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Article / Book Information

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（博士課程）
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論 文 要 旨

THESIS SUMMARY

専攻： Department of	化学工学　専攻	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of　（　工学　）
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Cyanobacteria are capable of carbon dioxide fixation under the light, utilizing water as a primary electron donor, and generating free oxygen, ATP and low potential reductive compounds NADH, NADPH and FADH2 for metabolism. Some species of cyanobacteria are capable of hydrogen production by supplying NAD(P)H from glycolysis to hydrogenase during dark anaerobic condition and photohydrogen production during photosynthesis. The unicellular to cyanobacterium *Synechocystis* sp. strain PCC 6803 has been used as a model study to develop biological hydrogen production. Transferring *Synechocystis* sp. strain PCC6803-Glucose tolerant cells from photoautotrophic condition to dark anaerobic HEPES buffer solution, it generates hydrogen (H_2) on bidirectional [NiFe] hydrogenase by receiving sufficient amount of NAD(P)H that is generated from the breaking-down of stored glycogen. This strain produces high hydrogen production under dark-anaerobic condition and nitrate-free condition. Increasing H_2 energy consumption and a search to establish alternative energy to replace depriving fossil energy have urged scientist to find an environmental-friendly process for H_2 production. And one of them is biological H_2 from cyanobacteria. This thesis has gathered the experimental observation on hydrogen production by *Synechocystis* sp. strain PCC 6803, one of the most studied cyanobacteria. Research strategies have been carried based on several tools from basic methods of optimized media to genetic modification in order to increase H_2 production from cyanobacteria.

Firstly, to increase NAD(P)H which normally cells receive from glycolysis or glycogen breakdown by supplying monosaccharides to cell suspension. Observations of H_2 production and other parameters during hydrogen fermentation were carried out. The primary purpose of this study was to evaluate the amount of H_2 produced by *Synechocystis* sp. strain PCC 6803-GT in dark HEPES buffer solution supplemented with either glucose, fructose, galactose, mannose or xylose and without monosaccharides. There has been no previous report of works carried out fermentation using fructose, galactose, mannose and xylose. Therefore, this work was the first work demonstrated other reducing sugar fermentation. This work has demonstrated that fructose was important for hydrogen production from *Synechocystis* sp. strain PCC 6803. The abundant fructose found widely in nature could be used simply to increase hydrogen production effectively in dark anaerobic condition. By adjusting nitrate free and fructose environment, cells yield high hydrogen production. It shows high hydrogen production with low consumption of fructose. This has made it to be an economical reducing sugar for biological hydrogen production process. The amount of hydrogen generated from logarithmically growing cells in HEPES buffer solution containing 50-70 $\mu mol\ mL^{-1}$ fructose yielded 6.5 folds hydrogen production than none-fructose fermentation.

Secondly, to optimize cellular NAD(P)H by selecting cells prepared from various growth phases in photosynthesis, fermented cells from various growth phase have been monitored. The assumption is raised that when cells are grown and divide themselves in media. Dense and diluted population cells in glass tube reach different light intensity. This different reachable light intensity will drastically change enzyme activity inside The Calvin cycle. Searching the growth phase of the inoculum suited for use in dark anaerobic nitrate-free hydrogen seems to be a good possibility to elevate hydrogen production. The results showed that dark anaerobic hydrogen production was strongly dependent on the growth phase of inoculum cells in the prior photosynthetic cultures. The inoculum cells from stationary phase were most suitable for hydrogen production. The stationary phase cells were capable of maintaining high level in cellular glycogen content. However this result was different, if reducing sugar was applied then the most suitable growth phase was logarithmic phase.

Thirdly, to remove competitive pathways competes with hydrogenase enzyme by elimination of NAD(P)H

consumption reactions that compete NAD(P)H with NiFe-hydrogenase in dark anaerobic nitrate-free condition, metabolic engineering was implemented. The disruption was not only genes coding for D-lactate dehydrogenase (ddh) but also genes coding for alcohol dehydrogenase (adh). Constructed mutant was proved to be an effective method for H_2 production, especially when fructose and growth phase selection were applied, the result has achieved more than 10-folds increase in H_2 production. Redox homeostasis network appears to be changed by genetic modification of NAD(P)H related reactions. Those mutants represent a high hydrogen production potential.

Finally, using all data to draw the protocol of how to adjusting reaction for the best H_2 production from cyanobacteria. The purposed process and protocol for cyanobacterium hydrogen enhancement method has been constructed based on experimental data using *Synechocystis* sp. strain PCC 6803 as a study subject. Cyanobacteria play a major role in carbon cycling on earth. Implementation of cyanobacterium hydrogen energy will not only be an alternative energy production process, their photosynthesis activity means CO_2 capture from atmosphere via photosynthesis. Biological H_2 production from cyanobacteria remains a highly important challenge and target in the development of new sustainable H_2 energy sources.