THE STUDY OF HYDROGEN PRODUCTION USING PHOTOSYNTHETIC BACTERIA *RHODOSPIRILLUM RUBRUM*

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A thesis submitted in fulfillment of the
requirement for the award of the degree of Bachelor of
Chemical Engineering

Faculty of Chemical Engineering
Kolej Universiti Kejuruteraan & Teknologi Malaysia

NOVEMBER 2006

"Saya akui bahawa saya telah membaca karya ini dan pada pandangan saya karya ini adalah memadai dari segi skop dan kualiti untuk tujuan penganugerahan Ijazah Sarjana Muda Kejuruteraan Kimia"

Tandatangan	: ....
Nama Penyelia	: Asmida binti Ideris
Tarikh	: 20 November 2006

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I declare that this thesis entitled "*The production of hydrogen using photosynthetic bacteria Rhodospirillum rubrum*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree**.**

Signature	: ....
Name of Candidate	: Norbaya binti Mat Rani
Date	: 20 November 2006

Special dedication to my family members that always love me,
my supervisors, my friends, my fellow colleague and all faculty members
for all your support, care and believe in me

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ABSTRACT

Hydrogen has been considered a potential fuel for the future since it is carbon-free and oxidized to water as a combustion product. Photoproduction of hydrogen from synthesis gas has been carried out in a batch culture. Rhodospirillum rubrum, an anaerobic photosynthetic bacterium was used in this research to catalyze water gas shift reaction which is oxidize CO and water to CO_2 and hydrogen in batch fermentation. The synthesis gas (CO) was used as a source of energy along with tungsten light supplied for growth and bioconversion of the photosynthetic bacteria. The bacteria were grown under anaerobic condition in liquid media; acetate was used as a carbon source in presence of synthesis gas. The effect of different light intensity and shaking frequency on production of hydrogen were determined. The maximum hydrogen produce at intensity of 1600 lux that is 0.43 mmol H_2 while shaking frequency of 300 rpm produce 0.58 mmol H_2

ABSTRAK

Hidrogen boleh di anggap sebagai bahan bakar yang berpotensi pada masa hadapan kerana bebas karbon dan di oksidakan kepada air sebagai hasil pembakaran. Fotoproduk hidrogen daripada gas sintesis telah di lakukan dalam kultur berkelompok. *Rhodospirillum rubrum*, anaerobik fotosintetik bakteria telah di gunakan dalam penyelidikan ini untuk memangkinkan tindak balas air gas di mana CO dan H_2O di oksidakan kepada CO_2 dan H_2 dalam penapaian berkelompok. Gas sintesis (CO) di gunakan sebagai sumber tenaga di samping cahaya tungsten yang di bekalkan untuk pertumbuhan dan penukaranbio bakteria fotosintetik. Bakteria membesar di bawah keadaan anaerobik dalam medium cecair; asetat di gunakan sebagai sumber karbon yang hadir dalam gas sintesis. Kesan perbezaan kepekatan cahaya dan frekuensi goncangan terhadap penghasilan hidrogen di tentukan.

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INTRODUCTION

1.1 Introduction

Nowadays, the pollution of the air resulting from the consumption of fossil fuels are become one of the serious global problem. It has been widely discussed and it is important for us to note that when the fossil fuels become depleted, their cost will certainly escalate. This is because the fossil fuels are not renewable and also get decreased day to day. Therefore, fossil fuels has become more expensive. For this reason, much attention and effort must be given to develop a new clean energy with the potential to supplement and even substitute the fossil fuel based energy production. One of the alternatives as an environmentally acceptable fuel is hydrogen gas that has been considered a potential fuel for the future. Hydrogen has been recognized as clean fuel which oxidized to water as a combustion product and return to the atmosphere without causing any air pollution (Najafpour et al., 2004).

Hydrogen is a clean fuel. After combustion water vapor is formed and returned to atmosphere without causing any air pollution. Preview of current researches shown that, hydrogen is produced by number of processes, such as: electrolysis of water, thermo-catalytic reformation of hydrogen-rich organic compounds, pyrolysis of lignocellulosic biomass and biological processes [1]. An excellent new area of fuel technology which has great potential to develop for future and practical applications is bio-hydrogen production. An alternative biological formation of H_2 from water was defined using photosynthetic bacteria via water-gas shift reacti while carbon monoxide is oxidized to carbon dioxide. In the reaction, hydrogen is released as the synthesis gas, CO is oxidized to CO2 in the presence of anaerobic bacteria. The water gas shift reaction is shown in Equation 1

$$CO_{(g)} + H_2O_{(l)} \longrightarrow H_2O_{(g)} + CO_{2(g)} \qquad [1]$$

The hydrogen production by photosynthetic chemolithotroph bacteria was considered as an environmental friendly technology that does not depend on fossil fuels [3]. Anaerobic bacteria are able to grow autotrophically on synthesis gas. The synthesis gas production from solid fuel and biomass will be an important technology in future contribution of clean fuel. The gas is derived from non-gaseous carbonic matters such as coal, oil shale, tar sands, heavy oil residues and biomass [4

The photosynthetic purple non-sulfur bacteria, *Rhodopseudomonas gelatinosa* and *Rhodospirillum rubrum* are well-known to perform the water–gas shift reaction to produce hydrogen. *R. rubrum* is more preferable because of the high carbon monoxide (CO) uptake rate and high yield of hydrogen production [6]. Many photosynthetic bacteria such as *Rhodopseudomonas palustris, Rhodobacter sphaeroides, Rhodocyclus gelatinosus* and *Rhodospirillum rubrum* are able to catalyze the water-gas shift reaction under anaerobic condition at ambient temperature. However, the obtained results have shown that *Rhodospirillum rubrum* capable of producing hydrogen from synthesis gas with a very high yield, close to theoretical value [7].

It is also known that hydrogen maybe converted to liquid fuels with the biocatalytic activities of several photosynthetic bacteria. The potential bacteria in bioconversion of synthesis gas to liquid fuels and chemicals are *Clostridium aceticum, Acetobacterium woodii, Clostridium ljungdahlii*, and *Clostridium thermoaceticum* [8]. The purple non-sulfur phototrophic anaerobic bacteria, *R. rubrum* use light (photon) to produce hydrogen from CO. The organism maintains energy for growth and metabolism from organic acids or carbon monoxide. The growth and biocatalysts activity of *R. rubrum* have been investigated by many researchers. In this study, biological hydrogen production has been investigated using a photosynthetic anaerobic bacterium, *R. rubrum*. The purpose of this research was to investigate the effect of different light intensity and shaking frequency on the production of hydrogen.

1.2 Objectives

. The research was conducted to study the production of hydrogen using photosynthetic bacteria *Rhodosprillum rubrum*. The objectives of the study are:

1) To study the effect of different light intensity on the production of hydrogen

2)To study the effect of shaking frequency on the production of hydrogen

CHAPTER 2

LITERATURE REVIEW

2.1 Hydrogen overview

The latin words 'hydrogenium', from Ancient Greek translated as hydro: "water" and genes: "forming" is a chemical element in the periodic table that has the symbol H and atomic number of 1. At standards temperature and pressure hydrogen is a colorless, odorless, nonmetallic, univalent, tasteless, highly flammable diatomic gas (H_2). With an atomic mass of 1.00794 g/mol, hydrogen is the lightest element. It is also the most abundant, constituting roughly 75% of the universe's elemental matter[1]. Stars in their main sequence are overwhelmingly composed of hydrogen in its plasma state. Elemental hydrogen is relatively rare on Earth, and is industrially produced from hydrocarbon. Most free hydrogen is used captively with the largest markets about equally divided between fossil fuel upgrading (e.g. hydrocracking) and in ammonia production (mostly for the fertilizer market).

The most common naturally occurring isotope of hydrogen contains one electron and an atomic nucleus of one proton. In ionic compounds, it takes on either a positive charge (becoming a cation, a bare proton) or a negative charge (becoming an anion known as a hydride). Hydrogen forms compounds with most elements and presents in water and all organic compounds. It plays a particularly important role in acid-base chemistry, in which many reactions involve the exchange of protons between soluble molecules. As the only element for which the Schrödinger equation can be solved analytically, study of the energetics and bonding of the hydrogen atom has played a key role in the development of quantum mechanics.

2.1.1 Chemical And Physical Properties

The solubility and adsorption characteristics of hydrogen with various metals are very important in metallurgy and in developing safe ways to store it as a fuel. Hydrogen is highly soluble in many compounds composed of rare earth metals and transition metals and can be dissolved in both crystalline and amorphous metals. Hydrogen can combust rapidly in air, and was blamed for the disaster with the Hindenburg tragedy on May 6, 1937.Hydrogen gas is highly flammable and will burn at concentrations as low as 4% H_2 in air. The enthalpy of combustion for hydrogen is –286 kJ/mol.

When mixed with oxygen across a wide range of proportions, hydrogen explodes upon ignition. Hydrogen-oxygen flames are nearly invisible to the naked eye, as illustrated by the faintness of flame from the main Space Shuttle engines. Thus, it is difficult to visually detect if a hydrogen leak is burning. Although it is widely believed that the Hindenburg zeppelin burned because of a spark ignition of hydrogen gas, the flames are actually from the covering skin of the zeppelin which contained carbon and pyrophoric aluminium powder that may have started the fire. Another characteristic of hydrogen fires is that the flames tend to ascend rapidly with the gas in air, causing less damage than hydrocarbon fires.

H_2 reacts directly with other oxidizing elements. A violent and spontaneous reaction can occur at room temperature with chlorine and fluorine, forming the corresponding hydrogen halides, HCl and HF

2.1.2 Hydrogen Economy

Hydrogen economy is a hypothetical future economy in which the primary form of stored energy for mobile applications and load balancing is hydrogen (H_2). In particular, hydrogen is proposed as a fuel to replace the gasoline and diesel fuels currently used in automobiles.

Hydrogen production is a large and growing industry. Globally, about 50 million metric tons of hydrogen were produced in 2004 with the growth rate of 10% per year. The energy in the current flow corresponds to about 200 gigawatts. Within the U.S., production was about 11 million metric tons, or 48 GW About 10.8% of the average U.S. total electric production of 442 GW in 2003. Because hydrogen storage and transport are so expensive, most hydrogen is currently produced and subsequently utilized by the same company. As of 2005, the economic value of all hydrogen produced is about $135 billion per year.

Currently, hydrogen production is derived from natural gas (48%), oil(30%), coal (18%), and water electrolysis (4%.)

There are two primary uses for hydrogen today. About half is used to produce ammonia (NH_3) via the Haber process, which is then primarily used directly or indirectly as fertilizer. The other half of current hydrogen production is used to convert heavy petroleum sources into lighter fractions for fuels. The latter process is known as hydrocracking. As the world population and the intensive agriculture used are both growing, ammonia demand is growing. Hydrocracking represents an even larger growth area, as rising oil prices encourage oil companies to extract poorer source material, such as tar sands and oil shale. In the future, it is hoped that hydrogen will supplant current stored energy (oil), as a viable and clean source of mobile fuel for transport, manufacturing and the overall economy.

2.1.3 Application

Large quantities of H_2 are needed in the petroleum and chemical industries. The largest applications of H_2 is for the processing of fossil fuels, and in the production of ammonia. The key consumers of H_2 in the petrochemical plant include hydrodealkylation and hydrodesulfurization. Hydrocracking H_2 has several other important uses. H_2 is used as a hydrogenating agent, particularly in increasing the level of saturation of unsaturated fats and oils (eg margarine), and in the production of methanol. Hydrogen also been utilized in the manufacture of hydrochloric acid. and used as a reducing agent of metallic ores.

Apart from its use as a reactant, H_2 has wide applications in physics and engineering. It is used as a shielding gas in welding methods such as atomic hydrogen welding. H_2 is used as the rotor coolant in electrical generators at power stations, due to its high thermal conductivity. Liquid H_2 is used in cryogenic research, including superconductivity studies. Since H_2 is lighter than air,(1/15th of the density of air), it was once widely used as a lifting agent in balloons and airships.

Hydrogen's rarer isotopes also each have specific applications. Deuterium (hydrogen-2) is used in nuclear fission applications as a moderator to slow neutrons, and in nuclear fusion reactions. Deuterium compounds have applications in chemistry and biology in studies of reaction isotope effects. Tritium (hydrogen-3), produced in nuclear reactors, is used in the production of hydrogen bombs, as an isotopic label in the biosciences, and as a radiation source in luminous paints.

2.1.4 Hydrogen As An Energy Carrier

Having been used as an ingredient in some rocket fuels for several decades, H_2 is now widely discussed in the context of energy. H_2 is not an energy source, since it is not an abundant natural resource. However, it could become useful as a carrier of energy. As elucidated in the United States Department of Energy's 2003 report, among the various alternative energy strategies, building an energy infrastructure used hydrogen as the primary carrier that connects a host of energy sources, enable a secure and clean energy future for the Nation. The hydrogen would then locally be converted into usable energy either via combustion or by electrochemical conversion into electricity in a fuel cell. One theoretical advantage of using H_2 as a carrier, is the localization and concentration of environmentally unwelcome aspects of hydrogen manufacture. For example, CO_2 sequestration could be conducted at the point of H_2 production

2.2 Rhodospirillum overview

Rhodospirillum is a genus of photosynthetic bacteria of the family Rhodospirillaceae. Their cells are generally spiral-shaped, polarly flagellated and contain vesicular, lamellar of stacked photosynthetic membranes [14]. They range from three to ten micrometers in length and one-half to one and one-half micrometers in width. Cells divide by binary fission. One of the type species of this genus is *Rhodospirillum rubrum*, a purple nonsulfur bacteria. These bacteria falls under the Alpha subdivision of the kingdom Proteobacteria.

These purple-red *Rhodospirillum rubrum* contains vesicular photosynthetic membranes and a cell width of 0.8 to one micrometer. R. rubrum is a gram negative bacteria containing both saturated and unsaturated fatty acids. Its major carotenoids (pigments) are rhodovibrin and spirilloxanthin. Biotin is a required growth factor for R. rubrum and its GC content is 63.8 to 65.8 percent [10].

Unique in many ways, it grows both aerobically with oxygen or anaerobically using light for its energy metabolism. As a phototroph, R. rubrum can grow autotrophically or heterotrophically. Scince it does not produce oxygen as a by-product of photosynthesis, R.rubrum an anoxygenic phototroph [12]. Extracellular elemental sulfur is the final oxidation product in R. rubrum, [10]. This bacterium has been used in many studies, for example, to study radiation resistance of pigmented bacteria and nitrogen fixation.

This organism contains chlorophyll b, which is different than chlorophyll a found in plants. Chlorophyll b distinguished by a lower absorption spectra, absorbs maximally at 660 nm rather than at 680 nm, [11]. Anoxygenic phototrophs such as R. rubrum can contain several bacteriochlorophylls, and most purple bacteria have bacteriochlorophyll a, which absorbs maximally between 800 and 925 nm. Organisms with many different types of chlorophylls are at an advantage, because they can use more of the energy of the electromagnetic spectrum, [11].

In prokaryotes, photosynthetic pigments are integrated into internal membrane systems that arise from invagination of the cytoplasmic membrane (purple bacteria), [11]. The carotenoids found in R. rubrum not only give the microbe its purple-red color, they also help gather light energy for photosynthesis [12]. Carotenoids function as accessory pigments, play a photoprotective role against bright light and transfer energy to the reaction center to be used in photophosphorylation [11].

Rhodospirillum rubrum and other purple nonsulfur bacteria can be found in natural setting such as pond water, mud or a sewage sample [11]. Phototrophic purple nonsulfur bacteria are used in sewage treatment processes, for biomass production as a source of animal food or agricultural fertilizer, and production of molecular hydrogen by evolution from nitrogenase. They may be used also as a source of cell-free systems performing photosynthesis and ATP formation, and for the production of vitamins and other organic molecules [10].

Rhodospirillum rubrum has been the subject of a substantial amount of physiological and genetic analysis. It is widely distributed and is Gram negative, motile, and spiral shaped. It is capable of growth under an aerobical and anaerobical conditions under the latter conditions, fermentation was employed for the production of energy. Photoautotrophic growth also occurs. Areas of particular research interest have been nitrogen fixation, carbon monoxide oxidation, CO_2 fixation, H_2 production, the photosystem, and the ATP synthase.

The nitrogen fixation system is particularly well described, consisting of both a MoFe and an Fe-only nitrogenase. The system of post-translational regulation of nitrogenase activity is the best-understood. For example, the reversible ADP-ribosylation as a regulatory system in any organism. This system responds to both nitrogen and energy status signals. R. rubrum is able to grow on CO as sole energy source. The CO oxidation system has been extremely well-analyzed genetically and biochemically, largely through DOE support. The structure of the CODH that lies at the heart of this system, together with analyses of its catalytic mechanism, provide a model for more complex CODHs of other organisms. The CO-oxidation regulon also contains a novel CO-sensing protein, CooA, that is becoming a paradigm for gas-sensors and transcriptional regulators.

2.2.1 Bacteria characteristic

Table 2.1 : The characteristic of bacteria, *Rhodospirillum rubrum*

(1) gram-negative bacteria (all Proteobacteria are)	
(2) purple color - from cartenoid pigments rhodovibrin and spirilloxanthin	
(3) nonsulfur bacteria - can use sulfide as an electron donor for the reduction of carbon dioxide, but not at high concentrations like sulfur bacteria.	
(4) cell morphology - spiral shaped cells	- polarly flagellated - length 3-10 micrometers - width 0.8-1 micrometer - vesticular photosynthetic membranes
(5) carbon and energy metabolism	- grows aerobically with oxygen (chemotroph) - also grows anaerobically using light (phototroph) - can grow auto- and heterotrophically