Supplementary Materials for

Timing-dependent effects of fine-bubble CO_2 supplementation on mixotrophic growth of *Euglena gracilis* in glucose–ammonium sulfate medium

Shino Suzuki-Nagata*[1], Nobuyuki Mase[1], Jack C. Ng[2], Tetsuya Suzuki[3]

[1] Department of Applied Chemistry and Biochemical Engineering, Faculty of Engineering, Shizuoka University, 3-5-1 Johoku, Central Ward, Hamamatsu, Shizuoka 432-8561, Japan

postcode; 432-8561. Phone: +81-53-478-1196.

Email: suzuki.shino@shizuoka.ac.jp

[2] Queensland Alliance for Environmental Health Sciences (QAEHS)

The University of Queensland, 20 Cornwall Street, Woolloongabba, Brisbane Qld 4102 Australia

*Corresponding author: Shino Suzuki-Nagata, Email: suzuki.shino@shizuoka.ac.jp

Supplementary Materials are:

Figure S1, Calibration of Cell Population Conversion and Optical Density at 750nm
Figure S2, Calibration standard of glucose concentration measured with OneTouch Vario®.
Figure S3, Raw data of Zeta potential measurement
Figure S4, pH fluctuation during cultivation of *E. gracilis Z* on GAS medium

Supplementary Material

Fig S1. Calibration standard for the relation of Optical Density at 750nm versus cell numbers per ml

Dilution	O.D.750nm
1: x1	1.6
2: x1/2	1.34
3: x1/4	1.19
4; x1/8	0.79
5: x/16.	0.58

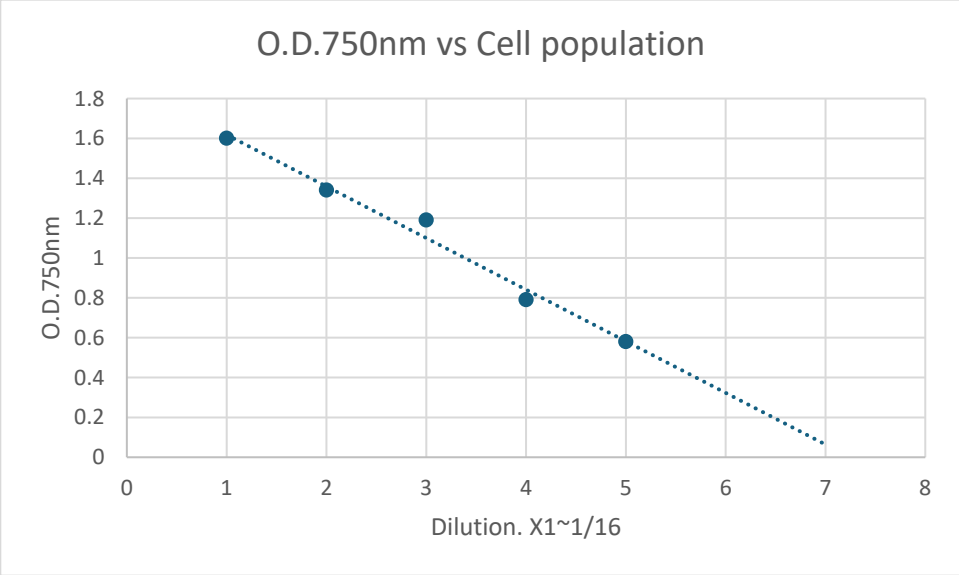


Fig S1a. Relation of O.D. 750nm vs E. gracilis Z cell dilution (x1 to 1/16 v/v)

Initial cell population (x1) is shown in the Table S2.

Table S2

O.D.at 750nm vs cell numbers/ml

O.D.750nm	cells/ml-(1)	cells/ml-(2)	cells/ml-(3)	Average cells/mol
1.6	6.50E+04	8.50E+04	7.10E+04	7.37E+04
1.34	4.50E+04	5.00E+04	4.80E+04	4.77E+04
1.19	4.00E+04	4.00E+04	4.00E+04	4.00E+04
0.79	1.00E+03	7.00E+03	3.00E+03	3.67E+03
0.58	500	500	400	4.67E+02

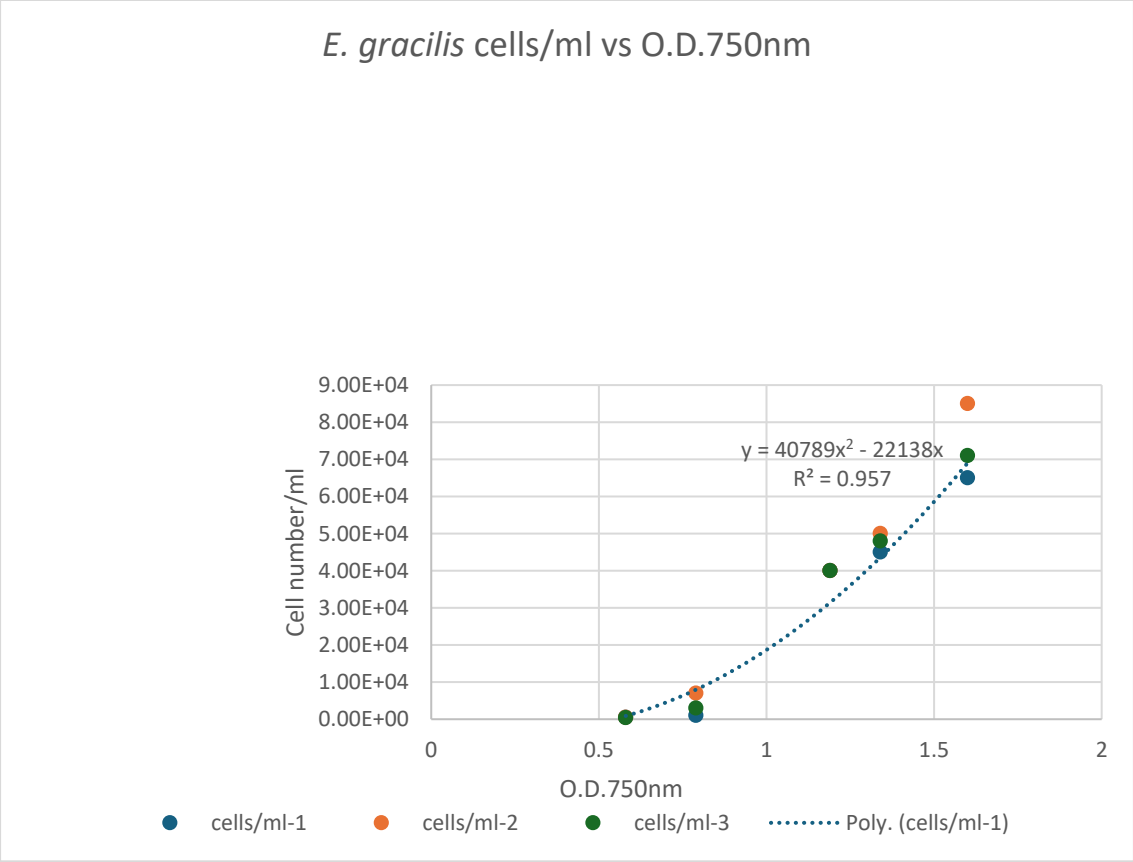


Fig S1b

By preparing a regression curve, the relation between cell numbers/ml and the optical density measured at 750nm is y =40789x2 – 22138x with a correlation coefficient of 0.9572, where y is cell numbers/ml and x optical density at 750nm.

Supplementary Material Fig. S2 Calibration standard of glucose concentration measured with OneTouch Vario® abbreviated by OT in table and Fig. S2.
based on FAD-glucose dehydrogenase.
OT1~3 presents glucose concentration data readings with OneTouch Verio®.
Av. OT data: average values of 3 measurements.

Glucose Concentration	Prepared Glucose concentration	Data read with One Touch Vario 1	Data read with One Touch Vario 2	Data read with One Touch Vario 3	Average Data read with One Touch Vario
	mg/dl	OT1	OT2	OT3	OT Average
	20	15	13	14	14
	40	29	26	28	27.7
	80	61	55	60	58.7
	160	118	115	117	116.7
	320	205	197	210	204
	640	285	289	291	288.3
	1300	355	348	349	350.7

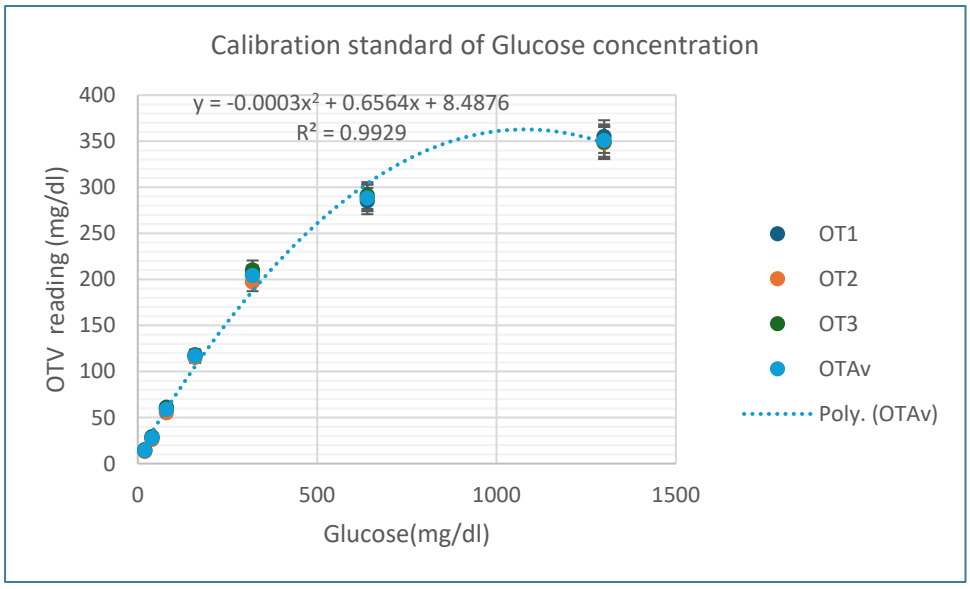


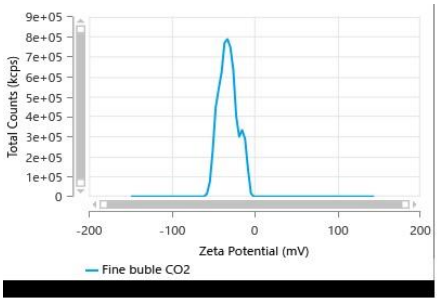
Fig S2.
X-axis: standard glucose concentration (mg/dl); Y-axis: glucose readings from One Touch Vario. Linearity is recognized up to 500 mg/dl glucose solution.

Supplementary Material S3

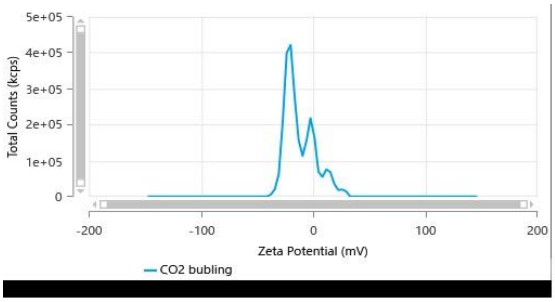
Raw data obtained with ZetasizerUltra for the Measurement of Zeta potential.

Zeta potential of an FB-CO_2-fed solution, which was prepared by feeding the FB-CO_2 for one minute at a flow rate of 1L/min, was provided for the determination of the potential. As a reference, ordinary CO_2 was fed for 1 min at 1 atm pressure. The device used for determining the zeta potential was a Zetasizer Ultra (Malvern Panalytical Ltd., Enigma Business Park, Grovewood Road, Malvern, Worcestershire, WR14 1XZ, England, UK).

Zetasizer Ultra is a device that uses an electrophoretic light scattering instrument utilizing capillary cells.



Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-31.2	-	-	-31.2	-31.2
Conductivity (mS/cm)	0.01147	-	-	0.01147	0.01147
Wall Zeta Potential (mV)	-77.1	-	-	-77.1	-77.1
Quality Factor	8.584	-	-	8.584	8.584
Zeta Peak 1 Mean (mV)	-33.92	-	-	-33.92	-33.92
Zeta Peak 2 Mean (mV)	-13.49	-	-	-13.49	-13.49



Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-12.12	-	-	-12.12	-12.12
Conductivity (mS/cm)	0.03105	-	-	0.03105	0.03105
Wall Zeta Potential (mV)	-40.3	-	-	-40.3	-40.3
Quality Factor	1.023	-	-	1.023	1.023
Zeta Peak 1 Mean (mV)	-20.59	-	-	-20.59	-20.59
Zeta Peak 2 Mean (mV)	-2.15	-	-	-2.15	-2.15

Fig. S3. Raw Data of Zeta Potential measured with ZetasizerUltra.

Upper Figure and data indicate raw data obtained from the FB-CO_2 fed water.

Lower Figure and data indicate raw data obtained from the CO_2 bubble fed water.

Supplementary Material S4. pH fluctuation during cultivation

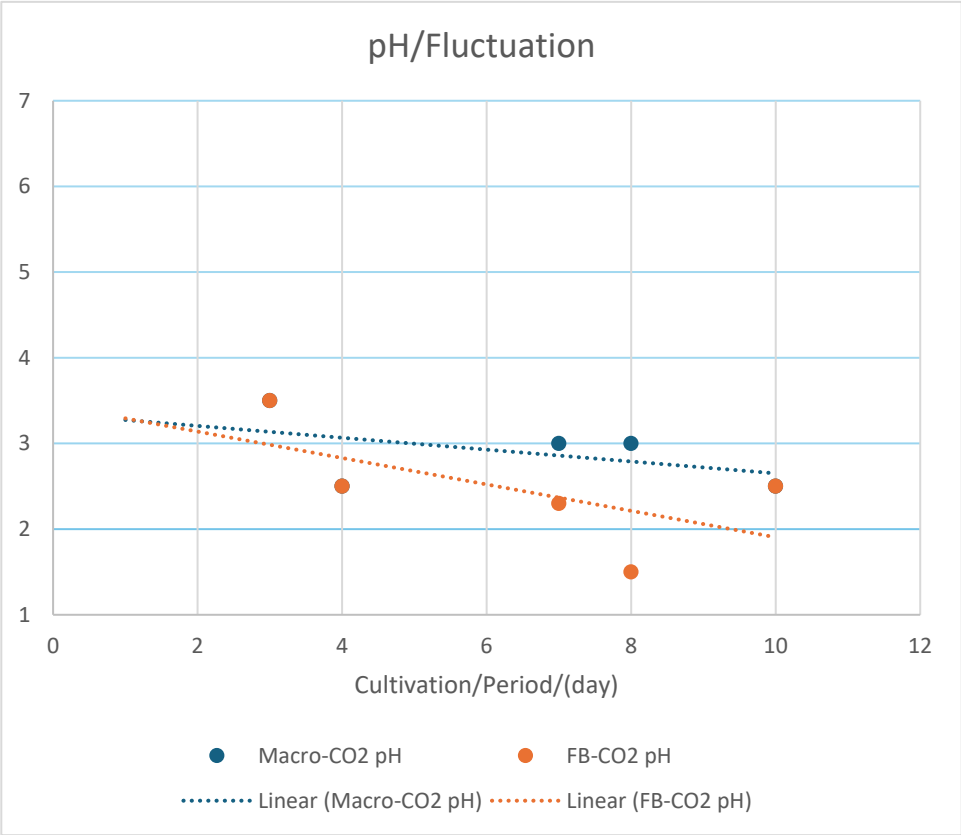


Fig. S4. pH fluctuation during cultivation of *E. gracilis Z* on GAS medium
X-axis, cultivation period (days); Y-axis, pH values.

Blue dots: pH value change under macro-CO_2-feeding and yellow dots: pH change under FB-CO_2-feeding.