Thesis Title	Hydrogen Production by Photosynthetic Bacteria *Rhodopseudomonas sphaeroides* 3701
Thesis Credits	12
Candidate	Miss Phutthachat Pansoomboon
Supervisors	Asst. Prof. Dr. Marasri Ruengjitchatchawalya
	Miss Niramol Catawatcharakul
Degree of Study	Master of Science
Department	Biotechnology
Academic Year	1998

Abstract

This study divided into two parts. The first part was hydrogen production by a photosynthetic bacterium, *R. sphaeroides* 3701, from different organic acids and nitrogen sources. The second part aimed to utilize waste from the agroindustries. The fermented products from the anaerobic digestion of squeezed juice from solid pineapple wastes were used for the production of hydrogen . The experiments were done under anaerobic light at 30 °C.

R. sphaeroides 3701 could use acetic, lactic, propionic and butyric acids as an electron donor for hydrogen production. Hydrogen production depended on substrate type and concentration. The experiment with varying butyric acid concentration showed that reduction of acid concentration from 48 mM to 10 and 20 mM resulted in increasing the amount of gas produced/mmole of substrate utilized from 68 to 125 and 123 ml, respectively. The conversion efficiency compared to the theoretical one increased from 30 to 55 %. With the initial concentration of 50 mM organic acid, the hydrogen productions from each substrate were 85, 78, 68 and 60 millilitre/mole of lactic, propionic, butyric and acetic acids, respectively. Hydrogen production from mixed acetic and lactic acids was higher than from lactic or acetic acid as substrate. Hydrogen production per mmole lactic, mmole acetic and mmole lactic and acetic were 59, 60 and 76 millilitre, respectively.

R. sphaeroides 3701 can use ammonium sulfate and monosodium glutamate as a nitrogen source. However, in the presence of ammonium sulfate, hydrogen production was repressed. The inoculum grown in medium containing ammonium sulfate produced hydrogen in

the media with or without monosodium glutamate slower than the inoculum grown in monosodium glutamate medium. Not only used as the nitrogen source but monosodium glutamate could be used as substrate for hydrogen production. Monosodium glutamate also enhanced the nitrogenase activity, so that acetylene reduction was much faster in the presence of monosodium glutamate.

When organic acid was depleted, hydrogen production stopped. The production rose again with the addition of organic acid. Though hydrogen production could prolong in the absence of nitrogen source, the production decreased after several rounds of addition due to low number of cells. The result suggested the nitrogen limitation leading to lower growth and enzyme synthesis. An increase in light intensity from 10,000 to 19,000 lux had no effect on hydrogen production from lactic acid. However, hydrogen/mmole substrate used increased from 44 to 108 ml at 10,000 and 19,000 lux, respectively, when butyrate was used as substrate.

The optimal concentration of cell inoculum was 1.11 mg dw / ml. Increasing the cell concentration, the conversion of substrate to hydrogen decreased.

The fermentation products from anaerobic digestion of juice squeezed from pineapple solid waste were acetic, lactic, alcohol and sugar. The fermented juice was tested to produce hydrogen under anaerobic-light by *R. sphaeroides* 3701. Acetic and lactic acids were consumed leading to hydrogen production. The remaining of other bacteria and the composition of fermented juice affected the hydrogen production. For the best case, it was found that the lactic acid present in the fermented juice was utilized in parallel with the degradation of sugar. The continuation of lactic acid production from sugar maintained the availability of substrate for hydrogen production and the maximum production of hydrogen could be achieved.

Keywords : Hydrogen production / Photosynthetic bacteria / *Rhodopseudomonas sphaeroides* / juice squeezed from pineapple solid waste