PB151

外部循環型フォトバイオリアクターを用いた耐塩性微細藻類*Dunaliella tertiolecta*によるグリセロール生産

SCEJ 53rd Autum Meeting

Glycerol production using halotolerant microalgae *Dunaliella tertiolecta* employing photobioreactor with external circulation loop

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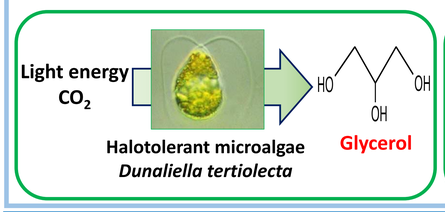
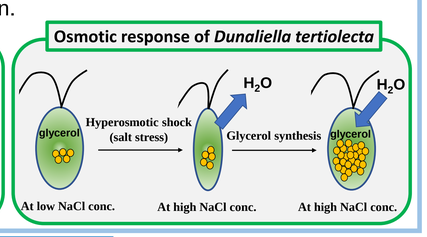
Purpose

Third-generation biorefineries using photosynthetic reactions by microalgae are drawing close attention from industries. We are working on a process development for efficient glycerol production from carbon dioxide using halotolerant microalgae *Dunaliella tertiolecta*, which accumulates glycerol to adapt to highly saline environments.

In this study, we examined the fundamental culture conditions for glycerol production using *D. tertiolecta.* Then, we challenged to enhance glycerol production by a novel photobioreactor with external circulation loop which increased the surface area for efficient light supply at high cell concentration.





Materials and Methods

Strain

***Dunaliella tertiolecta* NIES-2258**

Medium[1)]

Component	Conc.
NaCl	29.2-146 g/L
$MgCl_2 \cdot 6H_2O$	1.5 g/L
KNO_3	1.0 g/L
$MgSO_4 \cdot 7H_2O$	0.5 g/L
KCl	0.2 g/L
$CaCl_2 \cdot 2H_2O$	0.2 g/L
$NaHCO_3$	0.043 g/L
K_2HPO_4	0.495 g/L
KH_2PO_4	0.041 g/L
*Metal solution	1.0 mL/L
*$FeCl_3$ solution	1.0 mL/L

Metal solusion

Component	Conc.
H_3BO_4	0.61 g/L
$MnCl_2 \cdot 4H_2O$	0.023 g/L
$ZnSO_4 \cdot 7H_2O$	0.087 g/L
$CuSO_4 \cdot 5H_2O$	0.052 g/L
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.021 g/L
$CoCl_2 \cdot 6H_2O$	0.015 g/L
EDTA・2Na	1.89 g/L

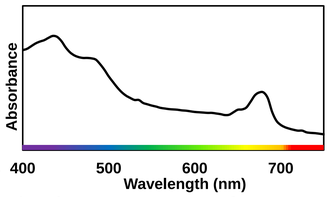
$FeCl_3$ solusion

Component	Conc.
$FeCl_3 \cdot 6H_2O$	0.050 g/L
EDTA・2Na	5.84 g/L

1) J. Horiuchi, *et al. J. Biosci. Bioeng.* 95: 412-415. (2003)

Analytical methods

Cell concentration	Absorption at 680 nm (OD_{680})
Glycerol concentration	F-kit (Roche/R-Biopharm)
Light intensity	Photosynthetic photon flux density (μE/m²/s)



The absorption spectra of NIES-2258

Cultivation methods

Temperature	27.0℃
Light	White fluorescent (100-800) μE/m²/s
Working volume	2000 mL
Airflow rate	1 vvm
Illuminated surface area	0.113 m²

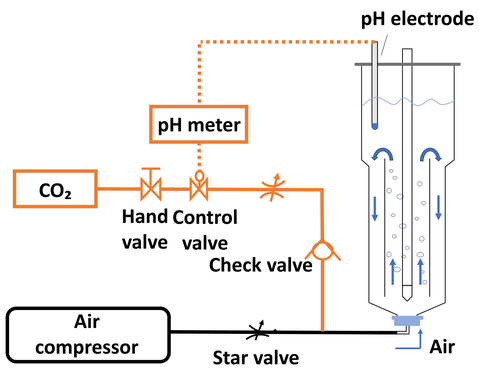
pH-stat system

$CO_2 + H_2O \rightleftarrows H^+ + HCO_3^- \rightleftarrows 2H^+ + CO_3^{2-}$

Microalgae growth⇒CO_2 consumption⇒Incresed pH

CO_2 addition

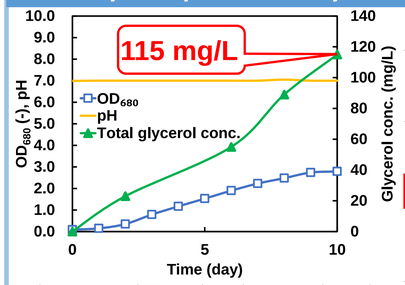
Culture pH was automatically controlled.



Flow sheet of air-lift bioreactor with pH-stat system

Results and Discussion

Glycerol production by air-lift bioreactor with pH-stat system



Time courses of OD_{680}, glycerol concentration and pH in cultivation of NIES-2258
(White fluorescent light: 100±10 μE/m²/s, NaCl: 29.2 g/L, pH7.0)

OD_{680} and glycerol concentration at various pH conditions

pH	OD_{680} (-)	Intracellular glycerol conc. (mg/L)	Extracellular glycerol conc. (mg/L)	Total glycerol conc. (mg/L)
Without pH control	1.63	33	46	79
7.0	2.79	53	62	115
7.5	2.73	27	2	29
8.0	1.32	7	30	37

○ **The highest glycerol production of 115 mg/L was obtained at pH 7.0.**

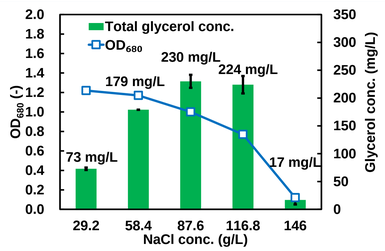
Effects of light intensity and NaCl conc. on glycerol production

Effects of light intensity on OD_{680} and glycerol production
(White fluorescent light, NaCl: 29.2 g/L, pH7.0)

Light intensity (μE/m²/s)	OD_{680} (-)	Intracellular glycerol conc. (mg/L)	Extracellular glycerol conc. (mg/L)	Total glycerol conc. (mg/L)
100	2.23	50	44	94
300	3.47	74	73	147
600	5.29	132	188	320
800	3.47	70	166	236

○ **NIES-2258 was tolerant to high light intensity.**

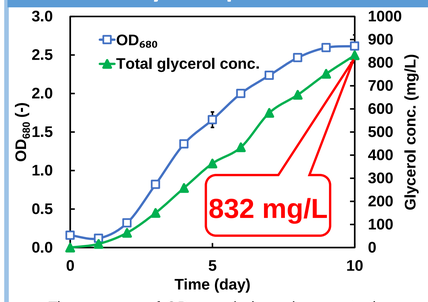
○ **Cell growth and glycerol production greatly enhanced at 600 μE/m²/s.**



Effects of NaCl conc. on glycerol production
(Flask culture, Red & blue LED: 100 ±10 μE/m²/s, 27.0℃, 100 rpm, n = 2)

○ **NaCl concentration suitable for glycerol production was 87.6 g/L.**

Glycerol production under optimal culture conditions



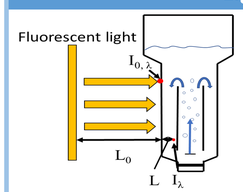
Time courses of OD_{680} and glycerol concentration in cultivation of NIES-2258

Comparison of cell growth and glycerol concentration

	Without pH control, 100 μE/m²/s, NaCl 29.2 g/L	pH7.0, 600 μE/m²/s, NaCl 87.6 g/L
OD_{680} (-)	1.63	2.62
Total glycerol conc. (mg/L)	79	832
Glycerol productivity (mg/L/day)	7.9	83.2
		10.5 times

[Culture conditions: pH7.0, Light intensity: 600 μE/m²/s, NaCl: 87.6 g/L]

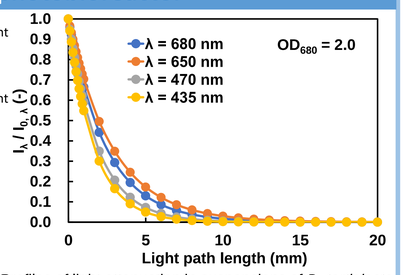
Light attenuation in photobioreactor



I (μE/m²/s): light intensity at light path length L
I_0 (μE/m²/s): incident light intensity
L (mm): light path length
L_0 (mm): light path length from a light source to illuminated surface
λ (nm): wavelength
C (kg/m³): cell concentration
ε (m²/g): attenuation coefficient

$$\frac{I_\lambda}{I_{0,\lambda}} = \frac{{L_0}^2}{(L+L_0)^2} \cdot 10^{-\varepsilon_{a,med,\lambda} L} \cdot 10^{-\varepsilon_{cell,\lambda} CL}$$

T. Katsuda, *et al. J. Chem. Eng. Japan* 35.5: 428-435 (2002)

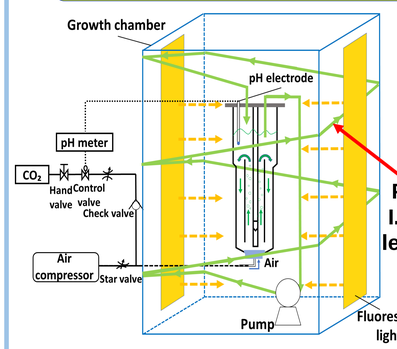


Profiles of light attenuation in suspensions of *D. tertiolecta*

✔ **Intensity of transmitted light falls below 10% at 5 mm depth from photobioreactor surface (OD_{680} = 2.0) .**

Development of photobioreactor with external circulation loop

Challenge: Efficient light supply to photobioreactor
Strategy: Increase surface area by external circulation loop



PVC tube
I.D.: 4 mm
length: 9 m

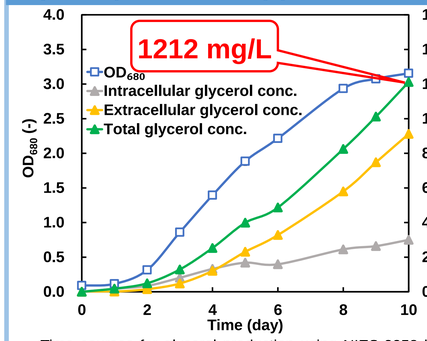
Photobioreactor with external circulation loop

Table Illuminated surface area in various reactors

Reactors	Illuminated surface area (m²)
Air-lift bioreactor	0.113
Photobioreactor with external circulation loop	0.226

2 times

Glycerol production by photobioreactor with external circulation loop

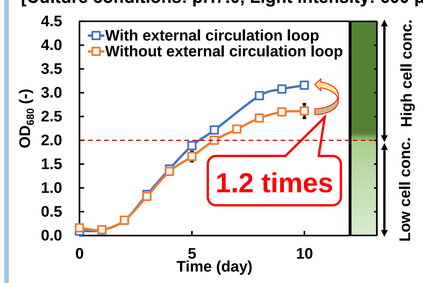


Time courses for glycerol production using NIES-2258 by photobioreactor with external circulation loop

OD_{680} and glycerol concentration by photobioreactor with external circulation loop

OD_{680} (-)	3.16
Intracellular glycerol conc. (mg/L)	300
Extracellular glycerol conc. (mg/L)	912
Total glycerol conc. (mg/L)	1212

[Culture conditions: pH7.0, Light intensity: 600 μE/m²/s, NaCl: 87.6 g/L, Linear velocity: 9 cm/s]



Comparison of cell growth and glycerol concentration

○ **Glycerol production was successfully increased by approximately 50% employing photobioreactor with external circulation loop.**

Conclusions

○ **The optimal pH, light intensity and NaCl concentration on glycerol production was pH7.0, 600 μE/m²/s and 87.6 g/L respectively.**

○ **Successful production of 832 mg/L glycerol was achieved by air-lift bioreactor under optimal culture conditions.**

○ **Photobioreactor with external circulation loop successfully improved glycerol production to 1212 mg/L.**