

ENHANCEMENT OF GREEN ALGAE HYDROGEN PRODUCTION BY LASER IRRADIATION

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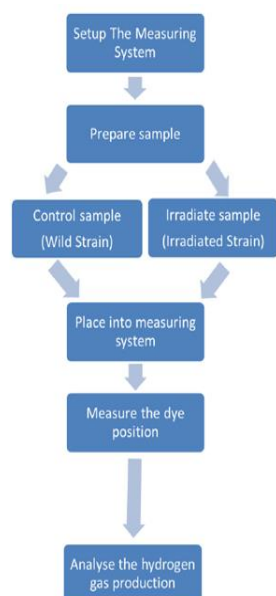
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Graphical Abstract



Abstract

Biohydrogen is an alternative to support the increasing hydrogen demand in the future. Biohydrogen is hydrogen gas produced by green algae and bacteria in certain quantity. The aim of this research is to enhance hydrogen gas production by green algae (*Closterium* sp.) using laser light. The laser used in this experiment was a diode laser operating in continuous mode with wavelength of 655 nm. Green algae are placed in a sulphur deprived medium so it will produce hydrogen gas. This algae is irradiated with diode laser for 30 minutes then stop before continue for the next 30 minutes. This process is repeated until the total irradiation is 120 minutes. Both strains of green algae are set up into measuring system under exposure of sunlight in a constant room temperature. The volume and rate of hydrogen gas produced is examined by measuring the dye position in capillary tube of 0.5 mm radius. The results showed that there is a 9.0% increase of hydrogen gas production in radiated strain of green algae compared to the wild strain. The rate of hydrogen gas production of radiated algae is faster than the wild strain. This showed that, red light laser has absorbed cell green algae and mutated its behaviour for producing more hydrogen gas. This result is in good agreement with other researcher.

Keywords: Hydrogen production, green algae, laser therapy

Abstrak

Biohidrogen merupakan alternatif bagi menampung permintaan terhadap gas hidrogen yang semakin meningkat pada masa hadapan. Biohidrogen ialah gas hidrogen yang dihasilkan oleh alga hijau dan bakteria secara biologi dalam kuantiti yang tertentu. Kajian ini bermatlamat untuk meningkatkan penghasilan gas hidrogen oleh alga hijau (*Closterium* sp.) menggunakan cahaya laser. Laser yang digunakan dalam eksperimen ini ialah laser diod yang beroperasi secara selanjur dengan panjang gelombang 655 nm. Alga hijau ditempatkan dalam medium yang kurang kandungan sulfur supaya ia dapat mengeluarkan gas hidrogen. Alga ini disinarkan dengan diod laser selama 30 minit kemudian berhenti dan sambung lagi untuk 30 minit yang berikutnya. Proses ini di ulang sehingga jumlah sinaran ialah 120 minit. Kedua-dua jenis alga hijau ini ditempatkan didalam radas pengukuran dan didedahkan dibawah sinaran matahari pada suhu bilik. Jumlah isipadu dan kadar penghasilan gas hidrogen diukur melalui kedudukan pewarna biru didalam tiub kapilari yang berjejari 0.5 mm. Hasil yang diperolehi menunjukkan terdapat peningkatan sebanyak 9.0 peratus penghasilan gas hidrogen oleh alga hijau yang telah dipancarkan berbanding alga hijau liar. Kadar penghasilan gas hidrogen oleh alga hijau yang disinarkan didapati lebih pantas berbanding alga hijau liar. Ini menunjukkan bahawa laser cahaya merah telah menyerap sel alga hijau dan merubah sifatnya sehingga is berkebolehan untuk menghasilkan lebih banyak gas hidrogen. Hasil ini menunjukkan persetujuan yang baik dengan penyelidik yang lain.

Kata kunci: Penghasilan hidrogen, rumpai hijau, terapi laser

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1.0 INTRODUCTION

Biohydrogen is hydrogen produced naturally by algae and bacteria. Hydrogen is an ideal alternative to replace fossil fuel for future energy application. Hydrogen can be produced from clean and renewable energy sources and thus, its life cycle is clean and renewable. There are many ways of producing hydrogen such as from electrolysis and thermolysis. However, these conventional methods consume intensive and high-energy process. Hence, new better methods should be developing in order to introduce hydrogen gas as clean and reliable alternative fuel. Scientists and researchers around the globe are working on biological hydrogen producer which is green algae.

Hydrogen produced through the action of living organisms is called biohydrogen. This is a type of biofuel, like bio-ethanol, bio-diesel or bio-gas or bio-oil. There are three classes of biofuels which is the first generation where the fuel is produced from food crop such as corn, the second generation where the fuel is produced from non-food crops and waste while the third generation is fuel produced by using microbes [1].

Advanced biofuels have several advantages over 1st and 2nd generation biofuels. Whereas first generation biofuels have caused increases in food prices, advanced biofuels would not. In comparison to second generation biofuels, advanced biofuels could capture sunlight energy 10 times more efficiently, meaning that smaller areas or land are needed to produce enough fuel.

Biohydrogen is an example of an advanced biofuel (or third generation biofuel). In advanced biofuel technologies, microbes are grown in special bioreactors and provided with the energy and nutrients that they need including, sunlight, waste organic material, CO₂ from the air or from conventional gas plants. As they grow the microbes produce the biofuel.

Among the advanced biofuels, biohydrogen is particularly attractive because of the excellent properties of hydrogen as a fuel and because biohydrogen is very easy to collect from the bioreactor. Conversely, biofuels such as bio-oils have to be purified from the microbial cells which are complex and expensive. Green algae are one of the organisms which have hydrogenase enzyme which is capable of producing hydrogen during photosynthesis [2].

Green algae do produce hydrogen but only in certain quantity. In this research, we would like to increase the hydrogen production by optically modifying the hydrogenase enzyme to enhance its abilities to produce hydrogen by irradiating green algae to a 655 nm laser beam. Low power light therapy is well known to have positive effect on organism down to cellular level because of its wave properties [5]. Hence, irradiated green algae could

produce greater amount of hydrogen compared to the wild strain green algae.

This research focusses on increasing the production of hydrogen gas by irradiating wild strain green algae with 655 nm continuous wave diode laser. The laser used has peak power of 150 mW. The green algae are placed into a sulphur deprived medium which is distilled water and being kept near sunlight to allow photosynthesis take place.

2.0 METHODOLOGY

The whole research activities in this project are summarized in Figure 1. Initially the work started by aligning the experiment set-up. This follows by preparing the algae sample. The hydrogen production will be collected into conditions; one as control sample whereby, the hydrogen collected direct by using algae only. Then, the hydrogen production was collected after the algae sample was irradiated by laser.

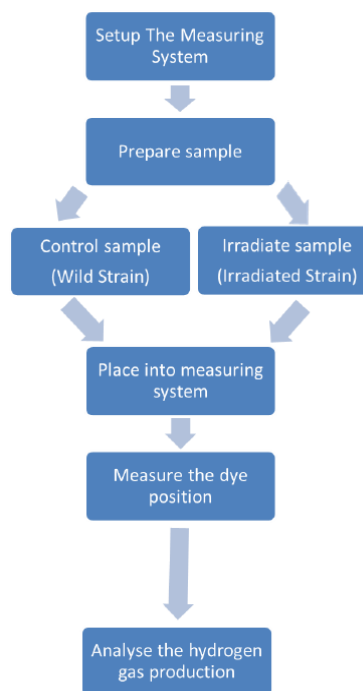


Figure 1 Flowchart of Research Activities

2.1 Measuring System

The experimental setup is shown in Figure 2. The transparent flask is used to contain green algae to allow maximum exposure of sunlight. The tube connecting the container into the capillary tube is 2 cm long and setup in inclined angle so that no hydrogen gas will be trapped at the middle of the tube. Then, there is cotton soaked with 1 mol of

sodium hydroxide (NaOH) to absorb possible oxygen and carbon dioxide produce from any microorganism left in the green algae. The capillary tube is 0.5 mm in radius and 30 cm long is positioned in horizontal axis associated with the spirit level to make sure the even distribution of hydrogen produced and the dye position is stable. The capillary tube was inserted with blue dye at length of 5 cm.

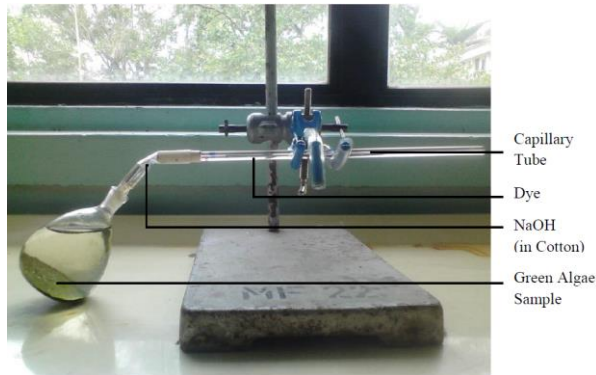


Figure 2 Measuring System Setup

2.2 Sample Preparation

A 2 liter of wild strain green algae is collected from clean natural resources at an unpolluted freshwater pond. The green algae sample is then mixed with distilled water which acts as a sulphur deprived medium. Two samples were used filled in into two different container labelled A and B. Container A will act as a control one while container B will be the radiated sample. Each container filled with 50 ml sample.

2.3 Irradiation Procedure

Container B is prepared by coating it with aluminum foil to increase the laser beam exposure. The sample is then radiated by using diode laser of 655 nm wavelength in a dark environment as shown in Figure 3. The radiation session took a total of 120 min by 30 minutes interval between the exposures to let the radiation effect takes places in the wild strain of green algae. The radiated strain of green algae is then immediately set up into measuring system for further observation along with wild strain green algae.

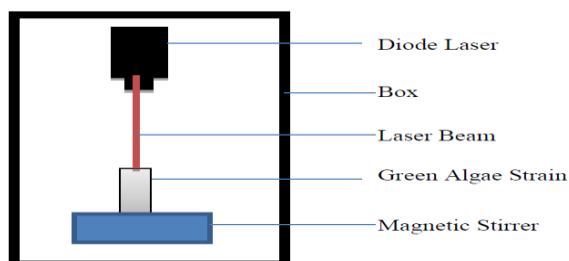


Figure 3 Irradiation Session

2.4 Data Collection

The collecting system is left near a window where they can get mild sun exposure. Excessive evaporation process might occur inside the flask and may disturb the reading. The data collection is done every 4 hour for 15 days. The position of the dye is recorded and Figure 4 shows the locations and measurement of dye position. The hydrogen volume produced by green algae is calculated by using volume equation.

$$v = \pi \times r^2 \times l \quad (1)$$

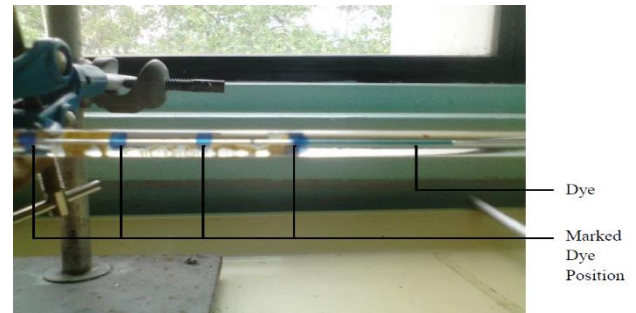


Figure 4 Locations and Measurement of Dye Position

3.0 RESULTS AND DISCUSSION

In cyanobacteria or photosynthetic bacteria, they do photosynthesis the same way plants do but they have a way to produce hydrogen which other plant cannot do. When light energy comes in, the first thing it encounters is a protein complex called photosystem II (PSII) where it split water by using sunlight energy into proton, oxygen and electron (Figure 5). The electrons then go into protein carrier in protein membrane and passes through series of protein complex component until it reach at photosystem I. Then, there a protein called ferredoxin where act as electron carrier. There are proteins called NADP+ pick up that electron to become NADPH. In other hand, the proton produced earlier is used to produce ATP which is later used to produce CO₂ and glucose and that is photosynthesis. But, in this case, the cyanobacteria have one unique component called Hydrogenase (H₂ese).

It uses ferredoxin to produce hydrogen gas directly [3]. Q, primary electron acceptor of PS II; plastoquinone PQ; cytochrome Cyt ; plastocyanin PC; ferredoxin Fd.

At the same time, coordinated photosynthesis and respiratory electron transfer process occurs as shown in Figure 6.

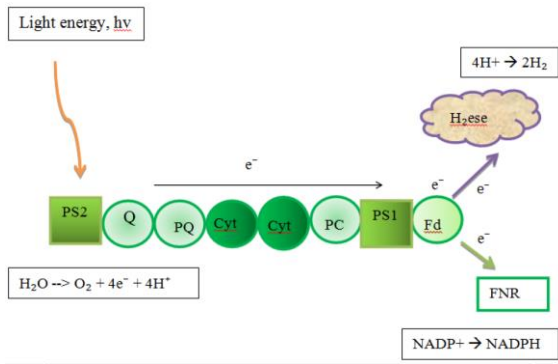


Figure 5 Hydrogen Production in Green Algae

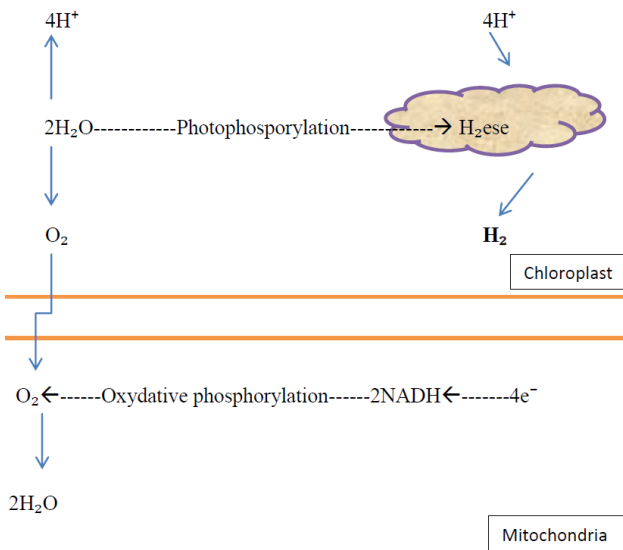


Figure 6 Photosynthesis and Respiratory Electron Transfer Process in Green Algae

However, there are certain conditions that will only enables the hydrogenase to operate which it is must be in sulphur deprived medium since the present of oxygen will distort hydrogenase [4]. Presence of sulphur will disrupt the internal oxygen flows inside the green algae. In this research, we want to enhance the hydrogenase capabilities by exposing to red light laser radiation in hope that it might positively modify the hydrogenase.

A photobiological reaction involves the absorption of a specific wavelength of light by the functioning photoacceptor molecule. The photobiological nature of photobiomodulation means that some molecule (photoacceptor) must first absorb the light used for the irradiation. After being light stimulated, primary molecular processes from these states can lead to a measurable biological effect. All light-induced biological effects depend on the parameters of the irradiation. According to action spectra, optimal wavelengths are 820-830, 760, 680, and 620 nm.

Naturally, absorption of low intensity light by biological system is purely non-coherent nature

because the rate of decoherence of excitation is higher than the rate of photoexcitation. Time for decoherence of photoexcitation determines the interaction with surrounding molecules. However, coherent light field can disturb the random distribution of the wave function phase of particles and previous experiments by using coherence light provide evidence that it has biological effect. Therefore, it is possible that the effect of coherence light manifested on the macroscopic level of green algae [4].

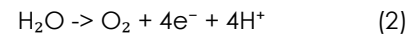
In this research, the spatial coherence of the light source is important in delivery of light in cellular component of green algae. It is important that the light has high temporal coherence to determine the volume of irradiated green algae. However, the high coherence of light cause formation of random interference of scattered light wave and formation of random non-homogeneities of intensity in space occur. These laser speckles causes a partially nonhomogeneous photochemical processes, changes in local pressure and deformation of cellular membranes.

3.1 Hydrogen Gas Production by Wild Strain Green Algae

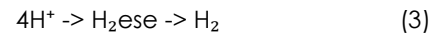
In sulphur deprived condition, green algae will quickly consume all dissolved oxygen and produce hydrogen gas in anaerobic condition under sunlight exposure. Hydrogen gas production is measured based on the observation of dye position in capillary tube for 360 hour. Volume of hydrogen yield is calculated by using formula (1).

Chemical equation occurs during this process is:

Water splitting:



Hydrogenase activity:



The hydrogen yield is plotted against time as shown in Figure 7. From the figure, there is no hydrogen gases recorded during first 210 hours. A rapid increase is realized after 210 hours duration of collection to 264 hours. At 264 hour, hydrogen yield is recorded about $8.56 \times 10^{-8} m^3$.

During the first 210 hour, in sealed cultures, imbalance in the photosynthesis-respiration relationship caused by sulphur deprivation resulted in net consumption of oxygen by the green algae causing anaerobic growth in the medium, where green algae slowly switch to hydrogen gas production. The process was considered as time consuming to produce hydrogen and pushed the dye. As result, we can see that after 210 hour, the increases in hydrogen gas yield recorded until it reach its maximum volume that is $8.56 \times 10^{-8} m^3$.

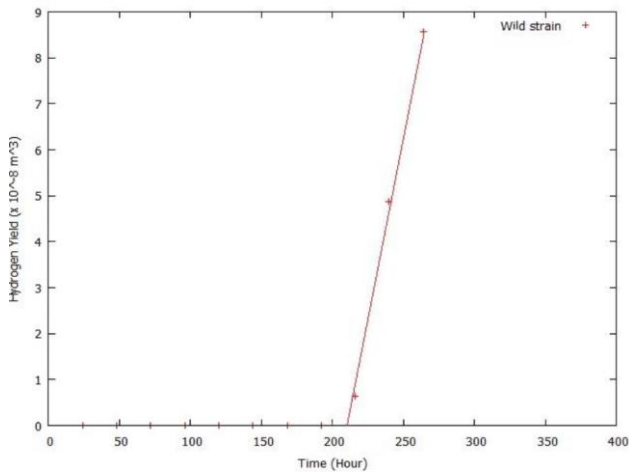


Figure 7 Graph of Hydrogen Gas Yield for Wild Strain Green Algae

After 264 hour, no more hydrogen gas yield recorded. This shows that green algae did not produce hydrogen gas since it did not survive for long time due lack of sulphur nutrient. The life time of green algae in sulphur deprived medium is about 264 hour.

3.2 Production of Hydrogen Gas by Irradiated Strain of Green Algae

The red light laser beam penetrated down to cellular level and modified the hydrogenase activity. Measurement of hydrogen gas produced by irradiated strain is done at the same time frame as the wild strain.

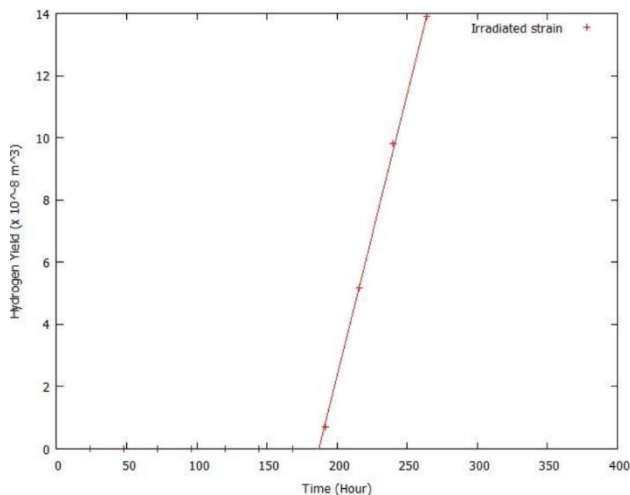


Figure 8 Graph of Hydrogen Gas Yield against Time for Irradiated Strain Green Algae

Hydrogen yield is plotted against time as shown in Figure 8. There are no hydrogen yields within the first 180 hour. The first increases in hydrogen gas yield

about $0.71 \times 10^{-8} \text{ m}^3$ occur at 190 hour and maxed out at 264 hour about $13.9 \times 10^{-8} \text{ m}^3$.

This resulted from the interaction between coherent light and cellular part in green algae, where 655 nm beam has enhance hydrogenase ability to produce hydrogen in greater amount compare to the wild strain.

3.3 Comparison Results Wild and Mutated Strain

Figure 9 combined the performance of hydrogen production from both irradiated and wild green algae sample.

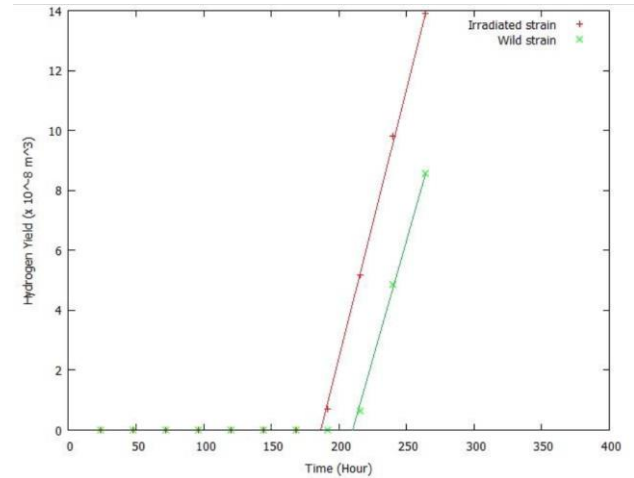


Figure 9 Graph of Hydrogen Gas Yield against Time for Wild and Irradiated Strain of Green Algae

The rate of hydrogen gas yield;

$$\text{Rate of Hydrogen Yield} = \frac{\Delta \text{Hydrogen Yield}}{\Delta \text{Time}} \quad (4)$$

For wild strain of green algae:

$$\begin{aligned} \text{Rate of Hydrogen Yield} &= \frac{8.56 \times 10^{-8} \text{ m}^3 - 0.6 \times 10^{-8} \text{ m}^3}{264 \text{ hour} - 216 \text{ hour}} \\ \text{Rate of Hydrogen Yield} &= 0.16 \times 10^{-8} \text{ m}^3 \text{ h}^{-1} \end{aligned}$$

For irradiated strain of green algae:

$$\begin{aligned} \text{Rate of Hydrogen Yield} &= \frac{13.9 \times 10^{-8} \text{ m}^3 - 5.2 \times 10^{-8} \text{ m}^3}{264 \text{ hour} - 216 \text{ hour}} \\ \text{Rate of Hydrogen Yield} &= 0.18 \times 10^{-8} \text{ m}^3 \text{ h}^{-1} \end{aligned}$$

Data obtained shows that there is a 9.0% increase of hydrogen gas production in radiated strain of green algae compared to the wild strain. The data

also shows that the rate of hydrogen gas production of radiated algae is 13% greater than the wild strain.

Green algae produced hydrogen gas in the presence of sunlight. This is because hydrogenase enzyme need electron to convert into hydrogen. During the data collecting process, there might be a rainy or cloudy day thus affected the sunlight exposure on the photoreceptor.

However, if there are continuous presence of light, green algae can yield more hydrogen gas. Ideal hydrogen gas yield (using maximum rate of hydrogen yield):

$$\text{Volume} = \text{Rate of Hydrogen Yield} \times 264 \text{ hour} \quad (5)$$

Wild strain:

$$\text{Volume} = (0.16 \times 10^{-8} \text{m}^3 \text{h}^{-1}) \times 264 \text{ hour}$$

$$\text{Volume} = 42.24 \times 10^{-8} \text{m}^3$$

Irradiated strain:

$$\text{Volume} = (0.18 \times 10^{-8} \text{m}^3 \text{h}^{-1}) \times 264 \text{ hour}$$

$$\text{Volume} = 47.52 \times 10^{-8} \text{m}^3$$

4.0 CONCLUSION

The main objective of this study is to see the effect of red laser irradiation in biohydrogen production. Strain of green algae was employed as biohydrogen sample. Diode laser 655 nm was used as a source mutation. The period to collect the biohydrogen production is 15 days. Blue dye filled in capillary tube was used to measure the amount of hydrogen gas production. The position of dye in the capillary tube is an indicator for the increment amount of gas.

The hydrogen production was collected in two conditions. First the hydrogen was collected in control wild strain green algae. The maximum amount of hydrogen after 15 days is $8.56 \times 10^{-8} \text{m}^3$. Secondly the hydrogen was collected from irradiated strain of green algae. The maximum hydrogen produced from such mutation biohydrogen material is $13.9 \times 10^{-8} \text{m}^3$. There is about 9.0% increase of hydrogen gas produced by irradiated strain compared to the wild strain. The data also shows that the rate of hydrogen gas

production of irradiated algae is 13% faster than the wild strain.

Comparing the hydrogen gas produced from both strain, we can deduce that 655 nm laser beam have mutated the green algae cellular activity. The cellular part responsible for producing hydrogen called hydrogenase manifest its interaction with coherent light from laser beam by producing greater amount of hydrogen.

The increasing demand and usage of hydrogen gas require a more quantity of hydrogen gas produced. A clean and energy-friendly method of generating hydrogen should be introduced to replace the current method. At the same time, the advancement of laser technology will produce much lesser energy required laser to be used in future. By using green algae, we have introduced green energy technology by using biomaterial where we believe that optically modified green algae to enhance its hydrogen production as a main strain of green algae will be used in hydrogen production at the industrial level.

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