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AEROBIC HYDROGEN PRODUCTION AIMED AT THE IMPROVEMENT IN THE YIELD OF HYDROGEN

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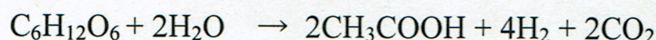
Many of facultative anaerobic bacteria evolve hydrogen to re-oxidize NADH under anaerobic condition. By this reason, the yield of hydrogen of anaerobic hydrogen production through NADH pathway is limited less than 2 moles from 1 mole glucose. Under aerobic condition, however, they may also evolve and yield much more hydrogen from the NADH produced at the TCA cycle if they were inhibited to use oxygen as the electron acceptor for the re-oxidation of NADH. That is, if they can use oxygen only for the re-oxidation of $FADH_2$ produced at the TCA cycle, the cycle could work by producing hydrogen for re-oxidation of NADH. This condition will be realized by inhibiting the NADH dehydrogenase complex of the electron transport chain under aerobic condition.

To prove this theory, inhibition examination was carried out by using facultative anaerobe Enterobacter aerogenes. After several hour cultivation under suitable pH for hydrogen evolution (approximately pH 6.0), inhibitor to the NADH dehydrogenase complex, Lauril gallate, was added on the culture liquid, simultaneously KNO_3 to make quasi-aerobic condition. As the result, the yield increased from the common value of 1 mol-H_2/mol- glucose to three times larger value of 3 mol-H_2/mol-glucose.

1. Introduction

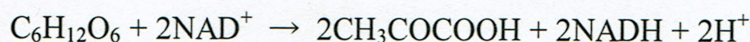
Fermentative hydrogen production is expected as one of the clean energy production method of the hydrogen age using biomass. At this production method, however, the amount of hydrogen from substrate glucose, namely the yield of hydrogen, is at most approximately 2 mol-H_2/mol-glucose[1] in case of strict anaerobe and only 1 in case of facultative anaerobe[2]. Tanisho estimated that the final energy conversion efficiency is nearly equal between alcohol fermentation and hydrogen fermentation if the hydrogen yield is 2 [3]. Since the fermentative alcohol production from sugar cane is hard as for the industrial energy production, it is needed to improve the hydrogen yield for the industrialization.

There are three representative pathway for fermentative hydrogen evolution, such as formate pathway, direct pathway and NADH pathway. According to stoichiometric evaluation, yield 4 by formate or direct pathway is the theoretical maximum in these.

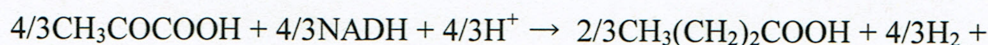


$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2$$

On the contrary, the actual maximum yield is 2.7 recorded by *Clostridium beijerinkii* [4] by NADH pathway. In this pathway, hydrogen is produced by using the residual NADH out of the 2 moles produced at the glycolysis as follows;



$$C_6H_{12}O_6 + 2NAD^+ \rightarrow 2CH_3COCOOH + 2NADH + 2H^+$$



$$4/3CH_3COCOOH + 4/3NADH + 4/3H^+ \rightarrow 2/3CH_3(CH_2)_2COOH + 4/3H_2 +$$

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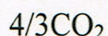
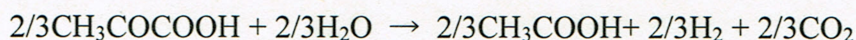
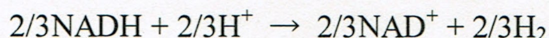


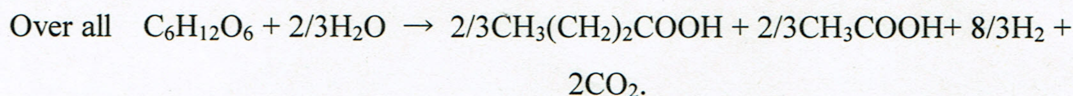
$$4/3CO_2$$



$$2/3CH_3COCOOH + 2/3H_2O \rightarrow 2/3CH_3COOH + 2/3H_2 + 2/3CO_2$$



$$2/3NADH + 2/3H^+ \rightarrow 2/3NAD^+ + 2/3H_2$$



Over all $C_6H_{12}O_6 + 2/3H_2O \rightarrow 2/3CH_3(CH_2)_2COOH + 2/3CH_3COOH + 8/3H_2 + 2CO_2.$

Therefore, if the more residual NADH could be gotten, the more hydrogen would be evolved through this pathway, consequently the higher yield would be gotten.
Because the facultative anaerobic *Enterobacter aerogenes* is known to be evolving hydrogen through NADH pathway [5] under anaerobic condition while under aerobic condition, it uses the NADHs for the production of ATP by means of the electron transport chain where protons from NADH are making H_2O. The authors have been studying how to use the large amount of NADH produced in the TCA cycle for the hydrogen production [6-8]. The theoretical result presented at the 13th World Hydrogen Energy Conference, Beijing, was that the yield of hydrogen could be improved by inhibiting the NADH dehydrogenase complex of the electron transport chain under aerobic condition. In this paper, we will show experimental results obtained under quasi-aerobic condition using NO_3- ion and inhibition.

2. Experimental method

The component of culture liquid is shown in Table 1. After inoculation of *Enterobacter aerogenes* on the sterilized 500 ml culture liquid, the culture were pre-cultivated for 16 hours under 38°C while stirred by magnetic stirrer. A 50 ml of this pre-cultivated liquid was added on a 2000 ml of regular culture liquid. Accumulated amount of hydrogen, mass of cells and concentrations of the metabolites were measured periodically during the regular cultivation, where the cell mass was measured by OD method (550 nm) and the metabolites with liquid chromatograph using a packed column Ultron PS-80H (Shinwa Kako Co. Ltd).
To make the quasi-aerobic condition, 1.0 g of potassium nitrate was added onto the regular culture after several hours cultivation.
A 0.09 g of Lauryl Gallate dissolved in 5 ml of ethanol was used as the inhibitor. The pH of culture liquid was kept at 6.0 at all experiments by automatic controller.

Maerial	amount
$C_6H_{12}O_6$	20.0 g
$MgSO_4$	0.4 g
Peptone	15.0 g
Na_2HPO_4	2.0 g
KH_2PO_4	2.0 g
Distilled water	2.0 L

Table 1. Composition of culture liquid.

3. Results and Discussions

Figure 1 shows the effects of KNO_3 and inhibitor on hydrogen evolution. These data were arranged so as to adjust the tendency of hydrogen evolution between three kinds of data by dividing the accumulated volume of hydrogen by cell mass at that moment. KNO_3 was injected at cultivation hours 5. The bacterium stopped hydrogen evolution when KNO_3 was injected, though it maintained the evolution until 7 hours, when the bacterium was cultivated normally.

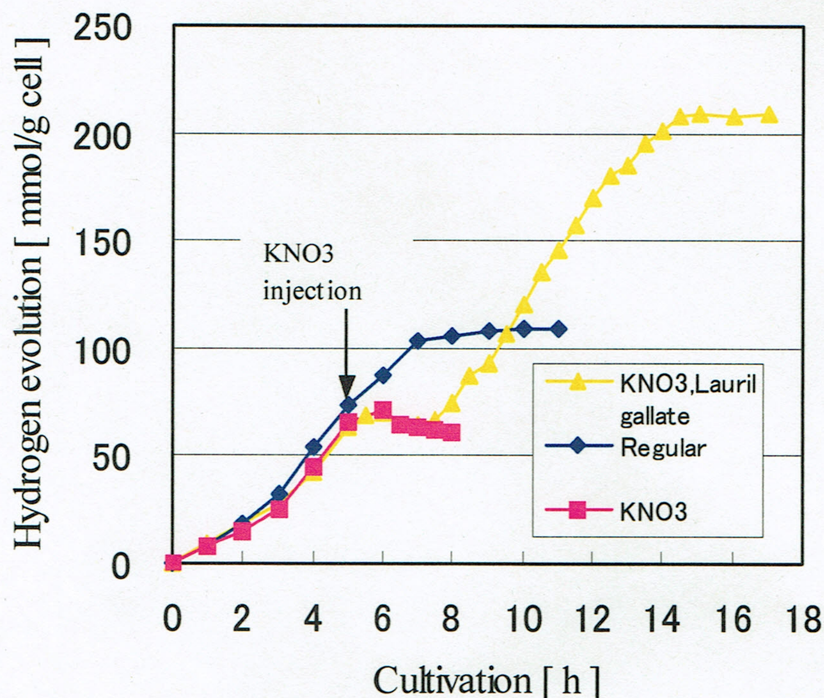


Fig. 1. Hydrogen evolution at different conditions

That is to say NADH was re-oxidized by KNO_3 and consequently H_2 production was stopped. This tendency was also seen in the result of inhibitor injection in Figure 1. The inhibitor was injected at 6 hours with KNO_3. The effect of KNO_3 appeared soon clearly and was kept for about 2 hours. Then the bacterium recovered the hydrogen evolving activity, i.e. the inhibitor required approximately 2 hours to become effective.

Figure 2 shows the development of the accumulated volume of hydrogen and the amount of consumed glucose in the case of the injection of inhibitor and KNO_3. The consumption rate of glucose increased from 4 hours to 6 hours rapidly by the growth of bacterium mass, the rate however decreased after 6 hours, i.e. after the injection of inhibitor and KNO_3, till the end of the cultivation. The phenomenon could be explained by such that the metabolic pathway changed from fermentation to respiration and since the productivity of ATP of respiration is several ten times larger than fermentation, glucose consumption would become slowly.

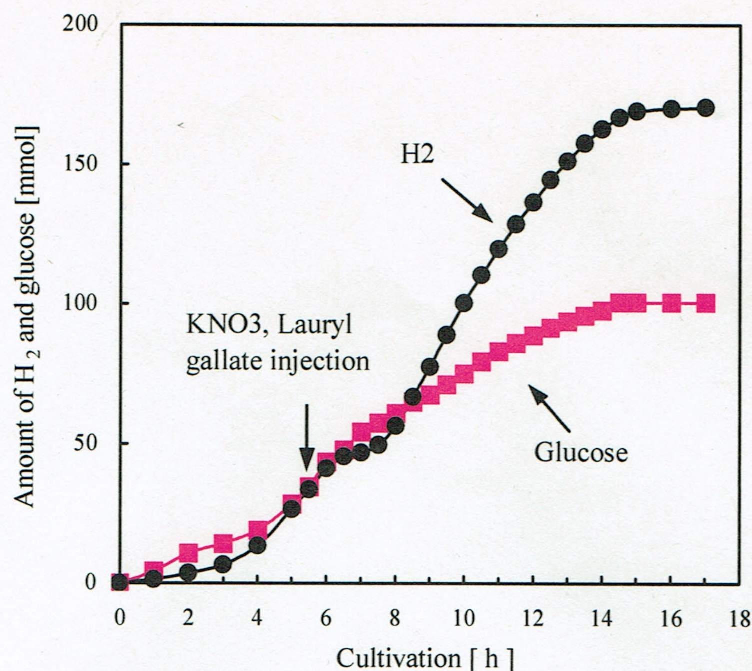


Fig. 2. Accumulated amount of hydrogen and amount of consumed glucose

On the yield of hydrogen from glucose by this case, the results are shown in Figure 3 where the yield was calculated by using short-term evolution and consumption at every measured point. Though data show unstable movement throughout the cultivation, before injection, data center on the yield 1 which is the regular yield of *E. aerogenes*, and after 2 hours from the injection till the last, data clearly center around 3.

From this figure, we can have confidence about the development of the yield of hydrogen by inhibiting the NADH dehydrogenase complex. One regrettable result is that we could not get such a high yield as 10 predicted by the theoretical consideration. We will clarify the reason in the near future.

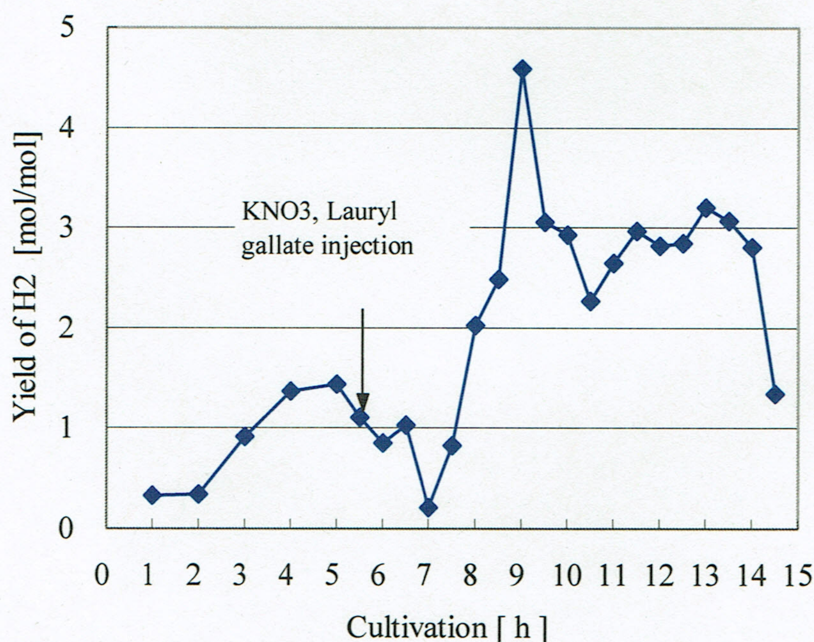


Fig. 3. Effect of inhibition on the yield of hydrogen

4. Conclusion

From the above experiments, the following facts have become clear on the bacterium *Enterobacter aerogenes*:

(1) KNO3 was able to be an electron acceptor for the bacterium and stop the hydrogen evolution of the bacterium.
(2) Glucose consumption rate decreased by injecting KNO3
(3) Lauryl gallate can be an inhibitor of the NADH dehydrogenase in the electron transport chain to living cell.
(4) The yield of hydrogen from glucose was improved from 1 to 3 by inhibiting the electron transport chain.
(5) More improvement of the yield depends on the future investigation.

5. References

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