Soft-sensors for monitoring *B. thuringiensis* bioproduction

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OUTLINE

1. Bioproduction of *B. thuringiensis*
2. Soft-sensors
3. Results
4. Conclusions

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HORIZON 2020 FUNDED

Marie Slodowska Curie Action

Research & Innovation Staff Exchange

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From Research
From Lab

...to Market
...to Field

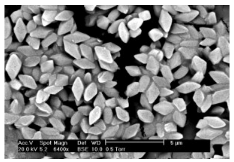
11 PARTNERS

6 COUNTRIES

4 YEARS DURATION

INTEGRATED PEST MANAGEMENT

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



STRAIN USED:
Bacillus thuringiensis kurstaki BLB1 and LIP

Citrus

TARGETED PEST:
insect larvae
Phyllocnistis citrella & Prays citri

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Interdisciplinary

Intersectoral

International

GOALS :

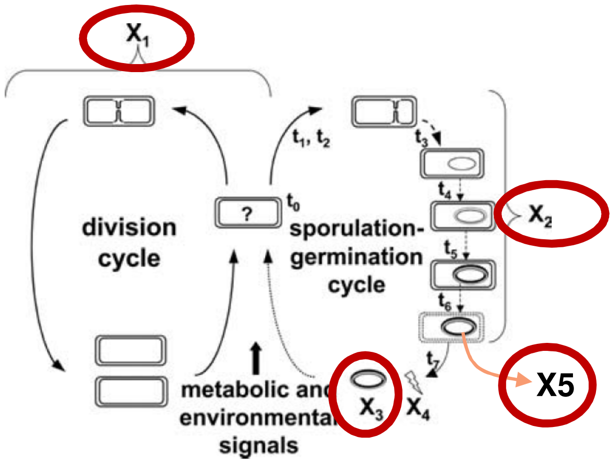
- Strengthening Academia & Industrial collaborations
- Optimising bioproduction processes
- Developing new biopesticides in the Mediterranean region

HOW :

- Feasibility study for future spin-off activities and new production lines,
- Benchmarking the opportunities & obstacles related to bringing innovative ideas to the market.

Research and innovation programme under Grant Agreement N° 734921

Physiological states in a spore-forming bacterium (application to Bt kurstaki)

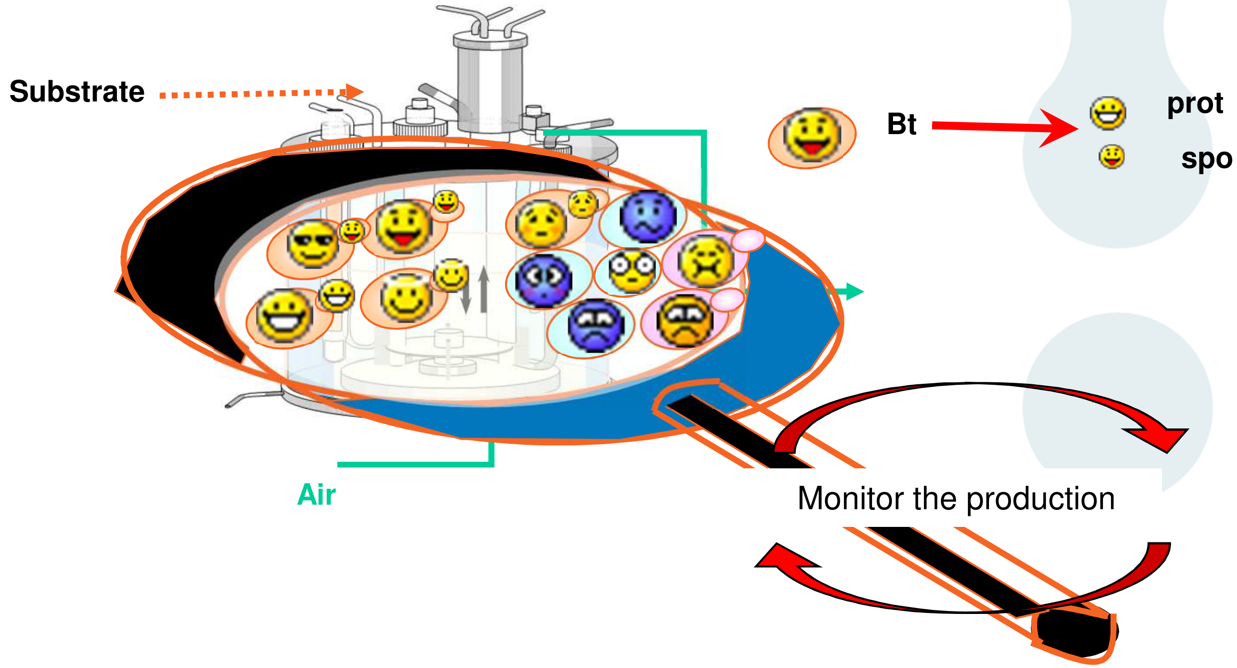


X1, X2, X3 and X4: cell quantification [cell],[DM] morphology

X5 : δ-endotoxin quantification [Crystal], [Protein] crystal morphology

Bioprocess Model Challenges

Scale-up of the production



Monitoring Bioproduction

Process

Evaluation of performance

Indicator of the state of the process
(Monitoring)

Fermentations

Commercial sensors

On-line

- pH
- Temperature
- Flow rate
- Gas composition
- Pressure

Off-line

- HPLC (Glucose)
- GC (Acids)
- Biomasse

DRAWBACKS
- Expensive
- Long time to have results

Software sensors

Software based monitoring systems

Measured Variables → Infrequently Measured Variables

Model-driven **Data-driven**

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(Hjersted and Hesnson, 2006; Valentinotti *et al.*, 2003; Riascos and Pinto, 2004)

Data driven soft-sensors

Support Vector Machine

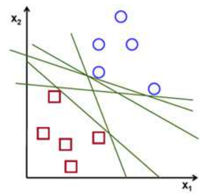
$$y = w^T \varphi(x) + b$$

Objective function

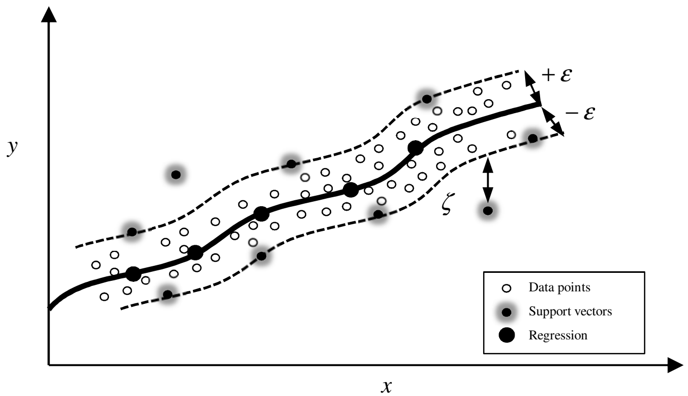
$$\min_{w,\,\xi,\,\xi^*} J = \frac{1}{2}\|w\|^2 + C\sum_{i=i}^{N}(\xi_i + \xi_i^*)$$

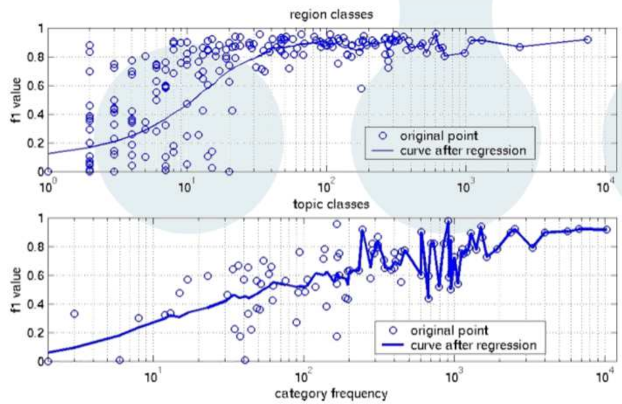
s.t.
$$\begin{cases} d_i \le \varepsilon + \xi_i \\ -d_i \le \varepsilon + \xi_i^* \\ \xi_i,\ \xi_i^* \ge 0 \end{cases}$$

$$d_i = y_i - w^T\varphi(x_i) - b$$



- **Statistical** learning
- **Low** quantity of **parameters** to define
- Good **generalization** of the solution
- **Explicit** knowledge of the **regression function**





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Support Vector Machine

New Objective function

$$\min_{\alpha,\,\alpha^*} W = \frac{1}{2}\sum_{i,j=i}^{N}(\alpha_i - \alpha_i^*).(\alpha_j - \alpha_j^*)\,K(x_i,\ x_j)$$

$$+\varepsilon\sum_{i=i}^{N}(\alpha_i + \alpha_i^*) - \sum_{i=i}^{N}(\alpha_i - \alpha_i^*)y_i$$

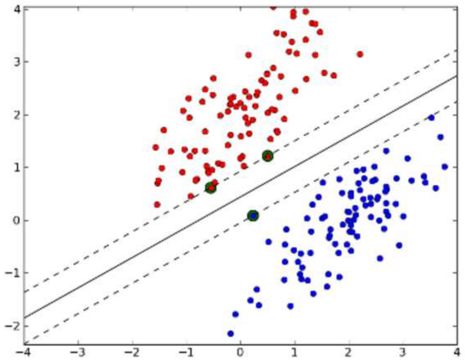
s.t. $$\begin{cases}\sum_{i=i}^{N}(\alpha_i - \alpha_i^*) = 0\\ 0 \le \alpha_i,\ \alpha_i^* \le C\,; \quad i = 1,2,\dots,N\end{cases}$$

Final regression function

$$y = \sum_{i=i}^{N}(\alpha_i - \alpha_i^*)\,\underline{K(x_i,\ x_j)} + b$$

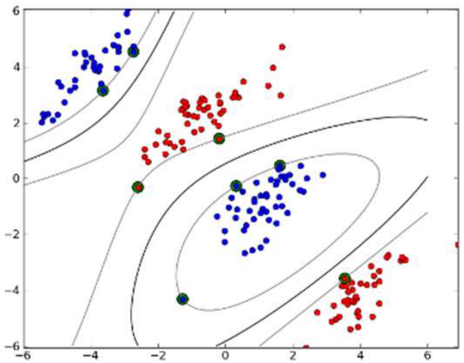
Kernel

Polynomial Kernel



$$K(x_i,\ x_j) = [(x.x_i) + 1]^d$$

Radial Basis Function Gaussian Kernel



$$K(x_i,\ x_j) = exp\left(-\left(\|x - x_i\|^2/2\,\sigma^2\right)\right)$$

The choice of Kernel impacts the results

(Vapnik, 1996; Desai *et al.*, 2006; Jianlin *et al*, 2006)

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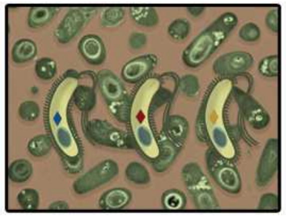
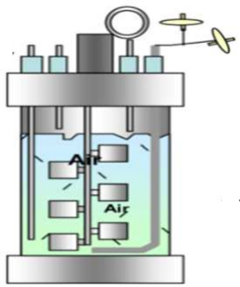
Application of soft sensors for *B. thuringiensis*

B. thuringiensis





Production of Protein

3 species

Measurements

Online
- Agitation
- pO2
- Aeration
- pH

Off-line
- Optical density
- Biomass
- Glucose
- Flora
- Spores
- Protein

The choice of Kernel impacts the results

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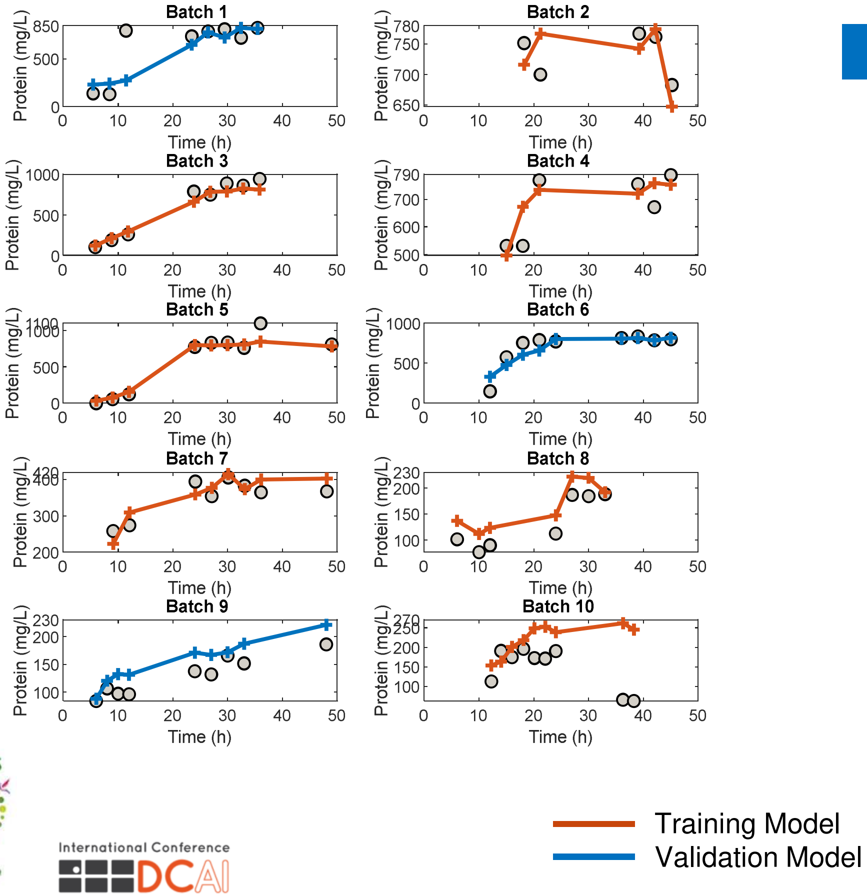
Application of soft sensors for *B. thuringiensis*

- 8 different combinations of variables
- Test of Kernel with SVM

$$RMSE = \sqrt{\frac{1}{N}\sum_{h\in N}\left(\frac{\hat{x_h}(t) - x_h(t)}{x_h(t)}\right)^2}$$

	Model							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	Gaussian Kernel							
RMSEt	189.35	64.96	66.18	57.41	65.18	66.22	61.26	66.42
RMSEv	207.50	174.57	133.62	152.31	129.26	126.65	165.01	156.05
	Quadratic Kernel							
RMSEt	200.74	128.01	103.62	94.75	50.45	53.40	191.29	185.30
RMSEv	194.30	316.80	166.32	175.14	138.05	151.54	228.61	235.21
	Linear Kernel							
RMSEt	218.98	208.41	186.31	175.43	122.89	134.42	145.46	148.65
RMSEv	199.95	184.66	174.75	189.71	218.33	162.90	168.17	172.29
Predictors								
OD	0	0	0	1	1	1	1	0
Bio	0	1	1	1	1	1	0	0
Glc	0	0	1	1	1	1	0	0
pO2	1	1	1	1	1	1	1	1
Agit	0	0	0	0	1	1	1	1
pH	0	0	0	1	1	1	1	1
Aer	0	0	0	0	1	1	1	1
Fl	0	0	0	0	0	1	0	0
Sp	0	0	1	1	1	1	0	0
Log(Fl)	0	0	0	0	0	1	0	0
Log(Sp)	0	1	1	1	1	1	0	0
Strain	1	1	1	1	1	1	1	1

Results



Model 5

- Performance for 3 strains
  - Low RMSE ✓
- Off-line variables ✗
  - OD
  - Biomass
  - Glucose
  - Flora
- On-line variables ✓
  - pO2
  - Agitation
  - pH
  - Aeration
  - Strain type

Difficult implementation

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Results

	Model							
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Agit	0	0	0	0	1	1	1	1
pH	0	0	0	1	1	1	1	1
Aer	0	0	0	0	1	1	1	1
Fl	0	0	0	0	0	1	0	0
Sp	0	0	1	1	1	1	0	0
Log(Fl)	0	0	0	0	0	1	0	0
Log(Sp)	0	1	1	1	1	1	0	0
Strain	1	1	1	1	1	1	1	1

Model 8

- Performance for 3 strains
  - Low RMSE ✓
- On-line variables
  - pO2
  - Agitation ✓
  - pH
  - Aeration
  - Strain type

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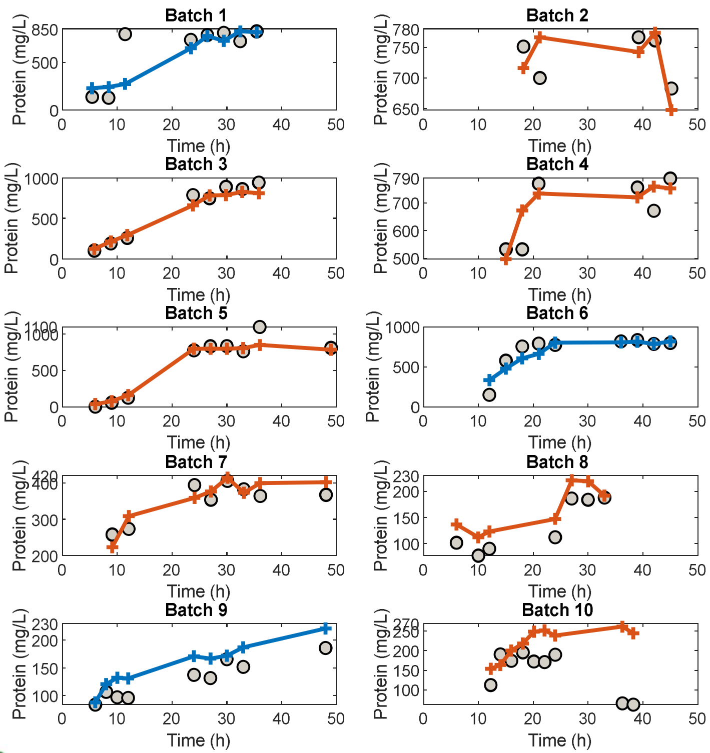
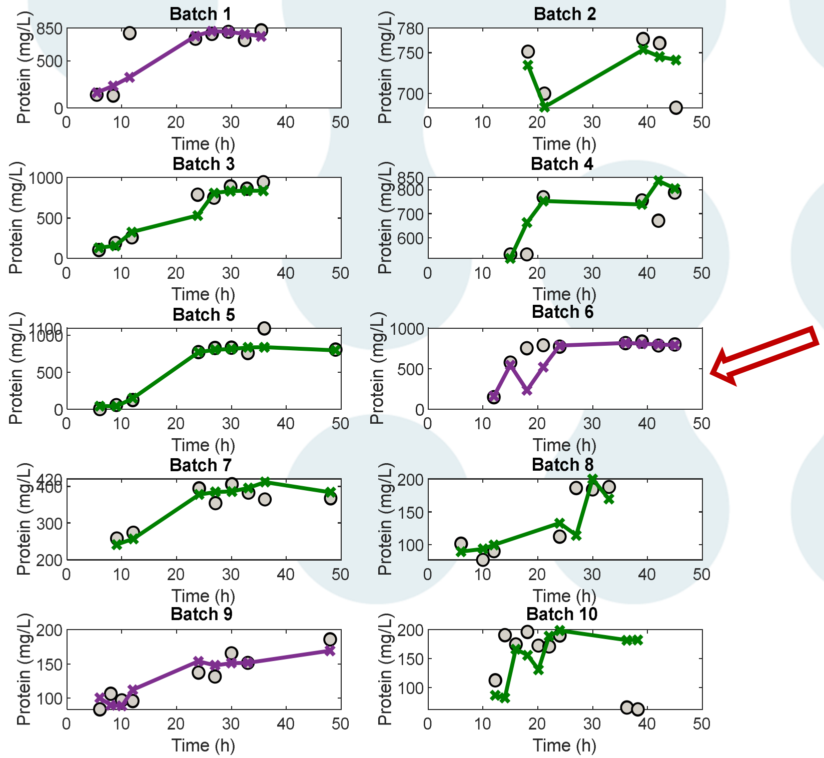
Results

Model 5



Model 8



Training Model

Validation Model

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Conclusions

- SVM was successfully implemented to estimate protein in fed-batch cultures with different strain types.
- The results proved that the developed soft-sensors are attractive and simple alternative for monitoring.
- The combination using only online measurements is good and preferred due to extrapolability to other conditions and industrial application

Perspectives

- Training and validation of SVM with other more experimental data.
- Training and extension to different conditions.
- Experimental validation of soft-sensors and control strategy

Thank you

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