

Chapter 17

A Feasibility Study of Large-Scale Photobiological Hydrogen Production Utilizing Mariculture-Raised Cyanobacteria

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and Kazuhito Inoue

Abstract In order to decrease CO₂ emissions from the burning of fossil fuels, the development of new renewable energy sources sufficiently large in quantity is essential. To meet this need, we propose large-scale H₂ production on the sea surface utilizing cyanobacteria. Although many of the relevant technologies are in the early stage of development, this chapter briefly examines the feasibility of such H₂ production, in order to illustrate that under certain conditions large-scale photobiological H₂ production can be viable. Assuming that solar energy is converted to H₂ at 1.2% efficiency, the future cost of H₂ can be estimated to be about 11 (pipelines) and 26.4 (compression and marine transportation) cents kWh⁻¹, respectively.

17.1 Our Need for Research and Development of Large-Scale Production of Renewable Energy

By 2005 the global atmospheric concentration of the greenhouse gas CO₂ had increased from a pre-industrial value of about 280 to 379 ppm (IPCC 2007). In order to mitigate global warming, the development of renewable non-polluting energy alternatives to fossil fuels on a worldwide scale is urgently needed (see Sakurai and Masukawa 2007; Sakurai et al. in press).

The amount of solar energy received on the earth's surface is vast (about $2,700,000 \times 10^{18}$ J/yr) and exceeds the present use of fossil fuel energy (404×10^{18} J/yr, in 2006) by more than 6,000 times. The technical challenge that must be overcome for solar energy to be an economically feasible alternative is the low intensity at which it is received on the earth's surface (about 1,500 kWh m⁻² yr⁻¹, at the middle latitudes). If we are able to convert solar energy into a usable form of energy

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at 1 and 2% efficiency, about 15 and 30 kWh m⁻² yr⁻¹, respectively, of renewable energy will be acquired in this region. If photobiological conversion of solar energy is to substitute for or supplement fossil fuels, economical energy production is essential. Considering that the amount of energy in foods accounts for only about 5% of the anthropogenic primary energy use (Sakurai and Masukawa 2007), we cannot expect large amounts of additional energy to be produced from land biomass, and thus we have proposed large-scale H₂ production utilizing mariculture-raised cyanobacteria. In proposing the system described below, we do not intend to criticize other systems such as the hydrogenase-based H₂ production and algal fuels (biodiesel).

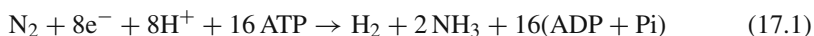
17.2 Nitrogenase-Based Photobiological Hydrogen Production by Cyanobacteria

17.2.1 *Hydrogenase and Nitrogenase as Hydrogen-Producing Enzymes*

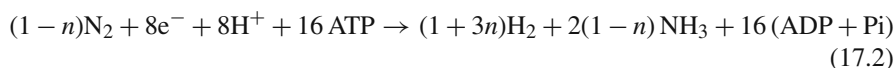
If large-scale H₂ production by mariculture is to be practical, the candidate photosynthetic organisms must use H₂O as the electron donor, thus narrowing the possibilities to cyanobacteria and eukaryotic microalgae. Both hydrogenase and nitrogenase are potential candidates as H₂-producing enzyme (review: Rao and Cammack 2001; for cyanobacteria: Tamagnini et al. 2002). In terms of the theoretical maximum energy conversion efficiency, hydrogenase (32.9% vs. 550 nm light (single-stage process), 22% (two-stage process)) is superior to nitrogenase (13.9–16.5%) (cf. C3 photosynthesis: 27.6%, C4 photosynthesis: 20.7–24.5%) (Sakurai and Masukawa 2007). However, hydrogenase catalyzes a reversible reaction and absorbs H₂ in the presence of O₂, when storage metabolites are exhausted, during the night or when shady conditions prevail. Hydrogenase-based processes therefore require frequent harvesting of H₂ or some measures to restrict H₂ reabsorption.

17.2.2 *Hydrogen Production by Nitrogenase*

Nitrogenase catalyzes the reduction of nitrogen to ammonia with reduced ferredoxin/flavodoxin as electron donors and with H₂ as the inevitable by-product. The reaction is expressed under the optimal conditions for nitrogen fixation, as

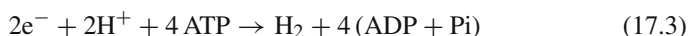


and more generally as



Nitrogenases typically bind a MoFeS cluster (Mo type) as the catalytic center, but some bind V (V type) or Fe (Fe-only type) instead of Mo. The latter types of enzymes are less efficient in nitrogen fixation, in other words, more favorable than the Mo type for H₂ production in the presence of N₂.

In the absence of N₂ (e.g., under Ar), all the electrons are allocated to H₂ production:



Although nitrogenase is less efficient in H₂ production than hydrogenase in terms of its theoretical maximum energy conversion efficiency as the reaction consumes large amounts of ATP, it has the merit of catalyzing a unidirectional production of H₂.

17.2.3 Heterocyst-Forming Cyanobacteria

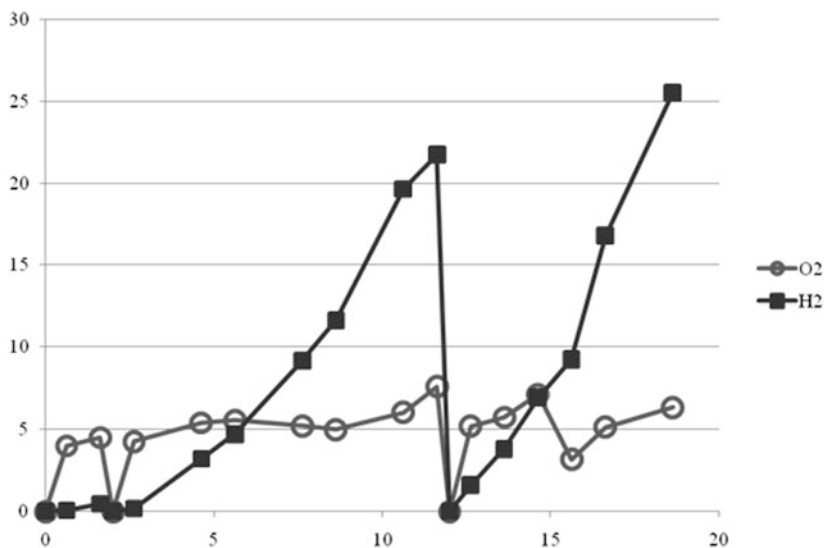
There are several types of strategies adopted by cyanobacteria in order to protect O₂-sensitive nitrogenase from the potentially dangerous O₂-evolving photosynthesis. We are using heterocyst-forming cyanobacteria because they are amenable to genetic engineering (Elhai and Wolk 1988) and because the whole-genome sequence of *Nostoc/Anabaena* sp. PCC 7120 strain was the first to be determined among the nitrogen-fixing cyanobacteria groups.

17.2.4 Effects of Inactivation of Hydrogenase Activity by Genetic Engineering

The entire process of photoinduced H₂ production is depicted as (1) production of organic compounds by ordinary C₃ photosynthesis accompanied by O₂ evolution in vegetative cells, (2) supply of organic compounds to cells specialized for N₂ fixation (heterocysts) that is devoid of O₂-evolving photosynthesis, (3) H₂ evolution (and N₂ fixation) by nitrogenase using organic compounds as electron donors. The presence of hydrogenases that reabsorb the H₂ is considered to be one of the major obstacles to achieving efficient solar energy conversion by a nitrogenase-based system, and a hydrogenase mutant of *Anabaena variabilis* ATCC 29413 generated by disrupting the *hup* gene was shown to have higher hydrogen-producing activity than the wild type (Happe et al. 2000). We have also created genetically defined hydrogenase-inactivated mutants of *Nostoc/Anabaena* sp. PCC 7120 and have shown that the mutants produced H₂ at four to seven times the wild-type rate (Masukawa et al. 2002).

Since our H₂ production system is based on photosynthesis and nitrogenase activities of cyanobacteria, we speculated that the wild-type strain with high nitrogenase activity under light might be a good candidate as the parent strain for further

improved photobiological H_2 production through genetic engineering. *Nostoc* sp. PCC 7422 was chosen from 12 other heterocystous strains because it has the highest nitrogenase activity. We sequenced the uptake hydrogenase gene (*hup*) cluster from the strain and constructed a mutant ($\Delta hupL$) by insertional disruption of the *hupL* gene (the wild-type cells of this strain showed almost no H_2 activity). The $\Delta hupL$ cells could accumulate H_2 to about 29% (Yoshino et al. 2007) in several days, in the presence of O_2 production (Fig. 17.1).



culture volume: 15 mL, gas volume: 10 mL,
1st N₂: 0.0%, 2nd N₂: 0.5%,

Fig. 17.1 Accumulation of H_2 by *Nostoc* sp. PCC 7422 Δhup mutant in the presence of evolved O_2 . A total of 15 ml of cells containing 30 μ g chlorophyll *a* grown in BG11₀ for 2 days were transferred to 25-ml flasks, and the H_2 (■) and O_2 (○) concentrations in the gas phase were determined daily. Light: 12-hour light–12-hour dark cycle (Kitashima et al. unpublished)

17.3 Outline of the Process Design of Large-Scale Hydrogen Production in the Future Utilizing Mariculture-Raised Genetically Improved Cyanobacteria

One of the plausible economical large-scale H_2 production systems for the future may be growth of cyanobacteria in large bioreactor floating on the sea surface, production of H_2 and its repeated harvesting (followed by H_2 gas separation), and

finally recycling of the waste cells as fish feed (Sakurai and Masukawa 2007). A plausible future process design is shown in Fig. 17.2.

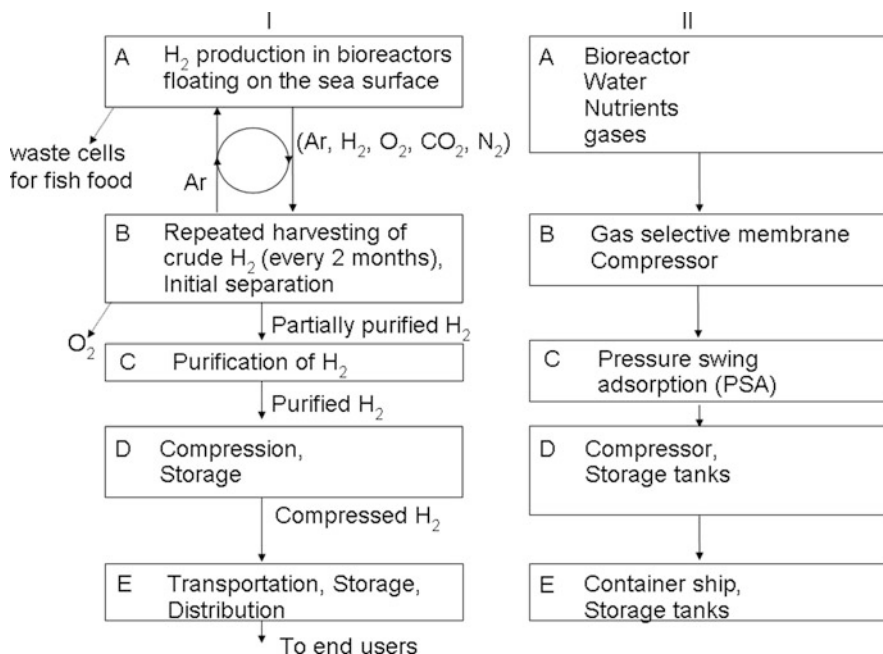


Fig. 17.2 Outline of photobiological H₂ production and transportation. (a) flowchart of process and (b) required process equipment. See text for (A–E)

17.3.1 H₂ Production in Bioreactors Floating on the Surface of the Sea

Cyanobacteria cells are first grown in a medium containing water and mineral nutrients under air plus CO₂ (e.g., 5%) fixing nitrogen in large plastic bioreactors consisting of several layers of plastic film, with at least one having low permeability to H₂. Each floating bag may be large (e.g., 25 m wide and 200 m long). Some areas of calm sea (such as inland seas) and ocean (e.g., the calm belts, the doldrums near the equator, and the horse latitudes of about 30° north or south) seem to be especially suitable for such large-scale mariculture in inexpensive plastic bags. If the medium is based on freshwater, the bioreactor would spread over the sea surface since the medium would have a lower density than the surrounding seawater. After a period of cell growth, simply decreasing the N₂ concentration (e.g., 1% N₂ in 5% CO₂ plus Ar) will prevent further growth while at the same time promote continuous H₂ production with concomitant evolution of O₂.

17.3.2 Repeated Harvesting of Crude H₂ and Initial Gas Separation

From the following assumptions, about 0.84 m³ (STP) of H₂ m⁻² of the bioreactor is produced in 2 months:

- Average solar energy received on the sea surface: 1,500 kWh m⁻² year⁻¹.
- Energy conversion efficiency by cyanobacteria: 1.2% (solar energy into H₂).
- H₂ produced in 2 months: 3 kWh m⁻² = about 0.84 m³ (STP) (with evolution of 0.42 m³ O₂) m⁻² of the bioreactor surface.
- If the volume of the initial gas phase is 0.5 m³ (STP) m⁻² of the bioreactor, then the final concentration of H₂ is about 48% (v/v) (0.84/(0.5 + 0.84 + 0.42)).

The gas mixture is harvested to a factory ship with hoses every 2 months with the assistance of working boats, and H₂ is initially separated from O₂ by gas-selective membranes (e.g., H₂ permeates a polychlorovinylidene film about 38 times faster than O₂, and the H₂ concentration can be increased to about 97% by a single operation). (This process of the initial separation is tentative.)

17.3.3 Further Purification of H₂

Contaminating O₂ (about 1.3%) is removed either by a second cycle of separation with gas-selective membranes or by using a catalyst (which would consume two volumes of H₂ for each volume of O₂). The H₂ is finally purified by pressure swing adsorption (PSA) on the factory ship.

17.3.4 Compression or Transformation to a Form Suitable for Transportation by Ship and Storage

For long-distance transportation of purified H₂ from the sea surface to the port, its volume should be greatly decreased by some means, possibly by compression (other possibilities: liquefaction, adsorption to alloy, etc). The H₂ is compressed into storage containers, transported by ships to final destination ports, unloaded, and stored awaiting final distribution.

17.4 Estimation of the Future Production Cost–Energy Balance

There are considerable uncertainties regarding the production processes and, therefore, the cost estimates of H₂ production are subject to change. Nevertheless, we present here an estimate so that the readers may understand the potential for such a

H₂ production system. We have detailed the costs of each item in the process separately so as to allow for the identification of the parts of the process that may be improved in order to reduce the total cost. With advances in relevant technologies, it will also be possible to recalculate the cost based on improved assumptions. In calculating the cost of H₂, the currency exchange rates assumed are US \$1 = 0.7 € = 95 ¥. As the value of energy of H₂, a high heating value (HHV, the oxidation product is condensed water) of 12.8 MJ m⁻³ is assumed (the low heating value (LHV, the product is vapor) is 10.8 MJ m⁻³, about 84% of HHV).

17.4.1 H₂ Production in Bioreactors Floating on the Sea Surface

A number of assumptions need to be made to estimate the net energy yield of the initial process. These can be divided into four areas.

17.4.1.1 Energy Conversion Efficiency (in the Future)

Cyanobacteria photobiologically convert solar energy (1,500 kWh m⁻² yr⁻¹, total radiation) into H₂ at 1.2% efficiency, resulting in 18 kWh or 64.8 MJ of H₂ m⁻² yr⁻¹.

17.4.1.2 Photobioreactor

The bioreactor is composed of three layers of plastic bags, for a total of six layers (sunny side and shady side) of transparent plastic film. The innermost bag holds the cyanobacterial culture, the middle bag has very low permeability to H₂, and the outermost bag serves as mechanical protection for the inner bags. The thickness of each film is 0.08 mm, and therefore 480 cm³ of plastic per m² of the bioreactor's sunny side surface is required. Assuming an average plastic price of \$2–4 kg⁻¹ (or liter), the material cost is 96–192 cents m⁻² of bioreactor. The used plastics can be recycled many times to regenerate plastic films at about half the price of the new materials. The above assumptions result in the cost of the bioreactor being about 48–96 cents m⁻² of bioreactor surface per year assuming once-a-year renewal.

Note that plastic film of 480 cm³ is assumed to be produced by consuming 360 ml of crude oil for processing, which is equivalent to 13.9 MJ (3.9 kWh) m⁻² year⁻¹. The plastics can be recycled at an energy cost of 20% of the feedstocks (about 0.78 kWh, 4.3% of H₂ produced). The amount of energy in feedstocks derived from fossil fuels can be decreased further because currently H₂ generated from fossil fuels is used as a part of the feedstocks for plastic film production, and photobiologically produced H₂ can replace some part of it.

17.4.1.3 Culture Medium

Nitrogen-fixing cyanobacterial cells can grow in liquid media without combined nitrogen. Cyanobacteria are cultured in liquid medium 20 cm in depth (200 l m⁻² or 0.2 ton m⁻² of the bioreactor) utilizing freshwater. Potentially growth-limiting

nutritional elements (especially the major ones, 18 mM K_2HPO_4 , 0.03 mM FeCl_3) are added to the medium as “fertilizers” akin to agricultural practices (5–20 cents). Once grown, cyanobacteria continuously produce H_2 allowing repeated harvesting, and further addition of nutrients and CO_2 is not necessary. If the medium is renewed twice a year, the cost of chemicals is calculated to be about 10–40 cents m^{-2} of the bioreactor per year (Sakurai et al. 2009). The cost of water for the medium (0.2 ton m^{-2}) is calculated to be 1.5–16 cents m^{-2} of the bioreactor surface from a reference price of water for industrial use sold by local governments in Japan at about 7.5–80 cents ton^{-1} . If the medium is renewed twice a year, the cost of water is 3–32 cents m^{-2} of the bioreactor per year.

As the water substrate for H_2 production, 18 g of H_2O can generate 22.4 l (STP) of H_2 , which corresponds to 1 kg of H_2O being converted to 1.24 m^3 of H_2 , with an energy content equivalent to about 0.35 l of crude oil (3.3 m^3 H_2 is equivalent to about 1 l of crude oil in enthalpy). The cost of water substrate used as the electron donor is thus negligible. Using eutrophic water could further reduce the cost of chemicals in the culture media.

17.4.1.4 Cost of Culture Gases

The initial gas phase composition is 5% CO_2 , 1% N_2 , and 94% Ar (0.5 m^3 m^{-2} of bioreactor surface). The price of Ar is assumed to be \$56 (a bulk rate), which leads to about 5 cents (2 cents with recycle, see below 17.4.2) m^{-2} of bioreactor. The costs of CO_2 and N_2 are small compared with Ar.

The sum of the Costs *AI-4* is calculated to be 63–170 (cents m^{-2}).

In addition to the Costs *AI-4*, the following costs will be incurred in the biological H_2 production stage: cyanobacteria growth costs, labor costs, the cost of ships, interest on capital goods, and the cost of marine transportation of production materials to the site of H_2 production.

17.4.2 Repeated Harvesting of Crude H_2 and Initial Separation

The gas mixture is harvested every 2 months (containing about 48% H_2) from bioreactors to a factory ship with the aid of a small group of boats. H_2 is partially purified in the initial separation process by gas-selective membranes, Ar is recycled to bioreactors, and O_2 is removed. We assume that 2% of energy in H_2 is lost in the initial separation process.

17.4.3 Further Purification of H_2

Contaminating O_2 (about 1.3%) is either removed by the catalyst consuming the two volumes of H_2 (2.6% of the energy). Thereafter, the H_2 is finally purified by pressure swing adsorption (PSA) on the factory ship with an overall energy efficiency of 85% with losses of 15% of energy. A subtotal of about 20% of energy is lost in 17.4.2 and 17.4.3.

17.4.4 Compression for Transportation by Ship

The purified H₂ is compressed to 35 MPa (about 15 kg/m³, energy content: 2.1 GJ/kg).

In the presentation “Well-to-Wheels Analysis,” Joseck and Wang (2007) estimated that 2,000 and 7,200 Btu (British thermal unit) of energy are required for compression (to about 35 MPa) and storage, respectively (about 8% in total), for the H₂ (116,000 Btu (100%)) originally generated by electrolysis powered by electricity from wind. By analogy, we assume that 8% of energy in 17.4.3 after PSA (80% energy yield) is lost in this process, which is equivalent to 6.4% of H₂ energy in the starting gas in 4.1A.

17.4.5 Marine Transportation and Storage

H₂ purified and compressed on a factory ship is transported to final destination ports in storage tanks by container ships. The landed H₂ can be transported either by pipelines or by trucks to end users. The compressed H₂ in containers is transported to ports by a container ship and delivered to final users. If the distance between the marine area of H₂ production and the port is 2,000 km, we assume that the energy lost is about 4% of H₂.

17.5 Estimation of Net Energy Production

We assume that 18×10^6 kWh of H₂ (100%) is produced per km² per year and 6% of energy is lost in the process A (including bioreactors), resulting in the subtotal energy losses (A–E) of about 36%: A (6%), B (5%), C (15%), D (6%), and E (4%). In addition to the above losses, fuels for a factory ship and a group of working boats will be required (estimated to be 4%). As a total of about 40% of energy in photobiologically produced H₂ is lost, the net energy of H₂ at the port is 10.8×10^6 kWh (270 ton) km⁻² year⁻¹ (equivalent to about 930 tons of gasoline (11.6 kWh kg⁻¹) or 980 tons of crude oil (10.8 kWh kg⁻¹)). This amount of energy will be more than enough to cover the energy cost required for manufacturing PSAs, compressors, storages, factory ships, cargo ships, etc., and therefore photobiological H₂ production would be able to produce a large quantity of net energy.

17.6 Estimation of Cost

17.6.1 Cost Analysis for Chlamydomonas-Based H₂ Production

The cost of hydrogenase-based photobiological H₂ production from the green alga *Chlamydomonas reinhardtii* was analyzed by Amos (2004). One of his assumed production systems is depicted roughly as follows: (1) growth of cells by ordinary

photosynthesis in ponds, (2) transfer of cells to anaerobic bioreactor (\$1 m⁻², ponds covered with transparent plastic films) and H₂ production (about 39 kWh m⁻² yr⁻¹, in Arizona; estimated solar radiation of about 2,600 kWh m⁻² yr⁻¹), (3) harvesting and purification of H₂ (compressor and PSA), and (4) compression of H₂ to 20 MPa (storage compressor and high-pressure storage). Assuming that ongoing improvements in technology are successful, he estimated a H₂ sale price of \$8.97 kg⁻¹ or 22.8 cents kWh⁻¹. The cost includes a 15% return on investment, and capital-related charges comprise about 90% of the cost. The largest cost arises from point 4, that is, compression and high-pressure storage (especially the latter), and is estimated to be \$7.75 kg⁻¹ of H₂, about 86% of the sale price. If the reactor is more expensive, the estimated H₂ sale price rises to 34.4 and 1,110 cents kWh⁻¹ for a reactor price of \$10 and 100 m⁻², respectively, indicating that reduction of reactor cost is very important in achieving economically viable production. By contrast, we are proposing a reactor consisting of three layers of plastic bags with a cost of about \$0.48–0.96 m⁻².

17.6.2 Estimation of the Cost of H₂ Production by Cyanobacteria

17.6.2.1 Comparison with Photobiological H₂ Production by *Chlamydomonas*

Overall, our cyanobacterial H₂ production system (System I) is rather similar to that of *Chlamydomonas* (System II) (Amos 2004) with some notable differences in H₂ production in the bioreactors.

Comparisons:

(1) *Cell culture*. The initial H₂ production costs from the bioreactors in System II are estimated to be about 1.45 and 9.34 cents kWh⁻¹ of the total capital costs assuming the reactors cost \$1 and 10 m⁻², respectively. In System I, the reactor cost is estimated to be \$0.48–0.96. In System I, a single type of bioreactor is required, and combined nitrogen can be omitted from the culture medium, which is renewed twice a year. In System II, a system with two continuous-flow reactors are used. Therefore, System II requires much more water and nutrient, notably combined nitrogen, than System I. In System I, ships and boats are required. The amounts of H₂ produced are 18 and 39 kWh m⁻² yr⁻¹ in System I and II, respectively. Overall, we simply assume here that the cost of the biological H₂ production stage is about the same (1.5 cents kWh⁻¹).

(2) *Harvesting of H₂ and initial separation*. In System I, initial separation of H₂ from O₂ is required, but not in System II. We tentatively assume a higher cost of 1 cent (about 4% of the final sale) kWh⁻¹ of H₂ as the final commodity. In System I, the gas is harvested at any time (typically every 2 months), but in System II, gas must be frequently harvested almost everyday or at least every week so that a higher number of backup storage systems and more labor would be required. In System I, no such backup system is required because the produced H₂ just inflates the plastic bags (see point 4).

(3) *PSA and high-pressure storage*. They are required in both systems, but the initial concentration of H₂ differs: about 48% in System I and nearly 100% in System

II. The pressure is higher in System I (35 MPa) than in System II. We assume an additional cost of 0.8 cent (about 3% of the final sale) kWh^{-1} of the final commodity H_2 for PSA and compression. As 15% more H_2 energy is lost in PSA in System I, about 0.2 cents should be added (the cost of this process in System II is about 1.5 cents kWh^{-1} of H_2).

(4) *Marine transportation*. In System I, marine transportation of the product H_2 is required, but not in System II. The cost is estimated from the following assumptions: (4.1) The distance from the site of H_2 production to port is 2,000 km. If the speed of the freighter is 800 km day^{-1} (4,000 km of a round trip requires 5 days), and if it takes a half-day each for uploading and unloading the high-pressure storage, a total of 6 days will be required for one round trip. Therefore, a freighter may carry H_2 about 60 times a year. (4.2) The mass percent of compressed H_2 is assumed to be 8% (% in weight of $\text{H}_2/(\text{H}_2 + \text{high-pressure storage})$). A 10,000 ton-class freighter carries 800 ton of H_2 (about $31.5 \times 10^6 \text{ kWh}$) to a port at a time or 48×10^3 ton of H_2 (about $1.9 \times 10^9 \text{ kWh}$) (collected from 180 km^2 of bioreactors) a year. The annual sale of 10,000 ton-class freighters is assumed to be \$20 million, and the cost of H_2 transportation is calculated to be 1.1 cents kWh^{-1} .

Because of the marine transportation used in System I, a higher capacity storage system is required than for System II. However, as discussed in Section 17.6.1, point 2, System II requires a greater number of storage backup systems. We assume here that the total storage capacity is about the same for the two systems.

17.6.2.2 Estimation of the Price of H_2 Produced by Mariculture-Raised Cyanobacteria

Amos (2004) estimated a H_2 sale price of 22.8 cents kWh^{-1} ($\$8.97 \text{ kg}^{-1}$) assuming the reactor cost of $\$1 \text{ m}^{-2}$. From the above-described comparisons, the sale price of H_2 produced by cyanobacteria (System I) is calculated to be 25.9 (22.8 + 1 + 0.8 + 0.2 + 1.1) cents kWh^{-1} plus costs of the factory ship and working boats and labor costs thereof. The cost of the factory ship itself is calculated to be very small (about 0.01 cent kWh^{-1}) from the assumptions; the price of a factory ship of 40,000 DWT (dead weight ton) is \$2 million, life: 40 years, annual interest: 5%, the ship produces $1.9 \times 10^9 \text{ kWh}$ of H_2 a year (see 17.6.2.1). The cost of working boats is also small. The labor of the crew is assumed to be 0.5 cent kWh^{-1} : 50 persons, annual salary of \$100,000 per person (including the cost of management), divided by $1.9 \times 10^9 \text{ kWh}$ of H_2 . From the above assumptions, the sale price of H_2 is calculated to be 26.4 cents kWh^{-1} .

In System II, the greatest cost arises from point 4, that is, compression and high-pressure storage (estimated to be 19.7 cents kWh^{-1} of H_2 ($\$7.75 \text{ kg}^{-1}$) of H_2). If this process can be omitted by directly connecting to H_2 pipelines, then the final price of H_2 produced by *Chlamydomonas* drops from 22.8 to 7.2 cents kWh^{-1} of H_2 (Amos 2004). With the cyanobacteria system, if the bioreactors are floated near land, and if a pipeline system is available, the final price of H_2 will drop to about 11 cents kWh^{-1} of H_2 .

17.7 Improvements Required in Biological Research

By increasing the energy conversion efficiency of photobiological H_2 production, the H_2 selling price drops due to reduced bioreactor cost and reduced labor charges per unit amount of H_2 . The theoretical maximum energy conversion efficiency of photobiological H_2 production is estimated to be 13.9–16.5% vs. 550-nm visible light (about 6.3–7.4% vs. total solar radiation assuming that visible light (photosynthetically active radiation, PAR) is 45% of the total solar radiation) (Sakurai and Masukawa 2007). Under laboratory conditions, the efficiency of around 3.8% (vs. visible light, which corresponds to about 1.7% vs. total solar radiation) was reported by several groups (e.g., Yoshino et al. 2007). These values apparently exceed our tentative target of 1.2%. However, these high efficiencies are only attained over a relatively short period (several hours) under low light intensities of about one twenty-fifth of full sunlight at the equator. Under outdoor conditions, a reported best efficiency over a relatively long period (days) is about 0.1% (Tsygankov et al. 2002). Thus, more research is needed to improve the long-term outdoor efficiency.

17.7.1 Potential Methods for Further Improvement in Efficiency

Potential methods for improvement of outdoor energy conversion include (1) reduction of antenna size, (2) improvement of nitrogenase (site-directed mutagenesis, use of V-type nitrogenase, reduced concentration of homocitrate essential for efficient nitrogen fixation; Masukawa et al. 2007), (3) improvement of culture conditions, (4) selection of promising wild-type strains followed by genetic engineering (e.g., Yoshino et al. 2007).

17.8 Conclusions

The future price of photobiologically produced H_2 at 1.2% energy conversion efficiency by mariculture-raised cyanobacteria is calculated to be 26.4 cents kWh^{-1} of H_2 . If H_2 pipelines were available the price would drop to 11 cents kWh^{-1} of H_2 . Although this is more expensive than the current price of crude oil, \$50–150 per barrel (about 159 l), equivalent to 2.9–8.8 cents kWh^{-1} , and gasoline (the retail price of \$1.5–4 per gallon is equivalent to about 4–11 cents kWh^{-1}), the price of H_2 could be further decreased by improving the light conversion efficiency and by advances in other relevant technologies. Research and development of photobiological renewable energy sources should be more earnestly pursued because photobiologically produced H_2 contributes to the reduction of the greenhouse gas CO_2 emission, and H_2 fuel cells are expected to be more energy efficient than internal combustion engines.

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References

- Amos WA (2004) Updated cost analysis of photobiological hydrogen production from *Chlamydomonas reinhardtii* green algae – Milestone completion report. NREL/MP-560-35593. www.nrel.gov/docs/fy04osti/35593.pdf. Accessed 5 August 2008
- Elhai J, Wolk CP (1988) Conjugal transfer of DNA to cyanobacteria. *Methods Enzymol* 167: 747–754
- Happe T, Schütz K, Böhme H (2000) Transcriptional and mutational analysis of the uptake hydrogenase of the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413. *J Bacteriol* 182:1624–1631
- Joseck F, Wang M (2007) Well-to-Wheels Analysis. Presented to HTAC on July 31, 2007. www.hydrogen.energy.gov/pdfs/htacjuly07_well_to_wheels.pdf. Accessed 5 August
- Masukawa H, Mochimaru M, Sakurai H (2002) Disruption of the uptake hydrogenase gene, but not of the bidirectional hydrogenase gene, leads to enhanced photobiological hydrogen production by nitrogen-fixing cyanobacterium *Anabaena* sp. PCC 7120. *Appl Microbiol Biotechnol* 58:618–624
- Masukawa H, Inoue K, Sakurai H (2007) Effects of disruption of homocitrate synthase genes on photobiological hydrogen production and nitrogenase of *Nostoc* sp. PCC 7120. *Appl Environ Microbiol* 73:7562–7570
- Rao KK, Cammack R (2001) Producing hydrogen as a fuel. In: Cammack R, Frey M, Robson R (eds) *Hydrogen as a fuel – Learning from nature*. Taylor & Francis, London and New York, pp 201–230
- Sakurai H, Masukawa H (2007) Promoting R & D in photobiological hydrogen production utilizing mariculture-raised cyanobacteria. *Mar Biotechnol* 9:128–145
- Sakurai H, Masukawa H, Inoue K (2009) A preliminary survey of the economical viability of large-scale photobiological hydrogen production utilizing mariculture-raised cyanobacteria. In: Gault PM, Marler HJ (eds) *Handbook on cyanobacteria: biochemistry, biotechnology and applications*. Nova Publishers, COMMACK, NY, pp 443–462
- Tamagnini P, Axelsson R, Lindberg P, Oxelfelt F, Wünschiers R, Lindblad P (2002) Hydrogenases and hydrogen metabolism of cyanobacteria. *Microbiol Mol Biol Rev* 66:1–20
- Tsygankov AA, Fedorov AS, Hisourov SN, Rao KK (2002) Hydrogen production by *c* cyanobacteria in an automated outdoor photobioreactor under aerobic conditions. *Biotechnol Bioengineering* 80:777–783
- Yoshino F, Ikeda H, Masukawa H, Sakurai H (2007) High photobiological hydrogen production activity of a *Nostoc* sp. PCC 7422 uptake hydrogenase-deficient mutant with high nitrogenase activity. *Mar Biotechnol* 9:101–112