



# Fermentative Hydrogen Production by a Newly Isolated Mesophilic Bacterium HN001

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# Summary of this presentation



- Obtained a very effective mesophilic bacterium named HN001.

at pH 6.0, batch cultivation:

Maximum H<sub>2</sub> production rate:

3.6 NL-H<sub>2</sub>/L·h (160mmol/L·h)

at 47°C from synthetic culture

H<sub>2</sub> yield : 2.6 mol-H<sub>2</sub>/mol-glucose

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

# Composition of the synthetic culture



## YNU anaerobic culture:

Casein peptone : 25g/L

Dried Yeast Extract-S : 22g/L

L-Cystein hydrochloride  
monohydrate : 0.3g/L

Mercaptoacetic acid : 0.3g/L

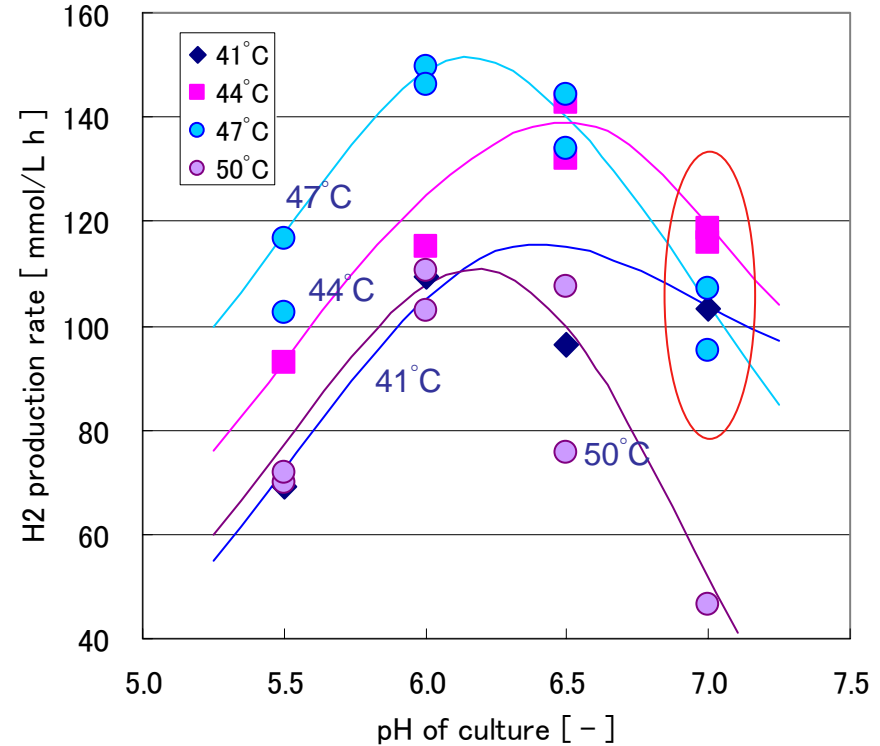
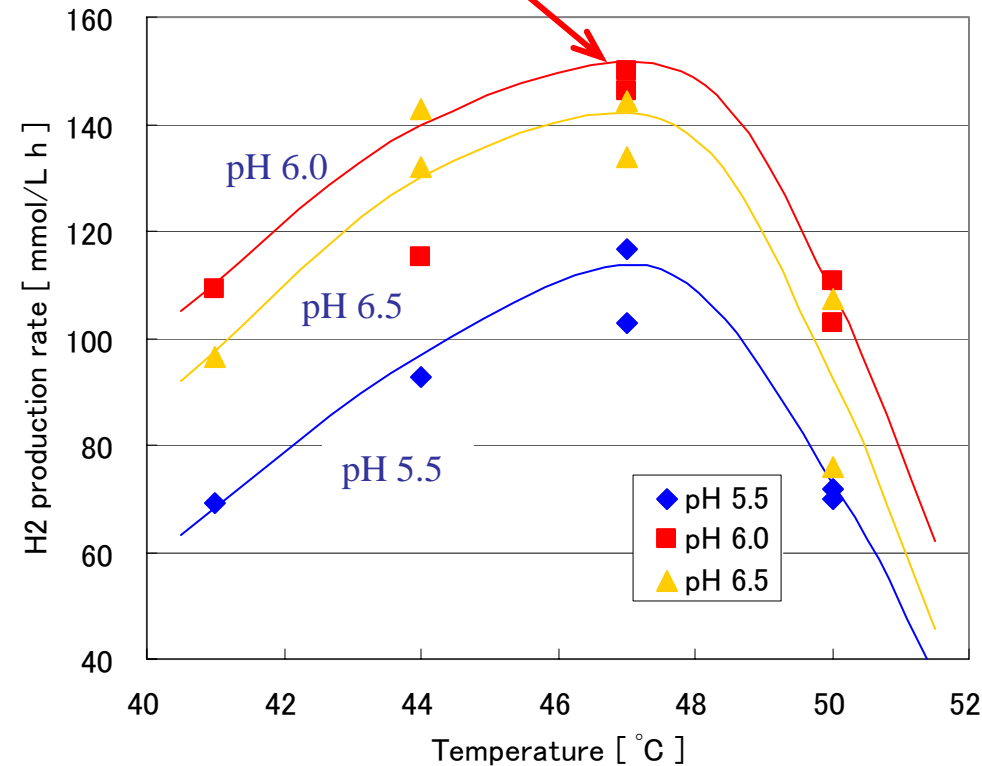
Glucose 15g/L

# Effect of temperature and pH on the H<sub>2</sub> evolution rate



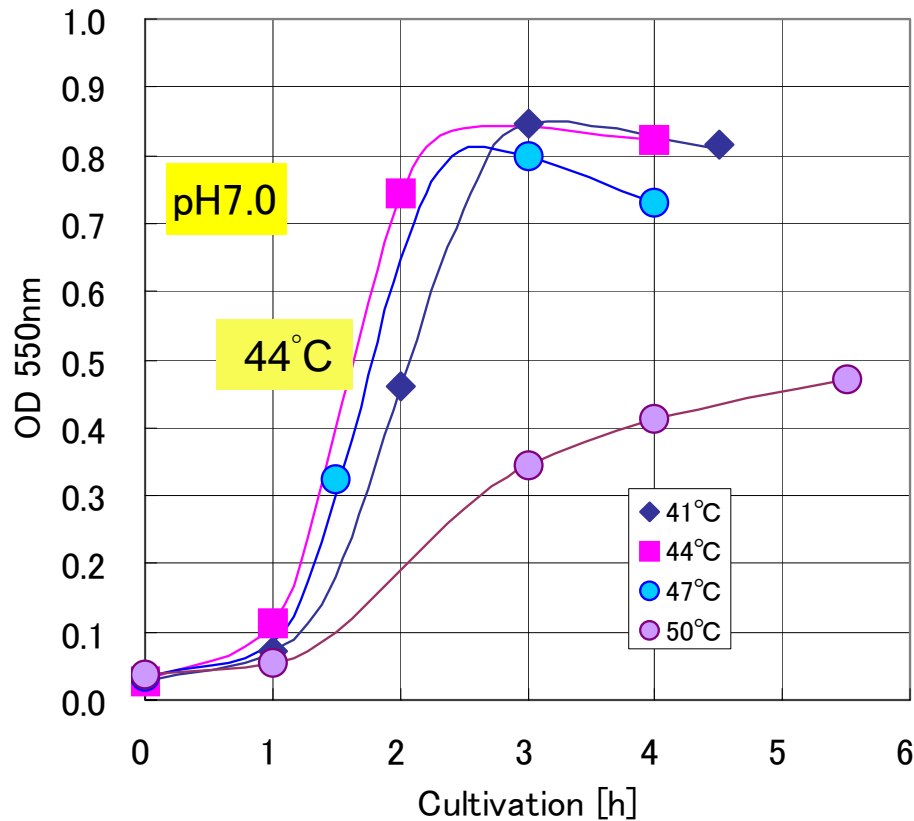
Glucose concentration : 1.5 % (83mM)

147 mmol/(L·h)

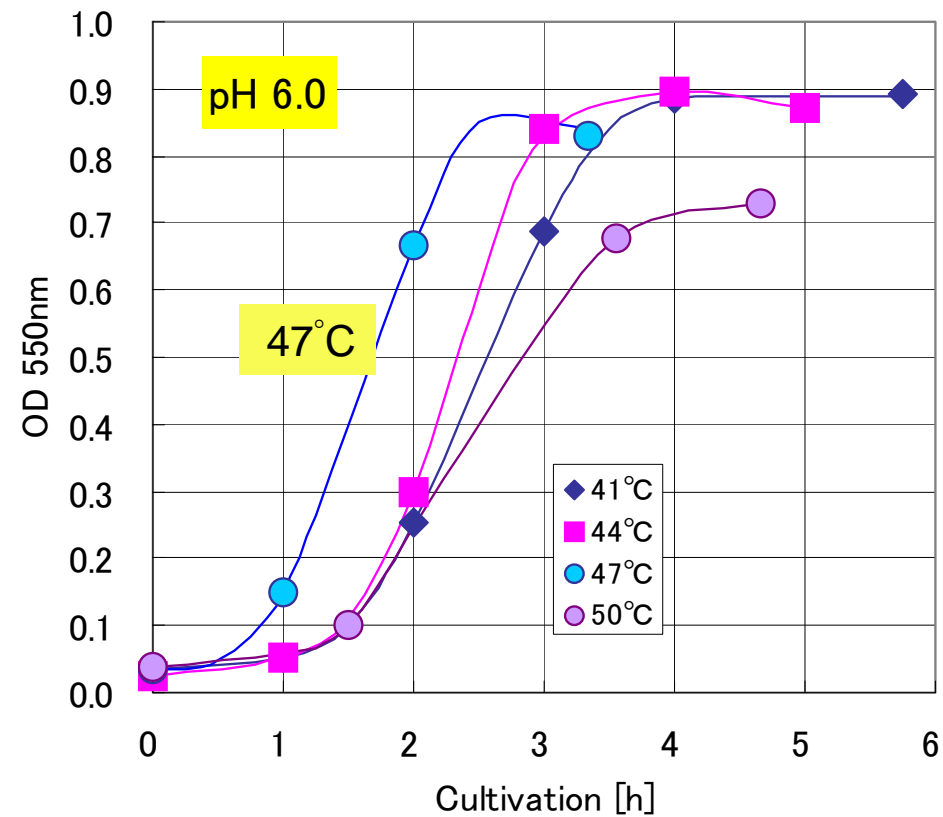


Maximum rate of H<sub>2</sub> production : 147 mmol/(L·h) at pH 6.0, 47° C

# Effect of temperature and pH on the cell production



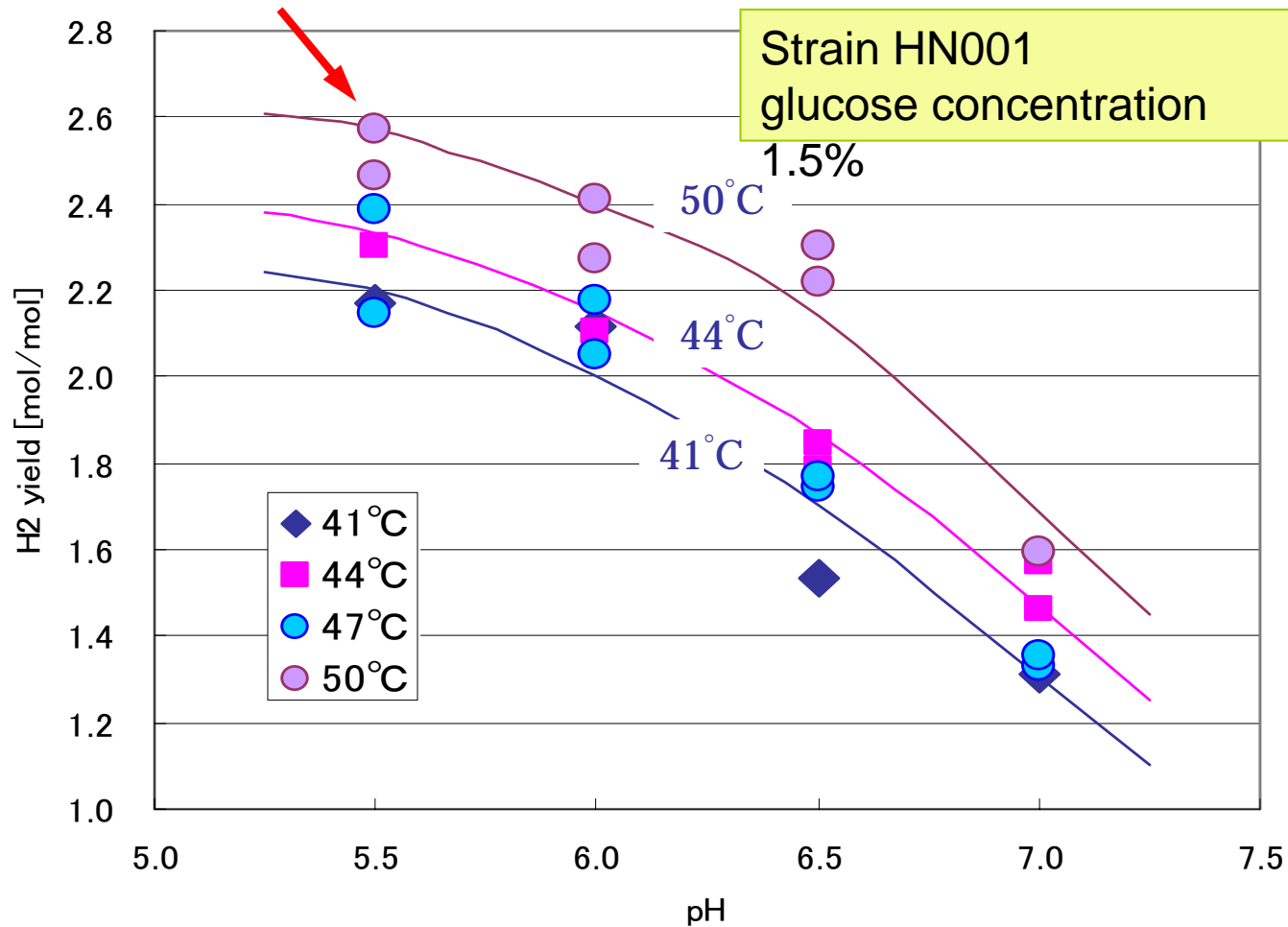
At pH 7.0; 44°C → 47°C → 41°C



At pH 6.0; 47°C → 44°C → 41°C

Suitable temperature for cell production changes with the culture pH.

# Effect of temperature and pH on the H<sub>2</sub> yield

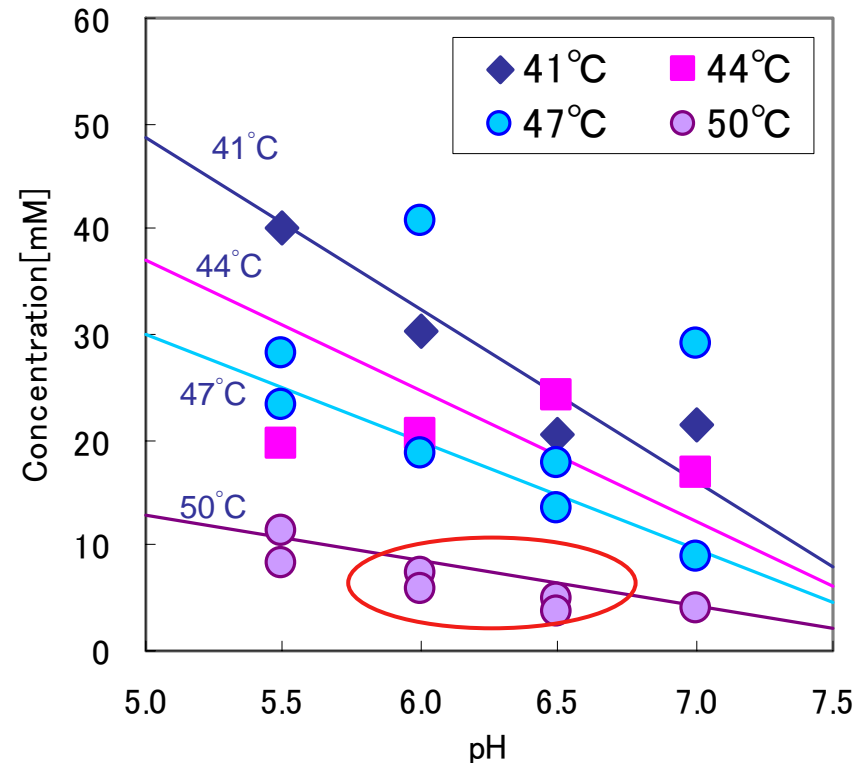
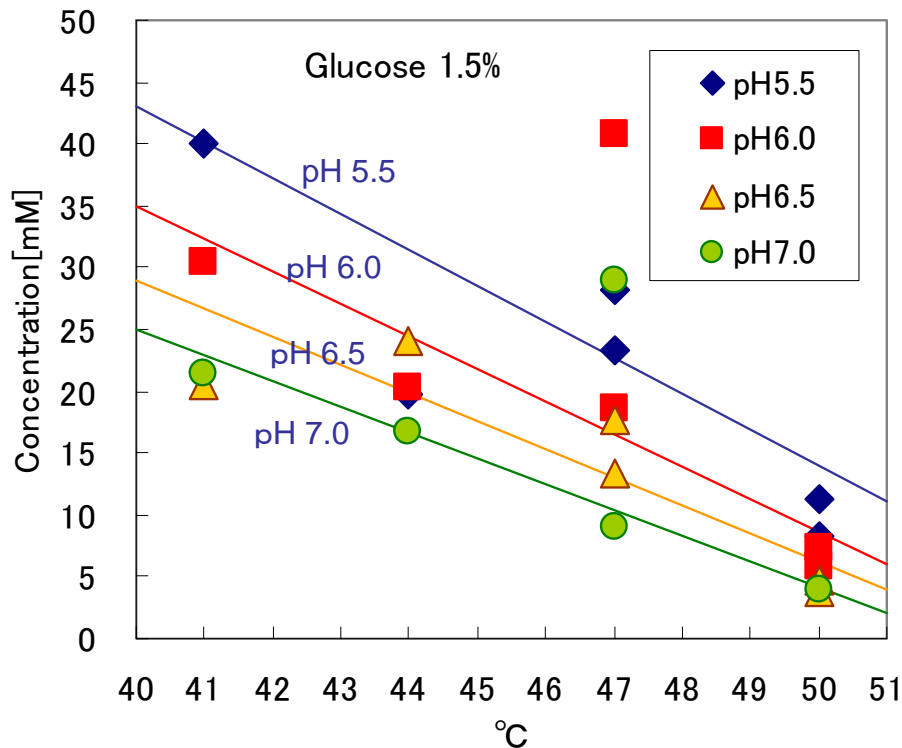


Maximum yield : 2.57 mol/mol at pH 5.5, 50° C

# Effect of temperature and pH on lactate production



Strain HN001, glucose concentration 1.5%



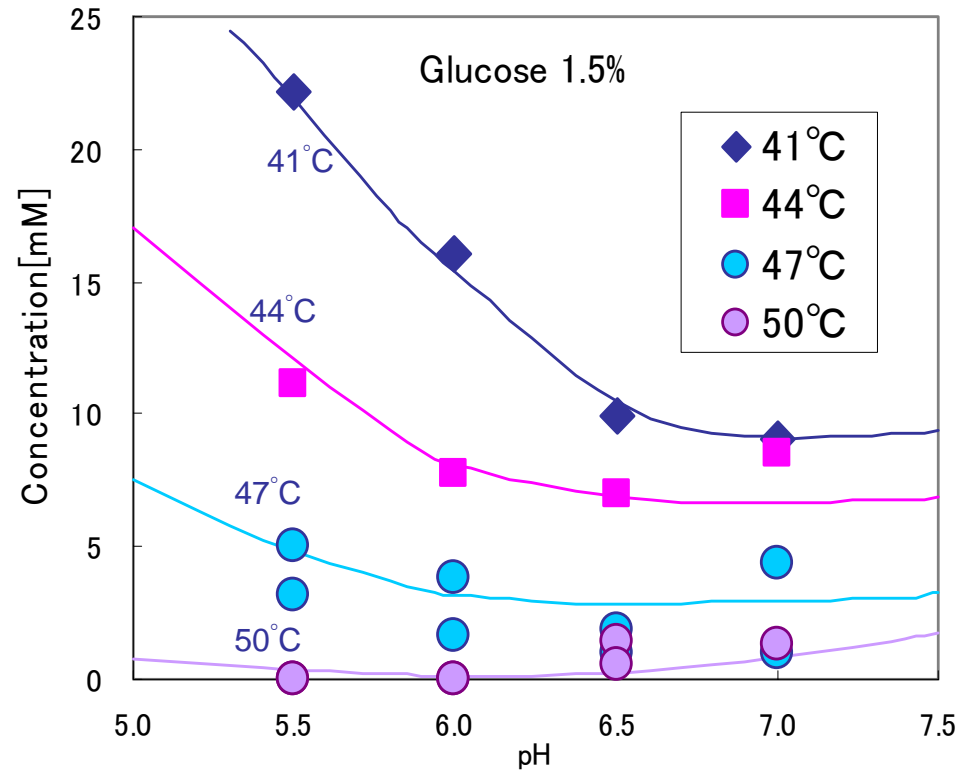
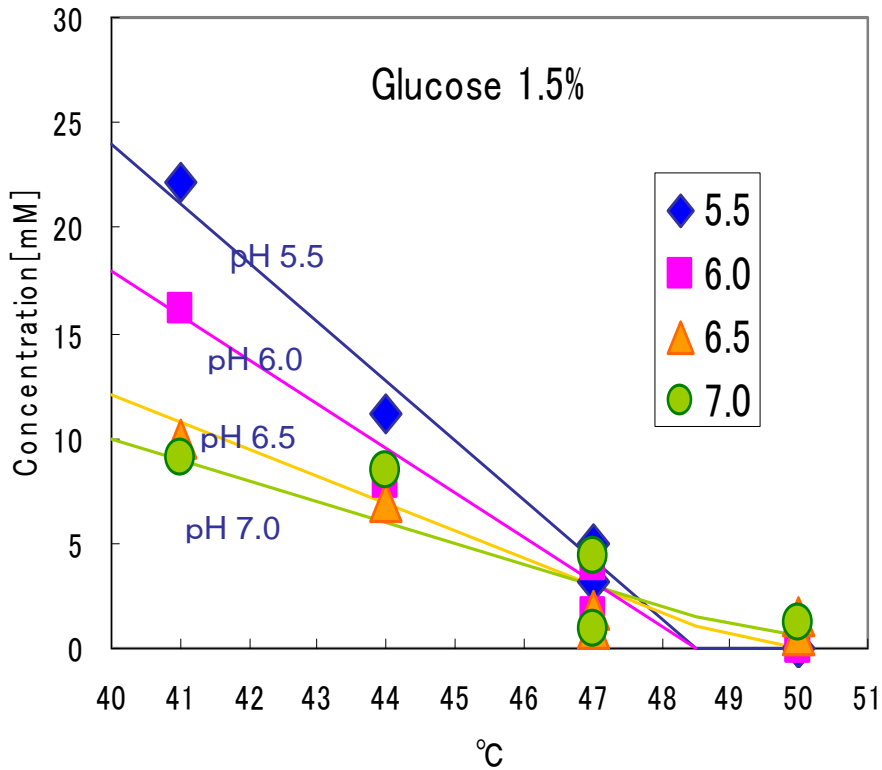
Lactate productivity decreased in accordance with the temperature increase and also with the culture pH increase.

At 50°C, the concentration was less than 10 mM.

# Effect of temperature and pH on butyrate production



Strain HN001, glucose concentration 1.5%

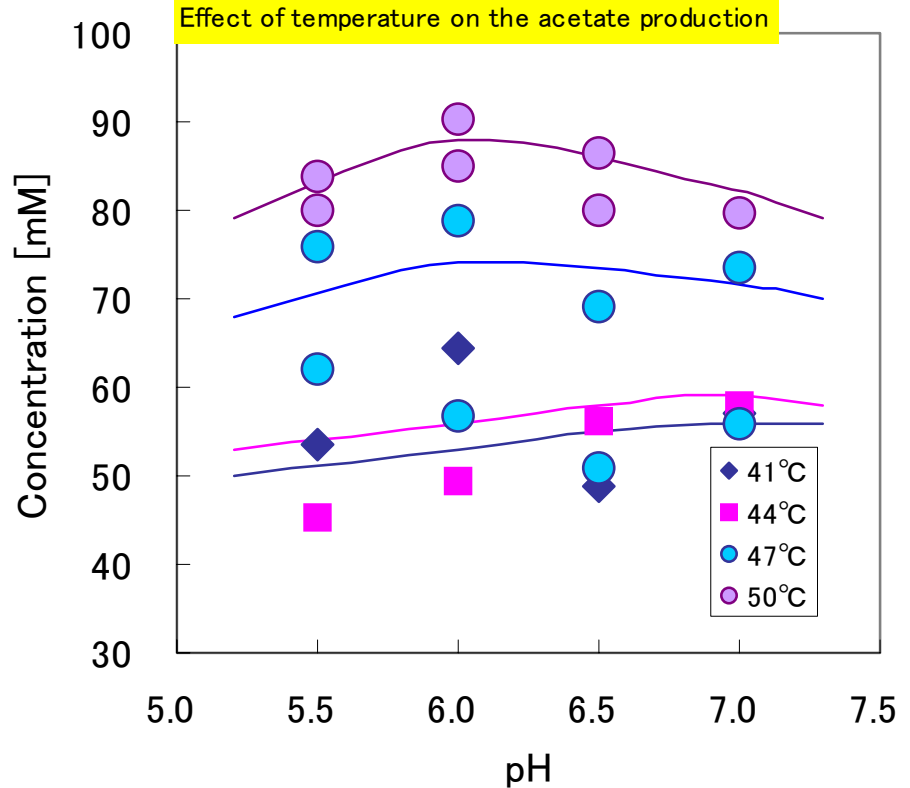
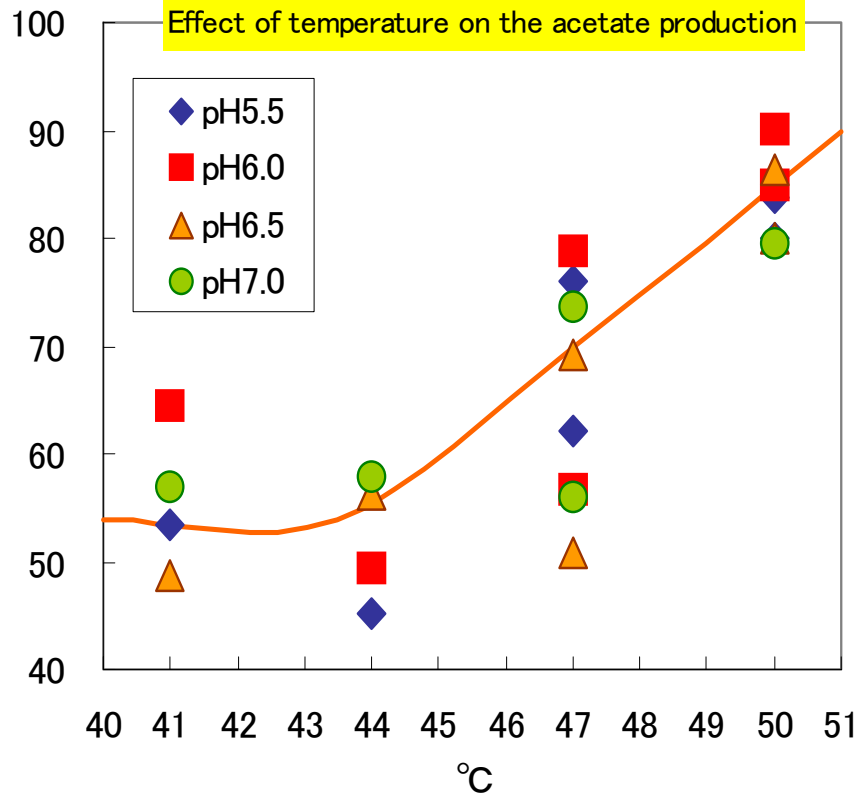




# Effect of temperature and pH on acetate and ethanol production



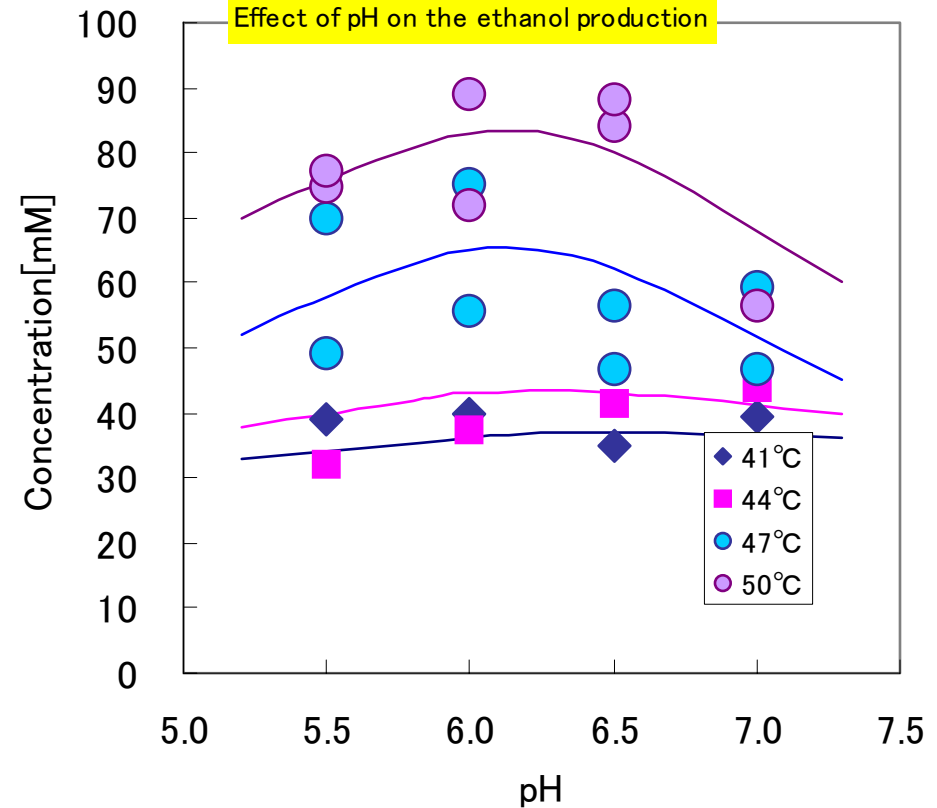
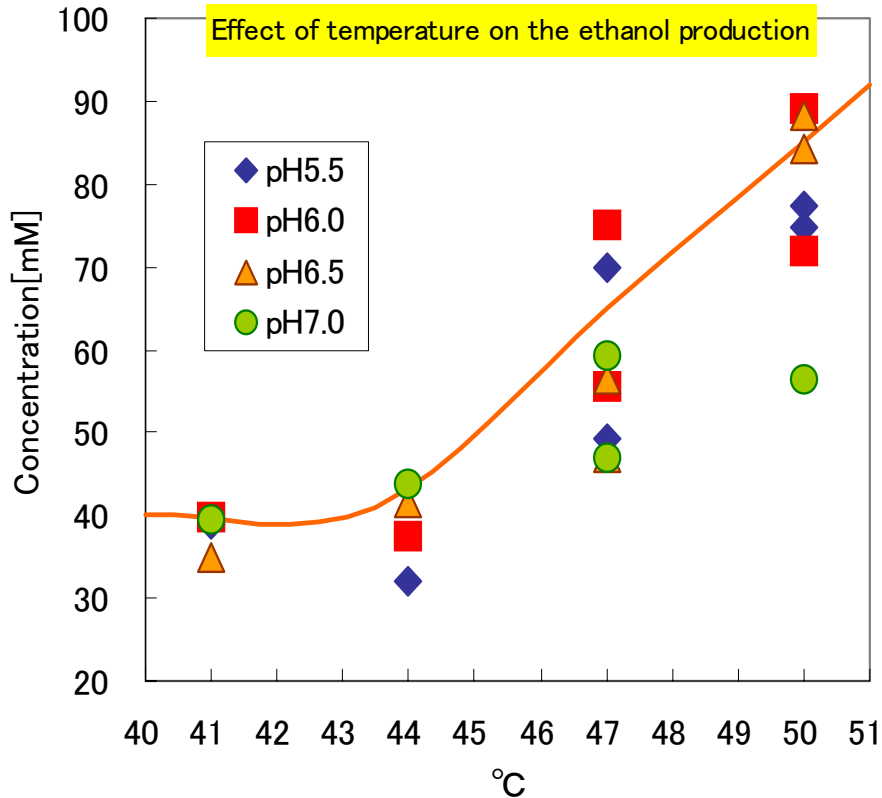
Strain HN001, glucose concentration 1.5%



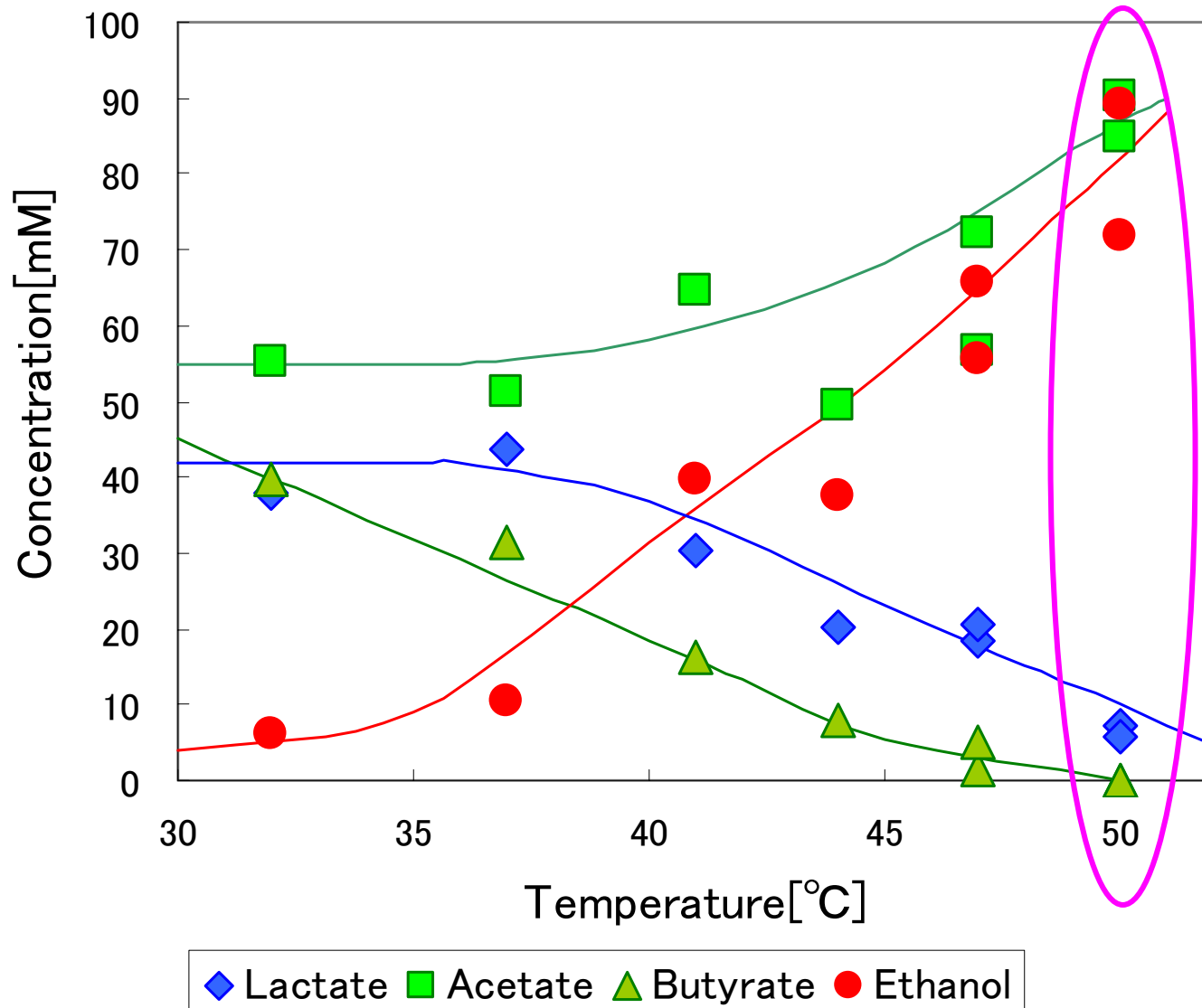
# Effect of temperature and pH on ethanol production



Strain HN001, glucose concentration 1.5%



# Change of metabolite formation by culture temperature



# Effect of temperature and pH on acetate and ethanol production

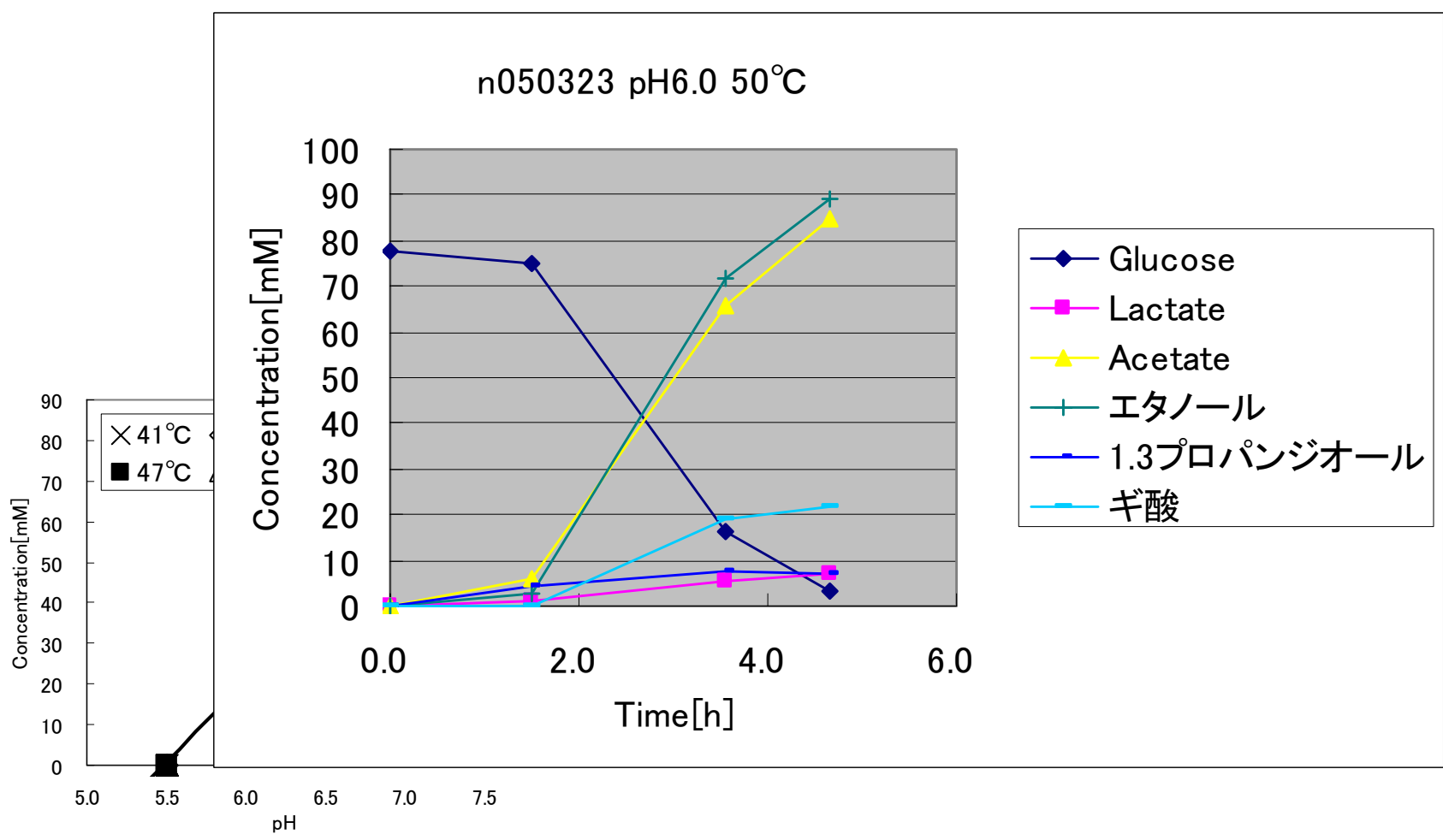
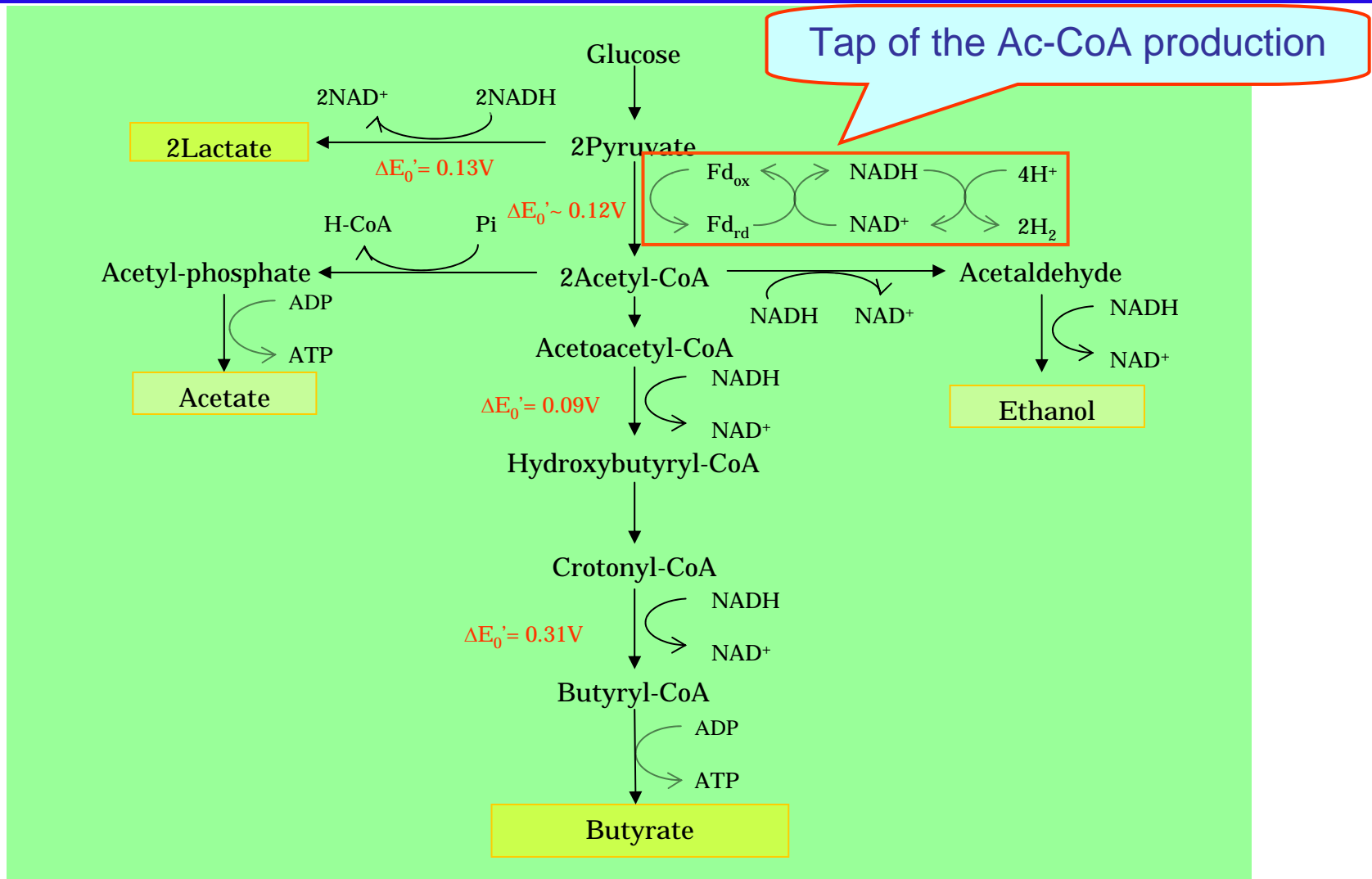


Fig.9. The effect of pH on formate production.

# Reaction pathways from pyruvate



# List of the H<sub>2</sub> yield and production rate by representative bacteria



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

	culture	pH [ - ]	Temp. [°C]	substrate	yield <sup>1)</sup> [mol/mol]	rate [mmol/L·h]	Author
<b>Strict anaerobes</b>							
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.
<b>Thermophiles</b>							
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.
<b>Facultative anaerobes</b>							
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.
<b>Co-culture or Mixed cultures from:</b>							
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.]

# List of the H<sub>2</sub> yield and production rate by representative bacteria



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

Microorganism	culture	pH	Temperature [°C]	Substrate	Yield [mol/mol-monosacch.]	Production rate [mmol/L·h]	Author
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-fermented soybean meal	C	6.0	35	glucose	1.4	8	2000 Mizuno et al.

147 mmol/L·h  
= 3.3 L/L·h

47 °C

65 ~ 80 °C

4 mol/mol

\* 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 rates of representative microorganisms



Category	doubling [ h ]	H <sub>2</sub> evolution rate [mmol/(L·h) ] [mmol/(g·h)]	
<b>A. Photochemical evolution</b>			
1. Oxygenic photosynthetic organisms	7 ~ 25		
<i>Oscillatoria</i> sp. Miami BG7		0.4	0.4
<i>Anabaena cylindrica</i>	25	1.2	1.3
2. Anoxygenic photosynthetic organisms	2.2 ~ 9		
<i>Rhodospseudomonas capsulata</i>		5.3	5.3
<i>Rhodospirillum rubrum</i>		3.0	2.5
<i>Rhodobacter sphaeroides</i> 8703		–	10.4
<b>B. Fermentative evolution</b>			
1. Strict anaerobe	0.16 ~ 2		
<i>Clostridium butyricum</i>		–	15 ~ 20
<i>Clostridium beijerinckii</i> AM21B		17	25
<b>Newly isolated bacterium HN001</b>		<b>160</b>	<b>44</b>
2. Facultative anaerobe			
<i>Citrobacter intermedius</i>		11	9.5
<i>Enterobacter aerogenes</i> E.82005	0.25	36	17





# 中温菌HN001の発酵水素生産特性

## Condition of H<sub>2</sub> and metabolites production by newly isolated bacterium HN001

