



Mass balance, cells, spores and δ -endotoxin distributions during *Bacillus thuringiensis kurstaki* bioproduction with wheat bran based medium

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IPM4Citrus activity:

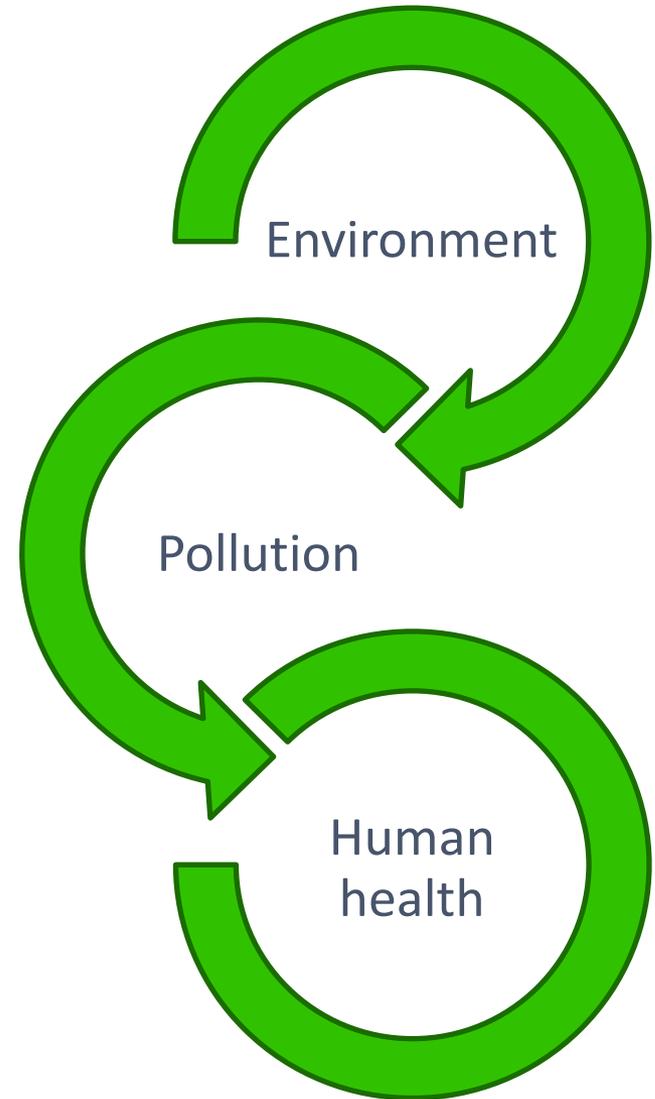
- WP2: Proof of concept, from *B. thuringiensis kurstaki* cultivation to δ -endotoxine production.
- T2.3. Bioprocess transposition (scale up 1-10L bioreactor scale)





Introduction

- ❑ Synthetic pesticides are chemicals products used to protect agriculture from pests.
- ❑ The pollution caused by these products affects the environment and subsequently human health.

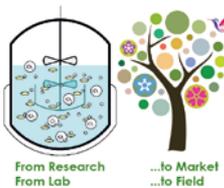




The solution to overcome the negative effects of chemicals and to protect agriculture is the use of:



- 1 Biological control
- 2 Biodegradable
- 3 Lasting effect



Bacillus thuringiensis

- Gram-positive
- Aerobic/ facultative anaerobic
- Endospore forming bacterium
- Vegetative cells have a stick shape
- Produce crystal proteins Cry

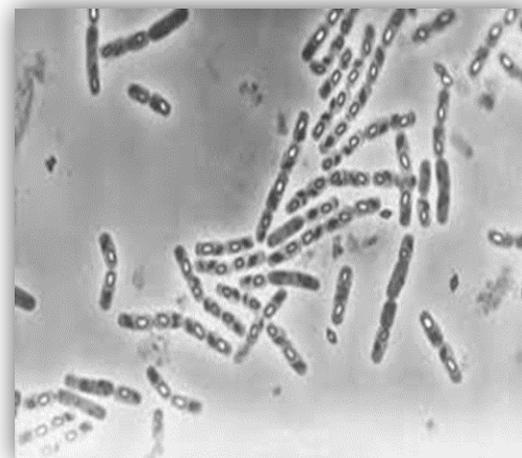
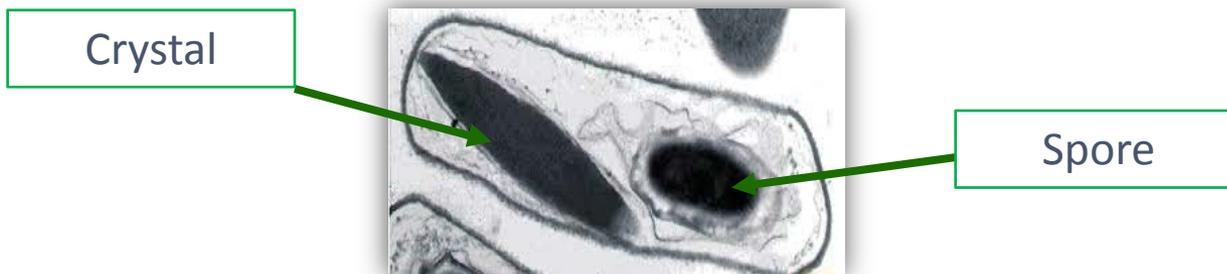
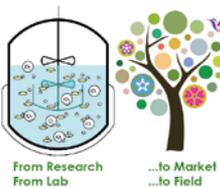


Figure 1: Microscopic observation of the vegetative cells of Bacillus thuringiensis during sporulation (Lacoursière J.O. et Boisvert J. Université de Québec à Trois Rivières, 2004)

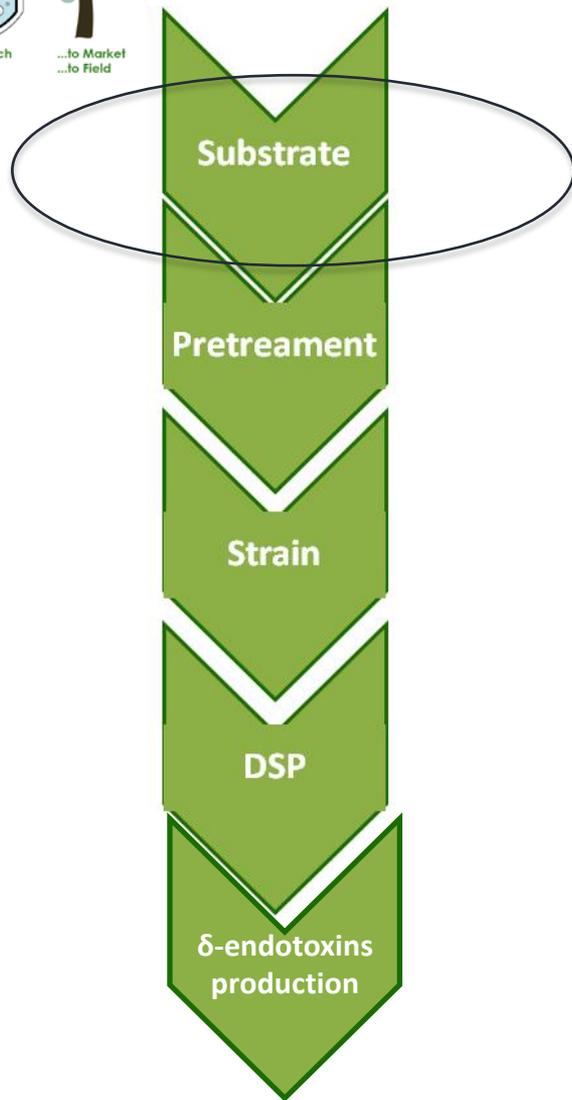




Aim and Strategy

- to characterize the mass balance (dry matter, chemical composition) :
 - Substrate: Wheat Bran
 - cells, spores and δ -endotoxin distributions between supernatant and pellet during *Bt kurstaki Lip* production
- to evaluate fermentescible fraction
- to select an adapted downstream processing (DSP).



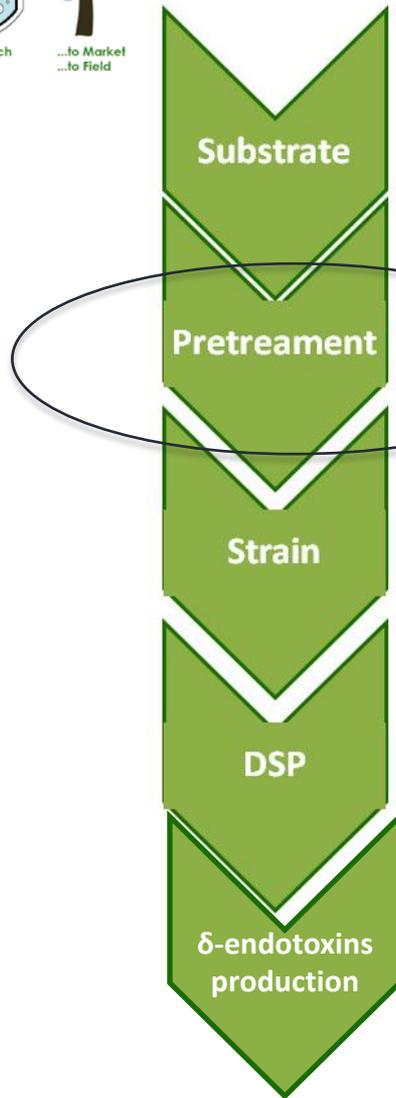


- Granulometry
- Water content, humidity, dry matter
- Composition
 - CHONS
 - Sugars
 - Ashes

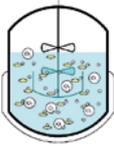


From Research
From Lab

...to Market
...to Field



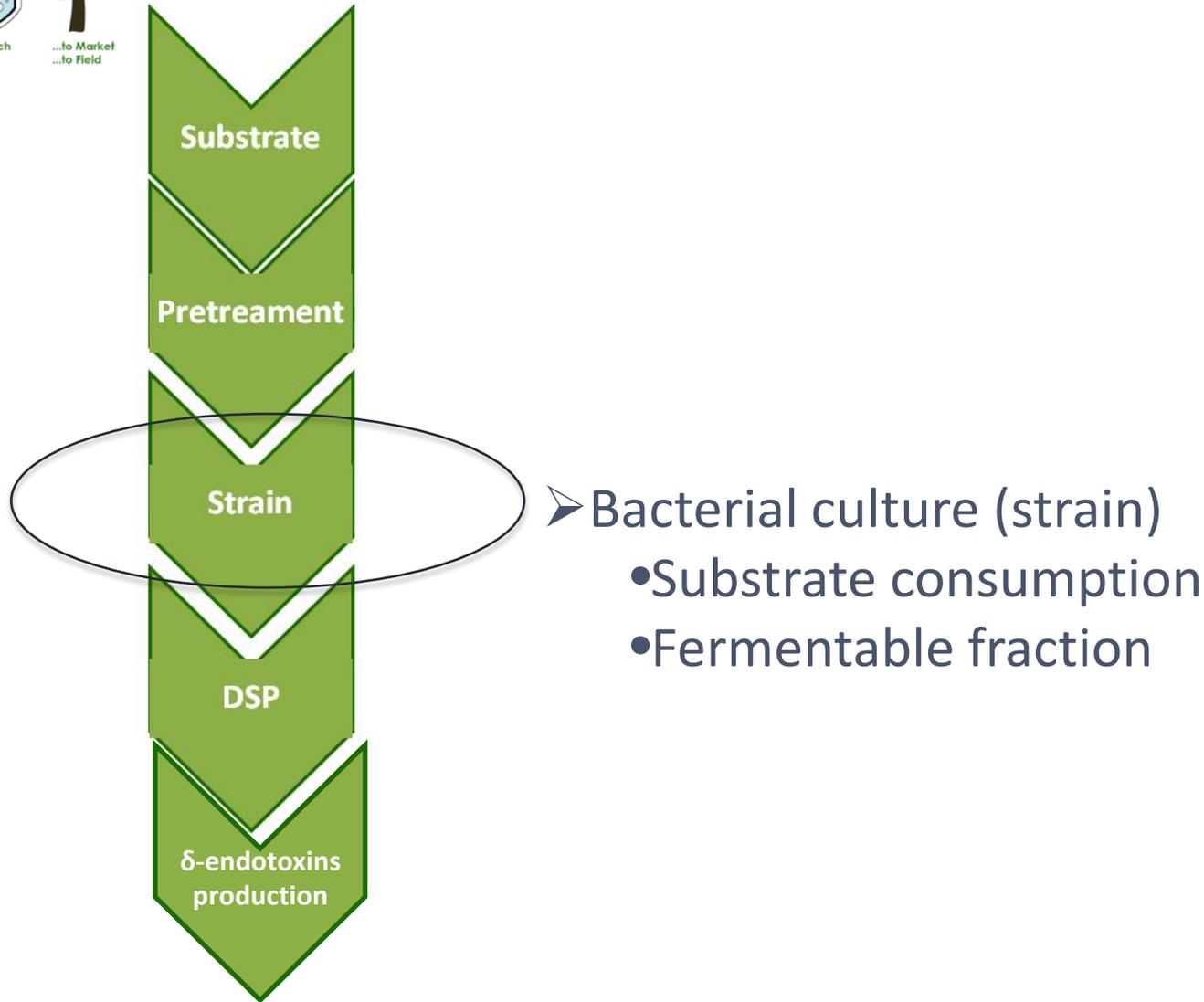
➤ Soluble fraction vs
Insoluble fraction

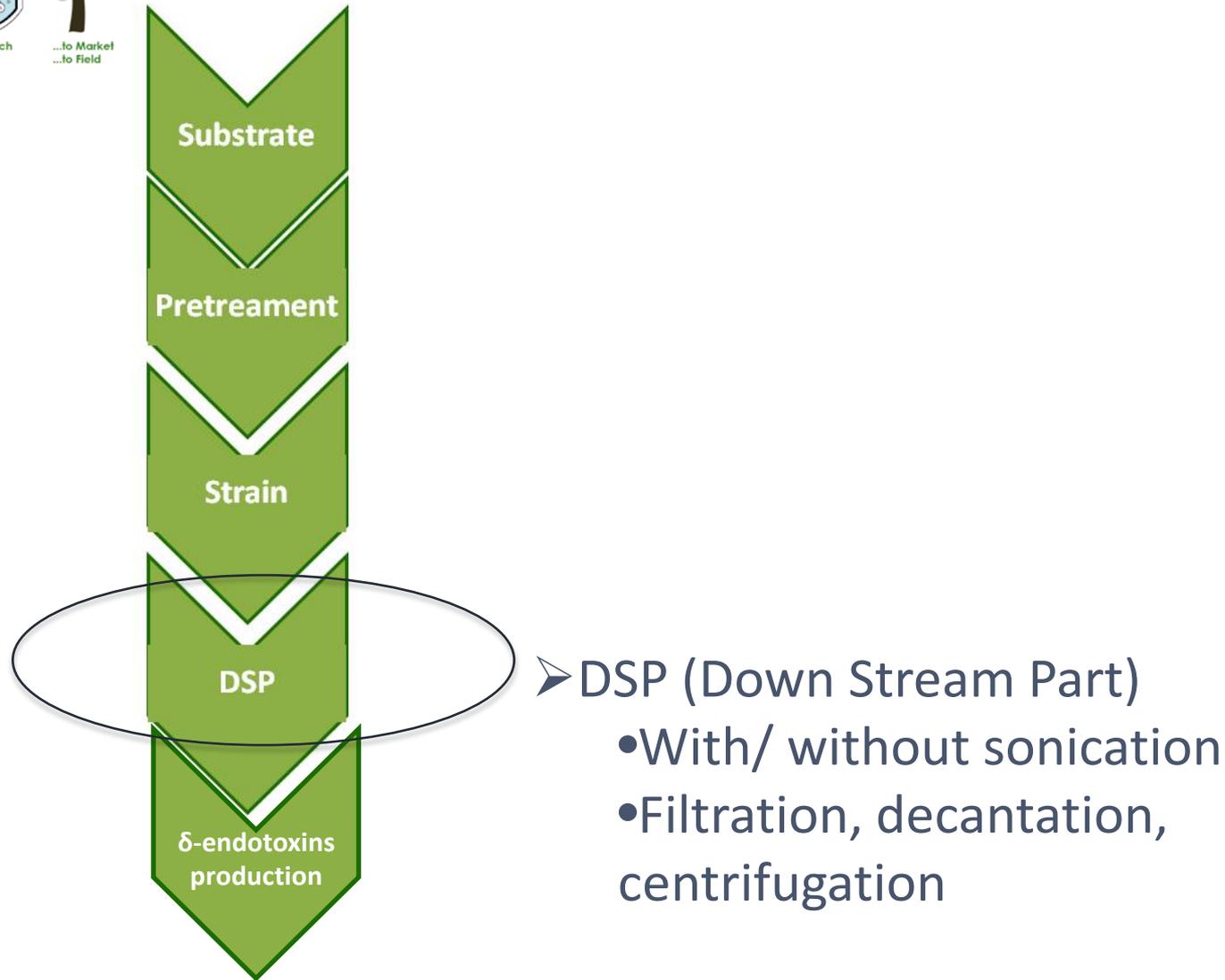


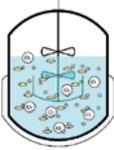
From Research
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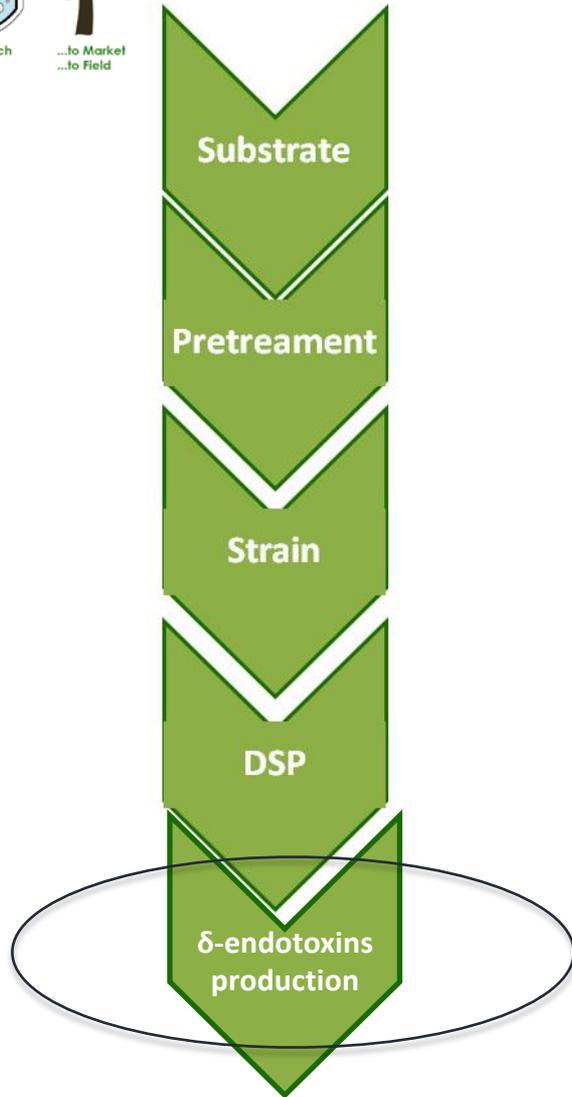




From Research
From Lab



...to Market
...to Field



- δ -endotoxins production
- Protein dosage
 - CFU (total flora and spores)



Substrate

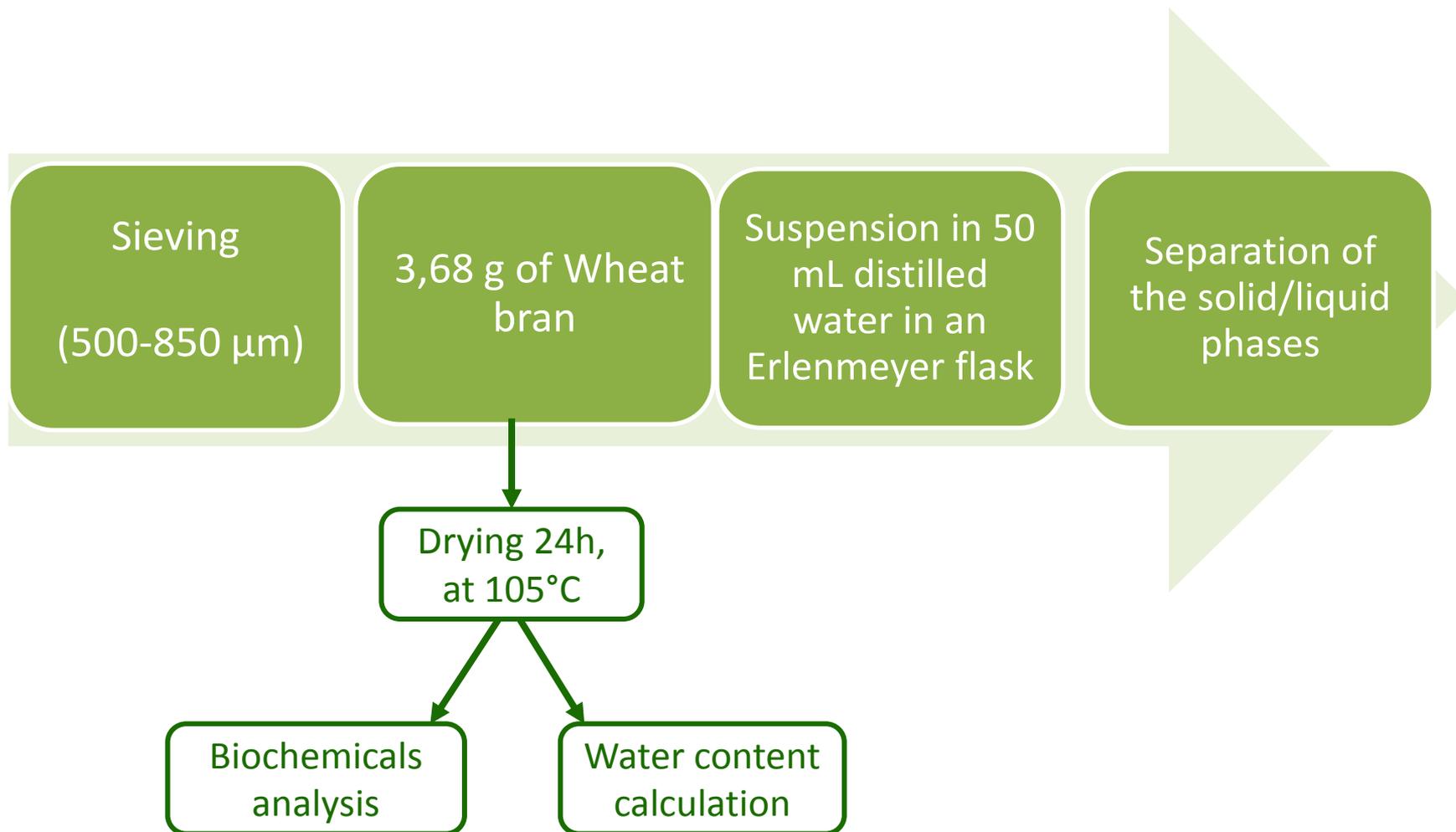


Table showing the biochemical composition of wheat bran

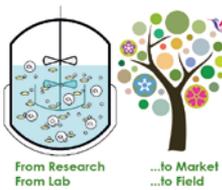
Analysis	Content
Dry matter(%)	89.58
Absolute humidity (n, gwater/gDM)	0.1164
Relative humidity (x, g water/gHM)	0.1042
Total Nitrogen[gNtot/ gDM]	0.0062
Mineral Nitrogen[gNmin/ gDM]	0.0028
Organic Nitrogen [gNorg/ gDM]	0.0034
Proteins [gProteins/ gDM]	0.0209
Sugars	n.r
Minerals [g minerals/ g DM]	0.049



Strains

- Rich medium (LB):
 - Genomic analysis
 - CHNS-O
- Industrial medium:
 - Mass balance
 - Fermentable fraction

Substrate + Oxygene+ Nitrogen + minerals + Vit → Biomass+ CO2 + water+ Energie



DSP (Lip)

- Lip strain was the reference for the DSP
- Effect of several separation techniques on the mass balance and the protein production
- Goal: Find the the technique which is as simple and cheap as possible (ASAP/ACAP)
- Lab scale => Bioreactor scale



DSP

Raw
Wheat
Bran

Strains
(LIP/BLB1/HD1)

Industrial
Medium
(IM)

IM
Sterilization

IM
Sterilization+
LIP Culture

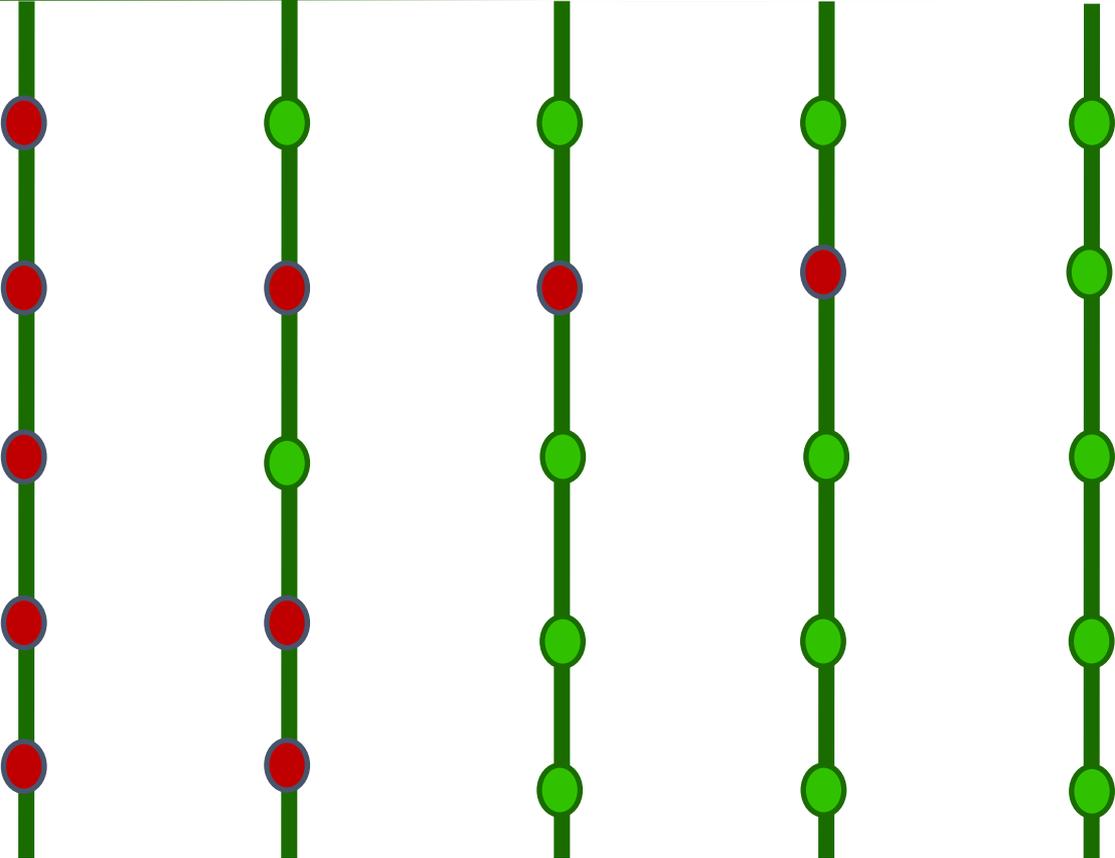
Decantation

D + S

F cut-off 1 and 2

F cut-off 1 + S

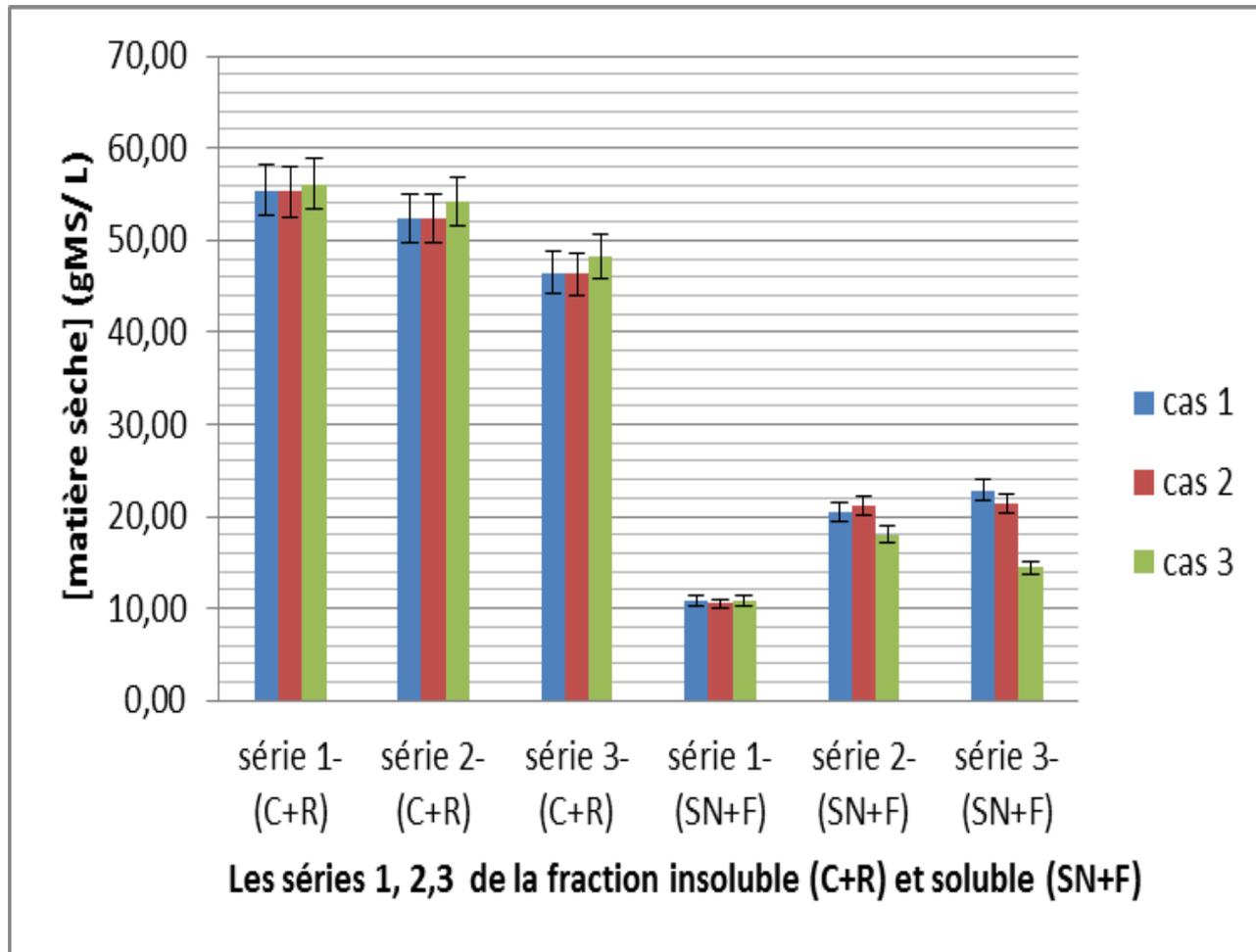
Centrifugation



D: decantation
S: sonication
F: filtration

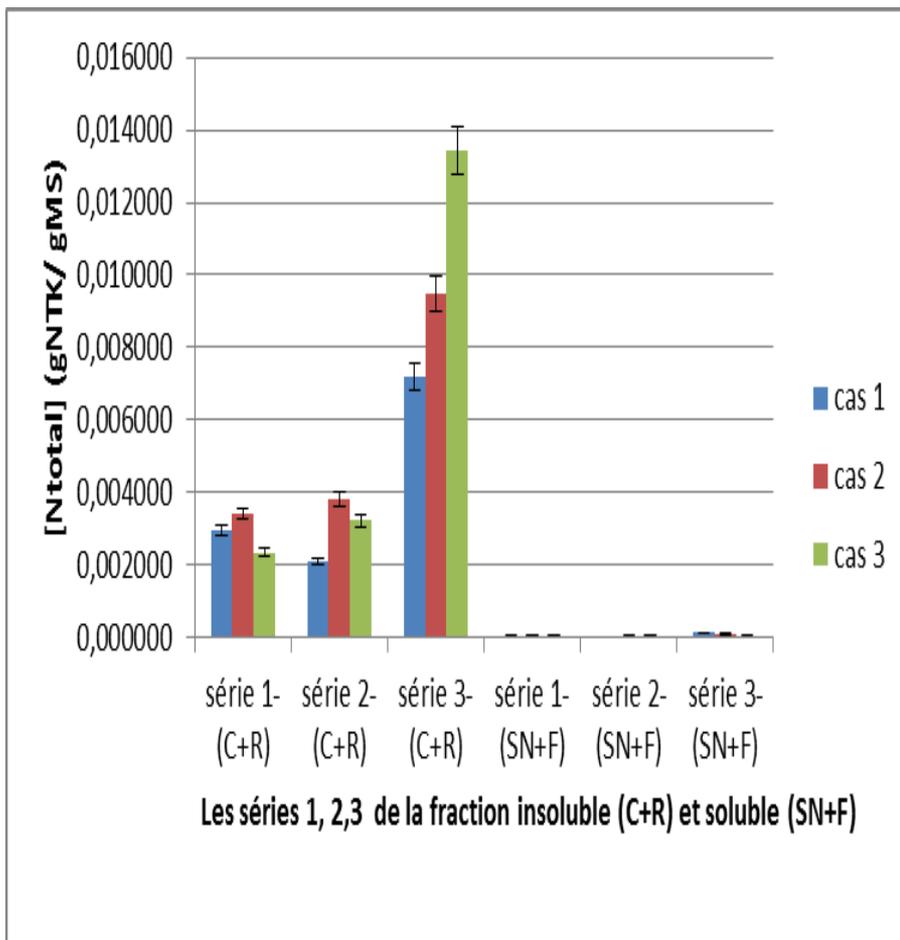


The variation of the dry matter concentration in the insoluble and soluble fractions.

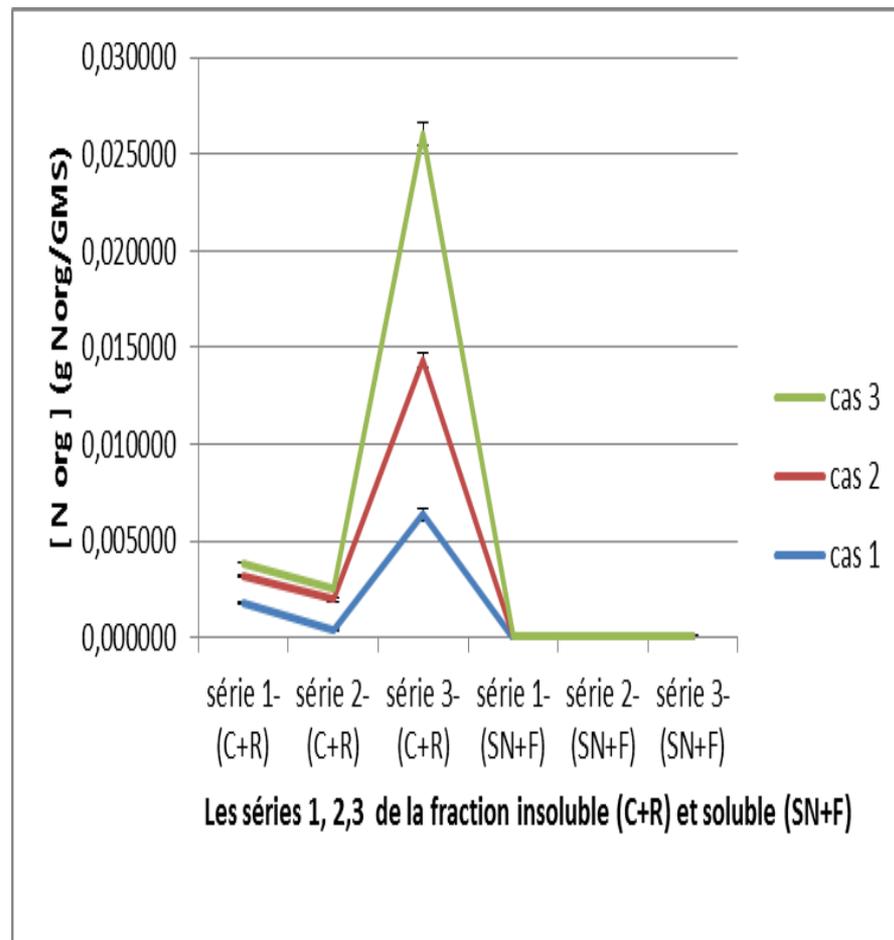


- Heat treatment increased solubilization.

The variation of the concentration of total nitrogen (N_{tot}) in the insoluble and soluble fractions.

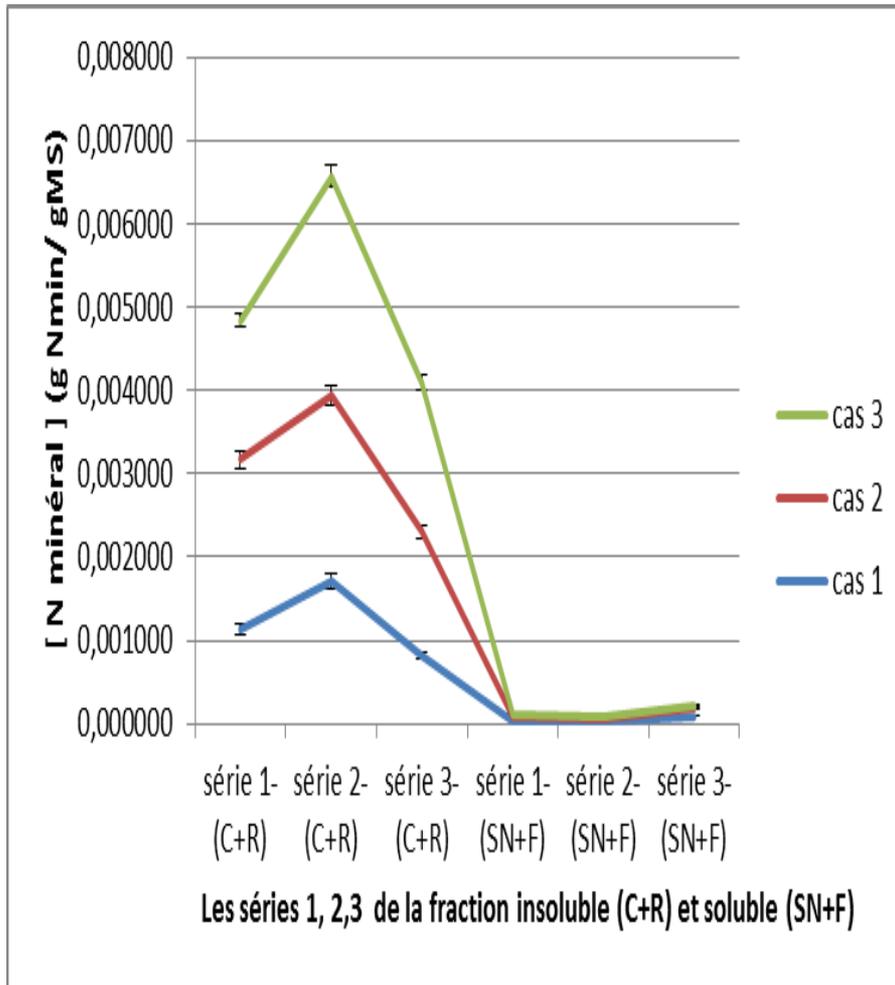


The variation of the concentration of organic nitrogen (N_{org}) in the insoluble and soluble fractions.

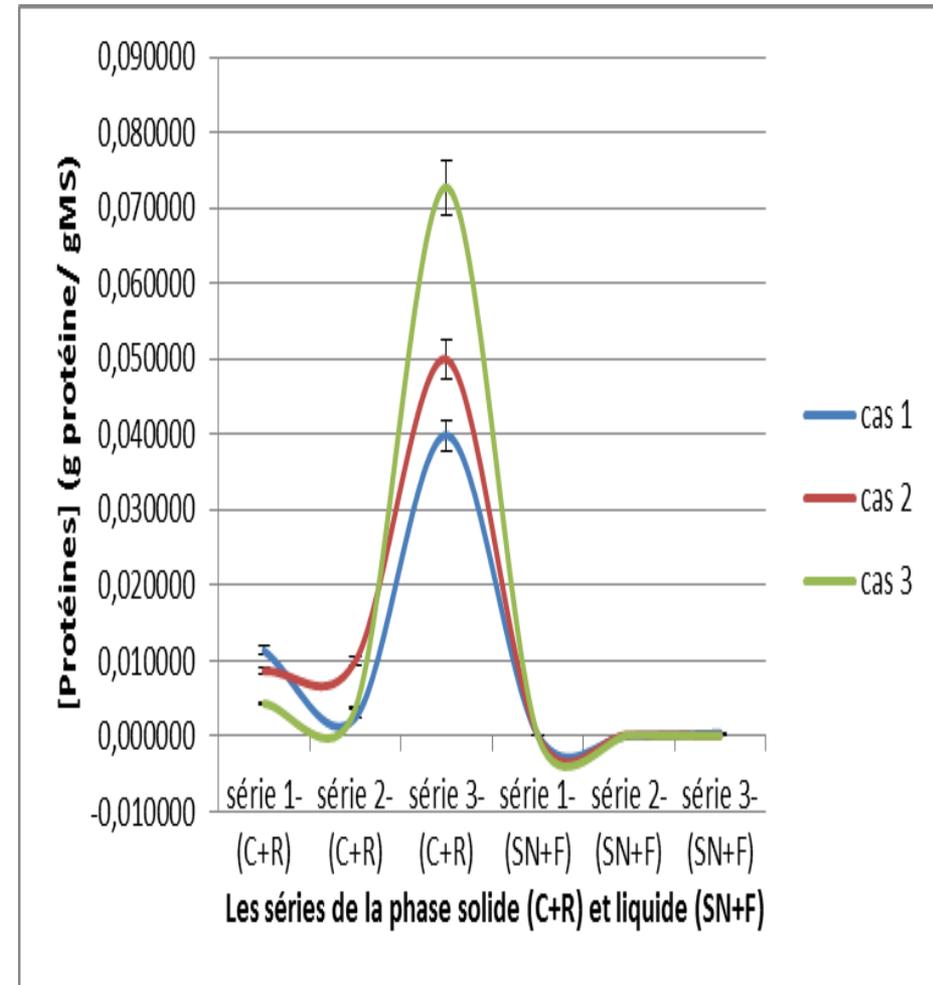


- Increase in the concentration of organic and total nitrogen in the presence of Bt culture.
- Not a significant difference with sonication.

The variation of the concentration of mineral nitrogen (Nmin) in the insoluble and soluble fractions.



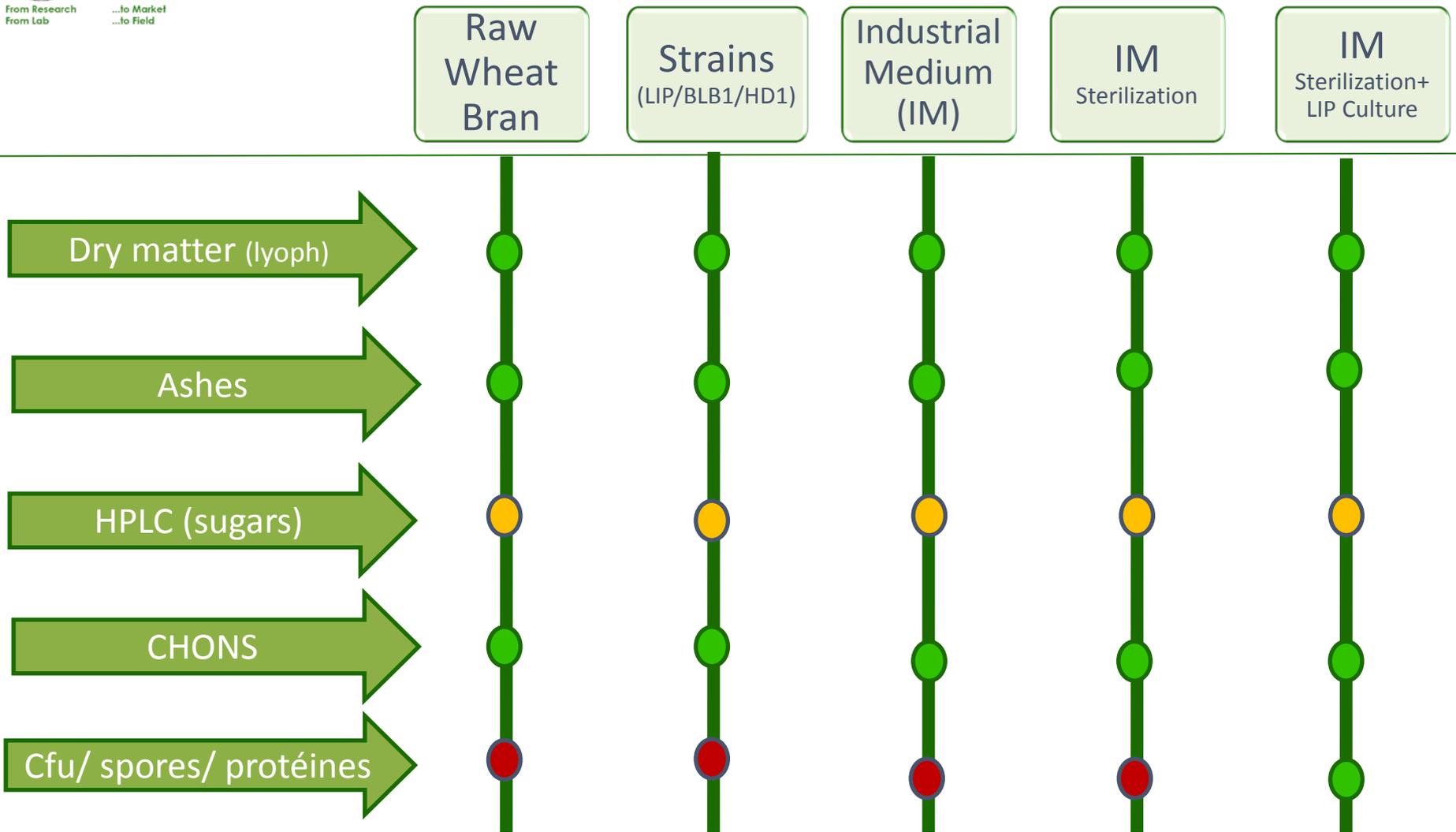
The variation of the protein concentration in the insoluble and soluble fractions.



- Mineral nitrogen is consumed by Bt, no effect of sonication, centrifugation has the highest concentration after autoclaving.
- Protein production, by Bt, higher following centrifugation



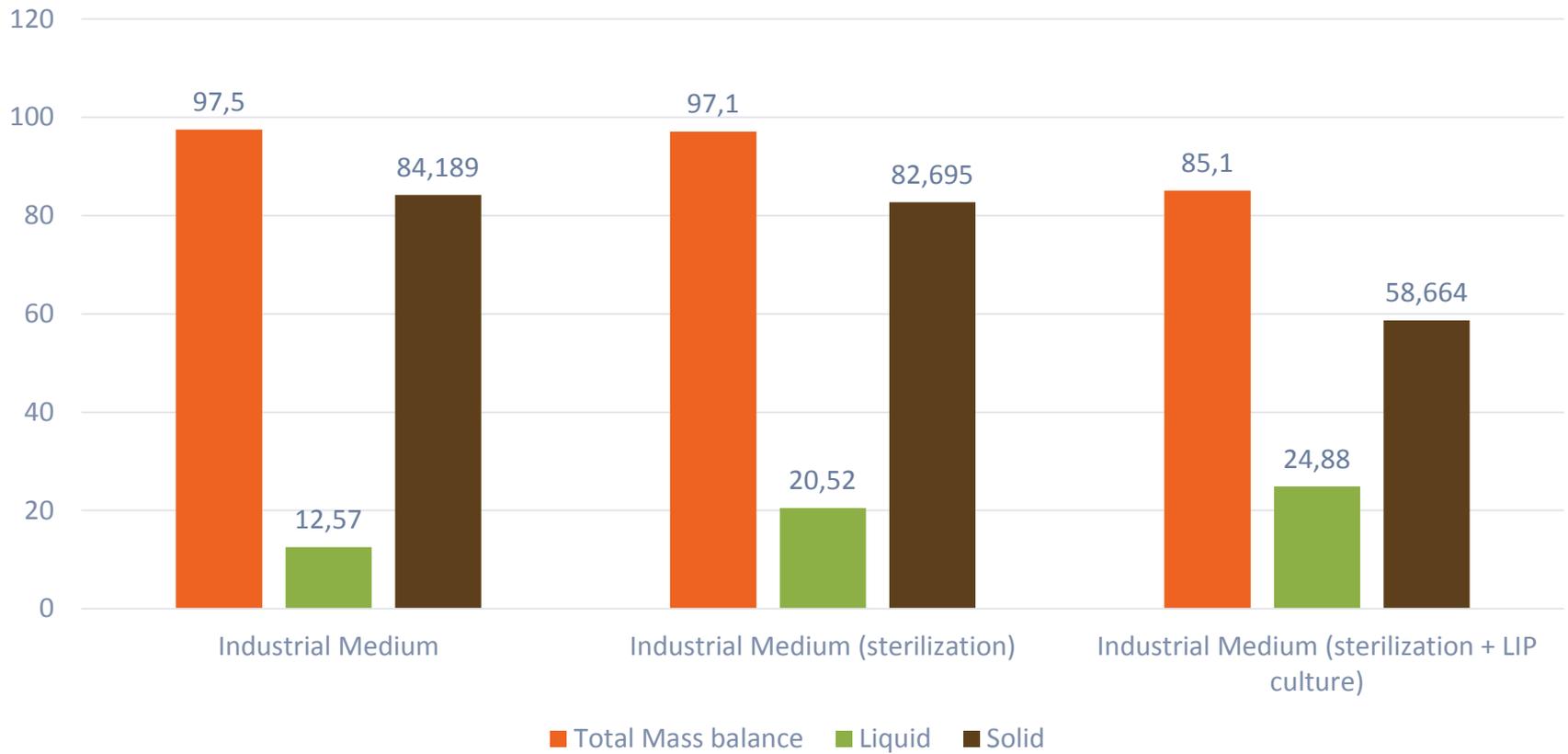
Physico-chemical and biological analyzes





Mass balance

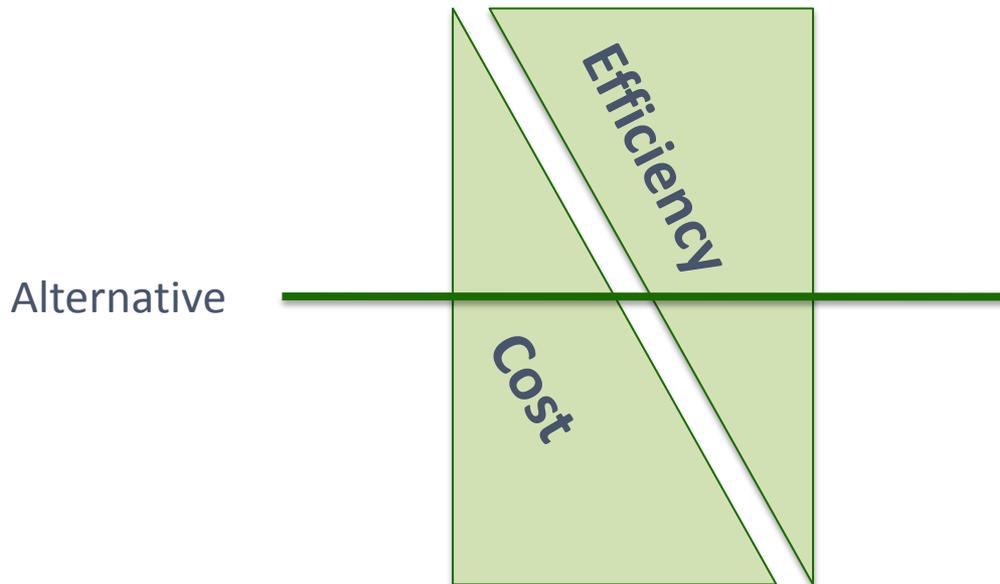
% DM in several medium





Protein production

- Industrial medium + Lip culture
- Technics used: Filtration with/without sonication, decantation with/without sonication





[Proteins], [Cells] and [Spores]

Medium	1D	2D	1F	2F
DO 595 nm	0.19	0.336	0.595	0.427
	0.446	0.22	0.531	0.476
	0.644	0.206	0.603	0.512
mean	0.427	0.254	0.576	0.472
[ctx] (mg protein BSA/ml)	0.731	0.428	0.993	0.810

1D : sonication + decantation
2D : decantation

1F : sonication + filtration
2F : filtration

- Larger production of crystalline protein from filtration with sonication.





[Cells] and [Spores]

	1D	2D	1F	2F
Nombre des cellules végétatives et spores (dilution 10⁻⁵)	83	90.333	109.667	85.667
[Flore total] (UFC/ml)	8.30E+0 8	9.03E+0 8	1.10E+0 9	8.57E+0 8

1D : sonication + decantation
2D : decantation

	1D	2D	1F	2F
Nombre des spores (dilution 10⁻⁵)	81	61.667	96.667	82.333
[spores] (Spores/ml)	8.10E+08	6.17E+0 8	9.67E+0 8	8.23E+0 8

1F : sonication + filtration
2F : filtration

- The population is suspended, no effect of sonication.



Conclusion

- ❑ Wheat bran, a low-cost substrate, can be used for the production of biopesticides.
- ❑ In terms of mass balance:
 - Sonication has no effect on the distribution of the basic components of the wheat bran medium.
 - The heat treatment increases the solubilization of the substrate. Higher protein concentration following centrifugation.
- ❑ The highest yield of δ -endotoxins requires the use of the most expensive production techniques.





THANK YOU!

DO YOU HAVE ANY QUESTIONS?



<http://www.ipm-4-citrus.insa-toulouse.fr/>

