Fermentative Hydrogen Production by a Newly Isolated Mesophilic Bacterium HN001

Hiroki NISHIYAMA, Shigeharu TANISHO[b]

Graduate School of Environment and Information Sciences, Yokohama National University
Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan
[b]tanisho@ynu.ac.jp

ABSTRACT:

Fermentative hydrogen production at high temperature has been tested using a newly isolated mesophilic bacterium strain HN001. Experimental temperatures and pHs were set at 41, 44, 47, 50C˚ and 5.5, 6.0, 6.5, 7.0, respectively. The rate of H_2 production from glucose showed the maximum at 47C˚ and pH6.0. The rate was approximately 150 mmol L-culture^{-1} h^{-1}. The yield of hydrogen from glucose increased along with the increase of temperature and the decrease of pH. The maximum yield of 2.4 mol-H_2 mol-glucose^{-1} was obtained at 50C˚ and pH5.5. This bacterium grew fast as cultivation temperature becomes high. The production of acetate and ethanol increased as the cultivation temperature increase while the production of lactate and butyrate decreased. The production of butyrate and lactate decreased also along with the increase of pH while the concentration of formate increased

KEYWORDS : *Hydrogen production, Fermentation, Mesophilic bacterium HN001, Cultivation temperature, pH*

Introduction:

Fossil fuels will be exhausted in near future and as a consequence, environmental problems become very serious. As an ultimate clean energy, hydrogen has been expected all over the world. Hydrogen fermentation is very promising H_2 production method from wet biomass such as food waste or molasses. But the rate of H_2 production was not fast enough for practical use. Recently, hydrogen fermentation at high temperature has been researched for the purpose of accelerating the rate of H_2 production. Moreover high H_2 yields were acomplished by some of thermophilc bacteria [1]. However, the rates of H_2 production of thermophilic bacteria were less rapid than regular bacteria. At the same time, keeping fermenter at high temperature such as 70°C requires large amount of energy. Accordingly, screening of new mesophilic bacteria was carried out. As the results, a new ideal mesophilic bacterium was obtained from sewage. This paper reports properties of this bacterium in hydrogen production from glucose such as effects of cultural temperature and pH.

Materials and methods:

Micro Organism and Culture medium:

A mesophilic bacterium newly selected from sewage was used in this experiment. This bacterium is called mesophilic bacterium HN001 in our laboratory.
Synthetic culture medium named YNU anaerobic culture was used for the pre- and main cultivation. The nutrient composition of the culture was as follows: casein peptone 25.0g, dried yeast extract-S (Nihonseiyakukougyo) 22.0g, L-cystein hydrochloride monohydrate 0.3g, mercaptoacetic acid 0.3g and glucose 15.0g per 1L liquid. This culture liquid was used without sterilization.

Cultivation and Analysis:

The mesophilic bacterium HN001 was pre-cultivated in a 16mL ABCM semisolid agar culture (Eiken Chemical) for 16 hours at 20 C˚ and kept at 50 C˚ for 30 min before the inoculation on the main culture to acclimatize to high temperature. Batch cultivation of the mesophilic bacterium HN001 was carried out with a 500 mL jar fermenter. The volume of culture liquid was 350 mL and the liquid stirred by stirrer at 30 rpm. To investigate the effect of cultivation temperature and pH on the hydrogen evolution, temperatures and pHs were set at 41, 44, 47, 50 C˚ and 5.5, 6.0, 6.5, 7.0, respectively. The pH was monitored by an automatic pH controller and adjusted to the setted up pH by 1M NaOH liquid. The accumulated volume of H_2 was measured by the liquid displacement method using 1M NaOH liquid to remove CO_2 from product gas. The maximum rate of H_2 production and the yield of H_2 were calculated by using the modified Gompertz equation

[2]. The metabolic products such as acetate, ethanol and lactate were analyzed by a liquid chromatograph (column: GL-C610H-S Hitachi Chemical).

Results and Discussion:

Effect of cultivation temperature:

Figure 1 shows the effect of cultivation temperature on the maximum rate of H_2 production making pH as parameter. The maximum rate was determined from one of the parameters of the modified Gompertz equation that is known as a growth curve. All data except at temperature 44˚C laid on lines taking pH for parameter. The lines showed convex curve to the cultivation temperature having a peak at 47˚C. By this, the optimum temperature for hydrogen production was found to be at around 47˚C for the strain HN001. The largest rate at the peak was ca. 150 mmol-H_2 L-culture^{-1} h^{-1} at pH6.0. This rate is the biggest among the known data listed in a paper [1]. From Fig.1, the optimum pH was also determined. The data on pH6.5 were a little lower than pH6.0 and the data on pH5.5 were lower than pH6.5. From these results, the optimum pH was found to be 6.0.

Fermentation products of this bacterium were mainly H_2, CO_2, acetate, ethanol, butyrate, lactate and formate. Figure 2 shows the effect of cultivation temperature on acetate production. The concentration of acetate increased as the temperature increase. The trend was clearly seen at the temperature over 45˚C. This phenomenon is very important for the biological hydrogen production, because many bacteria produce hydrogen by the NADH pathway [3] and surplus NADH is generated through acetate production.

$NADH + H^+ \rightarrow NAD^+ + H_2$
$C_6H_{12}O_6 + 2NAD^+ \rightarrow 2CH_3COCOOH + 2NADH$
$CH_3COCOOH + NAD^+ + H_2O \rightarrow CH_3COOH + CO_2 + NADH + H^+$

Therefore, more H_2 can be obtained when bacteria produce more acetate.

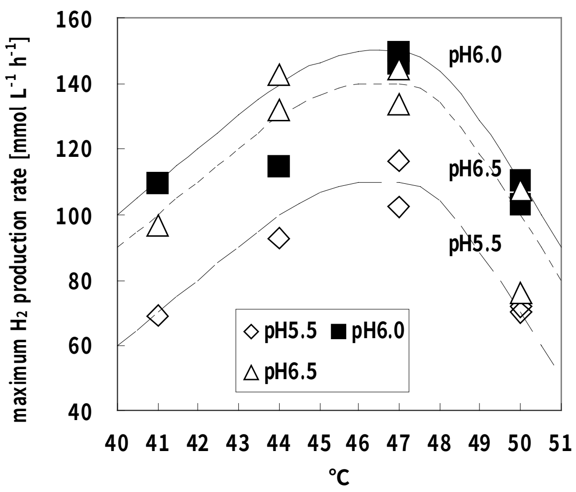

Fig.1. The effect of temperature and pH on the maximum H_2 production rate

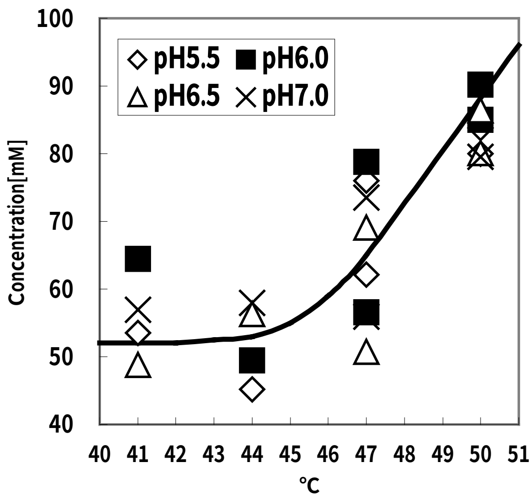

Fig.2. The effect of temperature on acetate production

Figure 3 shows the effect of temperature on ethanol production. Ethanol concentration also increased along with the cultivation temperature increase and the curve showed similar trend with the acetate production. Additionally, acetate and ethanol concentration became nearly equal at 50˚C. Production of ethanol from pyruvate requires 1 mole of NADH as the following reaction.

$CH_3COCOOH + NADH + H^+ \rightarrow CH_3CH_2OH + CO_2 + NAD^+$

So when bacterium produces ethanol, yield of hydrogen decreases in contrast. However ethanol is one of the good energy resources. Therefore, if it is separated from fermenter under reduced pressure, it may be used for energy resource.

Figure 4 shows the effect of cultivation temperature on butyrate production. The butyrate concentration was lower than the other main products as ca. 5 mM at 47C˚ while acetate and ethanol were high as ca. 60mM. It decreased along with the increase of temperature. The production of butyrate requires 2 moles of pyruvate. The reaction produces 2 moles of NADH but utilizes 2 moles of NADH simultaneously. After all, butyrate production consumes 2 moles of pyruvate without making surplus NADH like the following reaction.

$2\ CH_3COCOOH \rightarrow CH_3CH_2CH_2COOH + 2CO_2$

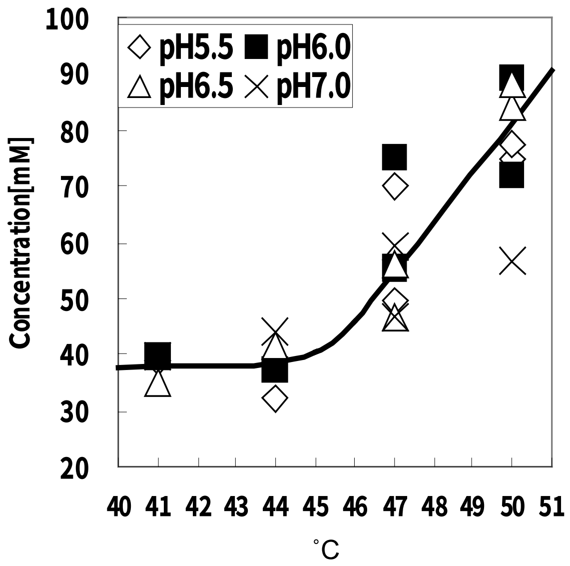

Fig.3. The effect of temperature on ethanol production

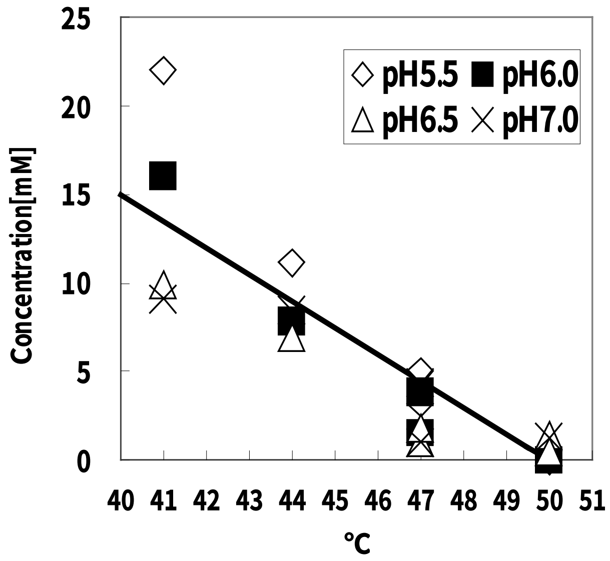

Fig.4. The effect of temperature on butyrate production

Figure 5 shows the effect of temperature on lactate production. Although there are wide spread data at 47C˚, the concentration of lactate decreased along with the increase of temperature. This trend is similar to the butyrate production. The pathway of lactate production requires 1 mole of NADH as follows, therefore when bacteria produce lactate, the yield of hydrogen decreases theoretically.

$CH_3COCOOH + NADH + H^+ \rightarrow CH_3CHOHCOOH + NAD^+$

Considering the above reaction, the decrease of lactate production at high temperature is a great advantage for hydrogen production.

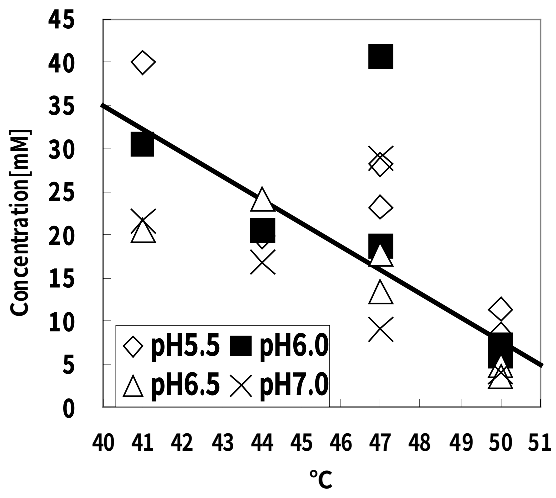

Fig.5. The effect of temperature on lactate production

Effect of pH on :

Figure 6 shows the effect of pH on the yield of hydrogen. The yield increased along with the decrease of pH. It steeply increased from pH 7 to over around pH 6. This might be resulted from formate decomposition. Since formate is known to be decomposed to H_2 and CO_2 at low pH [3]. Additionally, there was a trend of increasing yield in the increasing temperature. This is resulted from the lactate decrease and acetate increase at high temperature.

The effect of pH on acetate production was checked, but the correlation was not found between them. The same check was tried on the ethanol production but the correlation was not found also.

Figure 7 shows the effect of pH on butyrate production. The butyrate concentration decreased along with the increase of pH and it was constant over pH6.5. Although butyrate concentration was totally low, it is revealed that both higher pH and higher temperature repress butyrate production

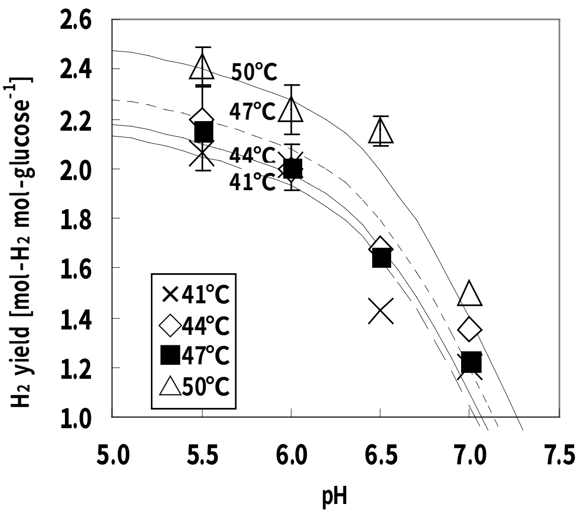

Fig.6. The effect of pH on H_2 yield.

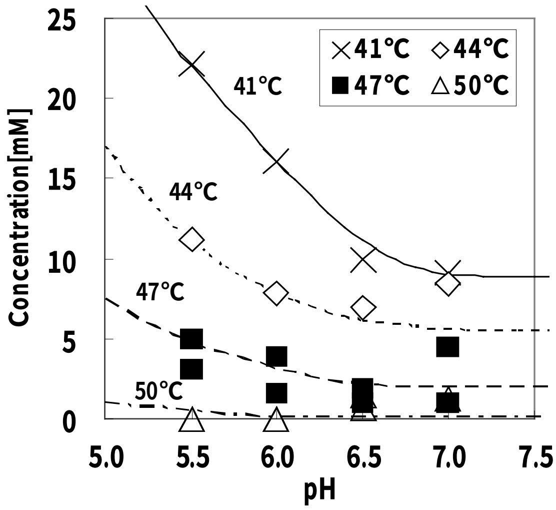

Fig.7. The effect of pH on butyrate production

Fig.8 shows the effect of pH on lactate production. Although there is a fluctuation, the concentration of lactate decreased along with the increase of pH. From Fig.8, lactate production was repressed from high pH and high temperature. The data except 44C˚ showed the clear its trend. So it is assumed that the data on 44C˚ also show the trend like straight line in Fig.8. To keep down the butyrate and lactate productions, higher pH (around pH7.0) and higher temperature are available but if pH is set at high, the H_2 yield becomes lower from Fig.6. Thereat high temperature (around 50C˚) is enough to repress the butyrate and lactate productions and the maximum H_2 production rate is fast (Fig.1, Fig.4 and Fig.5). From these results, the optimum conditions about H_2 production by Mesophilic bacterium HN001 seem to be lower pH and higher temperature.

At the same time, these results are very interesting because the reaction of NAD^+/NADH is affected by pH [3]. The redox reaction of NAD^+/NADH is concerned in acetate, ethanol, butyrate and lactate productions. Therefore the productions of acetate, ethanol, butyrate and lactate were expected to be affected by pH. But in this study, acetate and ethanol were not limited by pH.

Fig.9 shows the effect of pH on the formate production. The concentration of formate drastically decreased linearly along with the decrease of pH. From the previous study, it is revealed that formate is decomposed to H_2 and CO_2 at low pH (about under pH6.4)[3]. This is one of the reasons that higher H_2

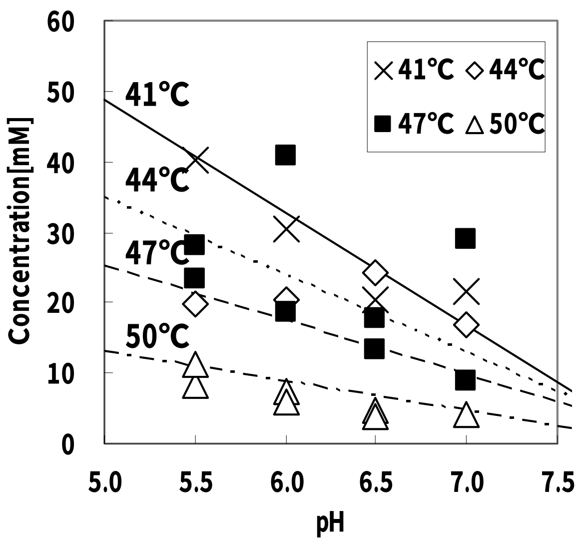

Fig.8. The effect of pH on lactate production.

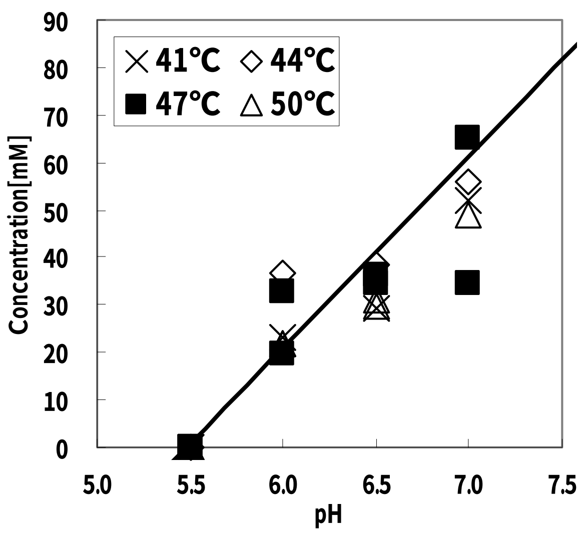

Fig.9. The effect of pH on formate production.

Conclusions:

This mesophilic bacterium HN001 showed a very high potential for hydrogen production by fermentation. The maximum rate of hydrogen production was 150 mmol-H_2 L-culture^{-1} h^{-1}. The result was obtained at 47C˚ and pH6.0. The reason was that 47˚C is the suitable temperature for growth for this bacterium and metabolic pathway changes to acetate and ethanol production at above this temperature. The yield of hydrogen increased along with the increase of cultivation temperature and the decrease of cultural pH. The highest yield of hydrogen was 2.4 mol-H_2 mol-glucose^{-1}, at 50˚C and pH 5.5. This bacterium was made clear to be the most expectable bacterium for hydrogen production by fermentation.

References:

[1] T.de Vrije and P.A.M.Classen “Dark hydrogen fermentations” in Bio-methane & Bio-hydrogen ed J.H.Reith et al. (2003) 103－123 ISBN: 90－9017165－7
[2] Samir Kumar Khanal *et al.* Biological hydrogen production: effects of pH and intermediate products. Int J Hydrogen Energy 29 (2004) 1123-1131
[3] Shigeharu Tanisho 2004. The Reason for Bacterial Evolution of Hydrogen by Fermentation and the Problems to be Solved for Utilization on Energy Production. The Hydrogen Energy Systems Society of Japan. 29 (1), 2－6