H_2 Production from Prokaryotic Algae Namely *Synechocystis sp.*, *Spirulina platensis*, and *Anabaena variabilis* under Direct Photolysis and Indirect Biophotolysis

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Abstract: - This study explores the potential of biohydrogen production from some prokaryotic Algae (*Synechocystis sp*., *Spirulina platensis*, and *Anabaena variabilis*) under some environmental factors the influencing microalgal hydrogen (H_2) production, determining the H_2 production yields. The main environmental conditions affecting H_2 production efficiency include the C/N ratio, pH, illumination intensity, incubation temperature, algae numbers and duration of H_2 production. The effect of C/N ratio (0.1, 0.2, 0.4, 0.8 and 1.0), pH (6.5, 7.0, 7.5, 8.0, 8.5 and 9.0), illumination intensity (120, 168, 180, 216 and 250 μmol/m^2.s), incubation temperature (20, 25, 30, 35 and 40^{o}C), algae numbers and duration of H_2 production (20, 40, 80, 100 and 120 hours), respectively, were examinec for the H_2 production yields with prokaryotic Algae namely *Synechocystis sp., Spirulina platensis,* and *Anabaena variabilis* under direct photolysis and indirect biophotoylsis processes.Algae cultivation is crucial for biohydrogen production. Maximum 99.40% H_2 production efficiency was measured at 0.1 C/N ratio, at pH=7.5, at 168 μmol/m^2.s illumination intensity, at 30^{o}C incubation temperature, at 35 EMS/100 ml *Synechocystis sp*., algae counts, after 100 h duration of H_2 production, respectively, with *Synechocystis sp*., prokaryotic algae under direct photolysis process. Maximum 99.54% H_2 production yield was obtained at 0.1 C/N ratio, at pH=9.0, at 180 mol/m^2.s, at 35^{o}C, at 88 EMS/100 ml *Spirulina platensis* algae counts, after 100 h, respectively, with *Spirulina platensis* prokaryotic algae under direct photolysis process. Maximum 99.44% H_2 production efficiency was found at 0.1 C/N ratio, at pH=7.5, at 216 mol/m^2.s, at 30^{o}C, at 172 EMS/100 ml *Anabaena variabilis* algae counts, after 40 h, respectively, with *Anabaena variabilis* prokaryotic algae under direct photolysis process. H_2 production yields in direct photolysis are higher than those in indirect biophotolysis. The responsible enzyme for H_2 evolution is a reversible hydrogenase because it catalyzes the reaction in both directions. The reversible hydrogenase and nitrogenase, however, are sensitive to the O_2 evolved in biophotolysis and promptly deactivated at quite low O_2 partial pressures (< 2%v/v), which results in a transient H_2 evolution. This intrinsic incompatibility of biophotolysis is a major barrier for sustained H_2 evolution. This is because direct energy is converted into H_2 energy in direct photolysis. Energy losses in direct photolysis are negligible compared to indirect biophotolysis. Therefore, H_2 production yields in direct photolysis are much higher. Additionally, the study conducts a Technical Economic Analysis (TEA) to evaluate the economic feasibility.

Key-Words: - Algae cultivation; *Anabaena variabilis*; Direct photolysis; H_2 production; Illumination intensity; Indirect biophotolysis; Prokaryotic algae; *Synechocystis sp*.; *Spirulina platensis*, Technical economic analysis (TEA).

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1 Introduction

Growing concerns about global warming and the limited amount of available fossil energy have increased the need to shift the energy production towards renewable sources. Hydrogen (H_2) is extensively proposed as a future source of alternative energy. Some species of microalgae are currently being investigated as potential sources of bioenergy and biofuels such as H_2, [1], [2], [3], [4], [5].

The limited source of fossil fuels and the simultaneous increase of their price along with the growing demand for energy sources, the existence of an alternative and environmentally friendly energy source is required to overcome the challenges of using fossil fuels in terms of waste issues and climate changes, [6],

[7]. Different types of renewable energy sources, such as wind energy, solar, biomass, and geothermal, can be considered a proper substitute, and geothermal can be regarded as an appropriate substitute for fossil fuels, [8], [9]. However, between all different types of renewable energy sources, the potential of utilization of biomass for H_2 production as one of the significant sustainable energy sources is unquestionable, [10], [11]. That is due to two reasons: first, the availability and diversity of the biomass in terms of type and conversion reaction routes, and second, the unique characteristics of H_2 as an energy (i.e., zero carbon emission and possessing the highest amount of energy per weight unit, [12]. Algae, among all various types of biomasses, is believed to be a promising source for H_2 generation mainly because of its straightforward cellular structure, fixing carbon dioxide (CO_2) by utilizing for their growth, fast growth rate, and ease of cultivation methods, [13], [14]. Due to the awareness of consumers about the numerous benefits and unique characteristics of algae, the microalgae market has been growing at a Compound Annual Growth Rate (CAGR) of 5.4%, which was valued at 3.78 billion in 2017 and predicted to reach 5.17 billion in 2023, [15].

On the other hand, due to the versatile application of H_2, an almost 6–10% increase in its utilization has been reported, with approximately 55 million tons generation per year, [16]. As of 2024, and according to H_2 Insights 2024 the market size has surpassed USD 6.49 billion and is anticipated to expand at a CAGR of more than 31% from 2024 to 2032. By 2030, global green H_2 deployment is expected to reach 150 GW, equating to roughly 63,750 tons/day. The cost for green H_2 production in 2020 was approximately 6 USD/kg based on the international hydrogen-promoting organization, the Hydrogen Council, [17].

Low-carbon hydrogen has emerged as a promising alternative among all sources of clean energy, [18]. Biohydrogen production methods have received attention owing to their potential to reduce reliance on fossil fuels and electricity compared with traditional H_2 production methods. Additionally, microalgae-based photosynthetic H_2 production does not release CO_2 into the environment. Instead, this photosynthetic H_2 production method captures CO_2 during the production process, making it an environmentally friendly method, [19]. Although, both prokaryotic and eukaryotic microalgae can produce H_2 through photosynthesis, their H_2 production processes and mechanism vary. These distinctions inevitably lead to the fact that the enhanced H_2 production strategies to prokaryotic and eukaryotic microalgae are significantly different. However, current reviews have paid little attention to the difference between prokaryotic and eukaryotic microalgae in H_2 production.

Microalgae can produce H_2 through various thermochemical or biological approaches, [20]. The thermochemical process possessed many advantages, including the capability of working under different types of feedstocks, [21], fast reaction, conversion rate, and high energy efficiency. It is worth mentioning that the yield and composition of the desired products can be significantly influenced by operating conditions such as temperature, pressure, concentration of feedstock, etc. Thus, specifying the optimum operating condition is paramount, [22]. On the other hand, biological approaches occur under ambient temperature and pressure. Hence, this reduces the final cost of the operation and provides an area for large-scale production, [23]. Furthermore, CO_2 can be fixed during the process, which is another merit of the biological approach. Nevertheless, the reaction takes longer than the thermochemical process and is highly dependent on the type of microorganisms, [24], [25].

The simplest most sustainable and efficient way to produce H_2 with microalgae is the so-called direct biophotolysis, which involves direct transfer of electrons from H_2O to the hydrogenase. However, until to date H_2 production of significant amounts of H_2 from direct biophotolysis is strongly limited by the O_2 sensitivity of hydrogenase which is the most challenging barrier to overcome, [26], [27]. Oxygen acts as a transcriptional repressor, an inhibitor of hydrogenase maturation, and an irreversible inhibitor of hydrogenase catalytic activity, [28], [29], [30].

Synechocystis sp. PCC6803 is a strain of unicellular, freshwater cyanobacteria. *Synechocystis sp.* PCC6803 image was given at Fig. 1.

* Figure 1 can be found in the Appendix section.

Synechocystis sp. PCC6803 is capable of both phototrophic growth by oxygenic photosynthesis during light periods and heterotrophic growth by glycolysis and oxidative phosphorylation during dark periods, [31]. Gene expression is regulated by a circadian clock and the organism can effectively anticipate transitions between the light and dark phases, [32]. Their observations that slr0388 (identified a hypothetical protein, encoded by the ORF slr0388 in CyanoBase, [33]), is involved in both phototactic motility and natural transformation provide further evidence that these two processes are intrinsically linked in *Synechocystis sp*. strain PCC 6803, [34]. Apart from the molecular machinery of the type IV pili (Tfp) genetic complement, the regulation of phototaxis and natural transformation is likely to involve other common genes that indirectly influence the biogenesis of the Tfp apparatus, as observed with slr0388 in this study. To that end, the identification of other factors specific only to the regulation of natural transformation should facilitate the elucidation of pathways that distinguish this process from phototactic motility, [34]. *Synechocystis sp*. strain PCC 6803 is one of the best-studied strains that can grow in the dark using glucose as the sole carbon source; however, it requires short, regular exposure to light for heterotrophic growth, [35].

Spirulina platensis is a filamentous, gram-negative cyanobacterium, [36]. *Spirulina platensis* image was shown at Fig. 2.

* Figure 2 can be found in the Appendix section.

This bacterium is non-nitrogen-fixing photoautotroph, [37]. *Spirulina platensis* is filamentous, motile bacterium. Motility has been described as a vigorous gliding without a visible flagella, [37]. As a photoautotroph the major carbon source is CO_2 and H_2O are a source of electrons to perform CO_2 reduction. *Spirulina platensis* has been found in environments with high concentrations of carbonate and bicarbonate. *Spirulina platensis* can also be found in high salt concentrations because of its alkali and salt tolerance. The temperature optimum for this organism is around 35°C, [38]. Based on environmental conditions, culture medium often has a pH between 9.0–10.0, inorganic salts, and a high bicarbonate concentration, [39].

Arthrospira is a representative filamentous non-N_2 fixing cyanobacterium that lacks any differentiation such as for the heterocyst, akinete or hormogonium, which develops in some filamentous N_2-fixing cyanobacteria. This cyanobacterium is also well known as '*Spirulina'* because of its useful property as a food. However, current taxonomy claims that the name '*Spirulina*' for strains used as food supplements is inappropriate, and there is agreement that *Arthrospira* is a distinct genus, [40], consisting of over 30 different species including *A. platensis* and *A. maxima*. *Arthrospira platensis* shows vigorous gliding motility of filamentous cells (trichomes) with rotation along their long axis. Gliding is a self-propulsion across a solid or semi-solid material without the aid of any visible flagellum, [41]; however, the mechanism of gliding motility is not fully understood. This organism also possesses ecologically very valuable characteristics such as alkali and salt tolerance and algal mat production on the periphery of lakes. *Arthrospira platensis* is also able to grow under high salt concentrations of 1.5-fold higher than sea water, [42]. Accordingly, it often dominates in lakes with high carbonate/bicarbonate levels and high pH levels, [43].

Arthrospira platensis has become an important industrial organic material as a health supplement, a source of beta-carotene and a natural coloring agent. It has been approved for treating symptoms of radiation sickness after the Chernobyl disaster in Russia, [44]. The presence of hydrogenase in its cells also makes this *Cyanobacterium* a useful material for clean energy production, [45]. Despite its various useful applications, very little is known about the biology, physiology and genetic system of *A. platensis*. For production of useful products, gene manipulation through genetic engineering should be considered. However, genetic transformation of *Arthrospira* has had limited success to date, [46], and thus commercial use of this organism has faced barriers due to difficulties in gene manipulation. To overcome these barriers, restriction-modification (RM) systems based on its genome sequence may prove useful.

Anabaena variabilis is a species of filamentous cyanobacterium. *Anabaena variabilis* prokaryotic algae was illustrated at Fig. 3.

* Figure 3 can be found in the Appendix section.

This species of the genus *Anabaena* and the domain *Eubacteria* is capable of photosynthesis. This species, though photoautotrophic like other cyanobacteria, can also be heterotrophic, meaning that it may grow without light in the presence of fructose, [47]. *Anabaena variabilis* also can convert atmospheric dinitrogen to NH_3 via N_2 fixation. *Anabaena variabilis* is a phylogenic-cousin of the more well-known species *Nostoc spirrilum*. Both of these species along with many other cyanobacteria are known to form symbiotic relationships with plants. Other cyanobacteria are known to form symbiotic relationships with diatoms, though no such relationship has been observed with *Anabaena variabilis*. *Anabaena variabilis* is also a model organism for studying the beginnings of multicellular life due to its filamentous characterization and cellular-differentiation capabilities, [48].

Two well-studied filamentous heterocyst-forming cyanobacterial strains, namely, *Nostoc punctiforme* ATCC 29133 and *Anabaena variabilis* ATCC 29413, are capable of true heterotrophic growth in complete darkness, [49], [50]. The former strain grows on glucose or fructose, while *A. variabilis* ATCC 29413 can use only fructose, [49]. In these two heterocyst-forming strains, sugars support not only growth in the dark but also nitrogen fixation, an energetically expensive reaction. *A. variabilis* is not known to be an endosymbiont; however, by morphology, phenotype, and genetics, it is virtually identical to many strains called *Anabaena azollae*, isolated from the symbiotic association of cyanobacteria with the H_2O fern Azolla, [51]. Fructose dramatically affects the physiology of *A. variabilis*. The cells grow faster, are bigger, and in filaments that have differentiated heterocysts, produce more and larger heterocysts, fixing more N_2 and producing more H_2 than do cells grown photo-autotrophically, [52]. [^{14}C] fructose, which is taken up almost immediately by vegetative cells in a filament, is quickly transported in some form to the heterocysts, where the ^{14}C compound accumulates and is metabolized to provide a reductant for N_2 fixation, [49]. Although, fructose supports nitrogen fixation in whole filaments, isolated heterocysts cannot use fructose as a source of reductant, suggesting either that fructose cannot be transported by heterocysts or that fructose is converted to another compound in the vegetative cell before it moves to the heterocyst, [53]. Growth with fructose results in increased respiration and decreased chlorophyll, [54]. In long-term, dark grown, fructose-adapted cells, there is an increase in photosystem II (PS II), resulting in a decrease in the ratio of photosystem I (PS I) to PS II, [47]. Cells grown with low CO_2 in the presence of fructose do not fix CO_2 well because of decreased carbonic anhydrase and decreased ribulose bis-phosphate carboxylase oxygenase, [55]. The decrease in O_2 production in fructose grown cells is thought to contribute to a micro-oxic environment that better supports N_2 fixation, [49]. Microarray analysis of Ribonucleic acid (RNA) from the non-N_2-fixing unicellular cyanobacterium *Synechocystis sp.* strain PCC 6803 under conditions of N_2 starvation shows increased expression of genes important in glycolysis, the oxidative pentose phosphate pathway, and glycogen catabolism and increased activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, two key enzymes of the oxidative pentose phosphate pathway, [56]. Uptake of fructose in *A. variabilis* and *Nostoc sp.* strain ATCC 29150 is constitutive but increases after exposure to fructose, [57], and is energy dependent in *A. variabilis,* [58].

The C/N ratio affects the level of microalgal NAD(P)H or plastoquinone (PQ) oxidation, which influences the expression of hydA genes, [59]. The pH level regulates the activity of hydrogen-producing enzymes, such as nitrogenase and hydrogenase, [60]. Illumination intensity significantly influences the transfer of electrons and the synthesis of Adenosine Triphosphate (ATP) and Nicotinamide Adenine Dinucleotide Phosphate [NAD(P)H]. As the illumination intensity exceeds the light saturation point, it inhibits the production of H_2 in microalgae, [61].

It's important to note that understanding the kinetics and fundamental reaction pathways is crucial for designing practical reactors, conducting lifecycle assessments, and performing techno-economic analyses. However, these areas are often overlooked due to their complex mechanics. To gain more insight into the phenomena and thermodynamics involved, it's helpful to model and optimize different processes, whether they are thermochemical or biological. Doing so makes it possible to predict how a process will behave on a lab and pilot scale before conducting actual experiments. On the other hand, the process economy is one of the most vital evaluation tools for any process scaling. To perform economic analysis of the entire algae to H_2 system, analysis and calculation of both mass and energy balances are needed. The simulation data will study the overall technical possibility and provide a technical and economic analysis (TEA) of the process's life cycle. The primary goal of TEA is to define and calculate the baseline of capital expenditures (CAPEX), [62], and cost expenditures (OPEX) of the whole integrated biorefinery. The economic analysis showed that the \$6–15/kg-H_2 provided allows market leverage to be a power system. If the proposed design works, the production cost will go below \$2/kg-H_2 due to higher revenues generated from the higher production rate of H_2. It will calculate the by-products' revenues (minerals rich in phosphorus and N_2 as fertilizer). Successful recovery of nutritious products would bring a gain of \$0.1/kg-H_2 produced, [63]. The TEA will be based on a specific and overall mass and energy balance (MEB) calculation for the whole process, from receiving wastewater, mixing food products, chamber operation, fuel cell, electricity transformation, and by-product recovery.

In this study, the potential of biohydrogen production from some prokaryotic Algae Namely *Synechocystis sp*., *Spirulina platensis*, and *Anabaena variabilis* under direct photolysis and indirect biophotolysis during some environmental factors the influencing microalgal H_2 production, determining the H_2 production yields. The main environmental conditions affecting H_2 production efficiency include the increasing C/N ratio (0.1, 0.2, 0.4, 0.8 and 1.0), pH (6.5, 7.0, 7.5, 8.0, 8.5 and 9.0), illumination intensity (120, 168, 180, 216 and 250 μmol/m^2.s), incubation temperature (20, 25, 30, 35 and 40°C), algae numbers and duration of hydrogen production (20, 40, 80, 100 and 120 hours), respectively. In addition to, the study conducts a Technical Economic Analysis (TEA) to evaluate the economic feasibility.

2 Materials and Methods

2.1 Bacterial Strains and Culturing Mediums

The positively phototactic strain of *Synechocystis sp.* strain PCC 6803 was purchased from Sigma-Aldrich, (Germany). Cells were grown either in BG-11 liquid medium, [64], or on BGTS agar (1%, w/v) plates consisting of BG-11 supplemented with 10 mM TES (pH=8.2 with KOH) and 0.3% (w/v) sodium thiosulfate, [Williams, 1988]. Where appropriate, antibiotics were added (7 µg chloramphenicol/ml, 5 µg kanamycin/ml or 5 µg spectinomycin/ml). Cultures were grown without shaking under continuous light (25 µmol photons/m^2.s) supplied by cool white fluorescent lamps at 28±1°C.

Arthrospira (Spirulina) platensis strain NIES-39 was obtained from Sigma-Aldrich, (Germany). The cells were grown in the SOT medium, [65], at 30ºC under continuous illumination at 30 μmol photon/m^2.s with aeration with 1% (v/v) CO_2.

Anabaena variabilis was purchased from Sigma-Aldrich, Germany. Strains of *A. variabilis* FD, a derivative of *A. variabilis* 29413 that can grow at 40°C and supports the growth of bacteriophages better than the parent strain does, [66], and *Anabaena sp.* strain PCC 7120 were maintained on agar-solidified Allen and Arnon (AA) medium, [67], supplemented, when appropriate, with 5 mM NH_4Cl, 10 mM N-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), pH 7.2, 25 to 40 μg/ml neomycin sulfate, or 3 μg/ml 1each of spectinomycin and streptomycin. Strains were grown photo-autotrophically in liquid cultures in an eightfold dilution of AA medium (AA/8) or in AA/8 supplemented with 5 mM NH_4Cl and 10 mM TES, pH 7.2, at 30°C, with illumination at 50 to 80 microeinsteins/m^2.s. Antibiotics were included as follows (when required): neomycin (3 to 5 μg/ml) and spectinomycin (0.3 μg/ml for liquid). For experiments to measure the growth of strains with fructose, cells were harvested at an optical density at 720 nm (OD_{720}) of 0.2, washed once in AA/8, and resuspended in AA/8 with the indicated concentrations of fructose at an OD_{720} of 0.02.

2.2 Photosynthetic H_2 Production by Microalgae

The studies on prokaryotic microalgae have mainly focused on three types involving *Synechocystis sp.*, *Spirulina platensis*, and *Anabaena variabilis*. Actually, there are advantages to utilizing both eukaryotic and prokaryotic microalgae for H_2 production. On the one hand, most hydrogen-producing cyanobacteria exhibit a simple cell structure. Pigments and enzymes involved in photosynthesis are directly distributed in the chromatin. This makes prokaryotic microalgae more sensitive to H_2 production reactions under anaerobic conditions, [68]. On the other hand, numerous chloroplasts were distributed in eukaryotic microalgae, and the site of photosynthetic H_2 production was mainly concentrated on the thylakoid membrane of chloroplasts, which meant that eukaryotic microalgae had a large H_2 production rate under hydrogen-producing conditions, [69].

2.3 The Electron Transfer Process of Prokaryotic Microalgae

During the process of cyclic photosynthetic electron transport, electrons pass through a structure that contains cytochrome, resulting in the generation of a gradient of protons. This proton gradient is subsequently utilized by ATP synthase to produce ATP, which is essential for the processes of N_2 fixation and H_2 synthesis. Externally derived organic matter is processed through metabolic pathways of organic carbon such as the glycolysis (EMP) and the tricarboxylic acid cycle (TCA) to yield protons, electrons, and CO_2. Electrons flow in a counter-current manner to ferredoxin (Fd), which in turn transfers electrons to nitrogenase to produce H^+ and e^-. Finally, electrons are transferred to nitrogenase, which results in ammonia (NH_3) and H_2 formation. In essence, to obtain substantial amounts of H_2, the quantity of N_2 and ammonium (NH_4^+) should be restricted, [70]. Additionally, prokaryotic algae accumulate excess protons and electrons, which are converted to H_2 and released.

2.4 Production of Hydrogen

Prokaryotic Algae cells were transferred in a 1 liter Pyrex Roux-type bottle photobioreactor (PBR) (5 cm light path) with a flat cross section (12 x 5 cm width), a flat bottom, and five ports. The PBR was fitted with three probes for the continuous monitoring of pH, redox potential and dissolved O_2 concentration. The main port at the top (2.5 cm I.D.) was sealed with a stopper equipped with tygon tubes that connected the PBR headspace to the gas-to-liquid conversion bottle. The illumination level of a photon-flux density (PFD) of 160 μmol photons/m^2.s was provided by cool white fluorescent lamps (Dulux L, 55W/840, Osram, Italy). Mixing of the culture was provided by a rotating impeller driven magnetically from the bottom of the reactor, [71].

2.5 Analytical Methods

The PFD value at the culture surface was measured with a quantum radio-photometer (LI-250A, Li-Cor Biosciences, Nebraska, USA) equipped with a cosine-corrected sensor. Culture parameters, such as pH, temperature, redox potential, dissolved O_2 concentration and H_2 gas production were monitored as described elsewhere, [71]. Culture's temperature was maintained at 26.0 ± 0.2ºC. The quantity of gas produced was monitored continuously according to Kosourov et al. [72]. Samples of the gas mixture produced were analyzed with a thermal conductivity detector (TCD) equipped gas chromatograph (model Clarus 500, PerkinElmer, Waltham, Massachusetts), with an at the following operating conditions: isothermal program at 35ºC for 2.25 min; N_2 carrier gas flow 30 ml/min; injection temperature 150ºC; detector temperature 150ºC. A packed column (model Carbosieve S-II Spherical Carbon, Supelco) was used. Calibration of H_2 was performed by injecting known amounts of pure gas.

In vitro hydrogenase activity was determined as described in Hemschemeier et al. [73]. The reaction mixture of this assay contains Triton-X 100 (a mild detergent which lyses the algal cells), methyl viologen as an artificial electron donor and sodium dithionite as an efficient reductant for methyl viologen. The hydrogenase activity of whole cells is defined as nmoles H_2 produced per hour and per µg chlorophyll.

2.6 Direct Photolysis Mechanism

One of the biological processes for H_2 production is direct photolysis. Within this pathway, H_2 will be generated from H_2O via two reactions, [74], [75], [76]. Splitting of H_2O molecules to H_2 protons, O_2, and electrons with the aid of Photosystem II (PS II) proteins as following equation (Eq. 1):

$$2H_2O \rightarrow 4H^+ + O_2 + 4e^- \quad (1)$$

Formation of H_2 from the combination of H_2 protons with electrons, which will be catalyzed by the iron-hydrogenase enzymes found in the microalgae as following equation (Eq. 2):

$$2H^+ + 2e^- \rightarrow H_2 \quad (2)$$

One of the main challenges via direct photolysis is the sensitivity of the iron-hydrogenase enzymes to O_2, a product of the photosynthesis process. To mitigate O_2 sensitivity, the algae growth medium could be purged with N_2. The performance of the enzymes will be hampered, and H_2 production will drop over time, [77]. Therefore, suppressing O_2 and creating an anaerobic environment is necessary. Anaerobiosis is applicable mostly via sulfur deprivation, [78], and through nutrients that control photosynthetic activity, [79].

The supercritical water gasification (SCWG) method is reported to be the most efficient method for H_2 production. However, tar formation and the requirement for the H_2 purification step hinder their widespread application, [80]. Therefore, direct photolysis, as biological pathways that occur at ambient temperature and pressure, can be assumed as an alternative method to alleviate the challenges of the SCWG method. Nevertheless, one of the significant obstacles via direct photolysis is the presence of O_2, which hinders H_2 production, [6].

Comparisons of the general advantages and disadvantages of H_2 production by direct photolysis and indirect biophotolysis processes from algal microbes are summarized in Table 1.

* Table 1 can be found in the Appendix section.

2.7 Indirect Biophotolysis Mechanism

Indirect biophotolysis is another route in biological pathway. Solar energy can be converted from H_2O to H_2 as chemical energy through different steps with the aid of microalgae photosynthesis, [81]. Different steps can be summarized as, [82]: Production of biomass by photosynthesis, Biomass growth, Production of 4 moles of H_2 per mol of glucose that stored in the algal cell through dark fermentation process, [83], along with the 2 moles of acetate and conversion of acetate to H_2.

The procedure consists of three reactions, two light dependent and one independent of light as described below, [84], [85].

First stage- aerobic equation as shown in Eq. 3:

$$6H_2O + 6CO_2 + light \rightarrow C_6H_{12}O_6 + 6O_2 \quad (3)$$

Second stage-anaerobic equations determined as following in Eq. 4 and Eq. 5:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2 \quad (4)$$

$$2CH_3COOH + 4H_2O + light \rightarrow 8H_2 + 4CO_2 \quad (5)$$

The overall reaction equation shown as following Eq. 6:

$$12H_2O + light \rightarrow 12H_2 + 6O_2 \quad (6)$$

Another alternative method to overcome the disadvantages of direct biophotolysis, the existence of O_2, is the indirect biophotolysis method. To evaluate H_2 production from microalgae *Scenedesmus sp.* via indirect biophotolysis process, a transient mathematical model was conducted inside the well-stirred photobioreactor within the Vargas et al. [86], work in pilot scale. The process is comprised of two different stages: the aerobic stage, where the algae growth section is, and the anaerobic one, where H_2 production takes place due to the lack of O_2 by cutting off the air supply. Differential mass balance equations were considered to determine all mass fraction distributions for specious and system temperature. Later, to minimize the computational time, all sets of equations were discretized using a three-dimensional cell-centered finite volume scheme, vibrationally excited molecules (VEM), where the required largest time through the simulation process was reported as less than 10 min. The proposed model consists of four subsystems (i.e., reservoir, bundle of transparent tubes, pump, and opaque tubes) where algae growth occurs inside the transparent tubes due to light radiation. Runge–Kutta–Fehlberg method was used for numerical solutions by coding in Fortran software. The model was validated with experimental data for the growth step. However, more validation is required, especially during the H_2 production step. Based on the results, as expected, the algae mass fraction within the aerobic section (10 days) will increase till the introduction of the anaerobic section, where it drops by 50% within later 8 days. On the other hand, for the H_2 mass fraction, no significant H_2 generation was observed at the initial stage.

Nevertheless, after 10 days, H_2 production begins, [86]. It is worthwhile to mention the highest mass fraction of H_2 can reach 8×10^{-7}, which is very low.

Table 1 provides a comparison of the general advantages and disadvantages of direct photolysis and indirect biophotolysis processes in H_2 production from algal microbes.

3 Results and Discussions

3.1 Effect of C/N Ratio

To measure the H_2 production efficiencies of the prokaryotic algae *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis*, different C/N ratios (0.1, 0.2, 0.4, 0.8 and 1.0) were applied under direct photolysis process, and the efficiency results are given in Table 2.

* Table 2 can be found in the Appendix section.

In *Synechocystis sp*., prokaryotic algae subjected to direct photolysis, H_2 production efficiencies ranging from 51.29% to 87.80% were measured at C/N ratios increasing from 0.2 to 1.0 (Table 2). When the C/N ratio increased from 0.2 to 1.0, H_2 production efficiencies decreased from 87.80% to 51.29% (Table 2). Maximum 99.40% H_2 production efficiency was found at 0.1 C/N ratio, with *Synechocystis sp*., prokaryotic algae under direct photolysis process (Table 2).

In *Spirulina platensis* prokaryotic algae that underwent direct photolysis, H_2 production efficiencies varied between 55.47% and 86.15% when C/N ratios varied between 0.2 and 1.0 (Table 2). It was observed that when the C/N ratio increased from 0.2 to 1.0, H_2 production efficiencies decreased from 86.15% to 55.47% (Table 2). Maximum 99.54% H_2 production efficiency was obtained at 0.1 C/N ratio, with *Spirulina platensis* prokaryotic algae under direct photolysis process (Table 2).

H_2 production efficiencies in the range of 61.20%-93.46% were measured at C/N ratios varying from 0.2 to 1.0 in the prokaryotic alga *Anabaena variabilis* under direct photolysis process (Table 2). With the increase in C/N ratios from 0.2 to 1.0, H_2 production efficiencies decreased from 93.46% to 61.20% (Table 2). 99.44% maximum H_2 production yield was found at 0.1 C/N ratio with *Anabaena variabilis* prokaryotic algae under direct photolysis process (Table 2).

In order to measure the H_2 production efficiency of prokaryotic algae *Synechocystis sp., Spirulina platensis* and *Anabaena variabilis*, different C/N ratios (0.1, 0.2, 0.4, 0.8 and 1.0) were applied under indirect biophotolysis process and the results are summarized in Table 3.

* Table 3 can be found in the Appendix section.

In *Synechocystis sp.* prokaryotic algae, H_2 production efficiencies in the C/N ratio range from 0.2 to 1.0 under indirect biophotolysis process vary between 40.17% and 75.68% (Table 3). When the C/N ratio increased from 0.2 to 1.0, H_2 production efficiencies gradually decreased to 40.17% (Table 3). Maximum 89.47% H_2 production yield was measured at C/N ratio=0.1, with *Synechocystis sp*., prokaryotic algae under indirect biophotolysis process (Table 3).

Using the prokaryotic algae *Spirulina platensis*, H_2 production efficiencies ranging from 44.36% to 75.04% were measured under indirect biophotolysis at C/N ratios ranging from C/N=0.2 to C/N=1.0 (Table 3). As the C/N ratio increased from 0.2 to 1.0, H_2 production efficiencies gradually decreased to 44.36% (Table 3). Maximum 89.63% H_2 production efficiency was observed at C/N =0.1 ratio, with *Spirulina platensis* prokaryotic algae under indirect biophotolysis process (Table 3).

H_2 production efficiencies ranging from 50.08% to 82.33% were measured under the indirect biophotolysis process at C/N ratios between C/N=0.2 and C/N=1.0 in the prokaryotic alga *Anabaena variabilis* (Table 3). As the C/N ratio increased from 0.2 to 1.0, H_2 production efficiencies gradually decreased to 50.08% (Table 3). 88.20% maximum C/N=0.1 ratio, with *Anabaena variabilis* prokaryotic algae under indirect biophotolysis process (Table 3).

Biophotolysis is the action of light on biological systems that results in dissociation of water into molecular H_2 and O_2, $H_2O \rightarrow H_2 + ½ O$. Direct biophotolysis refers to sustained H_2 evolution under light irradiation. The light energy is absorbed by the pigments at photosynthetic system II (PSII), or photosynthetic system I (PSI) or both, which raises the energy level of electrons from water oxidation when they are transferred from PSII via PSI to ferredoxin. A portion of the light energy is directly stored in H_2 gas.

3.2 Effect of pH

In order to observe the H_2 production efficiencies in the prokaryotic algae *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis*, different pH values (6.5, 7.0, 7.5, 8.0, 8.5 and 9.0) were run under direct photolysis process and the results obtained are detailed in Table 4.

* Table 4 can be found in the Appendix section.

For *Synechocystis sp.,* prokaryotic algae under direct photolysis process, H_2 production efficiencies are in the range of 59.74%-85.47% in the range of pH=6.5 to pH=9.0 (Table 4). When pH=8.0 increased to pH=9.0, H_2 production efficiencies decreased from 85.47% to 59.74% (Table 4). Maximum 99.40% H_2 production efficiency was measured at pH=7.5, with *Synechocystis* *sp*., prokaryotic algae under direct photolysis process (Table 4).

In *Spirulina platensis* prokaryotic algae, under direct photolysis process, H_2 production efficiencies in the pH = 6.5 - pH = 8.5 range vary between 49.51% and 91.87% (Table 4). When pH=8.5 decreased to pH=6.5, H_2 production efficiencies decreased from 91.87% to 49.51% (Table 4). 99.54% maximum H_2 production yield was obtained for pH=9.0 with *Spirulina platensis* prokaryotic algae under direct photolysis process (Table 4).

H_2 production efficiencies ranging from 65.57% to 97.09% were measured under direct photolysis in the prokaryotic algae *Anabaena variabilis* at pH values ranging from pH=6.5 to pH=9.0 (Table 4). It was observed that as pH=8.0 increased to pH=9.0, H_2 production efficiencies decreased from 97.09% to 65.57% (Table 4). Maximum 99.44% H_2 production yield was found at pH=7.5 with *Anabaena variabilis* prokaryotic algae under direct photolysis process (Table 4).

In order to determine the H_2 production activities in prokaryotic algae *Synechocystis sp., Spirulina platensis* and *Anabaena variabilis*, indirect biophotolysis was applied at different pH values (6.5, 7.0, 7.5, 8.0, 8.5 and 9.0) and the results are shown in Table 5.

* Table 5 can be found in the Appendix section.

For *Synechocystis sp*., one of the prokaryotic algae under indirect biophotolysis process, H_2 production efficiencies vary between 42.58% and 76.35% in the range of pH=6.5 and pH=9.0 (Table 5). Between pH=8.0 and pH=9.0, H_2 production efficiencies decreased from 76.35% to 42.58% (Table 5). Maximum 89.47% H_2 production yield was found at pH=7.5, with *Synechocystis sp.,* prokaryotic algae under indirect biophotolysis process (Table 5).

In *Spirulina platensis* prokaryotic algae subjected to indirect biophotolysis, H_2 production efficiencies were measured between 40.37% and 80.75% when pH changed between pH = 6.5 and pH = 9.0 (Table 5). When the pH increased from pH=6.5 to pH=8.5, the H_2 production efficiency was observed to be 80.75% (Table 5). Maximum 89.63% H_2 production efficiency was obtained at pH=9.0, with *Spirulina platensis* prokaryotic algae under indirect biophotolysis process (Table 5).

H_2 production efficiencies ranging from 56.46% to 75.07% were recorded under indirect biophotolysis process in the prokaryotic alga *Anabaena variabilis* at pH values between pH=6.5 and pH=9.0. (Table 5). When pH increased from pH=8.0 to pH=9.0, H_2 production efficiencies decreased from 75.07% to 56.46% (Table 5). Maximum 88.20% H_2 production yield was measured at pH=7.5, with *Anabaena variabilis* prokaryotic algae under indirect biophotolysis process (Table 5).

The pH is the most vital parameter that affects the growth rate and determines the H_2 yield. However, the optimal pH value varies depending on the algae species. It has been documented that most algae species grow at pH between 4.0 and 7.0, [87], [88]. A pH value lower than 4.0 provides excess protons that affect cellular activities by protonating essential enzymes' active side. This, in turn, lowers the kinetic activities of the cellular enzymes, which might lead to algae death. A pH above 7.0 directly affects the metabolism pathways, leading to cell death. A higher pH value than 7.0 provides a higher concentration of OH^- ions, providing a hostile environment for the undesirable oxidation reaction. Zhang et al. [87] found that when algae are grown in CSTR at a pH range between 4.0 and 7.0, the optimal pH value is 5.5. Zhang et al. [87] discussed the highest H_2 production attained merely when metabolic reactions followed an ethanol fermentation type that occurred at a pH close to 4.5.

3.3 Effect of Illumination Intensity

In order to compare the H_2 production efficiencies in the prokaryotic algae *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis*, different illumination intensities (120, 168, 180, 216 and 250 μmol/m^2.s) were examined under direct photolysis process and the results including the H_2 production efficiencies obtained are given in Table 6.

* Table 6 can be found in the Appendix section.

Synechocystis sp. from prokaryotic algae. Under the direct photolysis process, H_2 production efficiencies ranging from 65.48% to 99.40% were measured in the range of illumination intensities of 120 to 250 μmol/m^2.s, respectively. (Table 6). It was noted that when illumination intensity values varied between 120-250 μmol/m^2.s, H_2 production efficiency decreased from 87.10% to 65.48% (Table 6). Maximum 99.40% H_2 production efficiency was found at 168 μmol/m^2.s illumination intensity, with *Synechocystis sp*., prokaryotic algae under direct photolysis process (Table 6).

Under the direct photolysis process, H_2 production efficiencies ranging from 75.50% to 99.54% were observed between *Spirulina Platensis* prokaryotic algae and illumination intensities of 120 - 250 μmol/m^2.s. (Table 6). When the illumination intensity value increased from 216 to 250 μmol/m^2.s, H^2 production efficiencies decreased from 96.12% to 88.05% (Table 6). Maximum 99.54% H_2 production yield was obtained for 180 mol/m^2.s illumination intensity with *Spirulina platensis* prokaryotic algae under direct photolysis process (Table 6).

Under the direct photolysis process with *Anabaena variabilis* prokaryotic algae, H_2 production efficiencies ranging from 61.37% to 99.44% were measured for illumination intensities from 120 to 250 µmol/m^2.s (Table 6). It was observed that H_2 production efficiencies increased from 61.37% to 94.51% as illumination intensity values increased from 120 to 250 µmol/m^2.s (Table 6). 99.44% maximum H_2 production yield was found for 216 mol/m^2.s illumination intensity with *Anabaena variabilis* prokaryotic algae under direct photolysis process (Table 6).

In order to compare the H_2 production efficiencies in the prokaryotic algae *Synechocystis sp.*, *Spirulinaplatensis* and *Anabaena variabilis*, different illumination intensities (120, 168, 180, 216 and 250 µmol/m^2.s) were examined by applying indirect biophotolysis and the results including the obtained H_2 production efficiencies are given in Table 7.

* Table 7 can be found in the Appendix section.

Synechocystis sp. In prokaryotic algae, under the indirect biophotolysis process, illumination intensity values vary between 120 and 250 µmol/m^2.s, while H_2 production efficiencies vary between 54.37% and 89.47%. (Table 7). While illumination intensity values varied between 180 and 250 µmol/m^2.s, H_2 production efficiency decreased from 76.88% to 54.37%. (Table 7). Maximum 89.47% H_2 production efficiency was obtained at 168 µmol/m^2.s illumination intensity with *Synechocystis sp*., prokaryotic algae under indirect biophotolysis process (Table 7).

Under the indirect biophotolysis process, H_2 production efficiencies of 64.39% and 89.63% were measured between *Spirulina platensis* prokaryotic algae and illumination intensities of 120 - 250 µmol/m^2.s. (Table 7). When illumination intensity values increased from 180 to 250 µmol/m^2.s, H_2 production efficiency decreased from 87.42% to 77.09% (Table 7). Maximum 89.63% H_2 production yield was found at 168 µmol/m^2.s illumination intensity with *Spirulina platensis* prokaryotic algae under indirect biophotolysis process (Table 7).

By indirect biophotolysis with the prokaryotic algae *Anabaena variabilis*, H_2 production efficiencies ranging from 50.26% to 88.20% were observed for illumination intensities between 120 and 250 µmol/m^2.s (Table 6). H_2 production efficiencies increasing from 50.26% to 72.06% were seen when the illumination intensity values increased from 120 µmol/m^2.s to 180 µmol/m^2.s (Table 7). Maximum 88.20% H_2 production efficiency was measured at 216 µmol/m^2.s illumination intensity with *Anabaena variabilis* prokart-yotic algae under indirect biophotolysis process (Table 7).

Under limited light-intensity conditions, microalgae photosynthesis achieves a photon conversion efficiency of about 80%, [89]. This indirect biophotolysis, therefore, consists of two stages in series: photosynthesis for carbohydrate accumulation, and dark fermentation of the carbon reserve for H_2 production. In this way, the O_2 and H_2 evolutions are temporally and/or spatially separated, [89]. This separation not only avoids the incompatibility of O_2 and H_2 evolution (e.g., enzyme deactivation and the explosive property of the gas mixture), but also makes H_2 purification relatively easy because CO_2 can be conveniently removed from the H_2/CO_2 mixture, [90]. It is proposed a process of H_2 production via indirect biophotolysis by using natural light/dark cycles, [90]. According to this proposal, CO_2 is reduced to starch by photosynthesis in daytime, and the starch thus formed, is fermented to H_2 gas and organic acids under anaerobic conditions during nighttime. The organic acids and other fermentative products can be further used for H_2 evolution by photosynthetic bacteria under light irradiation.

3.4 Effect of Incubation Temperature

In order to comparatively define the H_2 production efficiencies of the prokaryotic algae *Synechocystis sp.*, *Spirulina platensis* and *Anabaena variabilis*, different incubation temperatures (20, 25, 30, 35 and 40°C) were applied under direct photolysis process, and the results of H_2 production efficiencies at these different inhibition temperatures are summarized in Table 8.

* Table 8 can be found in the Appendix section.

Synechocystis sp. from prokaryotic algae under the direct photolysis process, H_2 production efficiencies ranging from 71.22% to 99.40% were obtained at incubation temperatures between 20°C and 40°C. (Table 8). It was observed that H_2 production efficiency decreased from 84.34% to 74.09% as the incubation temperature increased from 35°C to 40°C (Table 8). Maximum 99.40% H_2 production efficiency was measured at 30°C incubation temperature, with *Synechocystis sp*., prokaryotic algae under direct photolysis process (Table 8).

Under the direct photolysis process with the prokaryotic algae *Spirulina platensis*, it was measured that H_2 production efficiencies increased from 59.68% to 99.54% in response to the increase in incubation temperatures from 20°C to 40°C (Table 8). H_2 production efficiencies increased from 59.68% to 94.32% when incubation temperatures increased from 20°C to 30°C (Table 8). Maximum 99.54% H_2 production yield was found at 35°C incubation temperature, with *Spirulina platensis*, prokaryotic algae under direct photolysis process (Table 8).

H_2 production efficiency ranging from 77.82% to 99.44% was measured with *Anabaena variabilis* prokaryotic algae under direct photolysis process at incubation temperatures ranging from 20°C to 40°C. (Table 8). Incubation temperatures increased from 35°C to 40°C when H_2 production efficiencies dropped from 95.08% to 79.89% (Table 8). Maximum 99.44% H_2 production yield was measured for 30°C incubation temperature with *Anabaena variabilis* prokaryotic algae under direct photolysis process (Table 8).

In order to compare the H_2 production efficiencies of the prokaryotic algae *Synechocystis sp., Spirulinaplatensis* and *Anabaena variabilis*, different incubation temperatures (20, 25, 30, 35 and 40°C) were applied under the indirect biophotolysis process, and the results of the H_2 production efficiencies at these different inhibition temperatures are collected in Table 9.

* Table 9 can be found in the Appendix section.

Synechocystis sp. with the indirect biophotolysis process, H_2 production efficiencies ranging from 60.01% to 89.47% were measured from prokaryotic algae at incubation temperatures between 20°C and 40°C (Table 9). It was noted that when incubation temperatures increased from 35°C to 40°C, H_2 production efficiency decreased from 73.22% to 64.04% (Table 9). Maximum 89.47% H_2 production yield was obtained at 30°C incubation temperature, weith *Synechocystis sp.,* prokaryotic algae under indirect biophotolysis process (Table 9).

Under the indirect biophotolysis process with the prokaryotic alga *Spirulina platensis*, it was measured that H_2 production efficiencies increased from 48.56% to 89.63% in response to increasing incubation temperatures from 20°C to 40°C (Table 9). It was noted that when incubation temperatures increased from 20°C to 30°C, H_2 production efficiencies increased from 48.56% to 83.20% (Table 9). Maximum 89.63% H_2 production efficiency was found at 35°C incubation temperature with *Spirulina platensis* prokaryotic algae under indirect biophotolysis process (Table 9).

H_2 production efficiencies ranging from 68.75% to 88.20% were measured during the indirect biophotolysis process with the prokaryotic algae *Anabaena variabilis* at incubation temperatures ranging from 20°C to 40°C. (Table 9). When incubation temperatures increased from 35°C to 40°C, H_2 production efficiencies decreased from 84.03% to 68.75% (Table 9). Maximum 88.20% H_2 production yield was measured at 30°C incubation temperature with *Anabaena variabilis* prokaryotic algae under indirect biophotolysis process (Table 9).

3.5 Effect of Algae Numbers at H_2 Production Efficiencies

In order to determine the effect of algae numbers on H_2 production efficiency in prokaryotic algae *Synechocystis sp., Spirulina platensis* and *Anabaena variabilis*, the algae numbers under direct photolysis process were examined.

For maximum H_2 production yields with prokaryotic algae, *Synechocystis sp*., a maximum of 35 EMS/100 ml algae counts were measured under direct photolysis process.

For maximum H_2 production yields with Spirulina platensis prokaryotic algae, a maximum of 88 EMS/100 ml *Spirulina platensis* algae count was measured under direct photolysis process.

To observe the maximum H_2 production efficiency from the prokaryotic algae *Anabaena variabilis*, a maximum of 172 EMS/100 ml Anabaena variabilis algae count was obtained using direct photolysis process.

The effect of algae numbers on H_2 production efficiency in prokaryotic algae *Synechocystis sp., Spirulina platensis* and *Anabaena variabilis*, the algae numbers under indirect biophotolysis process were operated.

For maximum H_2 production yields with prokaryotic algae, *Synechocystis sp*., a maximum of 22 EMS/100 ml algae counts were measured under indirect biophotolysis process.

For maximum H_2 production yields with Spirulina platensis prokaryotic algae, a maximum of 64 EMS/100 ml *Spirulina platensis* algae count was measured under indirect biophotolysis process.

To observe the maximum H_2 production efficiency from the prokaryotic algae *Anabaena variabilis*, a maximum of 151 EMS/100 ml Anabaena variabilis algae count was obtained using indirect biophotolysis process.

Genes such as sufC, petN, pabV, accD, and psaM were involved in photosynthetic hydrogen production in both eukaryotic and prokaryotic microalgae. These genes are mainly responsible for synthesizing subunits or cytochromes related to PSI/PSII and play a role in regulating the electron transport chain during photosynthesis. The regulation can affect the generation of NADPH and ATP, which influences the efficiency of photosynthetic hydrogen production. The different key genes related to photosynthetic H_2 production exist in eukaryotic and prokaryotic microalgae, respectively. In prokaryotic microalgae, the hyp gene synthesizes Fe–S clusters and is related to the maturation of hydrogenases, [90]. In both eukaryotic and prokaryotic algae, Fe–S clusters are crucial components for capturing light energy and participating in electron transfer processes. In prokaryotic microalgae, the key genes encoding hydrogenases are hox gene, [91], and hup gene, [92]. Variations in the expression levels and functions of these genes between eukaryotic and prokaryotic microalgae can lead to differences in the H_2 production capabilities of different microalgal species. Owing to these genetic differences, the [Fe–Fe] hydrogenase activity in eukaryotic algae is 10–100 times that of the [Ni–Fe] hydrogenase activity in prokaryotic algae, [93].

3.6 Effect of Duration of H_2 Production

In order to measure H_2 production efficiencies using prokaryotic algae *Synechocystis sp., Spirulina platensis* and *Anabaena variabilis*, direct photolysis process were applied at increasing the duration of H_2 production (20, 40, 80, 100 and 120 hours) and the results obtained are given collectively in Table 10.

* Table 10 can be found in the Appendix section.

Synechocystis sp. from prokaryotic algae under the direct photolysis process, H_2 production efficiencies ranging from 65.4% to 99.40% were obtained for duration of H_2 production between 20 and 120 h. (Table 10). When the duration of H_2 productions were increased from 20 h to 80 h, H_2 production efficiencies increased from 65.42% to 88.11% (Table 10). 99.40% maximum H_2 production yield was found for 100 h duration of H_2 production with *Synechocystis sp.*, prokaryotic algae under direct photolysis process (Table 10).

H_2 production efficiencies ranging from 61.44% to 99.54% were obtained for the duration of H_2 production between 20 and 120, respectively, with *Spirulina platensis* prokaryotic algae under the direct photolysis process (Table 10). It was observed that when the duration H_2 productions were increased from 20 h to 80 h, H_2 production efficiencies increased from 61.44% to 91.14% (Table 10). Maximum 99.54% H_2 production yield was found for 100 h duration of H_2 production, with *Spirulina platensis* prokaryotic algae under direct photolysis process (Table 10).

It was noted that under the direct photolysis process with *Anabaena variabilis* prokaryotic algae, H_2 production efficiencies ranged from 66.47% to 99.44% within the duration of H_2 production between 20 and 120 h (Table 10). It was observed that when the duration of H_2 production increased from 80 h to 120 h, H_2 production efficiency decreased from 93.65% to 66.47% (Table 10). 99.44% maximum H_2 production efficiency was observed for 40 h duration of H_2 production with *Anabaena variabilis* prokaryotic algae under direct photolysis process (Table 10).

In order to measure H_2 production efficiencies using prokaryotic algae *Synechocystis sp., Spirulina platensis* and *Anabaena variabilis*, indirect biophotolysis process were applied at increasing the duration of H_2 production (20, 40, 80, 100 and 120 h) and the results obtained are given collectively in Table 11.

* Table 11 can be found in the Appendix section.

Under the indirect biophotolysis process from *Synechocystis sp*., prokaryotic algae, H_2 production efficiencies ranging from 54.30% to 89.47% were obtained during the duration of H_2 production between 20 and 120 h (Table 11). It was observed that when the duration of H_2 production increased from 20 h to 80 h, H_2 production efficiencies increased from 54.30 to 76.00% (Table 11). Maximum 89.47% H_2 production yield was found at 100 h the duration of H_2 production with *Synechocystis sp*., prokaryotic algae under indirect biophotolysis process (Table 11).

H_2 production efficiencies ranging from 50.32% to 89.63% were measured with *Spirulina platensis* prokaryotic algae through indirect biophotolysis process for the duration of H_2 production between 20 and 120 h (Table 11). When the duration of H_2 production increased from 20 h to 80 h, H_2 production efficiencies increased from 50.32% to 80.04% (Table 11). Maximum 89.63% H_2 production efficiency was measured at 100 h the duration of H_2 production with Spirulina platensis prokaryotic algae under indirect biophotolysis process (Table 11).

It was noted that H_2 production efficiencies under indirect biophotolysis process with *Anabaena variabilis* prokaryotic algae varied between 55.38% and 88.20% within the duration of H_2 production between 20 and 120 h (Table 11). A decrease in H_2 production efficiencies from 82.51% to 55.38% was observed when the duration of H_2 production increased from 80 h to 120 h (Table 11). Maximum 88.20% H_2 production efficiency was found at 40 h the duration of H_2 production with *Anabaena variabilis* prokaryotic algae under indirect biophotolysis process (Table 11).

3.7 Technical Economic Analysis (TEA)

Despite significant industrial development and tremendous research on H_2 production from biomass feedstock, the process is still not yet on a commercial scale, [20], [94]. Successful H_2 enterprise depends greatly on technological innovation, technical feasibility, economic possibility, governmental policy, and environmental regulations. The financial and technical factors that affect the production cost of H_2 from algae was focused. The main challenges facing any renewable technology for fuels and energy production are the costs associated with process fabrication and operating costs, [20], [94]. Algae utilization for various purposes faces many challenges, such as production cost and environmental impact, [95], [96]. Several studies estimated the cost of growing algae on different types of biomasses, [96]. The cost calculation varies significantly from one study to another. H_2 production costs from algae depend on many factors, such as biomass price, algae productivity, wastewater treatment, bioreactor, maintenance, and labor cost, [96].

The process economy is one of the most vital evaluation tools for any process scaling. Perform a concerted economic analysis of the entire system, analysis and calculation of both mass and energy balances are needed, [97]. The simulation data will be used to study the overall technical possibility and provide TEA of the entire process life cycle. The primary goal of TEA is to define and calculate the baseline of capital expenditures (CAPEX) and cost expenditures (OPEX) of the integrated biorefinery. The TEA will be based on a specific and overall mass and energy balance (MEB) calculation for the whole process, from receiving feedstock to the final product's readiness for commercialization. The MEB will provide the foundation to build the first conceptual design with a detailed process: the conceptuy and lower production cost. Other factors that affect the final production cost, such as direct and indirect costs, will be considered. The payback period concerning the bank interest rate will also be calculated.

Artificial intelligence (AI) can aid in optimizing H_2 production from renewable resources in several ways, [96]:

(I). Data Analysis: Artificial intelligence (AI) algorithms can analyze vast amounts of data from various renewable energy sources, such as solar, wind, and hydroelectric power, to identify patterns and optimize H_2 production processes.

(II). Predictive Modeling: AI can create predictive models to forecast renewable energy availability and demand, enabling better planning and scheduling of H_2 production activities.

(III). Process Optimization: AI can optimize the efficiency of H_2 production processes by continuously analyzing and adjusting parameters such as temperature, pressure, and catalyst usage based on real-time data and environmental conditions.

(IV). Resource Allocation: AI algorithms can optimize the allocation of resources such as water, electricity, and raw materials to maximize H_2 production while minimizing waste and costs.

(V). Fault Detection and Maintenance: AI-powered systems can detect equipment malfunctions and predict maintenance needs, ensuring continuous operation of H_2 production facilities.

(VI). Integration with Energy Grid: AI can optimize the integration of H_2 production facilities with the energy grid, balancing supply and demand fluctuations and maximizing the use of renewable energy sources.

(VII). Overall, AI technologies offer significant potential to improve the efficiency, reliability, and sustainability of H_2 production from renewable resources.

The demand for green energy is expanding, and it seems that H_2 is the best option that can be produced and stored in large quantities, [94]. H_2 is a promising energy carrier that has various advantages compared to other energy sources. In addition to, H_2 is gaining significant attention as a green alternative for transportation, energy sector, and energy storage. The H_2-based energy system consists of four major stages: production, storage, safety, and utilization. Artificial neural networks (ANN) are effectively used in predicting optimal operational parameters for H_2 production from different methods, [94]. The progress done in the application of ANNs in H_2 production technologies to maximize the H_2 productivity and decreasing its cost. The coefficient of determination (R^2) and mean squared error (MSE) are used as performance criteria to evaluate the performance of the ANN applied in the different H_2 production methods, [95].

The main merit of using ANNs in H_2 production is the fast data processing in parallel implementations of the ANN model, [96]. ANNs are mostly employed for H_2 production approximations due to their excellent properties of flexibility, self-learning, fault tolerance, nonlinearity, and improved mapping of inputs to outputs, [96].

TEA detailed analysis of the annual cost of H_2 production from microalgae was calculated for Operating cost such as, 12,000 $/year (0.11 $/kg) of Feedstock costs, 6,000 $/year (0.06 $/kg) of Electricity costs, 18,000 $/year (0.16 $/kg) of De-watering costs, 24,000 $/year (0.22 $/kg) of Human resource costs, 59,000 $/year (0.53 $/kg) of Maintenance & washing costs, 1,360,000 $/year (12.44 $/kg) of Capital-related charges costs and 1,480,000 $/year (13.53 $/kg) of H_2 sales costs, respectively.

At Table 12 presents H_2 production costs from various algae-based technologies in comparison with studies in the literature.

* Table 12 can be found in the Appendix section.

The calculation cost of H_2 from algae is a relative cost, not an absolute one. This is due to variations in operating cost (OPEX) and capital investment from one region to another. For example, the OPEX in the USA is more significant than in China due to considerable differences in labor costs. However, we need to establish a minimum acceptable cost of H_2 production. The National Renewable Laboratory (NREL), USA, [98] defined the H_2 selling price as $13.53/kg to compete with H_2 production from conventional technologies. The NREL cost calculation is based on cycling algae cultivation based on a photobioreactor.

This cost includes the cost of pressure swing adsorption and high-pressure storage. The production cost could be reduced significantly to only \$3.68/kg from the reactor. The cost can be further reduced down to \$0.57/kg by minimizing the production area to 1 m^2. The CAPEX for H_2 that uses only a flat pond is estimated at \$1.90 million with no compression and storage. The system based on a 300 kg H_2/d stand-alone system would require a \$51k operating cost, [98].

The main cost of H_2 production from algae is directly proportional to two main factors. The first factor is the capital investment of the bioreactor. Several types of reactors exist, such as Photo-bioreactors, Continuous stirred tank reactors (CSTR), Fixed-bed bioreactors, Membrane bioreactors, multi-stage bioreactors, and Hybrid bioreactors. Due to the low density of H_2, minimizing the bioreactor's size is essential. This could be achieved by increasing the algae's productivity value and looking for new cheap fabrication materials. Therefore, the bioreactor cost varies significantly based on material types, thus making it hard to estimate the lowest bioreactor cost. The second cost factor is the compression and storage of the H_2 gas. Compression and storage are energy-intensive process steps. The photosynthetic efficiency capacity of microalgae is 10% for H_2, resulting in a new cost of \$50/m^2, [77], [99]. Moreover, the cost of thermal production corresponds to \$15/GJ. In the process of H_2 production, a large amount of wastewater is generated. The wastewater must be treated or disposed of in the local sewage at a cost determined by its chemical oxygen demand (COD). If H_2 as fuel replaced 5% of fossil fuel for energy production, a massive amount of wastewater is generated, corresponding to 123–143 trillion liters. This would contribute to 10-20% of the overall production cost, [77], [99].

Biomass (feedstock) used for biofuel production, such as ethanol, butanol, and H_2, contributes significantly to the economic feasibility. In the financial analysis of H_2 production from algae, the minimum purchase price needs to be defined. The sugar derived from biomass is mainly used as the primary feedstock for H_2 production from algae. The minimum prices vary but are within the \$1500–2400/ton, [100]. However, calculating the biomass cost is still problematic for cost estimators. Nevertheless, the agronomic approach is the best to estimate the biomass cost via the production of a sustainable crop. Developing and optimizing biomass to H_2 involves multidisciplinary research efforts spanning introductory chemistry, biology, physics, and engineering. Maximizing the H_2 yield per day, per area, and amount of biomass is the key step toward a sustainable and economically feasible process.

The estimated production cost varies from one technology to another. This is related to the feedstock type, reactor-type gas collection, and compression provisions. The H_2 production cost is calculated in most literature as \$/kg H_2 to benchmark it with another fuel cost. The cost varies between \$2–13/kg H_2 depending on the predefined process boundaries. Amos, [98], presented the minimum cost as low as \$2.04/kg H_2 but without any H_2 collection, storage, and transportation provision. In the same study, the cost can be as high as 13.54/kg H_2 when the H_2 collection storage and transportation are included in the calculation.

The cost estimate for algae-to-hydrogen production provided is relative, not absolute. Variations in cost estimates are common in the literature for most technical economic analyses. To account for these variations, it is important to consider factors such as technological advancements, regional differences, scale of production, and input costs. Sensitivity analyses can also be conducted to assess how changes in key parameters affect the overall cost estimate. This approach helps ensure a more comprehensive understanding and allows stakeholders to make more informed decisions based on their specific circumstances, [98], [101].

3.8 Future Prospects

Microalgae can absorb large amounts of CO_2 from the atmosphere as a carbon source under normal culture conditions, which is a carbon-reducing process. The onset of commercialization for photosynthetic H_2 production from microalgae is initiated by the sulfur-deficient anaerobic photo-culture. However, limitations within this process may affect the life cycle assessment (LCA) process. These drawbacks mainly include the lack of experience in developing enclosed large-scale photobioreactors, the high cost of consumed materials, and the large amount of energy required during the cultivation process, [102]. Furthermore, it is important to analyze the environmental advantages associated with the use of biohydrogen in power generation, alongside the efficacy of photosynthesis H_2 production. Biohydrogen, when utilized as a fuel for power generation, mostly generates water vapor as a byproduct during the combustion process. Consequently, it may be postulated that the carbon emission coefficient associated with the utilization of H_2 is negligible, [103].

By pre-cultivating microalgae in wastewater, economic costs can be reduced, and at the same time, the metabolism of microalgae can remove organic pollutants in wastewater. In addition to, traditional wastewater treatment process requires significant amounts of energy, resulting in the emission of greenhouse gases and worsening the issue of global warming. The utilization of algal water treatment technology is extensively employed in the field of water quality management and tail water treatment owing to its notable benefits in terms of efficacy and safety. The investigation of the mutualistic relationship between microalgae and bacteria is currently a prominent area of research.

During the first phase of microalgae cultivation in wastewater, a substantial quantity of organic debris might impede the growth of microalgae. Concurrently, the bacteria present in wastewater use this organic matter to proliferate extensively, resulting in the production of CO_2, [104]. Subsequently, the microalgae use solar energy, CO_2 produced by the bacteria, and N_2 and phosphorus in the wastewater to convert into their substances used for growth and reproduction, achieving both enhanced removal of pollutants from the wastewater and biomass energy recovery. Microalgae are autotrophic microorganisms and the O_2 produced by microalgae can be supplied to the bacteria as an electron acceptor, so there is no need for additional electrical aeration, [105]. As the O_2 levels in the co-culture system decrease, the culture will reach an anaerobic state quickly. This process guarantees the optimal functioning of hydrogenase within microalgae, resulting in the successful generation of H_2 through the utilization of microalgae, [106], [107], [108]. Through, this production method, not only can energy consumption of electricity and the pro duction costs be reduced, but greenhouse gas emissions can also be reduced, and clean energy H_2 can be produced.

4 Conclusions

Prokaryotic Algae is a particularly promising microbe that has the potential to produce other fuels and chemicals. Also, algae can benefit greenhouse gas assimilation, water desalination, and other purposes. By understanding the various technologies and techniques involved in biohydrogen production from algae, researchers can strategically use algae for H_2 production while utilizing it for other high-value products.

The studies on prokaryotic microalgae have mainly focused on three types involving *Synechocystis sp.*, *Spirulina platensis* and *Anabaena variabilis*. There are advantages to utilizing both eukaryotic and prokaryotic microalgae for H_2 production. The most hydrogen-producing cyanobacteria exhibit a simple cell structure. Pigments and enzymes involved in photosynthesis are directly distributed in the chromatin. These prokaryotic microalgae especially, more sensitive to H_2 production reactions under anaerobic conditions.

Maximum 99.40% H_2 production efficiency was observed at 0.1 C/N ratio, at pH=7.5, at 168 μmol/m^2.s illumination intensity, at 30°C incubation temperature, at 35 EMS/100 ml *Synechocystis sp.*, algae counts, after 100 h duration of H_2 production, respectively, with *Synechocystis sp.*, prokaryotic algae under direct photolysis process.

Maximum 99.54% H_2 production yield was measured at 0.1 C/N ratio, at pH=9.0, at 180 mol/m^2.s, at 35°C, at 88 EMS/100 ml *Spirulina platensis* algae counts, after 100 h, respectively, with *Spirulina platensis* prokaryotic algae under direct photolysis process.

Maximum 99.44% H_2 production efficiency was obtained at 0.1 C/N ratio, at pH=7.5, at 216 mol/m^2.s, at 30°C, at 172 EMS/100 ml *Anabaena variabilis* algae counts, after 40 h, respectively, with *Anabaena variabilis* prokaryotic algae under direct photolysis process.

H_2 production yields in direct photolysis are higher than those in indirect biophotolysis. The responsible enzyme for H_2 evolution is a reversible hydrogenase because it catalyzes the reaction in both directions. The reversible hydrogenase and nitrogenase, however, are sensitive to the O_2 evolved in biophotolysis and promptly deactivated at quite low O_2 partial pressures (< 2%v/v), which results in a transient H_2 evolution. This intrinsic incompatibility of biophotolysis is a major barrier for sustained H_2 evolution. This is because direct energy is converted into H_2 energy in direct photolysis. Energy losses in direct photolysis are negligible compared to indirect biophotolysis. In addition to, H_2 production yields in direct photolysis are much higher.

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The influence of environmental factors on the H_2 production efficiency of prokaryotic microalgae followed the order of C/N ratio > incubation temperature > duration of H_2 production > illumination intensity > pH values. Therefore, significant differences were observed in the environmental factors affecting H_2 production efficiency prokaryotic microalgae. The microalgae-based H_2 production faces two limitations. First, hydrogenase plays a crucial role in the photosynthetic production of H_2 by microalgae. However, the activity of hydrogenase is hindered by O_2, necessitating the maintenance of the reaction system in an anaerobic environment. Second, microalgae-based photosynthetic H_2 production exhibits low energy conversion efficiency owing to the inherent limitations of solar energy fixation and utilization by microalgae during photosynthesis. Generally, only a small fraction of the absorbed light energy is converted into chemical energy. However, maintaining an anaerobic environment and improving light conversion efficiency are crucial for optimizing environmental conditions for effective H_2 production.

It is observations that slr0388 is involved in both phototactic motility and natural transformation provide further evidence that these two processes are intrinsically linked in *Synechocystis sp.* strain PCC 6803. Apart from the Tfp genetic complement, the regulation of phototaxis and natural transformation is likely to involve other common genes that indirectly influence the biogenesis of the Tfp apparatus, as observed with slr0388 in the present study. As a result, the identification of other factors specific only to the regulation of natural transformation should facilitate the elucidation of pathways that distinguish this process from phototactic motility.

The photoautotrophic strain Anabaena sp. strain PCC 7120 to use fructose when growing in the light, suggest that strains that are naturally capable of sugar transport and utilization have evolved mechanisms that allow them both to use sugars efficiently and to overcome sugar toxicity. These are of course likely to be metabolically linked processes.

Per contra the heterotrophic growth requires the addition of glucose which is likely to be costly at large scale. Therefore, it will be important to consider potentially much cheaper sources (e.g., wastewater from sugar factories, and paper mills, baker's yeast and brewery). Yet, an important advantage of the O_2 consumption through respiration of organic substrates is represented by the purity of the H_2 produced (close to 98%), which strongly reduced the investment cost for H_2 purification, which can account for up to 50% of total cost.

The key advantage of using ANNs in H_2 generation is their ability to rapidly process data in parallel implementations of the ANN model. ANNs are widely preferred for H_2 generation approaches due to their excellent properties, such as flexibility, self-learning, fault tolerance, non-linearity, and improved mapping of inputs to outputs.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Prof. Dr. Delia Teresa Sponza and Post-Dr. Rukiye Öztekin took an active role in every stage of the preparation of this article.

The authors equally contributed in the present research, at all stages from the formulation of the problem to the final findings and solution.

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Conflict of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

APPENDIX

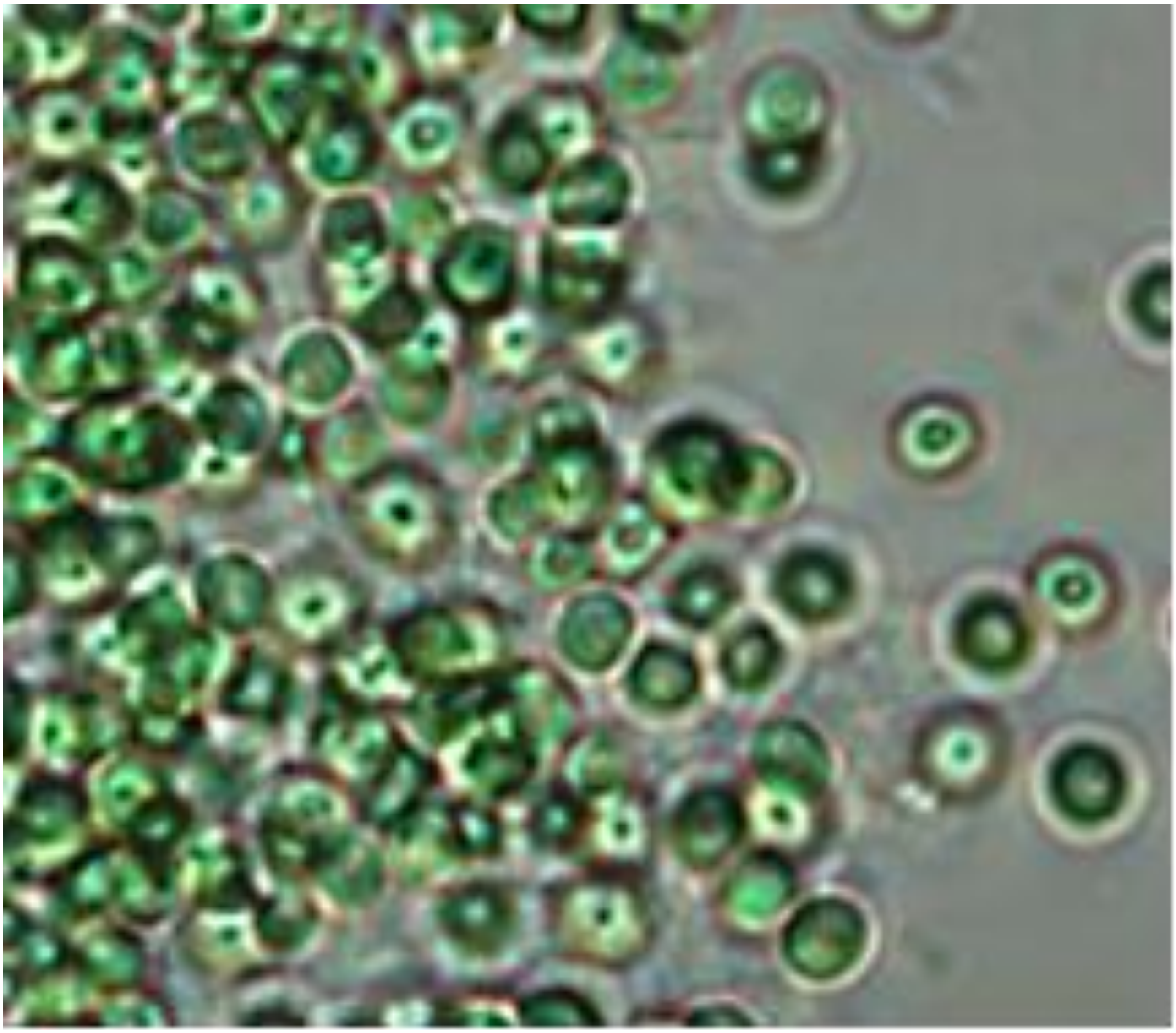

Figure 1. Electron microscopy image of *Synechocystic sp.* PCC 6803 prokaryotic algae (image size: 10 μm).

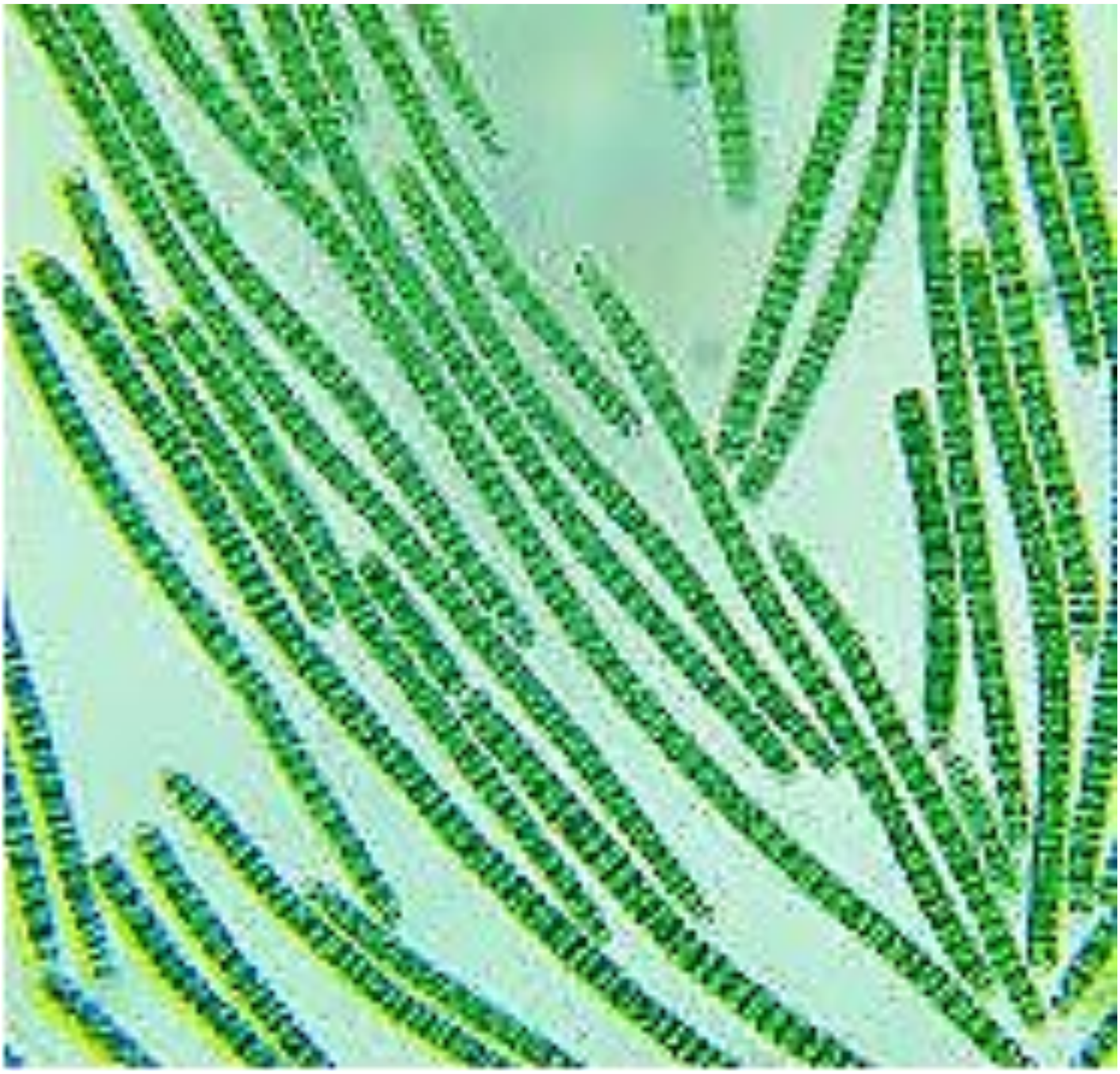

Figure 2. Elecron microscopy image of *Spirulina platensis* prokaryotic algae (image size: 10 μm).

Figure 3. Electron microscopy images of *Anabaena variabilis* prokaryotic algae (image size: 10 µm).

Table 1. Comparison of general advantages and disadvantages in H_2 production by direct photolysis and indirect biophotolysis processes from algal microbes

H_2 Production Processes	**Advantages**	**Disadvantages**
Direct Photolysis Process	Pure H_2 generations	Requirement of highlight intensity
	High solar conversions	Limitation of H_2 production due to the existence of O_2
	CO_2 consumptions	
Indirect Biophotolysis Process	Overcome the incompatibility problem of the simultaneous production of O_2 and H_2	Low H_2 productions
	Simple medium of microorganisms	The number of capable microorganisms is limited
	Cost effective	High substrate requirements

Table 2. The effect of C/N ratio for prokaryotic algae namely *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis* under direct photolysis for H_2 production efficiencies.

C/N Ratio	**H_2 Production Efficiencies (%)**		
	Synechocystis sp.	***Spirulina platensis***	***Anabaena variabilis***
0.1	99.40	99.54	99.31
0.2	87.80	86.15	93.46
0.4	72.54	73.40	86.11
0.8	64.03	65.79	73.40
1.0	51.29	55.47	61.20

Table 3. The effect of C/N ratio for prokaryotic algae namely *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis* under undirect biophotolysis for H_2 production efficiencies.

C/N Ratio	H_2 Production Efficiencies (%)		
	Synechocystis sp.	***Spirulina platensis***	***Anabaena variabilis***
0.1	89.47	89.63	88.20
0.2	75.68	75.04	82.33
0.4	61.42	62.29	74.98
0.8	53.91	54.68	62.29
1.0	40.17	44.36	50.08

Table 4. The effect of pH values for prokaryotic algae namely *Synechocystis sp.*, *Spirulina platensis* and *Anabaena variabilis* under direct photolysis for H_2 production efficiencies.

pH Values	**H_2 Production Efficiencies (%)**		
	Synechocystis sp.	***Spirulina platensis***	***Anabaena variabilis***
6.5	64.31	49.51	74.56
7.0	81.15	63.58	81.82
7.5	99.40	70.64	99.44
8.0	85.47	84.27	97.09
8.5	73.33	91.87	83.24
9.0	59.74	99.54	65.57

Table 5. The effect of pH values for prokaryotic algae namely *Synechocystis sp.*, *Spirulina platensis* and *Anabaena variabilis* under undirect biophotolysis for H_2 production efficiencies.

pH Values	**H_2 Production Efficiencies (%)**		
	Synechocystis sp.	***Spirulina platensis***	***Anabaena variabilis***
6.5	53.19	40.37	62.45
7.0	70.04	52.47	71.70
7.5	89.47	60.55	88.20
8.0	76.35	75.16	75.07
8.5	71.10	80.75	72.13
9.0	42.58	89.63	56.46

Table 6. The effect of illumination intensity for prokaryotic algae namely *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis* under direct photolysis for H_2 production efficiencies.

Illumination Intensity (μmol/m^2.s)	**H_2 Production Efficiencies (%)**		
	Synechocystis sp.	***Spirulina platensis***	***Anabaena variabilis***
120	82.36	75.50	61.37
168	99.40	94.34	74.90
180	87.10	99.54	83.05
216	76.17	96.12	99.44
250	65.48	88.05	94.51

Table 7. The effect of illumination intensity for prokaryotic algae namely *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis* under undirect biophotolysis for H_2 production efficiencies.

Illumination Intensity (μmol/m^2.s)	**H_2 Production Efficiencies (%)**		
	Synechocystis sp.	***Spirulina platensis***	***Anabaena variabilis***
120	71.25	64.39	50.26
168	89.47	89.63	61.78
180	76.88	87.42	72.02
216	55.05	84.01	88.20
250	54.37	77.09	82.41

Table 8. The effect of incubation temperature for prokaryotic algae namely *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis* under direct photolysis for H_2 production efficiencies.

Incubation Temperatures (ºC)	**H_2 Production Efficiencies (%)**		
	Synechocystis sp.	***Spirulina platensis***	***Anabaena variabilis***
20	71.22	59.68	77.82
25	88.05	77.84	92.45
30	99.40	94.32	99.44
35	84.34	99.54	95.08
40	74.09	97.11	79.89

Table 9. The effect of incubation temperature for prokaryotic algae namely *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis* under undirect biophotolysis for H_2 production efficiencies.

Incubation Temperatures (ºC)	**H_2 Production Efficiencies (%)**		
	Synechocystis sp.	***Spirulina platensis***	***Anabaena variabilis***
20	60.01	48.56	66.70
25	76.05	65.73	81.34
30	89.47	83.20	88.20
35	73.22	89.63	84.03
40	64.04	85.00	68.75

Table 10. The effect of duration of H_2 production for prokaryotic algae namely *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis* under direct photolysis for H_2 production efficiencies.

Duration of H_2 Production (hours)	**H_2 Production Efficiencies (%)**		
	Synechocystis sp.	***Spirulina platensis***	***Anabaena variabilis***
20	65.42	61.44	82.49
40	73.40	85.56	99.44
80	88.11	91.14	93.65
100	99.40	99.54	88.52
120	91.78	95.63	66.47

Table 11. The effect of duration of H_2 production for prokaryotic algae namely *Synechocystis sp*., *Spirulina platensis* and *Anabaena variabilis* under undirect biophotolysis for H_2 production efficiencies.

Duration of H_2 Production (hours)	**H_2 Production Efficiencies (%)**		
	Synechocystis sp.	***Spirulina platensis***	***Anabaena variabilis***
20	54.30	50.32	71.37
40	65.28	74.45	88.20
80	76.00	80.04	82.51
100	89.47	89.63	76.40
120	80.66	85.42	55.38

Table 12. Comparison of H_2 production costs from various algae-based technologies with studies in the literature.

Conversion chemistry	**Yields**	**Cost (\$/kg H_2)**	**Capital cost (MM\$)**	**H_2 Production Technology Readiness Level (TRL)**	**References**
Direct Thermal gasification	1239 kg/h	8.65	NA	5	[86]
		5.66	215	7	[83]
Indirect biophotolysis	2-5x10^{-7} mol/m^2.s	2.04-13.53	NA	5	[62]
Thermal alga	-	10			[28]
		2.60$	NA	7	[56]
			2	Target goal by NREL *	[62]
* Note: NREL is the U.S. Department of Energy's primary national laboratory for energy systems.					