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From Research ...to Market
From Lab ...to Field

Modeling and Dynamic Optimization of protein and spore production by *Bacillus thuringiensis*

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

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Consortium Meeting – IDB2022 – 19th to 22st December 2022

This presentation has 5 sections

1- IMP-4 Citrus aims

2- Model Process

3- Control Strategy

4- Results

5- Conclusions

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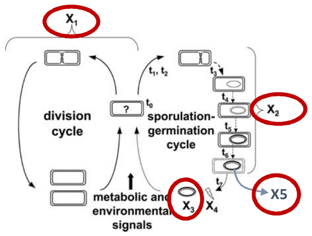
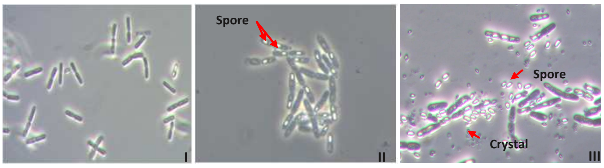
Physiological states in a spore-forming bacterium (application to Bt kurstaki)



- X1, X2, X3 and X4: cell
  - quantification [cell],[DM]
  - morphology
- X5 : δ-endotoxin
  - quantification [Crystal], [Protein]
  - crystal morphology



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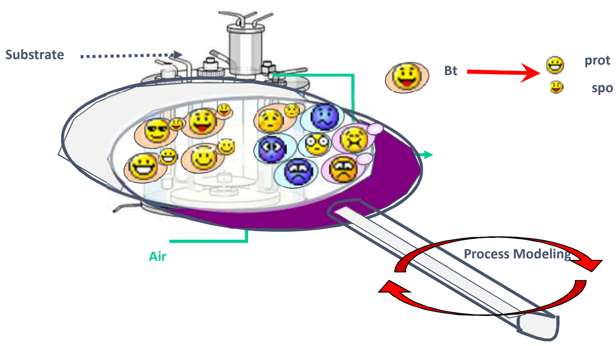
Bioprocess Model Challenges



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METHODES: Model for *B. THURINGIENSIS (FedBatch and Sequential Process)*

$$\frac{dX}{dt} = \mu * X - k_d * X - X\frac{Qin}{V} \quad (1)$$

$$\frac{dS}{dt} = -\frac{\mu * X}{Y1} - S\frac{Qin}{V} + Sin * Qin \quad (2)$$

$$\mu = \mu_{max}\frac{S}{(Kc * X) + S} \quad (3)$$

Model 1

$$\frac{dPro}{dt} = \frac{X * Y2}{Y1} - Pro\frac{Qin}{V} \quad (4)$$

$$\frac{dSpo}{dt} = \frac{X * Y3}{Y1} - Spo\frac{Qin}{V} \quad (5)$$

Model 2

$$\frac{dPro}{dt} = \frac{X * Y2}{Y1} + \alpha - Pro\frac{Qin}{V} \quad (6)$$

$$\frac{dSpo}{dt} = \frac{X * Y3}{Y1} + \beta - Spo\frac{Qin}{V} \quad (7)$$

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METHODES: Control Strategies

Case 1 (Feed batch Optimisation)

$$max_{Qin}\left(\frac{(Pro * V + Spo * V)}{t_{end}}\right)$$

Subject to: $\begin{cases} Eq.(1-7) \\ 1 < V < 10L \\ 0.01 < Qin < 0.4L/h \end{cases}$

Were t_{end} is fermentation final time.

t_c=2 h

Case 2 (Sequential Batch Optimisation)

$$max_{Gluin,V,t_{end1},t_{end2}}\left(\frac{(Pro * V + Spo * V)}{t_{end}}\right)$$

Subject to : $\begin{cases} Eq.(1-7) \\ 1 < V < 10L \\ 15 < S_{in} < 25 \\ 1 < t_{end1} < t_{end2} < 45h \end{cases}$

Were t_{end1} and t_{end2} are the fermentation final time for first and second sequential batches.

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Results

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Optimized parameter values from Lip strain (PSO)

Parameter	Lip		HD		LB	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
µ max (h^{-1})	0,3966	0,3916	0.5985	0.4024	1.1490	1.0720
Kc	0,6899	0,5794	1.4700	0.5140	4.7450	4.1250
Kd (h^{-1})	0,0189	0,0193	0.0458	0.0352	0.0437	0.0439
Y1 ($g_{Biomass}/g_{Glucose}$)	0,4866	0,4956	0.9612	0.8333	0.7136	0.7141
Y2 ($g_{Pro}/g_{Glucosa}/h$)	0,0005	0,0001	0.0056	0.0050	0.0067	0.0067
Y3 ($CFU*10^{-5}/g_{Glucose}/h$)	0,0213	0,0218	0.0281	0.0001	0.0537	0.0524
Alpha (g/L/h)	-	0,0042	-	0.0062	-	0.0001
Beta ($CFU*10^5/L/h$)	-	0,0002	-	0.1116	-	0.0001

$$min\left(\frac{\sum(\zeta - \hat{\zeta})}{\max(\zeta)}\right)$$

Subject to: $\{Eq. (1-7)$

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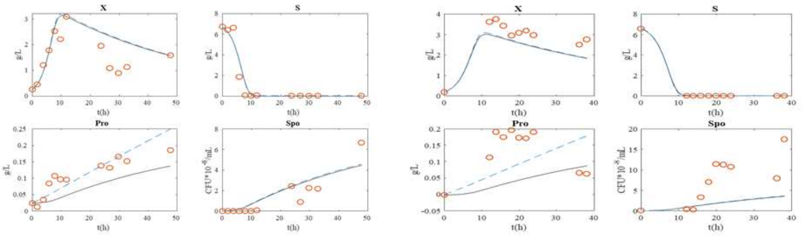
RESULTS: MODEL SIMULATIONS (LIP)



(-) Model 1, (- -) Model 2 and (o) **experimental data.**
X (biomass g/L), S (glucose g/L), Prot (Proteins g/L) and Spo (Spores CFU*10-8/mL)

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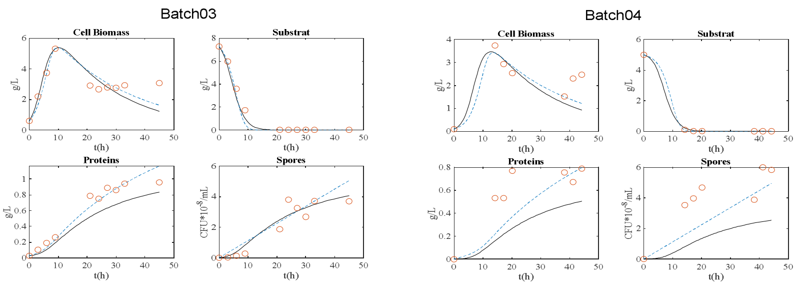
RESULTS: MODEL SIMULATIONS (HD)



(-) Model 1, (- -) Model 2 and (o) **experimental data.**
X (biomass g/L), S (glucose g/L), Prot (Proteins g/L) and Spo (Spores CFU*10-8/mL)

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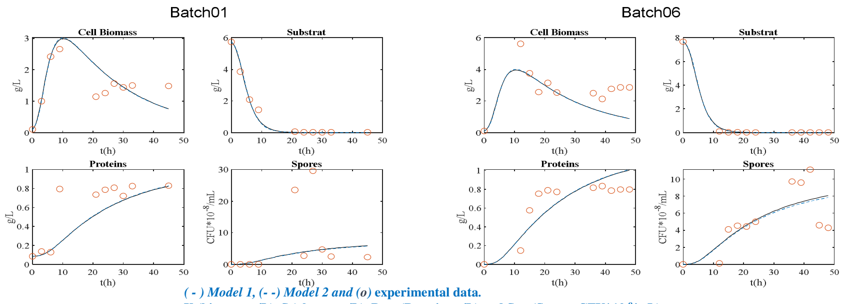
RESULTS: MODEL SIMULATIONS (BLB)



(-) Model 1, (- -) Model 2 and (o) experimental data.
X (biomass g/L), S (glucose g/L), Prot (Proteins g/L) and Spo (Spores CFU*10⁻⁸/mL)

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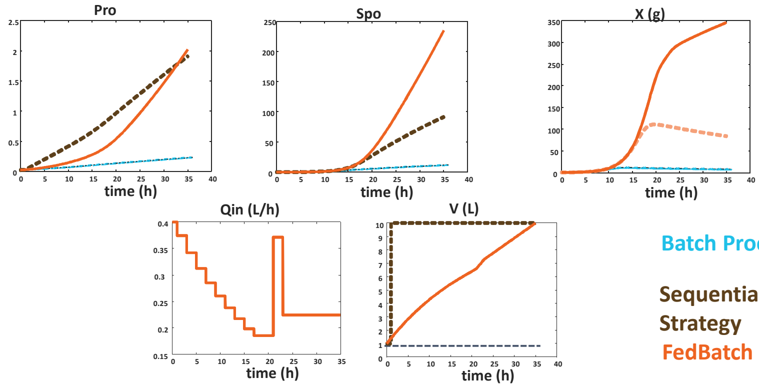
RESULTS: Control Strategy



Batch Process

Sequential Strategy

FedBatch Strategy

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CONCLUSIONS

Both models followed the biomass and substrate dynamics.

Model 2 fitted better all data specially the proteins production

Fed-batch strategy had the best proteins and spores productivity with high biomass productivity

Perspectives:

- To adapt control strategies with industrial substrates and Economic Control

- To propose some Soft Sensors to monitoring bioprocess

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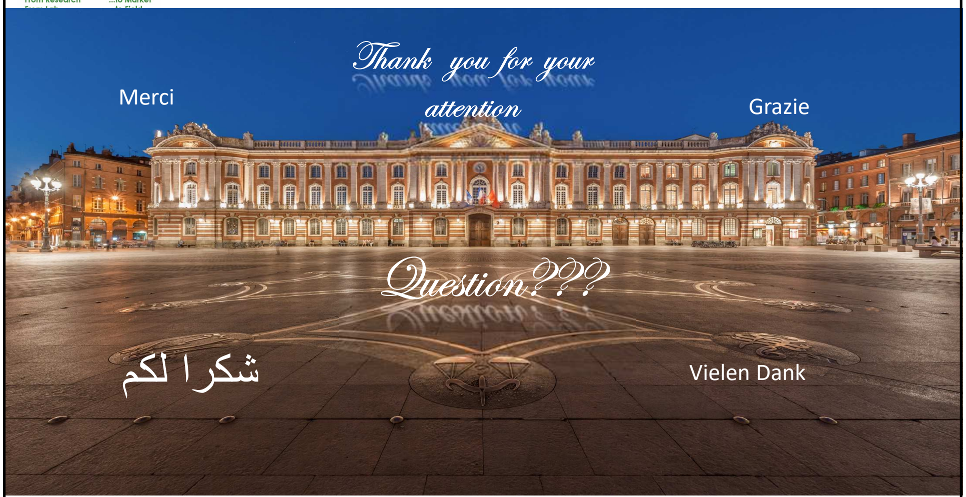

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