective with actives ingredients such as glycosyl polyphénol (taxifoline, acide caféique, acide rosmarinique, EGCG, oleuropéine, phlorétine, acide gallique and resvératrol) + Phosphorolyted glucose ($NAcGlcNH_2$ – 6P, alkylglucosides – 6P)

Successful application of biocatalyst for organic transformations in industries depends on factors that include availability of suitable enzyme.

To use or to introduce enzyme in microorganisms as processing aids

2 Fermentation to produce cosmetic actives such as HA
How can we improve it

1. Natural strain using specific enzyme such as Streptococcus zooepidemiccus to produce HA at HMW and today to obtain LMW we need a second step through an hydrolysis (Chemical or enzymatic)



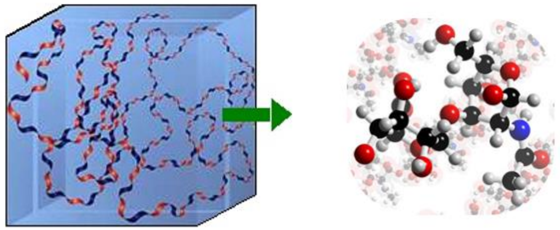
SODIUM HYALURONATE

*Viscosity (20° C): 5000 cPo approx at 1%DM

*Density : ~1 g/cm^3 at 1%DM

*Linear molecule / Helix form**

*Length = 5 to 25 µm *in vivo*



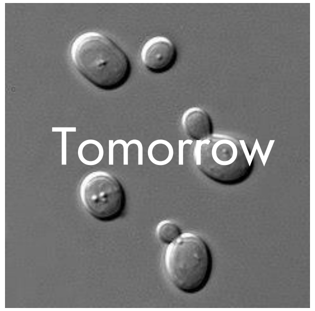
Hydrolysis

2. By using GMO Saccharomyces cerevisiae with genes codding hyaluronanes and hyaluronidases we can now doing both at the same time.

HA production

Limit is thermodynamic such as O2 dispersion and Heat transfer

Strain is to use carefully as pathogenic
Production of a biofilm at 37 °C (Exopolysaccharide)
Very high molecular weight > 2 M Da
Very viscous (at 1% > 5000 Cps)

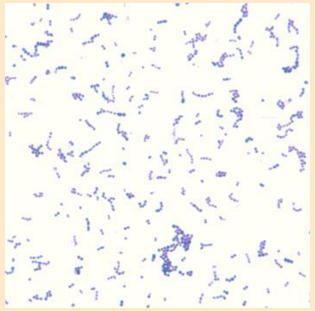
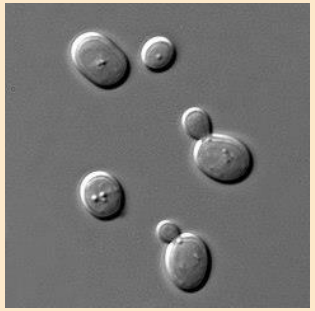


Our Strategy to use a GRAS strain
To introduce by GMO hyaluronane synthase
To fix enzyme selected "hyaluronidases"
 into the extra membranes to produce LMW

Production of oligosaccahrides
LMW < 0.1 MDa
No Viscosity
Lower T° 29 °C

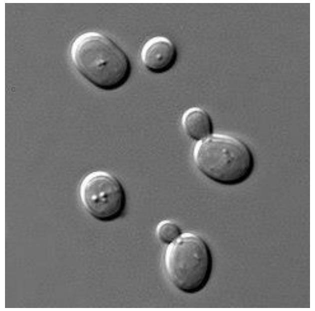
Why bio engineering is a game changer ?

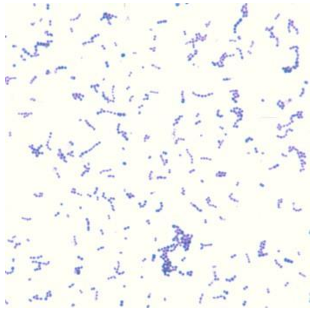
How can we improve the production of HA LMW

	Current production	Next way to produce
Strain	Bacteria (pathogenic)	Yeast (GMO)
T°	37 °C	29 °C
	Streptococcus zooepidemicus 	*Saccharomyces cerevisiae* 
Origin	Natural massal optimization	GMO patented
Type of MW	HMW	LMW
Raw Material	Glucose	sucrose

Fermentation DSP processing

Main avantages to use an GMO strain with intra Cell enzymes





Increase the concentration of HA in the broth **As less thermodynamic constraints**
Easier cell separation **As not viscous**
Easier purification
Cost reduction and less impact **Process steps from 8 to 5**

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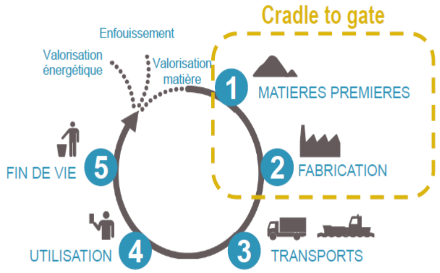
Focus on sustainability

Frontières du système



01 – Les indicateurs d'impact

Changement climatique (unité : *kg CO_2eq*) : prend en compte les émissions de gaz à effet de serre (GES) telles que le CO_2 , le CH_4 , le SF_6 , … *Méthode : IPCC 2021 100 years + quelques facteurs de caractérisation calculés par le JRC*

Acidification (unité : *mol H^+eq) :* prend en compte les émissions acidifiantes comme les Nox, SOX,. *Method : Accumulated Exceedance. Seppälä et al. 2006, Posch et al, 2008*

Ecotoxicité, eau douce (unité : *CTUe*) : prend en compte les émissions de substances bio-accumulable qui ont des conséquences néfastes sur les organismes vivants comme les métaux lours, POPs PCBs, etc. *Méthode : USEtox 2.1 (Rosenbaum et al 2008)*

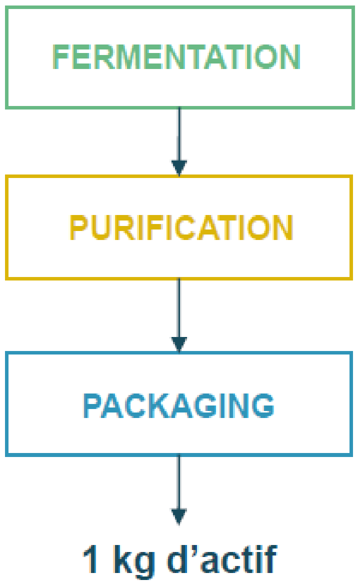
Eutrophisation terrestre (unité : *kg N eq*) : prend en compte les dépôt des émissions atmosphériques de composés azotés tels que les oxydes d'azote provenant des processus de combustion et l'ammoniac (NH3) provenant de l'agriculture. L'ajout de nutriments peut modifier la composition de la végétation en favorisant certaines espèces qui profitent de niveaux plus élevés de nutriments pour se développer plus rapidement que les plantes plus économes en nutriments. *Méthode : Accumulated Exceedance (Seppälä et al. 2006, Posch et al, 2008)*

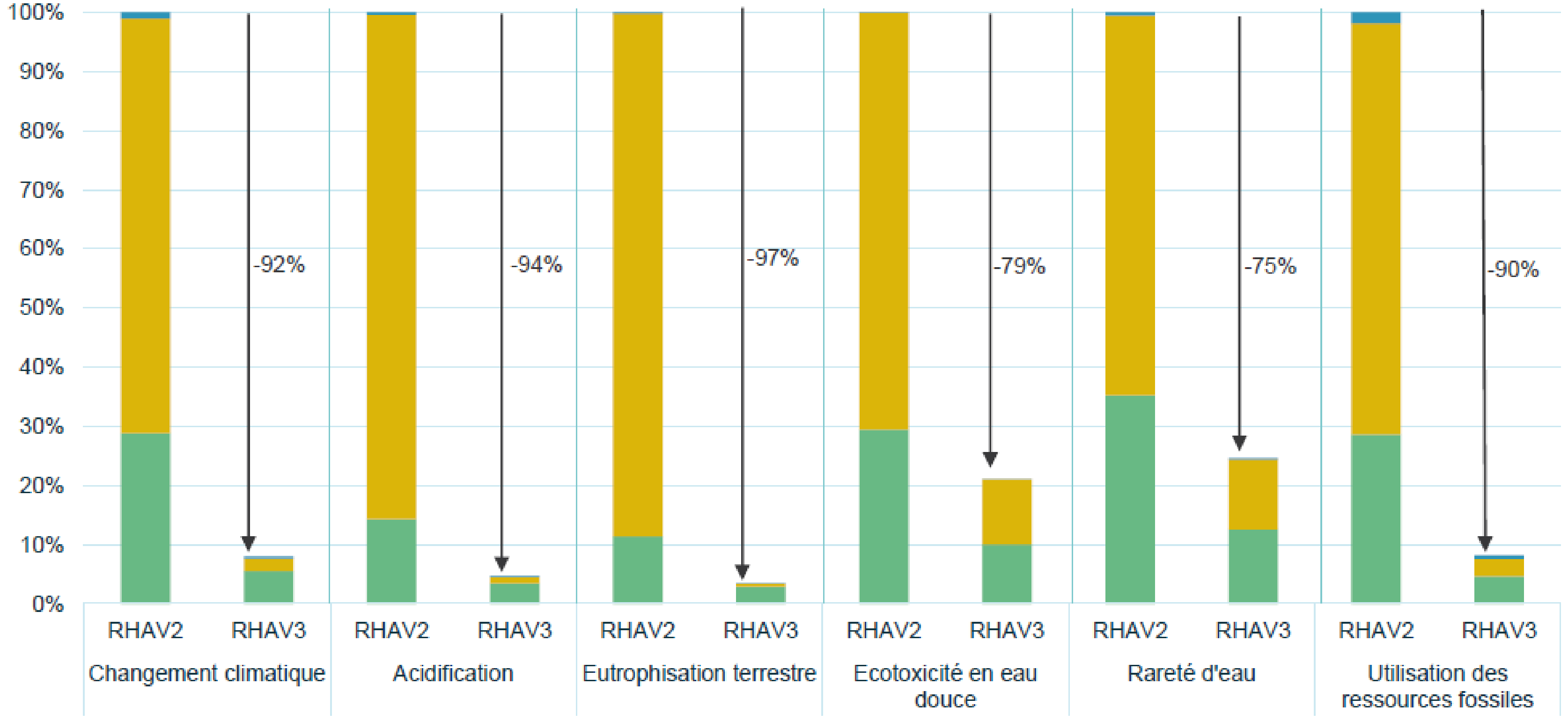
Consommation d'énergie non renouvelable (unité : *MJ*) : prend en compte les sources non renouvelables d'énergie telles que le fioul, le charbon, l'uranium et le gaz naturel. *Méthode : CML, v. 4.8 (2016) - Van Oers et al 2002*

Consommation d'eau (unité : *m^3 eq*) : prend en compte la consommation d'eau, pondérée par la disponibilité en eau du pays où le prélèvement est effectué. *Method : AWARE 2016 (relative Available Water Remaining)*

Impressive results by using intra-membrane enzyme

- **Impacts / 5**

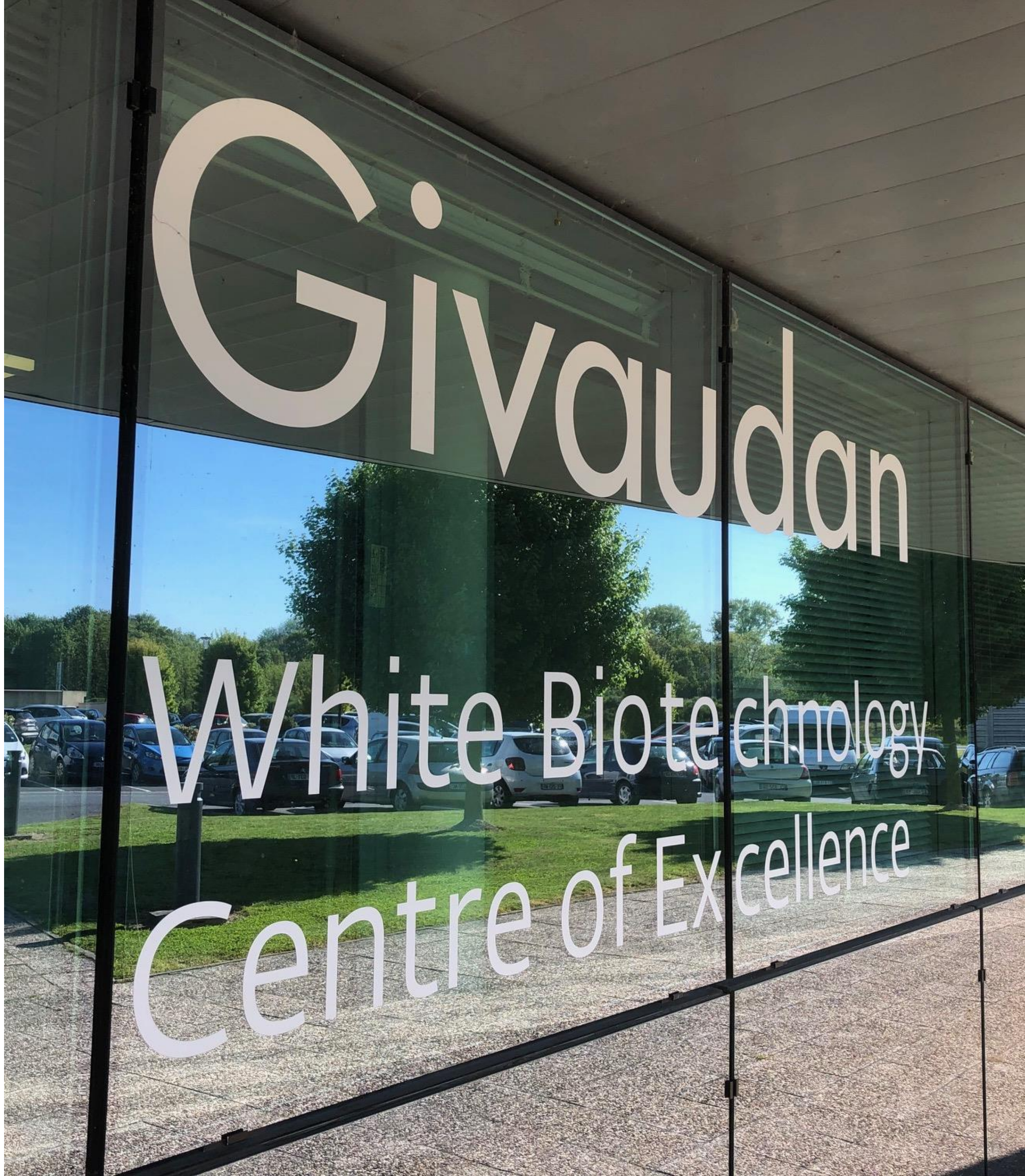




Conclusion

- Biotech need to perform productivity and efficiency.

- with enzymes we can improve both with also a better LCA result.



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