# Kinetics of phycocyanobilin cleavage from C-phycocyanin by methanolysis

Malwade, Chandrakant Ramkrishna; Roda Serrat, Maria Cinta; Christensen, Knud Villy; Fretté, Xavier; Christensen, Lars Porskjær

# Kinetics of Phycocyanobilin Cleavage from C-Phycocyanin by Methanolysis

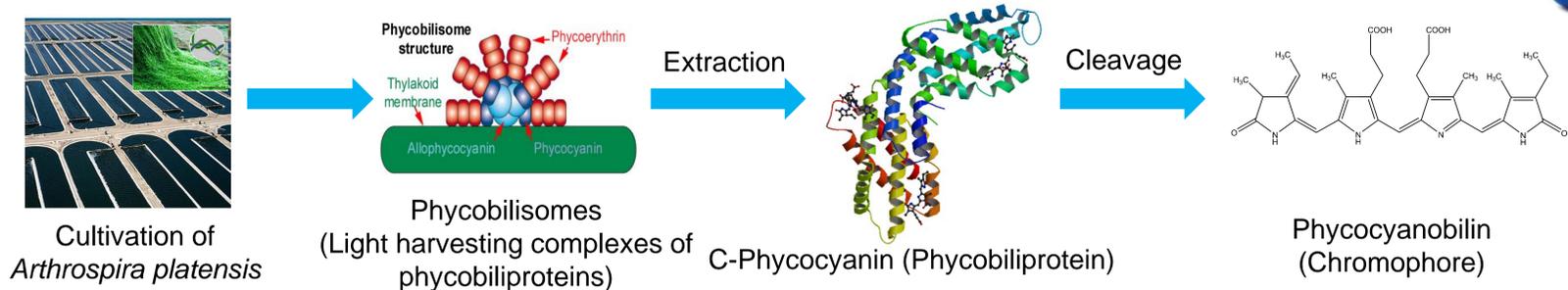**Chandrakant R. Malwade\*, Maria C. Roda-Serrat, Knud V. Christensen, Xavier Fretté, Lars P. Christensen**

Department of Chemical Engineering, Biotechnology and Environmental Technology
University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark
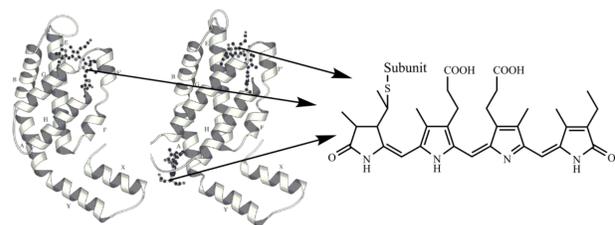crm@kbm.sdu.dk

ESCAPE26

SDU

## INTRODUCTION

Phycocyanobilin (PCB) is a linear tetrapyrrole chromophore covalently attached to protein subunits of phycobiliproteins, C-Phycocyanin (C-PC) and Allophycocyanin (APC), present in the light harvesting complexes of the blue-green algae *Arthrospira platensis*. PCB absorbs light in the red region of the electromagnetic spectrum, thereby exhibiting a vivid blue color. Therefore, it has great significance to the food industry due to its potential as a natural blue food color. The chemical synthesis of PCB is very complex and economically not feasible. Hence, there is a demand for the development of process to obtain PCB from phycobiliproteins. PCB is attached to the protein subunits through a cysteine residue with a thioether linkage. In this work, the kinetics of the cleavage process of PCB from protein subunits by methanolysis is investigated.



Cultivation of
*Arthrospira platensis*

Phycobilisomes
(Light harvesting complexes of phycobiliproteins)

Extraction

C-Phycocyanin (Phycobiliprotein)
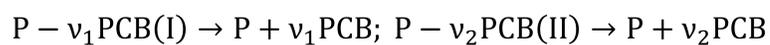
Cleavage

Phycocyanobilin
(Chromophore)

## KINETIC MODEL FOR CLEAVAGE OF PCB BY METHANOLYSIS



PCB attached to alpha (left) and beta (right) subunits of C-PC via thioether linkage

Cleavage of PCB can be described either as two first order reactions in parallel:

$$P - \nu_1 PCB(I) \rightarrow P + \nu_1 PCB; \quad P - \nu_2 PCB(II) \rightarrow P + \nu_2 PCB$$

Or two first order reactions in series:

$$P - \nu_1 PCB(I) - \nu_2 PCB(II) \rightarrow P - \nu_2 PCB(II) + \nu_1 PCB; \quad P - \nu_2 PCB(II) \rightarrow P + \nu_2 PCB$$

Where PCB(I) is easily accessible and PCB(II) is less accessible for cleavage, $\nu_1$ and $\nu_2$ are stoichiometric coefficients of PCB(I) and PCB(II), respectively. In a batch reactor the reactions in parallel will appear as a single first order reaction and can be represented by following set of equations:

$$\frac{dC_1}{dt} = -k_1 \cdot C_1 ; \qquad \frac{dC_2}{dt} = k_1 \cdot C_1 - k_2 \cdot C_2$$

Analytical solutions for set of equations above is:

$$C_1(t) = C_{10} \cdot e^{-k_1 \cdot t} ; \quad C_2(t) = \frac{k_1 \cdot C_{10}}{k_1 - k_2} \cdot \left(e^{-k_2 \cdot t} - e^{-k_1 \cdot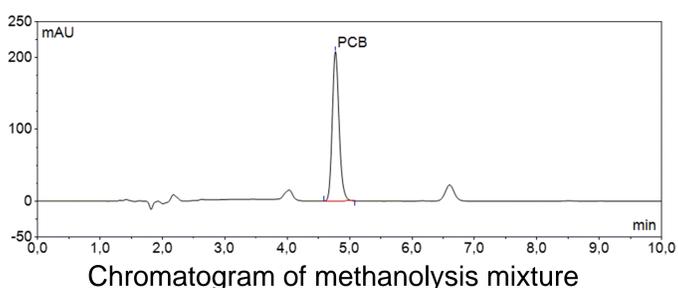 t}\right) + C_{20} \cdot e^{-k_2 \cdot t}$$ Where $C_{10}$ and $C_{20}$ are initial concentration of PCB(I) and PCB(II), respectively

Based on stoichiometry of reaction, the concentration of PCB can be expressed as:

$$C_{PCB}(t) = \nu_1 \cdot C_{10} \cdot \left(1 + \frac{\nu_2}{\nu_1} + \frac{\nu_2}{\nu_1} \cdot \frac{C_{20}}{C_{10}}\right) - \nu_1 \cdot C_{10} \cdot \left(1 + \frac{\nu_2}{\nu_1} + \frac{\nu_2}{\nu_1} \cdot \frac{k_1}{k_1 - k_2}\right) \cdot e^{-k_1 \cdot t} + \nu_1 \cdot C_{10} \cdot \left(\frac{\nu_2}{\nu_1} \cdot \frac{k_1}{k_1 - k_2} - \frac{\nu_2}{\nu_1} \cdot \frac{C_{20}}{C_{10}}\right) \cdot e^{-k_2 \cdot t}$$

## EXPERIMENTAL

- Linablue (Commercial extract of *Arthrospira platensis*) boiled in 400 mL methanol for 16 h at 65 °C
- Mixture samples are taken at regular interval for HPLC analysis
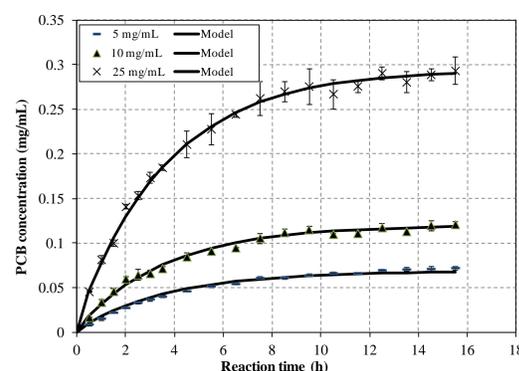- Three different initial concentration of Linablue used



Chromatogram of methanolysis mixture

## RESULTS

**Table 1.** Model data fitted to experimental data.

| Initial protein concentration (mg/mL) | $\nu_1 . C_{10}$ (mg/mL) | $\frac{\nu_2}{\nu_1}$ | $\frac{C_{20}}{C_{10}}$ | $k_1$ (h⁻¹) | $k_2$ (h⁻¹) |
|---|---|---|---|---|---|
| 5 | $2.7 \times 10^{-3}$ | | | | |
| 10 | $4.7 \times 10^{-3}$ | 24 | $1.0 \times 10^{-4}$ | 33 | 0.29 |
| 25 | $12 \times 10^{-3}$ | | | | |



Cleavage of PCB as a function of time. Fully drawn lines are calculated using the model with the parameters from Table 1.

## CONCLUSION

- Kinetic model describes the experimental data adequately
- The ratio between $\nu_2$ and $\nu_1$ is too large compared to the prior findings where a ratio 0.2 and 0.3 is more likely if all PCB is cleaved
- Although the model explains kinetic observations well, a two step model might be an over simplification

## ACKNOWLEDGEMENT