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# Kinetics of Phycocyanobilin Cleavage from C-Phycocyanin by Methanolysis

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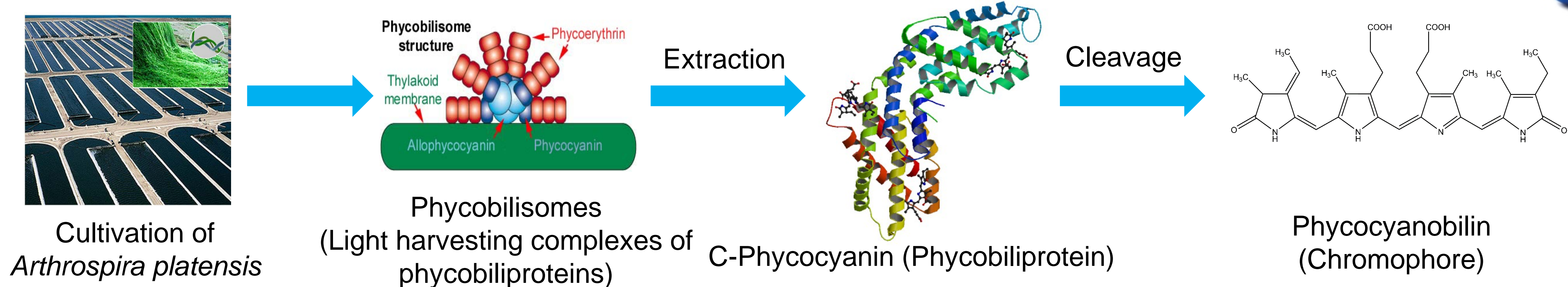
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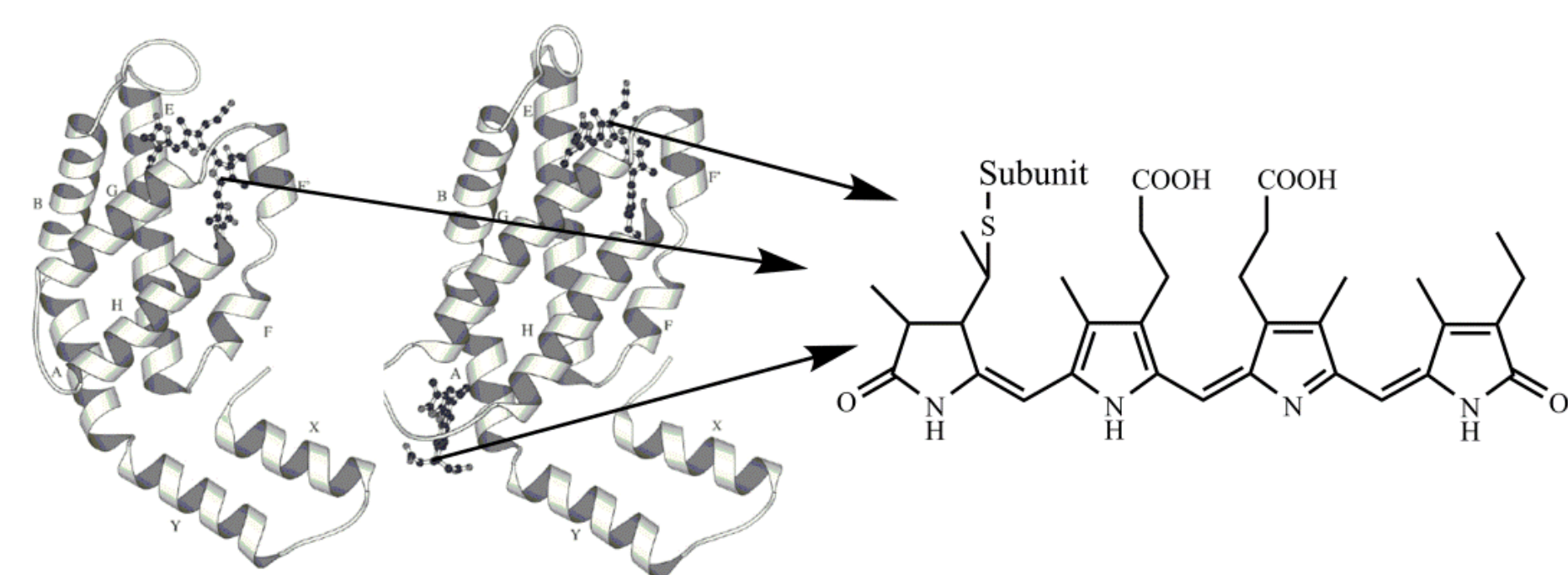


## INTRODUCTION

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:

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

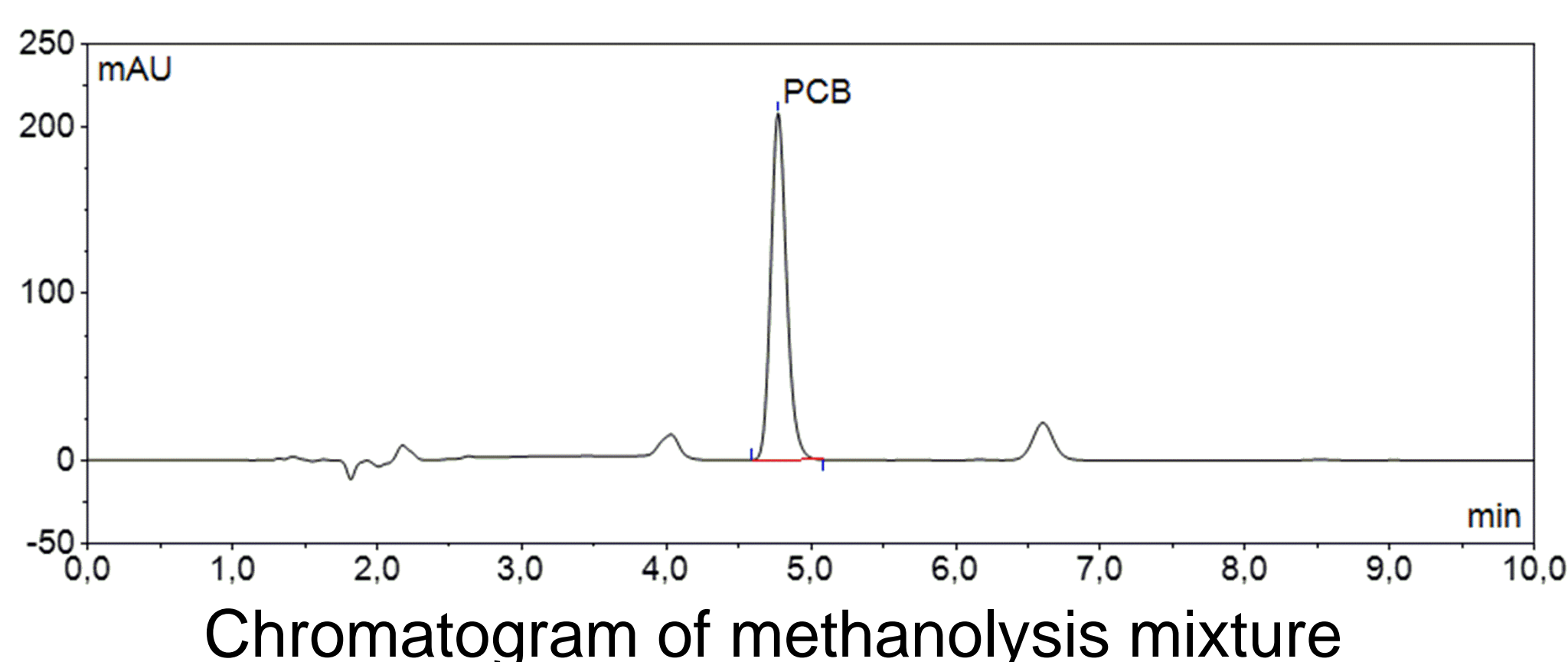
Where  $C_{10}$  and  $C_{20}$  are initial concentration of PCB(I) and PCB(II), respectively

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



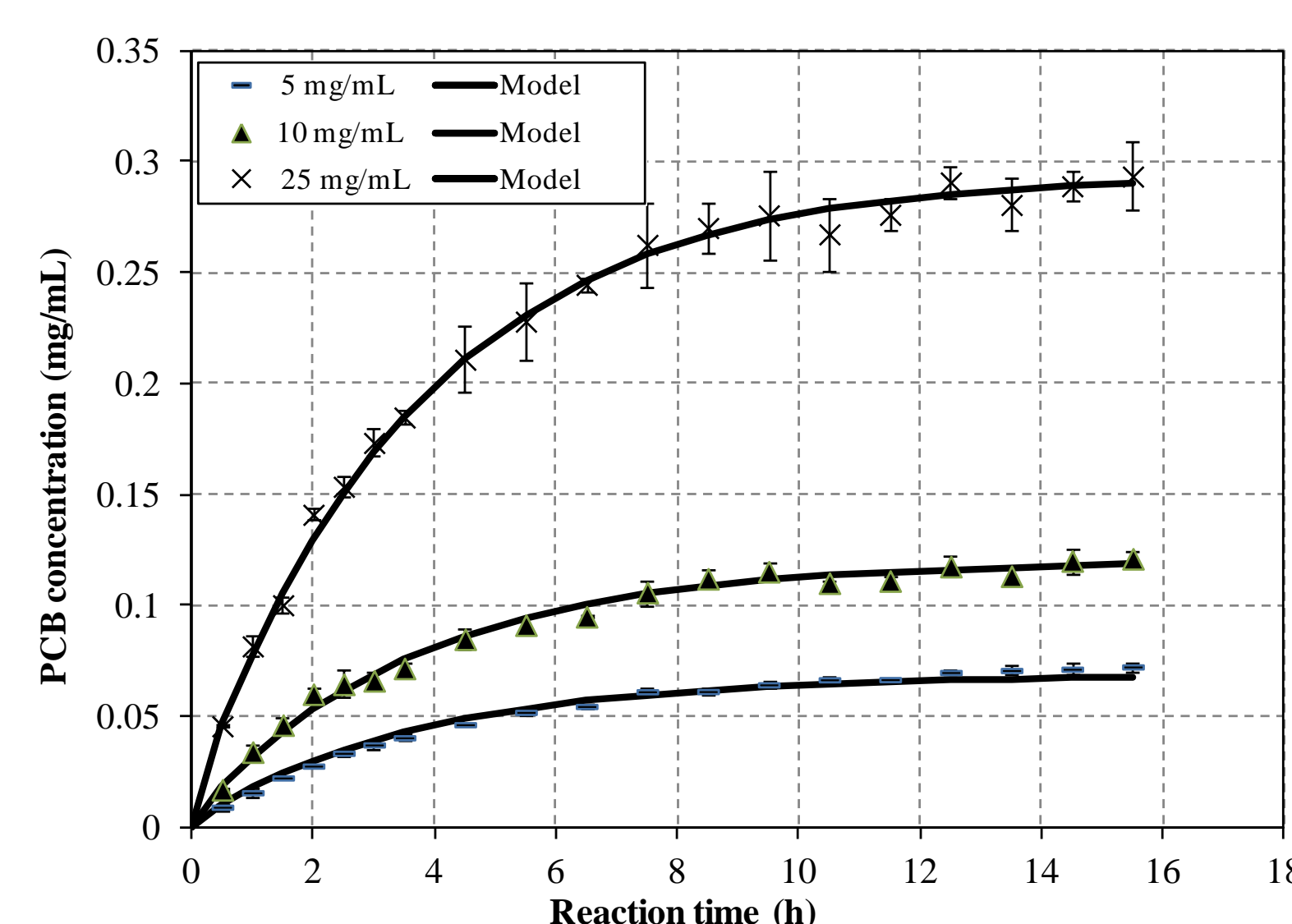
## CONCLUSION

- ❑ 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.

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