Hydrogen production in bioreactors: current trends.

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Abstract

It is possible to design bioreactors for large-scale H₂ production using microorganisms. Bioreactors can be distinguished based on the nature of H₂ production: bioreactors with H₂ production in the darkness by bacteria via water-gas shift reaction or fermentation and photobioreactors with H₂ production from H₂O using solar light by cyanobacteria or green algae. Hydrogen produced in a “water-gas shift reaction” or from glycerol is clean for injection into fuel cells. Fermenters or bioreactors with immobilized cells are used for H₂ production in the darkness. Research efforts are concentrated on designing tubular or plastic bag photobioreactors incorporating green algae and cyanobacteria.

Keywords: Biological hydrogen production; biohydrogen; photobioreactor; bioreactor

1. Introduction

The conventional industrial methods for H₂ production are costly and the problem has been to find a cheaper way to produce hydrogen. Certain microorganisms evolve H₂ either from organic materials (i.e., sugar or biomass) or from water in reaction catalyzed by nitrogenase or hydrogenase enzymes [1]. Biological H₂ (biohydrogen) production by microbial species has a number of advantages and it could be a cost-effective alternative to the current industrial methods of H₂ production [2]. Bioreactors are absolutely essential for industrial, large-scale H₂ production by microorganisms [3]. The bioreactor is a devise for cell growth (microbial, plant or animal cells) with practical purpose under controlled conditions.

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conditions. Bioreactors are closed systems and vary in size from the small (5 to 10 mL) laboratory scale to the larger one of more than 500,000 L industrial scale.

Microbial bioreactors for H₂ productions can be divided in two separate groups based on the nature of H₂ evolving reactions:

1. Bioreactors based on dark anaerobic H₂ production by bacteria.
   - Bioreactors based on "water-gas shift-reaction"
   - Bioreactors based on fermentation

2. Photobioreactors based on H₂ photoproduction.
   - Photobioreactors incorporating cyanobacteria.
   - Photobioreactors incorporating green algae

Photobioreactors are bioreactors that are made of an array of tubes, tanks or plastic bags, in which photosynthetic microorganisms including algae are cultivated and monitored [4, 5]. Since light is the essential component for growing photosynthetic microorganisms, these bioreactors are called photobioreactors.

Ten years ago, we published a paper on a similar topic [6]. The aim of this paper is to update on current developments in our laboratory in this field of study.

2. Bioreactors

2.1. Bioreactors based on "water-gas shift reaction"

"Water-gas shift reaction" bioreactors are based on unique type of H₂-producing activity originally found in a strain of purple bacteria by Uffen [7]. Cultures of Uffen’s strain in a complex organic medium carry out a water-gas shift reaction in darkness to produce H₂ according to the equation:

\[ \text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2 \]

During the shift reaction the bacterial cells produce hydrogen from water as verified by experiments with \(^3\text{H}_2\text{O} \) [8]. Since the time of Uffen’s discovery, a strain of nonsulfur purple bacterium <i>Rubrivivax gelatinosus</i> CBS, has been isolated from the mud water in Colorado (USA) by scientists from the National Renewable Energy Laboratory (NREL) [9,10]. This bacterium utilizes CO in darkness and does not require complex organic substrates.

We constructed a laboratory-scale hollow-fiber bioreactor (the total surface area of hollow fibers was 0.8 m²) for the production of H₂ by the immobilized purple bacterium <i>R. gelatinosus</i> (Figure 1). Bioreactor column was made from a 250 mm × 33 mm AM-4OM-SD cartridge with hydrophilic cuprammonium rayon hollow fibers (Asahi Medical CO. Ltd, Japan). The hollow fibers are made of semipermeable polymeric membranes. Gases (<i>i.e.</i>, CO) freely diffuse through the membrane. Bacterial cells, due to their larger size, cannot penetrate the membrane.

Mass transport of gaseous CO into an aqueous bacterial suspension is the rate-limiting step and the main challenge for bioreactor design. A simple method using hollow-fiber technology to enhance mass transfer of CO has proven effective. The bioreactor was designed such that CO (10% in N₂) passes from the inside of the fibers to the outside within the bioreactor. Bacteria were immobilized to the outer
surface of the hollow fibers. Hydrogen production from H₂O at an average rate of 125 ml·g dry weight⁻¹·h⁻¹ was observed for more than 8 months.

The gaseous product from our bioreactor, enriched in H₂ (20% H₂) and devoid of any remaining CO, was sufficiently clean for direct injection into a H₂ fuel cell. In fact, the effluent gas from the hollow-fiber bioreactor has been directly injected into small mV fuel cells and has shown capable of generating enough electricity to power small motors and lamps. No negative effect on the fuel cells was noted.

Carbon monoxide for the shift reaction can be produced by other microorganisms. A number of bacteria are able to degrade different molecules such as chlorophyll with the release of CO [11]. Additionally, R. gelatinosus can quantitatively shift the CO component of synthesis gas (e.g., from thermally gasified biomass) into H₂.

2.2. Bioreactors based on fermentation

Several bacteria can produce H₂ in anaerobic conditions from organic substrates according to the equation:

\[
[CH₂O]_n \rightarrow \text{Ferredoxin} \rightarrow \text{Hydrogenase} \rightarrow H₂
\]

organic substrate

Hydrogen production by fermentation has many advantages for industrial H₂ production. Among these advantages are: high production rates and high growth rates of bacteria [12]. Additionally, for this type of H₂ production, it is possible to use traditional bioreactors or fermenters (stirred or non-stirred tanks), such as used in ethanol production. Fermenters can be operated as continuous or batch culture. In continuous culture nutrients are continuously fed into the fermentation vessel, so that microbial cells can produce H₂ indefinitely.
In the past, the major problem that limited the successful implementation of H₂ production by dark fermentation was the low energy efficiency of substrate conversion: not all the energy of substrate was converted into H₂ energy, but instead some energy is still stored inside other products of fermentation such as acids or alcohols [12].

We used glycerol as a substrate for fermentative H₂ production by common bacterium Enterobacter aerogenes in bioreactors. Glycerol has recently become an abundant commodity due to its generation as a by-product of biodiesel production [13]. World biodiesel production is expected to reach 12 billion liters by the end of 2010 [14], with about one pound of glycerol created for every 10 pounds of produced biodiesel [14]. At present, a surplus of glycerol is destroyed by incineration [13]. However, burning glycerol produces nitrogen oxide as well as carbon dioxide (CO₂), the primary greenhouse gas. The over-production of glycerol considerably affects economic viability of the biodiesel industry [13]. Yet, using the surplus of glycerol for H₂ production offers a number of considerable benefits for biodiesel industry and the planet. Exact metabolic pathways of glycerol are not known for of E. aerogenes. It is well known, that this bacterium can ferment glucose via 2, 3-butanediol fermentation with main products such as 2, 3-butanediol, H₂, and CO₂, and minimal amounts of ethanol. Both 2, 3-butanediol and ethanol are valuable industrial commodities.

We used a traditional stirred-tank bioreactor BioFlo/CelliGen115 bioreactor/fermenter (10 L working volume) for glycerol conversion into H₂ by bacterium E. aerogenes. Hydrogen production from glycerol (2% glycerol, v/v) by E. aerogenes in our bioreactor was measured over the 6 days, showing the maximal H₂ rate at 650 mL·g⁻¹·h⁻¹. Hydrogen production rates and the glycerol uptake efficiency (65%) by E. aerogenes was exactly the same in the bioreactor and test tubes. This indicates the possibility that bioreactor for glycerol conversion into H₂ can be operated without agitation, which may offer a significant saving of energy for industrial applications of this technology. Hydrogen from this bioreactor has been directly injected into small mV fuel cells as well and shown capable of generating enough electricity to power small motors and lamps.

3. Photobioreactors

In cyanobacteria and green algae H₂ production is tied to photosynthetic water splitting reactions. Thus, it is possible to use solar (light) energy for the production of H₂ from water. The main challenge in photobioreactor design is to create a simple, inexpensive, with high volumetric productivity energy efficient photobioreactor, which is scalable to industrial capabilities. Photobioreactors should also provide the most efficient utilization of the solar energy, and allow monitoring culture purity and basic process parameters. To be used outdoors, such photobioreactor requires a cooling system (water sprinkler, heat exchange, or photobioreactor can be simply submerged into the ground) during summer and early fall periods, and the mixing of algal culture to achieve uniform illumination of all cells. Current research and development efforts are concentrated on designing tubular PVC or plastic bag photobioreactors incorporating cyanobacteria and green algae.

3.1. Photobioreactors incorporating cyanobacteria

Cyanobacteria are a diverse group of prokaryotic microbes which use plant-type O²-evolving photosynthesis. Cyanobacteria can be divided into heterocystous and nonheterocystous species. In heterocystous strains specialized cells, heterocysts, are the site of H₂ production under aerobic conditions. The enzyme nitrogenase is a major catalyst of H₂ production in heterocystous cyanobacteria.
to green algae, water involved in H₂ production by heterocystous cyanobacteria indirectly through a long chain of reactions according to scheme:

Reactions occur in vegetative cells:

\[ \text{O}_2 \rightarrow \text{H}_2\text{O} \rightarrow \text{Photosystem II} \rightarrow \text{Photosystem I} \rightarrow \text{Ferredoxin} \rightarrow [\text{CH}_2\text{O}]_n \rightarrow \text{Organic compounds} \]

Reactions occur in heterocysts:

\[ [\text{CH}_2\text{O}]_n \rightarrow \text{Photosystem I} \rightarrow \text{Ferredoxin} \rightarrow \text{Nitrogenase} \rightarrow \text{H}_2 \]

Several problems exist on a path to the cost effective H₂ production by cyanobacteria in photobioreactors, including:

1. N₂ interaction: cyanobacteria produce H₂ during nitrogen fixation according to equation:
   \[ \text{N}_2 + 8\text{e}^- + 8\text{H}^+ + 16\text{ATP} \rightarrow 2\text{NH}_3 + \text{H}_2 + 16\text{ADP} + 16\text{Pi} \]

2. H₂ uptake: cyanobacteria produce H₂, but in the same time consume H₂.

In our studies we tried to solve these problems (1 and 2) of cyanobacterial H₂ production.

1. Molecular nitrogen content in air can be reduced by using partial vacuum within a photobioreactor. The photobioreactor system was evacuated (250-300 torr) in our experiments and we achieved higher H₂ production rates of 20 mL g⁻¹ dry weight h⁻¹ than any reported so far for other cyanobacteria.

2. With the generation of a cyanobacterium *Anabaena variabilis* mutant PK84 [15], without uptake hydrogenase activity it was possible to build a tubular PVC photobioreactor for H₂ production by cyanobacteria without H₂ uptake. The photobioreactor was 2L total volume and 0.4 m high. The photobioreactor consisted of a transparent PVC tube, 7.9-mm internal diameter, which was wound helically on a vertical, transparent, cylindrical supporting structure. The cyanobacterial suspension in a photobioreactor was bubbled with a mixture of CO₂ and air to supply the cells with a carbon source and remove H₂. Hydrogen production rates up to 19 mL m⁻² h⁻¹ were observed under light intensities of 3 W m⁻².

3.2. *Photobioreactors incorporating green algae*

Green algae can produce H₂ directly from water in a reaction catalyzed by hydrogenase, but only for a short time under anaerobic conditions, since hydrogenase is very sensitive to O₂.

\[ \text{O}_2 \rightarrow \text{H}_2\text{O} \rightarrow \text{Photosystem II} \rightarrow \text{Photosystem I} \rightarrow \text{Ferredoxin} \rightarrow \text{Hydrogenase} \rightarrow \text{H}_2 \]
Because of oxygen sensitivity of green algal hydrogen production, only a few reports are available so far on photobioreactors incorporating green algae [16]. Green algae are one of the largest (with more than 7000 species) and most diverse class of algae in fresh waters, but also exist in marine environments.

For six months, we have operated a hollow-fiber photobioreactor (similar to the one described above) continuously for the production of H₂ using immobilized cell of a green alga *Chlamydomonas reinhardtii* CC-503 cw92 mt⁺. The growth medium was pumped into the photobioreactor column outside the hollow fibers, and the cells were immobilized on the outside surface of the fibers (Figure 2). The medium flowed into the hollow-fiber inner space and then out via external tubing to a gas trap, where H₂ was separated from the medium. Light intensity was 15 mol•m⁻²•s⁻¹. The photobioreactor utilized hydrogenase activity of the green alga *C. reinhardtii*. Hydrogen production was observed under partial vacuum at an average rate of 6.0 ml•g dry weight⁻¹•h⁻¹. Notably, H₂ production was achieved under ambient conditions in the presence of dissolved O₂.

Another possible design of algal photobioreactor for H₂ is a plastic bag photobioreactor. The bag is generally thin to allow deep light penetration. Such photobioreactors were used to grow microalgae with external light source. In particularly, we used a 50 L plastic bag (cell suspension working volume is 25 L) photobioreactors to grow green microalga *Neochloris oleoabundans* (Neo) in batch mode under artificial and natural illumination in order to study the pattern of algal growth. The photobioreactor was supplied with gas mixture (5% CO₂ and air). We exceeded our expectations by obtaining higher volumetric productivity values (3-4 g per L per day) compared to volumetric productivity data from other microalgae reported in published papers [17].
4. Conclusion

Hydrogen production in bioreactors in the darkness is ready for practical applications. Hydrogen produced by bacteria in the “water-gas shift reaction” or from glycerol by fermentation is sufficiently clean for direct injection into hydrogen fuel cells. Traditional fermenters (stirred tank bioreactor) or bioreactors with immobilized cells can be used for H₂ production by bacteria in the darkness. As for photobioreactors, our current research and development efforts were concentrated on photobioreactors with immobilized cells, PVC tubular photobioreactors, and plastic bag photobioreactors incorporating green algae and cyanobacteria.

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