



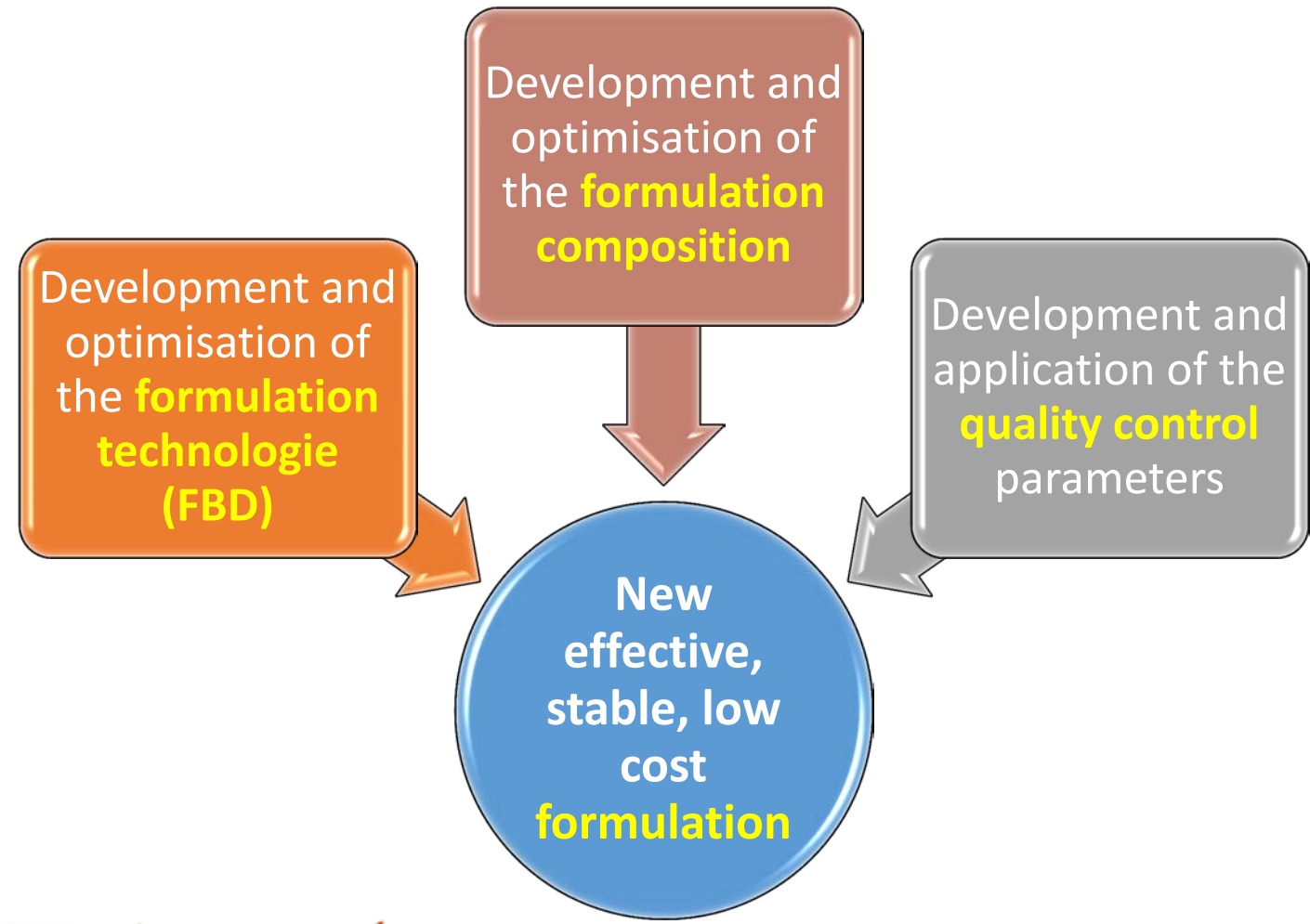
# Development of Biopesticide Formulations based on spores and crystals produced from two new *Bacillus thuringiensis* strains to control Citrus Pests

WP3: Activity Optimization

Presented by :  
Tayssir, AL HAMMAR (MEDIS)  
Stephanie, AL KHAWAND (USJ)



# Introduction

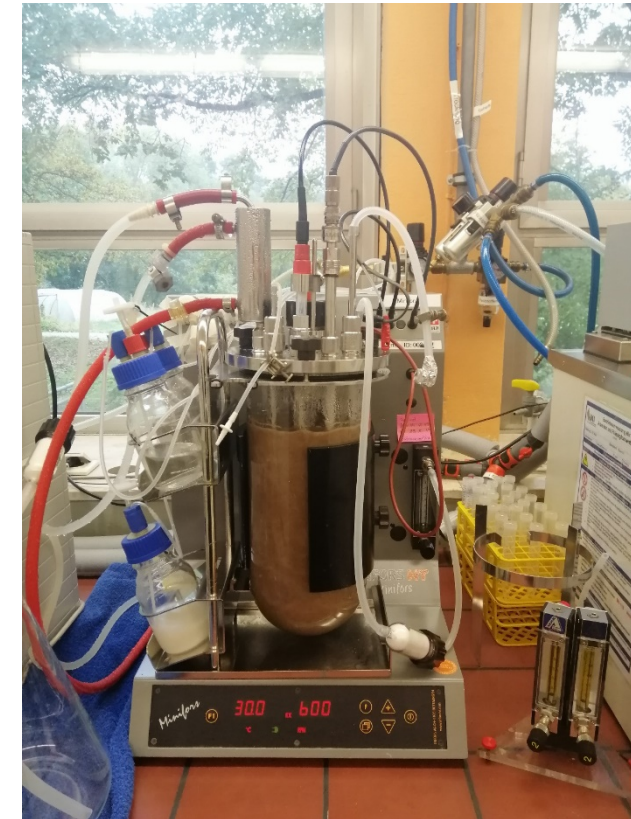


# Material and Methods

## 1. Fermentation:

The production of *Bt* spores and crystals is conducted following the optimized conditions.

*Conducted by our colleagues in Toulouse, at TWB, within the WP2*



5L Fermenter

## 2. Downstream Processing:



**Decantation**  
10 minutes



**Filtration**  
mesh of 106  $\mu\text{m}$



**Centrifugation**  
15 minutes at 13 000 rpm

# Material and Methods

## 3. Formulation:

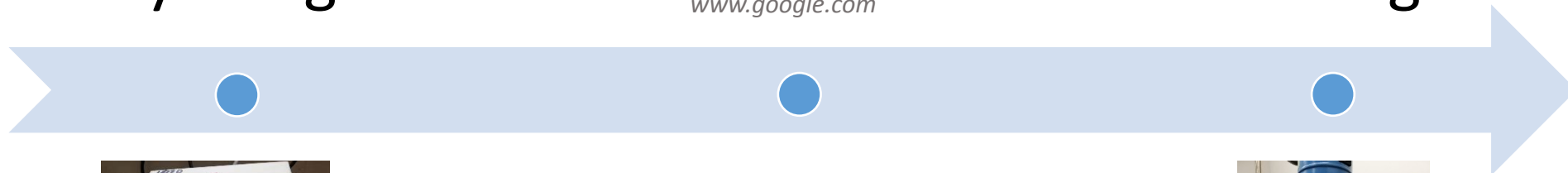
a. Preparation of the suspension:

Dry Weight



[www.google.com](http://www.google.com)

Mixing



Weighing





# Material and Methods

## 3. Formulation:

### b. Drying Process

- Equipment preparation and adding the carrier
- Set the pressure, air flow, and the inlet & outlet temperature
- Pump in the suspension
- Drying

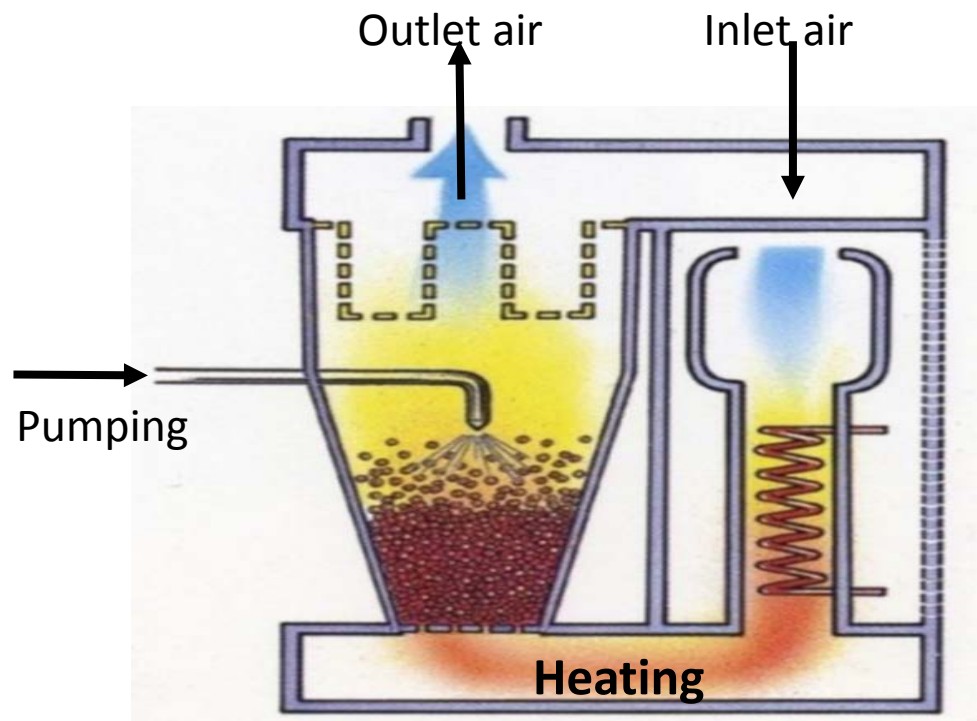


*Aeromatic-Fielder STREA-1*

# Material and Methods

## 3. Formulation:

### b. Drying Process



User Manual STREA-1

Process	Parameters to optimize	Experiment 1	Comments
Charging	Quantity of the Carrier (g)	81,9	
	Volume of Bt suspension (ml)	100	
	Quantity of the Active ingredient in the suspension (g)	7	
	Quantity of other component in the liquid suspension (g)	11,1	
	Wire mesh	200 mesh, 75 $\mu$ m	
Fluidization	Air stream ( $m^3/h$ )	110	Maintain the airstream constant during fluidization to stabilize the FBD
	Atomizing Pressure (Bar)	2	
	Blow up pressure (Bar)	6	
Spraying	Nozzle tip diameter (mm)	0,11	High spray effect
	Air stream ( $m^3/h$ )	110	8 on the ventilation scale
	Pump flow rate (ml/min)	1,11	the hose diameter=4 mm/ the hose length= 1 m
	Atomizing Pressure (Bar)	2	
	Blow up pressure (Bar)	6	
	Inlet temperature ( $^{\circ}C$ )	70	
	Outlet temperature ( $^{\circ}C$ )	45-55 (+/- 2 $^{\circ}C$ )	
Drying	Spraying time (min)	90	
	Air stream ( $m^3/h$ )	150	11 on the ventilation scale
	Atomizing Pressure (Bar)	2	
	Blow up pressure (Bar)	6	
	Inlet temperature ( $^{\circ}C$ )	70	
	Outlet temperature ( $^{\circ}C$ )	45-55 (+/- 2 $^{\circ}C$ )	
	drying time (min)	10	

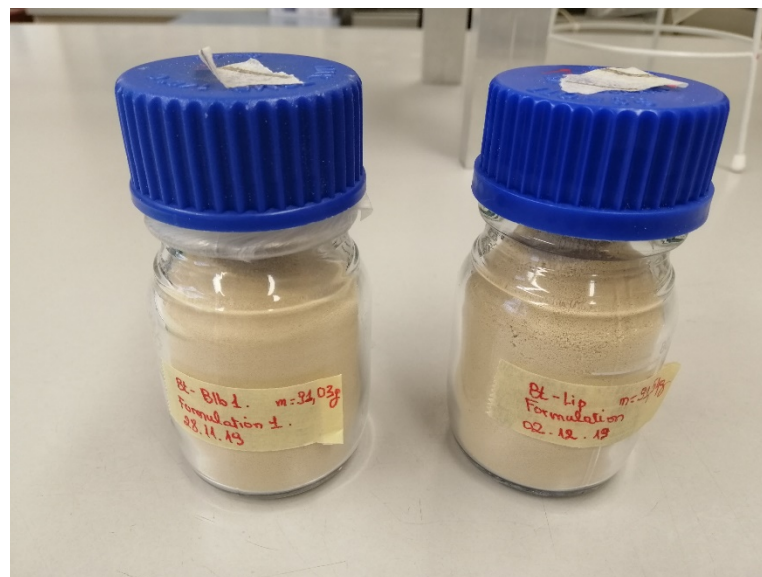
Table 1: Optimised drying process conditions.



# Material and Methods

## 3. Formulation:

### c. Powder Harvesting



*Blb1 and Lip Formulation*



# Material and Methods



## 4. Quality Control:

### 4.1 Proteins content determination, following the Bradford protocol

4.2 UV-test, using Bt-Cry1Ab/1Ac ELISA Kit

4.3 Suspendability

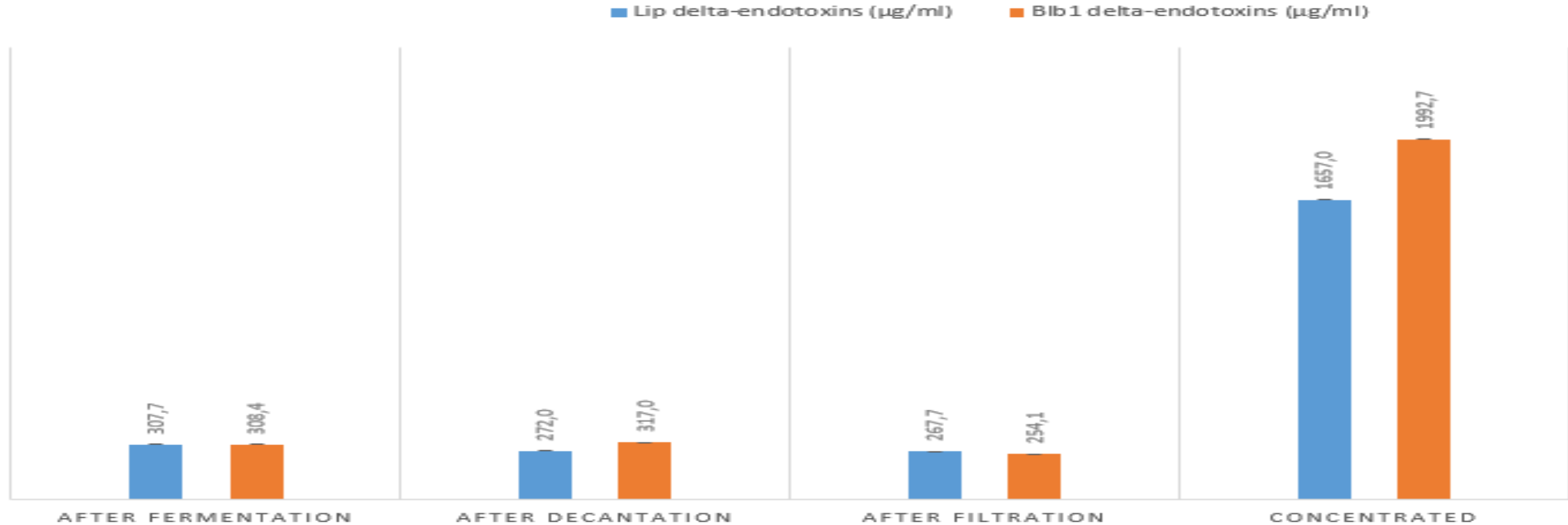
4.4 Wettability

4.5 Moisture content

4.6 Bioassay for the non-formulated filtrate

# Results and Discussion

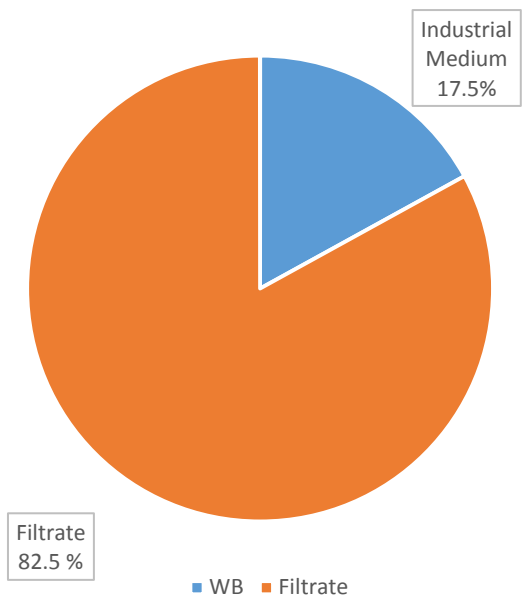
## 4.1 Proteins Content from fermentation to concentration



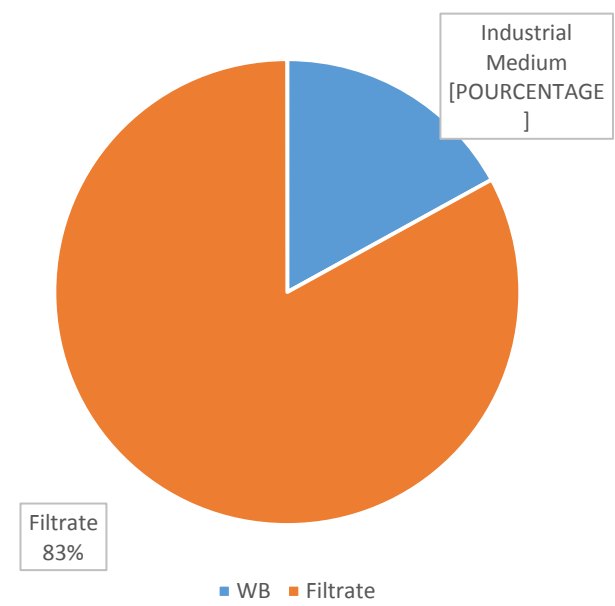


# Results and Discussion

## 4.1 Protein content (%) after filtration



*Bt - blb1*



*Bt - lip*

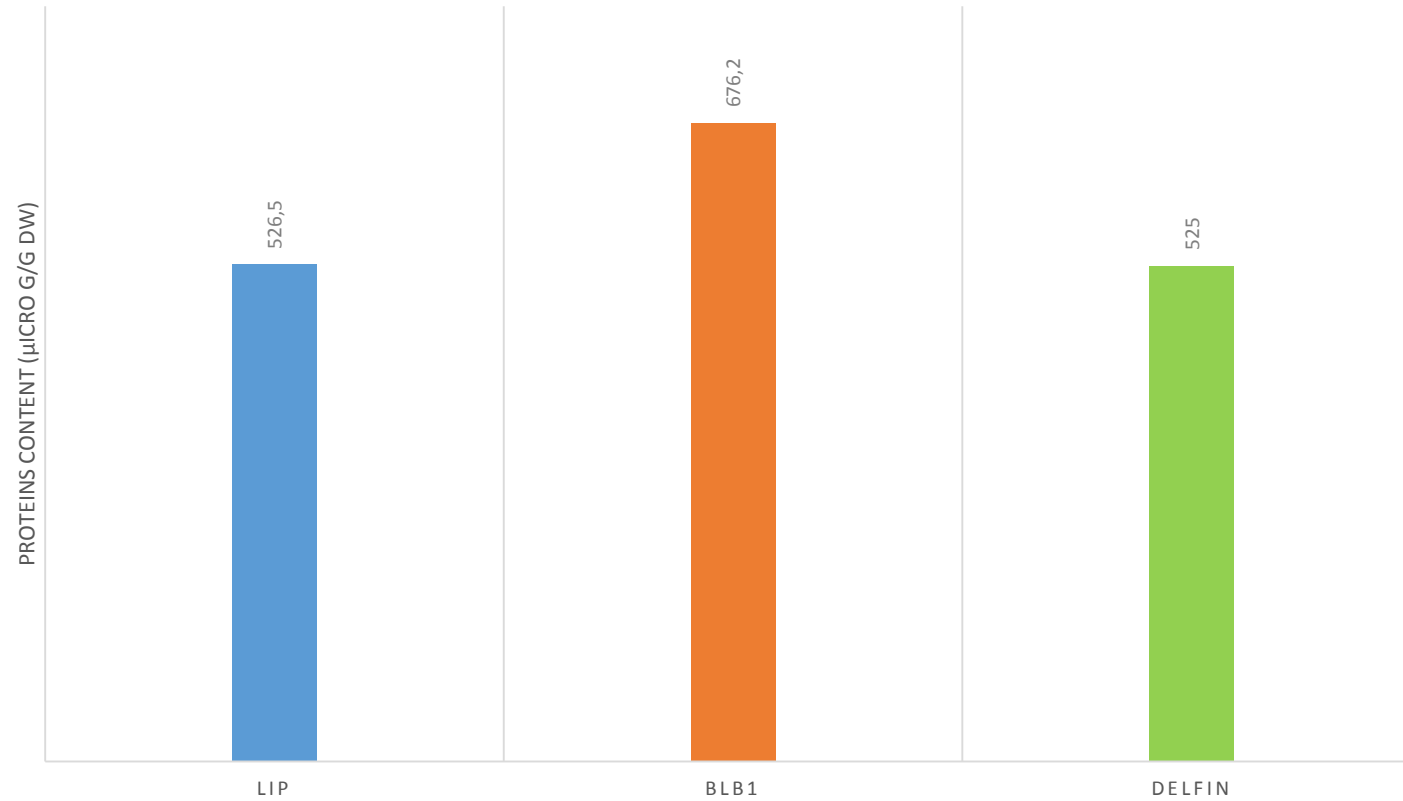
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 734921.



# Results and Discussion



## c. Proteins Content after Formulation





# Material and Methods



## 4. Quality Control:

4.1 Proteins content determination, following the Bradford protocol

**4.2 UV-test, based on direct double antibody ELISA technique**

4.3 Suspendability

4.4 Wettability

4.5 Moisture content

4.6 Bioassay for the non-formulated filtrate

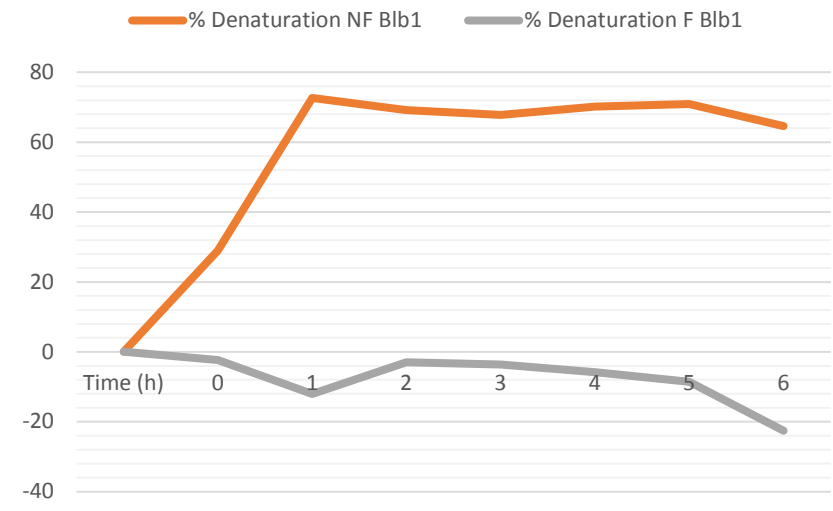


# Results and Discussion

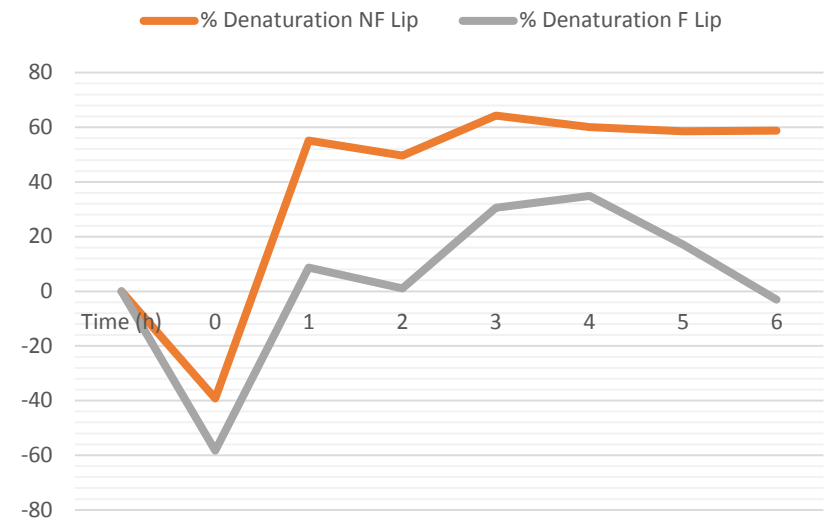


## 4.2 UV-test, using Bt-Cry1Ab/1Ac ELISA Kit

UV effect on Non Formulated (NF) and Formulated (F) *Bt blb1*



UV effect on Non Formulated (NF) and Formulated *Bt Lip*





# Material and Methods



## 4. Quality Control:

4.1 Proteins content determination, following the Bradford protocol

4.2 UV-test, using Bt-Cry1Ab/1Ac ELISA Kit

**4.3 Suspendability**

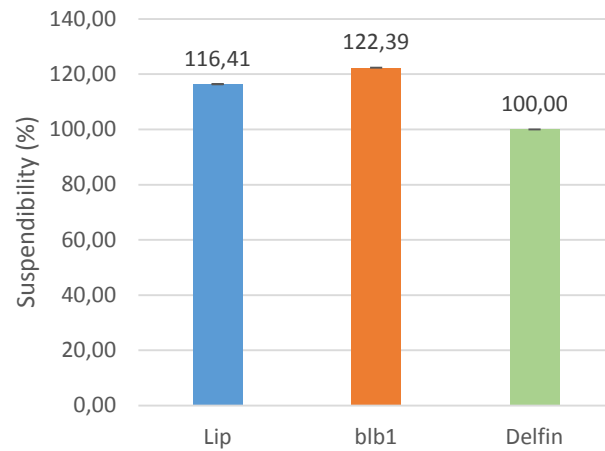
**4.4 Wettability**

**4.5 Moisture content**

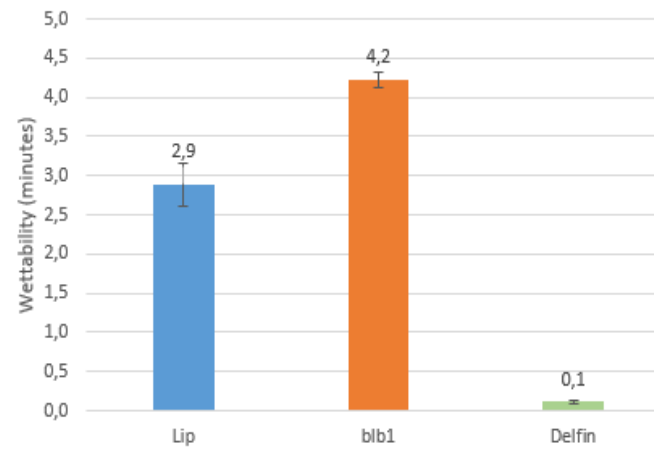
4.6 Bioassay for the non-formulated filtrate

# Results and Discussion

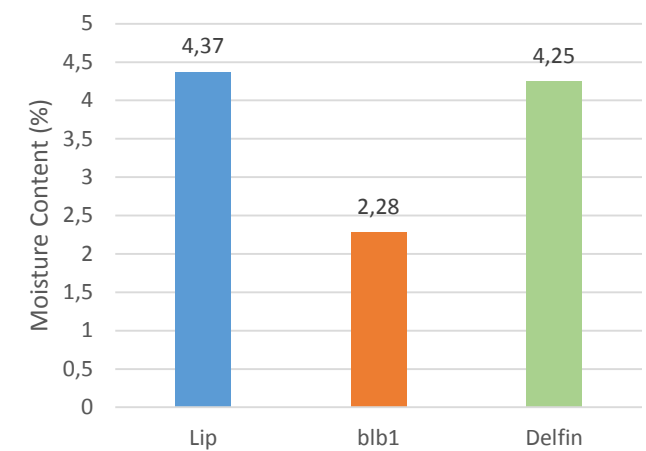
## 4.3 Suspendability



## 4.4 Wettability



## 4.5 Moisture Content

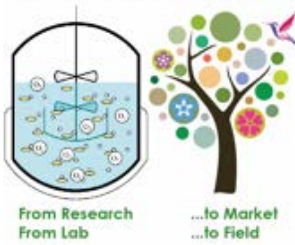


# Material and Methods

## 4.6 Bioassay for the non-formulated

- Proteins Concentration Range: from 1.6 to 55.2  $\mu\text{g}/\text{cm}^2$
- Temperature: 25°C
- Humidity: 30%  $\pm$  2%
- *Spodoptera frugiperda*: 3<sup>rd</sup> instar larvae





# Results and Discussion

## 4.6 Bioassay for non formulated Bt Blb1 and Lip

	<b>Blb1</b>	<b>Lip</b>
LC50	6.647 $\mu\text{g}/\text{cm}^2$	9.473 $\mu\text{g}/\text{cm}^2$
LC90	26.397 $\mu\text{g}/\text{cm}^2$	76.601 $\mu\text{g}/\text{cm}^2$
P-value	0.0006	0.007
Confidence Interval (95%)	[1.403 ; 2.870]	[0.575 ; 2.245]

	<b>Blb1</b>	<b>Lip</b>
LT50	2.3 days	4.5 days
P-value	0.068	0,119
Confidence Interval (95%)	[-0.107 ; 1.628]	[-0.123 ; 0.648]



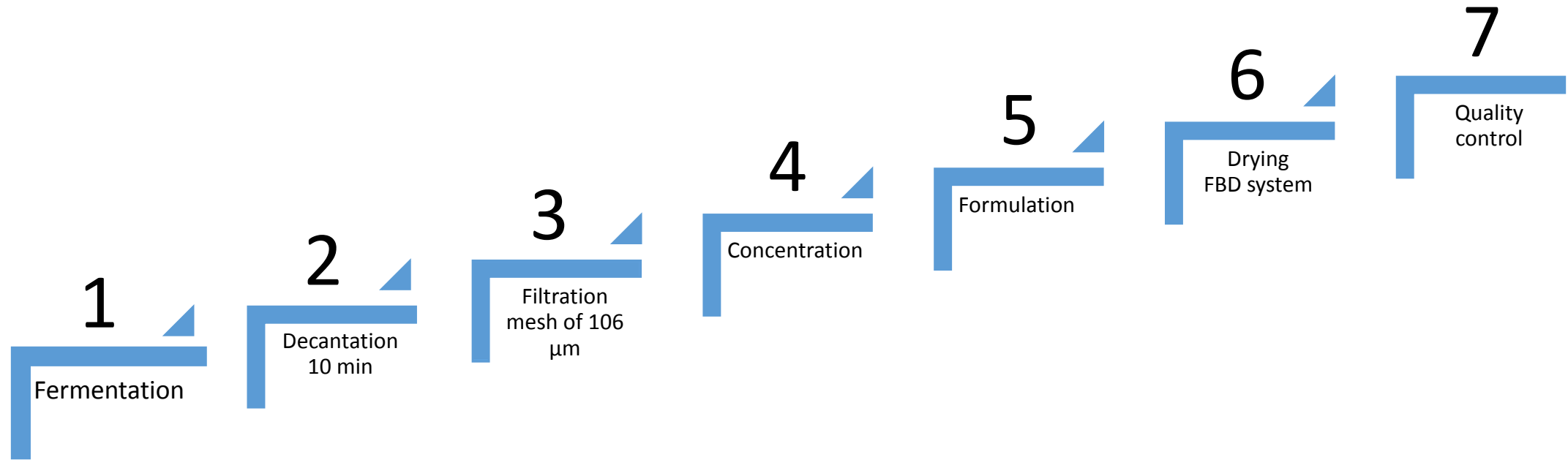
# Perspective



- ELISA test for the detection of UV effect should be optimized
- Wettability should be optimized (increasing the proportion of the wetter in the formulation/ Changing the wetter)
- Valorization of the industrial medium after downstream processing
- Next step: field trials...



# Recapitulation and Conclusion





Thank you!