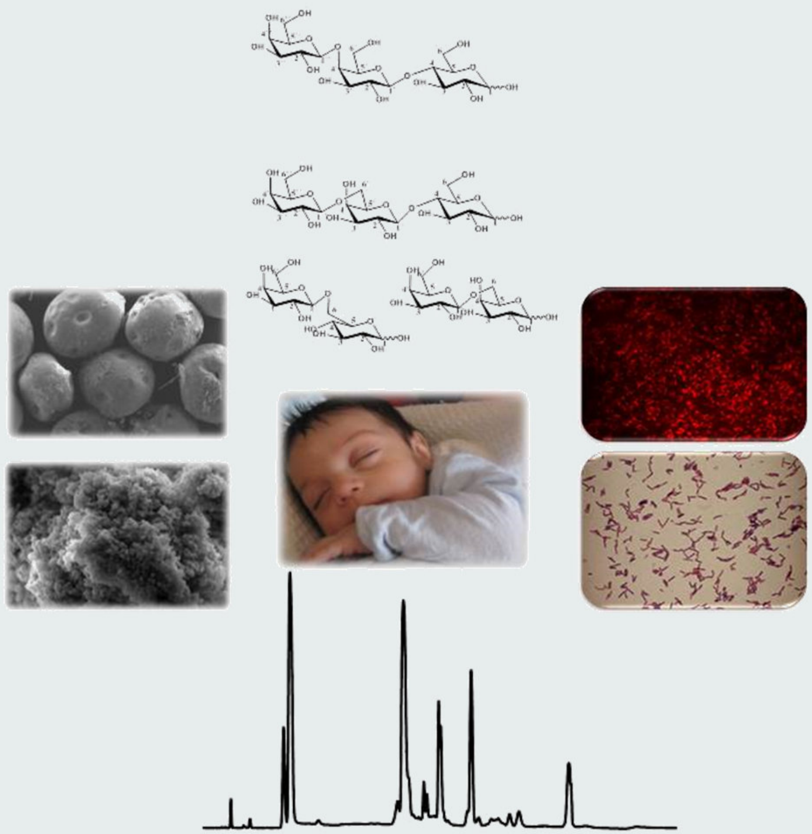
Transformations of carbohydrates into prebiotic oligosaccharides using free or immobilized glycosidic enzymes

Francisco J. Plou



Applied Biocatalysis Group
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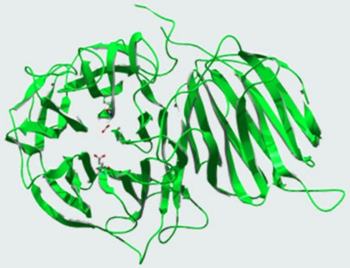
28049 Madrid (Spain)

Biotransformations catalyzed by glycosidic enzymes

Analysis and characterization of complex mixtures of carbohydrates

ABG

APPLIED BIOCATALYSIS GROUP



Immobilization of glycosidic enzymes

PREBIOTICS

"a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health''

Essential key:
balance between bacterial species

PREBIOTICS *vs.* PROBIOTICS

- Water solubility
- Sweetness
- Thermal resistance
- Good organoleptic properties
- Easy addition to foods
- No manipulation of live organisms

OUTLINE

Sucrose → *β-Fructofuranosidase* → **Fructooligosaccharides (FOS)**

Inulin, Agave fructans → *Inulinase* → **Fructooligosaccharides (FOS)**

Lactose → *β-Galactosidase* → **Galactooligosaccharides (GOS)**

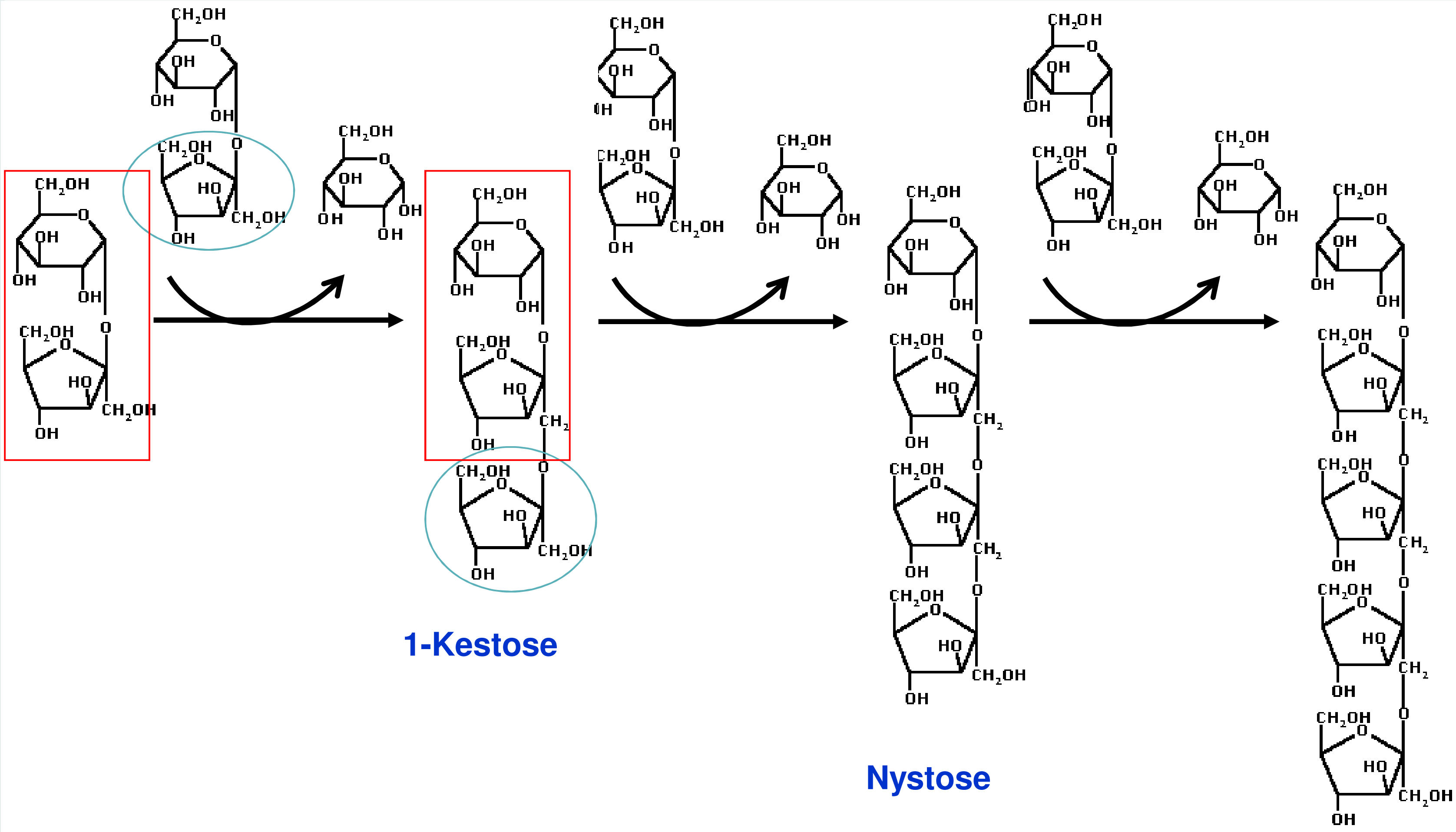
Starch → *α-amylase*, *β-amylase*, *α-glucosidase* → **Isomaltooligosaccharides (IMOS)**

Fructooligosaccharides

SYNTHESIS OF FRUCTOOLIGOSACCHARIDES (FOS)

Sucrose ⟶ **Glc-Fru$_n$ (n=2-5) + Glc**

Invertases
(β-fructofuranosidases)

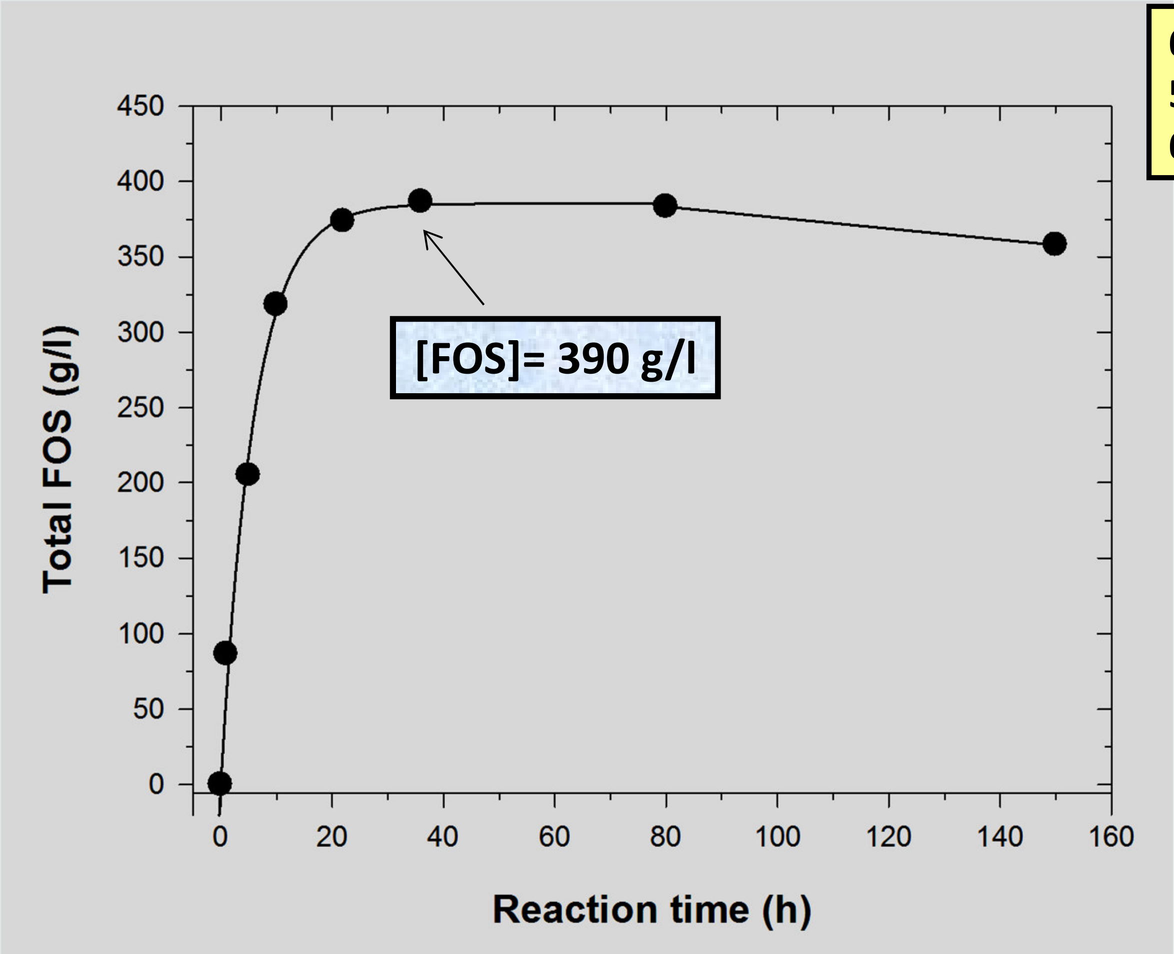


1-Kestose

Nystose

Fructosylnystose

FOS production with β-fructofuranosidase from Aspergillus aculeatus



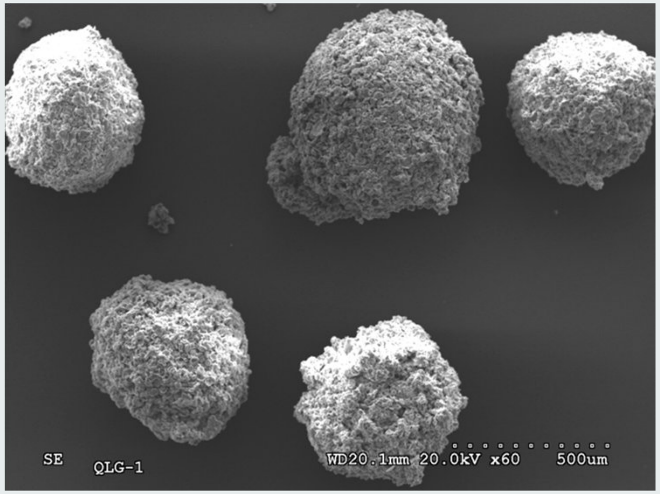
630 g/L sucrose
50 mM sodium acetate buffer pH 5.4
60 °C

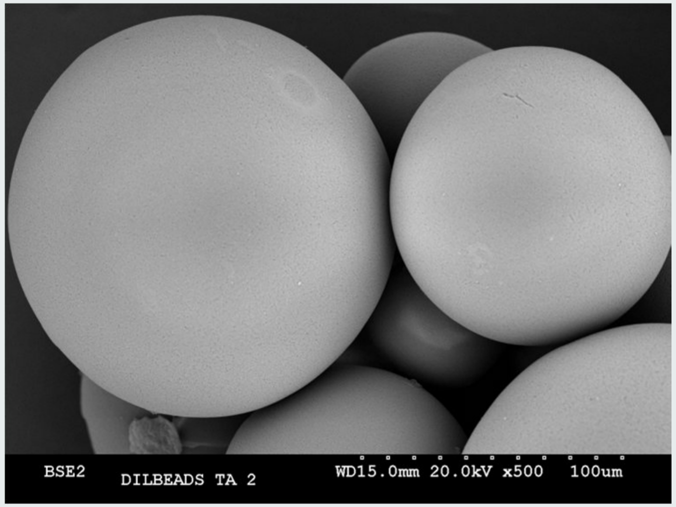
"Purification and kinetic characterization of a fructosyltransferase from Aspergillus aculeatus". I. Ghazi, L. Fernández-Arrojo, H. García-Arellano, M. Ferrer, F.J. Plou and A. Ballesteros. *Journal of Biotechnology*, 128, 204-211 **(2007)**

Molasses and sugar beet syrup as feedstock for FOS production

"Beet sugar syrup and molasses as *low-cost feedstock for the enzymatic production of fructo-oligosaccharides".* I. Ghazi, L. Fernández-Arrojo, A. Gómez de Segura, M. Alcalde, F.J. Plou and A. Ballesteros. *Journal of Agricultural and Food Chemistry*, 54, 2964-2968 **(2006).**

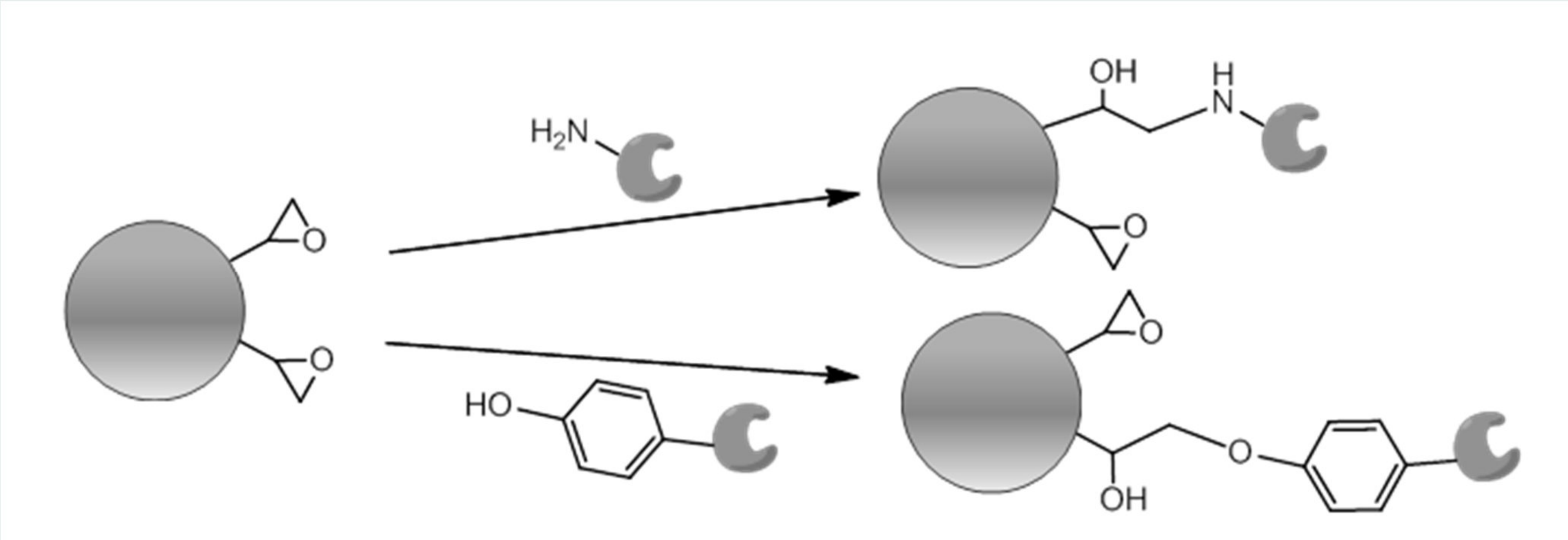
Enzyme immobilization for FOS synthesis

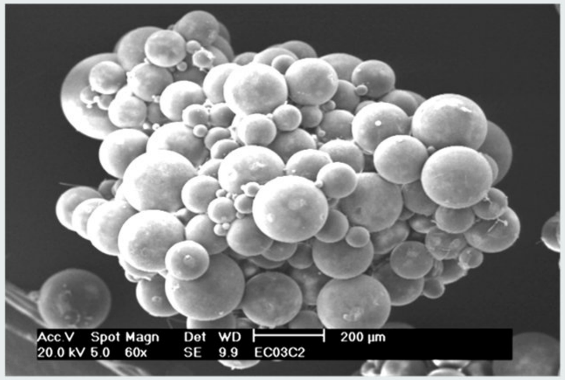




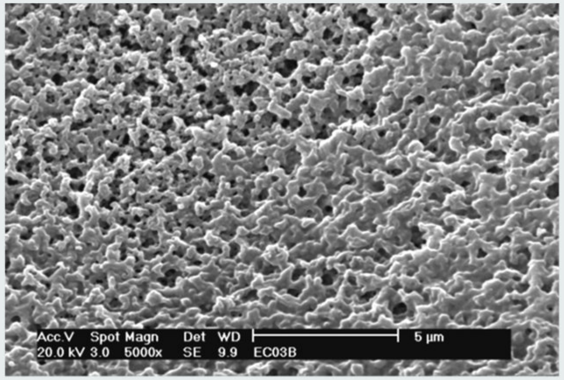
- **Separation of the biocatalyst**
- **Reuse of the biocatalyst**
- **Continuous reactors (CSTR, fixed-bed, etc.)**
- **Increase of stability**

Covalent immobilization in epoxy carriers

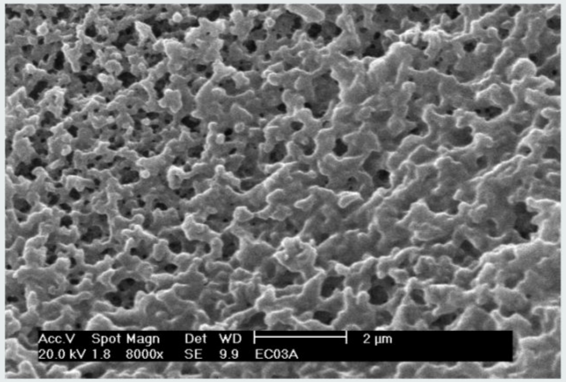




60x



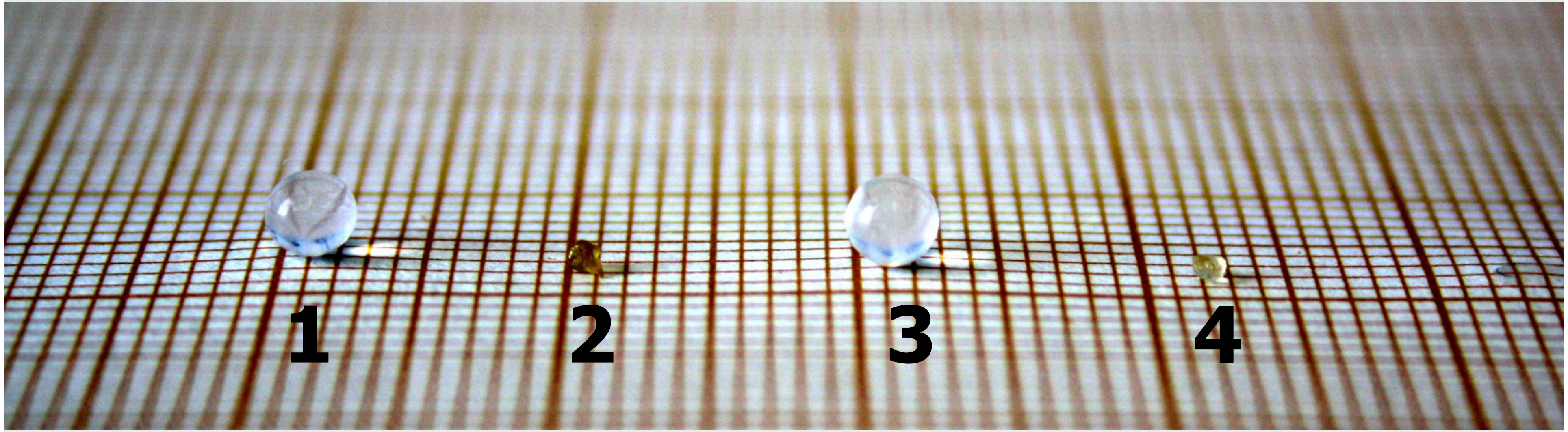
5000x



8000x

Sepabeads EC-3

Dried Alginate-Entrapped Enzymes (DALGEEs)

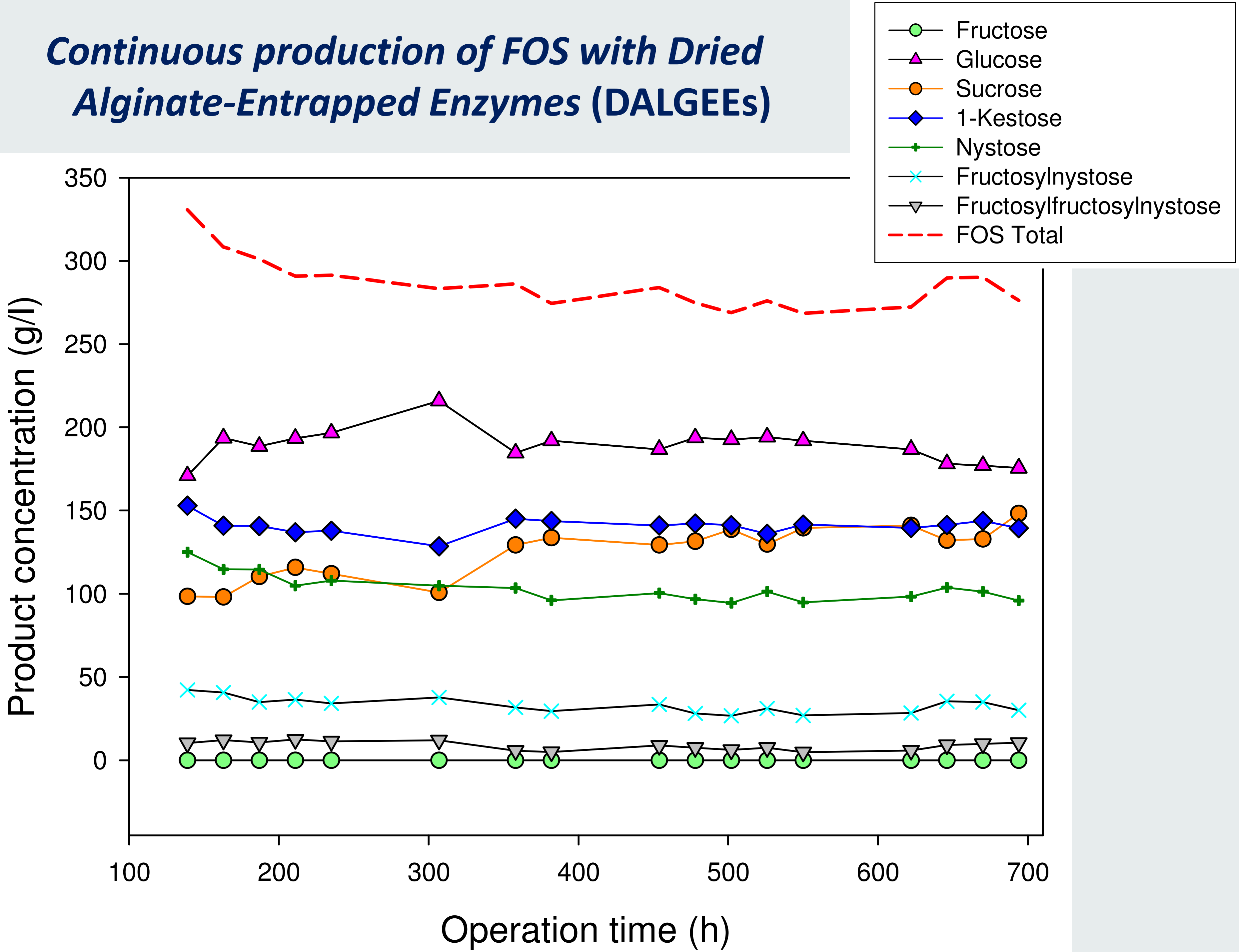


1: Alginate bead (wet)
2: DALGEE bead
3: DALGEE bead after contact with aqueous solution
4: DALGEE bead after contact with sucrose solution

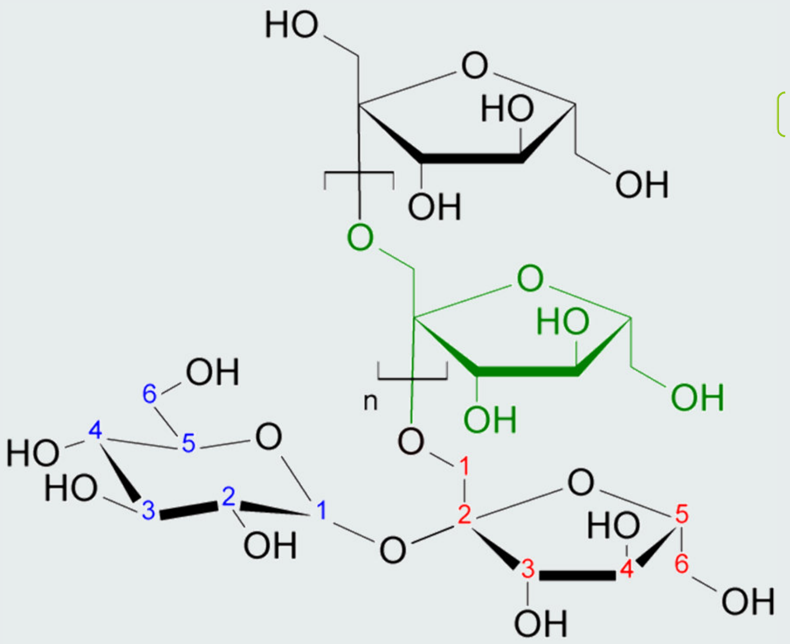
Continuous production of FOS with Dried Alginate-Entrapped Enzymes (DALGEEs)

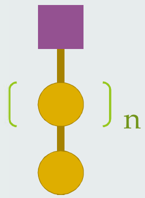
"Alginate-based immobilized biocatalyst of high operational stability for the transformation of carbohydrates in continuous reactors (DALGEE`s biocatalyst)". Spanish Patent 200930001 (2009). PCT/ES2010/070104

Continuous production of FOS with Dried Alginate-Entrapped Enzymes (DALGEEs)



Production of FOS by inulin hydrolysis





(2 ≤ n ≤ 60)

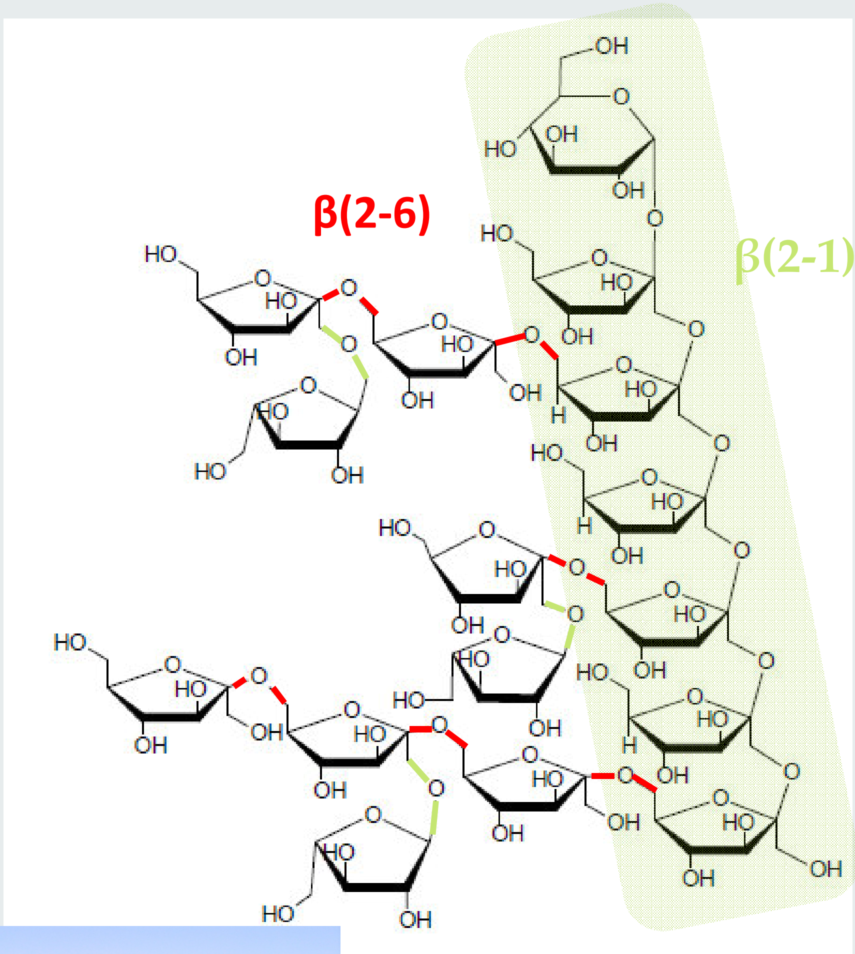
Chicory
(*Cichorium intybus*)


Mature roots

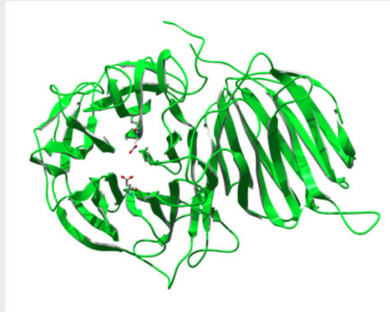
Pataca
(*Helianthus tuberosus*)


Tubers at base of plant

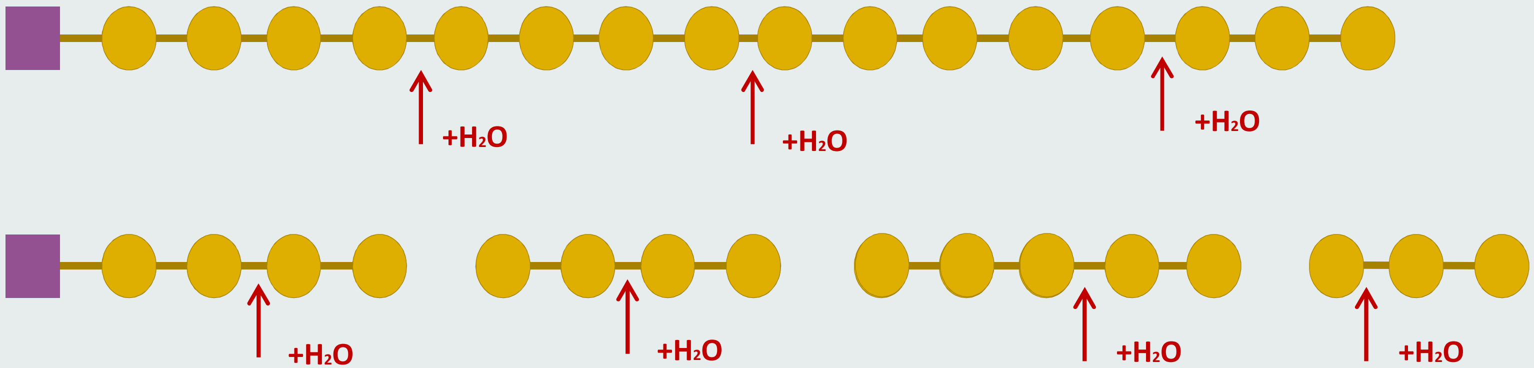
Agave fructans



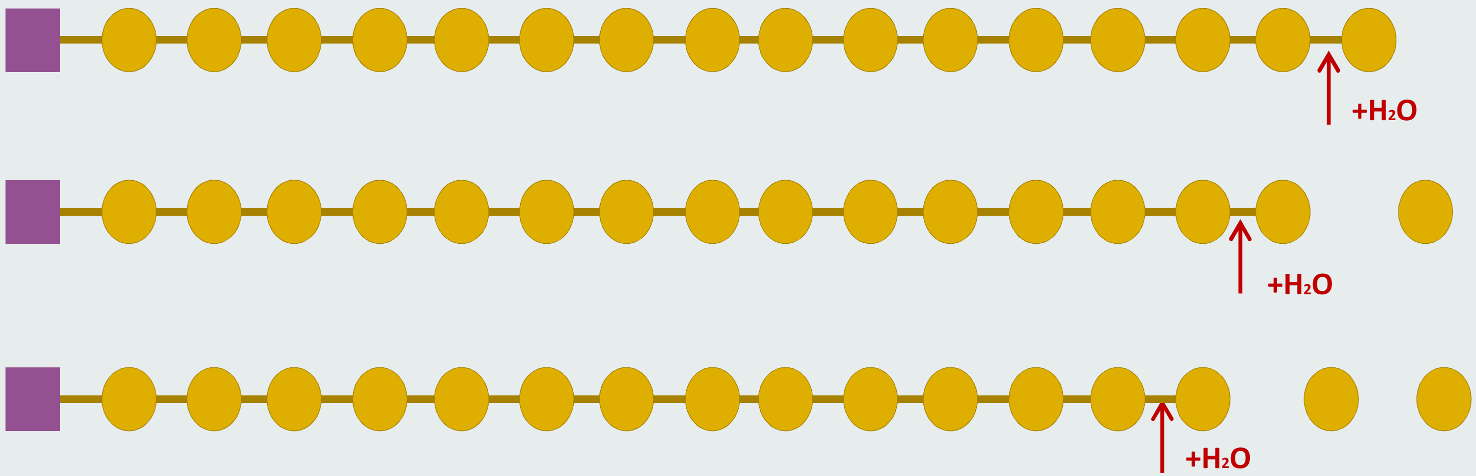
PRODUCTION OF FOS BY INULINASES



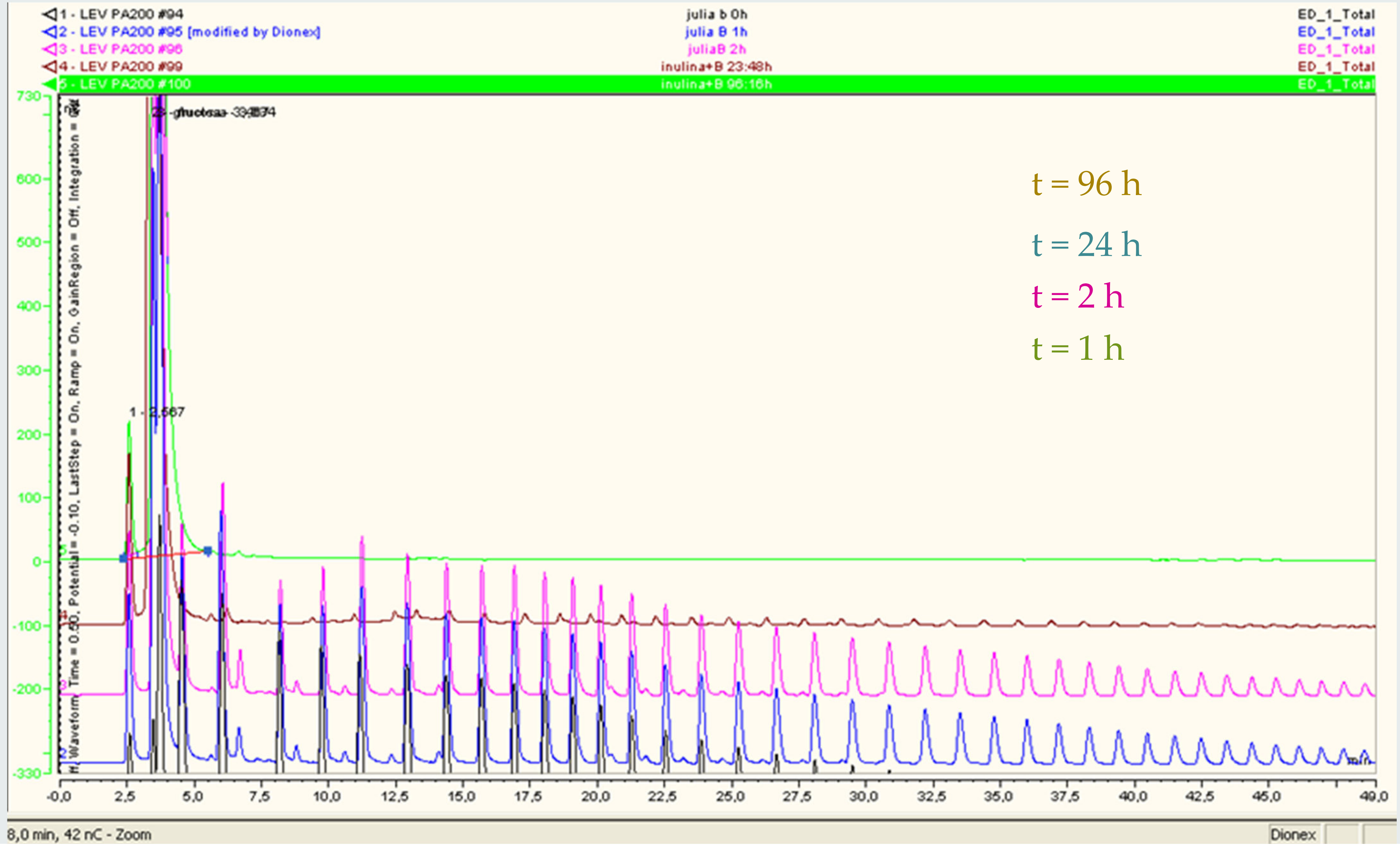
Endoinulinase (EC.3.2.1.7)



Exoinulinase (EC.3.2.1.80)

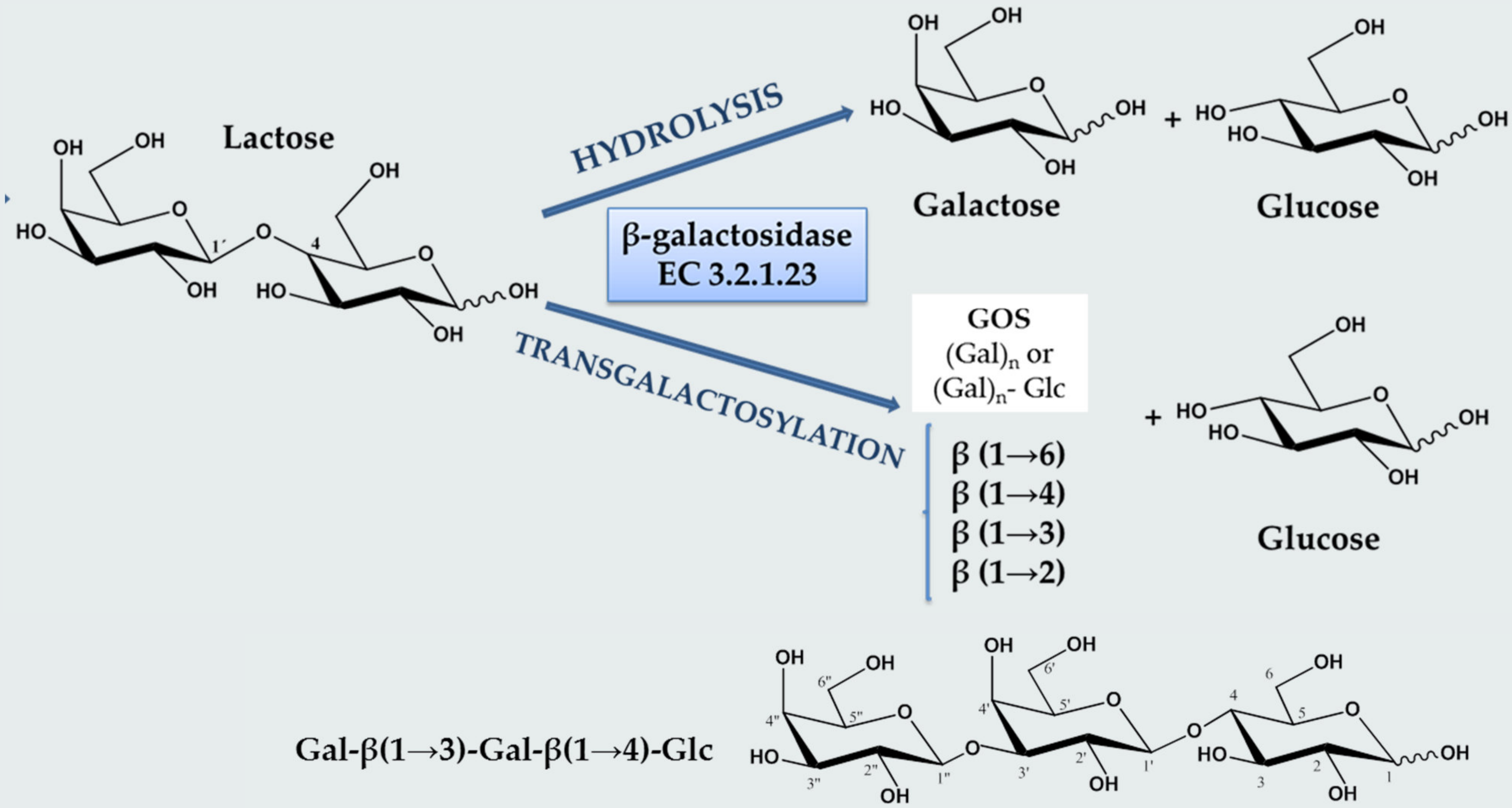


Inulin hydrolysis by inulinase from *Schwanniomyces occidentalis* followed by HPAEC-PAD (High Performance Anion Exchange Chromatography with Pulsed Amperometric Detector)



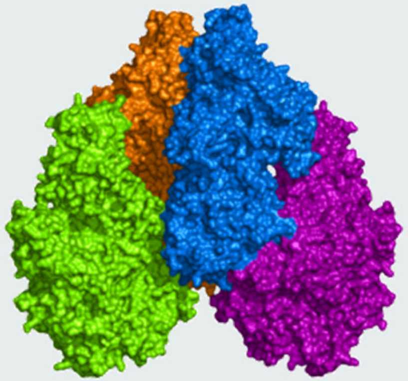
Galactooligosaccharides (GOS)

Lactose hydrolysis vs. GOS synthesis

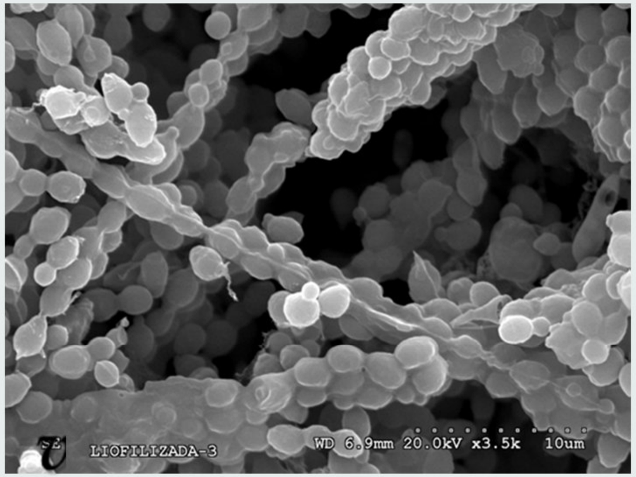


β-galactosidases

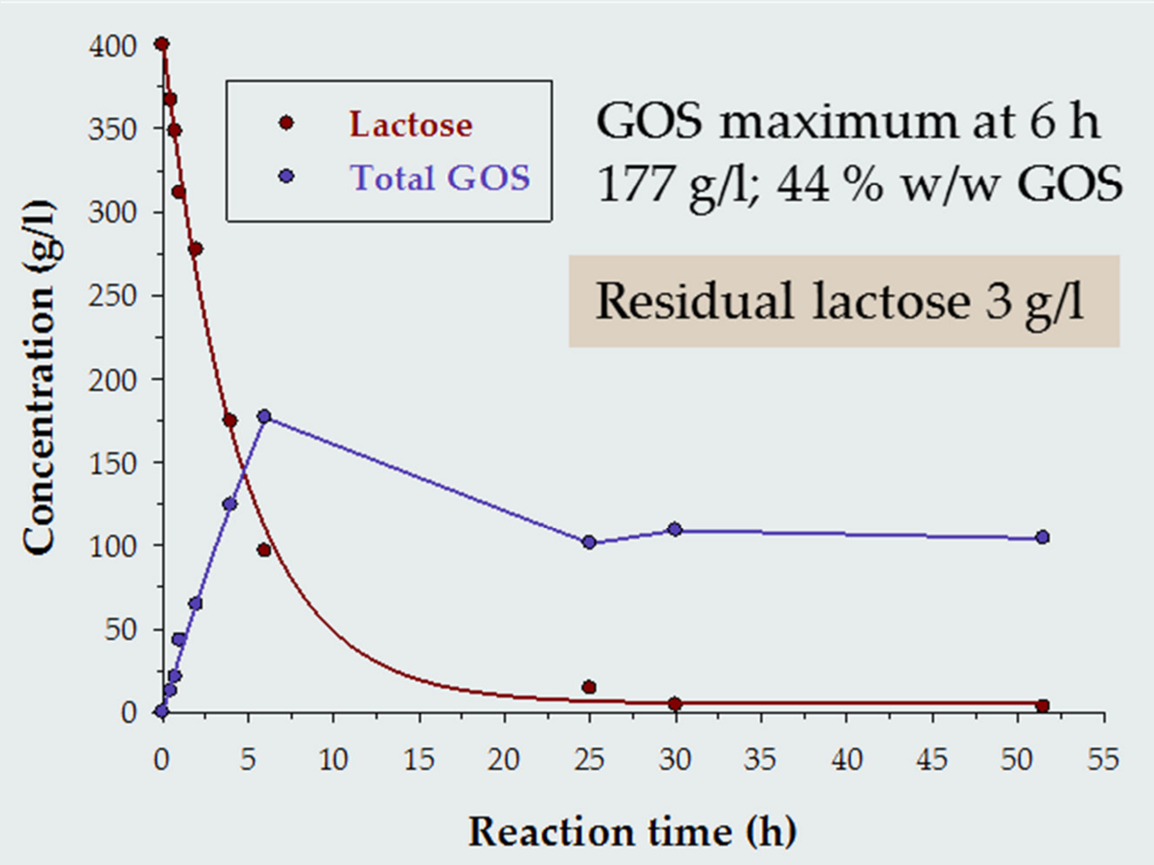
	Kluyveromyces lactis	*Bacillus circulans*	*Aspergillus oryzae*
Commercial name	Lactozym pure (Novozymes)	Biolactase (Biocon)	Lactase F (Amano)
Optimum temperature	40-50 °C	30-50 °C	45-55 °C
Optimum pH	6.8	5.5	4.5
Activity (0.1% lactose, pH 6.7)	1320 U/mL	550 U/mL	93 U/g



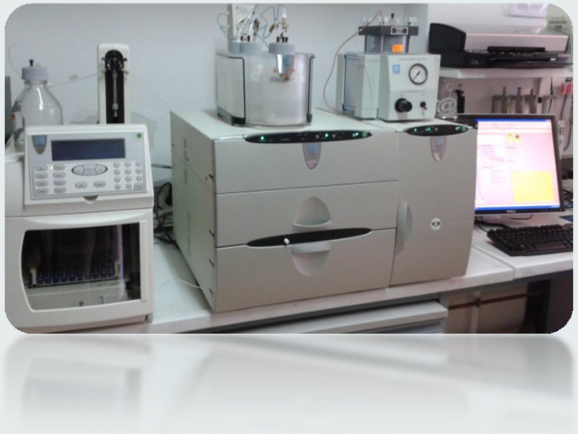
Production of GOS by the β-galactosidase from *Kluyveromyces lactis*



Permeabilized cells



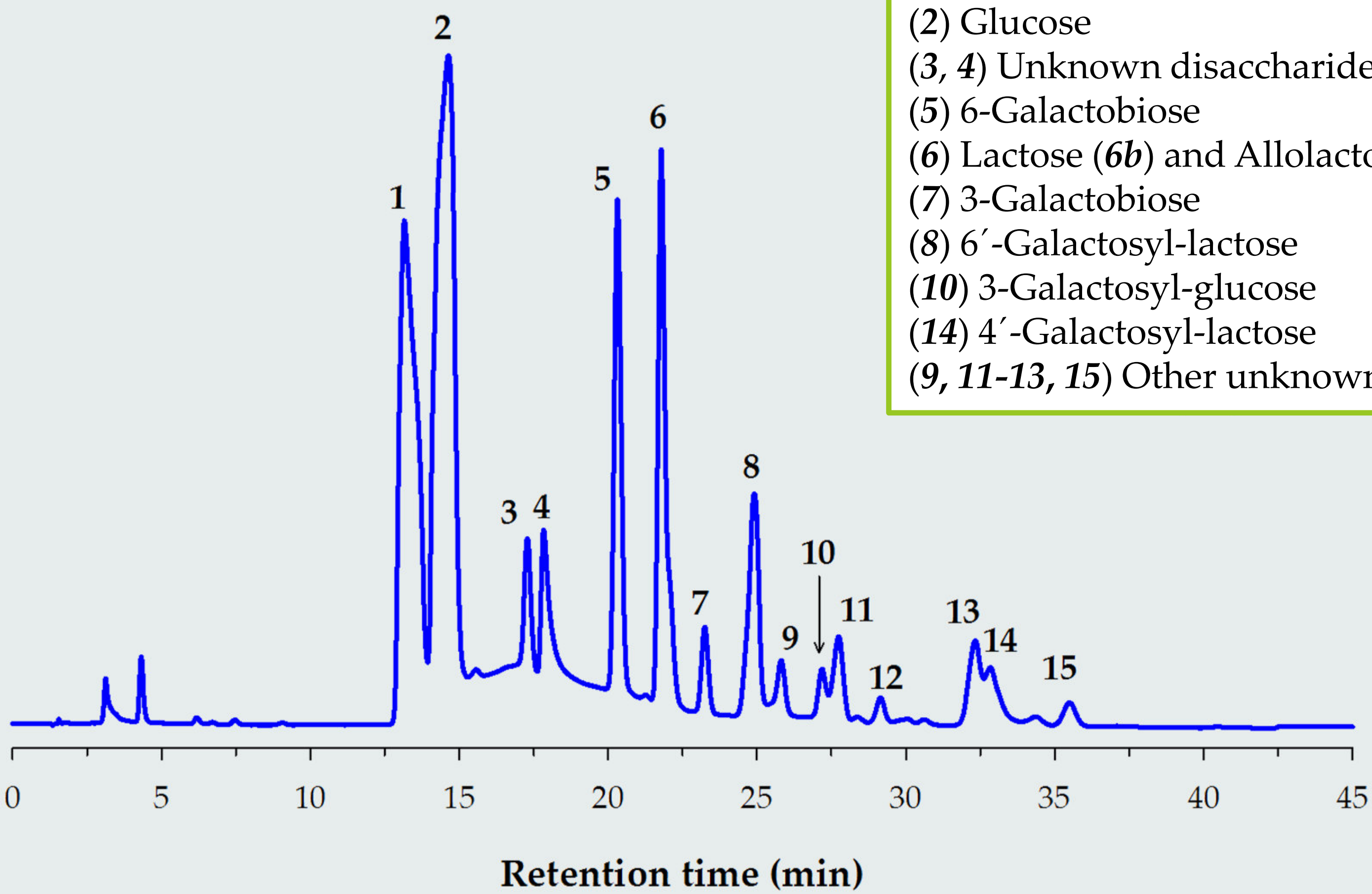
Reaction conditions:
400 g/l lactose
pH 6.8
1.2-1.5 U/ml
40 °C



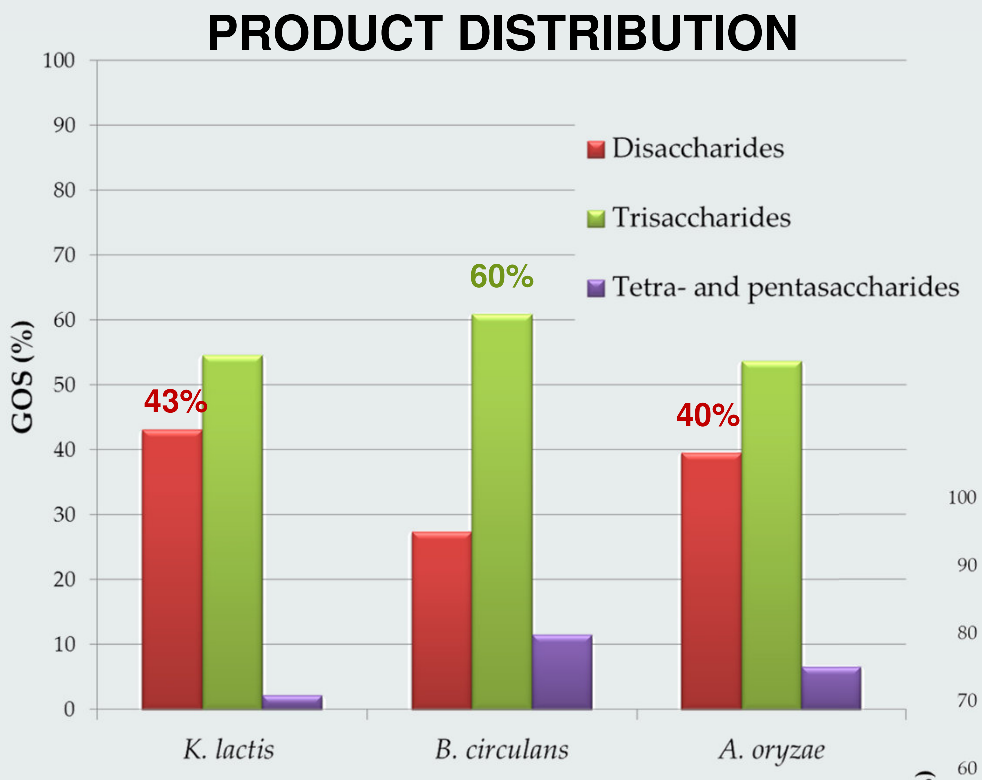
GOS production by permeabilized cells from *Kluyveromyces lactis*

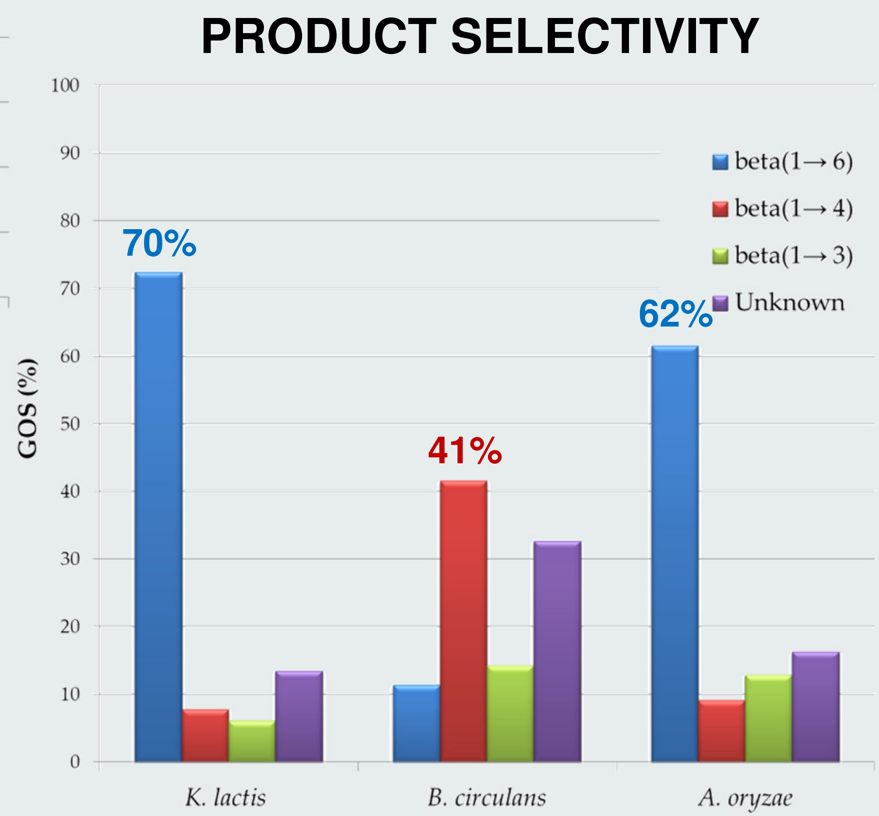
HPAEC-PAD analysis

(*1*) Galactose
(*2*) Glucose
(*3*, *4*) Unknown disaccharides
(*5*) 6-Galactobiose
(*6*) Lactose (*6b*) and Allolactose (*6a*)
(*7*) 3-Galactobiose
(*8*) 6´-Galactosyl-lactose
(*10*) 3-Galactosyl-glucose
(*14*) 4´-Galactosyl-lactose
(*9*, *11-13*, *15*) Other unknown GOS



Effect of enzyme source on GOS production





Formation *in situ* of GOS during the treatment of milk with β-galactosidases → "prebiotic milk" with a low content of lactose

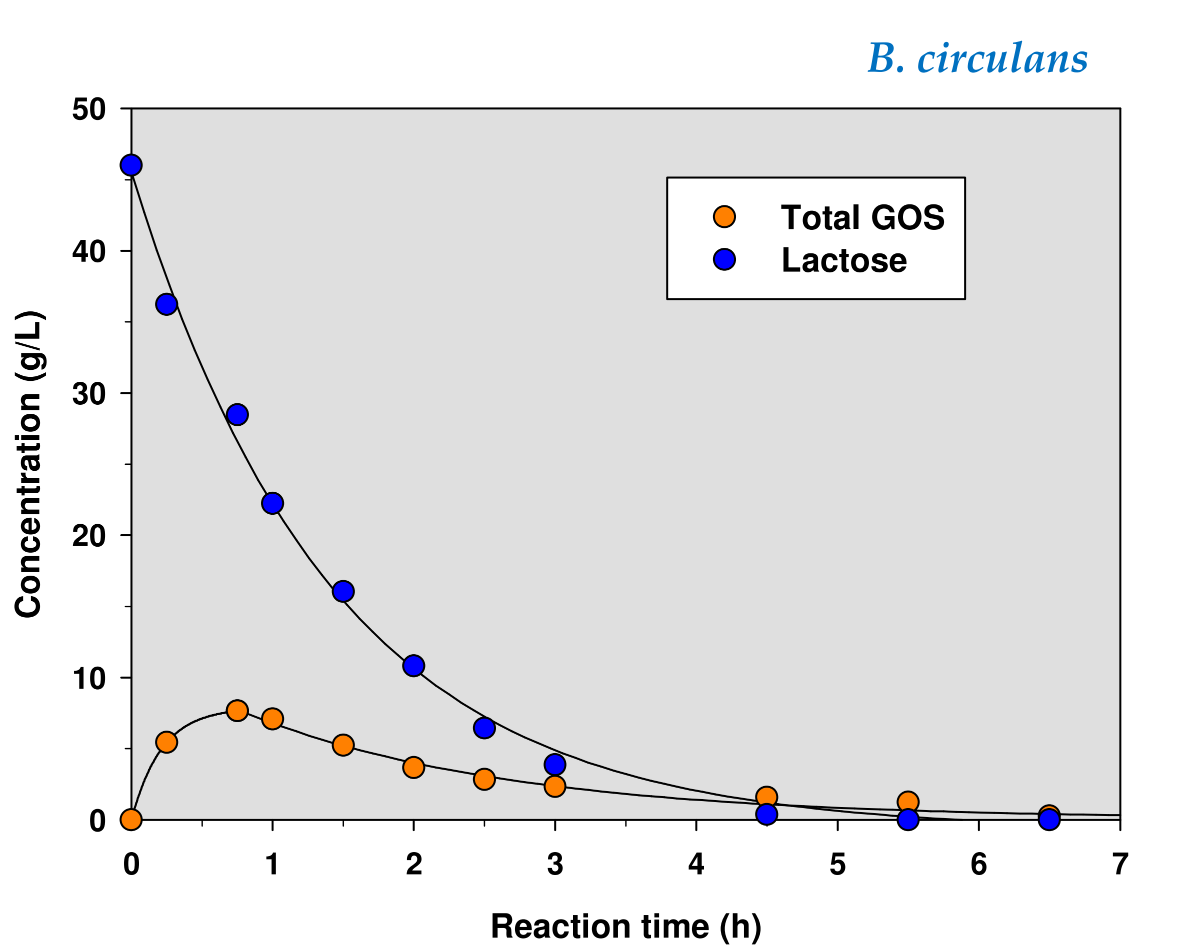
Reaction conditions:

46 g/L lactose
pH of milk 6.7
Enzyme dosage of 0.1% (v/v)
40°C and 4°C

Analysis by HPAEC-PAD

B. Rodriguez-Colinas, L. Fernandez-Arrojo, A.O. Ballesteros, F.J. Plou. ***Galactooligosaccharides formation during enzymatic hydrolysis of lactose: towards a prebiotic enriched milk.*** *Food Chemistry,* 145, 388-394 **(2014)**,

GOS formation during enzymatic hydrolysis of lactose in skim milk with *B. circulans* β-galactosidase

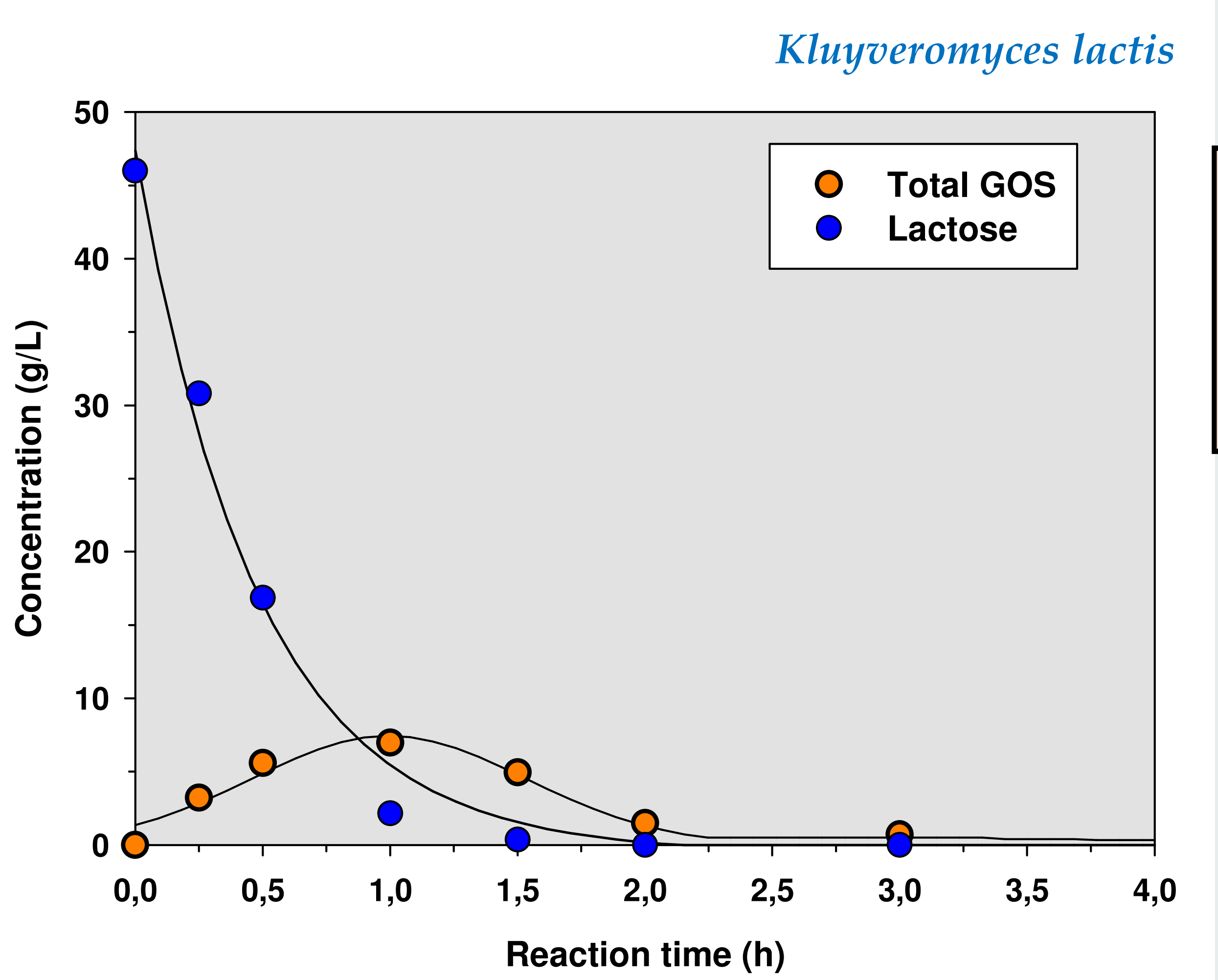


Maximum GOS at 0.75 h

Lact 28.1 g/L
GOS_{tot} 7.6 g/L

GOS maximum at 40-50% of lactose conversion

GOS formation during enzymatic hydrolysis of lactose with *K. lactis* β-galactosidase



Maximum GOS at 1 h

Lactose: 2.1 g/L
GOS_{tot}: 7.0 g/L

GOS maximum at 95% of lactose conversion

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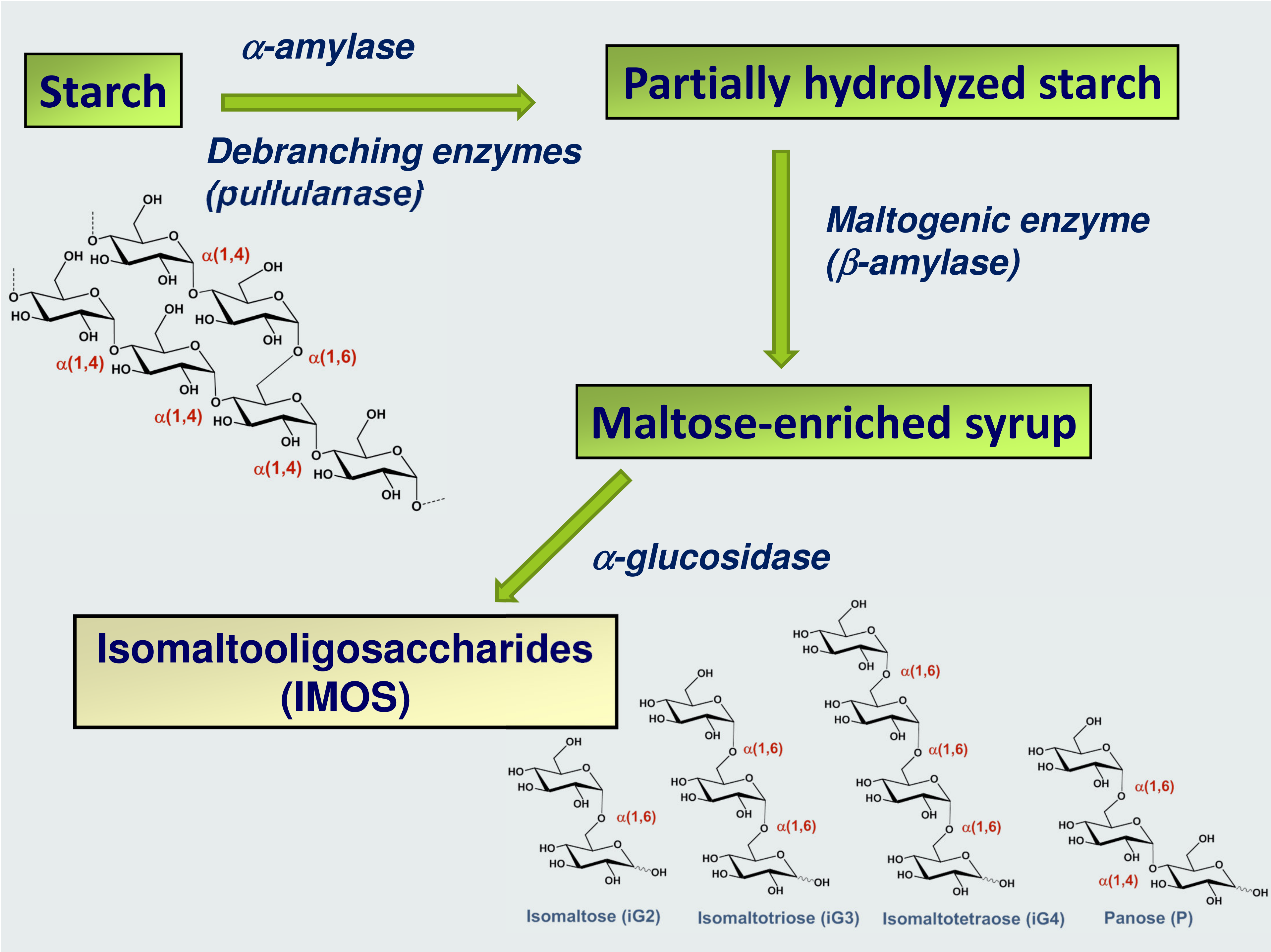
Will Isomalto-Oligosaccharides, a Well-Established Functional Food in Asia, Break through the European and American Market? The Status of Knowledge on these Prebiotics

DOROTHEE GOFFIN,[1,2] NATHALIE DELZENNE,[3] CHRISTOPHE BLECKER,[2] EMILIEN HANON,[4] CLAUDE DEROANNE,[2] and MICHEL PAQUOT[1]

Advantages over FOS and GOS

Higher pH and thermal stability

Lower levels of intestinal gas


Starch
α-amylase
Partially hydrolyzed starch
Debranching enzymes
(pullulanase)
α(1,4)
α(1,6)
Maltogenic enzyme
(β-amylase)
Maltose-enriched syrup
α-glucosidase
Isomaltooligosaccharides
(IMOS)
Isomaltose (iG2)
Isomaltotriose (iG3)
Isomaltotetraose (iG4)
Panose (P)

CONCLUSIONS

1. We have developed several processes for fructooligosaccharides (FOS) production from sucrose or inulin using β-fructofuranosidases or inulinases, respectively.

2. Operational stability of β-fructofuranosidase from *A. aculeatus* entrapped in dried alginated beads is very significant

3. For galactooligosaccharides (GOS) synthesis, *K. lactis* β-galactosidase forms basically β(1→6) linkages and gives rise to a mixture of di- and trisaccharides.

4. *K. lactis* β-galactosidase forms maximum GOS yield in milk of 7 g/L at 95% lactose removal, a value close to the HMOs content of human milk.

5. We have develop cascade strategies for isomaltooligosaccharides (IMOS) from starch involving several amylolytic enzymes.

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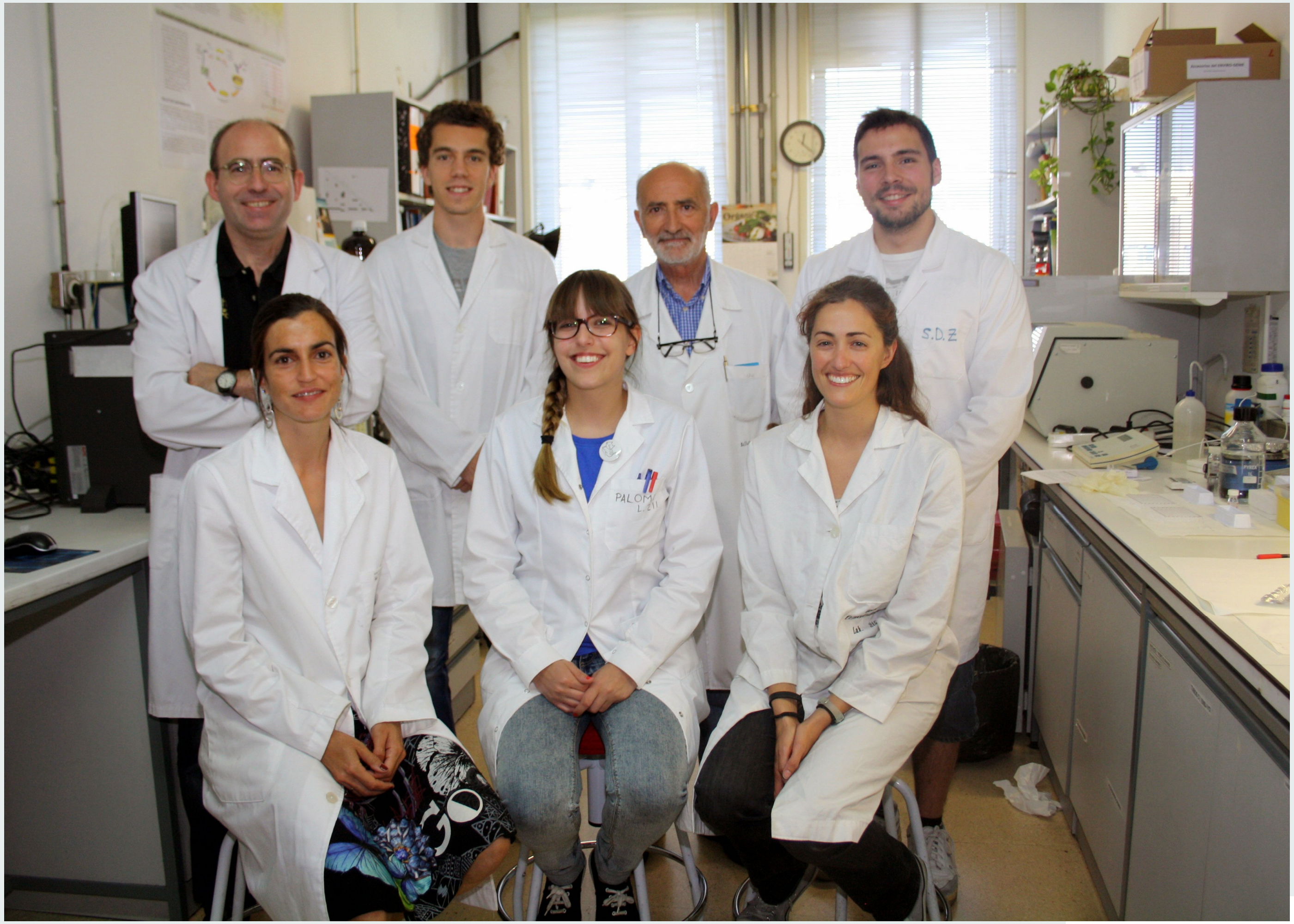
Dr. Andrés Illanes (Universidad de Valparaíso, Chile)
Ramiro Martínez (Novozymes)



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