



## Review Article

# Renewable Hydrogen Fuel from Photofermentation of Glycerol: Enhanced Reviews

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**Abstract:** The fluctuating fossil fuel price in the global energy sector and environmental destruction that comes with such fuels have paved ways for alternative fuels. Renewable hydrogen fuel is one of those alternatives which have generated massive interest in the world of renewable energy due to its unique property as a fuel-free of any pollutant. Biochemical conversion of waste materials of biomass origin to hydrogen is a sustainable technique for hydrogen production. Glycerol, a waste obtained during biodiesel manufacturing process has been found to be a suitable feedstock for hydrogen production using PF processes. The present work reviewed literature related to the PF process of glycerol to hydrogen. In the process, a methodical comparative study of recently available research reports on renewable hydrogen production as fuel from glycerol through PF was employed. The review emphasizes the challenges bedeviling PF of hydrogen from glycerol and suggested solutions to that effect with future recommendations on potential research areas needed to be undertaken to improve the process.

**Keywords:** Hydrogen Fuel, Photofermentation, Glycerol, Renewable Energy, Purple Non-sulfur Bacteria

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## 1. Introduction

Renewable hydrogen concomitant to fossil hydrogen is gaining importance as an alternative future fuel that is free of pollution [1]. This made wastes of biofuel for hydrogen production to be extensively researched, for instance, the byproduct of biodiesel production (glycerol). Biodiesel is one of the most investigated renewable fuel, it is produced through one of the easy routes of transesterification of fatty acids i.e. reacting a fat or oil (triglycerides) with alcohol in the presence of a catalyst (mostly alkali) at lower temperature and pressure with glycerol as an unwanted product [2–4]. Currently, according to findings, the global biodiesel production from cheap organic materials stood at 40bln liters, and to each 100 liters biodiesel produced is 10 liters of glycerol [5, 6].

Of the one tenth glycerol produced from every liter of biodiesel produced in the world, only two-third of it is

industrially harnessed with majors in food and beverage, pharmaceuticals, cosmetics, etc. [7]. An increasing trend of the emerging biodiesel industry has led to the flooding of glycerol causing new engineering and environmental challenges, hence, the necessity to explore alternative uses of glycerol [8, 9]. In doing so, researchers discovered glycerol as an excellent substrate for the production of biohydrogen, a type of renewable fuel.

Hydrogen is one of the common precursors in processing industries (e.g. chemical industry) and is recognized as a future renewable fuel and energy storage component. It is expected worldwide that an annual increase for hydrogen demand will be 4 – 5% [10]. Like most other production processes, hydrogen can be produced through various methods from different relevant substrates. However, of all such sources of hydrogen, fossil fuels still hold the largest

share as feedstock at (96%) and the remaining percentage are from electrolysis (3.9%) and other by-product sources of hydrogen (0.1%) [10, 11]. But attention is shifting towards renewable sources and processes of producing hydrogen as a result of environmental hazards, climate change, and global warming inflicted by non-renewables [12]. A typical renewable process of hydrogen production is the Photofermentation (PF) of biomass [2, 13].

Therefore, in this review, a methodical comparative study of recently available research reports on renewable hydrogen production as fuel from glycerol through PF using some carefully relevant published articles is presented and thereby, exploring the prospects in the research area. The review is presented according to the following steps: brief characteristics and treatment methods of glycerol, PF process, hydrogen production potentials from glycerol by PF process, challenges of PF process for hydrogen from glycerol, and lastly, strategies for improved hydrogen production by PF of glycerol.

## 2. Glycerol

Glycerol with IUPAC name (propane-1,2,3-triol) is a byproduct of biodiesel or soap production through transesterification, saponification, or hydrolysis process of fats and oils [8, 14, 15]. This typical oleochemical is with distinctive chemical and physical properties in its fold. Some of these properties include hygroscopic in nature, soluble in water and non-toxic in nature [15].

### 2.1. Characteristics and Compositions

Glycerol is a colorless, odorless, viscous liquid that tastes sweet. In its pure anhydrous condition and under normal atmospheric pressure glycerol has a specific gravity of 1.261 g/cm<sup>3</sup>, a melting point of 18.2°C, and a boiling point of 290°C [14]. Furthermore, crude glycerol tends to come in the company of nitrogen, sodium chloride, ash, and water. The composition of these constituents varies from biodiesel to soap industries [8, 15].

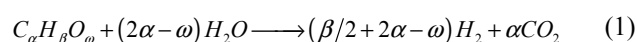
### 2.2. Treatment

Component composition of the glycerol, end-purpose use, and to some extent feedstocks and the process in which the glycerol was produced determine the purity of glycerol [15]. Although the glycerol treatment process varies due to recent technological breakthrough, basically, the purification process starts with neutralization, followed by filtration and centrifugation, and finally, glycerol is distilled under vacuum controlled conditions [6, 8, 14, 16, 17]. Substrates like lignocelluloses need to be treated first before harnessing them into hydrogen. However, for hydrogen production, glycerol needs not to be treated as its crude form possesses high energy content (i.e. 25.30 MJ/kg) than its purified form (19.00 MJ/kg) as such, the crude glycerol is very ideal substrate without much constraints for the production of hydrogen [4].

## 3. Photofermentation

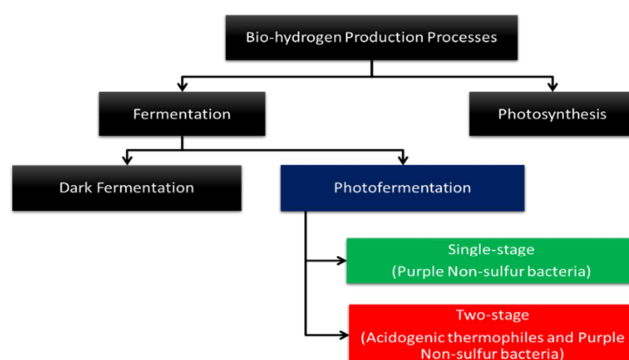
One of the many reasons for this review is to emphasize the PF process of hydrogen production because it depicts a very high proportion of the output of hydrogen compared to its sister processes like the dark- process [13, 18] as a result of good manipulation of factors like substrate inhibition, temperature, pH, the color of wastewater, light intensity and light wavelengths [13, 19]. However, the general perspective of photo-fermentation has been broadly covered by many previous research reviews, as such this review will not dwell much into it.

In the follow-up, the capability and process of converting substrates of organic origin and deriving from, hydrogen, with no oxygen evolve (anaerobic conditions) in the presence of light and purple non-sulfur bacteria (PNSB) while using nitrogenase and hydrogenase as enzymes is called photo-fermentation. The process is described according to the following general equation [13, 18, 20–23]:



### 3.1. Photofermentation Process

The process of renewable hydrogen production is broad. These processes are categorized and classified mainly on agents aiding the process. Given that, a cropped classification is depicted below.



**Figure 1.** Modified and emphasized biohydrogen production processes. [19, 24, 25].

### 3.2. Single-Stage Photofermentation Process

This process is usually termed PF. It uses direct photosensation only to carry out the fermentation process on glycerol to produce the desired product, hydrogen. To that effect, the single-stage conversion process is effective for glycerol to hydrogen, and such development is of good interest given the very large availability of glycerol being produced [18, 22].

### 3.3. Two-Stage Photofermentation Processes

This process takes place under anaerobic conditions and it is the combination of photo- and dark-fermentation (DF) processes to form the two-stage hydrogen production process from glycerol, as the organic substrate is completely

converted into hydrogen and a waste product of carbon dioxide [22]. Although, the latter part usually comes first in the two-stage process. However, the later part process favors substrates like sugars for the production of hydrogen with large quantities of unwanted byproducts (e.g. organic acids). Moreover, the dark fermentation process can only function at low molar yields, with some difficult thermodynamic challenges been encountered, in some instances not even feasible [18].

## 4. Photofermentative Hydrogen from Glycerol

As earlier mentioned, the review focused on photofermentative hydrogen from glycerol. Sabourin-Provost and Hallenbeck, in their research report, high yield conversion of a crude glycerol fraction from biodiesel production to hydrogen by PF, reported that a photosynthetic bacterium, *Rhodospseudomonas palustris*, converted glycerol to hydrogen through PF in both pure and crude glycerol samples with relatively high yields, up to 6 moles H<sub>2</sub>/mole glycerol (75% of theoretical, 8 moles of H<sub>2</sub>/mole glycerol) with no evidence of inhibition or toxicity noticed [2]. Lalitha et al in their findings have reported that floating glycerol-water mixture through highly stabilized and finely dispersed CuO/TiO<sub>2</sub> and Cu<sub>2</sub>O/TiO<sub>2</sub> catalysts gave a maximum range of hydrogen production of 0.0165 to 0.02006 mol/h. They also reported that the presence of the catalysts provided an unhindered ground for hydrogen production to be much more successful under the light sensation system [26].

In continuation of the field point, numerous approaches to convert glycerol into more usable products by a biochemical transformation in the presence of light have poured in. In their investigation using Response Surface Methodology, Gosh et al studied the interactive effects of process parameters like light intensity, the concentration of crude glycerol, etc. on the stoichiometric conversion of crude glycerol to hydrogen, 6.69 mol hydrogen/mole of crude glycerol were obtained, a 96% yield of theoretical assertion was achieved [27]. In the follow up of the aforementioned study, still, Gosh et al in another study, found that the effect of nitrogen source and different concentrations of crude glycerol yielded a 6.1 mol hydrogen/mole of crude glycerol under optimal conditions, depicting 87% of the theoretical assertion [28]. Furthermore, *Rhodospseudomonas palustris* was able to photo-degrade crude glycerol from biodiesel to hydrogen at a conversion efficiency nearing 90% of the theoretical maximum, however, inhibition during the process was observed and believed to have been caused by fatty acids as a result of saponification [29]. Another report yet indicated an optimized process using effluent of dark-fermentation in a two-stage method and *Rhodospseudomonas palustris* as fermentative bacteria, 80.21% of glycerol conversion rate to hydrogen was achieved [30].

Alternatively, Rodrigues et al (2020) demonstrated an effluent of a dark fermentation used in the PF by a phototrophic bacteria culture of *Rhizobiales* and *Clostridiales*

order, at a temperature little higher than the ambient and stable neutral pH which generated 3.94 mmol H<sub>2</sub>, with nearly averaged 90.0% advantageous destruction of ethanol, acetic acid, butyric acid, and methanol present in the substrate to add up for hydrogen evolved [31]. Furthermore, glycerol was photofermented to produce hydrogen in two designed models. It was found that the glycerol conversion efficiency was dependent on initial biomass concentration with hydrogen productivity of 37.7 mL/g biomass/h at a constant of 58% glycerol conversion efficiency [32].

### 4.1. Challenges of Photofermentative Hydrogen Production from Glycerol

Like other production processes, the PF of glycerol to hydrogen process tends to come with key challenges that require urgent attention to deal with. These challenges are explored in earnest.

#### 4.1.1. Inhibitors

During PF of glycerol to hydrogen, inhibitors have been found to emerge and play a great role in the retardation of hydrogen evolve. Of such inhibitors were soap (the highest inhibiting factor), methanol (with inhibition strength dependent on concentration level, as high concentration presents the high effect of inhibition) [33], and oxygen-evolving reactions (their presence attack enzymes aiding hydrogen production) [23].

#### 4.1.2. Light Source and Light Conversion Efficiency

The major challenging factor for photofermentative hydrogen production is the efficiency differences at which light is utilized by phototrophs under different magnitude of light concentrations [34] as recently reported that low-intensity electromagnetic field of extremely high frequencies affects hydrogen production by *Rhodobacter sphaeroides* [35]. This experience made the process to be a bit daunting to navigate through finding a suitable intensity condition in addition to alternative sources of light [36].

#### 4.1.3. Culture Cultivation and Condition

The selection of bacteria with a remarkable capability of producing hydrogen from waste like glycerol has been a challenging struggle [37]. To date, researchers are still trying to come up with an efficient way of cultivating such desired culture for a photo-fermentative hydrogen production process. Additionally, culture condition has been identified as one of the most impacting factors for the photofermentative hydrogen production process. Lo et al reported that delaying in culture growth was observed as a result of unfavorable conditions [38]. Meeting favorable conditions for culture in a process like PF of glycerol to hydrogen it's a huddle needed to be addressed [24].

#### 4.1.4. Photobioreactor Geometry

It has been understood that the relative growth rate of culture (i.e. PNSB) is lower in PF than that of the DF bacteria, therefore, a lower efficiency is obtained. To that effect, the PF process requires a larger reactor size [39]. However, Bolatkhan et al

(2019) reported that the size issue is becoming troublesome concerning light penetration as the deeper part of the reactor tends to lack availability of light and that prolongs the process at a snail pace of hydrogen evolution [24].

#### 4.2. Remediations

Having discussed the challenges of photofermentative hydrogen from glycerol in 4.1 above, it will be inconclusive of the review not to deal into some possible remediation of those challenges, therefore the remediation process are presented.

Based on the present review, inhibitors like soap can be removed and at the same time managing the pH of the process using sodium chloride and thereby improving hydrogen production from glycerol conversion [33]. However, the suitability of the inorganic salt must be verified further to ascertain its effects on cell growth during the PF process of glycerol. In another unique report by Kim *et al* (2014), it was observed that soluble microbial product was an additional factor restricting hydrogen yield during PF and that was dealt with through the addition of ethanol [40], however, the principle behind that effect is yet to be comprehended as no further explanation was reported.

Although PF is a striking way of producing renewable hydrogen. To improve hydrogen yield during PF of glycerol, it is necessary to intentionally modify cellular metabolism of the culture through recombinant technique [10] to obtain antenna mutants capable of harvesting light effectively [18]. In respect of light source, it has been found that artificial light sources like tungsten and luminescence type were effective as a source of light for both cell growth and hydrogen production during indoor PF [41].

One remarkable scenario to consider for improving hydrogen yield is to introduce photocatalysis during PF of glycerol. This may well prove effective for high yield of hydrogen from glycerol as it has been verified in other studies that used substrates other than glycerol. Finally, the performance rate of PF of hydrogen from glycerol system can further be exploited by integrating the system with other technologies. Moreover, its affinity to water, glycerol has gained an advantage as a component to design a low-cost process for enhanced hydrogen production [42]. Succeeding in such a trial will present a novel way of enhancing the future of renewable hydrogen from glycerol.

## 5. Conclusion

Here the renewable hydrogen production from glycerol by PF has been reviewed. Interestingly finding by the review revealed that the PF of glycerol to hydrogen phenomenon was dependent on crude glycerol concentration, as it tends to favor a high yield of renewable hydrogen.

Challenges like the sprout of inhibitors, instability of culture conditions, uncertainty of photobioreactor geometry, etc. in the conversion of crude glycerol to hydrogen by PF have proven a negative effect. Although the degree of effects by these challenges varies and independent of one another.

However, the challenges identified were solutions provided with some as concrete solutions while others were hypothetical solutions. These solutions were: modification of cellular metabolism of the culture; introduction of photocatalysis and integrating the PF system of glycerol with other technologies to improve hydrogen yield.

Furthermore, glycerol offers promising opportunities towards establishing glycerol as a platform for its conversion to fuels. Investing in crude glycerol for energy by the PF process would elevate the process and glycerol as a key raw material in the energy industry.

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