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Study of nutritional limitations of wheat bran (WB) based culture medium for scaling up *BTK* based biopesticide productions

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Scientific context

1.1. IPM-4-Citrus (MSCA RISE, No. 734921, 2017-2023)

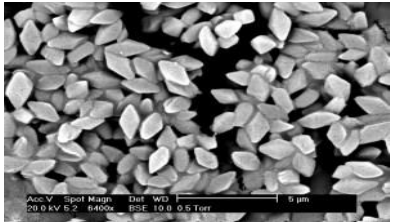
HORIZON 2020 FUNDED

Marie Slodowska Curie Action

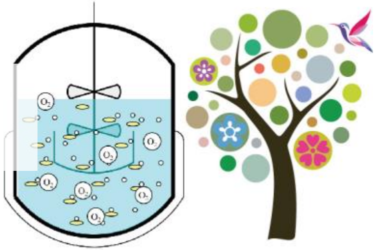
Research & Innovation Staff Exchange

INTEGRATED PEST MANAGEMENT

- ✓ **Understanding & sensitising stakeholders** about the health risks related to citrus pests
- ✓ **Developing an alternative IPM approach**
- ✓ based on biological control



IPM-4-CITRUS



CITRUS

11 PARTNERS

6 COUNTRIES

4 YEARS DURATION

TARGETED PEST:

insect larvae : Phyllocnistis citrella & Prays citri

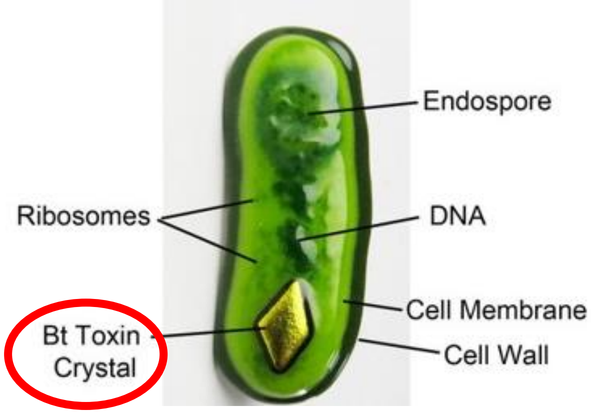
This project has received funding from the European Union's Horizon 2020 Research and Innovation program under Grant Agreement No 734921.

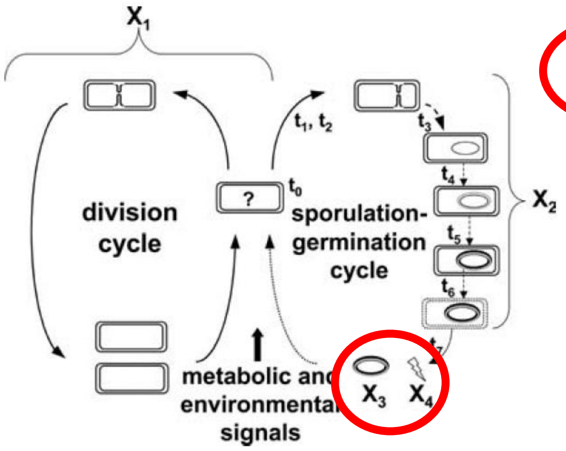
1.2. *Bacillus thuringiensis* kurstaki (Btk)

Bacillus thuringiensis kurstaki
(LIP, HD1, BLB1)

- **Bacilli**
- **Gram +**
- **Facultative aeroby**
- **Sporulant**
- **Endotoxin producer**

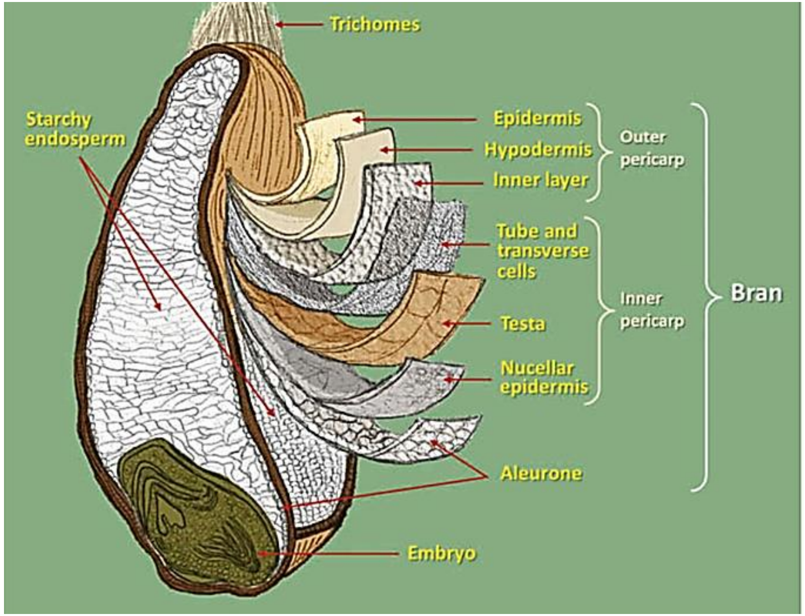
Bacillus thuringiensis (Bt) life cycle and endotoxins production (https://www.bpprc.org/)





(M. Sarrafzadehet al 2005)

1.3. Wheat bran (WB) structure & chemical composition



Wheat Bran Kernel structure

(Balandran et al, 2015)

Mean composition of Wheat Bran (WB)

(Hell et al., 2016, Sapirstein et al, 2016, Stoffel et al., 2019)

Component		**Content in WB (%w/w)**
Polysaccharides	**Reserve sugars** **- Oligosaccharides** **- Starch**	15-45 3,7 13-40
	Fibres (structure) **- Cellulose** **- Hemicellulose** **- Lignin**	35,7-62,3 6,5-11 20,8-33 9,8-16
Proteins		13,2-21,1
Ashes/Minerals		2,2-8
Water		9-12

1.4. Background

WB: good medium for Bt crystals production (Devi et al, 2005)

→

Effective low cost Btk crystal production in cheap WB based medium compared to semi synthetic medium (Mounsef et al, 2014)

→

Sieving of WB into 4 classes : class 1 (>850 µm), class 2 (500-850 µm), class 3 (250-500 µm) & class 4 (<250 µm).
Optimal Btk growth & crystal production in class 2, ([WB]=73.6 g/L), 248 rpm (Abboud et al, 2017)

1.4. Main Scientific Questions

1. Does the substrate elemental composition differ between one class and another?

2. Do the mass balance and the elemental composition inform us about the progress of the culture, the limiting nutrients and fermentable fraction?

3. In terms of scale-up approach, are the limiting components in bioreactor culture, the same as in flask culture?

4. Can the elemental composition help us interpreting the 3 phases of culture & biokinetics in bioreactor?

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Materials & Methods

2. Materials & Methods

Substrate

Raw WB
Class 1 (>850 µm):<1%
Class 2 (500-850 µm):19.8%
Class 3 (250-500 µm):51.4%
Class 4 (<250 µm) :28.2%

Suspension

HT without culture: Flask, Bioreactor
HT+ culture:
Flask (24h, 30°C, [WB]= 73.6 g/L, 250 rpm)
Bioreactor (48h, 30°C, [WB]= 73.6 g/L, pH7.5, pO_2> 25%)

Sample

Insoluble fraction (substrate)

Permeate (cells, spores, endotoxins)

Bioperformances	Chemical analysis
CFU (not shown) Spores (not shown) Endotoxins	Dry matter (gravimetry), Ashes (gravimetry) Starch: Colorimetry (I_2/KI), Nitrogen (Kjeldahl) CHONS (Combustion Flash + GC)

Determination of <u>mass balance</u> (soluble/insoluble fraction),
Studying the <u>elemental composition</u> of WB before and after culture

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Results & Discussion

3.1. Endotoxins production in WB vs Semi-synthetic medium (SSM)

Btk endotoxins yield in mg/mL

	Culture in bioreactor		Culture in flasks		
Btk Strains	SSM Sarrafzadeh et al, 2005	WB Raw Rahbani et al, 2015	WB Class 2	WB Class 3	WB Class 4
HD1	0,7	0,43	/	/	/
BLB1	0,73	0,63	/	/	/
Lip	0,19	0,43	0,549 ±0,117	0,547 ±0,071	0,432 ±0,062

- *Btk* Lip: **Higher yield** in WB vs SSM
- *Btk* Lip bioperformances ↑ on class 2 & 3

3.2. Raw and sieved WB composition

			Mass	Water content	Starch	Protein Kjeldahl	Elemental Composition [%w/w]					
Class	**Size (µm)**	**D [4,3] (µm)**	**[%w/w]**	**[g water /gdm]**	**[g/gdm]**	**[g eq.protein /gdm]**	**C**	**H**	**O**	**N**	**S**	**Ash**
Raw		598,5	100	0,119 ±0,07	0,207 ±0,008	0,138 ±0,012	44,79 ±0,44	6,74 ±0,20	36,53 ±0,37	2,50 ±0,24	0,00	4,43 ±0,12
2	500-850	865,4	19,80	0,116 ±0,04	0,173 ±0,35	0,145 ±0,012	44,21 ±0,19	6,50 ±0,12	35,62 ±0,45	2,60 ±0,23	0,00	3,70 ±0,12
3	250-500	531,1	51,41	0,116 ±0,04	0,144 ±1,35	0,127 ±0,014	45,34 ±0,08	6,46 ±0,04	37,89 ±0,44	2,48 ±0,04	0,00	4,28 ±0,12
4	<250	275,3	28,20	0,116 ±0,04	0,347 ±0,47	0,155 ±0,016	42,40 ±0,04	6,61 ±0,12	34,60 ±0,37	2,61 ±0,24	0,00	3,23 ±0,12

- WB granulometry ➔ ∅ effect on elemental composition
- WB molar formula (mol/C_mol): $CH_{1,74}O_{0,53}N_{0,04}$
- WB massic formula (g/g_C): $CH_{0,14}O_{0,71}N_{0,05}Ash_{0,1}$
- Class 4: highest starch [ ]

3.3. *Btk* elemental composition

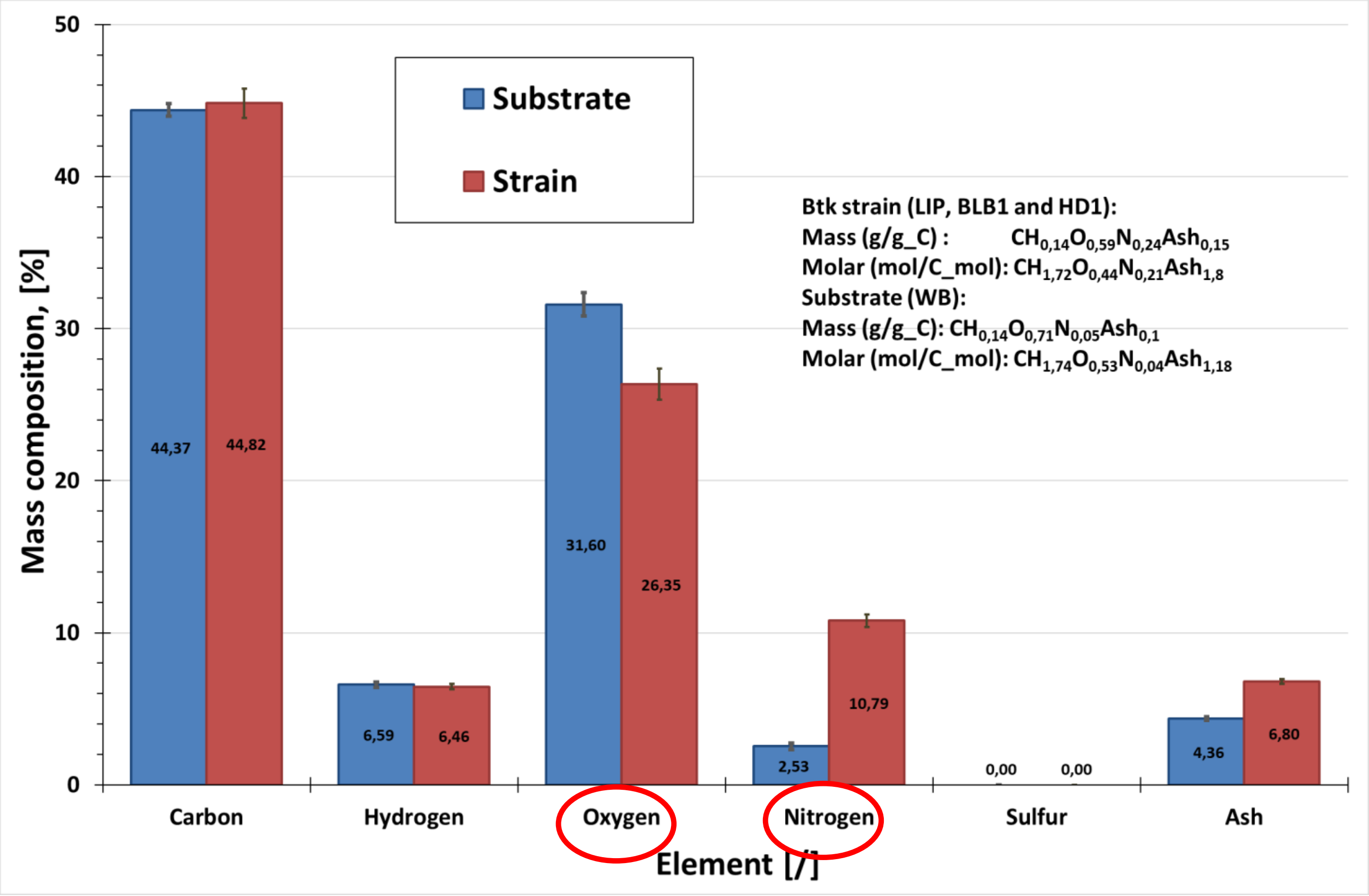
Btk Strains	C (%w/w)	H (%w/w)	O (%w/w)	N (%w/w)	S (%w/w)	Ashes (%w/w)
Lip	43,37	6,22	24,84	10,48	0, 00	7
BLB1	45,7 ±0,067	6,61 ±0,09	26,89 ±0,87	10,51 ±0,011	0, 00	6,7
HD1	44,65 ±0,3	6,42 ±0,072	26,56 ±0,68	11,23 ±0,068	0, 00	NA
Mean value	44,82 ±0,98	6,46 ±0,17	26,35 ±1	10,79 ±0,4	0, 00	6,8 ±0,17

- *Btk* strains: identical elemental composition
- **Molar formula of the strain (mol/C_mol):** $CH_{1,72}O_{0,44}N_{0,21}$
- **Massic formula of the strain (g/g_C):** $CH_{0,14}O_{0,59}N_{0,24}Ash_{0,15}$

Strain	**Elements (%m/m)**					**Chemical formula**	**Molar Mass**
	Ashes	C	H	O	N	(Cmol)	(g/mol)
Bt spp. kurstaki (MSc)	6,80	44,82	6,46	26,35	10,79	$CH_{1.72}O_{0.44}N_{0.21}$	25,52
Lactobacillus helveticus	9,03	47,54	6,25	24,39	12,79	$CH_{1,58}O_{0,39}N_{0,23}$	25,26
Flavobacterium Dehydrogenans	13,5	45,16	6,15	24,29	10,87	$CH_{1,63}O_{0,40}N_{0,21}$	26,60
Escherichia coli	11,3	47,83	6,95	21,65	12,3	$CH_{1,74}O_{0,34}N_{0,22}$	25,11
Bacillus cereus	9,98	46,05	5,73	26,26	11,98	$CH_{1,49}O_{0,43}N_{0,22}$	26,08

- *Btk*: elemental composition close to *Bacillus cereus* *(Popovic et al, 2019)*

3.4. *Btk* vs WB, Elemental composition

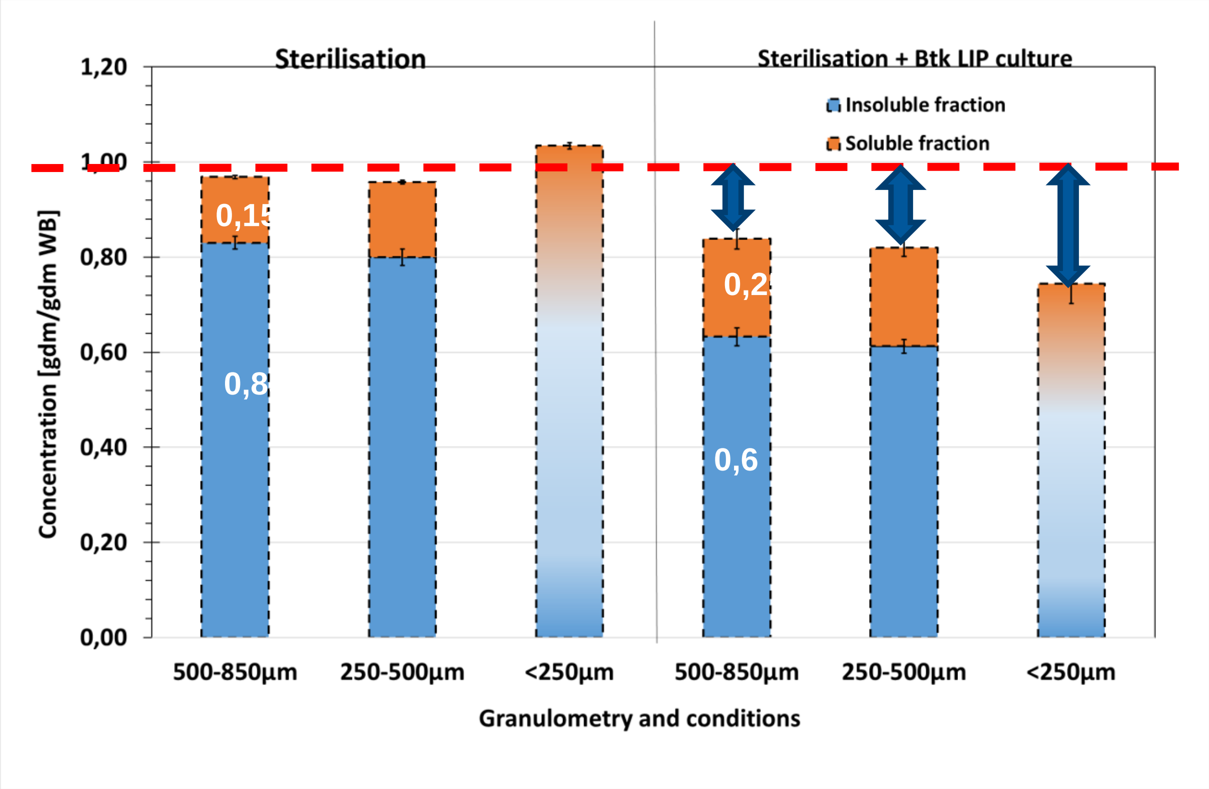


- Strains are more rich in Nitrogen and less in Oxygen than WB
- WB is the only source of Nitrogen during *Btk* culture

3.5.What is the fermentable fraction over classes?

Substrat + Oxygène + Azote + Oligo + … ➔ Biomasse + CO2 + produits (Spore, endotoxine) + eau + énergie

$1/6\ C_6H_{12}O_6 + \alpha\ O_2 + \beta\ NH_3$ + Sels +… ➔ $Y_{sx}\ X\ (CH_{1,8}O_{0,3}N_{0,2}) + \delta\ CO_2 + \lambda\ H_2O + \varepsilon$ Spores + ξ Endotoxine + Energie…



- ➚ fermentable fraction when granulometry➘
- If Yx/s = 0.5 fermentable fraction ~ Starch

➔ Class 2 : 32,3%

➔ Class 3 : 36,1%

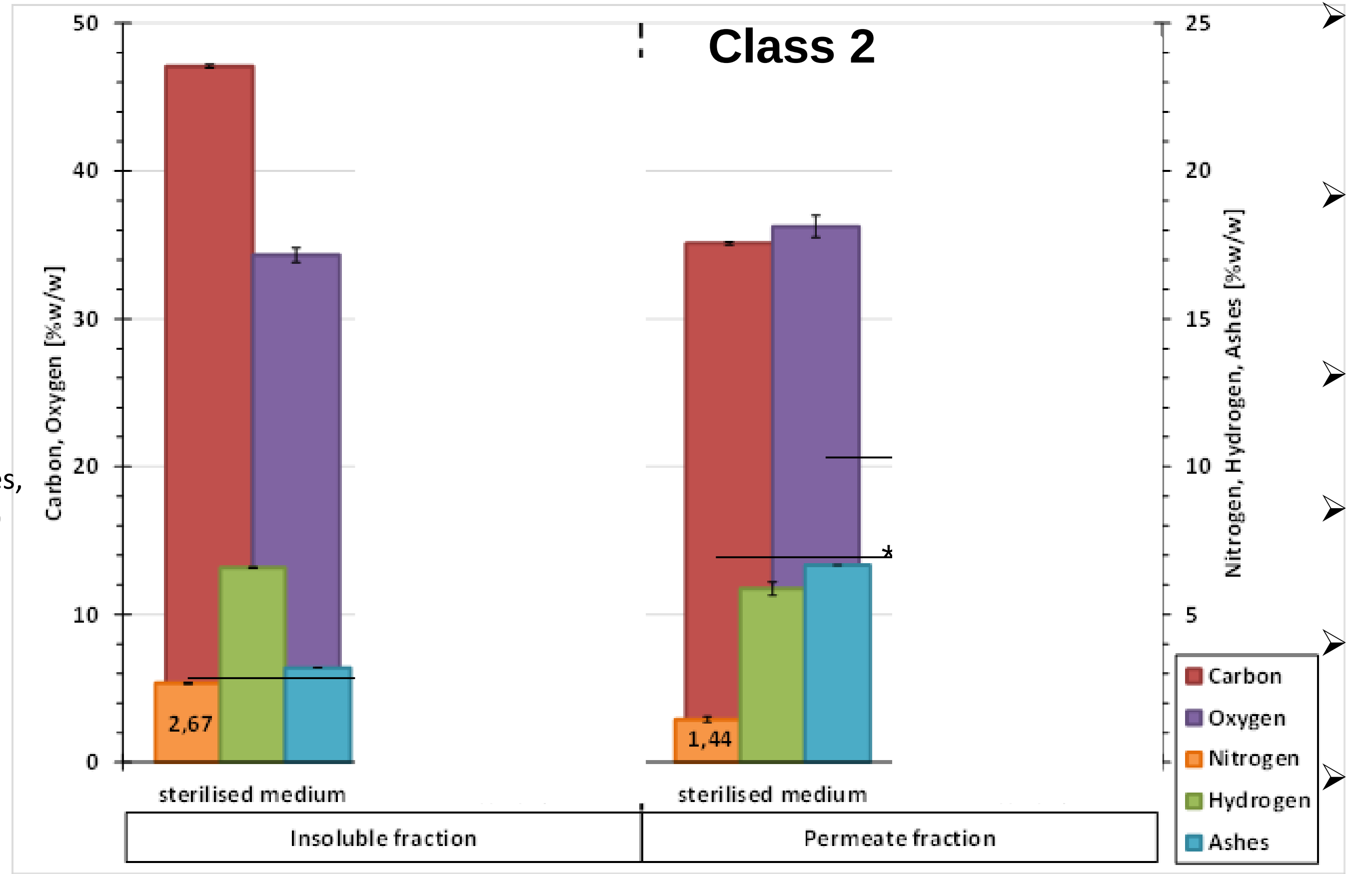
➔ Class 4 : 51,1%

- Partial consumption of lignocellulosic fraction ([Starch]= 0.173 gdm/gdmWB)

3.6. How does the medium elemental composition evolve?

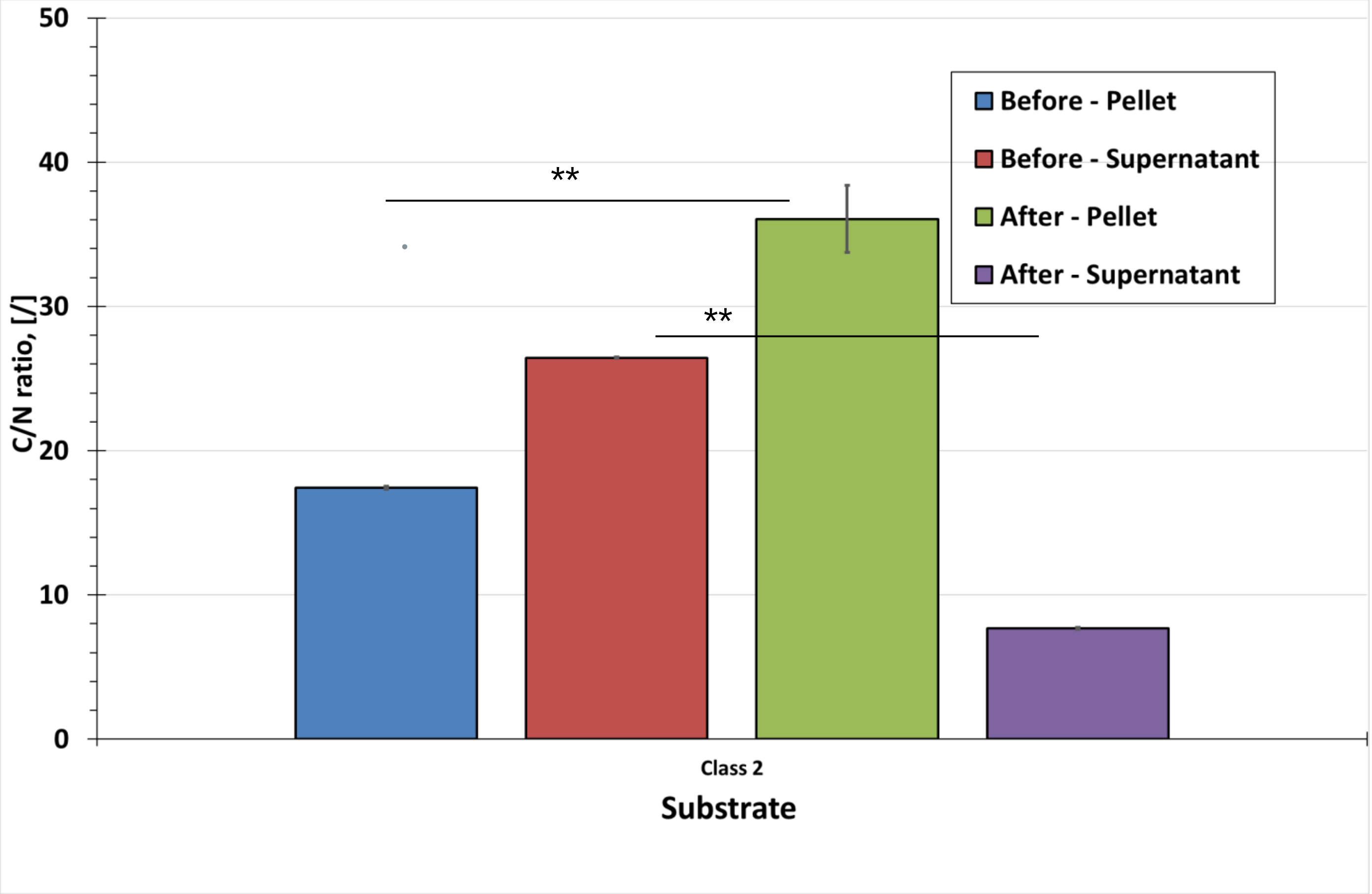
Insoluble fraction (substrate)

Permeate (cells, spores, endotoxins)



- Permeate: Biomass production
- Insoluble fraction: substrate proteins consumption
- N: limiting nutrient
- N disponible ~ 48% N accessible
- Class 3: same behaviour
- Class 4: No separation pellet/supernatant

3.7. C/N ratio: indicator of the process evolution in flask?

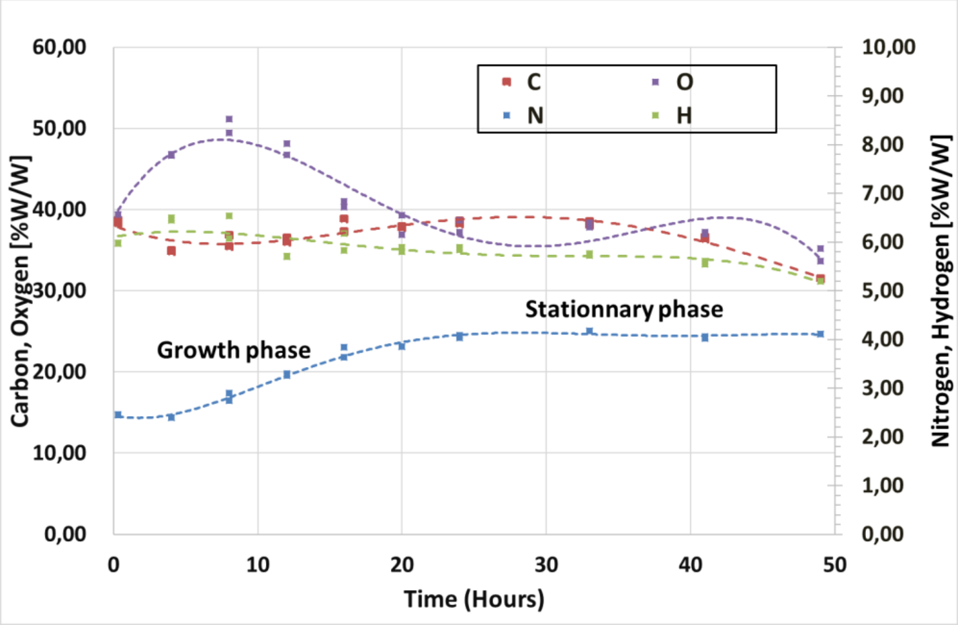


- C/N is a reliable indicator to interpret the process evolution & phases of culture

3.8. Monitoring of culture biokinetics through elemental composition analysis in bioreactor

Permeate

Insoluble fraction



- Same behaviour between flask and bioreactor.
- Biokinetics: 0-12h: growth phase, 20- 48h: stationnary phase followed by sporulation phase

4

Conclusion & Perspectives

4.1. Highlights & perspectives

- WB elemental composition: no evolution between classes
- Fermentable fraction: starch & lignocellulosic fraction
- N: culture limiting nutrient
- Residual N: Physically non accessible
- C/N ratio: reliable indicator of culture phases & bioperformances (flask & bioreactor scale)

Determination of chemical limitations → optimisation of biopesticide production in a low cost WB medium at large scale

4.1. Highlights & perspectives

Perspectives:

- **Study WB physical characteristics (morpho-granulometry, rheometry, settling speed)**
- **Define an appropriate mathematical model in order to better describe bioperfmances & optimise endotoxin production**

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Thank you for your attention

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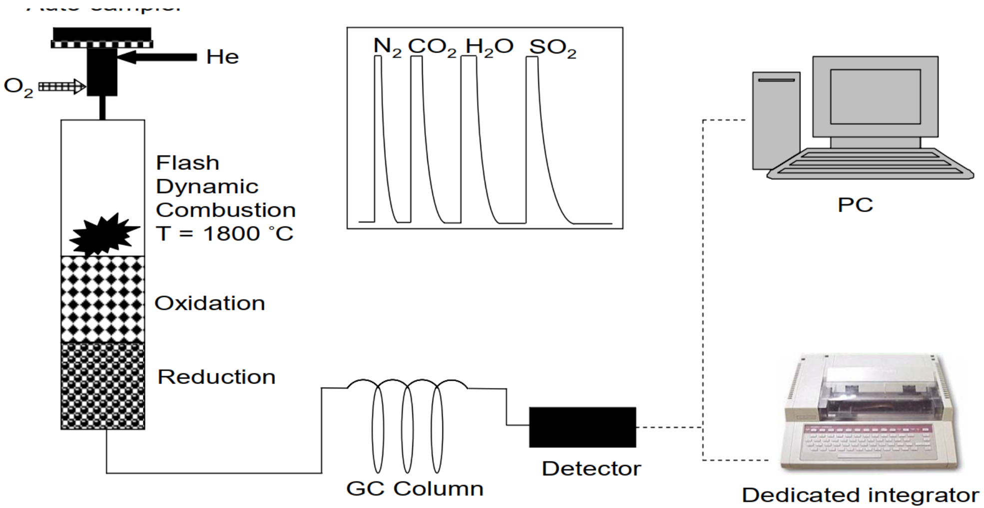
Back-up

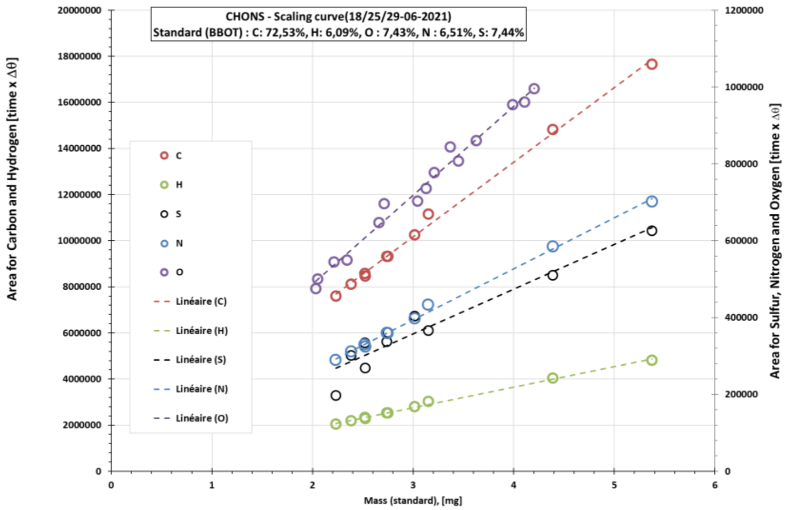
5. CHONS analysis method

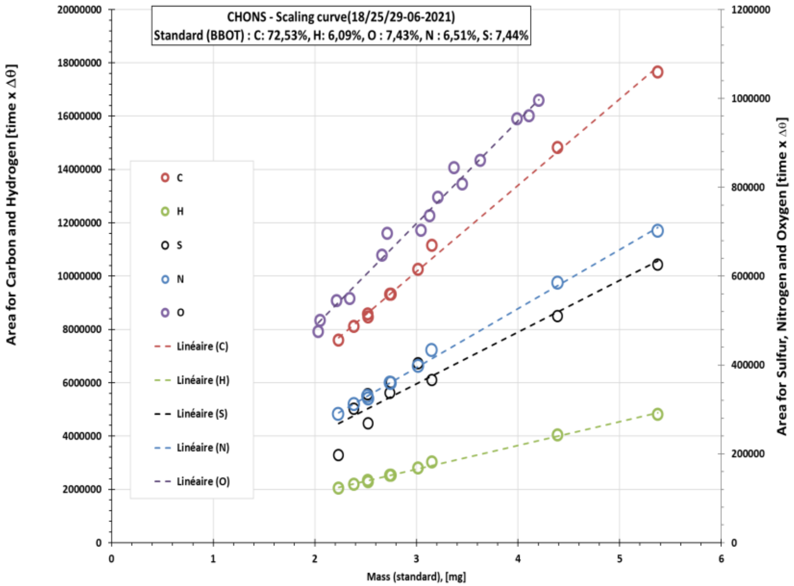
Flash smart 2000,thermofischer scientific



CHONS analysis mechanism

5. CHONS calibration curves





8. DM and ashes analysis method

6

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6.References

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