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Feasibility and viability of procuring biohydrogen from microalgae: An emerging and sustainable energy resource technology

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Abstract. As the world's population is increasing at an unprecedented rate, causing a severe impact on the limited and depleting petroleum reserves by their overexploitation and consumption. It is estimated that due to increasing socioeconomic and infrastructural advancements, we have already consumed about 50% of the petroleum reserves. Furthermore, the excessive usage of fossil fuels is believed to be a potential cause of global warming and a threat to environmental sustainability. This led the researchers to explore and study renewable and carbon-neutral sources of energy, which can be optimized as per the requirement and should be economically viable. Microalgae stand out momentous and materialized as feedstock to get all that we need at a single platform. Microalgae are the primary producers that utilize Carbone dioxide CO_2 and light for their growth. They can be grown in freshwater, saline water, and even in wastewaters due to their disparate biochemical metabolism. This urged microalgae to be exploited for obtaining various renewable energy-based fuels, as it has the following significant features: potential for CO_2 fixation; high biomass growth rate; its capacity to store carbon in lipids and carbohydrates to produce biofuels (bioethanol, biodiesel, biohydrogen, and biomethane). Recently, Hydrogen have gained interest as one of the most environmental friendly fuel. Hydrogen has numerous merits as compared with others fuel. The range of energy content is 120–142 MJ/Kg and it has high content (142 MJ/kg) as comparing with energy content of gasoline (47 MJ/kg), methane (56 MJ/kg), and natural gas (54 MJ/kg) while, the energy density is 8.5–10.1MJ/L. Furthermore, the yield is 92–485 mL/gVS and cetane number 50-53. This mini review provides an insight about the processes of biophotolysis, and fermentation utilized in the production of biohydrogen utilizing microalgae. It will incorporate the recent developments and innovations in biohydrogen production using microalgae. It will also give an overview of the challenges encountered in the production routes and the future perspectives.

1. Introduction

Hydrogen is regarded as one of the cleanest renewable fuels because water vapour is the only by-product during its combustion. It does not emit CO_2, while it can be converted to electricity using fuel cells. Energy content, energy density, cetane number, and yield as 120–142 MJ/Kg, 8.5–10.1MJ/L, 50–53, and 92–485 mL/gVS, respectively. It has high energy content (142 MJ/kg) as compared to gasoline (47

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MJ/kg), methane (56 MJ/kg), and natural gas (54 MJ/kg). These features make hydrogen an emerging and sustainable selection for future fuel. However, the method of procuring hydrogen involves the utilization of fossil fuels and has an alarming footprint in terms of greenhouse gases. This creates a conflict in the concept of achieving clean and sustainable fuel. Therefore, to overcome this problem, researchers worked on biological methods to obtain biohydrogen. Biohydrogen production from microalgae has been an alternate option to minimize the cost of production and environmental impact. Sunlight is the propulsive medium in splitting water to Hydrogen H_2 and Oxygen O_2. The idea of the production of microalgae-based biohydrogen was given by Gaffron and Rubin (1). Microalgae are the primary producers that utilize CO_2 and light for their growth. They can be grown in freshwater, saline water, and even in wastewaters due to their disparate biochemical metabolism. This urged microalgae to be exploited for obtaining various renewable energy-based fuels, as it has the following significant features: potential for CO_2 fixation (2); high biomass growth rate; can be grown with wastewaters (3); its capacity to store carbon in lipids and carbohydrates to produce biofuels (bioethanol, biodiesel, biohydrogen, and biomethane) and recovery of various value-added metabolites (pigments, polysaccharides, antioxidants, and biopolymers) (4). Furthermore it does not require land for its growth, as it can be cultivated in open systems and closed photobioreactors(PBRs) (5). Microalgae have the capability of well adapting their metabolism depending on the growth conditions to produce hydrogen. Biohydrogen from microalgae can be obtained from various production routes such as indirect and direct photolysis, dark fermentation, and photo- fermentation (6). These processes are illustrated in Figure 1.

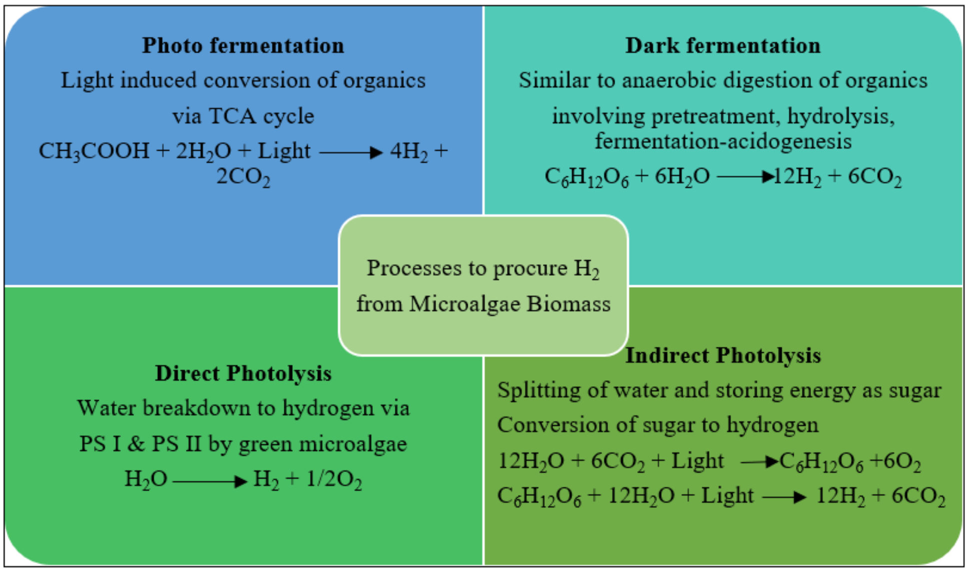

Figure 1. Different processes (production routes) to obtain biohydrogen from microalgae biomass

There are two steps to produce the Hydrogen by Direct photolysis. In the first step, the water is spilt into into proton (H+), electron (e-) and O_2 due to the photosystem II (PSII) is activated by sunlight. Then, the e- is across the photosystem I (PSI) and ferredoxin (Fd) from PSII. In the second step, e- is moved to Fd to [Fe-Fe]-hydrogenase, which the molecular H_2 is produced. e- is induced to move by light energy contribution. The production of biohydrogen through the above-mentioned processes from microalgae can be improvised by extensive research, to make these processes economically viable. This mini review provides an insight about the processes of biophotolysis, and fermentation utilized in the production of biohydrogen utilizing microalgae. It will incorporate the recent developments and innovations in biohydrogen production using microalgae. It will also give an overview of the challenges encountered in the production routes and the future perspectives.

2. Production routes to obtain biohydrogen

Biophotolysis is the bio-electrolysis of water by photosynthetic microorganisms (breaking down of water to H_2 and O_2). Microalgal species of *C. reinhardtii* and *Scenedesmus obliquus* are promising species to produce H_2. Furthermore, *C. reinhardtii* is considered a very productive species and researchers are also working on the genetic modification this species and some of its mutants resulted in the production of 300 mL/L (7). While in fermentation, the carbohydrates contained in microalgae are converted to H_2. Some of the microalgal species studied to produce H_2 by dark fermentation are *Chlorella* sp., and *Scenedesmus* sp. The productivity of hydrogen in terms of yield ranges from 0.35 mL H_2/g VS (untreated microalgae) to 340 mL H_2/g VS (thermally pre-treated microalgal biomass) (8).The comparison between the dark and photo fermentation is incorporated in Table 1.

2.1 Biophotolysis

During direct biophotolysis, cyanobacteria (*Synechocystis*) or microalgae (*Chlamydomonas reinhardtii*) lead to the conversion of water into O_2 and H_2 in the presence of CO_2 and light by photosynthesis (9) *Acutodesmus obliquus* was cultivated by utilizing the exhaust gases emitted from diesel engines and it was found that there was a considerable reduction in (Nitrogen oxides) NOx and CO_2 content (10). It can be concluded from the studies that direct bio photolysis has tremendous potential to produce H_2, but it also possesses some bottlenecks like light requirement, higher O_2 sensitivity, and the release of H_2-O_2 mixture from the process, which is considered explosive. While indirect biophotolysis is different from direct biophotolysis, the O_2 is evolved in a stage separated from H_2 production. The initial stage involves photosynthesis in which H_2O and CO_2 gets converted to O_2 organic substances (10). In the next stage a light independent reaction takes place which break down the organic matter produced in the first stage into CO_2, H_2, and other metabolites (11). This implies that the separating of H_2 production phase from O_2 evolution phase helps to overcome two of the limitations in direct photolysis i.e., O_2-H_2 mixture formation and O_2 inhibition (12). However, to facilitate the two stages, a complex system is required that results in high CAPEX and OPEX-Capital cost and Operational Cost (11).

2.2 Fermentation

Photo-fermentation PF-H_2 is produced by using microalgal biomass in the presence of light. The bacterias (Purple Non-Sulfur) having non-sulfur nature works robustly (*Rhodobacter sulfidophilus, Clostridium sp., Rhodopsuedomonas palustris, Rhodobacter sp.*, etc) in the PF-H_2 process (13).Since, PNS contains pyruvate ferredoxinoxidoreductase (PFOR), it helps to catalytically transform pyruvate to CO_2 and acetyl-CoA (14).This route generates reduced Fd, that is re-oxidized by [Fe]- hydrogenase or [Fe-Fe] and produces PF-H_2 (15). Thus, PF-H_2 is considered an effective and efficient process compared to other modes (Direct photolysis DP-H_2, Indirect photolysis IP-H_2 and Dark Fermentation DF-H_2) (16). Moreover, the substrate transformation capability is higher as compared to DF-H_2 process (17). Thereby it gives high PF-H_2 productivity. But at the same time the need of illumination, anaerobic bioreactor, and larger area elevates the total process cost, indeed making it a drawback (16).

DF-H_2 has gained notable attention as it used different types of organic biomass as a source. In the DF-H_2 the feedstock (organic) is exploited by the facultative bacteria during hydrolysis (18). This is a subsequent process and economical than PF-H_2 (14). In the first stage microalgal biomass having polymeric compounds (proteins, lipids and carbohydrates breaks down into fermentation substrates by utilizing various pretreatment strategies (19). In the second stage the fermentative feedstock is degraded by the extracellular enzymes secreted by the microorganisms. In the Acidogenic phase, the fermentative bacteria consumes microalgal biomass and helps produce CO_2, DF-H_2, organic acids, and acetate (14). The main limitation of the process is the utilization of DF-H_2 (e-donor) by the methanogenic bacteria in the production of methane (6). The criteria of selecting fermentative microorganisms is based on DF-H_2 production efficiency, temperature of fermentation, and pretreatment conditions of microalgae (15). A study reported the use of *C. vulgaris* (ESP6) biomass after pre-treating with 1.5 HCL and *C. butyricum* CGS5 as fermentative bacteria in the production of DF-H_2 (20). Direct fermentation has the following advantages: economical and eco-friendly process; produces a variety of organic acids (acetic acid, butyric acid, and lactic acid) and it can be commercialized easily. Furthermore, it does not need any aeration and illumination that require additional costs. However, the bottleneck of DF is the generation of CO_2 and other gases making DF-H_2 recovery more expensive and tedious.

Table 1. Comparison between different types of processing to obtain BioH_2 (16, 18)

Active Parameters	Dark fermentation	Photo Fermentation	Direct Biophotolysis	Indirect Biophotolysis
Oxygen requirement	No	No	No	Yes
Light requirement	No	Yes	Yes	Yes
Enzymes involved	CoA, acetyl-CoA	PSII, [Fe]-hydrogenase	[FeFe]-hydrogenase	PSI, PSII, PQ
Microorganisms contributing	Anaerobic microbes i.e., *Clostridium, Thermotoga* sp., and microalgal species	photosynthetic microorganisms i.e., *sphaeroides, Rhodobacter* etc.	green algae i.e., *Scenedesmus obliquus, Chlorella Vulgaris, Tetraspora sp., Chlamydomonas reinhardtii,*	*dry Cyanobacterium Anabaena variabilis*
Photobioreactors	Flat-panel PBRs, Glass bottles	Raceway pond	2-L algae culture tank, Culture bottles	1.2 L culture volume flat glass photobioreactor
Duration of Mechanism	(2 – 3 days)	(4 – 5 days)	(5 – 10 days)	(6 – 7 days)
Yield in Range	0.0165 – 0.14 L H_2/ g (VS)	0.13 L H2/ g (VS)	1.2 – 73.5 mL H_2/ L	6.625 – 243 mL H_2/ L
Advantages	Economic and have high yield	Greater range of feedstocks can be exploited, Broad light spectrum	Simple nutrient requirements of microalgae and easily cultivated	Eliminating that O_2 inhibition and the formation of the H_2–O_2 mixture
Disadvantages	Unwanted and harmful gaseous by products(e.g., CO_2,H_2S, CO, CH_4), effluent treatment is required	Costly photobioreactors, higher light dependency, low photosynthetic efficiency	High O_2 sensitivity, light requirement, and production of the explosive H2–O_2 mixture	High investment and operational costs, and H2 production is quite low
Increasing the BioH_2 production efficiency	NA	NA	Approaching efficiency 10% by direct water photolysis	The efficient use of microorganisms for improved light led to in the range of 10-13%

3. Limitations, opportunity, and future trends of BioH_2

3.1 Limitations

The low productivity is causing a major limitation and is believed to be a main drawback in large scale H_2 production. Some of the limitations of the conventional microalgae based H_2 systems are low biomass conversion rate, lower yield, and lack of information about the reactions and enzymes involved. In the DP-H_2 process the sensitivity of enzymes in terms of O_2 may reduce the yield of H_2 (16). DP-H_2 production is considered to be the highly energy-efficient process but the high CAPEX is the main bottleneck in the commercialization of the process (21). Additionally, the other potential drawback is the problems encountered in the transportation and storage of H_2 (22). The most alarming is the safety concern in direct biophotolysis as O_2 and H_2 can form an explosive mixture (23). To adapt with the production and usage of H_2 following parameters should be focused and worked upon to stabilize the feasibility of H_2 as a fuel: public acceptance, safe transport and storage, regulation and formulation of policies, implementation of standards and codes. On the other hand, the yields of H_2 is too low when hydrogen is acquired through bio photolysis because the sensitivity to O_2 and inefficient light conversion (10). Studies revealed that depriving the medium of certain nutrients such as nitrogen and sulfur can result in the decreased evolution of O_2 and enhancement of H_2 yield (24). According to (10); the substrates being used for fermentation can increase the cost of H_2 production, therefore to overcome this limitation food, agricultural and livestock wastes can be used . At the same time using wastewater and sewage sludge as substrates can contribute towards energy production and bioremediation. It is of paramount importance to select the appropriate microorganism for the fermentation as it effects the yield of H_2. The biogas generated during fermentation is a mixture of CO_2 and H_2. While the DF process is faster than either photofermentation or biophotolysis, its primary disadvantage lies in its low H_2 yield caused by the various by-products formed in the reaction (25). H_2 production by using biophotolysis have been studied to include that; increasing the efficiencies of light utilization (26), improvement the bioreactor design (27), modification the microalgae genetic (28) and optimization the parameter of microalgae culture (29).

3.2 Opportunities

H_2 is considered to be an up-and-coming fuel, since it has low heating value and high energy yield as compared to fossil fuels (9). The drive to find alternative fuels for the fossil fuels which are environmentally friendly and sustainable has led the researchers to study different types of options. In the recent time hydrogen fuel cells are constantly in debate to become a considerable option in the energy sector (10). The significant advantage of consuming BioH_2 as a fuel source is that its combustion leads to the generation of H_2O (30). The cultivation of microalgae is not dependent on agricultural land, thus solving fuel vs food feud (30). Microalgae is having the advantage of being cultivated throughout the year in open or closed systems unlike the terrestrial plants which can be grown on seasonal basis (30). Microalgae also possess other advantages such as high photosynthetic efficiency, enhanced yield and short life cycles as compared to other photosynthetic terrestrial plants (31). Even some of the microalgal species are having so fast growth that they can double in just few hours under optimized conditions, thus facilitating small harvesting cycles. Different types of microorganisms works for the synthesis of hydrogen by acquiring various transformation routes, such as Photo fermentation, dark fermentation, combined photo-dark fermentation for the production of BioH_2; genetic engineering; indirect and direct photolysis for the production of BioH_2 (30). Hydrogen is currently utilized in various industrial applications, but in most of the cases the production of hydrogen is achieved by the technologies such as gasification, steam reforming, electrolysis, and gas separation. But all these processes depend on the consumption of fossil fuels (32). BioH_2 possess immense potential to be utilized for the generation of power from internal combustion engines or fuel cells (10, 33). BioH_2 is currently being employed as a source of energy to run various industries and buses in various countries. Joint efforts of engineers and researchers are required to make H_2 as a future source of energy (34) US has about 44 fuelling stations for hydrogen (HFS), while most of them are in California (35). Europe is targeting to have 139 hydrogen fuel stations by 2025. Currently Toyota is having ***Mirai*** fuel cell vehicle in line of production, at the same time other brands like Honda and BMW are also working on fuel cell vehicles (30). Germany is planning to make 400 HFS by 2023, Japan is targeting 320 HFS by 2025 (30, 36).

3.3 Future trends of BioH_2

Many countries are not matching the standard criteria to produce H_2 as fuel. This issue needs to be solved by the effective coalition of government policies implementation and public awareness (37). Hence, the assimilation of different advanced approaches can contribute towards improved microalgal hydrogen production. The future of H_2 production based on microalgae is based not only on optimum design of bioreactors and efficient metabolic pathways but also on the economic aspect and public acceptance (6). For example, the biophotoylsis processes which are light driven can either work when the microalgae are exposed to sunlight or when artificial light is provided. The provision of artificial light may lead to higher cost, thus making the process uneconomic (10). Furthermore, the DP-H_2 productivity can be increase through the following measures: selection of optimum microalgae species, genetic modification of metabolic routes or enzymes, reduction in O_2 sensitivity etc. Higher DP-H_2 production can be accomplished by designing an improvised bioreactors and executing optimum physiological parameters (16) (22).The use of specialized mutant bacterial strain for fermentation have also shown significant increase in the productivity of DF-H_2 (38).

4. Techno-economic analysis TEA of Biohydrogen production from microalgae

The integration of hybrid systems for H_2 production helps in reducing the OPEX and increasing the yield (39) . At the same time the use of biological enzymes to pre-treat biomass can contribute towards process economic viability (19). In the production of economically viable H_2, it is important to visualize and consider following parameters: pretreatment cost, labour, PBRs, cultivation cost, and biomass cost (14). The main focus is on the cost analysis to deploy the Photo-bioreactors PBRs to the industrial scale, whether it be BioH_2 production using photolysis or biodiesel production using microalgae (30). The TEA of various H_2 production routes is elaborated in the subsections. The most important parameter of the H_2 production system is based on the cost effectiveness of the technology for their implication for the real world (40).The administration of TEA contribute towards effective collection of gas, optimization of yield, and minimization of CAPEX and OPEX. There are many studies focusing the production of BioH_2 but few of them deal with the economic aspects (6).The critical parameters evaluated for the production of H_2 using microalgae are: cost of bioreactor system comprising of reactor design & fabrication, illumination, aeration, bioreactor maintenance, nutrients and culture mixing mechanism (6). The commercial objective to produce H_2 is 0.30 USD/kg/H_2, identical with the price of gasoline (2.5 USD/GJ) (14). It was investigated that the cost of IP-H_2 production is \$1220 per Gj/year, while the cost of DP-H_2 production is \$7.24/kg (41). The preliminary investment cost of IP-H_2 and DP-H_2 are 140 ha and ≤100 ha respectively (6). Furthermore, the capital cost of IP-H_2 poses 90% and 80% for DP-H_2 [3]. Other percentages are estimated for operation cost and maintenance. The commercial scale of H_2 production for IP-H_2 is (243 mL L^{-1}) and (11.65 mL L^{-1}) for DP-H_2 (42, 43). Commercial level H_2 production of 20 kg/1000 m^2 /day was estimated through the utilization of DP-H_2 production. The cost of producing DP-H_2 was estimated to be 2.80/kg USD (6). It is suggested that the commercial price of H_2 production should be equivalent that 1 kg H_2 production = 3.785 kg of gasoline, so the cost of the generated DP-H_2 price is matching with gasoline price (6). Table 2 summarize the active parameter of TEA for both IP-H_2 and DP-H_2 as comparison.

Table 2. The comparison of techno-economic analysis TEA between indirect and direct of *biophotolysis*

Active Parameters	IP-H_2	DP-H_2	Ref.
Cost of production	\$1220 per Gj/year	\$7.24/kg	(41)
preliminary cost	140 ha	≤100 ha	(6)
Capital cost	90%	80%	(14)
operating costs	12 million USD	10% (50/m^2 USD)	[3](40)
plant produces	1200 TJ/year	-	(40)
H2 production cost was estimated	10 USD per unit GJ	15/GJ USD	(14)
commercial scale H2 production	(243 mL L^{-1})	(11.65 mL L^{-1})	(42, 43)

5. Hybrid hydrogen production systems

Recently there is an increasing trend on building hybrid systems to produce BioH_2. The advanced research is based on two point strategy: (i) to overcome the drawbacks of traditional methods and to use the advantages and unique features of the available methods to enhance the yield and reduce the cost of BioH_2 (30). Combining PF with DF might be a good strategy for improving H_2 yield by utilizing microalgal biomass(14). Therefore, to combine DF with PF can be a good methodology to improve the yield of BioH_2 by using microalgal biomass (44). The integration of PF-DF-H_2 gives increased yield compared to the single-stage method, but very few studies were published showing the PF-DF-H_2 by using microalgae biomass (44). The combined generation of PF-DF-H_2 with Bio-CH_4 is well known. The study examined three stages of process by PF, DF, and methanogenesis using the biomass obtained from *C. pyrenoidosa*. While the biomass pretreatment was achieved by steam heating with diluted acid (44). Furthermore, One of the hybrid systems to produce hydrogen has the combination of photofermentation and biophotolysis, and both of the processes utilize the organisms that work with light to produce H_2 (10). If the required carbon can be retrieved from water, CO_2, and nutrients at reduced cost, the economy of producing BioH_2 can be considerably improvised (45). The hybrid photoheterotrophic degrading system supplemented with dark-fermentation is believed to have lower CAPEX and OPEX as compared to highly efficient conventional systems (30). Furthermore if wastewater is used as a source of CO_2, nutrients and water in the microalgal degradation of dark fermentation it will considerably lower the cost of the process (46) . Figure 2 shows the summery of PF-DF-H2 and DP-PF-DF-H_2 hybrid systems to produce BioH_2 from microalgae cultivation with the requirements of producing BioH_2 such as CO_2, nutrient, and sunlight.

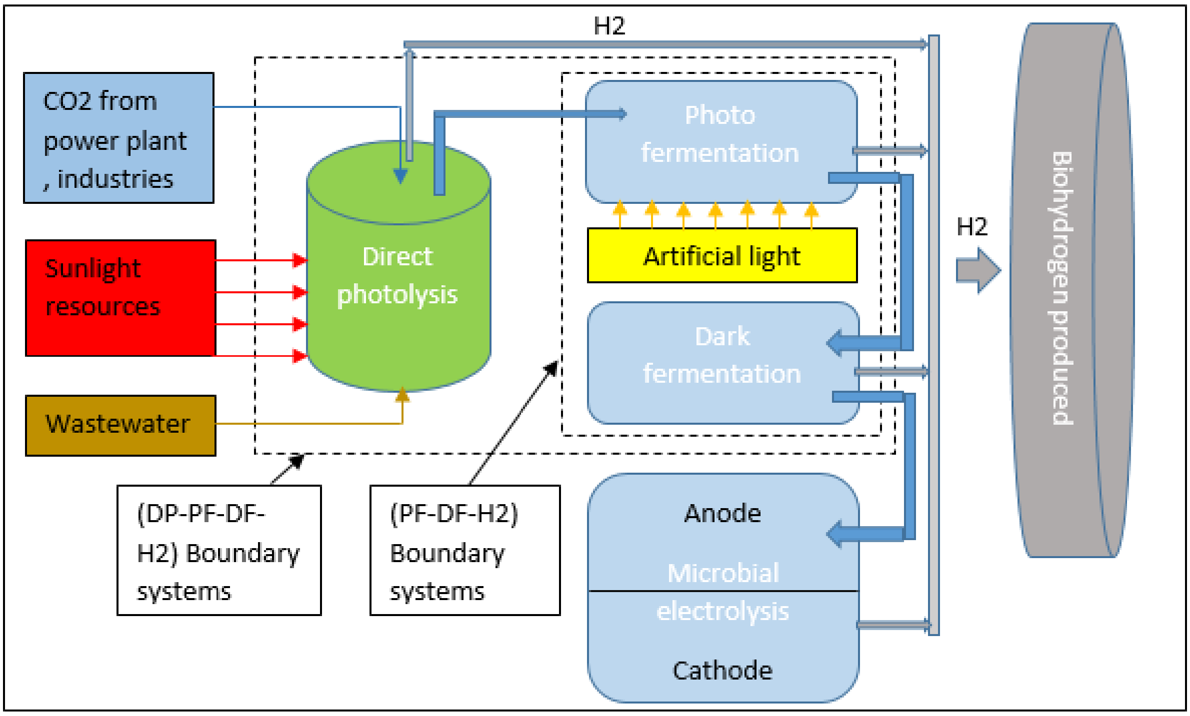

Figure 2. Hybrid integrated system bioreactor approach for biohydrogen production from microalgae

Microbial electrolysis cells are defined as bioelectrochemical systems (BESs) that apply a voltage to drive the bioelectrochemical reactions to the cells (47). During the process of microbial electrolysis cell (MEC), organic compounds undergo catalytic oxidation at the anode, while the hydrogen gets chemically evolved at the cathode. Therefore the production is directly proportional to the hydrogen production anaerobically (48). Hydrogen production in MFC like reactor is facilitated by the exoelectrogenic bacteria as they assist in the release of electrons. The process is termed as electrohydrogenesis because the release of electrons is made possible by the bacteria but it is simultaneously done with protons forming hydrogen gas, instead of electricity as in the case of MFC (49).

6. Conclusion

Production and storage of hydrogen have gained interest significantly these days. Hydrogen is regarded as one of the cleanest renewable fuels because water vapour is the only by-product during its combustion. Biohydrogen is the alternative production of H_2 that can be produced from microalgae. Production of $BioH_2$ from microalgae cultivation is more environmentally friendly and safe. This paper summarises the different types of processes to obtain $BioH_2$ from microalgae, such as direct and indirect photolysis, dark fermentation, and photo fermentation. However, biohydrogen production has many limitations: low productivity, feasibility at large scale, and high CAPEX and OPEX. Techno-economic analysis (TEA) mainly contribute to producing $BioH_2$ with more feasible to be implemented and invested. Furthermore, hybrid systems of $BioH_2$ production are considered one of the best solutions to reduce the cost of production and increase the productivity of $BioH_2$.

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