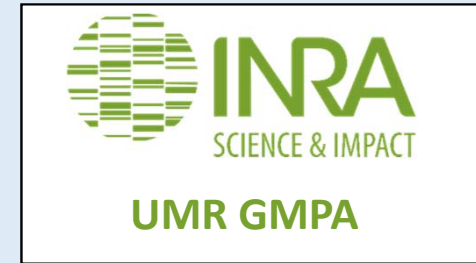


CHAIRE AGROBIOTECHNOLOGIES INDUSTRIELLES - AGROPARISTECH



Coupling of production and separation processes in biotechnologies: focus on the case of 3-hydroxypropionic acid

F. Chemarin^{1,2}, M. Moussa², F. Allais¹, A. Teixeira¹, I.C. Trelea², V. Athès²

¹ Chaire Agro Biotechnologies Industrielles AgroParisTech, Pomacle, Grand-Est

² UMR AgroParisTech-INRA, Génie et microbiologie des procédés alimentaires, Thiverval-Grignon, Île-de-France



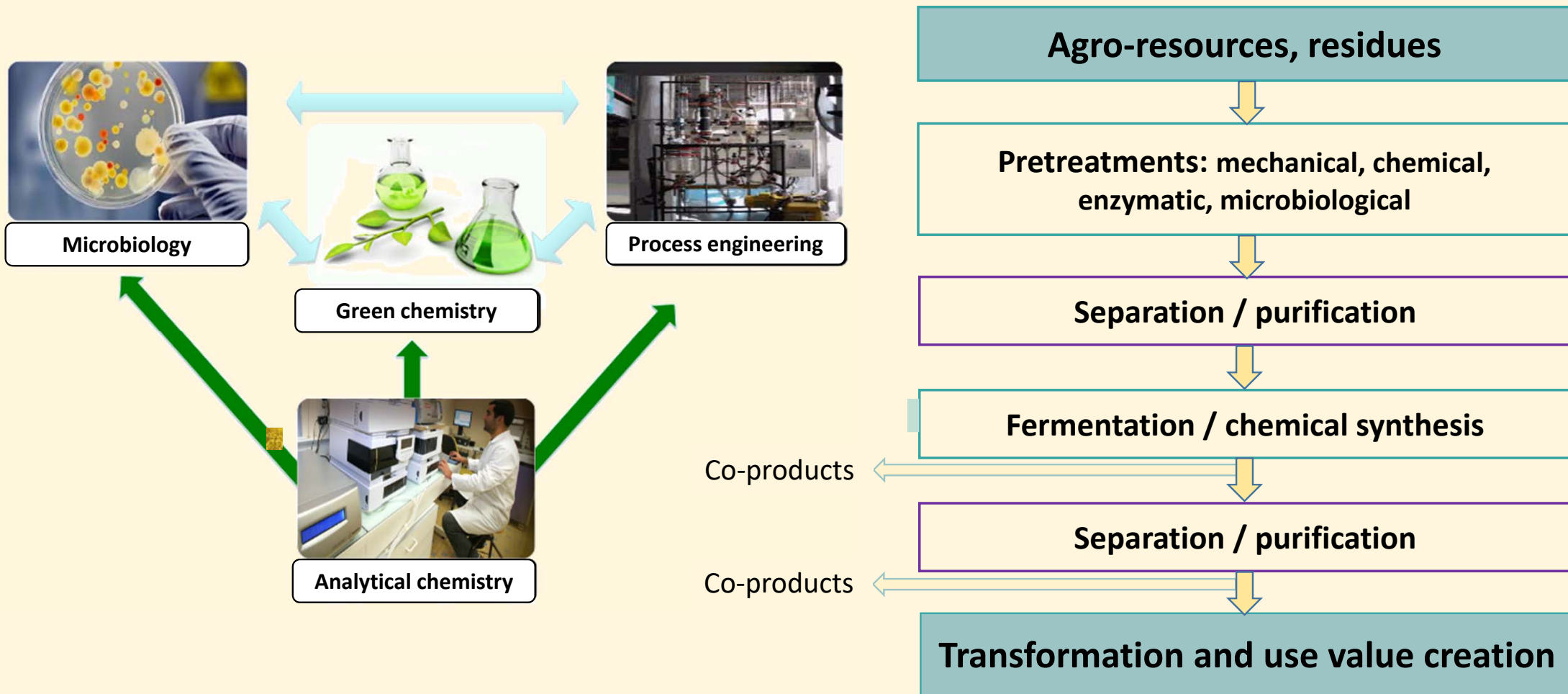
16^{ème} Congrès de la Société Française de Génie des Procédés
Nancy, 11 juillet 2017

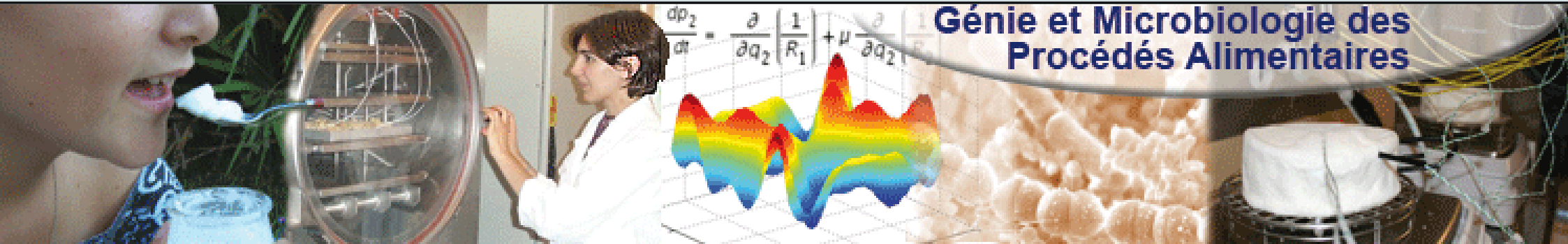


F. Chemarin: Coupling of production and separation processes in biotechnologies

CENTRE EUROPEEN DE BIOTECHNOLOGIE ET BIOECONOMIE







Génie et Microbiologie des Procédés Alimentaires

FOOD SCIENCE
(biophysics, Sensory Analysis)

PROCESS ENGINEERING
(automatic control, instrumentation, thermodynamics, transport phenomena...)



Applied MATHEMATICS
Modeling

MICROBIOLOGY
microbial ecology and physiology

MICROBIAL ENGINEERING



BIOPROCESSES
Food and bio-products (real matrices)

INTRODUCTION

Industrial biotechnology: use of microorganisms and enzymes for the sustainable processing and production of chemicals

Drivers of industrial biotechnology:

- price swings of crude oil
- diversifying resources
- Consensus to reduce GHG emission
- Stricter environmental legislation

Move to large volume, low-priced, chemicals with the necessity to decrease costs

Disruptive technologies: developments that are likely to cause big changes and provide business opportunities

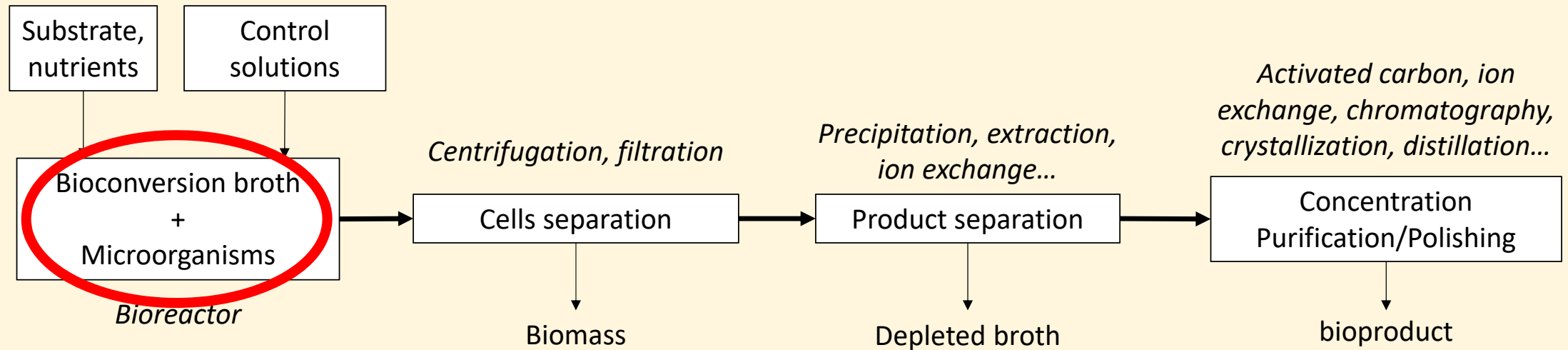
→ biomass, bioprocess engineering, microbial engineering, platform chemicals...

→ **INTEGRATION !**

Downstream processes 50-70% of total production costs

→ **Integration of reactions and separation operations** (whole cell bioconversion)

CONVENTIONAL BIOCHEMICALS PRODUCTION PROCESS



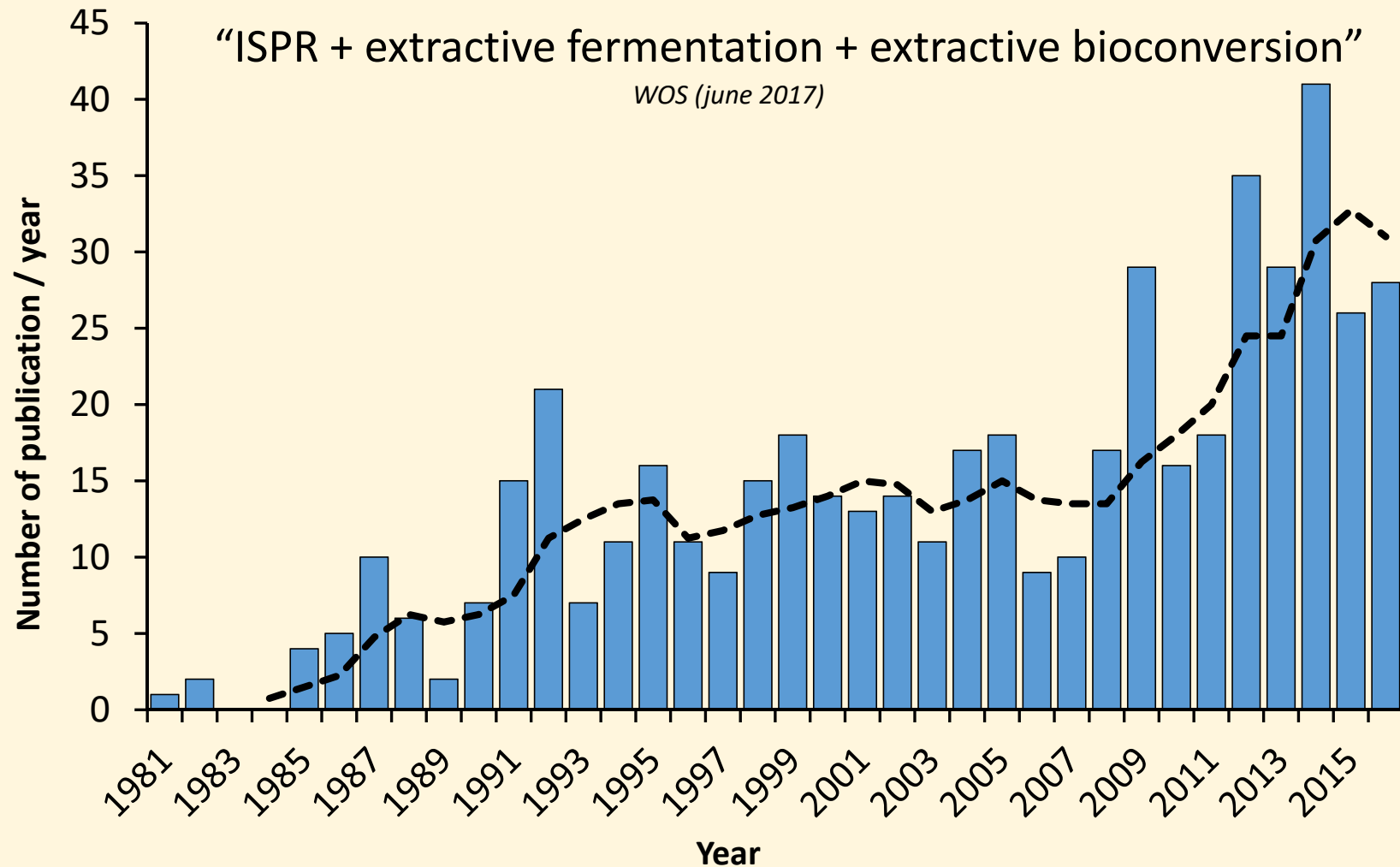
Product accumulation → inhibitory effect

Recovering the product directly when it is produced could improve the process

→ *In-situ* product recovery

ISPR IN BIOTECHNOLOGIES

COUPLING BIOPRODUCTION AND SEPARATION: A TRENDING TOPIC



ADVANTAGES AND EXPECTATIONS OF IN-SITU PRODUCT RECOVERY

EXPECTED ADVANTAGES

Higher titers

Increased productivity

Increased yield

Decreased Volume (compacity, water footprint, evaporation needs ...)

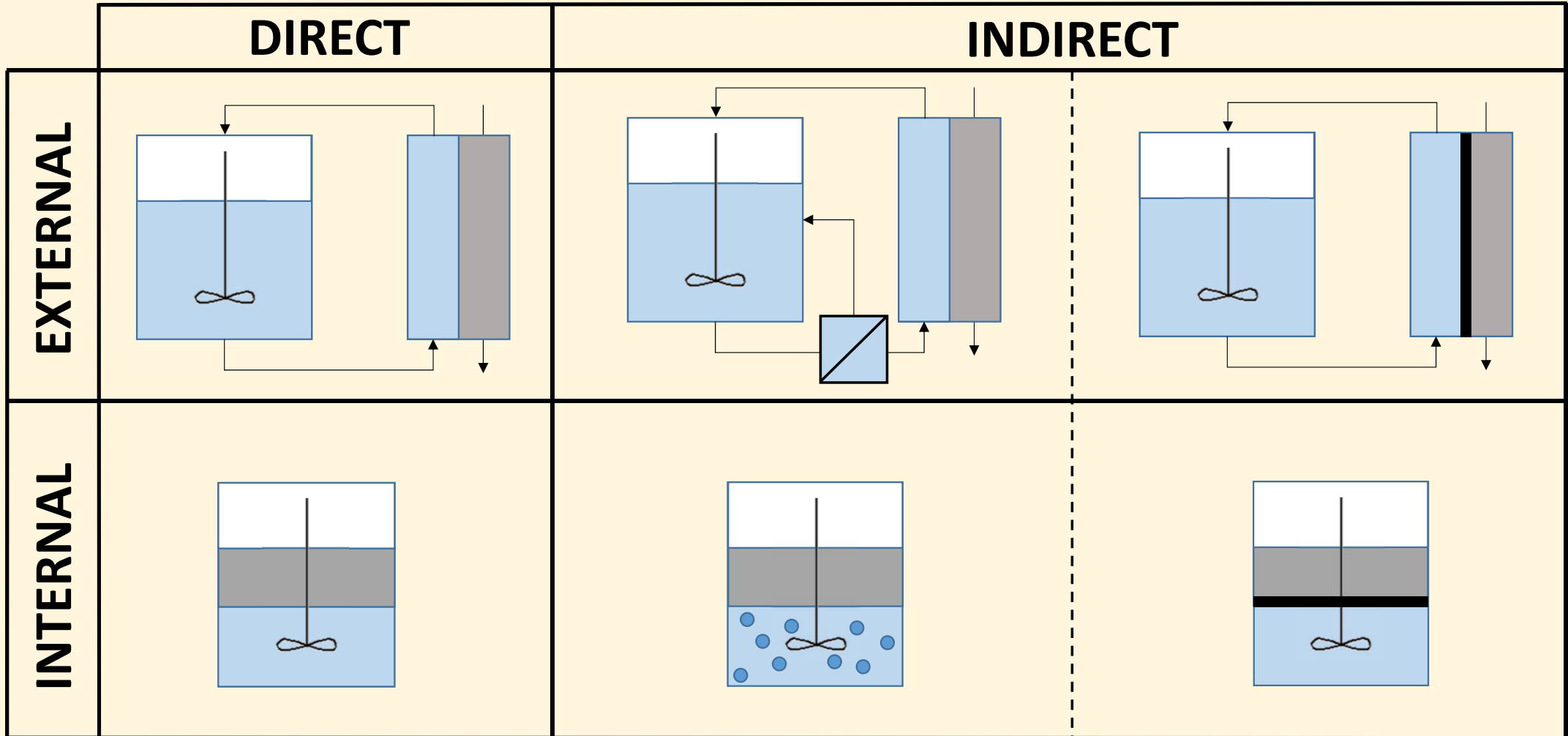
INDUSTRIAL REQUIREMENTS

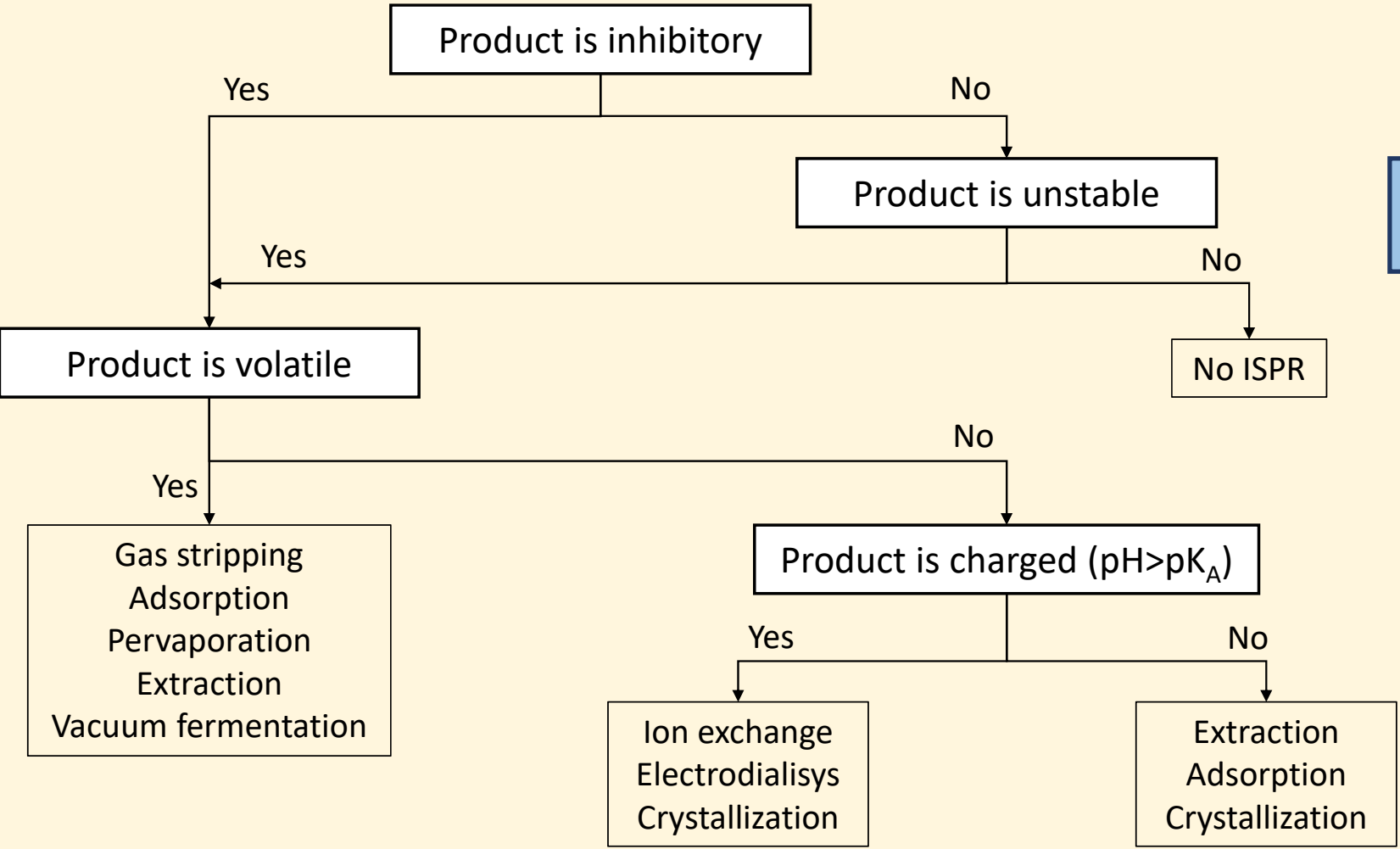
Scalibility

High product recovery

Stability and robustness

Expected increase in sustainability





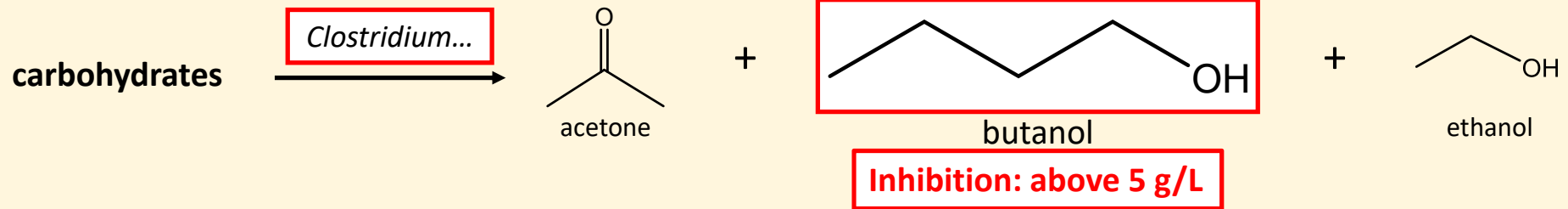
Kinds of products of interest

Solvents

Organic acids

Aroma compounds

TYPICAL EXAMPLES

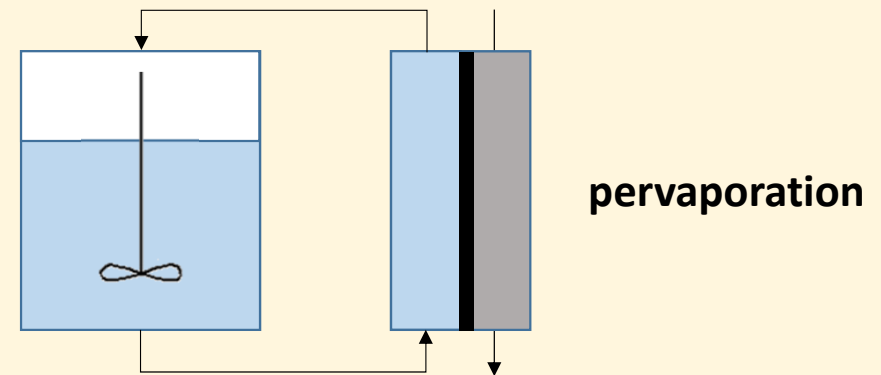


Max fed-batch (butanol): ~ 12 g/L in 60h

Pervaporation (continuous): PDMS membrane

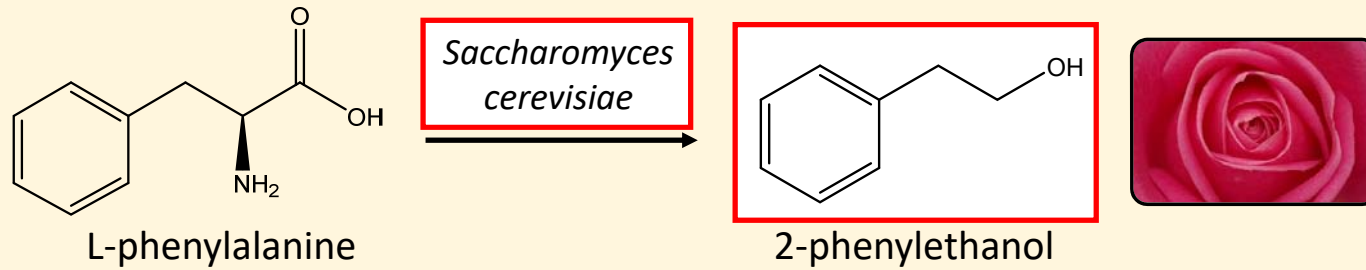
→ ~ 160 g/L butanol in permeate

→ ~ 8 g/L residual butanol in broth

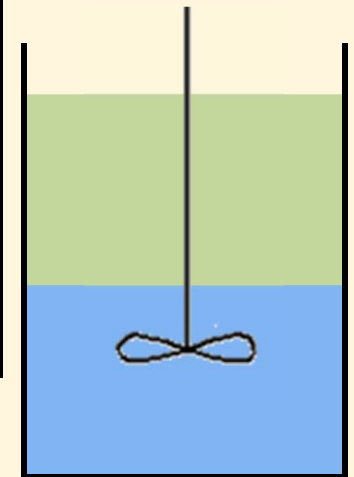


AROMA COMPOUND: 2-PHENYLETHANOL BIOPRODUCTION

FC15



	Fed-batch bioconversion	Final titer (g/L)	Yield (mol/mol)	Av. productivity (g/L/h)
(1)	Optimized without ISPR	3.8	83 %	0.18
(2)	Internal direct LLE (oleic acid)	12.6	91 %	0.26
(3)	Internal indirect* LLE (pentane)	21.2	~100 %	0.27



- (1) Stark et al., Biotechnol Prog, 2002
 (2) Stark et al., Biotechnol Prog, 2002
 (3) Mihal et al., Chemical Papers, 2014

*Membrane contactor

Diapositive 15

FC15

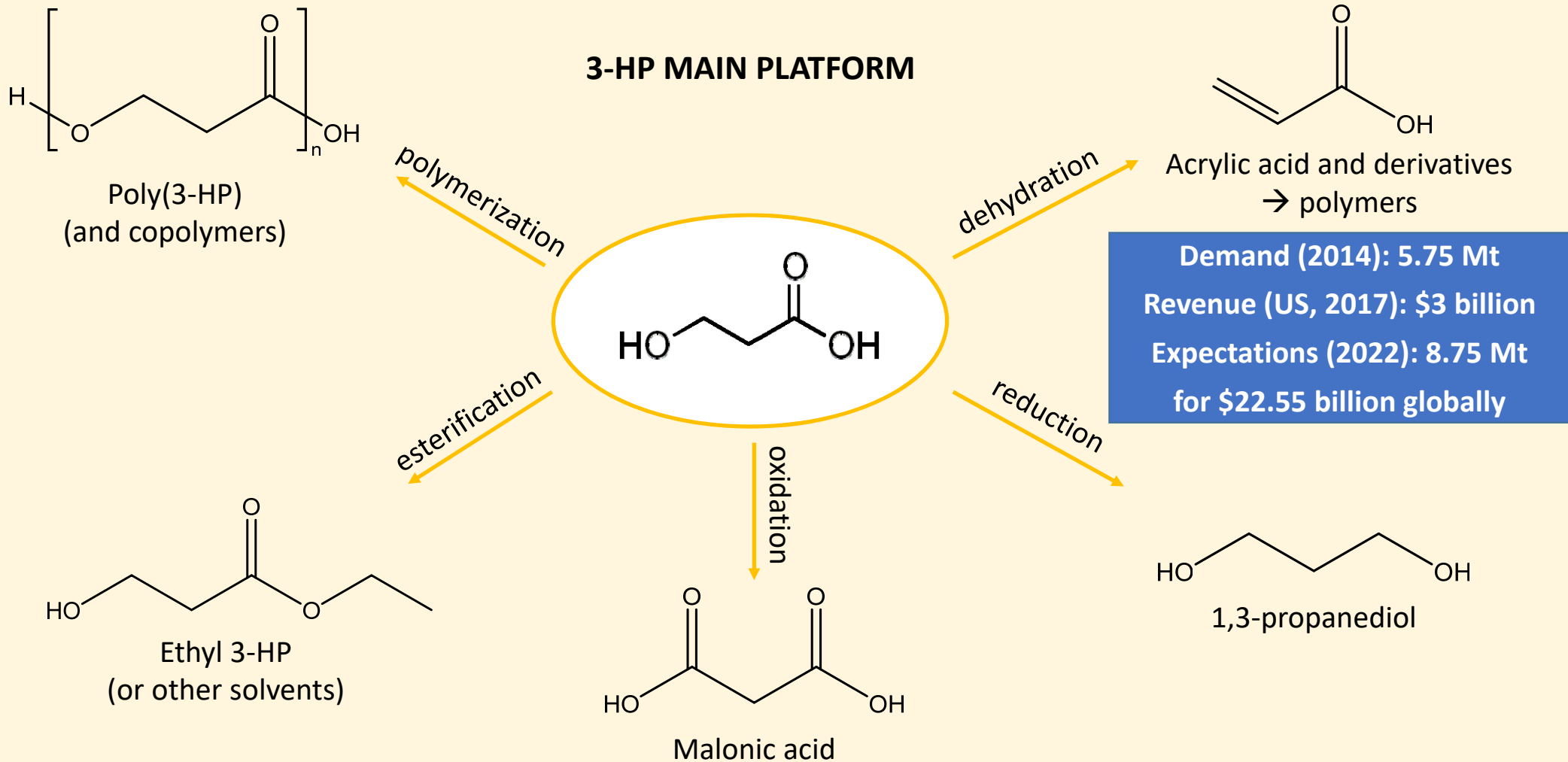
expliquer à l'oral membranes immergées qui permettait de réaliser le contact à l'intérieur bioréacteur sans dispersion

Florian Chemarin; 30/06/2017

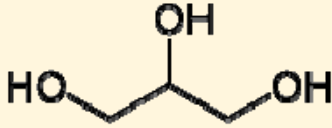
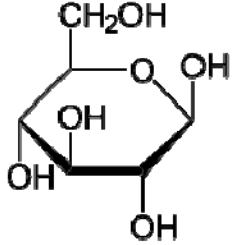
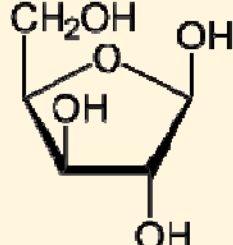

FOCUS ON
3-HYDROXYPROPIONIC ACID

3-HYDROXYPROPIONIC ACID BUILDING-BLOCK

(Werpy et al., Top value-added chemicals from biomass, 2004
Grand View Research, Acrylic acid market analysis, 2016)

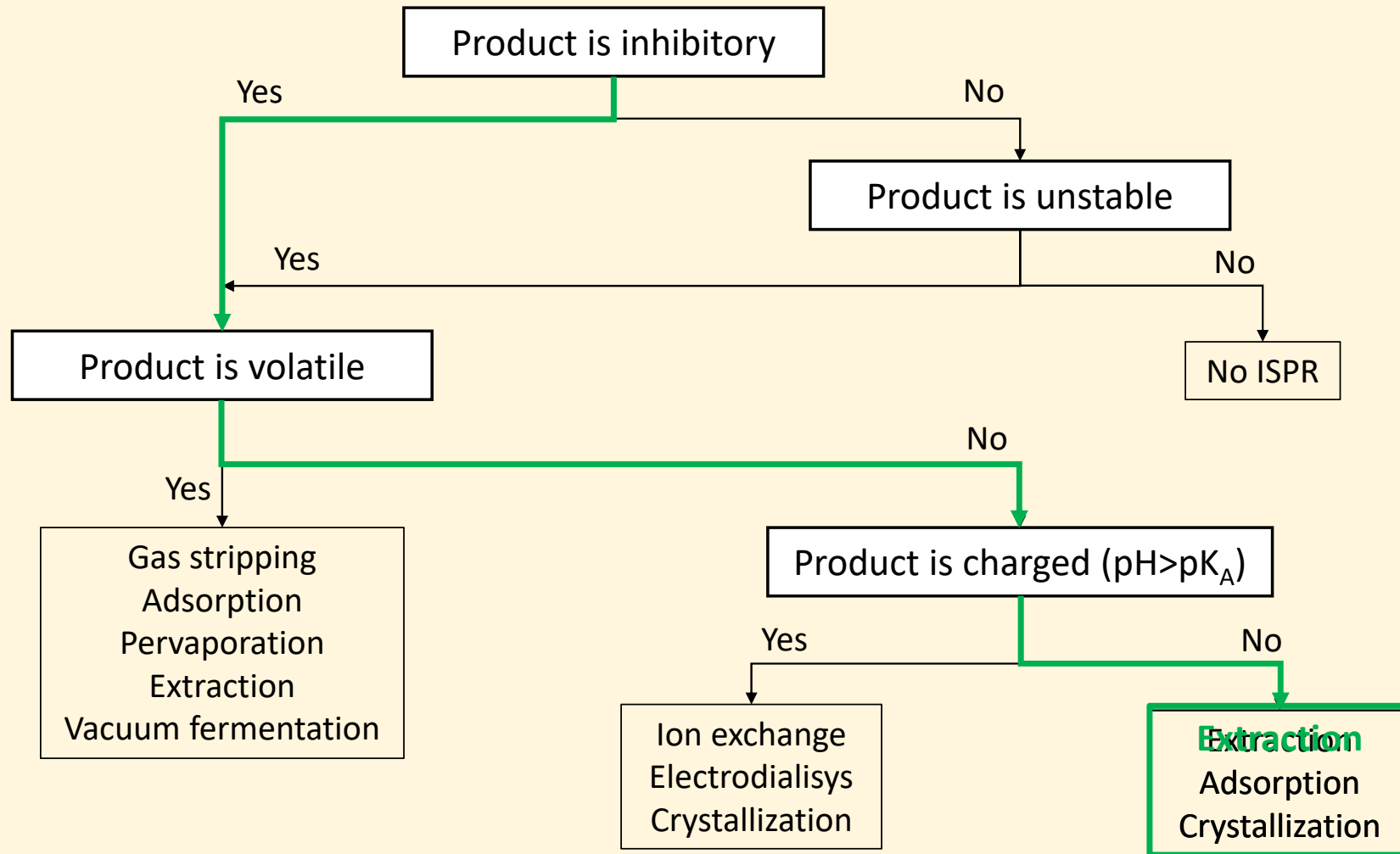


WHICH BIO-BASED SUBSTRATES ?

	 <p>Glycerol</p>	 <p>Glucose</p>	 <p>Xylose</p>	$O=C=O$ <p>Carbon dioxide</p>	 <p>1,3-propanediol</p>
Max final titer (g/L)	83.8 (73.4)	100	7.4	< 1	72.3
Productivity (g/L/h)	1.16 (1.53)	2.5	0.06	< 0.01	(>) 2.5
Microorganism	<i>K. pneumoniae</i>	yeast	<i>S. cerevisiae</i>	<i>Synechocystis</i> (cyanobacteria)	<i>G. oxydans</i>
Reference	Li et al, <i>Scientific Reports</i> (2016)	Cargill patent, US 2016/0060204 A1 (2016)	Kildegaard et al, <i>Metabolic Engineering Communications</i> (2015)	Wang et al, <i>Metabolic Engineering</i> (2016)	Zhao et al, <i>Bioprocess and biosystems engineering</i> (2015)

Low pH tolerant !

CHOICE OF SEPARATION PROCESSES FOR 3-HP RECOVERY



REACTIVE LIQUID-LIQUID EXTRACTION: A SOLUTION TO EXTRACT 3-HP

PROBLEM: water insoluble solvents unable to extract efficiently short chain carboxylic acid

→ Decanol (volume ratio 1:1): 2% extraction yield

→ Oleyl alcohol (volume ratio 1:1): ~ 0% extraction yield

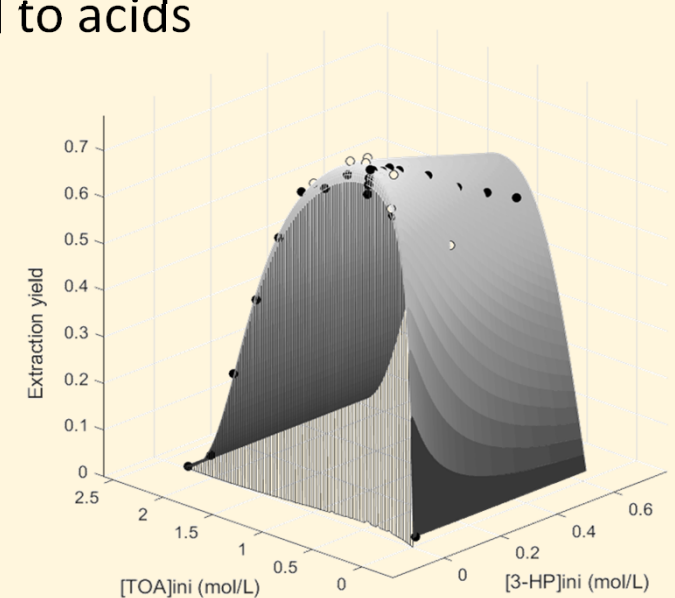
SOLUTION: using a hydrophobic species able to selectively bind to acids

→ Reactive liquid-liquid extraction

→ Aliphatic amines widely studied



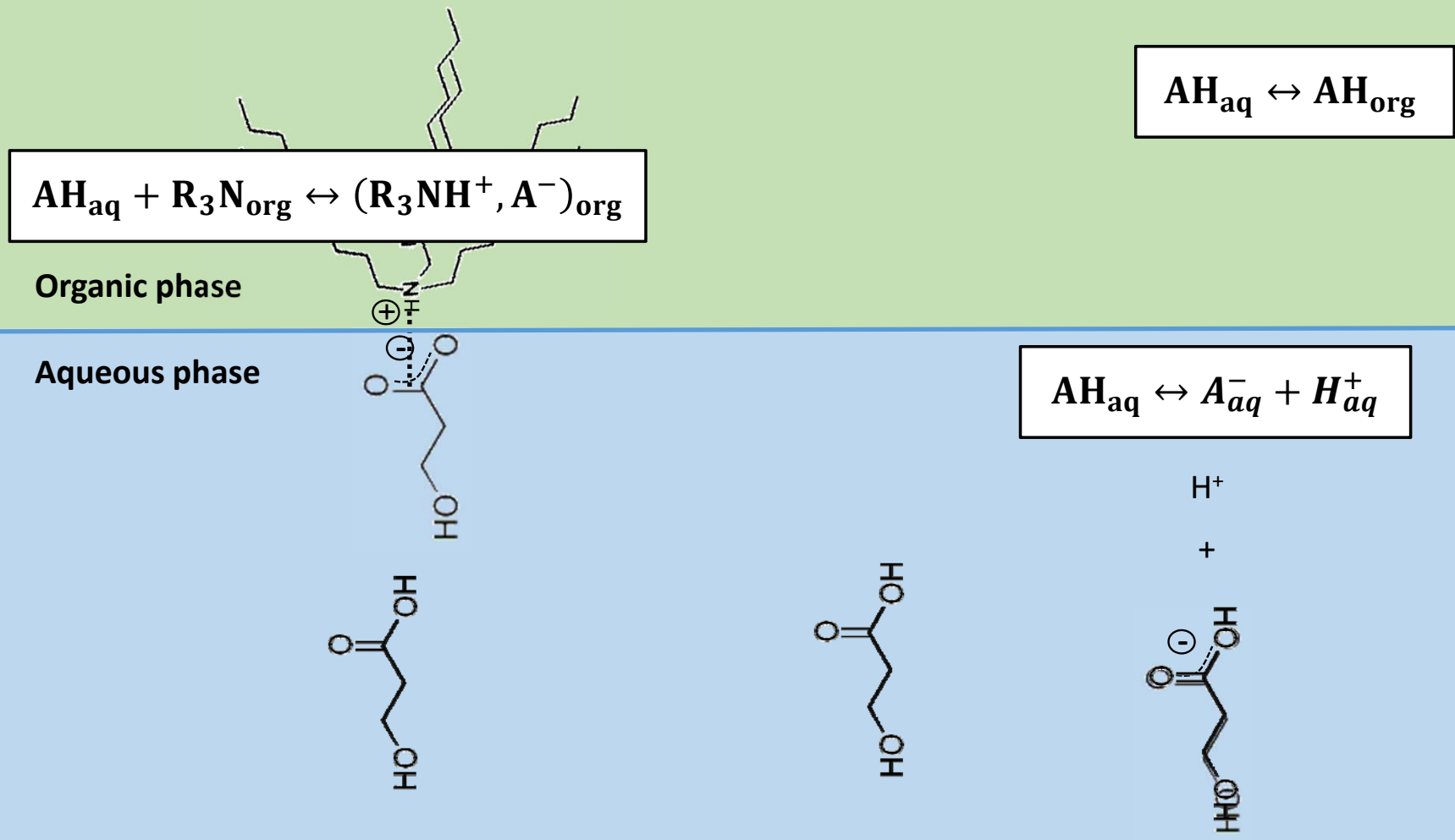
(Chemarin et al., *Sep Purif Technol*, 2017)



(Chemarin et al., *Sep Purif Technol*, in Press)

REACTIVE EXTRACTION MECHANISM

(Chemarin et al., Sep Purif Technol, in Press)



High specific surface area without dispersion

→ No emulsion

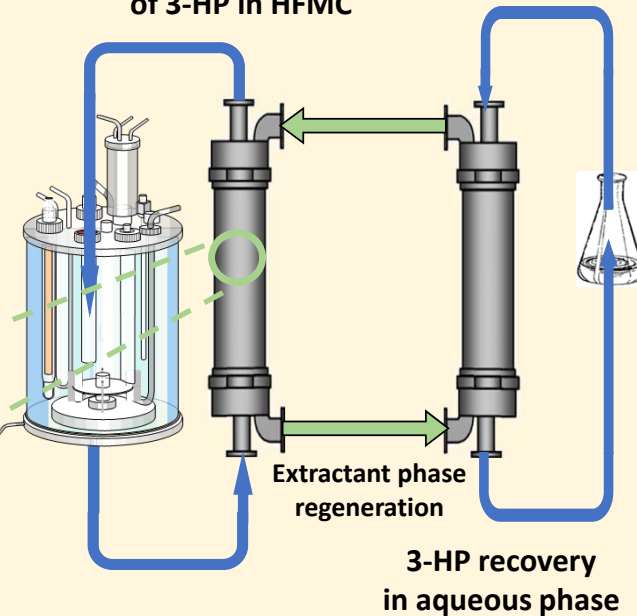
Prevents cells from contacting the organic phase

→ Potentially avoids contact toxicity with solvents

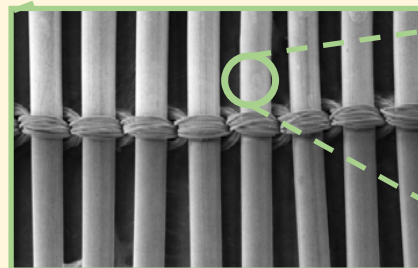
Continuous primary purification

→ Product concentration possible

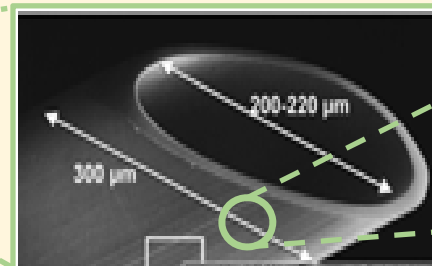
Reactive L-L extraction
of 3-HP in HFMC



3-HP recovery
in aqueous phase



Hydrophobic membrane
(porosity = 30-40 %
pore diameter = 30 nm)

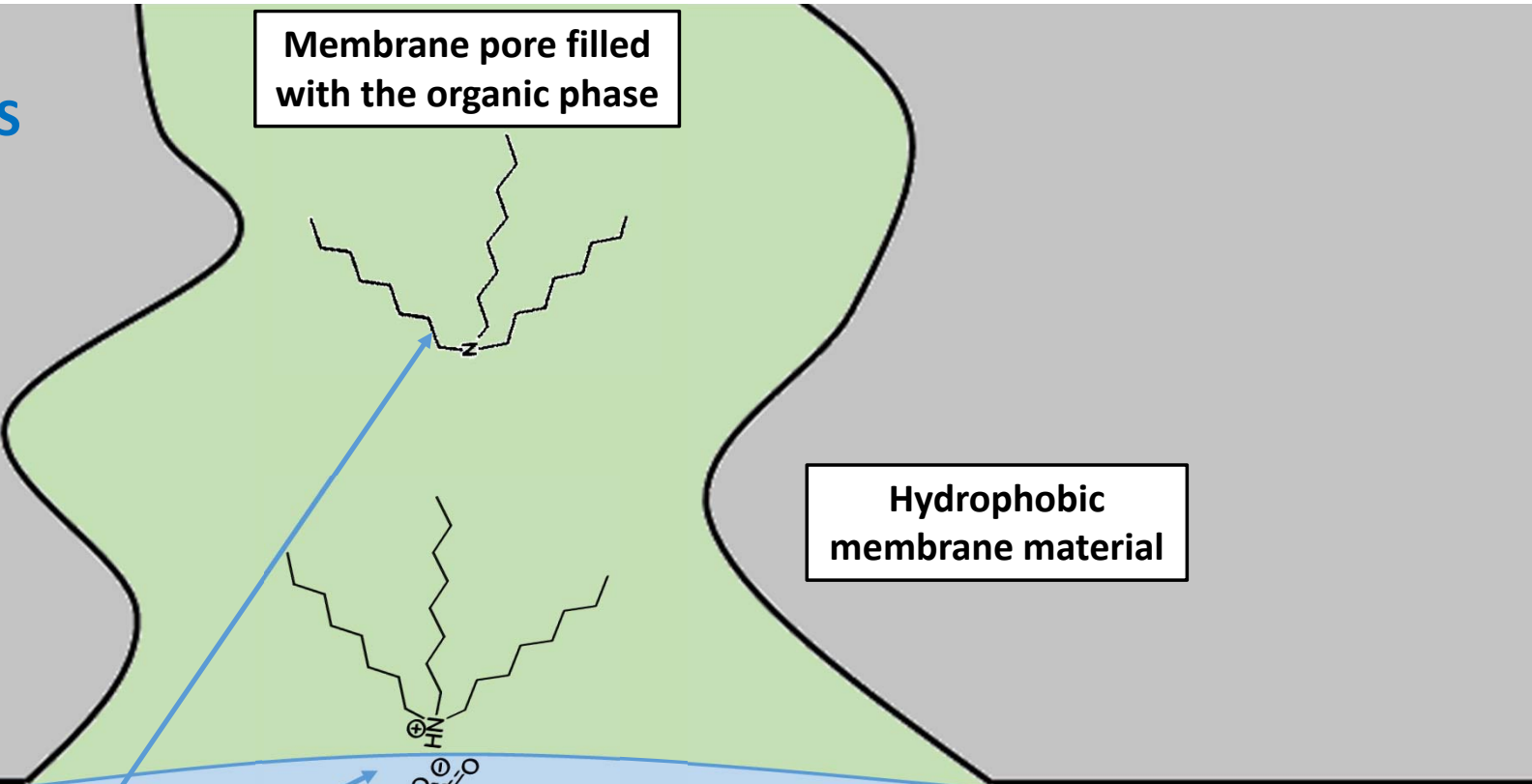


Diapositive 22

FC27 retain/separate from
Florian Chemarin; 30/06/2017

FC28 retourner le schémas, faire un premier zoom du module vers les fibres
Florian Chemarin; 30/06/2017

MASS TRANSFER IN MEMBRANE CONTACTORS

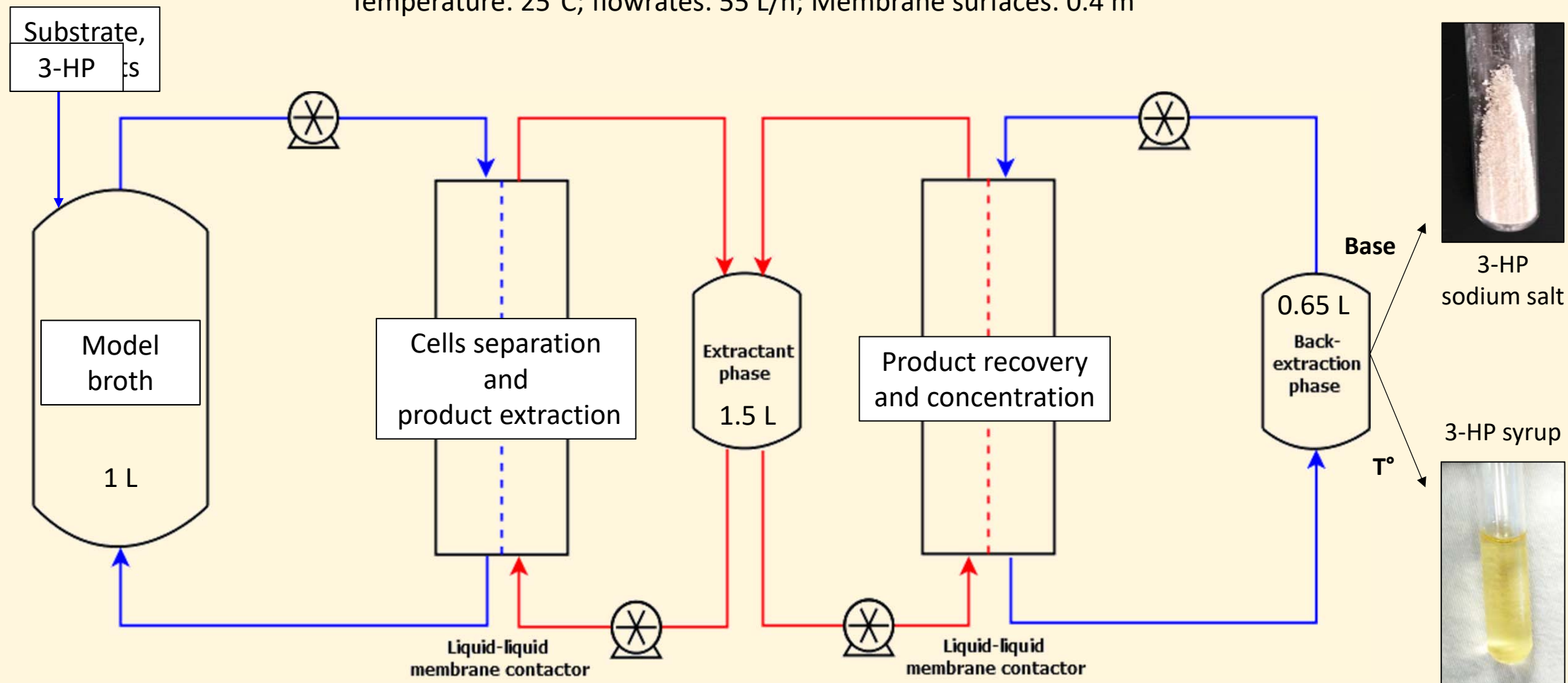


- Diffusion limiting step in membrane pores
- Major role of chemical reactions

Development of a predictive model based on reactions, diffusion and boundary layers

3-HP INTEGRATED PROCESS OF EXTRACTIVE BIOCONVERSION

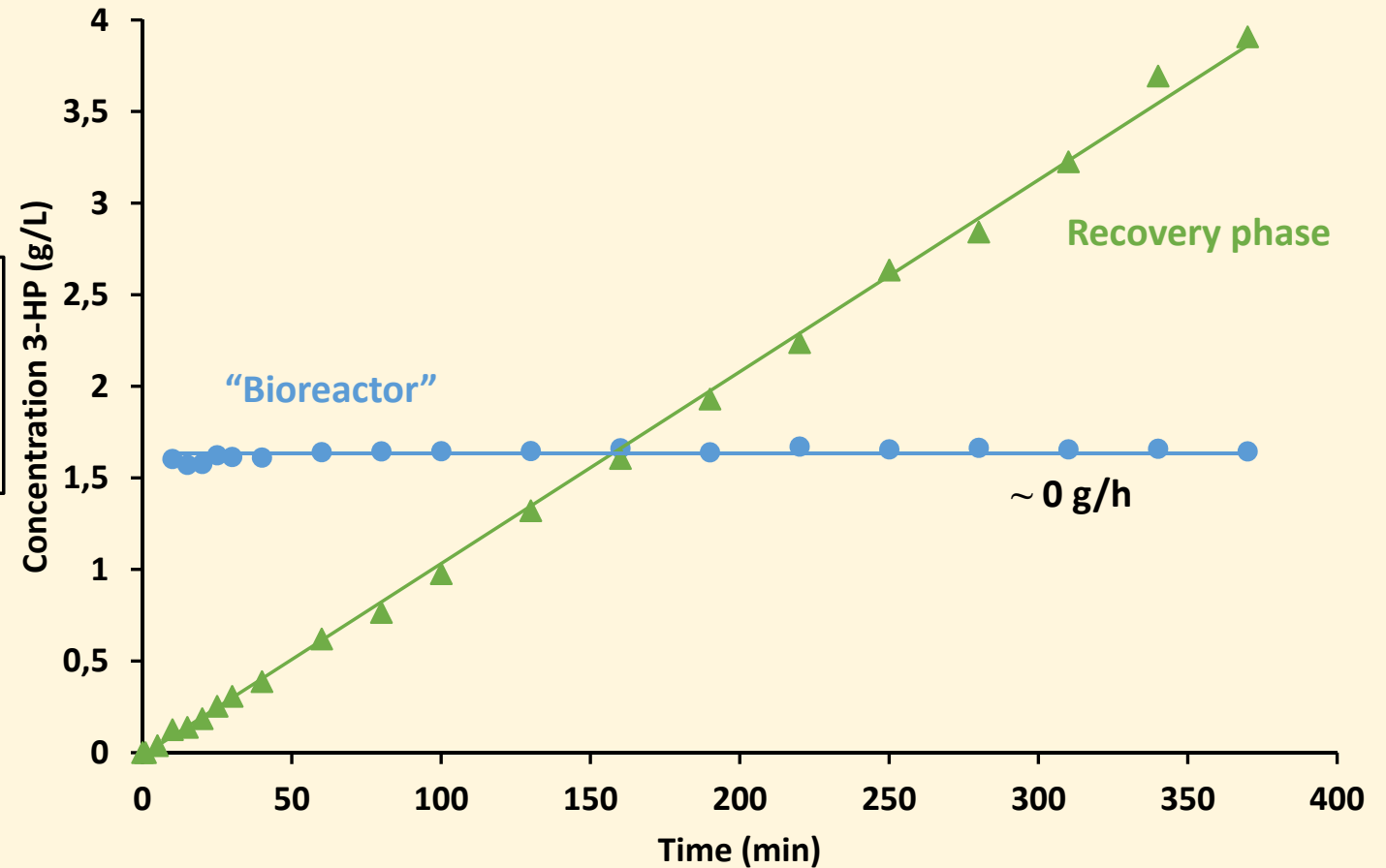
Temperature: 25°C; flowrates: 55 L/h; Membrane surfaces: 0.4 m²



SIMULATED EXTRACTIVE BIOCONVERSION IN “STEADY STATE”

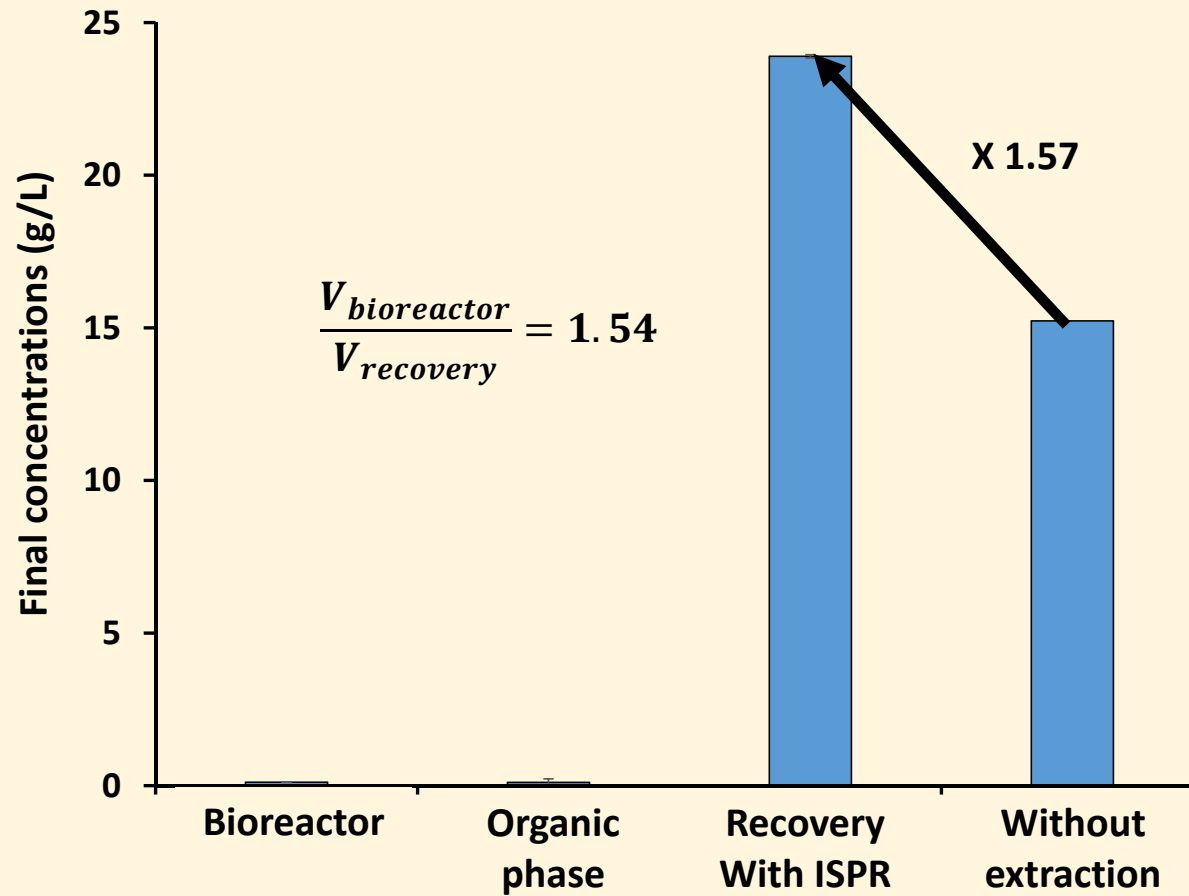
Initial conditions (3-HP):

- Bioreactor: 1.54 g/L
- Organic phase: 2.55 g/L
- Fed-batch bioreactor: 0.53 g/h



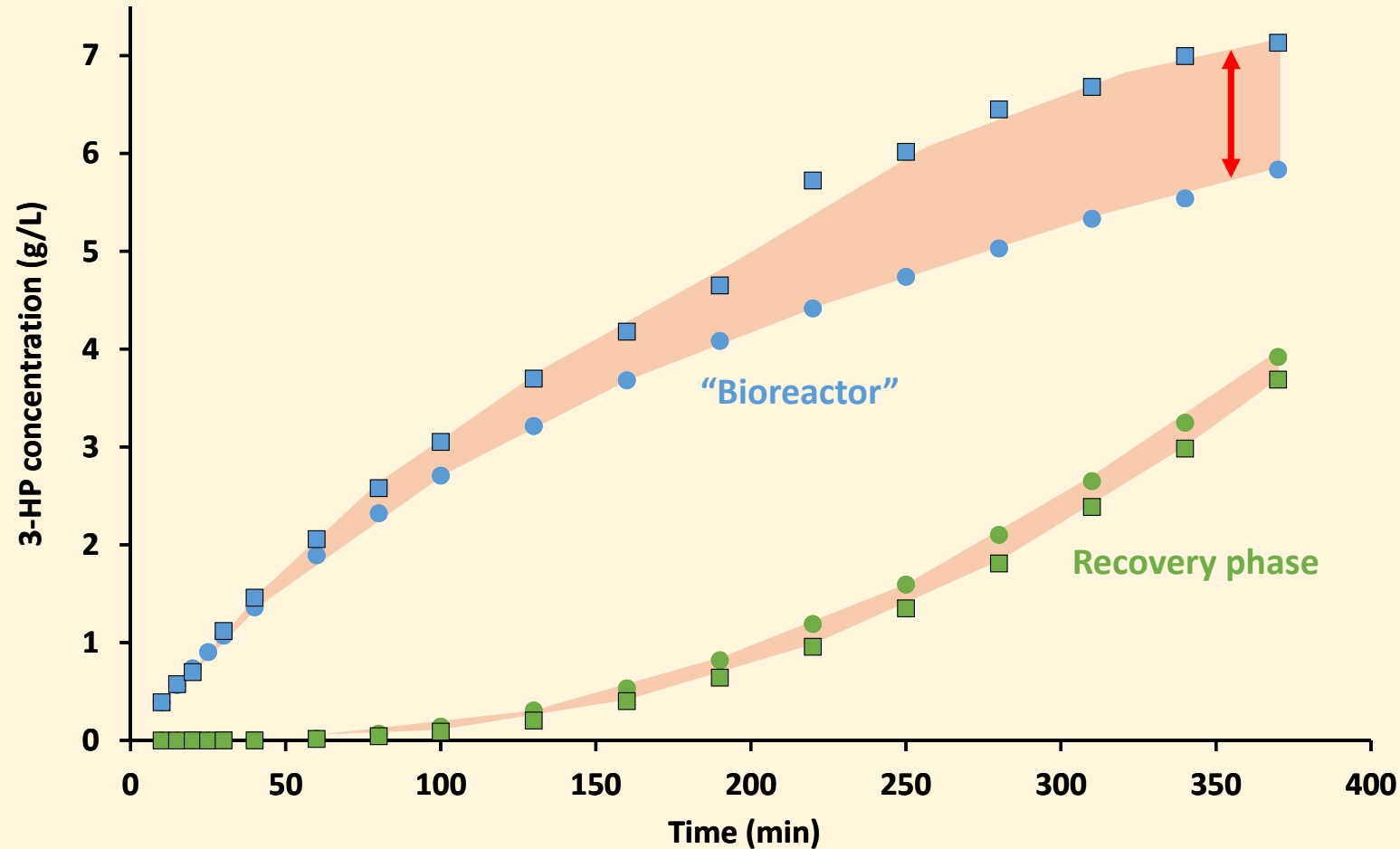
RECOVERY EFFICIENCY

Final concentrations after feed stop



Easy concentration possible

INFLUENCE OF A BIOCONVERSION BROTH



Interactions with:

- Salts → Anion exchange
- yeasts → Buffering power
- yeasts → Reduced available surface

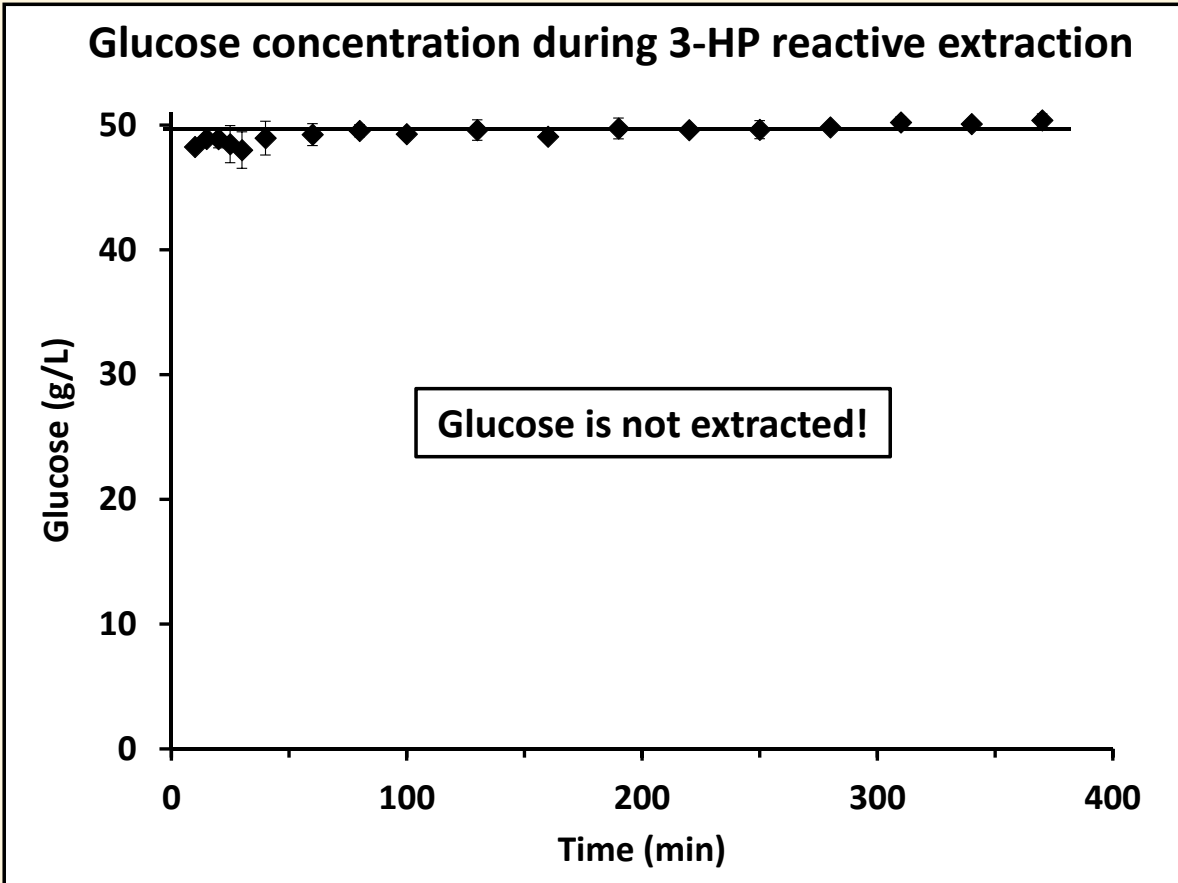
Initial composition:

- 50 g/L glucose
- 4 g/L *S. cerevisiae* (dry matter)
- 1.7 g/L urea
- 1.5 g/L KH_2PO_4
- 0.12 g/L MgSO_4

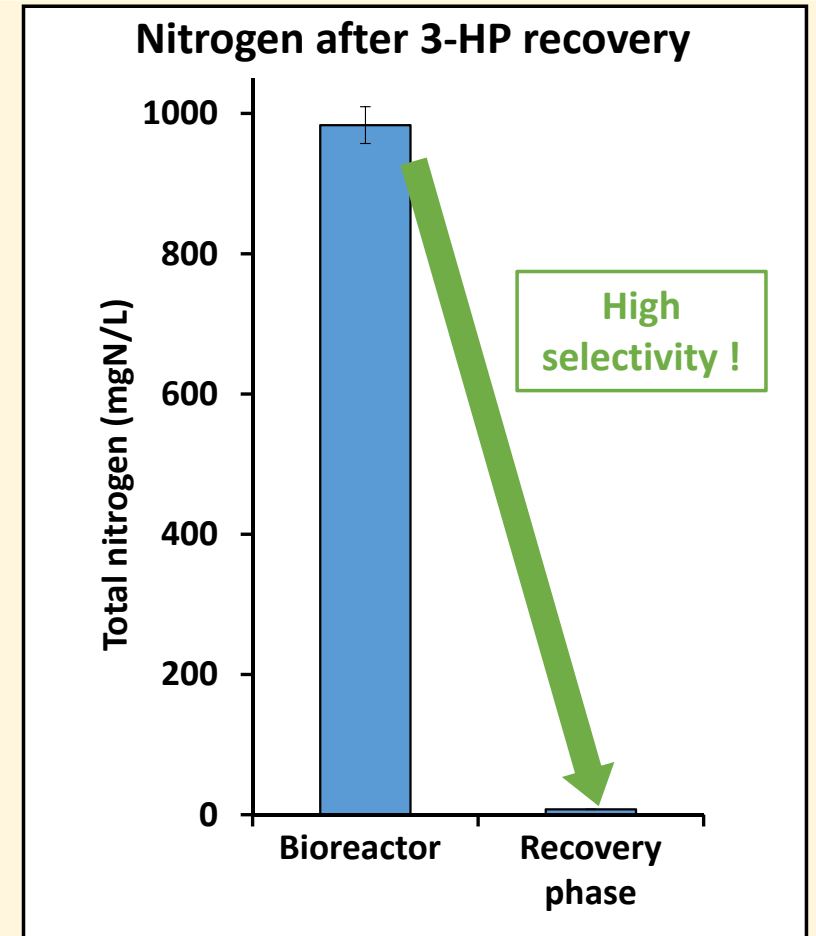
Fed-batch bioreactor:

2.5g/L/h

SELECTIVITY IN REACTIVE EXTRACTION



Initial medium composition: 50g/L glucose, 1.7 g/L urea, 1.5 g/L KH_2PO_4 , 0.12 g/L MgSO_4



Initial medium composition: see left + 4 g/L *S. cerevisiae* (dry matter)

CONCLUSION

Membrane-based reactive liquid-liquid extraction for organic acids removal

- Intensive (reaction/separation, highly compact, concentration, continuous)
- Selective (microorganisms, substrates, coproducts, proteins, cations)
- Combine inhibitory product removal, first purification and concentration

Prospects

- Ageing with long-term use
- Biocompatible system selection
- Membrane materials and ISPR configuration

Global considerations

- Increase sustainability and performances to be more competitive
- Intensification and innovation in down(in)-stream processes
- Integrated processes, modeling and novel processes to be presented

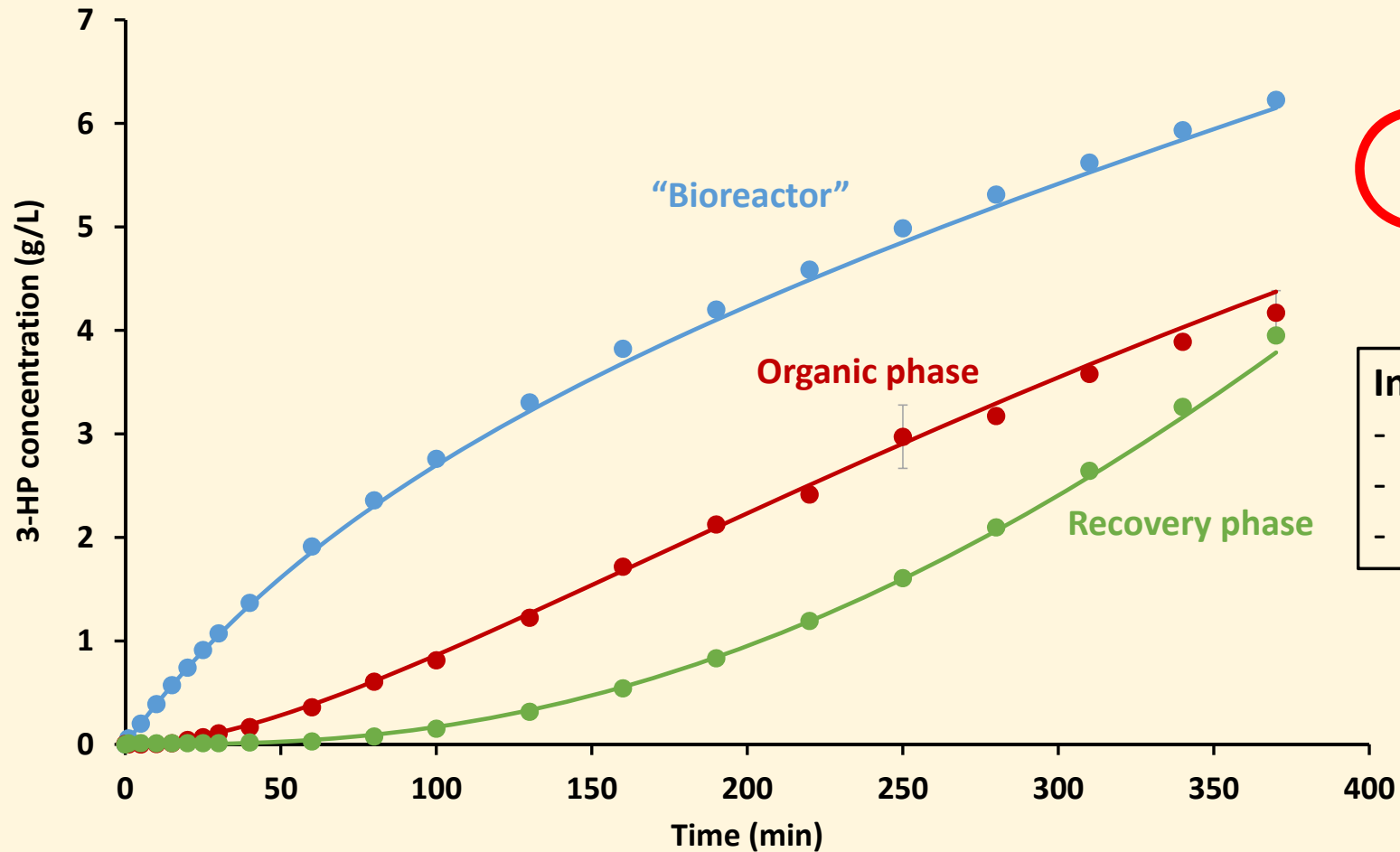
THANK YOU FOR YOUR ATTENTION



florian.chemarin@agroparistech.fr

TIME FOR QUESTIONS

SIMULATED EXTRACTIVE BIOCONVERSION IN "TRANSIENT STATE"



Initial conditions (3-HP):

- Bioreactor: 0 g/L
- Organic phase: 0 g/L
- Fed-batch bioreactor: 2.5 g/L/h