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Production of Hydrogen as a Potential Source of Renewable Energy from Green Algae – A Review

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Abstract. Today, as the world is facing a crisis in terms of energy economization, different studies are being carried out to promote different sources renewable energy in future. The objective of this study is to promote one such extremely efficient and clean source of renewable energy – the green algae. The basic principle is – the green algae, e.g. *Chlamydomonas reinhardtii* can photosynthetically produce molecular hydrogen under sulphur deprived conditions. They offer a biological route to decarbonized hydrogen production via a renewable source, hence strengthening hydrogen's claim to become the sustainable fuel of the future. Molecular hydrogen is an energy carrier that has the potential to provide the clean energy required for transport, heating and electricity.

The hydrogen production is initiated under anaerobic conditions when the hydrogenase enzyme is released. During normal photosynthesis the production of oxygen and hence the presence of oxygen during respiration around the microorganism inhibits the release of hydrogenase enzyme. When the anaerobic conditions prevail, the oxygen release during photosynthesis is consumed by algae itself and hence, hydrogenase is released. Experiments have shown that if the enzyme is added externally, hydrogen production increases 400%. Algal hydrogen production does not generate any toxic or polluting by-products and could potentially offer value-added products derived from algal biomass. We conclude that as this microorganism is abundantly found on the earth (approximately 50,000 microalgae can be caught on an average human finger easily), this non-polluting and clean source of renewable energy is very much capable of meeting with the ever increasing world's energy requirements in the future.

Keywords: Algae, Anaerobic, By Product, Enzyme, Hydrogen, Renewable.

1. Introduction

Hydrogen gas holds the promise of providing mankind with a clean and renewable energy carrier that does not stress our environment. The advantage of using hydrogen as an energy carrier to power vehicles is that when it combines with oxygen the only byproducts are water and heat. Thus, the use of hydrogen greatly reduces pollution. Molecular hydrogen is energy carrier that has the potential to provide the clean energy required for transport, heating and electricity. It is the optimum feed for fuel cells, where water is the sole product of the electrochemical cycle. At present, the production of hydrogen is expensive and hardly reaches a greater output than input of energy. Today, basically hydrogen is produced by steam methane reforming process at high temperatures in large central facilities, a process that negates many of the benefits of using H_2 as a fuel. This lays the importance of finding new alternatives for production of hydrogen as a source of energy in future.

Photobiological H_2 production by green algae provides a promising prospect of the source of energy for our future. The fact that it is a clean fuel generated from light and water (both of which occur as plentiful resources in nature) can be put into use. Algal fuels do not require arable land and do not affect fresh water resources, can be produced using ocean and wastewater, and are biodegradable and relatively harmless to the environment if spilled. Other advantages of using green algae for production of hydrogen include – less

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doubling time required in case of microalgae (4-6 hours), easy gene manipulation to increase yields is possible and conditions can be controlled to maximize yields. It can be observed that algae have the potential to yield greater volumes of biofuel per acre of production than other biofuel sources. In fact, algae can produce up to 300 times more oil per acre than conventional crops, such as palms and soybeans.

2. The Process of Green Algal Hydrogen Production

Molecular hydrogen has the potential to become the fuel of the future, but only if it is produced by a sustainable process. The aim is to generate carbon-free H_2 using unlimited resources, sunlight and water. The green alga *Chlamydomonas reinhardtii* (*C. reinhardtii*) has the ability to photosynthetically produce H_2 under anaerobic conditions. Initially, photons are absorbed within the chloroplasts of *C. reinhardtii*. This solar energy facilitates the photochemical oxidation of water into protons and oxygen molecules by photo system II (PSII) proteins. Electrons generated in this process are transported by Ferredoxin to the iron-hydrogenase enzyme. The process of proton and electron recombination is catalysed by the [Fe] hydrogenase enzyme to produce H_2. Hydrogenase activity is inhibited in the presence of molecular oxygen (O_2), which implies that the direct bio photolysis process is self-limiting. In order to maintain a continuous H_2 process, it is necessary to remove the oxygen as it is being produced. Sulphur deprivation of *C. reinhardtii* diminishes its ability to repair PSII proteins, thus reducing photosynthetic O_2 production below the level of respiratory O_2 consumption so that overall, O_2 is being used up. Algal metabolism is therefore responsible for creating an anaerobic environment that leads to sustained H_2 production. The main barriers to the commercialisation of green algal H_2 production technologies are the low photochemical conversion efficiencies and the prohibitive photobioreactor (PBR) costs.

In green algae, the [Fe]-hydrogenases are nuclear encoded, monomeric enzymes that are localized in the chloroplast. Ferredoxin PetF, the natural electron donor, links the [Fe] hydrogenase to the photosynthetic electron transport chain. Electrons from reducing equivalents that are generated during fermentation are supplied into the photosynthetic electron transport chain via the plastoquinone pool. Thus, the [Fe] hydrogenase serves as an electron valve and enables the algae to survive under anaerobic conditions.

2.1. Algal growth-materials and methods

C. reinhardtii is a unicellular green alga commonly found in soil and fresh water, with a diameter of approximately 10 mm. Laboratory wild-type *C. reinhardtii* strains (such as CC-124) are used as model organisms for studying chloroplast-based photosynthesis. *C. reinhardtii* has a relatively large chloroplast, which allows it to efficiently harvest the solar energy it needs for photosynthesis. It is consequently a robust organism that can outgrow most competing microalgae and bacteria. Algal growth was monitored by utilising the optical properties of the culture to measure either its chlorophyll content or optical density (OD). Chlorophyll content is a function of light absorption at the photosynthetically active wavelengths of 645 nm and 663 nm. It provides an indication of the culture health, since damaged cells tend to decolourise. OD is a direct measure of the light scattering in the near infra-red spectrum. OD measuring probes are widely available and therefore favoured for use in PBRs. Some of the key parameters that have been shown to influence *C. reinhardtii* growth are wavelength, light intensity; agitation and pH. Cultures were grown to PBR volumes of 1E3 L using the Tris acetate phosphate (TAP) growth medium. The TAP medium provides all the necessary nutrients (nitrogen, carbon, sulphur and trace elements) for *C. reinhardtii* to grow efficiently, but it also contains expensive ingredients such as acetate, ammonium and phosphate.

2.2. Sulphur deprivation

Sulphur deprivation results in metabolic changes within *C. reinhardtii*, with an initial accumulation of starch and breakdown of endogenous protein and fat. It also reduces photosynthetic rates as sulphur is required to repair PSII damage. The result is that O_2 evolution gradually decreases while respiration levels are maintained, causing the culture to become anaerobic. The algal cells have the ability to switch to an anaerobic fermentative metabolism which involves both the use of solar energy to break down water, generating adenosine triphosphate and H_2, and the breakdown of starch into formic acid, ethanol, acetic acid, malic acid, carbon dioxide and H_2. Sulphur deprivation was achieved by replacing the sulphur rich TAP

growth medium with the sulphur-deplete TAP-S medium. *C. reinhardtii* cells were extracted by centrifugation and then re-suspended in the TAPS medium.

2.3. Centrifugation

Centrifugation is commonly used for lab-scale systems, but it is a time consuming process that results in the loss of algal cells and would be difficult to scale-up. A more efficient ultra filtration system is under development. It is also possible to dilute a growing culture in the TAP-S medium and hence drastically reduce the sulphur concentration of the system. This culture gradually uses up the remaining sulphur supply and becomes anaerobic within days. Continuous H_2 production could therefore either be attained by using separate PBRs, linked with ultra-filtration units, for the growth and H_2 production stages, or with one smart PBR that can monitor and control the sulphur content of the culture.

2.4. Hydrogen measurement

After 24 hours of sulphur deprivation Hydrogen evolution was quantified after separation by gas chromatography or by means of water displacement system. Dissolved H_2 can be measured by a reversed Clark electrode or by membrane inlet mass spectrometry. The gas is verified by injecting a small sample into mass spectrometer.

2.5. The photobioreactor

Green algae can be cultured in open ponds but since many parameters need to be controlled and assessed a closed system serve the purpose better. The following four photo bioreactors (PBR) are considered: 1. the vertical column reactor 2. the stirred tank batch reactor 3. tubular flow reactor and 4. flat plate reactor.

A vertical column reactor is inexpensive and appropriate for initial algal growth, but cannot sustain the high surface to volume ratio required for H_2 photoproduct ion. The stirred tank batch reactor has a high degree of back-mixing and a residence time distribution unsuitable for photolysis of hydrogen.

The best surface to volume ratio is provided by the flat plate reactor, which is known to generate high algal cell densities and H_2 production yields.

2.6. Energy calculations and efficiency enhancement

From the conducted experiments, results have shown that 10 micro mole of H_2 can be produced per hour (roughly 50% of peak maximum but extended for an hour) per mg of chlorophyll.

Additionally, a density of 10% of the top 1 cm (or 100% of top mm) of the system would be populated by chlorophyll, for a density of 1 mg chlorophyll per square cm of collector.

This leads to 10,000 cm multiplied by 10 mg chlorophyll per centimeter for a total of 100,000 mg chlorophyll.

Multiplying 100,000 mg chlorophyll by10 micromole H_2 generated per hour per mg chlorophyll yield 1 mole of hydrogen gas per meter per hour.

Combusting one mole of H_2 with one half mole of oxygen yields 286K Joules or 68 Kcal

Using any of the following conversions yields KWatt hours or watts:

1 calorie = 4.184 Joules

1 calorie = 0.0011622 KwHr

1 Joule = 0.0002778 Watt hours

1 K Joule = 0.2778 watts

286 KJoules X 0.2278 Watts / KJoules = 79 Watts

68,355 calories X 0.0011622 KwHr per calories = **79 KwHr**

So it looks like 1 square meter of hydrogen producing algae (modified for continuous production) yields about 79 watts, or enough to run a 75 watt light bulb at full power.

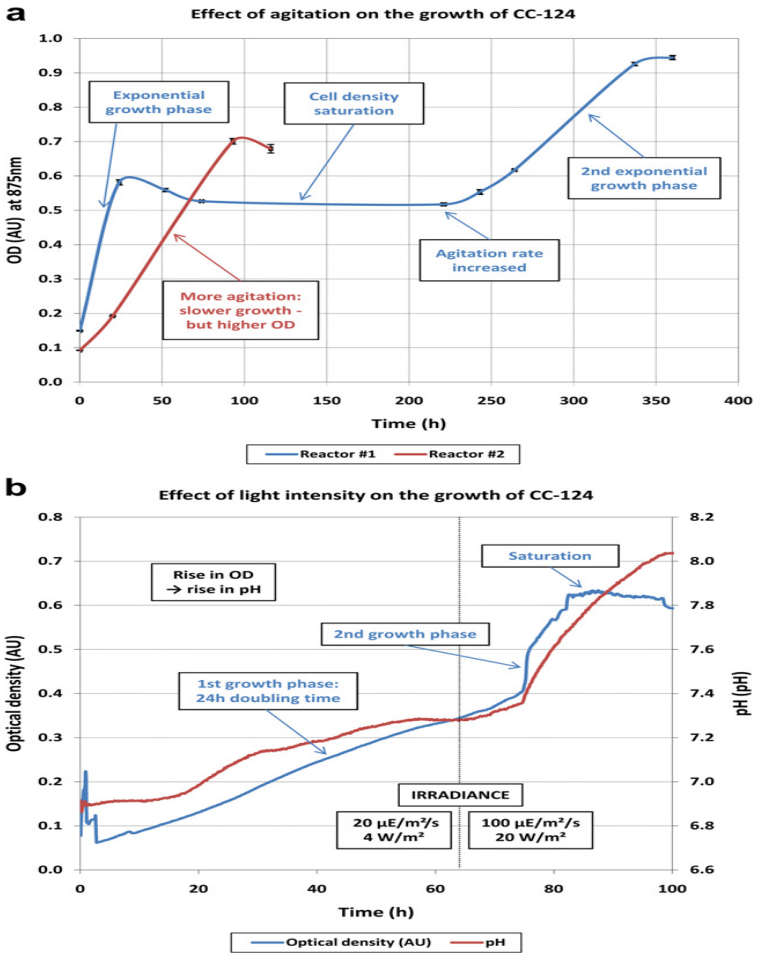

Fig. 1: Effect of agitation and light intensity on growth of CC-124.

3. Observations, Results and Inferences

3.1. Growth kinetics

C.reinhardtii cells reproduce by meiosis. It has been observed that under ambient conditions, the cell density increases exponentially with a doubling time of about 24hrs. In a Photobioreactor (PBR), the algal cell density is governed by the intensity of light penetrating through the culture. From a series of experiments carried out, the graph has been plotted of OD vs Time. Here it is found that the cell density saturates and eventually stabilizes at maximum OD, in the range of 0.5-1.5 absorbance units (AU). Healthy cells of *C.reinhardtii* can be stored at this stable OD for 2-4weeks before fouling, dead cell accumulation and bacterial growth. At this point the cells must be re-suspended in a fresh TAP. Two factors affected the maximum attainable OD: Agitation and light intensity. The graph shows that increasing the agitation rate decreased the algal growth rate but increased the maximum attainable OD. A light intensity of 100µE/m^2/s was found to be more favorable than 20 µE/m^2/s. Increasing the light intensity or agitation rate of a stable culture leads to a secondary growth phase and the establishment of a new maximum OD.

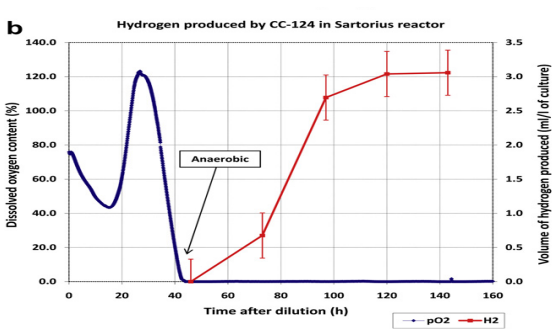

Fig. 2: Graph of Dissolved O_2 content vs time.

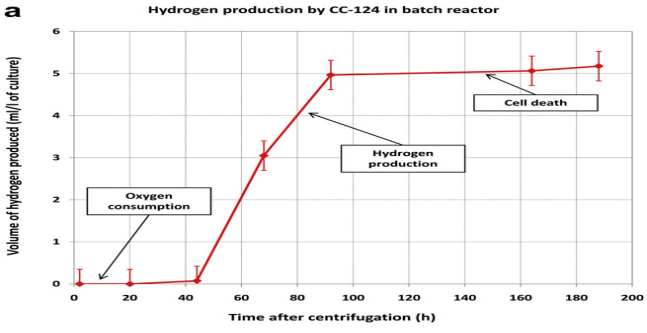

Fig. 3. : Graph of Volume of H_2 vs time

3.2. H_2 production

The unique ability of to produce H_2 under anaerobic conditions brought about by sulphur deprivation was measured using a stirred tank batch reactor where sulphur deprivation was introduced by centrifugation and re suspension and in a Sartorius tubular flow reactor where the dilution method of sulphur deprivation was used. The process was divided into three distinct phases: oxygen consumption, H_2 production, and cell death. Overall, H_2 production was maintained for a period of approximately 5 days. At this point, the *C. reinhardtii* cells could no longer produce enough energy (adenosine triphosphate) to satisfy their metabolic needs and they began to die. Algal lifetime could be extended by sulphur (or oxygen) re-insertion. The centrifugation process of sulphur deprivation resulted in a total yield of 5.2±0.3ml of H_2 from 1L of culture compared with 3.1±0.3ml/L for dilution process. More H_2 was produced by centrifugation due to the higher initial cell density of the culture and the correspondingly larger number of PSII reaction centres. Additionally, the dilution process had a longer start up time approximately 2 days, compared with 1 day for centrifugation. On the other hand, dilution was significantly easier to implement and scale-up. The Sartorius reactor was used to continuously measure key parameters such as pH, dissolved oxygen (pO_2) and OD and was therefore well equipped to monitor the process kinetics. Modifications are being made to improve the H_2 seal of the Sartorius reactor and incorporate membrane inlet mass spectrometry system for continuous in situ dissolved H_2 measurement.

3.3. Comparison of H_2 production ability of various green algae

In studies about algal fermentation, several species were described to produce hydrogen upon anaerobic adaptation, such as *C. reinhardtii*, *Chlamydomonas moewusii, Chlorella fusca (Scenedesmus vacuolatus), and Scenedesmus obliquus*. Such species were subjected to experiments to find the potential of hydrogen evolving species. The in vitro activities of *C. reinhardtii* (200 nmol H_2) *S. obliquus* (150 nmol H_2) and *S. Vacuolatus* (155 nmol H_2) confirmed the values of former investigations , thus demonstrating that anaerobic incubated cultures of *S. vacuolatus* and *S. Obliquus* generally produce hydrogen with lower rates than C. reinhardtii. Two other Chlorophyceae, *Chlorella vulgaris* and *Dunaliella salina*, developed no detectable amounts of hydrogen during anaerobic incubation, indicating the lack of a hydrogenase. The largest hydrogenase of was found in the marine green alga *Chlorococcum littorale* , but the specific enzyme activity is very low compared with *C. reinhardtii*. In fact, cultures of *C. moewusii* evolve in vitro the highest quantities of hydrogen.

4. Hydrogen Storage

Hydrogen storage, a topical goal of hydrogen economy, describes the methods for storing H_2 for subsequent use. This can be carried out in the following three ways.

As Compressed hydrogen: Compressed hydrogen can be stored in hydrogen tanks at high pressures (350-700 bar). But, the energy density per unit volume is very low and this process requires much larger tanks.

As liquid hydrogen: Liquid hydrogen must be stored in cryogenic tanks (<20K), which are spherical or cylindrical in shape (to reduce heat loss). Liquefaction of hydrogen has the disadvantage of requirement of a large expenditure of energy.

As Metal Hydrides: This new method uses the principle that certain metals alloys (like MgH_2, $NaAlH_4$) absorb hydrogen to form a metal hydride at a relatively lower temperature and when the hydrogen gas is needed, the gas is recaptured by lowering the pressure, or by raising the temperature of the metal hydride above the absorption process. The advantages include low pressure requirement, more storage capacity and economic benefits.

5. Hydrogen Safety

As hydrogen is colorless, odorless and has low ignition energy, hydrogen safety is considered to be an important issue. Apart from that, wide range of explosive limits and invisible flame in daylight conditions necessitates this issue to be taken care of. The hazards of leakage, diffusion, and buoyancy result from the difficulty in containing hydrogen. For detection, hydrogen sensors may be used. UV/IR flame detectors may also be used to show the hydrogen flames. Liquid hydrogen poses additional challenges due to its increased

density and extremely low temperatures. It requires complex storage technology, like the special thermally insulated containers.

6. Conclusion

Concerns about global warming and environmental pollution due to the use of fossil fuels, combined with projections of potential fossil fuel shortfall toward the middle of the 21st century, make it imperative to develop alternative energy sources that are clean, renewable, and environmentally friendly. The recently developed single-organism, two-stage photosynthesis and H_2 production protocol with green algae is of interest because significant amounts of H_2 gas were generated for the first time, essentially from sunlight and water. Further, this method does not entail the generation of any undesirable, harmful, or polluting byproducts and it may even offer the advantage of value-added products as a result of the mass cultivation of green algae.

Ultimately, the advent of hydrogen will bring about technological developments in many fields, including power generation, agriculture, the automotive industry, and other as yet unforeseen applications. This will have a positive impact on the environment in which atmospheric pollution is all but alleviated and the so-called greenhouse effect is mitigated.

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