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# Hydrogen Production by *Anabaena* sp. CH1 with Two-stage Process

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## 1 Introduction

Hydrogen can be produced by cyanobacteria normally under anoxic conditions without nitrogen [1, 2]. *Anabaena* sp. Ch1 (cyanobacteria) can produce hydrogen with extra addition of fructose as energy source [3]. During hydrogen production process, nitrogen is exhausted gradually and chlorophylls may break down to provide the nitrogen. This phenomenon is unfavorable for a long term operation of bio-hydrogen production by cyanobacteria. In this research, this problem can be solved through a two-stage operation (hydrogen production stage and recovery stage as Fig. 1).

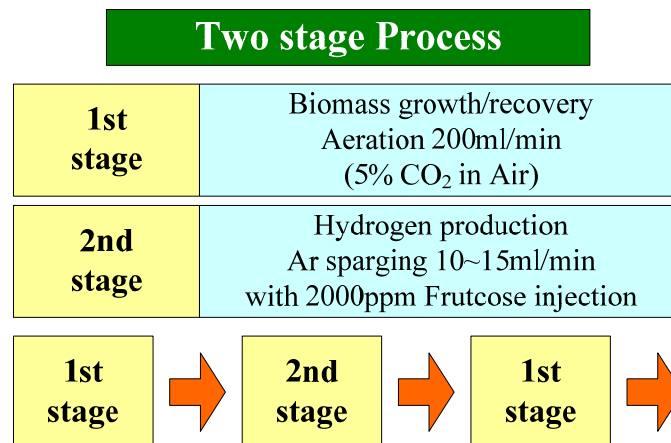
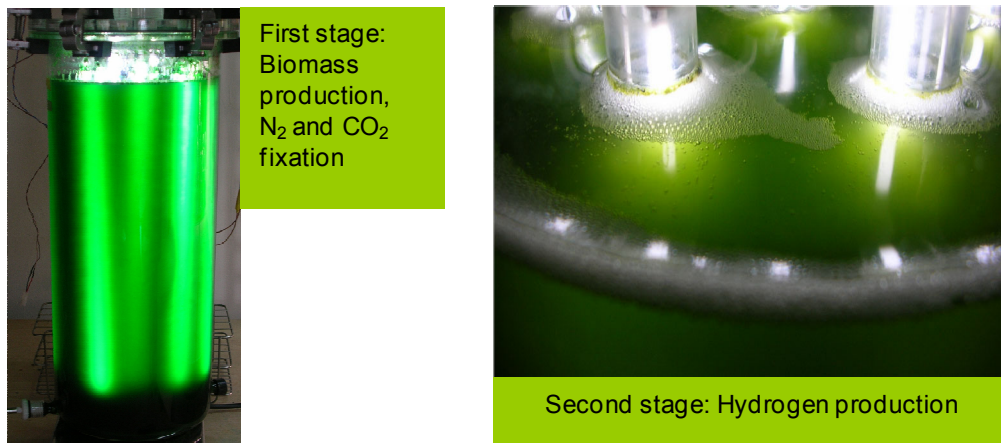


Figure 1: Conditions and sequences of two stage process.

## 2 Material and Methods

1. Cyanobacterium *Anabaena* sp. CH1 was isolated from Taiwan paddy soils. CH1 cells are cultivated under artificial Arnon medium with 5% CO<sub>2</sub> and 250 μmole/m<sup>2</sup>/s light intensity [4]. CH1 will take nitrogen gas in ambient air as nitrogen source and produce nitrate through nitrogenase for cell growth. CH1 will also take CO<sub>2</sub> to produce sugar through photosynthesis [5]. So, in the first stage, we call it biomass growth or recovery stage and in the second stage the hydrogen production stage (Fig. 2).

2. When CH1 cells grow to late log-growth phase, the upper part is flushed with argon gas to create an anaerobic condition in order to change to hydrogen production stage when 2000 ppm fructose is added (Fig. 2).
3. During the two stage process, chlorophyll a, fructose, COD, MLSS and gas carbon dioxide, hydrogen was measured [6, 7, 8, 9].
4. Five liter photo-bioreactor is designed by our laboratory.



**Figure 2: Purpose and image of each stage.**

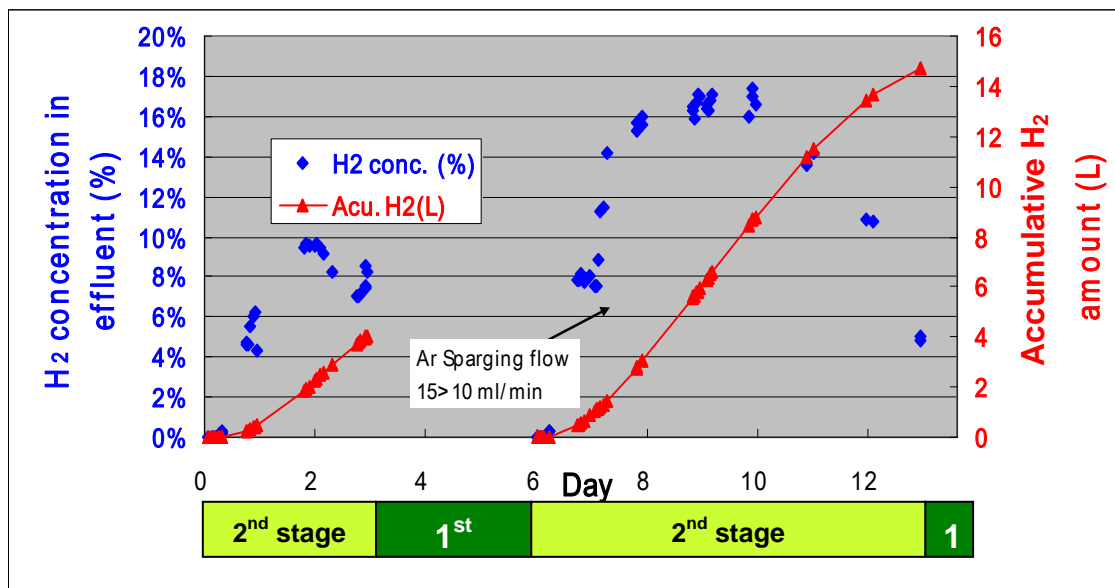
### 3 Results

During the H<sub>2</sub> production stage, chl-a and specific chl-a (chl-a to MLSS ratio) decreased to 77.2% and 67.5% after aeration with argon for 3 days (Table 1). After operation of hydrogen production for 3-7 days, the cells shifted to normal growth condition (aeration with air+5% CO<sub>2</sub>). Within 4 days, cells recovered completely, and then cells shifted again to H<sub>2</sub> production stage. The H<sub>2</sub> production rate was as high as the original rate. This indicated that the effects of nitrogen deprivation on the photosynthetic mechanisms were recoverable and the cells were able to produce H<sub>2</sub> in a cyclic way. The hydrogen production rate is 76.4 μmol/mg chl-a/h. After two cycles, that is twice 2000 ppm fructose is exhausted, the accumulative hydrogen amount is 18.7 liter in a five liter bioreactor, i.e. 1.9 L per day (Fig. 3).

At the recovery stage strain CH1 captured CO<sub>2</sub> and transformed to carbohydrates or cell materials by photosynthetic reaction [10]. The average carbon fixing rate of strain CH1 with 5% CO<sub>2</sub> and illumination was 933.3 mg CO<sub>2</sub>/L/day. However, the average carbon release rate at H<sub>2</sub> production stage was 406.4 mg CO<sub>2</sub>/L/day. The net carbon sequestration of two-stage process is positive. Therefore, this kind of energy production is useful to eliminate CO<sub>2</sub> emission.

**Table 1: Variation of Chlorophyll a content, MLSS content and specific chl-a ratio at the end of each stage in two operation runs.**

End of each stage	Chl-a (mg/L)	MLSS (mg/L)	Specific chl-a ratio (%)
1st Stage of 1st Run	12.66	738	1.72
2nd Stage of 1st Run	10.27	795	1.29
1st Stage of 2nd Run	18.44	1079	1.71
2nd Stage of 2nd Run	15.36	1244	1.23



**Figure 3: The H<sub>2</sub> concentration and accumulative H<sub>2</sub> amount during two-run operations.**

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