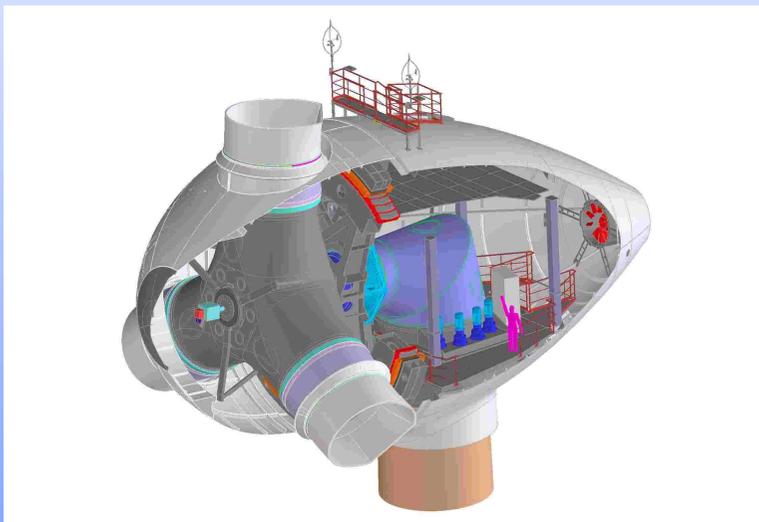


Development Plan in High Speed, High Yield Hydrogen Production by Fermentation

IPHE Renewable Hydrogen Workshop
Seville 24-26 October 2005



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Dept. of Environmental Sciences
Yokohama National University
JAPAN

General Information

(partners, duration, budget, etc...)

x **Babcock-Hitachi K.K.**

**National Institute of Advanced Industrial Science and
Technology (AIST)**

✓ **From 09/03**

□ **NEDO Project**

**“Development for Safe Utilization and
Infrastructure of Hydrogen”**

Objectives

- x Utilization of Biomass Wastes as Substrates for Hydrogen Producing Fermentative Bacteria.**

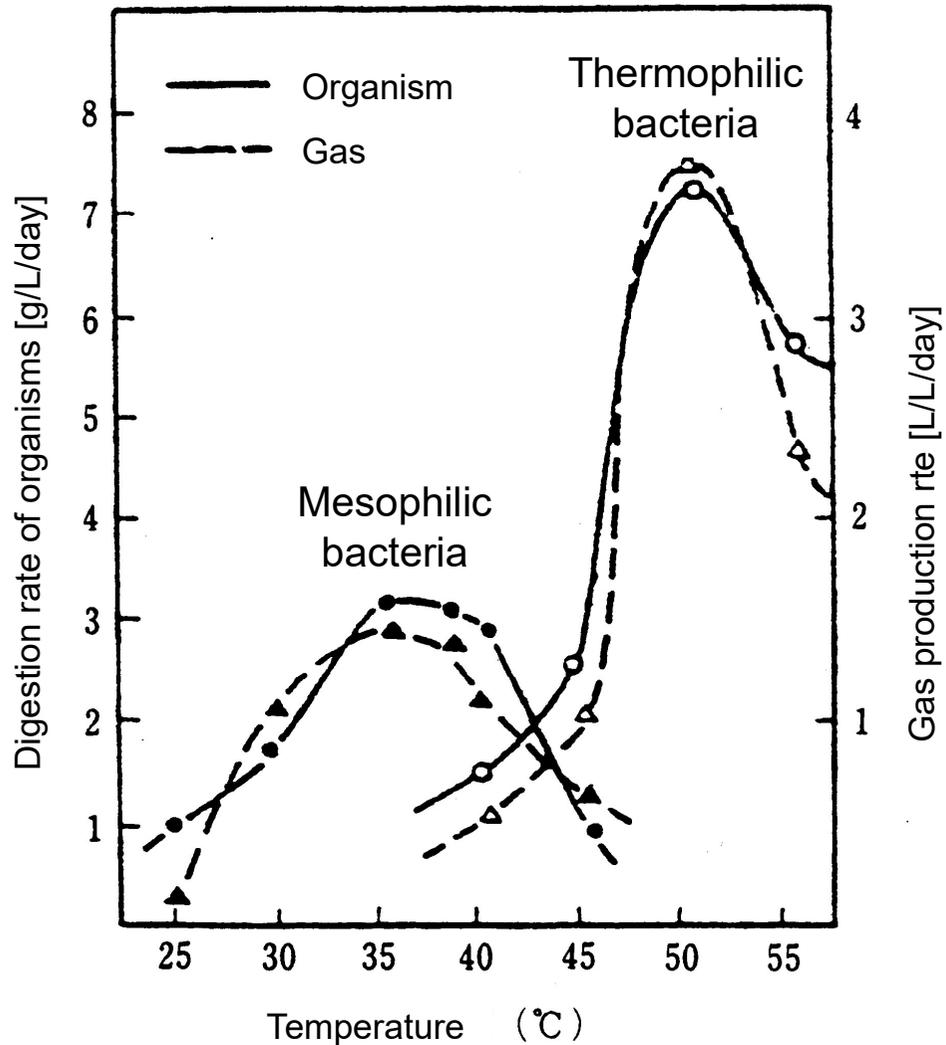
in Japan:

Excreta & urine of livestock	91 Mton/y
Food wastes	19 Mton/y
Straws of rice, wheat, etc.	13 Mton/y

Main challenges

- × **Screening of Thermophilic, High Speed Hydrogen Producing Bacteria.**
- × **Improvement of Hydrogen Yield by Gene Manipulation.**
 - Inhibit NADH dehydrogenase placing at the NADH dehydrogenase complex in the electron transport chain to use NADH for H₂ production under aerobic condition.**

Arrhenius effect on the production rate of H₂

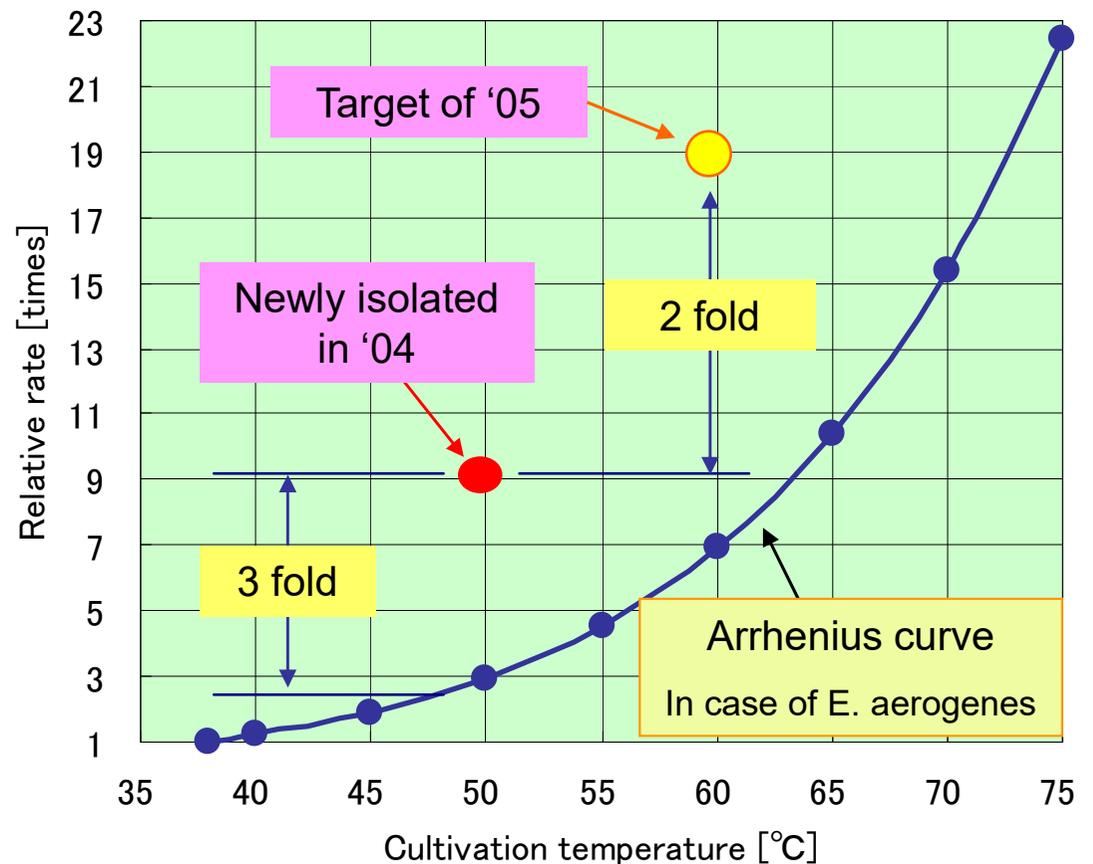


Effect of temperature on methane fermentation

Arrhenius equation

$$\frac{d \ln k}{dT} = \frac{E}{RT^2}$$

$$k = A \exp\left(-\frac{E}{RT}\right)$$



Main Achievements

- × **Obtained a Very Effective Mesophilic Bacterium.**

at Batch cultivation:

Max. H₂ production rate:

3.6 NL-H₂/L·h at 47°C from synthetic culture

1.4 NL-H₂/L·h at 37°C from artificial garbage

H₂ yield : 2.5 mol-H₂/mol-glucose

55 NL-H₂/kg-wet artificial garbage

By-product yields (mol/mol-glucose):

Ethanol 0.92 and Acetate 0.88 at 50°C

Ethanol 0.10 and Acetate 0.51 at 37°C

Main Achievements

× at Fed Batch cultivation:

using artificial garbage (exchanged 80% culture at after every 10 h cultivation, 37°C)

Concentration of feed:

0.1 kg- wet artificial garbage/L-culture

Max. H₂ production rate:

1.4 NL-H₂/L·h at 37°C

H₂ yield : 55 ~ 60 NL-H₂/kg-wet artificial garbage

By-product ratio:

Ethanol 13% , Acetate 38% and Butyrate 24%

Hydrogen Production by Fermentation



Japanese road map for hydrogen production from renewable energy

Thema (~2003)	Task		
	Short term (2004~2007)	Middle term (2010)	Long term (2015~2020)
Hydrogen from renewable energy	<ul style="list-style-type: none"> •Photo catalitic : Conversion efficiency 1% •Biological : above 1.0 m³/h·m³ 	<ul style="list-style-type: none"> •Photo catalitic : Conversion efficiency 5% •Biological : above 2.0 m³/h·m³ 	<ul style="list-style-type: none"> •Photo catalitic : Conversion efficiency 10% •Biological : above 5.0 m³/h·m³
<Long-term subjects>	<ul style="list-style-type: none"> •Biological hydrogen production from biomass : Screening of new bacteria, Development of effective fermenters, hydrogen production rate, Pyrolysis, Gassification •Photo-biological hydrogen production : Direct production by photo-catalyst, Development of effective photo-bioreactor, conversion efficiency •Utilization of cell function : Biomolecular devices, Utilization of photosynthetic protein as devices, Development of yield, etc. •Hydrogen production by using natural energy such as solar, wind, geothermal, etc. 		

Hydrogen Production by Fermentation



Japanese road map for hydrogen production from renewable energy

2015~2020

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<Long-term subjects>	<p>1.0 m³/h·m³ 2.0 m³/h·m³ 5.0 m³/h·m³</p> <ul style="list-style-type: none"> Direct production by photo-catalyst, Development of effective photo-bioreactor, conversion efficiency Utilization of cell function : Biomolecular devices, Utilization of photosynthetic protein as devices, Development of yield, etc. Hydrogen production by using natural energy such as solar, wind, geothermal, etc. 		

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•Biological hydrogen production from biomass :
 Screening of new bacteria,
 Development of effective fermenters and
 hydrogen production rate

 •Utilization of cell function :
 Biomolecular devices,
 Utilization of photosynthetic protein as devices,
 Development of yield, etc.

Hydrogen Production by Fermentation



Table. Hydrogen yields and production rates by microorganisms as reported in the literature.

	culture	pH	Temp.	substrate	yield ¹⁾	rate	Author
		[-]	[°C]		[mol/mol]	[mmol/L·h]	
Strict anaerobes							
Clostridium sp. no 2	B	6.0	36	glucose	2.0	24	1992 Taguchi et al.
C. paraputrificum M-21	B	-	37	GlcNAc	2.5	31	2000 Evvyernie et al.
C. butyricum LMG1213tl	C	5.8	36	glucose	1.5	22	1986 Heindrichx et al.
Clostridium sp. no 2	C	6.0	36	glucose	2.4	21	1990 Taguchi et al.
Mesophilic bacterium HN001	B	6.0	47	glucose	2.3	147	2004 Nishiyama et al.
Thermophiles							
Thermotoga maritima	B	-	80	glucose	4.0	10	1994 Schroder et al.
Thermotoga elfii	B	7.4	65	glucose	3.3	3	2002 van Niel et al.
Caldicellulosiruptor saccharolyticus	B	7.0	70	sucrose	3.3	8	ibid.
Facultative anaerobes							
E. aerogene E.82005	B	6.0	38	glucose	1.0	21	1983 Tanisho et al.
E. cloacae IIT-BT 08 wt	B	-	36	glucose	3.0	35	2000 Kumar et al.
E. aerogenes E.2005	C	6.0	38	molasses	0.7	36	1993 Tanisho et al.
E. aerogenes HU-101 m AY-2	C	-	37	glucose	1.1	58	1998 Rachman et al.
Co-culture or Mixed cultures from:							
C. butyricum IFO13949 + E. aerogenes HO-39	C	5.2	36	starch	2.6	53	1998 Yokoi et al.
-sludge compost	C	6.8	60	waste water	2.5	8	1996 Ueno et al.
-sewage sludge	C	5.7	35	glucose	1.7	30	1999 Lin et al.
-fermented soybean meal	C	6.0	35	glucose	1.4	8	2000 Mizuno et al.

* Vrije & Claassen, "Dark hydrogen Fermentation", in Bio-methane & Bio-hydrogen, ed. Reith et al. (2003), ISBN:90-9017165-7

1) [mol/mol-monosacch.]

Hydrogen Production by Fermentation



Table. Hydrogen yields and production rates by microorganism and substrate at different temperatures.

Microorganism	culture	pH	Temperature (°C)	Substrate	Yield (mol/mol)	Production rate (mmol/L·h)	Author
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147 mmol/L·h
= 3.3 L/L·h

47 °C

65 ~ 80 °C

4 mol/mol

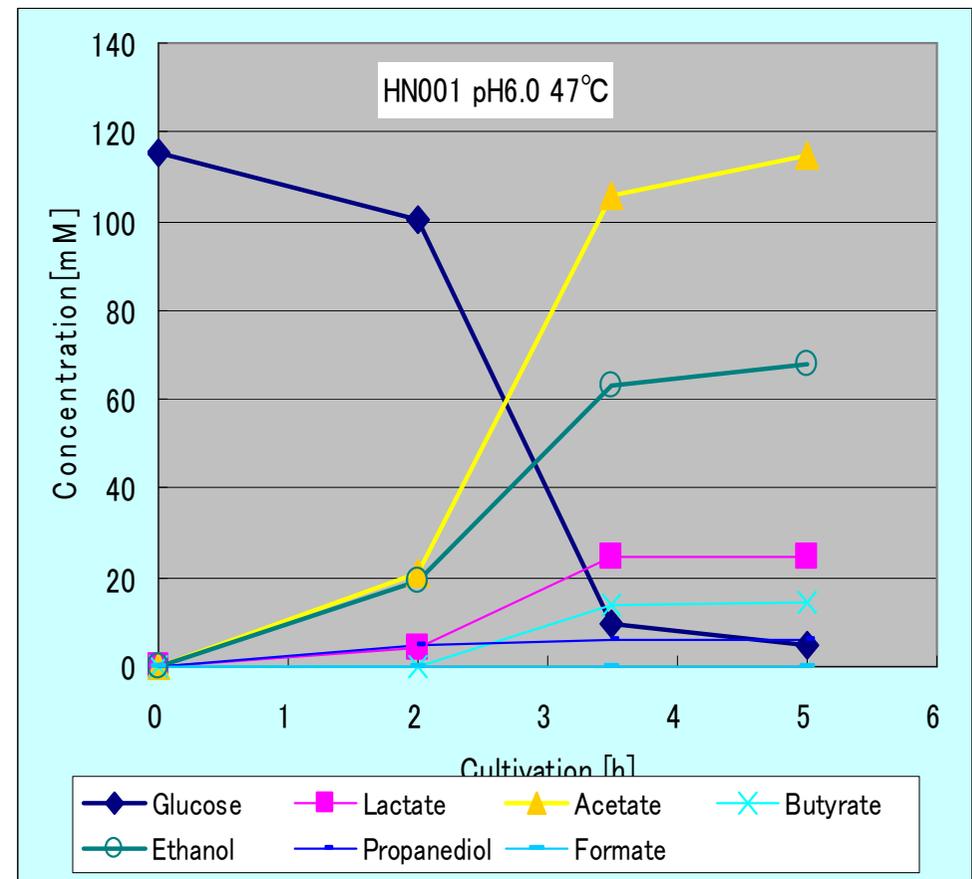
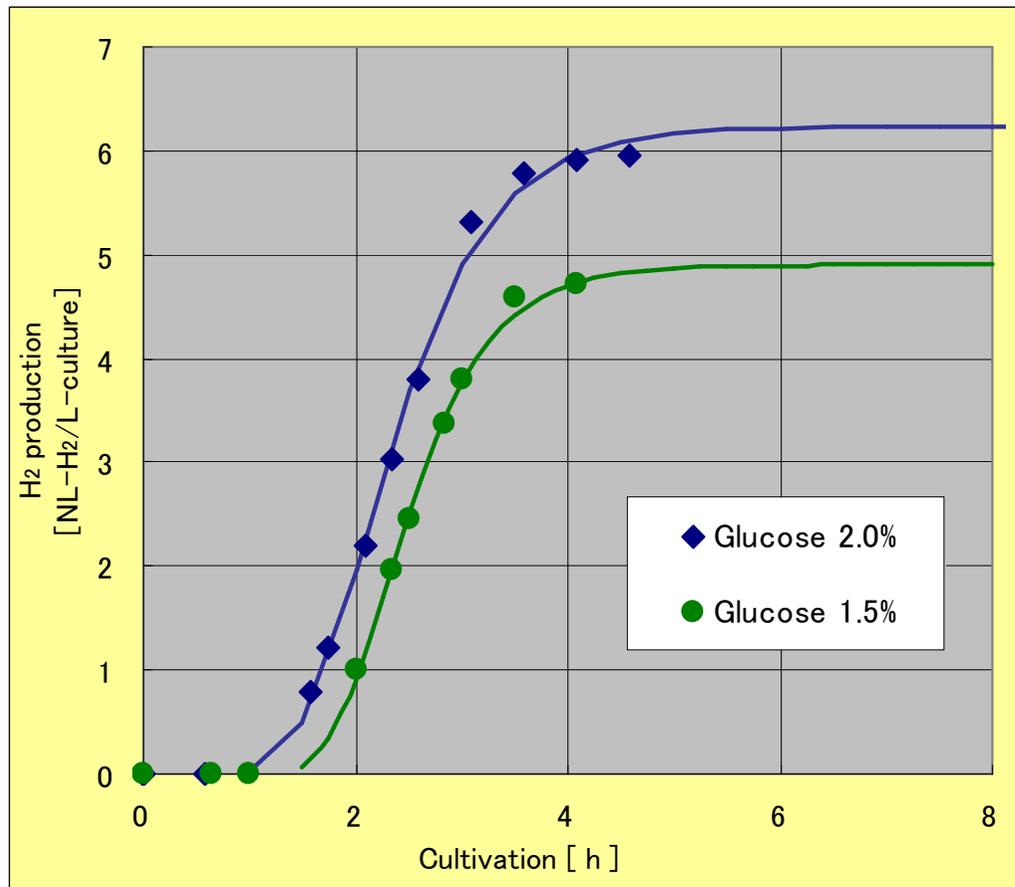
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1) [mol/mol-monosacch.]

Hydrogen Production by Fermentation



Condition of H₂ and metabolites production by newly isolated bacterium HN001



Hydrogen Production by Fermentation



List of newly isolated strain

Sample name	Culture [L]	pH [-]	Temp. [°C]	Feed Conc. [%]	H2 Prod. [L/L]	Max rate [L L ⁻¹ h ⁻¹]	Yield [mol-H ₂ /mol]
HN001	0.35	6.00	47	2.0	6.23	3.61	2.18
HN001	0.35	6.00	47	1.5		3.34	2.25
MZ9-2	0.7	6.00	48			2.93	2.59
HH06-2-2	0.7	6.00	48			2.92	2.1
HH01-4	0.7	6.00	48			2.77	
3F-3-2	0.7	6.00	48	1.5	4.61	2.69	
3F-3-1	0.7	6.00	48	1.5	3.73	2.37	1.76
ON2-1-3	0.7	6.00	48	1.5	3.83	2.36	1.84
HH06-2	0.5	6.00	49	1.5	5.49	2.22	2.40
HH01-2	0.7	6.00	48	1.5	5.02	1.98	2.48
ON2-3	0.6	6.00	49	1.5	4.79	1.96	2.24
HH01-1-2	0.7	6.00	48	1.5	4.06	1.93	1.96
HH06-2-1	0.7	6.00	48	1.5	4.47	1.91	2.18
MZ11-1	0.7	6.00	48	1.5	4.72	1.75	2.32
ON2-1	0.6	6.00	49	1.5	4.80	1.65	2.24
HO10-1-3	0.7	6.00	48	1.5	4.85	1.51	2.38
HH01-1	0.5	6.00	49	1.5	4.95	1.49	2.15

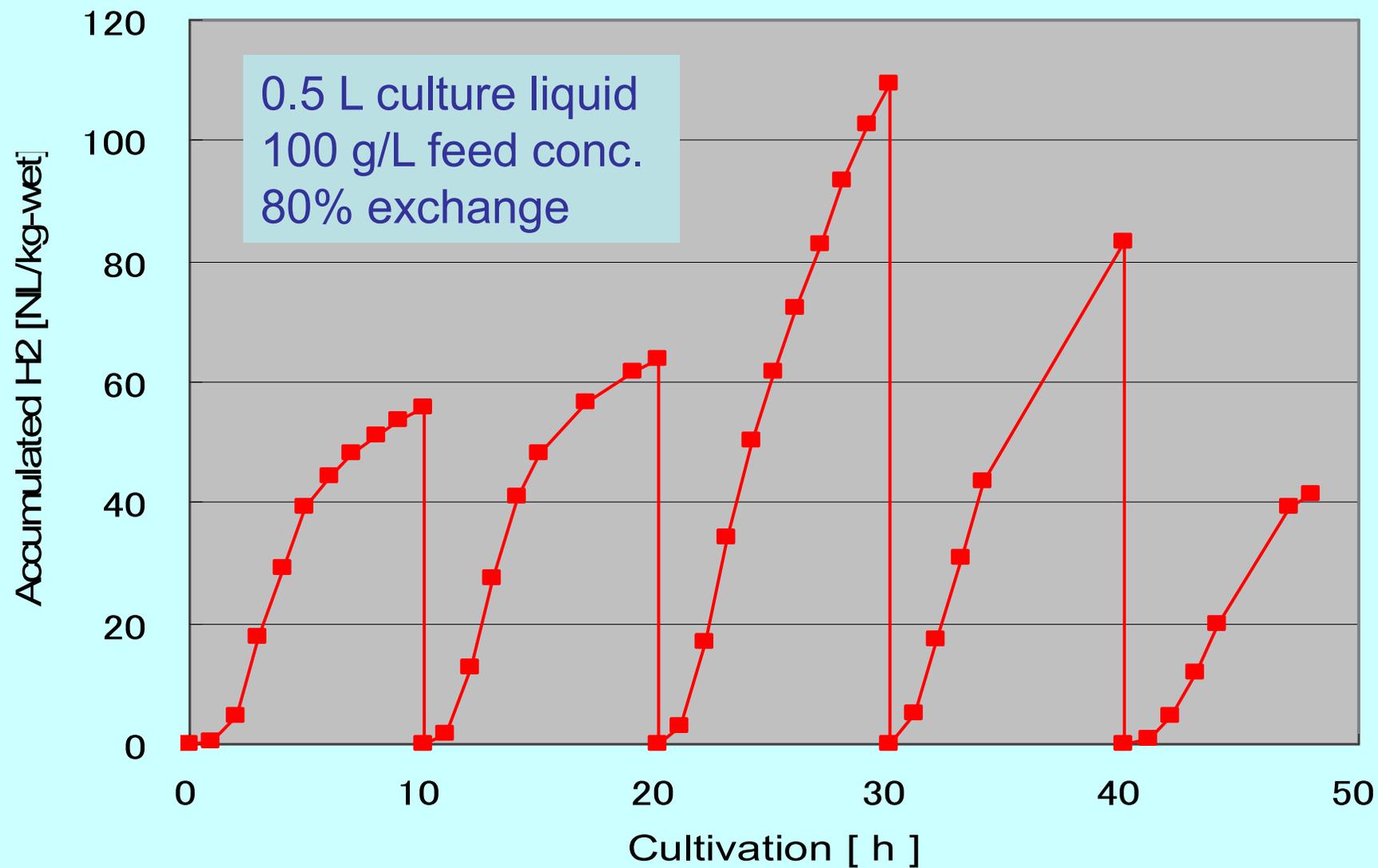
3.6 L/L·h

2.6 mol-H₂/mol

Hydrogen Production by Fermentation



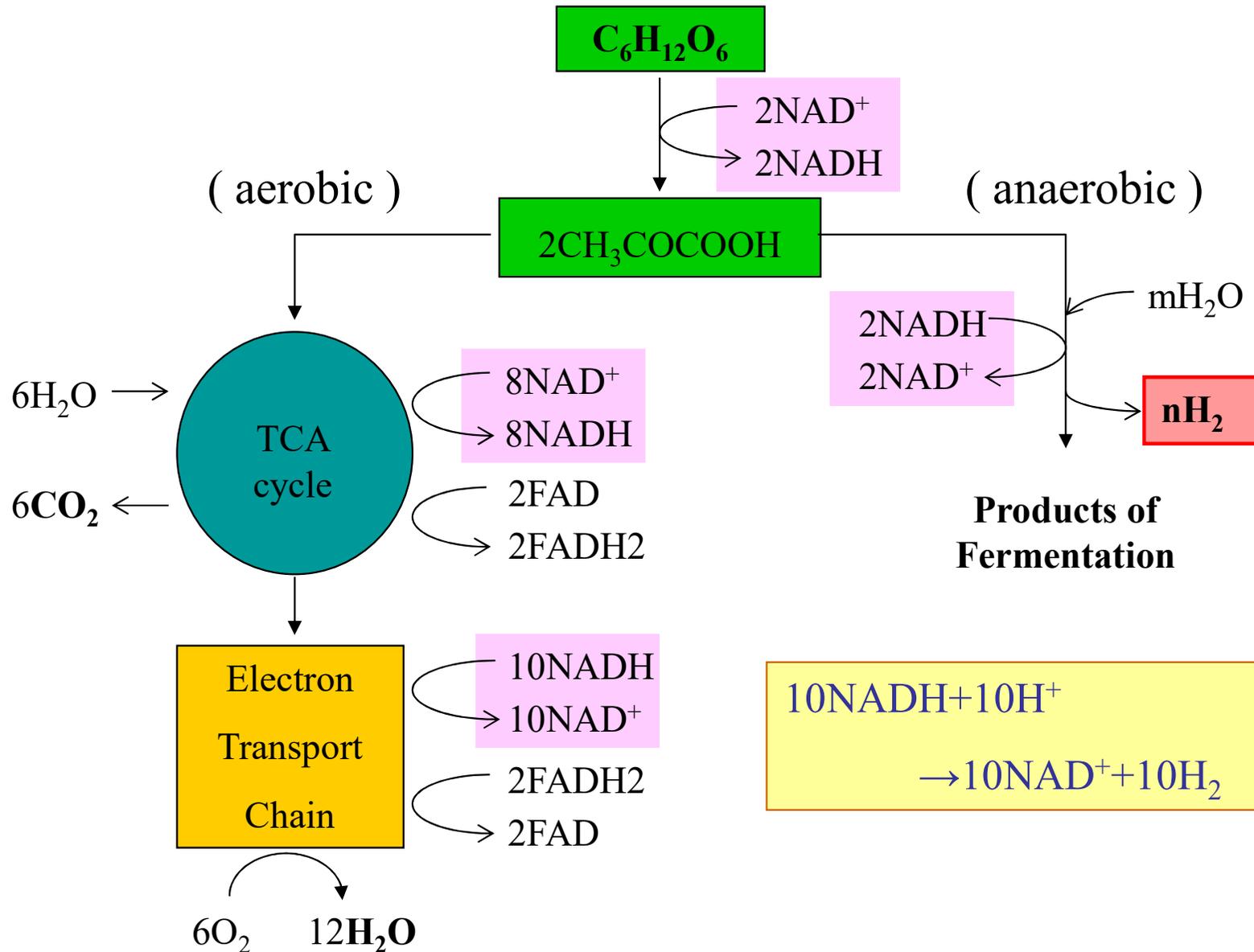
Fed batch hydrogen production using artificial garbage
(Mesophilic bacterium HN001, 37°C, pH 6.0)



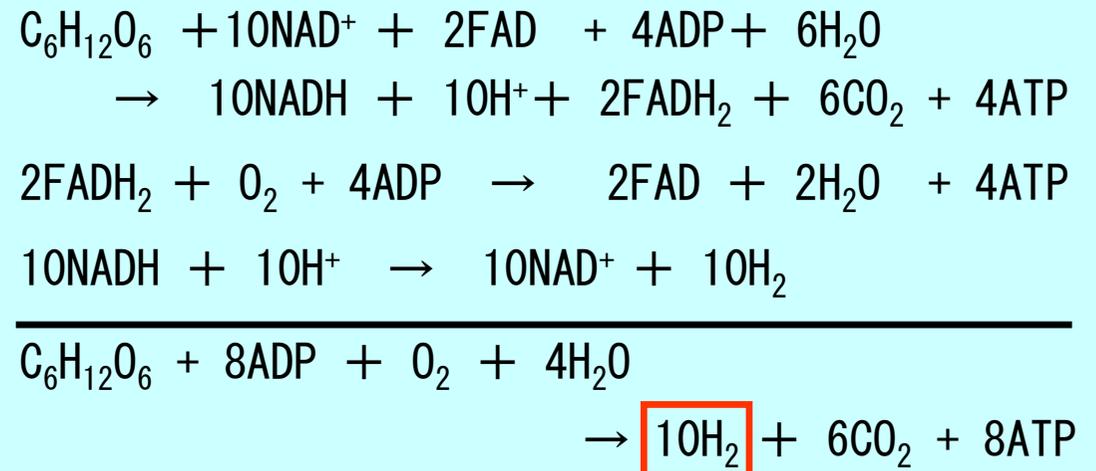
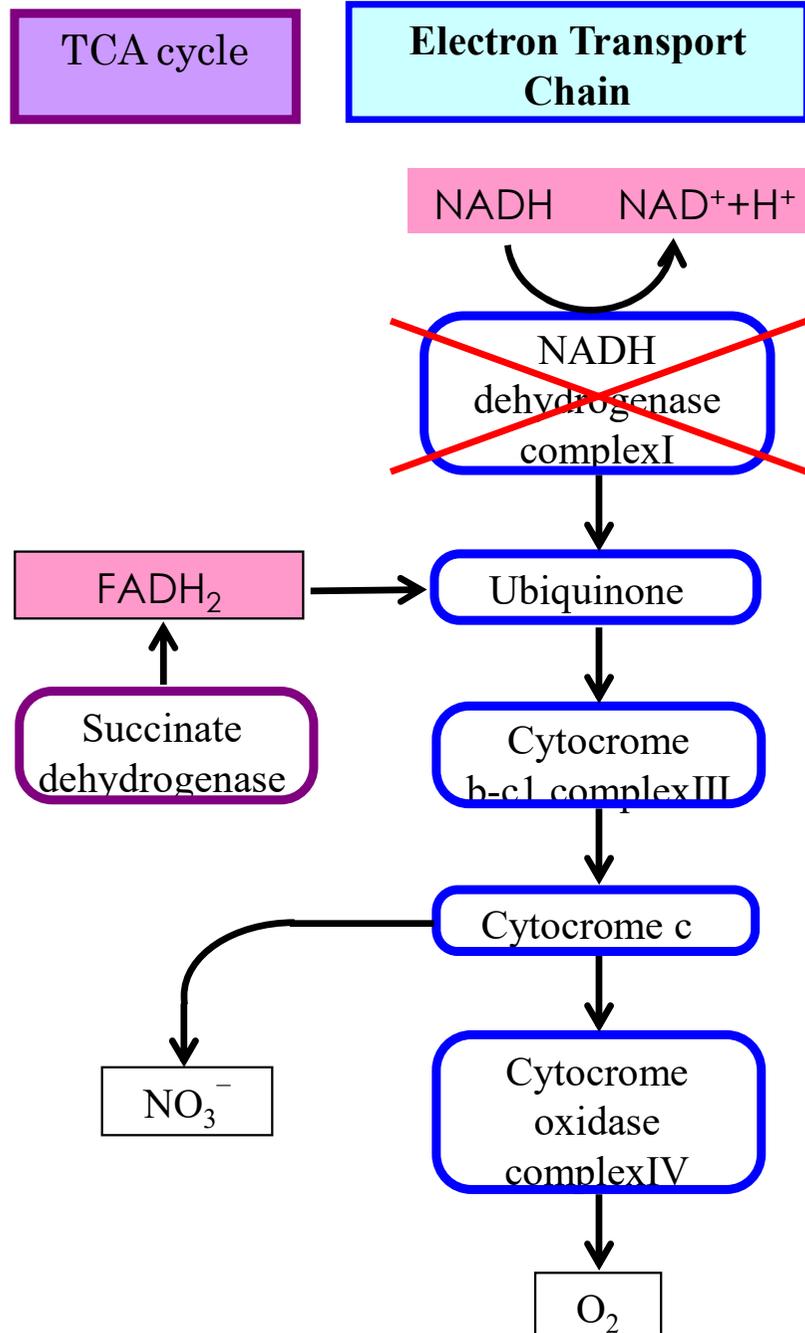
Main challenges

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- × **Improvement of Hydrogen Yield by Gene Manipulation.**
 - Inhibit NADH dehydrogenase placing at the NADH dehydrogenase complex in the electron transport chain to use NADH for H₂ production under aerobic condition.**

NADH production/re-oxidation at aerobic/anaerobic digestion of glucose by facultative anaerobes



Strategy to circulate the TCA cycle while evolving H₂ through NADH pathway



Not yet succeeded!

Future Work

- × **Examination of Various Biomass.**

 - Garbage, Food industry waste, Palm oil mill effluent,
Glycerine from bio-diesel oil waste, etc.**

- × **Gene Manipulation for High Yield.**

 - Continue the inhibition plan of NADH de-hydrogenase
complex**

- × **Reduction of the Waste Liquid from Fermenter.**

Possibility of Co-operation under IPHE

- × **Fermentation is a very old technology, and there is no new technology in our work.**
- × **We may however cooperate on the study for reduction of waste liquid from fermenter.**