Biological and Bioelectrochemical Hydrogen and Ethanol Production from Waste Glycerol Discharged from a BDF Plant

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Biodiesel Fuel (BDF)

Esterification of fatty acids such as vegetable oils, used food oils and animal oils with methanol

BDF

$$\begin{array}{l} CH_2COOR_1 \\ | \\ CHCOOR_2 \\ | \\ CH_2COOR_3 \end{array} + 3CH_3OH \longrightarrow \begin{array}{l} R_1COOCH_3 \\ R_2COOCH_3 \\ R_3COOCH_3 \end{array} + \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array}$$

Glycerol-containing wastes from esterification of used food oils could not be sold, and BDF production will increase through the world.

Chemical material?

New treatment methods of glycerol will be needed.

Glycerol ⟶ various compounds, especially to 1,3-propanediol

Our colleagues of this study, Prof. Tanisho and Prof. Nishio found:
In hydrogen fermentation of glycerol in ***Enterobacter aerogenes***,
a representative hydrogen-producing bacteria

$$C_3H_5(OH)_3 \longrightarrow H_2 + C_2H_5OH + CO_2$$

Objectives

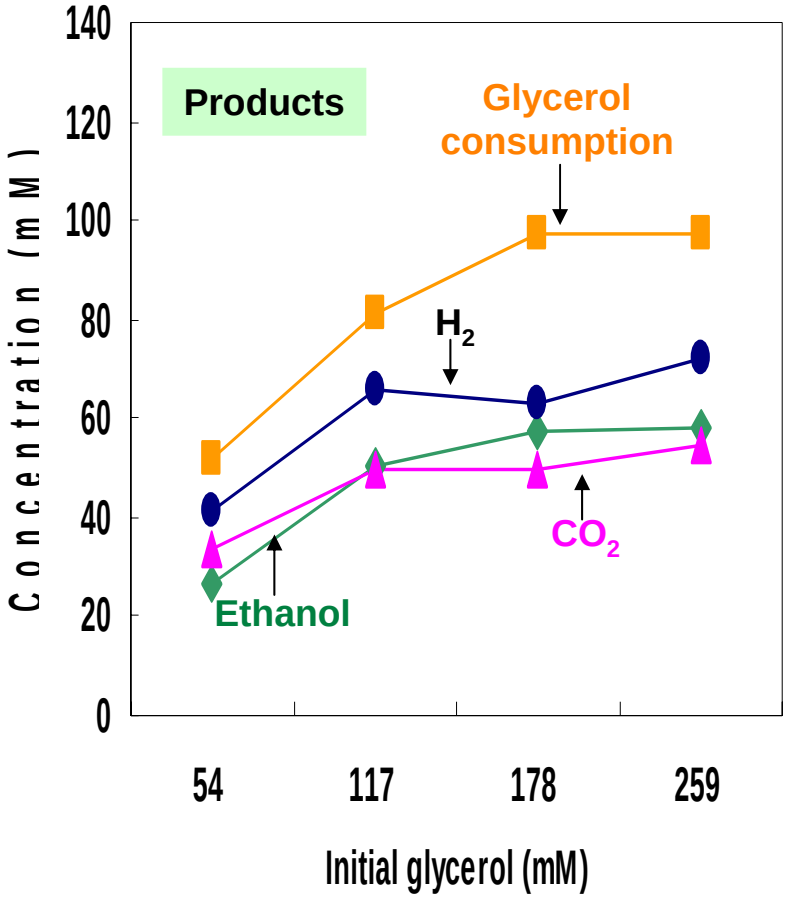
- Clarify a possibility of hydrogen and ethanol production in *E. aerogenes* from glycerol wastes discharged from a BDF plant using used food oils
- Bioelectrochemical hydrogen production to activate the cell of *E. aerogenes*

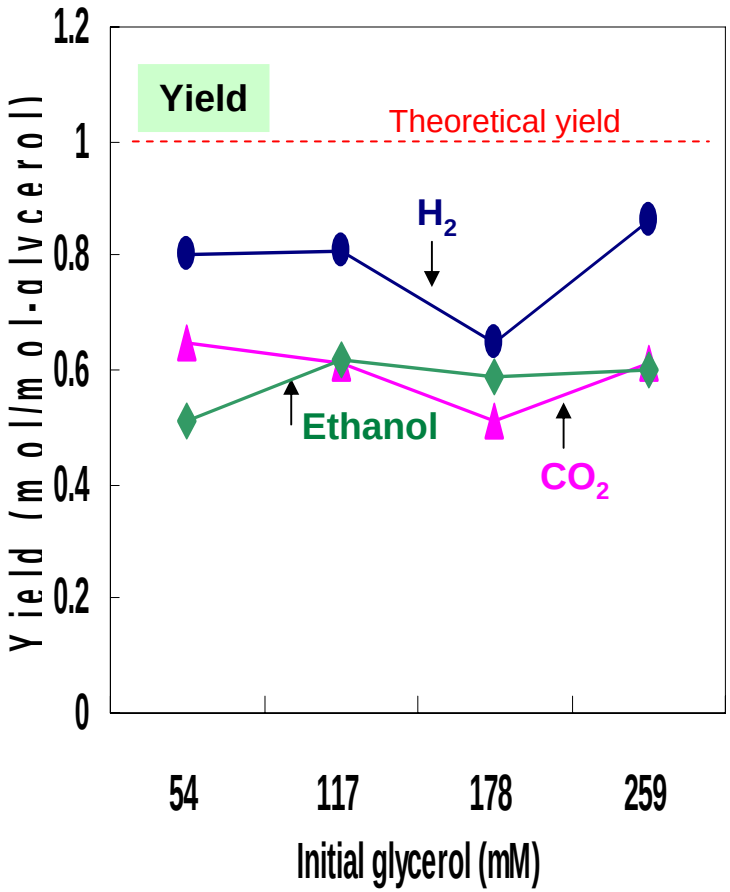
Bacteria employed in this study was *Enterobacter aerogenes* NBRC12012 obtained from National Institute of Technology and Evaluation in Japan

E. aerogenes NBRC12012 was pre-cultured in the NBRC702 medium (pH7.0) composed of polypepton (10 g/l), yeast extract (2 g/l) and $MgSO_4 \cdot 7H_2O$ (1 g/l) at 30ºC.

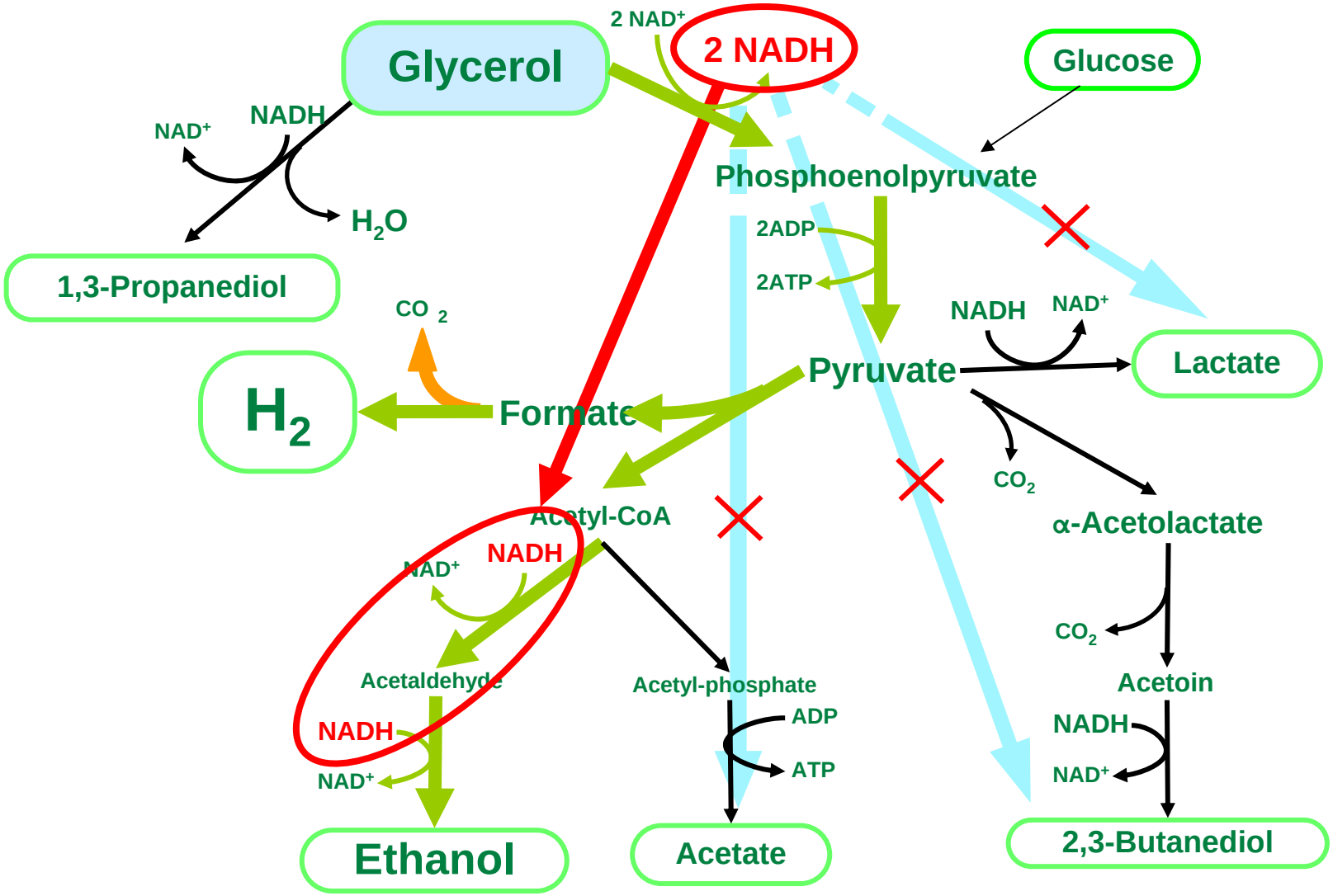
For the evaluation of fermentation of glycerol in *E. aerogenes* NBRC12012, 4% (v/v) of the pre-culture suspension and 1% (v/v) glycerol were inoculated into the NBRC702 medium (50 ml) in the serum bottles sealed with butyl rubber sttopers.

The bottles were incubated at 30ºC with agitation. The pH of the solution in these bottles was not controled during experiments.





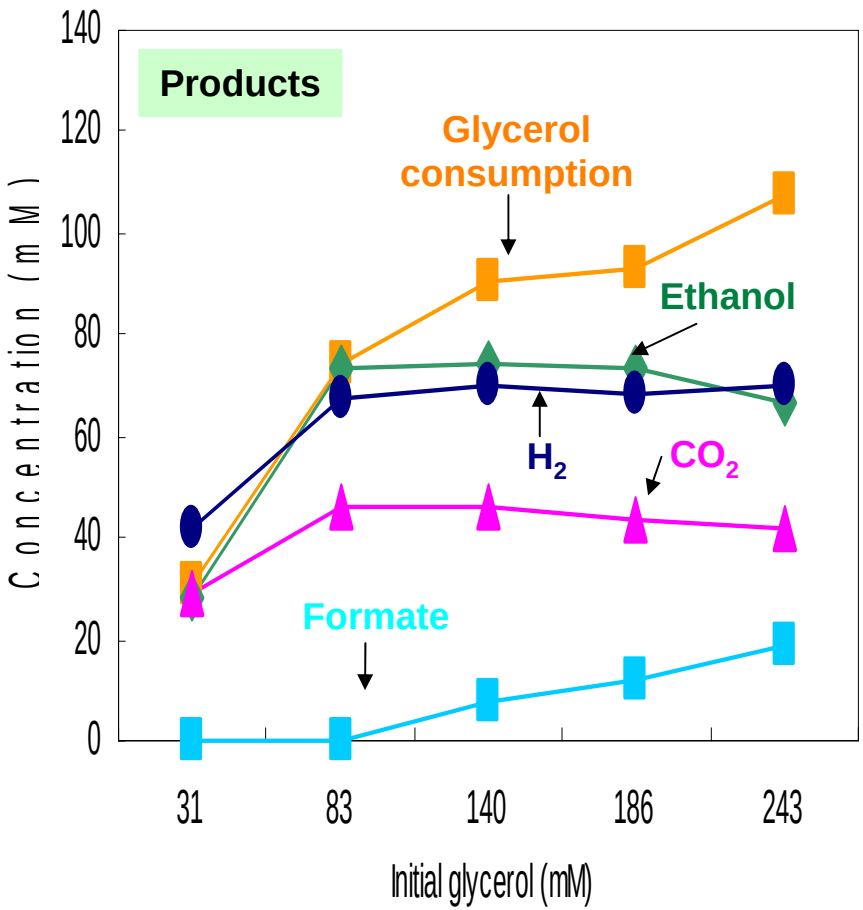
Effect of initial concentration of commercially available glycerol on glycerol consumption, products and their yields after 24h incubation

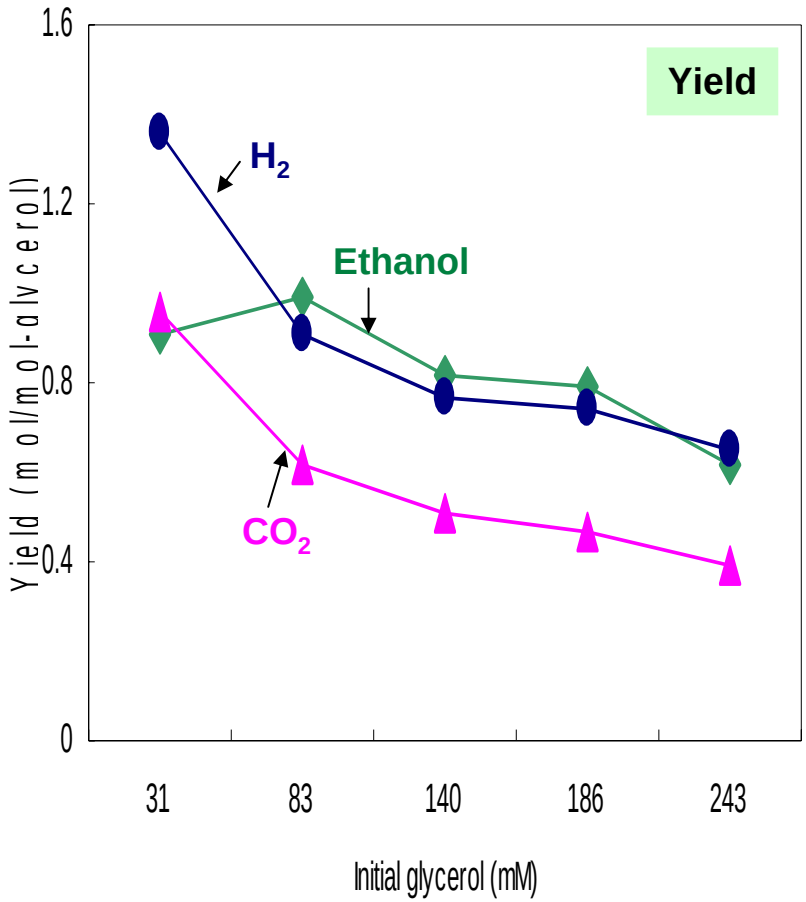


Metabolic pathway from glycerol to H_2 and ethanol in *E. aerogenes*

Composition of glycerol containing wastes discharged from a BDF plant using used food oils in Ishikawa Prefecture in Japan

Constituent	Concentration (g/l)
Glycerol	445
Methanol	175
Formate	0.23
Acetate	0.67
Propionate	0.68
Butylate	0.68
Soluble TOC	471





Effect of initial concentration of glycerol-containing biodiesel wastes on glycerol consumption, products and their yields after 24h incubation

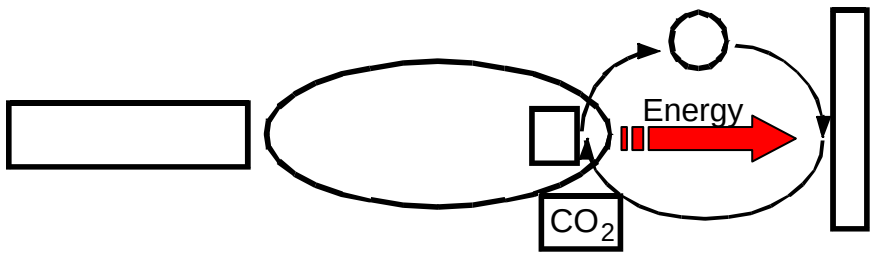
The wastes discharged from the BDF plant contained 44.5% (w/w) glycerol.

However, glycerol consumption in hydrogen fermentation was suppressed at more than 83 mM initial glycerol concentration, corresponding to about 0.8% (w/w) glycerol solution.

Dilution of 50 times is needed!

This means heavy load of waste water treatment.

In previous study, hydrogen production rate per microbical cell was improved in bioelectrochemical cells using *E. aerogenes* NBRC12012, suggesting that the cells of *E. aerogenes* would be activated under such conditions.



Reducing equivalents generated from carbohydrate in microbial cells were squeezed by a redox mediator.

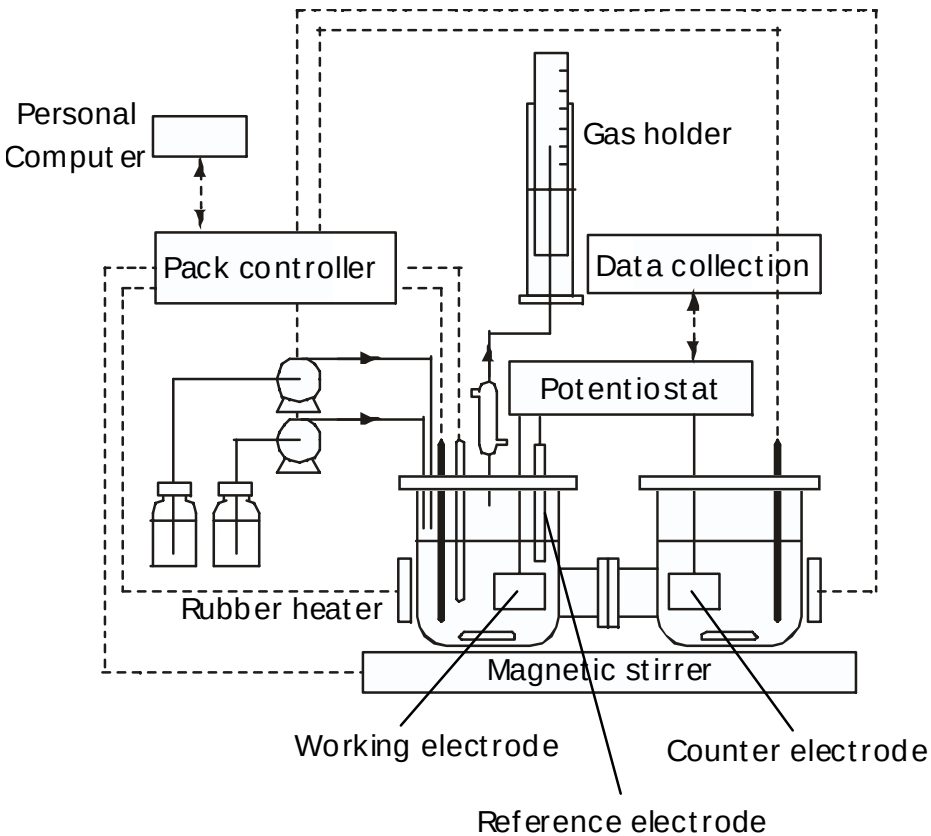
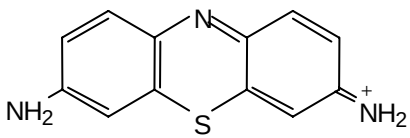


Diagram of Bioelectrochemical reactor

Thionin



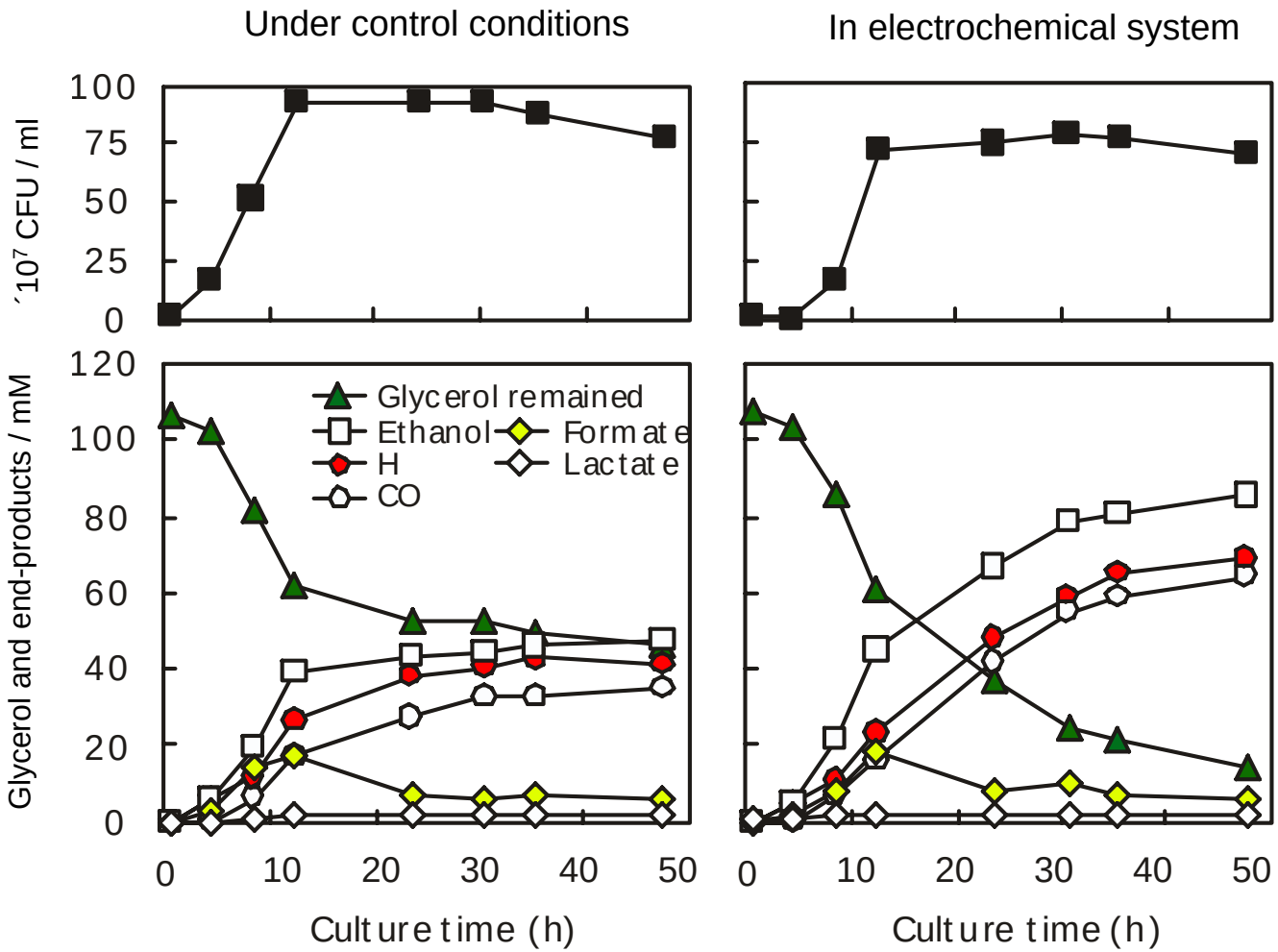
Three electrode method
- Working electrode: carbon cloth
- Reference electrode: Ag/AgCl
- Counter electrode: Pt mesh

The working electrode was imposed +0.2 V vs. Ag/AgCl reference electrode by a potentiostat.

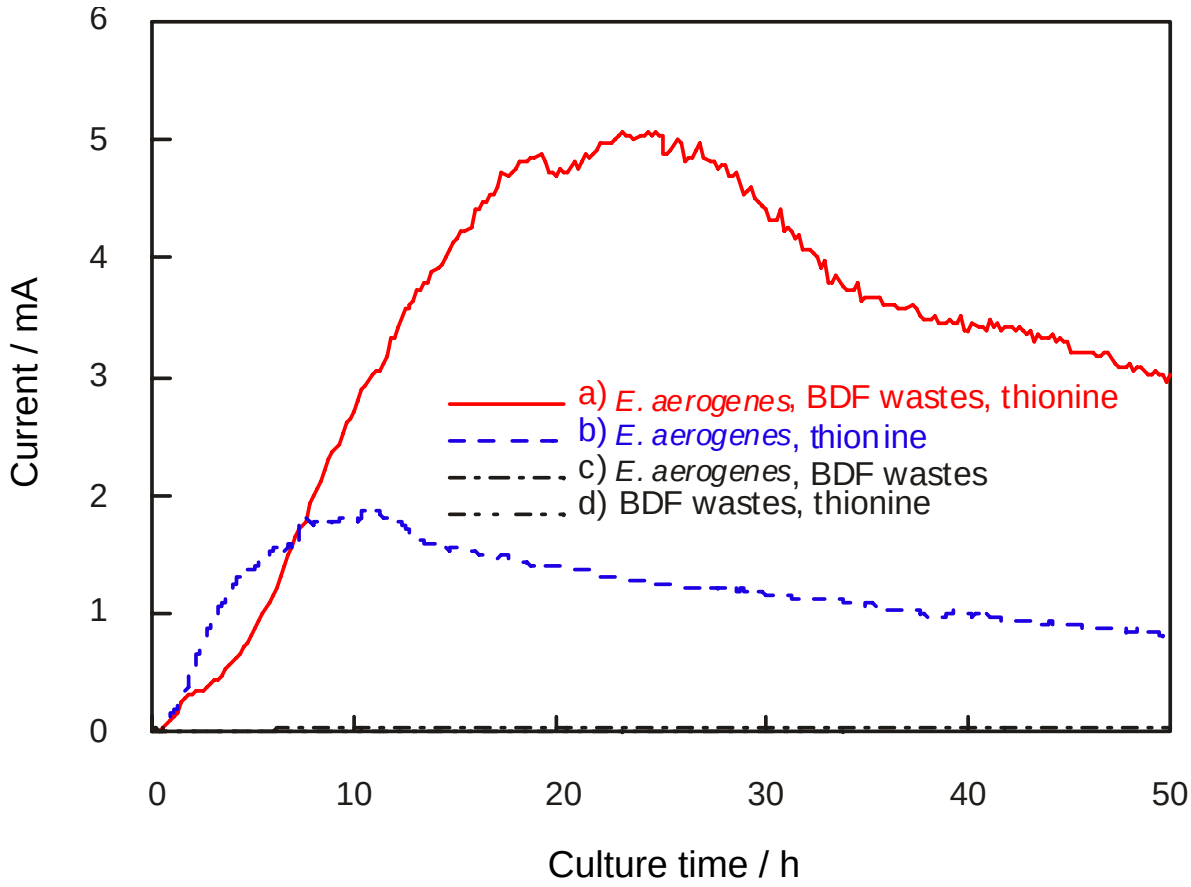
The anode solution contained:
- 4% (v/v) bacteria suspension
- Waste glycerol solution
- 1 or 2 mM thionin
- in NBRC702 medium except for yeast extract

The cathode solution was 0.1M phosphate buffer

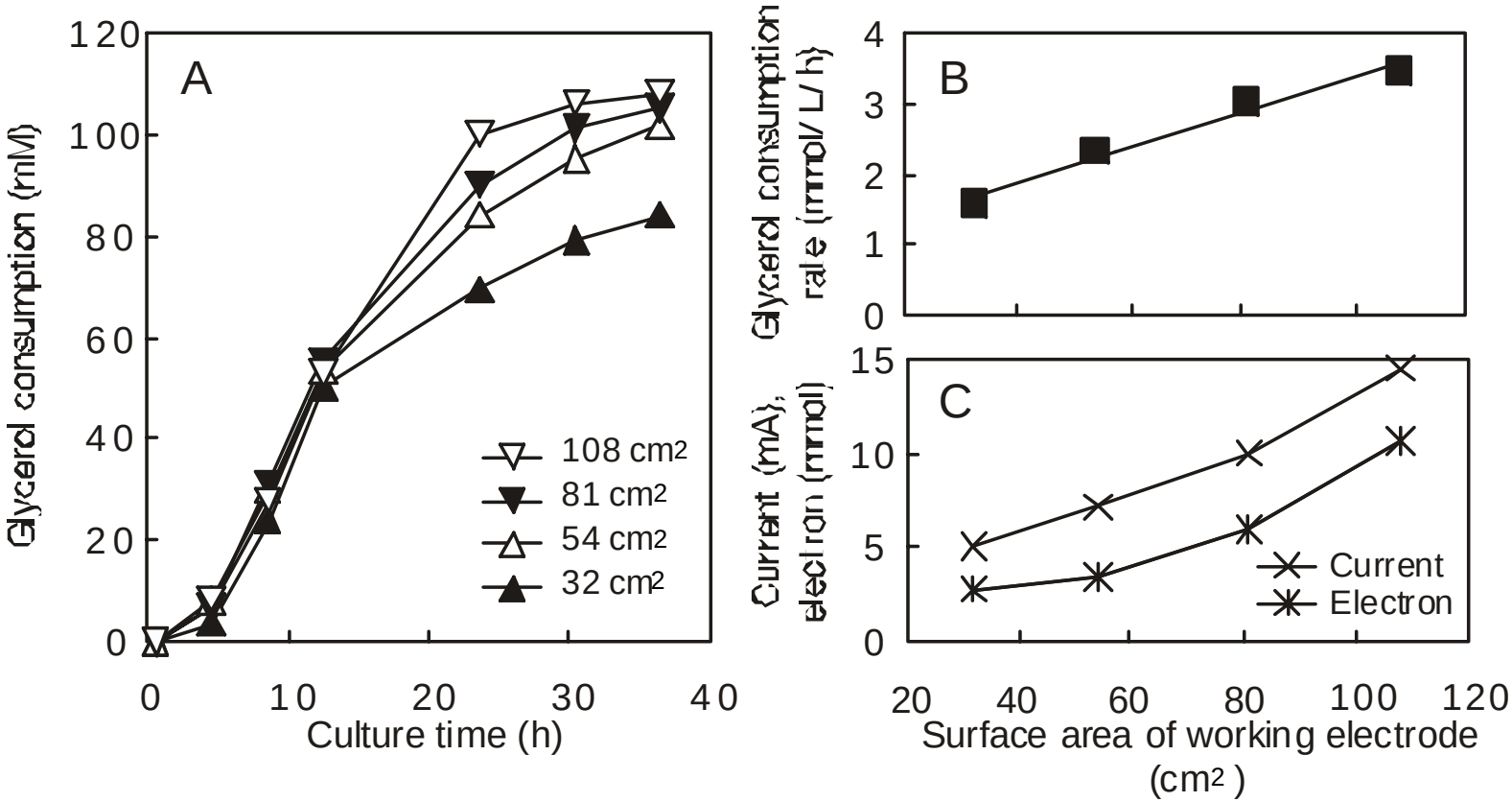
These experiments were performed at 30ºC with rubber heaters with continuous stirring with a magnetic stirrer, and its pH was monitored and controlled by a pH electrode controller to keep pH6.0.



Typical time courses of batch cultures of *E. aerogenes* under control conditions (without thionine and electrode) and in electrochemical system with modified NBRC702 medium (removed yeast extract)



Typical current-time curves in the batch cultures of *E. aerogenes* in electrochemical system



Comparison of batch cultures of *E. aerogenes* in electrode system with four types of working electrodes

A: Time course of glycerol consumption, B: glycerol consumption rate at 24 h cultivation, C: maximum current and total electrons during 36 h cultivation

Fermentation profiles in the batch cultures of E. aerogenes NBRC 12010 grown at the initial concentration of waste glycerol containing 110 and 160 mM glycerol in the electrode system with thionine.

Parameter	with a working electrode of			
	32 cm^2 [a]		108 cm^2 [b]	
Initial glycerol concentration	107	160	108	161
[mM (g/L)]	(9.9)	(14.7)	(9.9)	(14.8)
Culture time (h)	48	60	36	60
Glycerol consumption (mM)	92.9	103	108	154
Yield (mol/mol-glycerol)				
H_2	0.74	0.63	0.77	0.66
CO_2	0.69	0.66	0.80	0.78
Ethanol	0.92	0.86	0.88	0.85
Formate	0.07	0.06	0.04	0.05
Lactate	0.03	0.04	0.03	0.03
Electron	0.16	0.19	0.36	0.41
Recovery (%)				
Carbon	89	86	90	87
Electron	94	89	92	89

[a] Thionine concentration was 2 mM. [b] Thionine concentration was 1 mM.

Conclusion

- Hydrogen and ethanol were produced from *E. aerogenes* NBRC12012 with glycerol-containing wastes discharged from a BDF plant using used food oils.
- The hydrogen yield was higher than that with commercially available glycerol. However, it declined with initial glycerol concentration in BDF wastes.
- Glycerol consumption substantially increased in the bioelectrochemical reactor.

Future studies,

- Identification of inhibitors in glycerol-containing wastes for hydrogen and ethanol production
- Screening of thermophilic bacteria to facilitate distillation of ethanol, and bacteria with activities of the treatment of high concentration glycerol (Ethanol may be returned to the BDF plant to produce fatty acid ethyl ester.)
- Optimization of operation conditions of bioelectrochemical reactors

Acknowledgement

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Thank you for your attention!