IDB2022 THE 20TH INTERNATIONAL DAYS OF BIOTECHNOLOGY

IPM-4-Citrus final meeting- 19th and 20th December 2022

IPM-4-CITRUS

Physical limitations induced by a wheat bran-based medium for the production of biopesticides by *Bacillus thuringiensis serovar kurstaki*

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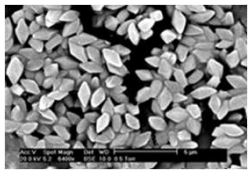
Biopesticide production: Why and how?

IPM-4-CITRUS

Citrus plants

PROTECTION



***Btk* Lip and BLB1**

PHYTOPHAGEN

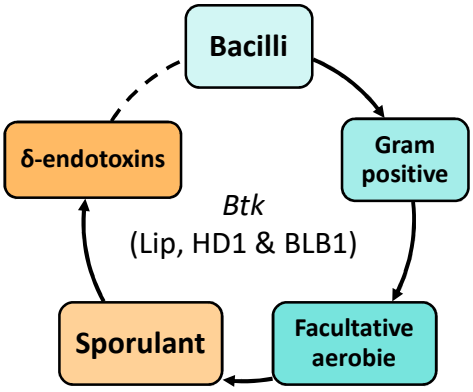
BIOPESTICIDE

Pests

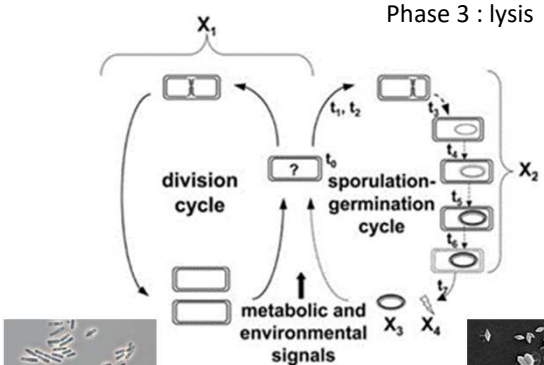
Phyllocnistis citrella* & *Prays citri

This project has received funding from the European Union's Horizon 2020 Research and Innovation program under Grant Agreement No 734921.

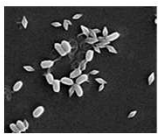
Bacillus thuringiensis var kurstaki (Btk)



Phase 1: oxydative growth Phase 2 : sporulation
Phase 3 : lysis



Sarrafzadeh et al, 2005



Loutfi et al, 2021

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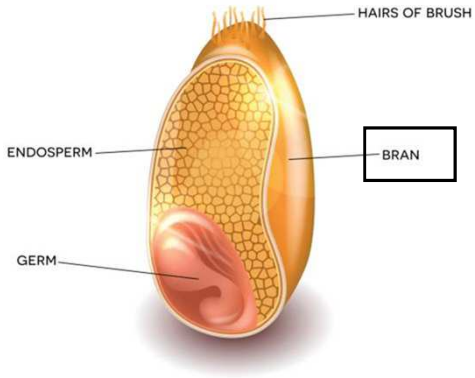
Wheat bran (WB): a good medium?



LOW COST

HUMAN NUTRITION
(Balandran et al, 2015)

HIGH YIELD
(Rahbani et al, 2014)

Meets *Btk* full nutritional requirements *(Onipe et al, 2015)*

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Scientific background & objectives

Production of *Btk* δ-*endotoxins in a wheat bran based medium*

Comparison of the bioperformances in different media (SSM & WB)

(Mounsef et al. 2014)

Impact of WB granulometry

WB sieving → flask culture → optimal growth conditions → [WB]=73.6 gmh/L, pH=7, 248 rpm, Tp=30°C

(Abboud et al. 2017)

Identification of nutritional limitations

Biochemical analysis of the WB : starch, proteins, elemental composition
Determination of the fermentable fraction & the limiting nutrient

(MSc Al Kassis 2020, MSc Barssoum 2021 MSc Nasserdinne 2021)

Investigation of the process physical limitations

Granulometry, rheometry, settling kinetics and scale-up

(Barssoum et al, 2022)

Studying of the physical limitations generated by the media :
(i) During the crucial steps of the process (oxydative growth, sporulation and lysis)
(ii) Simulation of the process using a dynamic model
→ Parameters : morphology, rheometry and solid-liquid separation

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The technological itinerary of the substrate (WB)

Sieving

- Class 1 (>850 µm): <1%
- Class 2 (500-850 µm): 19.9%
- Class 3 (250-500 µm): 60.5%
- Class 4 (<250 µm): 19%

Culture

- WB suspension
- Heat treatment (HT)
- Inoculation (Flask)
- Solid-liquid separation (insoluble fraction, permeate)

Analyses

- Chemical: elemental composition (± culture), proteins, **starch** (- culture)
- **Physical: Morpho-granulometry** (- HT, + culture), **solid-liquid separation** (± HT, + culture), **rheometry** (+ HT, - culture)

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Chemical characterization of the WB

Class	Diameter	starch	Proteins (Nitrogen Kjeldahl)	Elemental composition (%w/w)				
	(µm)	(g/g DM)	(g proteins /g DM)	C	H	O	N	Ashes
2	500-850	**0.173 (±0.35)**	0.145 (±0.012)	44.21 (±0.19)	6.50 (±0.12)	35.62 (±0.45)	2.60 (±0.23)	3.70 (±0.12)
3	250-500	**0.144 (±1.35)**	0.127 (±0.014)	45.34 (±0.08)	6.46 (±0.04)	37.89 (±0.44)	2.48 (±0.04)	4.28 (±0.12)
4	<250	**0.347 (± 0.47)**	0.155 (±0.016)	42.40 (±0.04)	6.61 (±0.12)	34.60 (±0.37)	2.61 (±0.24)	3.23 (±0.12)

- **Proteins** & **elemental composition**: **independants** from the **particle size**
  - **Starch** concentration ↘ when the **particle size** ↗

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Physical characterization of the WB

Physical analyses (raw, class 2, class 3, class 4)

- **Morpho-granulometry**
  - Optical microscopy (Mastersizer G3s, Malvern instruments)
  - **En, Ev (diameter of equivalent circle)**
- **Solid-liquid separation**
  - Pictures: t=2.5 min (-HT), t= 24 min (+HT), t=45 min (+culture)
  - **Settling velocity (U max)**
  - **Water retention capacity (WRC)**
- **Rheometry**
  - Rotational viscosimetry (Mars 3, ThermoFischer Scientific)
  - **Np vs Re → Metzner & Otto → kp, ks, µeq**
  - **Rheological behavior of WB suspensions**

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Technological itinerary and down stream process (DSP)

Substrate (WB)
- Granulometry
- Rheometry

Bioconversion
- Nutritional limitations

Solid-liquid separation
- Settling velocity

Formulation
- Composition
- Drying technology

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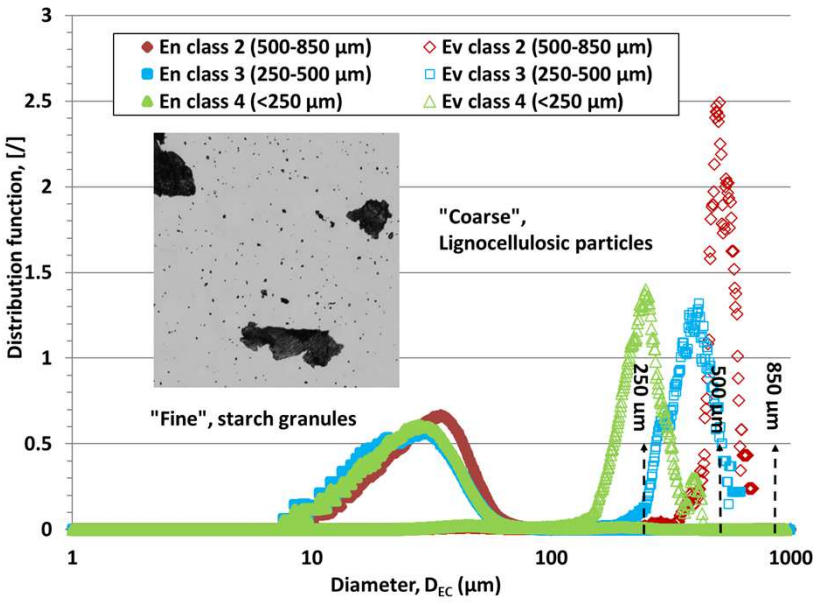
Morpho-granulometry of the WB particles



- **Two** populations: fines (**ovoidal** shape) and coarse (**angular** shape)
- **Distribution :**
  En(dec) → Fine population
  Ev(dec) → Coarse population
  (effect of sieving)

- **Fines** → **starch** granules
- **Coarse** → **lignocellulosic particles**
- **Heat treatment** → **starch** granules **liberated**

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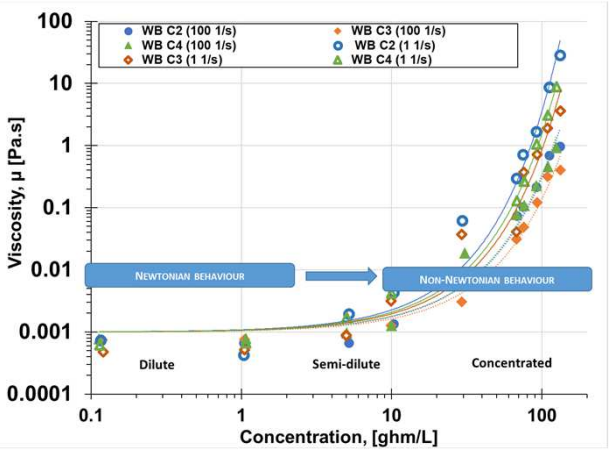
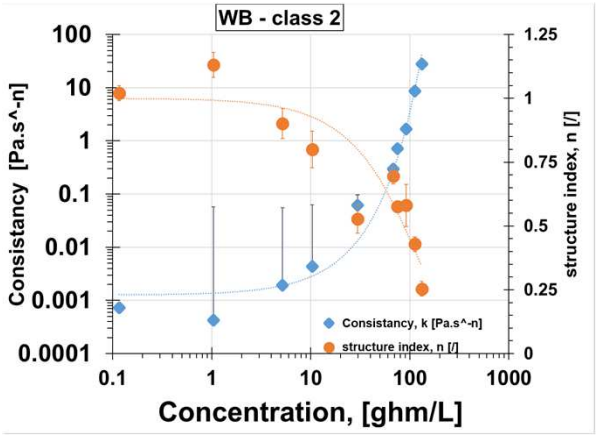
Rheological behavior of WB suspensions

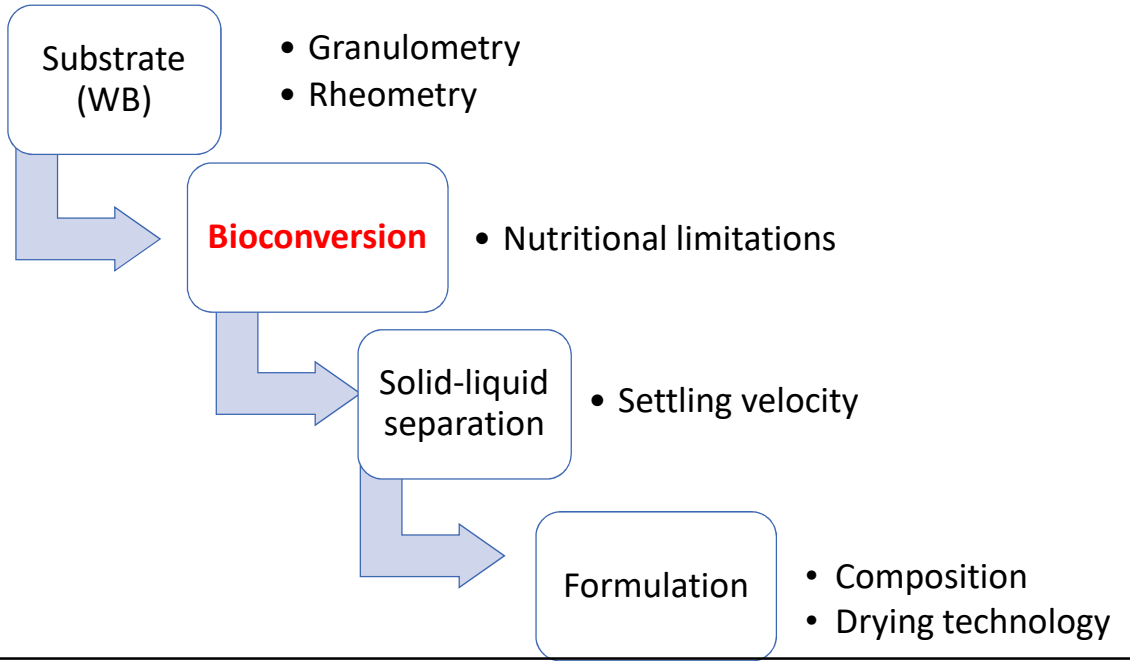




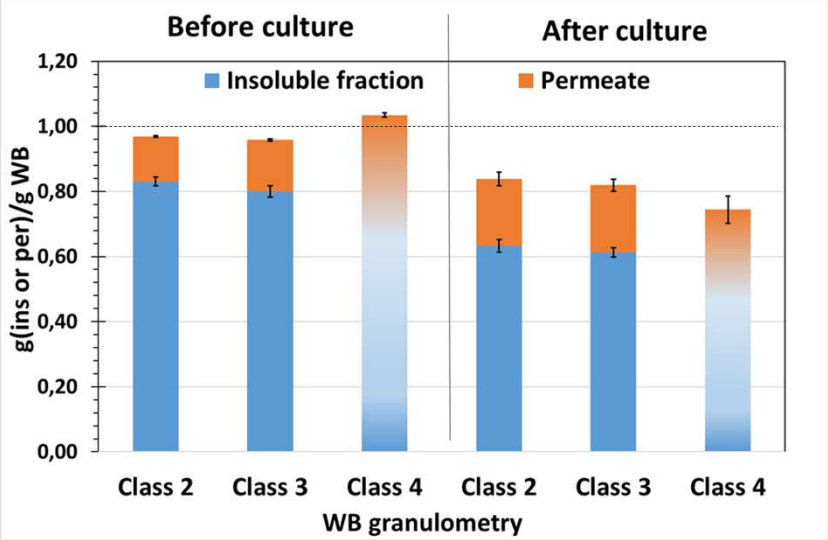
- **Dilute** regime: **Newtonian** behavior
- Concentrated regime : **shear- thinning** behavior
  $\mu_{CI2} > \mu_{CI3} > \mu_{CI4}$
- **Balance** between **starch** and **granulometry effects**

- Concentration ↗ → K ↗
- Concentration ↘ → n ↗
- Incertitude (methodology)

Technological itinerary and down stream process (DSP)



What is the fermentable fraction?

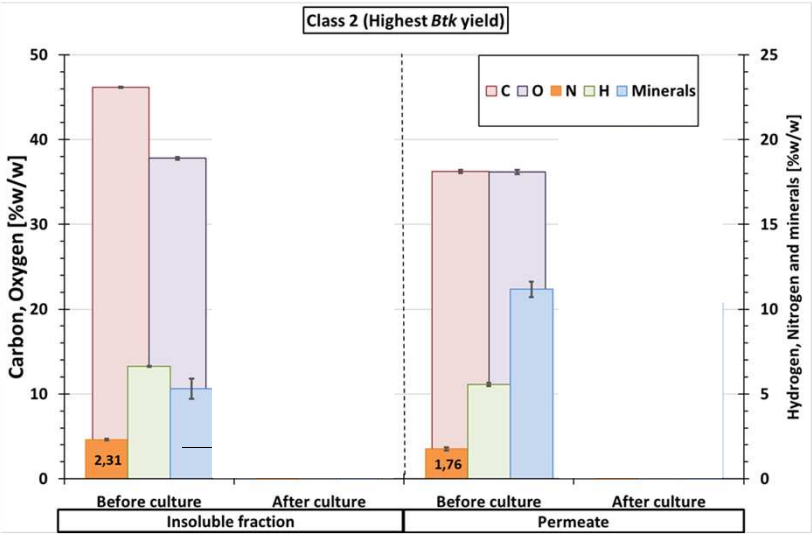


- Insoluble fraction: concentration ↘
- Permeate: concentration ↗
- Fermentable fraction ??

	Fermentable fraction (g/ gdm WB)	Starch (g/ gdm WB)
Cl 2	0.32	0.17
Cl 3	0.36	0.14
Cl 4	0.51	0.34

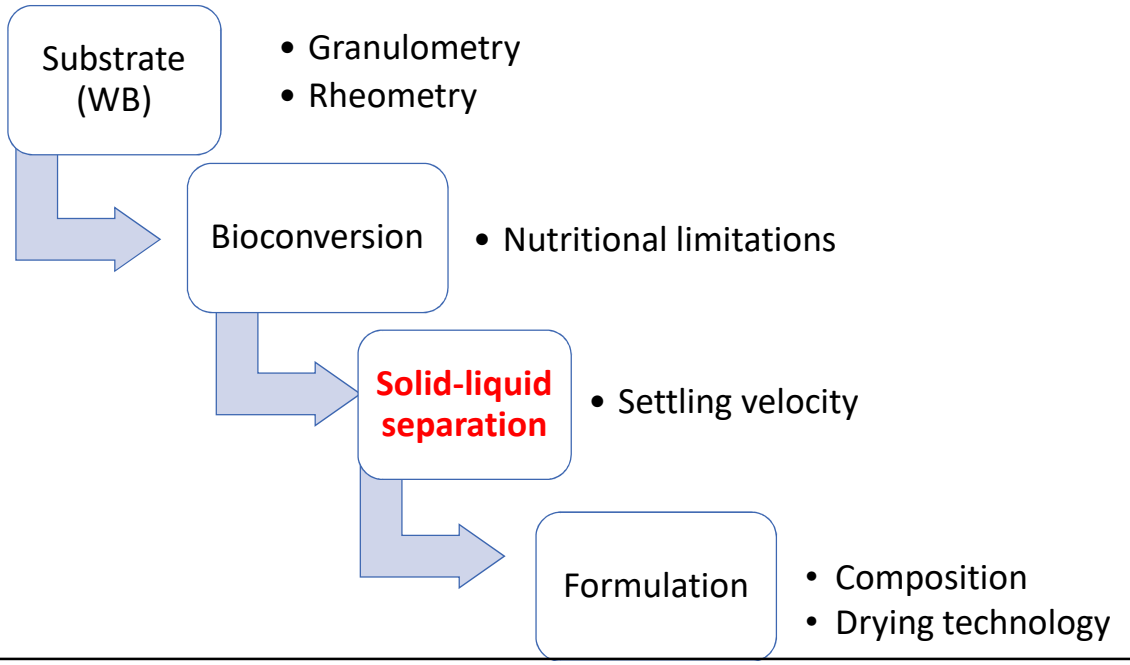
- **Lignocellulosic fraction:** partial consumption

Is there a nutritional limitation during the culture?



- Insoluble fraction: N ↘ → substrate proteins consumption
  → N in substrate
  > 40% N accessible
- Permeate: N ↗ → biomass production
- **N: limiting nutrient**

Technological itinerary and down stream process (DSP)



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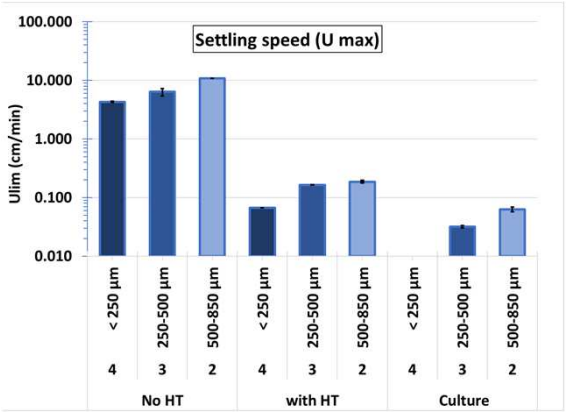
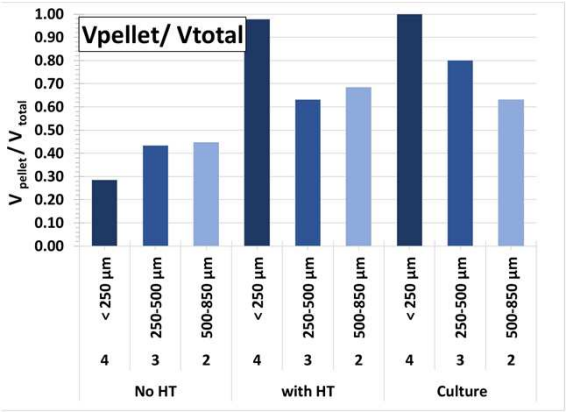
Focus on DSP: solid-liquid separation





- Particle size ↗ → U_{lim} ↗
- Heat treatment → **starch colloids** → U_{lim} ↘
- Culture → Starch consumption by *Btk*

- Heat treatment: Vp/Vt
  class 4 > class 3> class 2
- Culture → Vp/Vt increases

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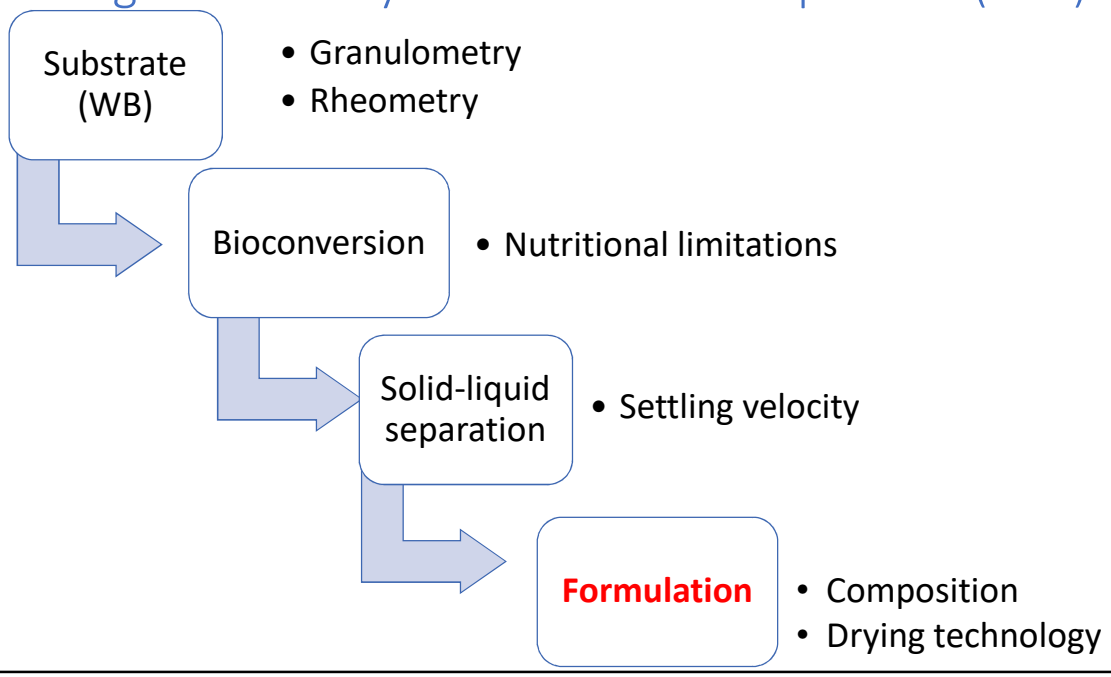
Technological itinerary and down stream process (DSP)



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Focus on DSP: formulations & drying technology

Strain	Number of dry formulations	Composition of the dry formulation	Drying technology
Btk Lip	Two	• with molasses (one formulation) • without molasses (one formulation)	Fluid bed drying
Btk BLB1	Two	• with molasses (one formulation) • without molasses (one formulation)	Freeze drying +mechanical grinding

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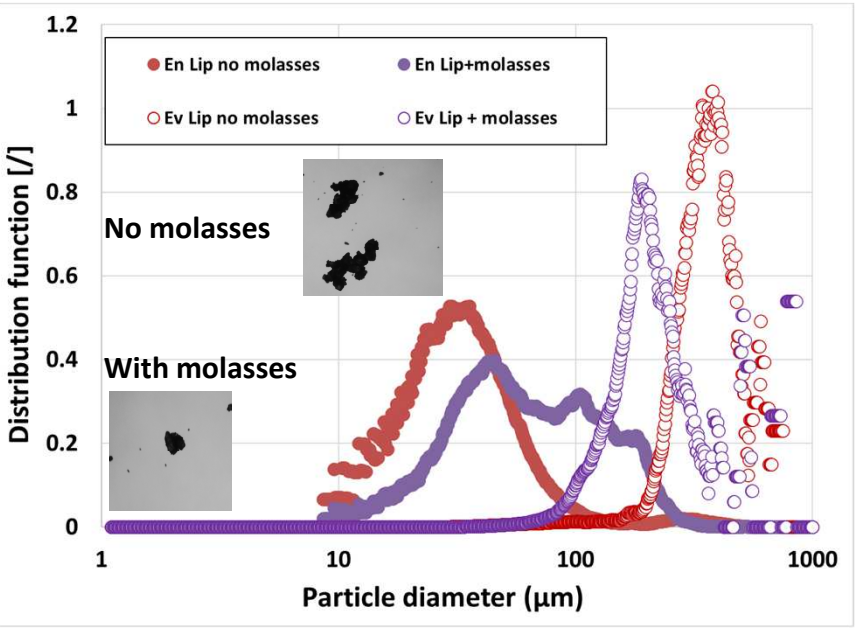
Focus on DSP: effect of molasses on formulations



- **Two populations: fines & coarse**
- **En: fines** (10-100 µm)
- **Ev**: **coarse** (100-1000 µm): **aggregates** of lactose
- En: molasses → **bigger aggregates**
- Ev: molasses → **smaller aggregates**
- Addition of **molasses** → **dispersion** of **coarse particles**

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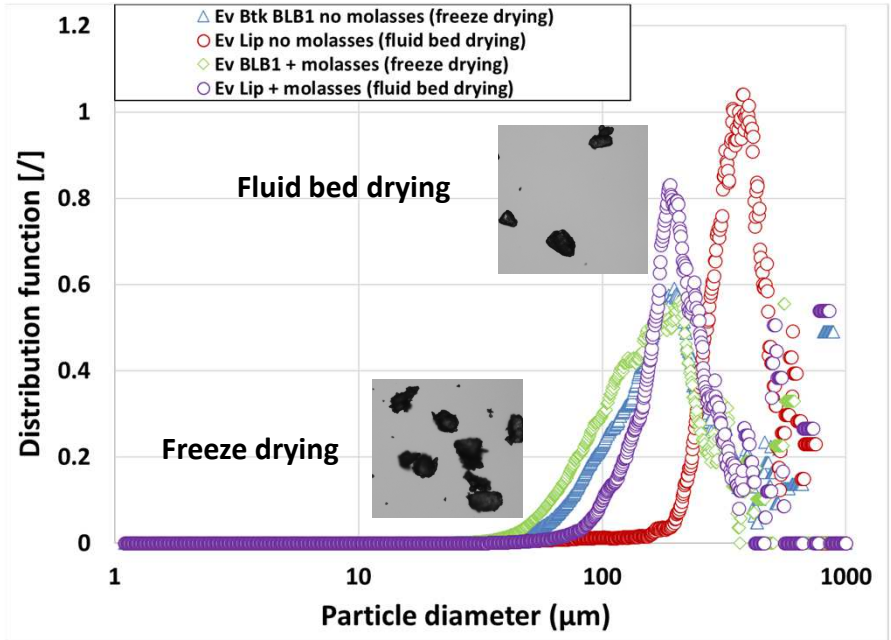
Focus on DSP: effect of the drying technology



- **Fluid bed drying** → no mechanical attrition → **bigger aggregates**
- **Freeze drying** → mechanical attrition → **smaller aggregates**
- **Freeze drying:** breaks the particles **heterogeneously**
- **Fluid bed dryer** is **more adapted** for the **process**

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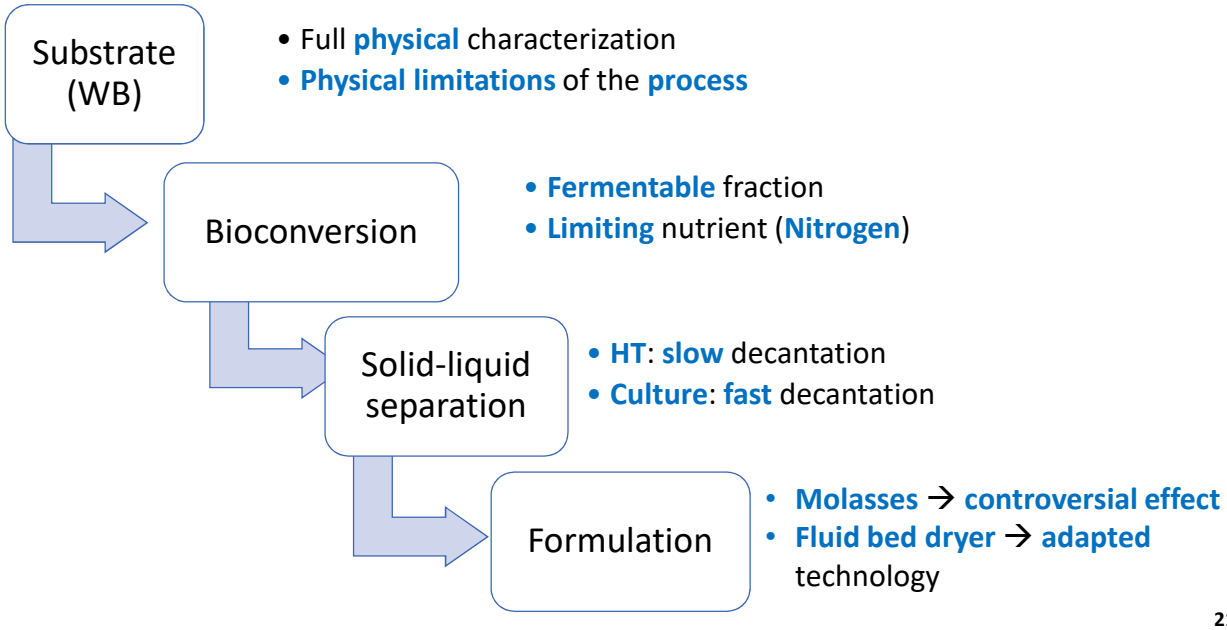
Conclusions



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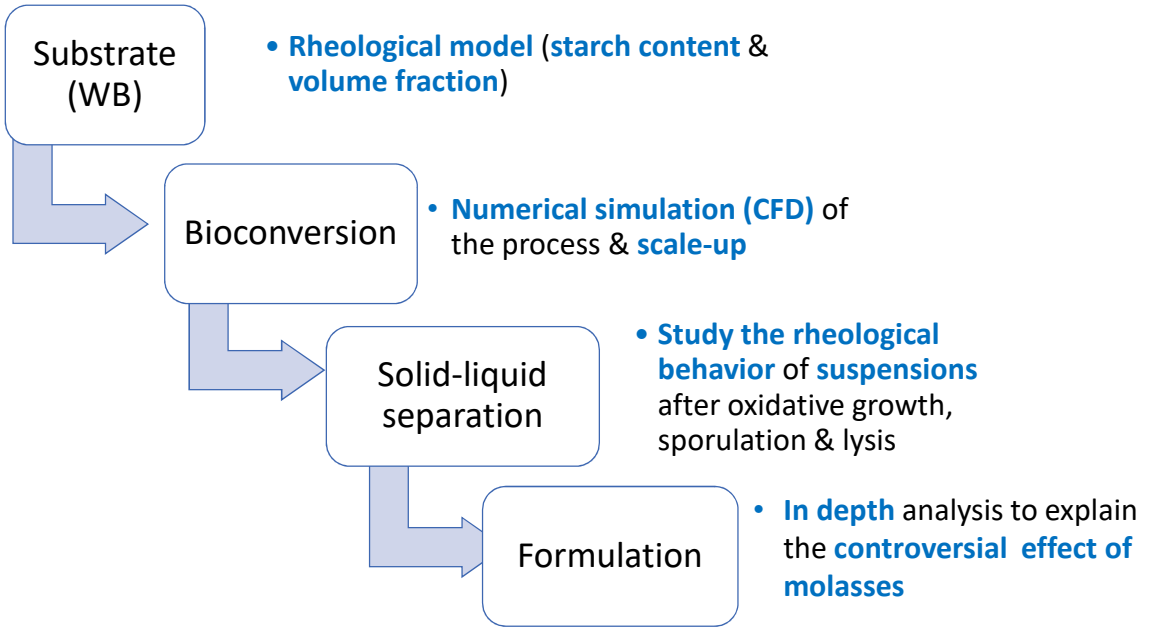
Perspectives



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Toulouse White Biotechnology
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The organizers of JIB 2022

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Thank you for your attention

21. RHEOMETRY: EXPERIMENTAL STRATEGY & SOLUTIONS

Solution	1st step: CP60/1°	1st step: PP35	1st step: CC B27DG	2nd step: Vanne	2nd step: Impeller HR
Water	OK	OK	OK	OK	OK
Glycerin 10%	OK	OK	OK	OK	OK
Glycerol 25%	OK	OK	OK	OK	OK
Glycerol 50%	OK	OK	OK	OK	OK
Glycerol 100%	OK	OK	OK	OK	OK
Xanthane 0,15%	OK	OK	OK	OK	OK
Xanthane 0,5%	OK	OK	OK	OK	OK
Xanthane 1%	OK	OK	OK	OK	OK
WB vs cc (0 to 150 gdm/L) and class	/	/	/	OK	OK

N and NN fluids
With CP/PP/DG

Power consumption curve (Np, Re) → Kp, Np0
With Vanne / HR

Metzner and Otto concept
Generalised Re → Ks

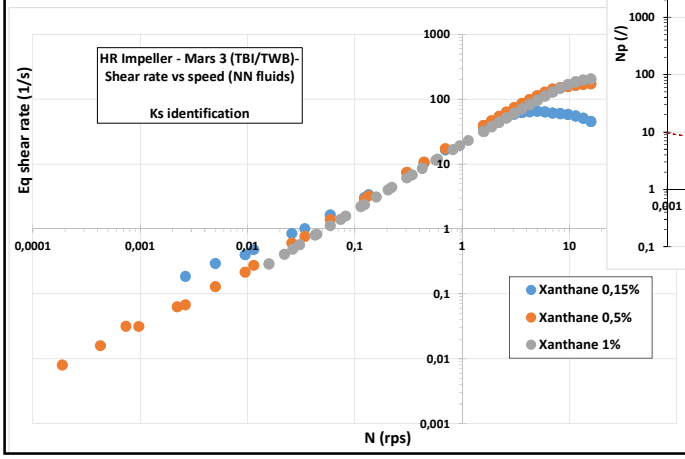
With WB suspension
(Torque, Mixing) → μ and shear rate

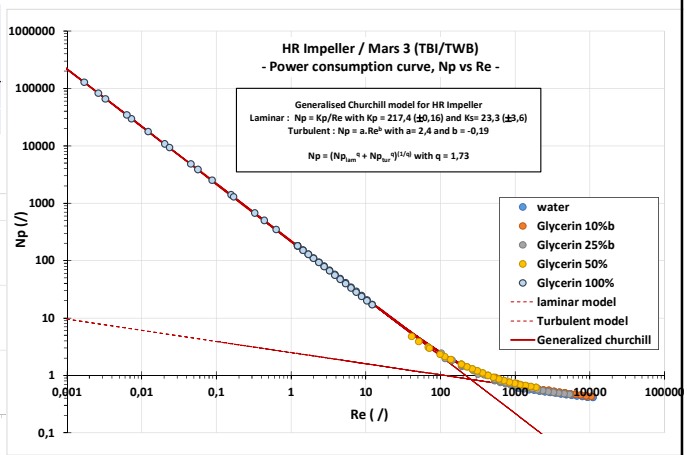
Rheological behaviour of WB suspensions
Vs CC and Class

Modelling vs cc, granulometry & starch content

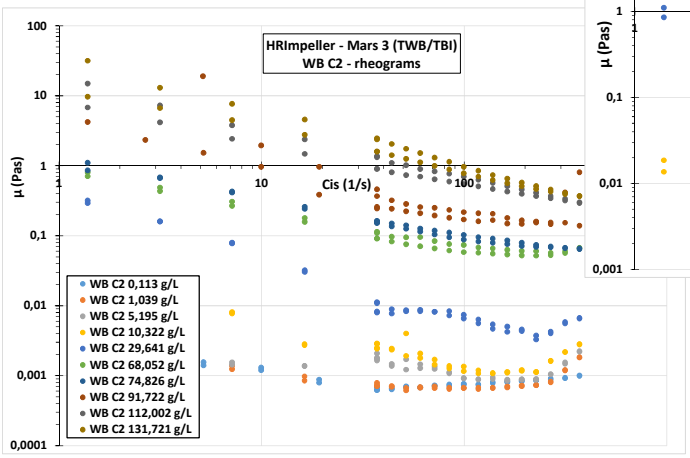
41. RHEOMETRY: POWER CONSUMPTION CURVES (HR IMPELLER)

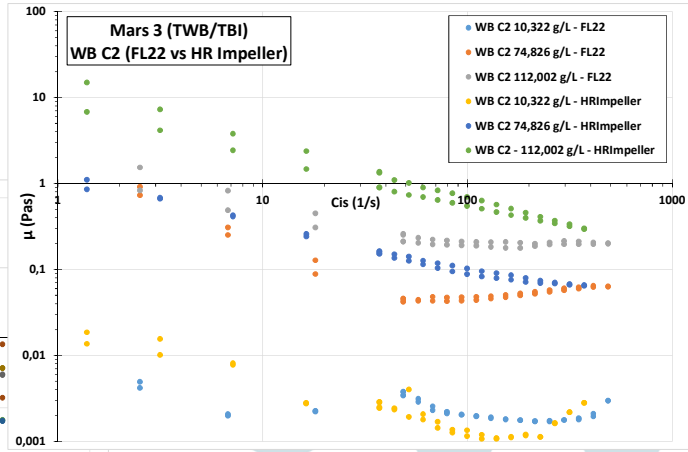
Impeller / Batch	Diameter (mm)	Diameter (m)	Height fluid (mm)	Height impeller / Batch (mm)
FL22	22	0,022	35	16
HRImpeller	26	0,026	42	25,8





42. WB RHEOGRAMS (CLASS 2): FL22 & HRIMPELLER



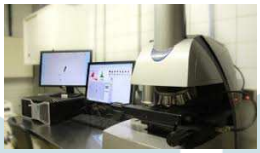


1. CONTEXT OF STUDY & STRATEGY

Wheat Bran suspension (Raw, class 2, 3 and 4)

- Morphology (Dry & Wet Way) → Laser Light Diffraction (Malvern Mastersizer 3000)

Optical microscopy (Morphology G3s, Malvern instruments)

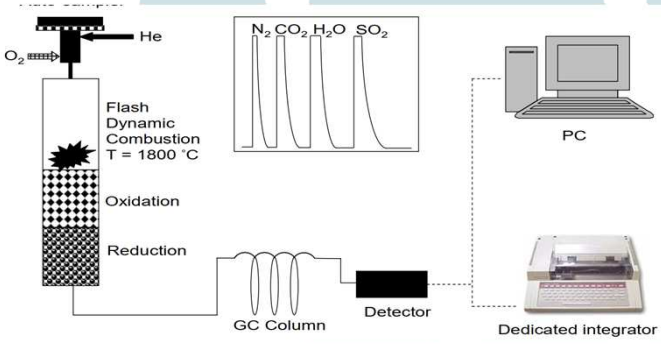


- Rheometry (Wet way) → Rotationnal viscometry (Mars III, Thermofischer Scientific)

- Decantation kinetics (Wet way) → Turbiscan Lab (Formulaction)

5. CHONS analysis method

Flash smart 2000.thermofischer scientific



CHONS analysis mechanism

Active ingredients of the formulation

Ingredient	%	Quantity/100g	function
Molasses	5	5.95 g	UV protectant
water		4 mL	
sorbitol	2	2 g	Suspending agent
Corn steep	2	2 g	phagostimulant
Tween 80	0.10	0.1 g = 2 drops	emulsifier
Calcium stearate	2	2 g	lubricant
water		2 mL	
Bt pellet (spores+proteins)	7 or 14	Depends on the desired proteins content and the dry weight of the pellet	Active ingredient
water		48 mL	
Lactose (in the fluid bed dryer)	81.9 or 74.9	81.9 or 74.9g	Carrier and protection of the active ingredient