Model-based design of experiments and photobioreactor-on-a-chip technology for rapid and reliable modelling of microalgae growth

Elisa Cimetta¹, Tomas Morosinotto², Fabrizio Bezzo¹

PAR-Lab (Padova Algae Research Laboratory)
¹Department of Industrial Engineering & ²Department of Biology
University of Padova

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Outline

- Background
- Modelling microalgae systems
  - A multiscale approach
- The need for suitable experimental protocols
- The potential of lab-on-a-chip technologies
- Conclusions
Some background
Some improvements required

Light theoretical conversion efficiency is $\sim 13\%$

In reality: 1-3%
So what?
Natural and artificial environments

Natural environment vs. Artificial environment
Motivation
Setting up reliable models for process optimisation

- A detailed and reliable **modelling approach** of the growth and metabolic mechanisms required to:
  - **Process understanding**
    - quantifying the effect of key variables and operation parameters
  - Simulate and **optimise** design and operation
    - Define **control** strategies
    - Optimise process **economics**
  - Drive **selection and genetic modification** of microalgae strains
The modelling challenge
A true multiscale problem

- Photosystem \( \sim 10^{-9} - 10^{-7} \text{ m} \)
- Cell \( \sim 10^{-6} - 10^{-4} \text{ m} \)
- Mixing phenomena \( \sim 10^{-4} - 10^{-1} \text{ m} \)
- Light optical path \( \sim 10^{-2} - 10^{-1} \text{ m} \)
- Cultivation plant \( \sim 10^1 - 10^3 \text{ m} \)
- Photoproduction \( \sim 10^{-3} \text{ s} \)
- Photoregulation \( \sim 10^1 - 10^2 \text{ s} \)
- Photoinhibition \( \sim 10^2 - 10^3 \text{ s} \)
- Photoacclimation \( \sim 10^3 - 10^5 \text{ s} \)
- Nutrient utilisation \( \sim 10^3 - 10^5 \text{ s} \)
- Cultivation process \( \sim 10^5 - 10^6 \text{ s} \)
Complex models…
…imply identification challenges, too

- Large number of parameters
- High correlation between parameters
- Limited system observability
- Noisy measurements
- Variable biological response
An illustrative example
Photoproduction, photoregulation and photoinhibition

- Photoproduction
- Photoinhibition

**Han model**

- Photoregulation

**Semi-empirical model**

\[ F = S_P \sigma \Phi_f \]
\[ \Phi_f = \left( \frac{A}{\Phi_f^A} + \frac{B}{\Phi_f^B} + \frac{C}{\Phi_f^C} \right)^{-1} \]
\[ \dot{A} = -I_\sigma_{PS2} A + B/\tau \]
\[ \dot{B} = I_\sigma_{PS2} A - B/\tau + k_r C - k_d \sigma_{PS2} IB \]
\[ A + B + C = 1 \]
\[ \sigma = \frac{\sigma_{PS2} N}{\nu \Phi_f^A} \]
\[ \Phi_f^A = \frac{\eta_P}{1 + \eta_D + \eta_{qE} + \eta_P} \]
\[ \alpha_{SS} = \frac{I_n}{I_{\Phi_f}^n + I_n} \]
\[ \dot{\alpha} = \xi (\alpha_{SS} - \alpha) \]
\[ \Phi_f^A = (1 + \eta_P + \eta_D + \eta_{qE})^{-1} \]
\[ \Phi_f^B = (1 + \eta_D + \eta_{qE})^{-1} \]
\[ \Phi_f^C = (1 + \eta_{qE})^{-1} \]
\[ \eta_{qE} = \eta_{qE}^\alpha \]

(Han et al., 2000. *J Plankton Res*, 22, 865)

(Nikolaou et al, 2015, *J. Biotechnol.*, 194, 91)
The model

Need to describe complex photoregulation mechanisms

Complex NPQ:

\[
\alpha_F(I, t) = \xi_F(\alpha^{SS}(I) - \alpha_F)
\]

\[
\alpha_S(I, t) = \xi_S(\alpha^{SS}(I) - \alpha_S)
\]

\[
\eta_{qE} = \alpha_F(\hat{\eta}_{qE}^F + \alpha_s \hat{\eta}_{qE}^{int}) + \alpha_S \hat{\eta}_{qE}^S
\]
The model
Identification

The new model requires the **identification of 15 parameters**

**Structurally non-identifiable!**

However, it can be demonstrated that by including in the experimental dataset:

- photosynthesis – irradiance (\(PI\)) curves
- antenna size measurements

the model becomes **identifiable**
Identification
Structural identifiability is not practical identifiability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimated value</th>
<th>95% conf. int.</th>
<th>t-value 95%</th>
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<tr>
<td>$\xi_F$</td>
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<td>$1.64 \times 10^{-1}$</td>
<td><strong>0.011</strong> *</td>
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<tr>
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<td><strong>0.46</strong> *</td>
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<td>$1.05 \times 10^{-3}$</td>
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Two parameters cannot be identified with standard experiments:
- **biological variability**
- measurement **noise**
- low sensitivity to measurements
- high **correlation** between parameters
Model-based design of experiments
General sequential procedure

**MBDoE:** sequence of 3 key activities

1. **Design of the dynamic experiment**
2. **Optimal input design**
3. **Parameter estimation and statistics**

**Is it satisfactory?**

(YES, STOP)

(No, go back to 1)

Optimal design problem

\[ \phi^{\text{opt}} = \arg\min_{\phi} \left\{ \psi \left[ V_{\theta} (\theta, \phi) \right] \right\} = \arg\min_{\phi} \left\{ \psi \left[ H_{\theta}^{-1} (\theta, \phi) \right] \right\} \]

subject to

\[ f \left( \dot{x}, x, u, w, \theta, t \right) = 0 \]
\[ \hat{y} = h(x) \]

\[ \widehat{C} = z(\dot{x}(t), x(t), \dot{u}(t), u(t), w, \theta) \leq 0 \]

DESIGN OPTIMALITY

MODEL

FEASIBILITY CONDITIONS
(Constraints on State Variables)

Design vector

\[ \phi = \begin{bmatrix} y(t_0), u(t), w, t^{sp}, \tau \end{bmatrix} \]

The optimal design vector is defined in terms of initial conditions, inputs dynamics, sampling times, experiment duration.
MBDoE
State of the art

- Optimal experiments for model discrimination
  - Assessing different modelling hypotheses
- Optimal experiments for model identification
  - Estimating the model parameters in a sound way
- Advanced techniques
  - Online redesign to “correct” the experiment as soon new data are available
  - Accounting for uncertainty to guarantee experimental feasibility
  - Designing simultaneous experiments to exploit different directions in information
MBDoE
A new experiment
### Practical identifiability

Identification results after MBDoE

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### Additional Information

All parameters can be identified in a **statistically satisfactory** way!

(Bernardi et al (2016), *PLOS ONE, 11*, e0152387)
Model development
Higher integration in experiments and modelling

- Model development and identification require an effective experimental approach
  - optimal design of experiments (e.g., model based)
  - suitable experimental facilities

- Importance of getting targeted information (e.g., typical growth data are blurred by mixing and shadowing effects)
- Need for rapid generation of data
- Need for assessing interaction between different inputs
Typical lab experimental facilities are quite **time consuming**, possibly **expensive** and possibility to run many experiments simultaneously is generally quite limited.

**Difficult to guarantee that experiments starts from the same cultivation batch.**
Lab-on-a-chip technology
Microphotobioreactors for rapid data generation

- Platform was designed with the aid of 3D CAD software
- Mould produced via stereolithography rapid prototyping
- Microdevice built in PDMS
  - **45 wells** (40 μl of working volume each)
  - compatibility with **on-line imaging**
  - **CO₂ permeability**
  - **No shear** environment
  - **No shading**
MicroPBR

Some characteristics

Possible to generate stable concentration gradients if fluid flow is applied

Easy to mask portions of the device so as to filter light at run experiment at different light intensities simultaneously

Transparent case with microtubes for efficient temperature control
Monitoring system
Exploiting fluorescence measurements

Fast monitoring using fluorescence measurements correlated to cellular concentrations
Experimental results
Growth at different light intensities

In a single trial:
• 3 light intensities (6/60/360 μE)
• 15 biological replica per light intensity
• 5 days

Potential for on-site monitoring of photosynthetic features of microalgae (e.g., NPQ)

Preliminary results show the possibility to establish nutrient (NO$_3^-$) concentration gradients and to study the interactions between nitrogen availability and irradiation.
Conclusions
And future work

- **Models** are useful for simulation, optimisation, control
  - very important tools to boost microalgae industrial potential
- Microalgae-based processes are complex **multiscale** systems
  - modelling is complex and model identification can be challenging
- Optimal design of experiments can support the modelling effort
  - **Model-based design of experiments** represents the state-of-the-art to exploit the experimental potential dynamically
- **Lab-on-a-chip** technologies represent a valuable companion for MBDoe applications (and for rapid experimental investigation)
  - A **microPBR** prototype design has been tested and potential is highly promising
  - Future work will aim at improving the design for flow (and possibly steady-state) applications
Thank you for your attention