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To cite this article: K S Maanav Charan *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **573** 012043

IOP Conf. Series: Earth and Environmental Science **573** (2020) 012043 doi:10.1088/1755-1315/573/1/012043

Multiple Reactor System for Biohydrogen And Bioethanol Production

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Abstract. Hydrogen gas has proven to be an ideal green fuel. Its use is limited at present due to inefficient and harmful methods of production like steam-methane reformation and coal gasification. Systems that utilize hydrogen as a fuel can be considered practical only with the development of better, cost-efficient methods of its production. This study focuses on efficient mass production of bio-hydrogen (and bio-ethanol as a byproduct) for commercial utilization with a theoretical multiple reactor system. The process involves culturing of modified photosynthetic algae (such as *Chlamydomonas reinhardtii*) in bioreactors for bio hydrogen production. This process produces some waste algal cells. These can be sent to subsequent reactors for production of bio-ethanol by fermentation. Also, the waste algal cells need to undergo pyrolysis before the fermentation. Hydrogen is produced in this process too. All these factors put together improve the overall yield of the complete process and reduce its cost substantially. Though the model is theoretical, it can be adapted for commercial production of hydrogen. The hydrogen can, in turn, be used as fuel for powering fuel cells which have zero emission and hence, are environment-friendly.

1. Introduction

The world demand for oil and other fossil fuels is rising tremendously and is expected to see a rise of 60% from the current level by 2025. [1]. Owing to this pressing situation, we need to start focusing more on sustainable sources of energy like wind, biomass, hydro and solar worldwide [2]. Production of biofuels like biodiesel [3], bio-oil [4], biogas [5], bio hydrogen [6] from bio masses of various sources such as forestry, agriculture, and aquatic biomass were taken into consideration. Besides exhaustion of biomass without appropriate compensation, most of these fuels have adverse impacts on the environment (especially the carbon cycle due to production of greenhouse gases). Hence these bio fuels led to bio mass scarcity, deforestation and loss of bio diversity [7].

Hydrogen gas can be utilized as a clean fuel in power plants, vehicles and other similar industries [8] as the energy production from it is manifold compared to conventional fuels. Also, no green-house gas is produced upon combustion like the traditional carbon-based fossil fuels [9]. But as of now, the commercial and economic viability of hydrogen fuel is very limited as 94% of the production of hydrogen is from coal gasification, steam methane reforming and partial oxidation methods. All these methods use non-renewable sources of energy such as coal or oil for production of hydrogen which make it expensive and pollute the environment.

Due to these drawbacks the focus started shifting towards hydrogen production using green algae. These algae display huge ecological and genetic diversity i.e., they can be found on land, water and even on snow. This shows that they are easily available, can withstand harsh conditions and, therefore require very less maintenance. Such photo biological production of hydrogen ensures its sufficient availability and so, makes it economically viable. It is not only efficient but environment friendly too.

2. Methodology

2.1. Production principle

The photosynthesis process in micro algae is similar to that in plants in which the water supplied for the process is taken up and split into respective hydrogen ions, electrons and oxygen. This process occurs in the photo system II. These electrons are then transferred to photo system I where the electrons are used for NADPH synthesis by the process of photo-phosphorylation. During this process, the biomass is kept under FE-FE hydrogenase enzyme to utilize the hydrogen ions (produced during hydrolysis of water) for production of hydrogen gas simultaneously. [8]

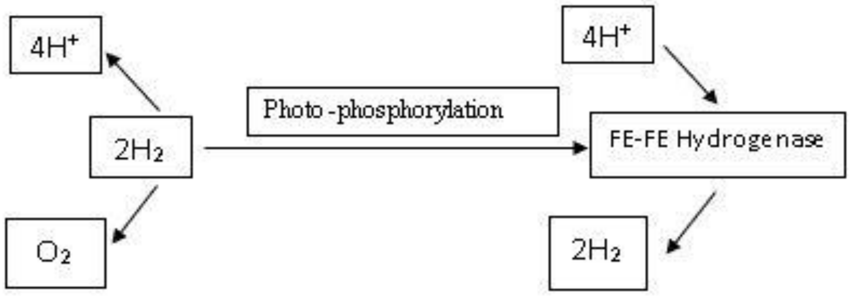

Fig 1 –Production of hydrogen from the algal biomass during photosynthesis

Various other by-products such as biodiesel, bioethanol etc. can also be extracted from the algal biomass in order to reduce the total cost of production. Bio-ethanol production has been chosen in this particular process due to its high economic value. For this production, the algal bio mass is sent to a tank in which it is pre-treated and broken down into simpler components so that the process of hydrolysis is aided. After the sugars are broken down by hydrolysis, it is sent for fermentation and distillation for ethanol production.

2.2. Strain selection

Under laboratory conditions, various strains of algae might produce hydrogen, but for large scale production they might not be efficient or it may not be possible to culture them due to various external factors. The algae have a large genetic pool, a production strain which can be utilized for such large-scale production efficiently must be found out first. *Chlamydomonas reinhardtii* is one such strain that has been identified after extensive research. It is a single cell green alga (flagellate) whose cell wall majorly consists of hydroxyproline rich glycol proteins. It can not only be cultured in large amounts but can also be genetically modified as per our needs easily [10].

Researches have proven that the *Chlamydomonas reinhardtii* strain can be enforced to produce hydrogen under sulphur free conditions in an anaerobic reactors. . This principle could be applied to increase the hydrogen production efficiency of the algae [8]. Also, the algal biomass has more than 60% carbohydrate content. Most of the polysaccharides and starch found in their cell wall can be utilized for ethanol production by pyrolysis (pre-treatment), hydrolysis and fermentation. [16]

Considering all these advantages of the strain, it can be used efficiently for production of hydrogen and ethanol (by product). It is due to all these advantages that *Chlamydomonas reinhardtii* is chosen for the process.

2.3. Multiple reactor production system:

This is a continuous flow process where the micro algae is grown in a photobioreactor and then moved on to various other reactors and tanks for production of hydrogen and subsequent production of ethanol. The waste cells being produced in this process are utilized for ethanol production by keeping them in the presence of recombinant *Saccharomyces cerevisiae* (yeast). This increases production quantity and reduces cost thereby decreasing the market rate of hydrogen up to several times.

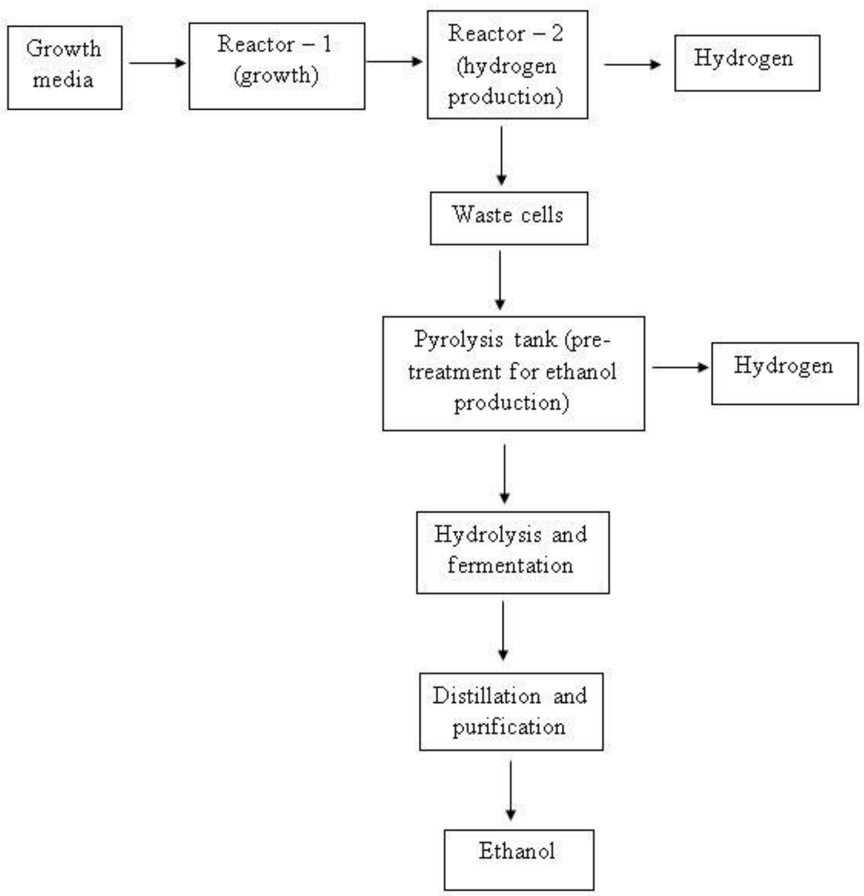

Fig 2- schematic representation of the flow in multiple reactor system

Initially, two continuously stirred tanks are used. The first step is culturing of the cells in reactor 1. This is done in a nutrition media which has low levels of Sulphur. The deficiency of Sulphur slows down photosystem-II and ensures that oxygen is consumed at a rate faster than its production for cellular respiration. The solution is also bubbled with limited amounts of oxygen and carbon dioxide so that the cells can always remain in the reactor. These cells obtain energy from both photosynthesis and respiration in the acetate solution.

The cells from reactor 1 are moved to reactor 2 after adequate growth of the algae, which is maintained under anerobic conditions (as the cells have the photosystem-11 suppressed, reactor 2 does not become aerobic). Even the residue oxygen in the reactor will be consumed by them. The anaerobic environment of the reactor will pressurize the cell to produce hydrogenase, which will convert the H^+ ions produced to hydrogen gas. Simultaneously some cells from the reactor 2 are removed before they completely stop hydrogen production, while new cells with high production rate are added. Successful activity is seen at a dilution rate of 0.5/day for the cells, which is equal to an average stay of 2 days for them. [12]

As a recent study suggests, to improve the efficiency of production of hydrogen, the genes that control the amount of chlorophyll in the algae, chloroplast can be manipulated. This will reduce the naturally occurring chlorophyll molecules in a chloroplast from 600 to 130 molecules. At this point, dense cultures of algae in such big reactors could make three times as much hydrogen as now [13].

The cells with low rates of productivity are withdrawn from reactor 2 to prevent the accumulation of inert biological materials that can't be re-consumed by new cells. Instead of wasting these cells, they are sent to other reactors for production of bio ethanol. This will improve the efficiency of the process and reduce production cost.[11]. At first it is sent to Pre-treatment tank (pyrolysis) in order to obtain sugars directly. This prevents the loss or degradation of the sugar produced, and also reduces the process's energy requirements (thus production cost is reduced). Pyrolysis is the most suitable pretreatment for this system as it requires minimal energy input. In this process of pyrolysis, the algal biomass is treated at temperatures over 300 degree centigrade. The cellulose is degraded to char and other gases like hydrogen and carbon mono oxide. The hydrogen produced in this again is then collected separately. This increases the overall hydrogen production of the plant.[14]

This char is now either washed with diluted acid or water, and there is enough carbon source in the residual solution to sustain microbial growth for bioethanol production. It is reported in a study that at this stage about 55 % of the biomass weight is lost [10]. Now this solution will be taken for the next step, i.e., process of hydrolysis and fermentation. In this step, a method called simultaneous saccharification and fermentation(ssf) is implemented as both fermentation and enzymatic hydrolysis can be performed in the same reactor. This reduces the overall cost to a greater extent compared to other processes as it is carried out in just one reactor. The hydrolysis process is very efficient when dilute acid or warm water is used [11]. Unlike other methods of enzymatic hydrolysis where the entire process takes place in separate tanks, a small amount of cellulase enzyme is required in this process. It is because inhibition of enzyme activity is very low as glucose and cellulose fermenting units are present in the same reactor.

During this process of fermentation, the fermentable sugars are converted into ethanol and carbon di oxide by a microorganism called *Saccharomyces cerevisiae*. In this method, an average of 0.51kg bioethanol can be obtained from glucose or sugar per kg in theory but with genetically modified recombinant yeasts and improvement in production technology this can be achieved in practice too in the near future. Since both saccharification and fermentation take place in the same reactor, the temperature of the reactor must be monitored and regulated frequently in order to have high efficiency. For this step to have higher yields the *Saccharomyces cerevisiae* cultures must be held at a pH of 4.5 and temperature of 37 degree Celsius [15].

At the final stage, the mixture produced after fermentation is sent to the distillation process to separate the pure ethanol from it. This separation process is achieved by heating the mixture of ethanol and water. As ethanol has lower boiling point, it vaporizes before water. This evaporated bio ethanol is collected in a distillation column.

In this multiple reactor system, hydrogen is produced not only in the reactor but also during the process of pyrolysis. Also, the wasted algal cells which cannot be reused for hydrogen production are used for producing ethanol as a by-product. All these factors put together improve the overall efficiency and reduce the overall cost of production, thereby reducing the market cost of both hydrogen and ethanol fuel phenomenally.

3. Results and Discussions

This production method involves usage of genetically engineered *Chlamydomonas reinhardtii* such that there is a low amount of chlorophyll molecules in their chloroplasts. This ensures algal cells present on the top layer consumes lesser amount of sunlight and more sunlight is made available for the cells present in the deeper layers of the reactor. This one particular feature of the genetic engineering alone can increase the hydrogen production manifold. As the biomass is kept in a photo bio reactor, the optimum conditions required for the growth and production of hydrogen and ethanol can be maintained throughout the process. This ensures a high rate of productivity. It is observed that during the pre-treatment process for ethanol production (pyrolysis), when the biomass is heated at low temperature there is production of hydrogen. This this increases the overall quantity of hydrogen produced from the system. In order to improve the production efficiency of bioethanol, genetically modified recombinant yeast such as the *Saccharomyces cerevisiae* is used. Such yeast helps in fermenting the hexose sugars and pentose sugars to ethanol simultaneously and decreases the production time. [17]. Pyrolysis requires a low amount of energy for treating the biomass and is very efficienct too. Hence, hydrolysis does not need to be done separately. This allows us to carry out the fermentation process along with the hydrolysis process. As both the processes take place in the same reactor, the overall capital cost for this system is substantially reduced.

. This system recycles the algal cells having low production rates of hydrogen for ethanol production and so, there is no need to expend more energy or invest separately for the ethanol production. This use of algal biomass for bio ethanol production not only reduces the cost of ethanol but also, reduces the conventional usage of sugar cane (first generation crop) for ethanol production.

The average cost of petrol or diesel is around $ 0.90 whereas that of hydrogen fuel is around $15 per kg (equivalent on price per energy basis to $ 5.60 per gallon of gasoline). Due to this reason many people do not prefer hydrogen fueled cars. By using this multiple reactor system to produce hydrogen and ethanol, the overall cost of production can be reduced manifold. Hydrogen fuel cell cars can become much more affordable and therefore, a popular choice in the market. This will spark various companies to make more affordable hydrogen fuel cells in the future eventually decreasing the consumption of oil phenomenally. Apart from this the ethanol produced in this process can also be used in direct alcohol fuel cells (DAFC) which can be used for automotive purpose in future and for various other purposes.

4. Conclusion

The production of hydrogen using *Chlamydomonas reinhardtii*is theoretical at present. However, as the future encompasses technologies like green fuel and hydrogen systems to scale a more sustainable and environmentally obligable model, it is of immense importance. It is the strain's distinctive characteristics that make the process practically possible. Also, the fact that the strain can be modified easily to increase the output manifold ensures commercial success, if developed properly. The waste cells from the multiple reactor system are used to produce ethanol, another important commercial product. This small cost optimization in production of fuels from green algae can make hydrogen more affordable as a fuel thereby reducing the 4.6 metric tons of carbon dioxide produced to even 1 metric ton annually. This initiative has the potential to pave the way for mankind to attain carbon neutrality.

Given the rapid depletion of fossil fuels and their adverse effects on the environment, such a process could bring about a whole new fossil fuel-like, but much more sustainable and eco-friendlier era.

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