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Production of Biohydrogen by *Scenedesmus obliquus* and Yield Comparison with Various Pre-Treatment Methods

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ABSTRACT

The exploration of new energy resources has been a major challenge in the last decades for urban development. Biohydrogen is one of the most efficient renewable sources because the consumption of fossil fuels is highly polluting the environment. The production of biohydrogen from microalgae is a very attractive approach that helps to achieve bioenergy sustainability and carbon neutrality. However, the low yield of biohydrogen production appears to be the main challenge with microalgae. The aim of this research was to study the production of biohydrogen by *Scenedesmus obliquus* and various pretreatment methods such as physical, chemical and biological to increase the yield. The chemical pre-treatment process such as the alkaline method produces more hydrogen than other methods.

1. Introduction

The world is currently facing serious energy challenges as a result of the excessive burning of fossil fuels that has caused global climate change. Exploration for a cleaner form of energy has increased, resulting in a decline in traditional oil wealth and many environmental circumstances. To improve the country's economy through carbon neutral emissions and a self-sustaining culture of energy, the exploration of cleaner forms of energy has been increased. Biomass, which is considered renewable energy solutions, is currently marketed and operational to meet current energy demand. Biofuel is a promising alternative energy source, amongst other things. With respect to energy and fuel production, commercial enterprises are now gradually accepting technologies with a carbon-neutral process. Part of the carbon neutral approach to alternative energy production is *Azolla* (duckweed fern), water hyacinth (an invasive plant species), and microalgae (which trap CO_2 by assimilating it in their metabolic system to increase their growth), which helps to achieve the circular economy [1].

Hydrogen has recently been used as an alternative domestic fuel as it can enhance long-term energy security and reduce the impacts of air pollution and greenhouse gas emissions. By 2024, it is expected that the hydrogen market will exceed $191.80 billion [2,3]. Biohydrogen is a clean, renewable, low-emission, energy-intensive energy carrier that does not contribute to the accumulation of greenhouse gases, it has the potential to be used as an alternative to non-renewable energy [4]. There are several methods such as natural gas reforming, coal and biomass gasification, water splitting photoelectrolysis, bacterial fermentation (dark fermentation) and photobiological production to produce hydrogen. However, for the commercialization of hydrogen as a fuel abides major bottlenecks but reduces some of the disadvantages of old methods in terms of the technical, economic and environmental feasibility [5], storage related challenges [6], and transportation and fossil fuels dependance [7]. There is a need to significantly improve plant efficiencies, reduce capital costs and operating flexibility, and improve the reliability of hydrogen production processes. The use of microalgae to synthesize hydrogen is a biological technique that can effectively reduce these limitations. In comparison with other biological sources, microalgae have many advantages, such as a high growth rate [8], a lower environmental impact [9], low energy needs and lack of expensive pre-treatment processes due to lack of lignin [10]. Recently, the production of biohydrogen from microalgae has attracted due to mild fermenting conditions and its high rate of degradation of organic matter. Among all the process, the production of biohydrogen, micro-algae photofermentation is extensively studied. But there are some limitations such as susceptibility to oxygen, difficulties in process engineering, less microalgae production and insufficient information on strain capacity in the commercialization of micro algal bio hydrogen production, research is being carried out to overcome this restriction. Some pre-treatment steps like converting polymeric carbohydrates into monomeric sugars and increasing microbial accessibility can be included in biohydrogen production. Physical, chemical and biological pre-treatments are generally used to depolymerise carbohydrates. pH, temperature, partial pressure, nutrient content and substrate used for fermentation, etc. are some parameters involved in the production of hydrogen [11].

In this study, the microalgae used is *Scenedesmus obliquus* to produce biohydrogen in the laboratory. Physical methods such as ultrasound, chemical methods such as acidic and alkaline treatment and enzymatic treatment using pectinase enzyme are used in this study for the pretreatment of microalgae for improved production and its comparison.

2. Experimental Methods

2.1 Algal Strain and Cultivation

Scenedesmus obliquus NCIM 5586, microalgae was used in this study and was procured from National Collection of Industrial Microorganism (NCIM), Pune. The algae was cultured in Erlenmeyer flask containing 100 mL BG11 medium (composition in gL^{-1}) $NaNO_3$ 1.5; KH_2PO_4 0.4; $MgSO_4.7H_2O$ 0.75; $CaCl_2.2H_2O$ 0.36; Na_2CO_3 0.2; EDTA 0.01; $C_6H_8O_7.H_2O$ 0.06; $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$ 0.06; H_3BO_3 0.286; $MnCl_2$ 0.181; $CuSO_4$ 0.008; $Co(NO_3)_2$ 0.005; Na_2MoO_4 0.0391. The pH of the medium was set as 7.1. The medium was autoclaved and inoculated using 5% inoculum. The inoculated medium was incubated at 30 °C with intermittent shaking under the illumination of white fluorescent lamps with a photoperiod of 12 h light and 12 h dark.

2.2 Biohydrogen Production Setup

To produce biohydrogen, algae must grow anaerobically. To make it oxygen-free, a low sulphur medium was used which helps to increase hydrogenated activity, as this magnesium sulphate has been replaced by magnesium chloride. The production of the bio-hydrogen at lab-scale was

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prepared with a 500 mL conical flask and closed with a tight cap. A silicone tube is attached to the cap with a glass pipette. The end of the tube was closed and all sides were sealed by paraffin wax and aluminum foil. The total production of biohydrogen was calculated by water displacement method. The displacement of the water was done using a vessel and a measuring cylinder. The other gases that microalgae should produce are oxygen and carbon dioxide. To check whether the gas produced was hydrogen, dissolved oxygen was measured using the Winkler method and carbon dioxide was measured through pH changes before and after water displacement method.

2.3 Pretreatment Methods

2.3.1 Chemical Pretreatment

For chemical treatment, acid and alkaline treatments have been carried out. For acid pretreatment and alkaline treatment, microalgae was grown in a basal medium at pH 5.5 using HCl and pH 7.5 using NaOH respectively. The algae was maintained with proper growth conditions. The medium has been maintained in both light and darkness for the production of biohydrogen.

2.3.2 Physical Pretreatment

For physical treatment, the ultrasonication method was applied. This was done by means of a water bath and precautions were taken to prevent contamination. The amplitude was set to 35% and the time was 3 minutes.

2.3.3 Biological Pretreatment

The enzyme pectinase was used for biological treatment method. 0.1 mg of enzyme was dissolved in 10 mL Milli-Q water. The reaction mixture consists of algal biomass, enzymatic solution and phosphatic buffer (pH 7.5) and incubated for 72 hours. The reaction was stopped by keeping in water a bath for five minutes.

3. Results and Discussion

3.1 Microalgal Growth and Biohydrogen Production

The algae was grown in the BG 11 medium by replacing $MgSO_4$ with $MgCl_2$, and on the third day the culture was kept in light and darkness by covering with dark sheets. Starting from the third day, the algae showed growth; so that the third day experiment was done for biohydrogen production. The production of biohydrogen from microalgae that have been cultivated at pH 6.5 has been defined as a control since microalgae normally grow at that pH. The production of biohydrogen was measured by water displacement method. The maximum yield obtained was on the sixth day and the yield in light was 24 cc and in dark it was 28 cc (Fig. 1). The biohydrogen production during dark fermentation is more than light, because in light, PS 11 activity has been more compared to darkness, which increases the oxygen content and thus decreases the hydrogenase enzyme activity necessary for hydrogen production. The dissolved oxygen and pH were the same before and after the water displacement method.

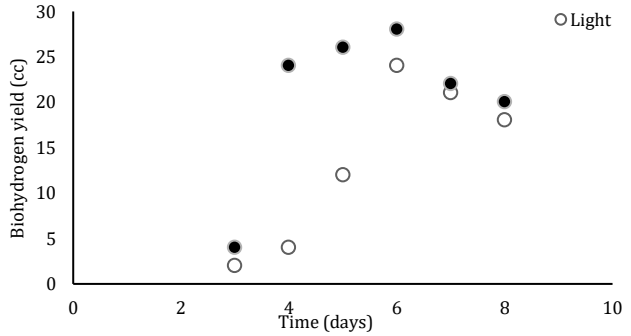

Fig. 1 Biohydrogen yield in dark and light process

3.2 Pretreatment Methods

In order to improve biohydrogen yield, various pretreatment methods have been carried out, including chemical, physical and biological methods.

3.2.1 Chemical Pretreatment

3.2.1.1 Alkali Pretreatment

The alkaline treatment was carried out at pH 7.5 using NaOH. The production of biohydrogen was estimated using the water displacement method. After alkali treatment, the maximum yield obtained was on the sixth day and the yield in light was 98 cc and in dark it was 242 cc (Fig. 2). The production of biohydrogen during dark fermentation is more than light. The dissolved oxygen and pH were the same before and after the water displacement method. The yield of biohydrogen production after the alkali pretreatment process has increased, because in alkali treatment, the growth of microalgae was less to that of other pretreatment methods therefore, PS11 activity decreased and the activity of the enzyme hydrogenase was more likely to increase hydrogen yield.

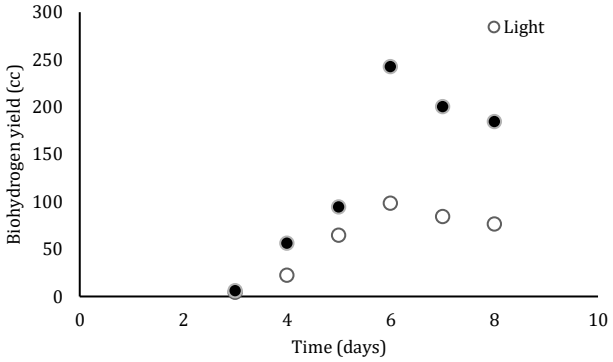

Fig. 2 Biohydrogen yield after alkali pre-treatment in dark and light process

3.2.1.2 Acid Pretreatment

Acid pretreatment was performed at pH 5.5 using HCl and biohydrogen yield was estimated by the water displacement method. After acid pre-treatment, the maximum yield obtained was on the sixth day and the light yield was 72 cc and in the dark it was 98 cc (Fig. 3). In acid pretreatment the production has increased which means that there is a cellular solubility which leads to the exposure of the biomass inside the microalgae. The glucose produces pyruvate after glucose catabolism which is oxidized to acetyl Co A. Acetyl Co A is reduced to ferredoxin which is re-oxidized with the enzyme hydrogenase and produces ferredoxin and releases electrons and molecular hydrogen [12].

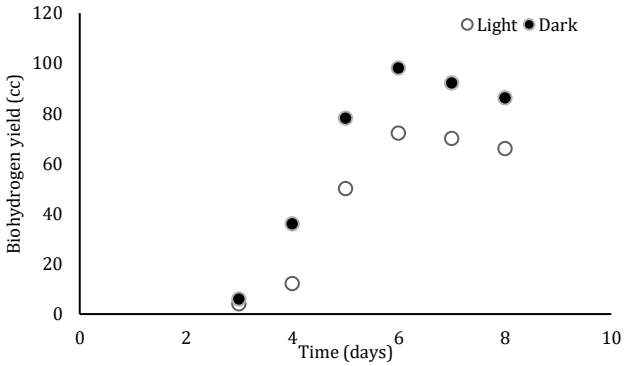

Fig. 3 Biohydrogen yield after acid pre-treatment in dark and light process

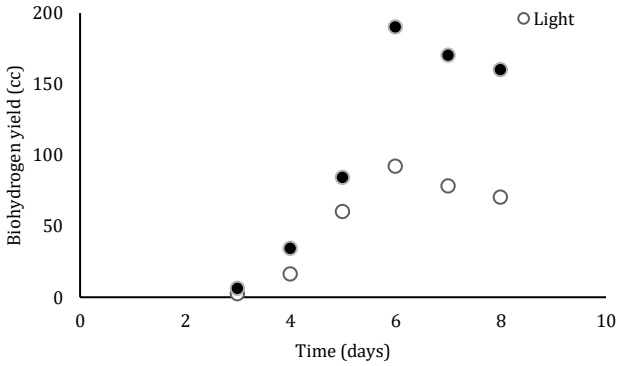

Fig. 4 Biohydrogen yield after ultrasonication in dark and light process

3.2.2 Physical Pretreatment : Ultrasonication

Ultrasonication was carried out for a physical treatment at 35% amplitude for 3 minutes and the biohydrogen yield was estimated using the water displacement method. The yield of biohydrogen production after ultrasonication has increased. The yield of post-ultrasonication biohydrogen production using a light process was 92 cc and in the dark 190 cc on the sixth day (Fig. 4). The yield of biohydrogen in the course of

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dark fermentation is more than light. During ultrasonication, the cell wall of microalgae is solubilized and releases the biomass that produces hydrogen using a hydrogenase enzyme.

3.2.3 Biological Pretreatment

The enzyme pectinase was used in the biological pretreatment method and the biohydrogen yield was estimated using the water displacement method. After enzyme pretreatment, the yield was in light is 64 cc and in dark is 94 cc on the sixth day (Fig. 5) The cell wall is solubilized in the enzyme pre-treatment method, which exposes the cell biomass for the production of molecular hydrogen.

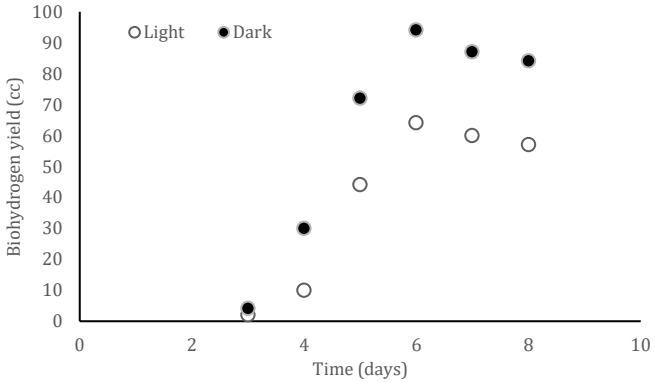

Fig. 5 Biohydrogen yield after enzyme pretreatment in dark and light process

4. Conclusion

Hydrogen is carbon-free, it has a good chance of highlighting significant changes in the use of fossil fuels and making dramatic changes in weather conditions. Commercial production of biohydrogen is an important way to improve the biofuel industry. Compared to other hydrogen production methods, biohydrogen production is environmentally sound. With no greenhouse gas emissions, it can displace traditional fossil fuels. Pretreatment is an important step in the production of biohydrogen and has an impact on yield. All pretreatments, biological is reported as the most cost-effective and environmentally friendly, but because it produces less hydrogen as compared to other methods. Algae and other microorganisms are a good option for economically producing biohydrogen. Algae play a major role in photosynthetically producing biohydrogen. This method helps to reduce the costs as well as to increase the yield of biohydrogen. Throughout the process, the dark process produces more biohydrogen than the light process. The pre-treatment of algal biomass increases the production of biohydrogen. Alkaline pretreatment produces more hydrogen, whereas enzymatic pretreatment has less. Dissolved oxygen turned out to be the same before and after, indicating that the gas produced was not oxygen. The pH was also the same before and after that the resulting gas was not carbon dioxide. It confirms that the gas produced was biohydrogen. Day six showed the maximum generation of biohydrogen in all processes.

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