

Supplementary Material

Optimization of heterotrophic culture conditions for the microalgae *Euglena gracilis* to produce proteins

Weiying Xie^{1,2,3,4}, **Xiaojie Li**^{2,3}, **Huo Xu**^{1,4}, **Feng Chen**^{2,3}, **Ka-Wing Cheng**^{2,3}, **Hongbin Liu**^{4,5**}, **Bin Liu**^{2,3**}

¹ SZU-HKUST Joint Ph.D. Program in Marine Environmental Science, Shenzhen University, Shenzhen, China

² Shenzhen Key Laboratory of Marine Microbiome Engineering, Institute for Advanced Study, Shenzhen University, Shenzhen, China

³ Institute for Innovative Development of Food Industry, Shenzhen University, Shenzhen, China

⁴ Department of Ocean Science, The Hong Kong University of Science and Technology, Hong Kong, SAR, China

⁵ Hong Kong Branch of Southern Marine Science & Engineering Guangdong Laboratory, The Hong Kong University of Science and Technology, Hong Kong, SAR, China

Correspondence:

Bin Liu: liubin@szu.edu.cn

Hongbin Liu: liuhb@ust.hk

1 Supplementary Figures and Tables

1.1 Supplementary Figures

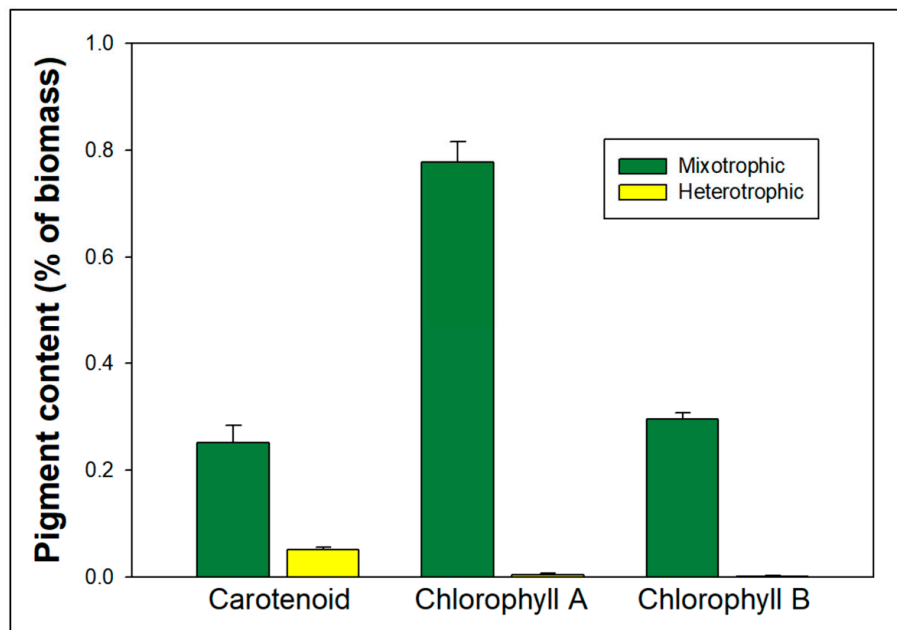


Figure S1. Carotenoid, chlorophyll a and b content of *E. gracilis* under mixotrophic and heterotrophic cultivation.

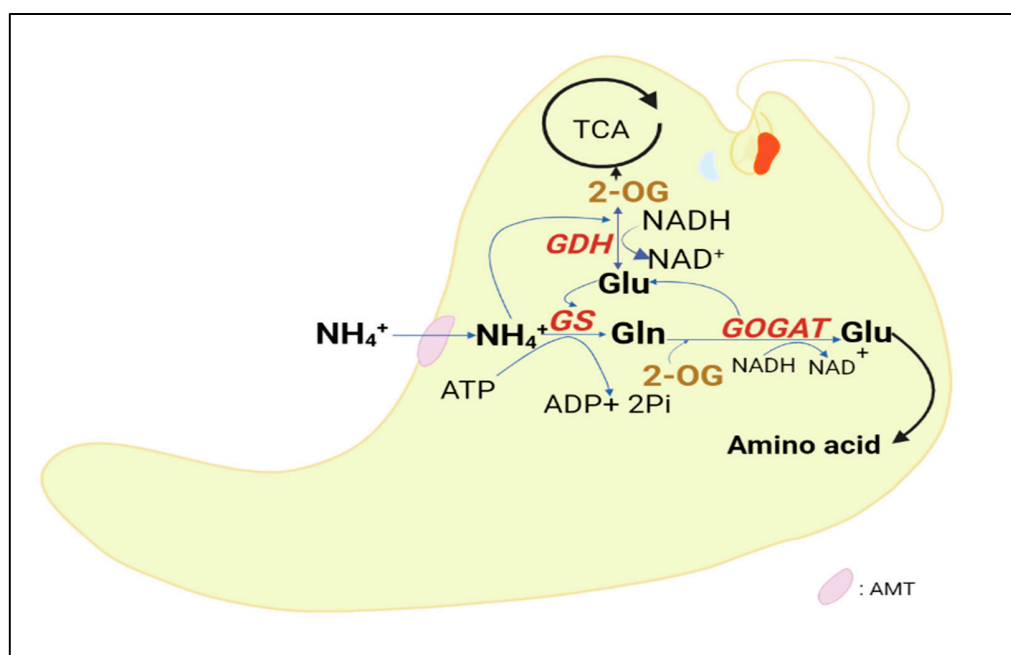


Figure S2. Nitrogen assimilation pathway (GS: glutamine synthase; GOGAT: glutamate synthase; GDH: glutamate dehydrogenase; Gln: Glutamine; Glu: glutamic acid; 2-OG: 2-oxoglutarate; MSG: monosodium glutamate; AMT: ammonium transporter).

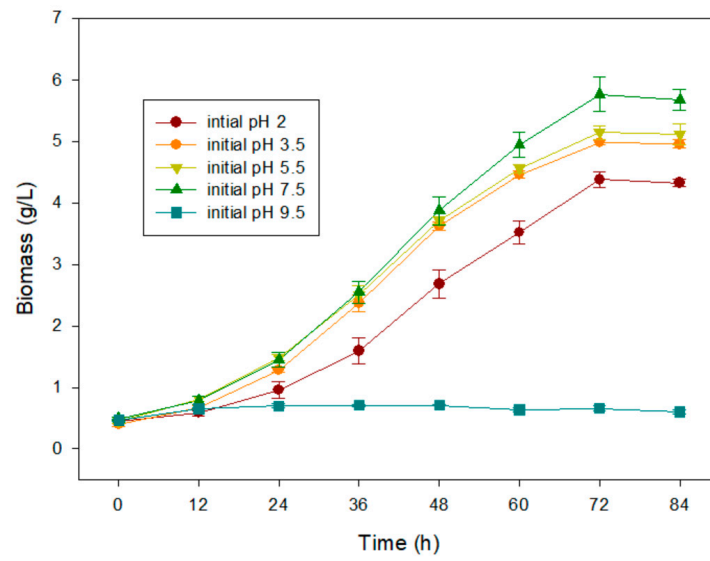


Figure S3. Growth curve of *E. gracilis* cultured under different initial pH.

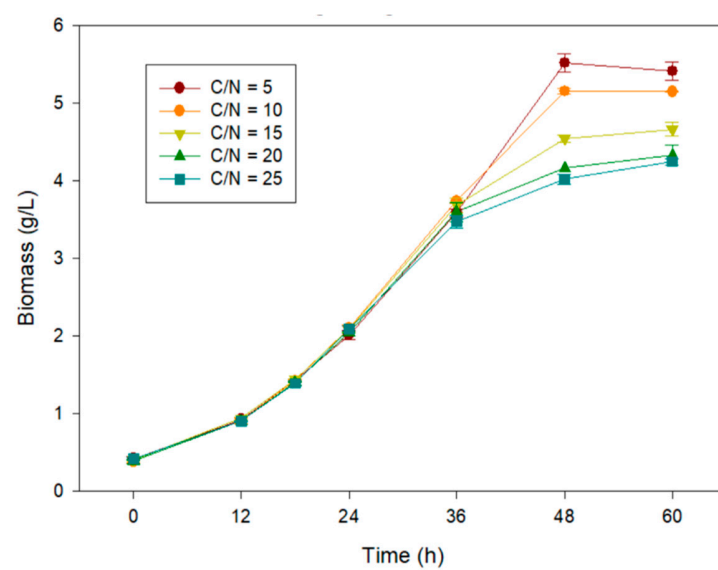


Figure S4. Growth curve of *E. gracilis* cultured under different C/N.

1.2 Supplementary Tables

Table S1. The maximum specific growth rate of heterotrophic *E. gracilis* under different temperature

Temperature (°C)	25	28	32	35
Maximum specific growth rate (/h)	0.049 ± 0.001^a	0.058 ± 0.001^b	0.069 ± 0.002^c	0.061 ± 0.003^b

Note: ^{a-b} Row values with same superscript letter displays not significantly different ($p < 0.05$).