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Application of Helium-Neon Red Laser for Increasing Biohydrogen Production from Anaerobic Digestion of Biowastes

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Abstract

Biohydrogen has significant feasibility since biological processes are much less energy intensive compared with electrolysis and thermo-chemical processes. It is widely recognized that considerable amounts of hydrogen (H2) can be produced from renewable resources without using energy from fossil fuels. Biological processes and bacterial fermentation are considered as the most environmentally friendly alternatives for satisfying future hydrogen demand. Biohydrogen production from agricultural and agro-industrial solid waste and wastewater is considered as highly advantageous as materials of this kind are abundant, cheap and biodegradable. The combustion of H2 with oxygen produces water as its only product: Unlike other fuels, the combustion of H2 does not produce carbon dioxide (CO2), carbon monoxide (CO), nitrogen oxides (NOx), sulfur dioxide (SO2), hydrocarbons or particulate matter (PM). Therefore, hydrogen is an environmentally friendly fuel where endeavors focus on producing specially designed internal combustion engines that can use H2 as fuel. The results showed that laser irradiated inoculum increased biohydrogen production by 1.2 times of the control. Therefore, in this research, it was hypothesized that exposing purple non-sulfur bacterial (PNSB) mix consortium to Helium-Neon red laser for 2 hours increased cell activity and consequently the biohydrogen production from food wastes through photo-fermentation process.

Keywords: Biohydrogen; Photobiostimulation; Anaerobic treatment; Biomass; Biofuels; Laser irradiation.

1. Introduction

Energy became a multi aspect problem all over the world. One aspect is the rising demand for energy as an outcome of population increase, modern life standards and the massive expansion in industry [1]. On the other hand, fossil fuels –which considered as one of our major sources of energy now, will be depleted in the becoming few tens of years if the current consumption rate remains the same or increased [2]. The environmental catastrophe is another aspect of this energy dilemma; fossil fuels are a main cause of the hazardous emissions and greenhouse gases such as CO_2. The concentration of CO_2 in the atmosphere is 380 ppm now compared to 270 ppm before the industrial revolution [3,4].

Finding alternative sources of energy became an urgent need to be used in parallel to fusel fuel in the near future and to completely replace fossil fuel eventually. These alternatives should be sustainable, clean and have no dangerous impact on the environment or at least have an impact that we can deal with or contain. Renewable energy such as solar energy, Biofuels, Geothermal energy, etc. are strong candidates [5].

Hydrogen is a promising source of energy for the following reasons: (1) it is an environmentally friendly

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source of energy since the combustion of hydrogen results in a good amount of energy and H_2O ($2H_2+O_2 \rightarrow 2H_2O$). (2) The high energy density of 122-142 MJ/Kg. (3) can be directly used to generate electricity instead of direct combustion. Producing hydrogen through biological processes especially when we use the biological wastes such as agricultural wastes, food wastes, etc. can maximize the benefits of this rising candidate known as: Biohydrogen [6,7,8].

Biohydrogen can be produced using of these methods: (1) photo-fermentation by using photosynthetic bacteria, (2) dark fermentation method by using anaerobic bacteria, (3) Hybrid systems including dark and photo-fermentation processes, (4) bio-photolysis of water by using algae/ cyanobacteria, (5) microbial electrolysis cell [9,10]. Through the intensive research to find out the best method to produce Biohydrogen from the previous methods, Photo- fermentation is considered one of the best and more reliable methods for these reasons: (1) High substrate conversion ratio, (2) High Biohydrogen yield, (3) wide spectrum of light can be used, and different types of biological wastes can be utilized [11,12].

Purple non-sulfur bacteria (PNSB) are a very interesting species. They are gram-negative photosynthetic bacteria which is gluttonous for light [13]. PNSB can grow heterotrophically and produce biohydrogen from simple organic acids such as or volatile fatty acids such as (acetic acids, propionic acid, butyric acid, and valeric acid) under anaerobic conditions in the presence of light. However, biohydrogen production depends on specific conditions such as; light intensities (3 - 10 klux) and wavelength (between 400 and 1000 nm), temperature (31 - 36°C) and optimum pH (6.8 -7.5) since the PNSB have multiple metabolic pathways. Purple non-sulfur bacteria (PNSB) can be found in wastewater, Pig manure, alkaline lakes and earthworm drops but enrichment techniques are required after isolation to reach the needed concentration [14, 15].

Several types of organic wastes can be used to produce Biohydrogen such as agricultural waste, food waste, etc. Food wastes are organic solid wastes that accounting for 15-63% of total municipal solid wastes worldwide. Additionally, according to the Food and Agriculture Organization (FAO), United Nation, more than 1.3 million tons of food is being wasted. Food waste is considered as s perfect candidate for Biohydrogen production since food wastes are rich in in carbohydrates, proteins, fats, nitrogen, minerals, cellulose and hemicelluloses [8, 16].

Light amplification by stimulated emission of radiation known as LASER is just a normal light but has a very important characteristic which is spatial coherence [17], the LASER device emits coherent light within a narrow wavelength range (spectrum) that is focused to a spot which means that the light energy is concentrated in that spot. There are many types of LASER, such as; solid state laser (Ti:Saph), gas laser (He-Ne), liquid laser (Dye), etc., each type or material gives a certain range of spectrum and has different applications [18-21]. LASER application in biological sciences is a promising major study and many research works have been conducted on the bio-stimulating of bacterial cells using LASER radiation [20, 23-27]. Light saturation of photo-fermentation is an important parameter that impacts fermentation efficiency and hydrogen yield, especially in mass culture. In other words, unsteady light irradiation negatively affects biohydrogen production. On the other hand, less information exists on the light-to-H_2 energy conversion efficiencies of photosynthetic bacteria. In this approach, we expose biohydrogen-producing bacteria to helium-neon red laser with wavelength of 632.8 nm that causes cells photobiostimulation making these cells more active and increase the growth rate, therefore, increase the biohydrogen production from biological wastes.

The main objective of the present study is to increase biohydrogen production from food wastes via Photobiostimulating of hydrogen-producing bacteria using laser irradiation.

2. Material and methods

2.1. Cultivation of purple non-sulfur bacterial (PNSB) mix consortium and preparation of inoculum

Purple non-sulfur bacteria were isolated from a stagnant water samples collected from both Teraat El-Marioutya (El-Marioutya Canal) in and a small water canal in a village called Shabramant (Giza, Egypt) in sterile containers. all samples were kept in the fridge (at 5ºC) till use. Collected samples were aseptically inoculated in sterile Acetate Yeast Extract (AYE) basil medium (2 ml or g sample + 18 ml medium). AYE medium consisted of K_2HPO_4, 1.0 g; $MgSO_4$, 0.2 g; $CaCl_2$,0.02 g; $Na_2S_2O_3$, 0.10 g; Na-Acetate, 2.2 g; Yeast Extract, 4.0 g; 1 L distilled water [11,22]. The final pH was 7.0 ± 0.2. The medium ingredients were dissolved in distilled water, then distributed in screw cap tubes (18 ml) and autoclaved at 121 ºC for 15 min at 1 atm pressure. . Afterwards, the inoculated culture tubes were completely filled with the media and sealed with the screw cap. The tubes were then incubated under a 60 W incandescent bulb lamp at room temperature. The tubes placed at a distance of 25 cm from the light source. After 4 weeks of incubation, formation of red blooms will be noted, indicating growth of PNSB (Fig 1).

Fig. 1: PNSB consortium enrichment in AYE medium. A) Sterile medium and B) Growth of PNS bacterial consortium.

2.2. Installing and adjusting the biohydrogen production setup

A batch anaerobic system was designed and installed as shown. The experimental setup consists of: (1) High transparency glass flasks of volume 1L with well-sealed screw caps (photobioreactors) and gas outlet connected to gas holder with 1/4″ connectors through polyurethane hose (4 mm internal diameter and 6 mm external diameter). (2) The temperature control; a thermostatic water bath (Homemade, 90×60×25 cm, 135 L, 0 - 100 ºC). (3) Biohydrogen storage; the biohydrogen produced can be stored in ultra-clear polypropylene graduated cylinder (250±2 ml, Azlon, Staffordshire, UK) connected to gas outlet by 6 mm polyurethane hose at its base and placed upside down in another polypropylene cylinder (500 ml, Azlon, Staffordshire, UK) filled with water (Fig. 2).

Fig. 2: Biohydrogen production setup after installation.

2.3. Experimental design

The prepared PNSB mixed inoculum was injected in each photobioreactor, 125 ml for each. The treatment was photo-biostimulation of PNSB with He-Ne red laser -in triplicates- and the control.

2.4. Irradiation with LASER

He-Ne red laser radiation with a wavelength of 632.8 nm (model HNL 100L, Thorlabs Inc., New Jersey, USA) was used to irradiate the PNS bacterial consortium inoculum for 2 hours (Fig. 3).

Fig. 3: irradiating the PNS bacterial consortium inoculum with red laser.

2.5. Waste pretreatment

Food wastes were collected from many restaurants and mixed together then chopped using a chopper (40544, TOUCH®, ElZenouki Electrical Appliances, El Obour City, Egypt) to a very fine size until it became in doughy form. Distilled water was mixed with the chopped wastes in ratio 2:1. Basic chemicals were used to adjust the initial pH of the mixture to 7.0. Each photobioreactor was filled with 900 ml (125 ml of PNSB mixed inoculum and 775 ml of food waste-distilled water mixture. A vacuum pump was used to create anaerobic conditions inside the photobioreactor. The properties of food wastes and 2:1 food waste mixture were listed in Table 1.

Table 1: Chemical composition of mixed food wastes.

Parameters	Mixed Food Waste (2:1)*
Total Solids [TS%]	6.42
Volatile Solids [VS%	5.46
VS [% as TS]	85.04
Total Nitrogen [TN%]	1.49
C/N Ratio	33.1 : 1
Ash [%]	0.96
pH	4.50
Organic Carbon [OC%]	49.33

* Water to fresh waste ration is 2:1

2.6. Running the experiment and conducting the measurements

The photobioreactors were perfectly sealed and so the rest of the setup components to avoid any leakage after running the experiment. The photobioreactors were inserted in the water bath to keep the temperate 35 °C as long as the experiment is running. a white Light Emitting Diode (LED) with a luminous flux of 3600 lumen was installed above the photobioreactors. The total biohydrogen yield was measured by water displacement method. The hydrogen percentage was measured using H_2 gas detector (XP-3140, New Cosmos Electric Co., Ltd., Tokyo, Japan). The components of the yielded gas were determined using a gas chromatography (GC, Shimadzu 2014, Japan) equipped with a thermal conductivity detector (TCD) included: hydrogen, carbon dioxide, methane, and nitrogen. Initial sugar (mol) was determined using HPLC (Shimadzu LC-10A, Japan) with a Shimadzu RID-10A differential refractive index detector.

2.7. Methodology of statistical analysis

Statistical analysis was applied by SPSS (IBM SPSS Statistics for Windows) version 24 and the figures were generated using GraphPad prism 8 software (GraphPad Software Inc., San Diego, CA, United States). Data were expressed as mean ± standard error mean (SEM). Statistical comparisons were performed using Student’s t-test or mixed model ANOVA test followed by a post hoc Sidak's multiple comparisons test. The results indicate a statistical significance when P value < 0.05.

3. Results

The results show that red laser irradiation on purple non-sulfur bacteria have a positive impact on biohydrogen percentage, daily biohydrogen production and cumulative biohydrogen production values. Additionally, the results show a positive impact on daily gas production values and cumulative gas production values, which means a positive impact on PNSB activity.

3.1. Effects of red laser radiation on biohydrogen production

A significant increase in biohydrogen percent following red laser irradiation was noticed on day 9 ($p < 0.01$) and days 12 and 13 ($p < 0.001$) compared to the control group (Fig. 4A). A significant increase in daily biohydrogen production following red laser irradiation was noticed on days 7-14 ($p < 0.001$) compared to the control group (Fig. 4B). A significant elevation in cumulative biohydrogen production following red laser irradiation was noticed on day 6 ($p < 0.05$) and days 7-14 ($p < 0.001$) compared to the control group (Fig. 4C).

3.2. Effects of red laser radiation on gas production

A significant elevation in both daily and cumulative gas production following red laser irradiation was noticed on days 4-14 ($p < 0.001$) compared to the control group. (Fig. 5A,B).

3.3. Effects of red laser radiation on specific gas production

Significant elevation in total gas and biohydrogen yield ($p < 0.001$) as well as specific H_2 production ($p < 0.001$) were noticed compared to the control group (Fig. 6A,B).

4. Discussion

This research represents one of the unique solutions of many problems facing the world these days. In this approach food wastes are being used as a substrate for biohydrogen production. The world produces millions of tons of food wastes and kitchen leftovers every year around the world which demands a radical solution to not only get rid of these wastes in an ecofriendly method but also to convert these wastes into a high-value product and source of energy, this will be positively reflected on the economy.

Purple non-sulfur bacteria (PNSB) are a strong candidate in photo-fermentation process as photosynthetic bacteria, it has a good conversation ratio, that means PNSB can convert most of the organic acids from food wastes are rich in into biohydrogen [23]. Using PNSB consortium inoculum would be more beneficial as it make the process of photo-fermentation more stable and resistible to change in the fermentation environment.

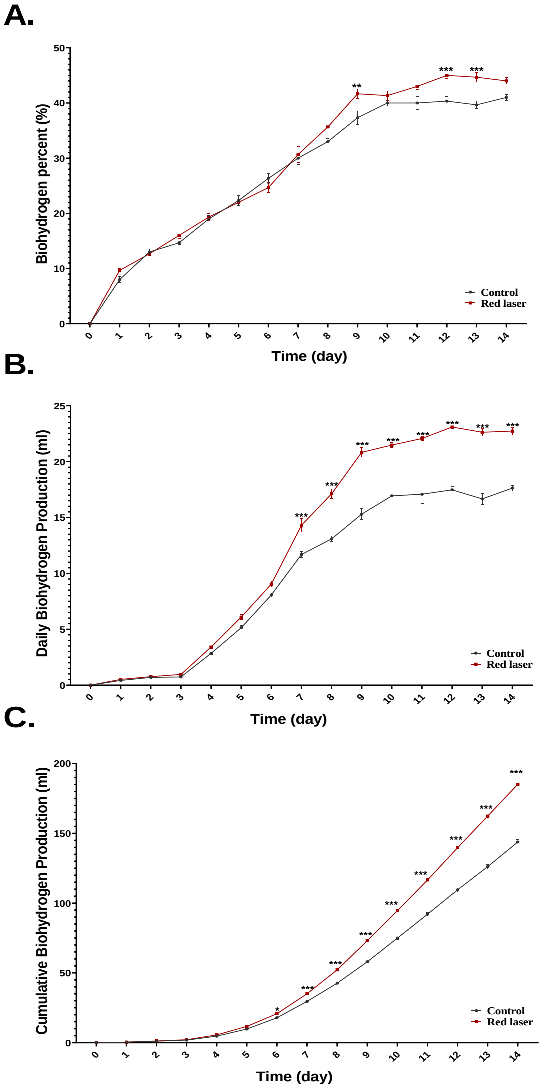

Figure 4: Effect of red laser radiation on biohydrogen production (A-C). A. Biohydrogen percent (%), B. Daily biohydrogen production (ml) and C. Cumulative biohydrogen production Values are mean ± SEM. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs control.

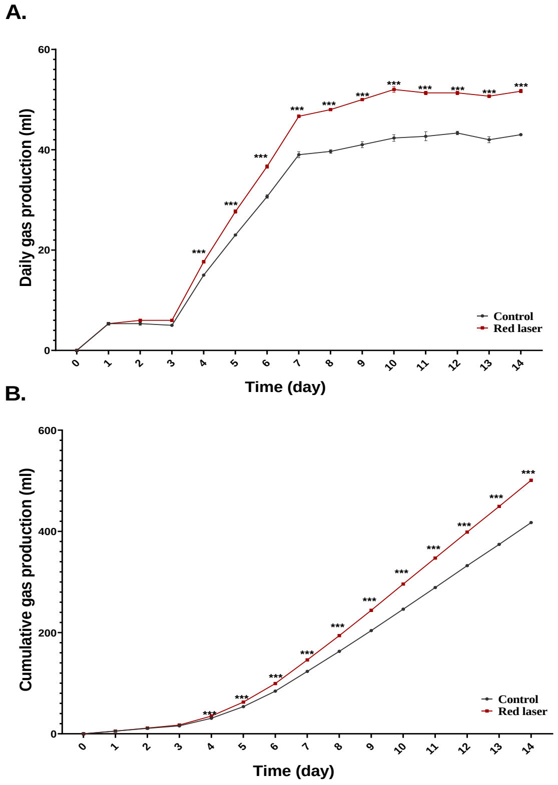

Figure 5: Effect of red laser radiation on gas production (A-B). A. Daily gas production (ml) and B. Cumulative gas production Values are mean ± SEM. *** $P < 0.001$ vs control.

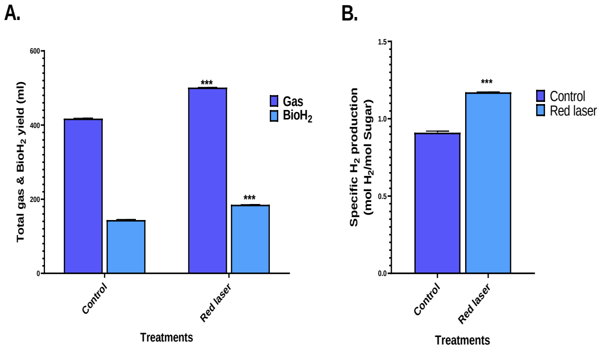

Figure 6: Effect of red laser radiation on specific gas production (A-B). A. Total gas and biohydrogen yield (ml) and B. Specific H_2 production (mol H_2/mol sugar). *** $P < 0.001$ vs control.

Based on the results of this study, exposing the purple non-sulfur bacteria (PNSB) inoculum to laser radiation is an easy and affordable technique with a very good outcome. Laser has high energy in unit area helps to increase the activity of cells in both growing and gas production sides [24]. The definite wavelength of lasers opens the gate for further investigations to know the most beneficial type/wavelength that can be used with PNSB to maximize biohydrogen production.

Maximizing biohydrogen production from food wastes using this environmental-friendly method will encourage to scale-up the process to an industrial scale to generate clean energy using an ecofriendly technique and solve the food wastes issue as well.

A study investigated the effects of nanomaterials irradiation using laser source on the biohydrogen production [23]. However, the bacteria were not irradiated. In contrast, the present study investigated the direct irradiation of bacteria using laser source and without nanomaterials. Besides, several studies have investigated the effects of laser radiation on the methanogenic bacteria [24-27].

5. Conclusion

It can be concluded from this study that:

1- Purple non-sulfur bacteria (PNSB) exposure to helium-neon red laser led to significant increase in cell activity, therefor, conversion ratio.
2- The irradiation of purple non-sulfur bacteria (PNSB) consortium inoculum helium-neon red laser source for 2 hours increases the biohydrogen production by 1.2 times of the control.
3- Using mixed inoculum of purple non-sulfur bacteria (PNSB) increases the stability and the resistance of inoculum to any variance in the experiment conditions and the fermentation process itself.

6. Conflicts of interest

There are no conflicts to declare.

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