Fermentative Hydrogen Production from Artificial Food Wastes

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ABSTRACT:

Fermentative hydrogen production from artificial food wastes was studied by using a newly isolated mesophilic bacterium HN001. This bacterium could produce H_2 from starch directly. Since there is much of rice in food wastes in Japan, we firstly investigated the characteristics of hydrogen production from starch by HN001 at batch cultivation. The maximum rate of H_2 production decreased along with the increase of temperature from 1.2L-H_2 L-culture^{-1} h^{-1} at 37°C to 0.3L-H_2 L-culture^{-1} h^{-1} at 50°C. It was also increased from 0.6L-H_2 L-culture^{-1} h^{-1} to 1.8L-H_2 L-culture^{-1} h^{-1} along with the increase of culture pH from 5.5 to 6.5. However it did not increase linearly along with the increase of starch concentration but approached nearly fixed rate at 1.5L-H_2 L-culture^{-1} h^{-1} at over 2.0%-starch. This bacterium produced H_2 approximately 66L at 37°C, pH6.0 from 1kg of wet artificial food waste containing cooked rice and frozen vegetables with entrails of fishes. The hydrogen production experiment was also carried out at fed batch continuous cultivation.

KEYWORDS : *Hydrogen production, Fermentation, Mesophilic, Starch, Entrails of fish*

Introduction:

In Japan, food wastes appear approximately 20 million tons per year. Most of them are incinerated and/or used for landfill. However, these dispositions have caused new problems such as harmful emissions accompanying incineration and limited capacity of landfill. Depending on these conditions, it is remarked the food wastes as energy sources. Because food wastes become the substrate for hydrogen fermentation. Since hydrogen is expected as ultimate clean energy for next generation, many papers on fermentative hydrogen production from food wastes have been reported recently. Digestion of starch in food wastes is comparatively easy for many bacteria. In this paper, hydrogen production from starch and artificial food wastes is reported by using Mesophilic bacterium HN001.

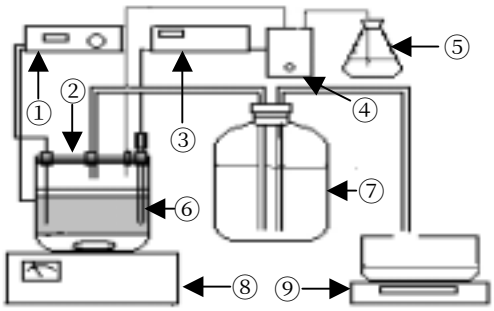
Materials and methods:

Micro organism and culture medium:

Mesophilic bacterium HN001 found out by screening was used in experiments. This bacterium could produce H_2 at a high speed, 3.3L-H_2 L-culture^{-1} h^{-1} from glucose at 47℃[1]. It was precultivated in an approximately 15ml of ABCM semisolid agar culture (Eikenkizai Co., Ltd, Tokyo). The composition of medium was as follows: starch 5.0, 10.0, 15.0, 20.0, 25,0 30.0, 40.0 or 50.0g; casein peptone 25.0g; dried yeast extract-S (Nihon Pharmaceutical Co., Ltd, Tokyo) 22.0g; l-cysteine hydrochloride monohydrate 0.3g; mercaptoascetate 0.3g; per 1L of ion exchanged water. The culture medium was sterilized at 120℃ for 15 minutes.
An artificial food wastes contained cooked rice 25.0g, mixed vegetables containing corn carrot and green pias 25.0g. To investigate effect of entrails of fish, they were added to artificial food wastes. Other nutrients did not added in the artificial wastes medium. The culture medium was crushed with 500ml of tap water for about 5 minutes by a blender and used without sterilization.

Bioreactor system:

Batch bioreactor was fabricated like the overview flowchart (Fig.1). The volume of bioreactor was approximately 860ml with 500ml of culture medium. The medium was stirred at 200rpm with magnetic stirrer. The pH of it was automatically regulated with 2N NaOH solution. Cultivation temperature was adjusted by PID controller. Biogas containing H_2 and CO_2 was collected in a bottle filled up with 1N NaOH solution. Replaced NaOH solution was measured by an electric scale as the volume of H_2.



① PID controller
② Fermenter
③ pH controller
④ Peristric pomp
⑤ 2N NaOH solution
⑥ pH electrode
⑦ Gas holder
⑧ Magnetic stirrer
⑨ Electric scale

Fig.1 Schematic diagram of the bioreactor system

Analysis:

Metabolic products like volatile acid and alcohol were analyzed by a high performance liquid chromatograph (HITACHI 655A). Optical density of the cultivated liquid was measured at 550nm by spectrophotometer.

Results and Discussion:

Effect of temperature on starch medium:

In this experiment, the temperature of culture medium was set at 32, 37, 41, 45 and 50℃. The pH of it was regulated at 6.0 and starch concentration was kept at 1.0%. Fig.2 shows the effect of temperature on the maximum rate of H_2 production. Fig.3 shows the effect of temperature on the production of H_2. The maximum rate of H_2 production and the production of H_2 were 1.2L L-culture^{-1} h^{-1} and 2.9L L-culture^{-1} at 37℃ respectively and decreased along with the increase of temperature at over 37℃. Starch consumption and OD decreased along with the increase of temperature at over 41℃. Since HN001 could live at 50℃ and evolved H_2 at very high speed as 2.5L L-culture^{-1} h^{-1} from glucose medium[1], these result read us to the conclusion that this bacterium did not decompose starch sufficiently at over 45℃.

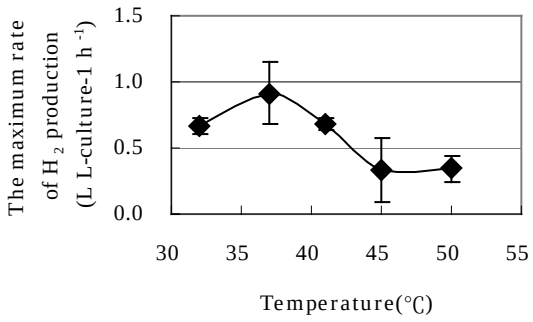


Fig.2 Effect of temperature on the maxium rate of H_2 production

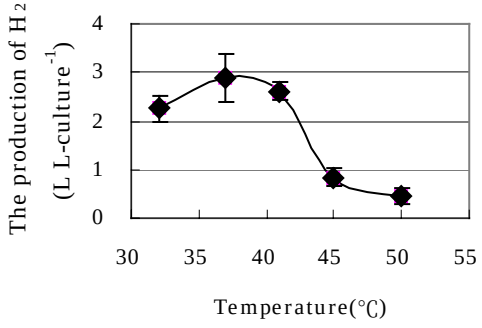


Fig.3 Effect of temperature on the production of H_2

Effect of pH on starch medium:

In this experiment, the pH of culture medium was set at 5.5, 6.0 and 6.5. The temperature of it was regulated at 37℃ and starch concentration was kept at 1.0%. Fig.4 shows the effect of pH on the maximum rate of H_2 production. The maximum rate of H_2 production was increased from 0.6L L-culture^{-1} h^{-1} to 1.8L L-culture^{-1} h^{-1} along with the increase of culture pH from 5.5 to 6.5. Because cell growth rate was fast along with the increase of culture pH. Fig.5 shows the effect of pH on the production of H_2. The production of H_2 tended to increase along with the decrease of culture pH from 6.5 to 5.5.

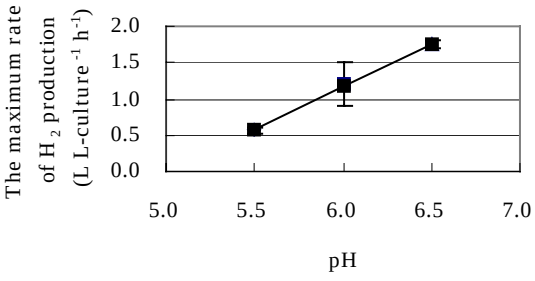


Fig.4 Effect of pH on the maximum rate of H_2 production

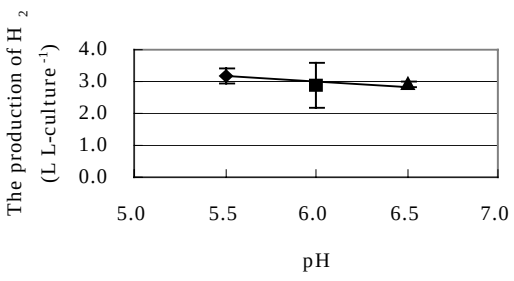


Fig.5 Effect of pH the production of H_2

Effect of starch concentration on starch medium:

In this experiment, starch concentration was set at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0%. The temperature and pH of culture medium was regulated at 37℃ and 6.0. Fig.6 shows the effect of starch concentration on the maximum rate of H_2 production. The maximum rate of H_2 production did not increase linearly along with the increase of starch concentration but approached nearly fixed rate at 1.5L-H_2 L-culture^{-1} h^{-1} at over 2.0%-starch. The reason for peak out of the maximum rate of H_2 production at high starch concentration was that OD peaked out at high starch concentraion. Fig.7 shows the effect of starch concentration on H_2 yield. H_2 yield decreased linearly along with the increase of starch concentration. Fig.8 shows the amount of lactic acid, acetic acid and butyric acid. Lactic acid increased along with high starch concentration. On the other hand, acetic acid and butyric acid tended to peak out at high starch concentration. The production of lactic acid, acetic acid and butyric acid from glucose under anaerobic conditions can be explained by the following chemical equations.

$$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH \quad ①$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad ②$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad ③$$

H_2 produces together with acetic acid and butyric acid as the equation ② and ③. From this figure, the reason that H_2 yield decreased linearly along with the increase of starch concentration is that all excess starch were metabolized only lactic acid. High starch concentration might inhibit H_2 production.

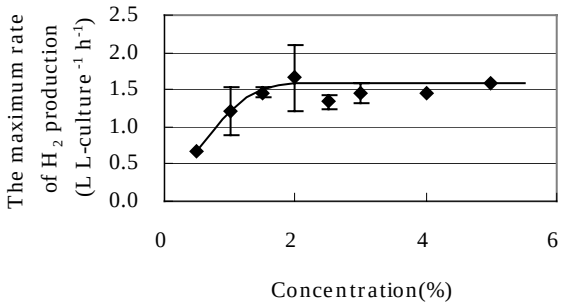


Fig.6 Effect of starch concentration on the maximum rate of H_2 production

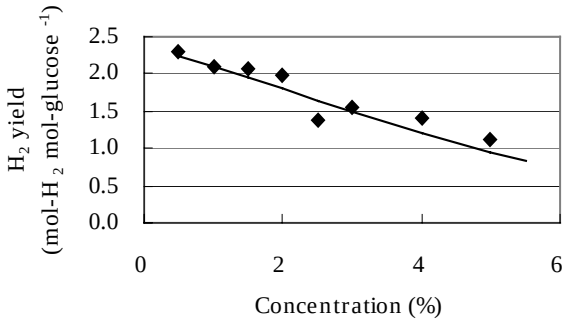


Fig.7 Effect of starch concentration on H_2 yield

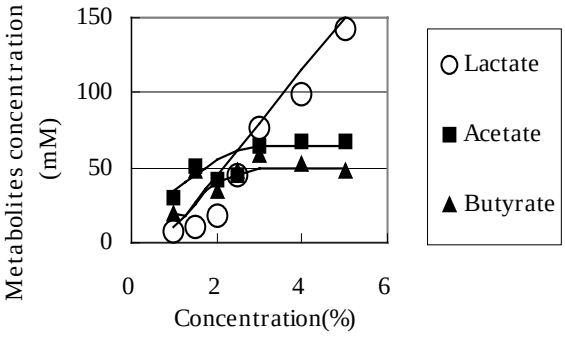


Fig.8 Effect of starch concentration on lactic acid, acetic acid and butyric acid concentration

Effect of entrails of fish on artificial food wastes medium:

In this experiment, the temperature and pH of culture medium was regulated at 37℃ and 6.0. Fig.9 shows the production of H_2 from artificial food wastes. Accumulated volume of H_2 after experiment finished from artificial food wastes with entrails of fish was 66L kg-wet^{-1}. Without entrails, it was only 22L kg-wet^{-1}. Fig.10 shows the effect of entrails of fish on percentage of metabolic products. Though lactic acid productivity was over 40% without entrails, it was only 8% with entrails. Acetic acid and butyric acid productivity were increased with entrails. These products produce together with hydrogen as the equation ② and ③. On the other hand, lactic acid produces together without hydrogen as the equation ①. From this result, it was obvious that accumulated volume of H_2 increased with entrails. Entrails of fish might have some ingredients

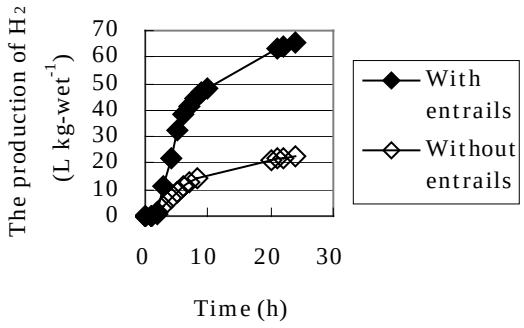


Fig.9 H_2 production from artificial food wastes

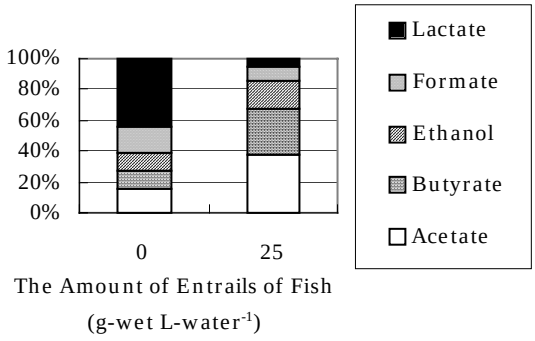


Fig.10 Effect of entrails of fish on percentage of metabolic products

to inhibit lactic fermentation. Fig.11 shows the effect of amount of entrails of fish on maximum rate of H_2 production. Entrails of fish were added to culture medium 0, 25, 50, 75, and 100g per 500ml. The maximum rate of H_2 production without entrails was 0.3L L-culture^{-1} h^{-1}. With 25, 50, 75 and 100g of entrails, it was 1.0, 0.8, 1.2 and 1.2 L L-culture^{-1} h^{-1}, respectively. The maximum rate of H_2 production did not increase along with the increase of entrails. But it was found that the maximum rate of H_2 production was made fast with entrails. It is known that entrails of fish are good nitrogen sources for *Enerobacter aerogenes*[2]. From these results, it appears that entrails have a benefit effect on H_2 production.

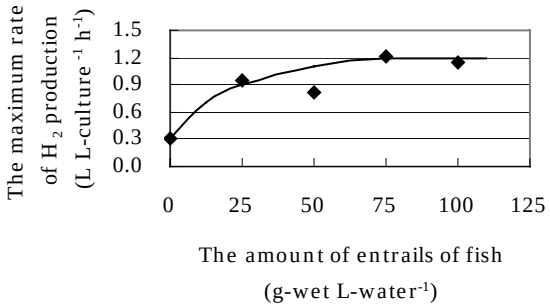


Fig.11 Effect of amount of entrails of fish on the maximum rate of H_2 production

Continuous hydrogen production from artificial food wastes in repeated batch culture:

Continuous H_2 production from artificial food wastes in repeated batch culture was carried out. The temperature and pH of culture medium was regulated at 37℃ and 6.0. The culture was drew 80% of culture medium and added equal amount of fresh culture medium at 10-hour intervals. Fig.12 shows continuous hydrogen production from artificial food wastes in repeated batch culture. An average volume of H_2 was 71L kg-wet^{-1}.

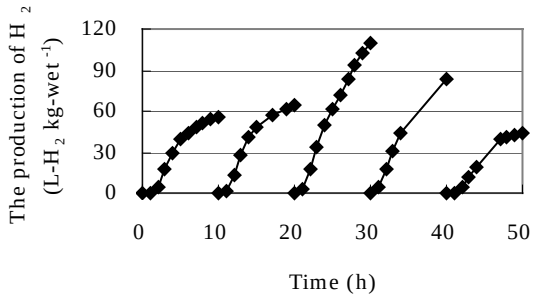


Fig.12 Continuous H_2 production from artificial food wastes

Conclusions:

Mesophilic bacterium HN001 could produce H_2 from starch efficiently. It was found that hydrogen productivity from starch is influenced by culture of temperature, pH and substrate concentration. Optimal temperature is approximately 37℃ to produce H_2 from starch by this bacterium. But this bacterium could not produce H_2 at over 45℃ efficiently. The maximum rate of H_2 was increased from 0.6L-H_2 L-culture^{-1} h^{-1} to 1.8L-H_2 L-culture^{-1} h^{-1} along with the increase of culture pH from 5.5 to 6.5. It did not increase linearly along with the increase of starch concentration but approached nearly fixed rate at 1.5L-H_2 L-culture^{-1} h^{-1} at over 2.0%-starch. H_2 yield decreased linearly along with the increase of starch concentration because of the increased lactic acid production. From 1kg of wet artificial food waste containing cooked rice and frozen vegetables with entrails of fishes, HN001 could produce hydrogen approximately 66L at 37°C, pH6.0. The maximum rate of H_2 production with entrails was 4 times faster than it without entrails. Entrails of fish have a benefit effect on H_2 production.

References:

[1] H. Nishiyama and S. Tanisho Fermentative hydrogen production by a newly isolated Mesophilic bacterium HN001, 25th Hydrogen Energy System Society Meeting Proceeding, 171-174, 2005

[2] S. Tanisho and Y. Fujii Hydrogen production from garbage by the bacterium *Entrobacter aerogenes*. *Journal of The Hydrogen Energy System Society of Japan*, Vol.20, 10-15, 1995