

Syddansk Universitet

## Kinetics of phycocyanobilin cleavage from C-phycocyanin by methanolysis

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

*Published in:*  
Computer - Aided Chemical Engineering

*DOI:*  
[10.1016/B978-0-444-63428-3.50015-1](https://doi.org/10.1016/B978-0-444-63428-3.50015-1)  
[10.1016/B978-0-444-63428-3.50015-1](https://doi.org/10.1016/B978-0-444-63428-3.50015-1)

*Publication date:*  
2016

*Document version*  
Other version

### *Citation for published version (APA):*

Malwade, C. R., Roda Serrat, M. C., Christensen, K. V., Fretté, X., & Christensen, L. P. (2016). Kinetics of phycocyanobilin cleavage from C-phycocyanin by methanolysis. In Z. Kravanja, & M. Bogataj (Eds.), *Computer - Aided Chemical Engineering* (pp. 61-66). Amsterdam: Elsevier. (*Computer - Aided Chemical Engineering*, Vol. 38). DOI: 10.1016/B978-0-444-63428-3.50015-1, 10.1016/B978-0-444-63428-3.50015-1

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Kinetics of Phycocyanobilin Cleavage from C-Phycocyanin by Methanolysis

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

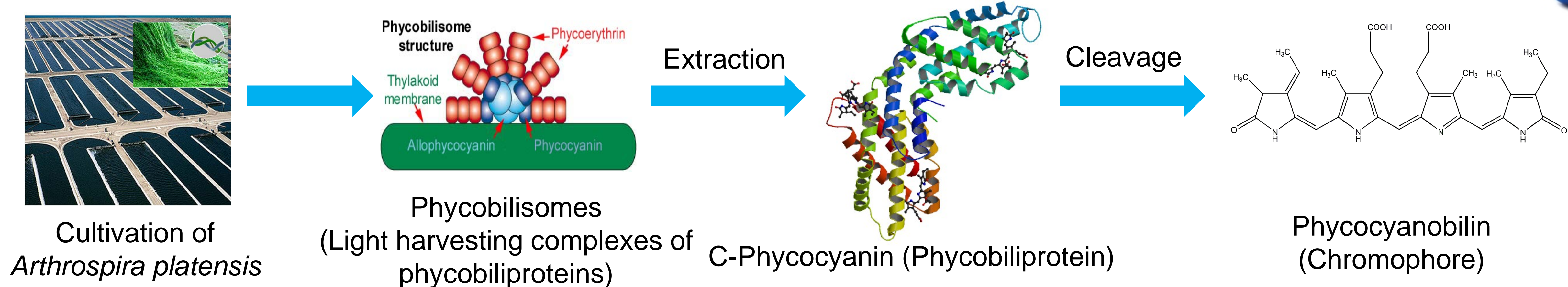
Department of Chemical Engineering, Biotechnology and Environmental Technology  
University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark  
crm@kbm.sdu.dk

ESCAPE26

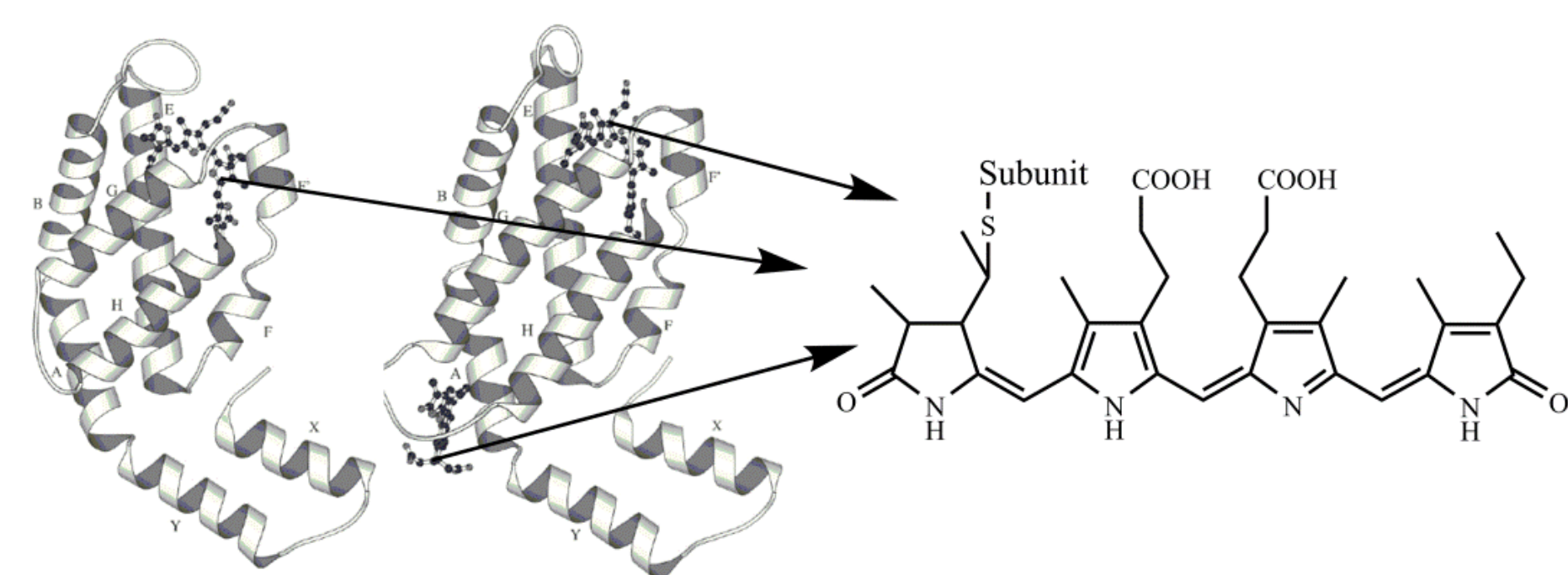


## INTRODUCTION

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.



## 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:



Or two first order reactions in series:



Where PCB(I) is easily accessible and PCB(II) is less accessible for cleavage,  $v_1$  and  $v_2$  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:

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

Analytical solutions for set of equations above is:

$$C_1(t) = C_{10} \cdot e^{-k_1 \cdot t}; \quad C_2(t) = \frac{k_1 \cdot C_{10}}{k_1 - k_2} \cdot (e^{-k_2 \cdot t} - e^{-k_1 \cdot t}) + C_{20} \cdot e^{-k_2 \cdot t}$$

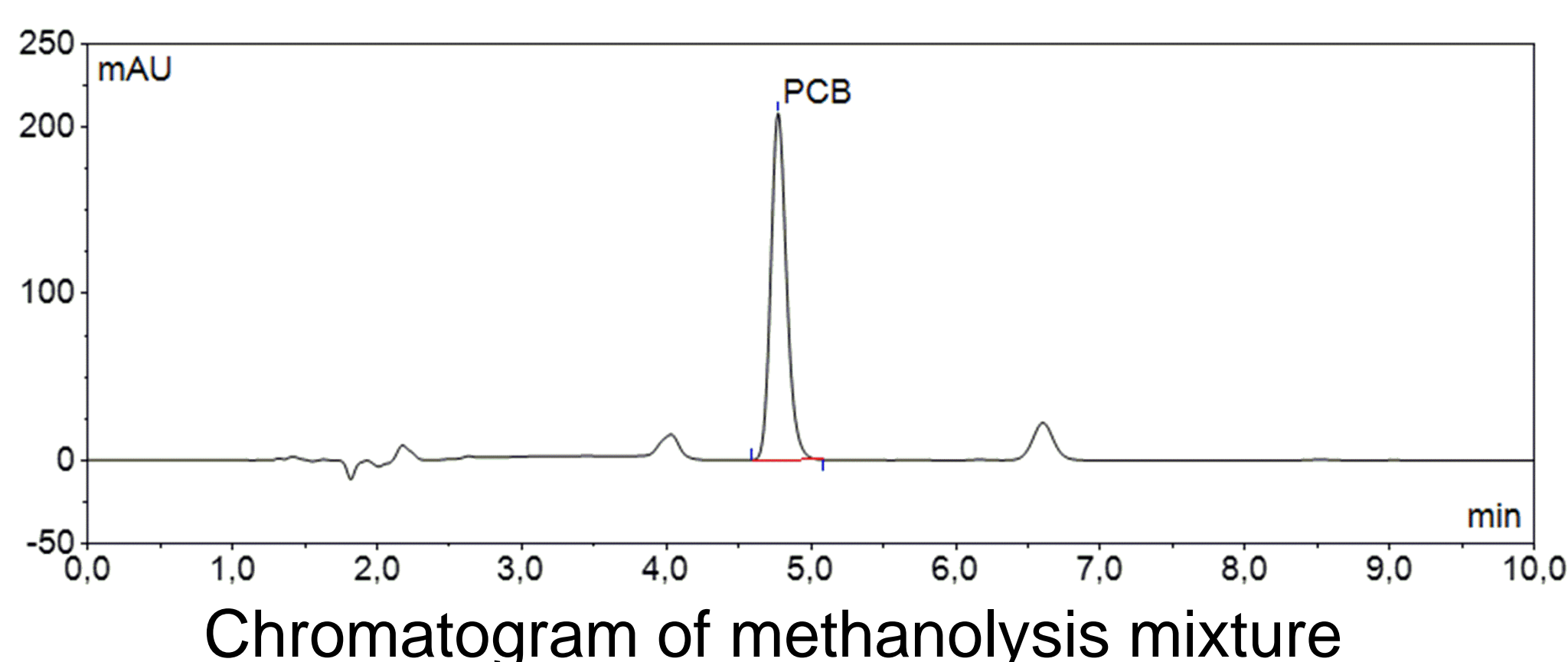
Where  $C_{10}$  and  $C_{20}$  are initial concentration of PCB(I) and PCB(II), respectively

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

$$C_{\text{PCB}}(t) = v_1 \cdot C_{10} \cdot \left(1 + \frac{v_2}{v_1} + \frac{v_2}{v_1} \cdot \frac{C_{20}}{C_{10}}\right) - v_1 \cdot C_{10} \cdot \left(1 + \frac{v_2}{v_1} + \frac{v_2}{v_1} \cdot \frac{k_1}{k_1 - k_2}\right) \cdot e^{-k_1 \cdot t} + v_1 \cdot C_{10} \cdot \left(\frac{v_2}{v_1} \cdot \frac{k_1}{k_1 - k_2} - \frac{v_2}{v_1} \cdot \frac{C_{20}}{C_{10}}\right) \cdot e^{-k_2 \cdot t}$$

## 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



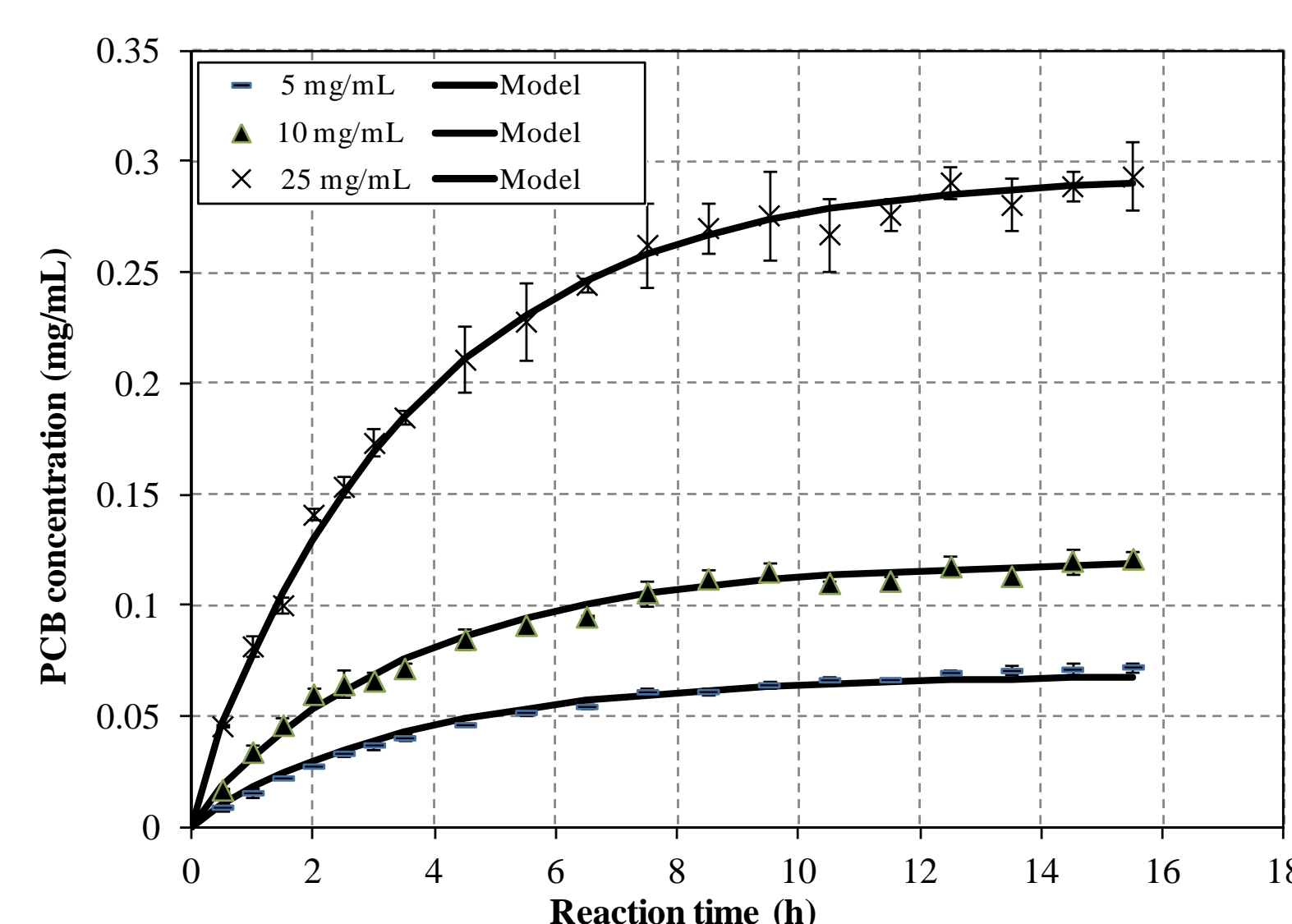
## CONCLUSION

- ❑ Kinetic model describes the experimental data adequately
- ❑ The ratio between  $v_2$  and  $v_1$  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

## RESULTS

Table 1. Model data fitted to experimental data.

Initial protein concentration (mg/mL)	$v_1 \cdot C_{10}$ (mg/mL)	$\frac{v_2}{v_1}$	$\frac{C_{20}}{C_{10}}$	$k_1$ (h <sup>-1</sup> )	$k_2$ (h <sup>-1</sup> )
5	$2.7 \times 10^{-3}$	24	$1.0 \times 10^{-4}$	33	0.29
10	$4.7 \times 10^{-3}$				
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.

## ACKNOWLEDGEMENT

This work is a part of the research within the Project Natural Blue Colorant for Food Applications financially supported by Danish Agency for Science Technology and Innovation to whom the authors are indebted