Proceedings of the 5th International Symposium on Green and Smart Technologies for a Sustainable Society
Ube, Yamaguchi, JAPAN, 25th -27th, March, 2019

Green and Smart Technologies for a Sustainable Society

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Green and Smart Technologies for a Sustainable Society

2019 年 3 月 20 日印刷
2019 年 3 月 25 日発行

編集　山口大学大学院創成科学研究科
熊切　泉

発行　Organizing Committee at
Yamaguchi University
〒755－8611
宇部市常盤台 2 丁目 16 番 1 号

印刷　有限会社　三共印刷

Printed in Japan

MOBILITY PROGRAM REPORTS

POSTER PRESENTATIONS

Biohydrogen Production by Halophilic Bacteria from Samut Sakhon Salt Pan in Thailand

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Previous studies have shown that biohydrogen production from lignocellulosic biomass was possible through dark fermentation by bacteria. But lignocellulosic biomass requires pretreatment and enzyme hydrolysis into fermentable sugars prior to dark fermentation process. The high concentration of NaOH used after pretreatment process makes it unfavorable for most of bacteria to live. However, halophilic bacteria can survive in high salinity condition, whereas this condition increase osmotic pressure and provide inhibition to most other bacteria's activity. The bacteria in this study have been cultivated from soil sample of commercial salt pan field in Samut Sakhon, Thailand and kept at anaerobic saturated (26%) sodium condition for three years. These halophilic bacteria are capable to produce hydrogen under saturated salt (NaCl) condition without methane (CH4) by-products. In this study, experiments at different pH and temperature conditions were done to determine the optimum condition for biohydrogen production.

Improvement of Oxygen Transfer by increasing Contact area of Gas-Liquid Interface

Passaworn Warunyuwong, Tsuyoshi Imai

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In the current study, an apparatus called air-water interface generator was tested for oxygen transfer enhancement by increasing a contact area between air and water. The effect of this apparatus and its optimal installation were investigated. The total volumetric oxygen transfer coefficient (kLaT) in this study was divided into volumetric oxygen transfer for the bubble transfer (kLaB), volumetric oxygen transfer for the inner interface transfer inside the apparatus (kLaI) and volumetric oxygen transfer for the free water surface transfer (kLaS) The result shows that the apparatus could increase the oxygen transfer caused by the presence of inner interface. However, this interface was ineffective when the apparatus was installed more than one layer or at the positions other than on water surface. Even though the more number of apparatus could increase the contact area between gas phase and liquid phase, the bubble dispersion was limited, resulting in the decrease of bubble transfer which has the significant contribution in oxygen transfer.

Development of cellulose nanofiber membranes

Keisuke Fujii, Izumi Kumakiri, Kazuhiro Tanaka, Hidetoshi Kita

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Cellulose nanofibers are one of the new bio-materials attracting attentions. The hydroxyle groups in the cellulose nanofibers can easily be modified, which changes the adsorption ability of the matarial. In this study, composite nanofiber membranes were prepared using cellulose nanofibers and mullite supports.

BIOHYDROGEN PRODUCTION BY HALOPHILIC BACTERIA FROM SAMUT SAKHON SALT PAN IN THAILAND

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1. Introduction

Hydrogen gas has the highest energy density of common fuels expressed on a mass basis


Palm oil shell


Disposable chopsticks waste


Sugarcane bagasse

Lignocellulosic biomass

Requires alkali/acid pretreatment & enzyme hydrolysis to release fermentable carbohydrates

High concentration of NaCl → inhibition for fermentation by most bacteria

Extremely halotolerant bacteria

- Produces H_2 without producing any methane
- Leans toward more efficient acetic acid pathway
- Can reduce the cost of water, oxygen & sterilization

Objectives:

To investigate optimum hydrogen production by extremely halotolerant bacteria under different temperature and pH conditions after 3 years acclimatization in 26% NaCl condition.

To determine prevalent biohydrogen producer in acclimatized cultures of extremely halotolerant bacteria

2. Materials and Methods


Seed microorganism were collected from Samut Sakhon salt pan, Thailand

Cultivation of fermentation bottles (A)

Gas volume checked periodically with glass syringe

500 mL serum bottle
Liquid + solid volume ± 350 mL

Substrate is changed periodically for each bottle. Mixed liquor is taken out by glass syringe and centrifuged. The supernatant is removed, and pellets are mixed with new substrate

After substrate change, bottle is flushed with N_2 gas to ensure anaerobic condition

Cultivation bottles are kept in 37°C incubator

Experiment of hydrogen production: Basic condition, different pH & T conditions

- 75 ml serum bottles
- 15 mL substrate
- 15 mL seed microorganism from (A)

Bottle filled with N_2 gas to ensure anaerobic condition

pH was adjusted with 0.1 N, 1 N & 3 N NaOH

Gas production checked every 24 hours with wetted glass syringe method

Experiment serum bottles are kept in shaking water bath incubator in controlled temperature, 100 rpm shaking speed

Process development of H_2 production directly from lignocellulosic waste

H_2, N_2, CH_4, and CO_2

Gas chromatography (GC-8APT/TCD; Shimadzu Corp. Japan), 60/80 activated charcoal mesh column, Argon as carrier gas, operation temperatures of the injector, column, and detector of 50 °C, 60 °C, and 50 °C

Volatile Fatty Acids (VFAs)

Gas chromatography (GC-8APF/FID; Shimadzu Corp. Japan), Unisole F-200 30/60 glass mesh column, Nitrogen as carrier gas, operation temperatures for the injection port, FID detector, and oven were 250°C, 140°C, and 140°C

PCR-DGGE

- 16s-rRNA PCR using 27F & 1492R primers, V3 region PCR using 341F-GC & 518R primers, PCR after band excision using 341F & 518R primers
- 8% Gel, 30-70% denaturant, 1xTAE buffer, 60°C, 70V for 16 hours, SYBR Gold staining

Substrate composition

$NaHCO_3$ (buffer)		2 gram/L
K_2HPO_4 (buffer)		2 gram/L
Yeast extract		1 gram/L
Glucose		10 gram/L
NaCl		26% w/v (26% =351.35 gram/L)
Trace elements	A	2 mL/L
	B	10 mL/L
	C	1 mL/L

B	KCl	75 gram/L
	NH_4Cl	85 gram/L
	$FeCl_3 \cdot 6H_2O$	42 gram/L
	$MgCl_2 \cdot 6H_2O$	81 gram/L
	$MgSO_4 \cdot 7H_2O$	25 gram/L
	$CoCl_2 \cdot 6H_2O$	1.8 gram/L

A	$(NH_4)_2HPO_4$	350 gram/L

C	$CaCl_2 \cdot 2H_2O$	150 gram/L

Theoretical cumulative hydrogen calculation

$$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2 \quad (1)$$

$$C_6H_{12}O_6 + 2H_2 \rightarrow CH_3(CH_2)_2COOH + 2CH_3COO^- + H^+ + 2H_2 \quad (2)$$

Equation (1): 1 mol of glucose will produce 4 mol of hydrogen → 1 g of glucose STP conditions produce 498 mL of H_2 via the acetic acid (HAc) pathway
Equation (2): 1 mol of glucose will produce 2 mol of hydrogen → 1 g of glucose at STP conditions produce 249 mL of H_2 via the butyric acid (HBu) pathway

$$\textit{Hydrogen yield}\ (\%) = \frac{\textit{Observed cumulative hydrogen}\ (mL)}{\textit{Theoretical cumulative hydrogen}\ (mL)} \times 100$$

pH experiment (7.5, 8.0, 9.0, 9.5 &10) at 37°C (cultivation temperature) → T experiment (35, 40, 42 & 45°C) at optimum pH of 9 → % NaCl experiment (15%, 20% & 26%) at 35°C, pH9

Maximum theoretical cumulative hydrogen for this experiment: 74.67 mL (HAc pathway)

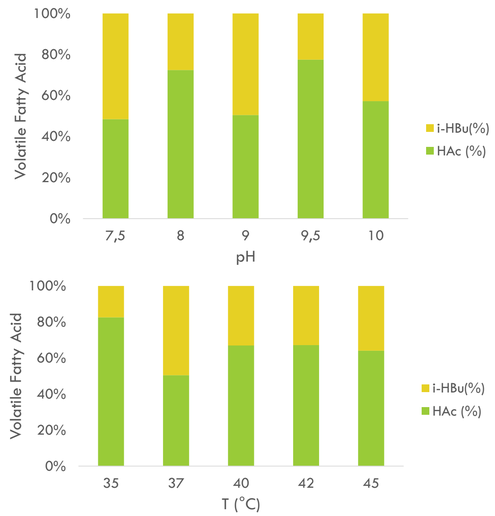
3. Results and discussion

Biohydrogen production at 26% (w/v) salinity after two years of acclimatization

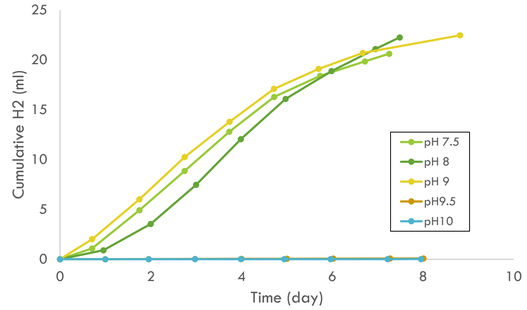
Soil Sample	Biohydrogen production (ml)	H_2 yield (HAc pathway)	Yield ($molH_2/mol_{glucose}$)
Samut Sakhon salt pan	13.44	18%	1.08
Khon Kaen salt damaged soil (1)	14.31	23%	1.20
Khon Kaen salt damaged soil (2)	8.22	1%	0.78
Glucose 0.12 g (5 g/L), inoculum 3,000 mg/L VSS, T 37°C, initial pH 6.6 (unadjusted)			

Biohydrogen production at 26% (w/v) salinity of Samut Sakhon salt pan microorganism after three years of acclimatization

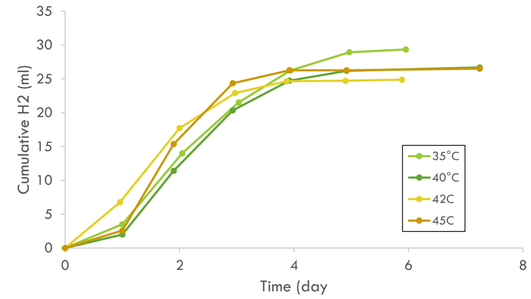
T (°C)	pH	Biohydrogen production (ml)	H_2 yield (HAc pathway)	Yield ($molH_2/mol_{glucose}$)
37	7.5	20.61	28%	1.11
37	8	22.25	30%	1.19
37	9	22.47	30%	1.20
37	9.5	0.08	0%	0.00
37	10	0.02	0%	0.00
35	9	29.35	39%	1.57
40	9	26.69	36%	1.43
42	9	24.88	33%	1.33
45	9	26.52	36%	1.42
Glucose 0.15 g (10 g/L), inoculum 465 mg/L VSS				



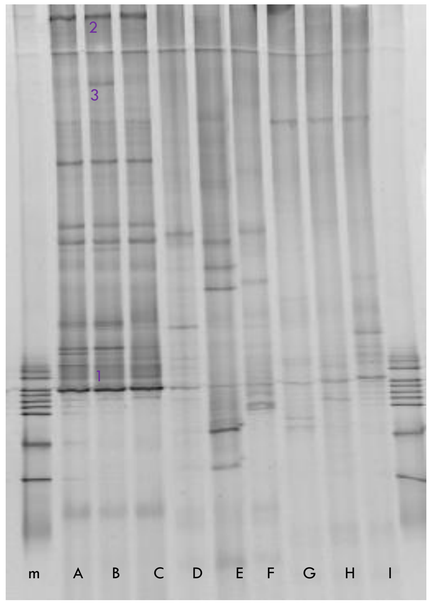
VFA composition for 9 F/M ratio at 26% salinity of Samut Sakhon salt pan microorganism after three years of acclimatization (HAc= acetic acid, i-HBu = isobutyric acid)



Cumulative hydrogen production for different pH conditions (T 37°C)



Cumulative hydrogen production for different temperature conditions (pH 9)

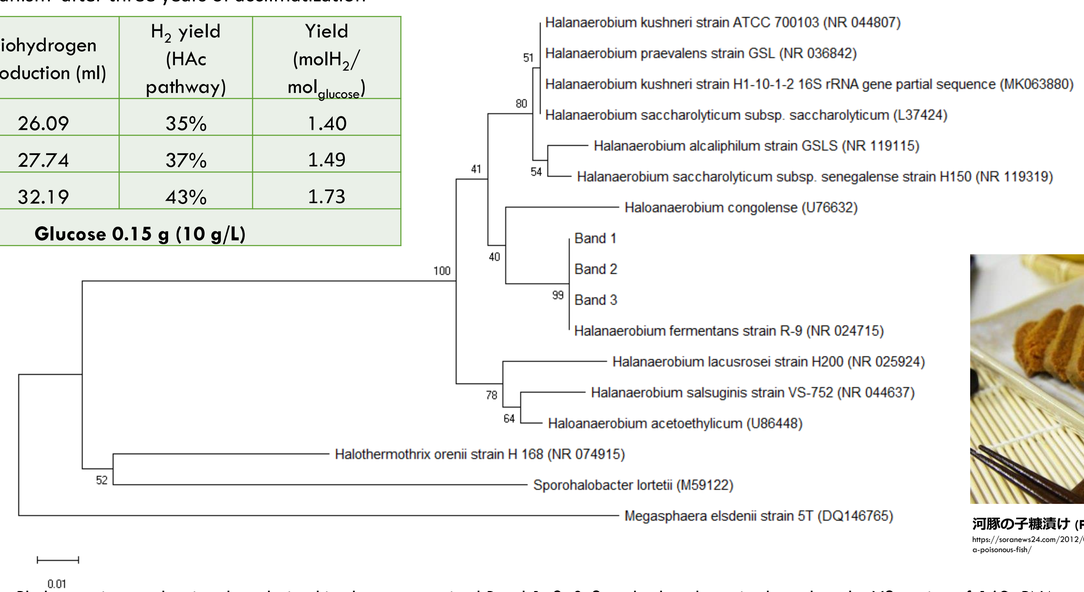


PCR-DGGE Result

(m. Nippon Gene DGGE marker II, A. Khon Kaen 1 cultivation bottle, B. Samut Sakhon cultivation bottle, C. Khon Kaen 2 cultivation bottle, D. Anaerobic digester of Yagi Bioecology Center, E. UASB reactor granules, F. Soil sample from the shore of salt damaged soil from Khon Kaen, G. Soil sample Salt damaged soil from Khon Kaen, farther from the shore, H. Soil sample from the surface of salt pan in Samut Sakhon, I. Soil sample from the surface of dry salt pan in Samut Sakhon)

Biohydrogen production at 35°C, pH 9 of Samut Sakhon salt pan microorganism after three years of acclimatization

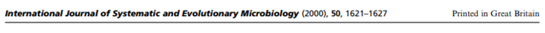
NaCl	Biohydrogen production (ml)	H_2 yield (HAc pathway)	Yield ($molH_2/mol_{glucose}$)
15%	26.09	35%	1.40
20%	27.74	37%	1.49
26%	32.19	43%	1.73
Glucose 0.15 g (10 g/L)			



Phylogenetic tree showing the relationships between excised Band 1, 2, & 3 and related species based on the V3 region of 16S rRNA gene. The tree was based on Jukes-Cantor distance and constructed using Neighbor-Joining method with 1,000 bootstrap using Mega X. The scale bar represents 0.01 substitutions per nucleotide position. *Megasphaera elsdenii* was used to root the tree. The percentage of replicate trees clustered together in the boostrap test are indicated at the nodes. Reference sequences in the dendrogram were obtained from NCBI's GenBank.

河豚の子糠漬け (Fugu no ko nukazuke)

https://soranews24.com/2012/06/29/treat-yourself-to-this-japanese-delicacy-if-you-dare-ovaries-from-a-poisonous-fish/

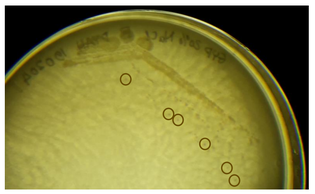


International Journal of Systematic and Evolutionary Microbiology (2000), 50, 1621–1627 Printed in Great Britain

Haloanaerobium fermentans sp. nov., a strictly anaerobic, fermentative halophile isolated from fermented puffer fish ovaries

Takeshi Kobayashi, Bon Kimura and Tateo Fujii

"To isolate and purify the new isolate, we used GYP agar containing (l^{-1} distilled water): 10 g glucose, 10 g yeast extract, 5 g Bactopeptone, 2 g meat extract (Kyokuto), 2 g $CH_3COONa.3H_2O$, 5 g $CaCO_3$, 5 ml salt solution (l^{-1}: 40 g $MgSO_4.7H_2O$, 2 g $MnSO_4.4H_2O$, 2 g $FeSO_4.7H_2O$, 2 g NaCl), 0·25 g Tween 80, 200 g NaCl and 12 g agar. A GasPak anaerobic system (BBL) was used for cultivation."



Colonies were observed after 4 days

4. Conclusions

- Optimum condition for biohydrogen production by microorganism from Samut Sakhon salt pan after 3 years of acclimatization was at 35°C, pH 9, and 26% NaCl.
- From PCR-DGGE, sequencing and BLAST results, the strong bands in acclimatized halotolerant cultivation bottles showed 100% similarities of 16s-rRNA's V3 regions *Halanaerobium fermentans* strain R-9.
- 16s-r RNA colony PCR with EZBioCloud's 16s-based identification confirmed 99.36% similarity with *Halanaerobium fermentans* strain R-9.

5. Acknowledgments

The authors thank Yoshiki Tsuda for his valuable assistance in data collection. The first author would like to gratefully acknowledge the Indonesian Endowment Fund for Education (LPDP) and The Directorate General of Higher Education (DIKTI) for providing BUDI-LN scholarship. This work was supported by JSPS Core-to-Core Program, A. Advanced Research Networks which was financially supported by Japan Society for the Promotion of Science (JSPS).