

## REVIEW




# Sustainability considerations in bio-hydrogen from bio-algae with the aid of bio-algae cultivation and harvesting: Critical review

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## ABSTRACT

*Interest in developing renewable fuels, such as hydrogen from biomass, has surged due to escalating energy demands and the imperative to curtail greenhouse gas emissions. Overview of bio-hydrogen production pathway, reactor designs, and configurations for bio-hydrogen production from bio-algae were explored. Environmental, social sustainability and economic feasibility have been reviewed.*

Microalgae present an enticing alternative to conventional fossil fuel-dependent technologies for producing hydrogen, offering an intriguing and sustainable energy source. Numerous strains of microalgae are under investigation for their capacity to generate hydrogen, alongside various techniques and breakthroughs being developed to optimize the process. However, significant hurdles must be addressed for commercial viability, including the high manufacturing costs and the necessity for efficient harvesting and sorting methods. This paper delves into several aspects concerning hydrogen synthesis in algae, encompassing microalgae anatomy and physiology, hydrogen synthesis via photosynthesis and dark fermentation, and the integration of microalgal hydrogen synthesis with other renewable energy sources. The potential for microalgal hydrogen generation is considered pivotal in transitioning toward a future reliant on more renewable and sustainable energy sources. This review aims to serve as a valuable resource for researchers, decision-makers, and anyone interested in the advancement of environmentally conscious energy technology. The primary objective of this research paper is to scrutinize the challenges, opportunities, and potential outcomes associated with eco-friendly bio-hydrogen production through algae. It evaluates the current technological hurdles facing bio-hydrogen synthesis from algae.

**Keywords** biological synthesis · biomaterial · energy generation · environmentally protective · renewable

## Discussion

- Will bio-hydrogen from bio-algae be a future renewable energy?
- Which is the best pathway to produce bio-hydrogen from bio-algae?
- Regarding greenhouse gas emissions, how does the generation of bio-hydrogen from bio-algae compare to conventional hydrogen production techniques?
- What difficulties lie in increasing the amount of bio-hydrogen produced by bio-algae to satisfy major energy demands?

## Introduction

The contemporary energy landscape is largely dominated by fossil fuels—such as natural gas, petroleum, and coal—that significantly exacerbate warming temperatures and air pollution due to their limited resources.<sup>1</sup> Given the escalating energy demands, exploring sustainable alternatives that fulfill these needs without causing harm to the environment is imperative. The biomass industry, inclusive of sewage and organics, is being converted into various biofuels, notably bio-hydrogen. The production of bio-hydrogen from bio-algae offers several advantages, including high biomass yield, rapid development, and minimal land requirements.<sup>2</sup> Moreover, integrating the creation of bio-hydrogen from algae with other renewable energy techniques—such as carbon capture and wastewater treatment—shows promise. In essence, utilizing bio-hydrogen and algae as viable sources of clean energy could provide a long-term substitute for gasoline and diesel, contributing to mitigating climate change and reducing environmental pollution. However, several challenges need resolution to establish sustained bio-hydrogen (BH) production from algae<sup>3</sup>:

- *Poor BH Yield* the difficulty in producing bio-hydrogen from algae is attributed to its poor yield. The low conversion efficiency is due to the high oxygen sensitivity of hydrogen-producing bacteria.<sup>4</sup>
- *High Production Cost* the costs involved in growing and harvesting microalgae result in relatively higher expenses for bio-hydrogen production compared to traditional methods.<sup>5</sup>
- *Lack of Standardization* the absence of standardized processes for bio-hydrogen generation makes it challenging to compare the effectiveness of different systems and technologies. Identifying opportunities and potential solutions to enhance the sustainability and efficiency of bio-algae-based bio-hydrogen production is crucial.<sup>6</sup>

This study primarily focuses on synthesizing bio-hydrogen from bio-algae, exploring both the opportunities and challenges associated with this technology.<sup>7</sup> It analyzes the current status of bio-hydrogen production from algae, existing technological challenges, and potential remedies to improve efficiency and sustainability.

Furthermore, the study examines how bio-hydrogen derived from bio-algae contributes to a less polluting form of energy.<sup>8</sup> Nevertheless, this analysis has limitations. It solely concentrates on the creation of bio-hydrogen from bio-algae without encompassing other renewable energy sources.<sup>9</sup> Additionally, it focuses solely on the present state of the technology, excluding potential advancements. Lastly, the research article does not offer an extensive economic evaluation of the costs associated with producing bio-hydrogen from bio-algae.<sup>10</sup>

## Bio-algae cultivation harvesting

The cultivation and harvesting of algae for various purposes, including bioenergy production, such as bio-hydrogen, are encompassed within the practice of bio-algae cultivation and harvesting.<sup>11</sup> A concise guide follows on the methods involved in algae cultivation and harvesting: The effectiveness of bio-harvesting operations can be influenced by the choice of algae strains due to their diverse traits and properties.<sup>12</sup> When selecting algae strains for bio-harvesting, the following key factors should be considered:

- *Growth Rate* algae strains with rapid growth rates are preferred for bio-harvesting due to their ability to multiply quickly and achieve high biomass concentrations, resulting in enhanced productivity.<sup>13</sup>
- *High Lipid Content* strains with high lipid content are particularly favored for bio-harvesting, as these lipids can be harvested and converted into renewable biofuels like biodiesel.<sup>14</sup>
- *High Bulk Density* algae strains with higher biomass densities offer advantages by maximizing resource utilization and space efficiency, leading to increased productivity and reduced production costs.<sup>15</sup>
- *Nutritional Needs* understanding the nutritional requirements of specific algae strains is crucial for optimizing growth and productivity, as different strains thrive in varying nutrient conditions.<sup>16</sup>
- *Environmental Adaptability* algae strains adaptable to diverse environmental conditions, including pH, temperature, salinity, and light intensity, enable cultivation across various geographic and climatic zones.<sup>17</sup>
- *Resistance to Contamination* strains displaying resistance or tolerance to contaminants such as mold, bacteria, or other algae forms reduce the risk of crop loss and help maintain culture purity.<sup>17</sup>
- *Harvesting Ease* strains facilitating efficient and cost-effective harvesting methods, based on characteristics like cell size, shape, and tendency to aggregate, are desirable.<sup>18</sup>
- *Intended Use* selection of algae strains may depend on their intended application, as different strains produce substances useful in various products, such as proteins, colorants, or antioxidants used in food, medicines, cosmetics, etc.<sup>18</sup>

Table 1 illustrates the Optimization of Algal Cultivation for Sustainable Biofuel Production. It is essential to note the abundance of available algae strains, each with unique properties. Therefore, thorough research, consideration of project goals and expert consultation are recommended when selecting appropriate algal strains for harvesting.<sup>19</sup>

## Overview of bio-algae cultivation methods

To harvest algae, the biomass must be separated from the growth media. Harvesting can occur through various methods,

including: filtration: this process involves separating the algae cells of the culture from its liquid media using filters. Sedimentation, achieved by minimizing mixing and allowing adequate settling time, allows the algae to form a layer at the bottom of the culture system.<sup>40</sup> This procedure is crucial for obtaining high-quality biomass suitable for numerous applications after further processing.

The role of filters in bio-harvesting is outlined. Purpose of filtration: in bio-harvesting, the primary objective of filtration is to separate algal organisms from the liquid media. The culture broth, typically comprising nutrient-rich water and elements essential for algae growth, is eliminated through filtration, compressing the algae biomass. This makes handling and continued processing more manageable.<sup>41</sup> Different filtration technologies can be employed for algae harvesting, depending on the properties of the algal strain, desired biomass quantity, and specific downstream processing needs.

Various filtration techniques include:

- *Gravity Filtration* this method involves allowing the culture broth to pass through a filter media. It is commonly used for managing relatively low biomass concentrations and is suitable for large-scale operations.<sup>42</sup>
- *Vacuum Filtration* to expedite the filtration process, vacuum filtration employs vacuum pressure. It is often used when larger biomass concentrations need to be achieved more rapidly.
- *Pressure Filtration* in this method, positive pressure forces the culture broth through the filter matrix. High biomass concentrations can be obtained using this technique, which can also be combined with other filtration methods to enhance efficiency.
- *Crossflow Filtration* also known as tangential flow filters, this technique involves transferring culture broth radially across an opaque membrane. It is frequently used when dealing with vulnerable or shear-sensitive algae strains to reduce blockage and fouling of the filter.<sup>43</sup>
- *Filtration Media* the choice of filtration medium depends on the size of the algal cells, required biomass concentration, and filtration degree.

Typical filter media include

- *Microfiltration (MF) membranes* ideal for removing larger algal cells, detritus, and suspended particles (0.1–10 µm).
- *Ultrafiltration (UF)* these membranes can filter tiny particles such as bacteria, viruses, and microscopic algae cells due to smaller pores (0.001–0.1 µm).
- *Nanofiltration (NF) and reverse osmosis (RO)* employing even smaller pore sizes, these membrane processes concentrate algae biomass further or remove dissolved materials from the culture broth.<sup>44</sup>

Optimizing filtration parameters such as flow rate, pore pressure, and screen media choice is necessary to achieve the desired

separation efficiency and biomass concentration.<sup>45</sup> During optimization, considerations such as the viscosity of the culture broth, the presence of aggregating agents, and the filtration system's design should be taken into account.

After filtration, depending on the intended application, the concentrated algal biomass might undergo further processes. This may involve lipid extraction, drying, cell rupture for releasing intracellular components, or additional purification. Filtration stands as an essential process in the bio-harvesting of algae, aiding in the separation and concentration of algae cells within the liquid growth media.<sup>46</sup> Effective filtration techniques can enhance the productivity and profitability of algae-based technologies across various applications, such as biofuels, bioplastics, animal feed, and wastewater treatment.

Flocculation involves aggregating algae cells using flocculants to aid their separation from the growth medium.<sup>47</sup> Here is a description of flocculation's role in bio-harvesting: The primary function of flocculation in bio-harvesting is to encourage the formation of larger, denser algal clumps or flocs. By clustering individual algal cells, flocculation improves the settling or flotation properties of the biomass, facilitating its efficient removal from the growth medium.<sup>48</sup>

Several mechanisms can trigger flocculation. Chemical flocculants or substances are added to the growth medium to promote the aggregation of algal cells. These flocculants can be organic polymers like chitosan or cationic polymers or inorganic salts like aluminum sulfate or ferric chloride. They function by balancing the surface charges of the algae cells, enabling them to aggregate into flocs and come closer together.<sup>49</sup> Certain algae types naturally produce extracellular polymers, known as bioflocculants, aiding the flocculation process. These bioflocculants can be generated by bacteria in the growth medium or by the algae cells themselves. Physiological flocculation can be enhanced by improving growth conditions, such as nutrient accessibility or light intensity.

Physical techniques like mixing or stirring can induce flocculation by encouraging collisions and interactions among algae cells. These techniques can be employed alone or in conjunction with chemical or biological flocculation to enhance floc formation.<sup>50</sup> The success of flocculation relies on various factors that need optimization. Optimizing the dosage of flocculants or coagulants in the growth medium is crucial to achieve the desired floc size and strength. Inadequate dosage can lead to weak, unstable flocs, while excessive dosage can produce an excess of flocs, making separation challenging.<sup>51</sup> The pH and alkalinity of the culture medium can influence the flocculation process. Within a reasonable range, altering these parameters can promote flocculation by favoring surface charge neutralization and enhancing flocculant bonding or adsorption. Appropriate mixing or stirring conditions aid in uniformly distributing flocculants and promoting interactions among algae cells. Enhancing mixing parameters such as speed and duration can boost flocculation effectiveness.

Following flocculation, the resulting algal flocs can be removed from the growth media using techniques like

**Table 1.** Optimizing algal cultivation for sustainable biofuel production.

Types of algae	Cultivation method	Operating conditions	Applications	References
<i>Chlorella</i>	Photo-bioreactor	Temperature 25–35 °C	Biofuel	20
<i>Spirulina</i>	Open Pond	pH 9–11	Nutritional Supplement	21
<i>Dunaliella</i>	Raceway Pond	Salinity 20–30 g/L	Beta-Carotene Production	22
<i>Haematococcus</i>	Closed System	Light Intensity 5000–10,000 lux	Astaxanthin Extraction	23
<i>Nannochloropsis</i>	Vertical Tubular Photo-bioreactor	CO <sub>2</sub> Concentration 5–10%	Omega-3 Fatty Acid Production	10
<i>Porphyridium</i>	Flat Panel Photo-bioreactor	Light/Dark Cycle 12/12 h	Pigment Extraction	24
<i>Isochrysis</i>	Bubble Column	Temperature 18–25 °C	Aquaculture Feed	25
<i>Tetraselmis</i>	Vertical Bag	Nitrate Concentration 20–30 mg/L	Bioremediation	26
<i>Botryococcus</i>	Open Raceway Pond	Nutrient Ratio: N:P = 20:1	Hydrocarbon Production	27
<i>Ulva</i>	Land-Based Tank	Light/Dark Cycle 14/10 h	Bioremediation	28
<i>Scenedesmus</i>	Horizontal Tubular Photo-bioreactor	pH 6.5–8.5	Bioplastics Production	29
<i>Dictyochloropsis</i>	Vertical Column	Temperature 20–25 °C	Biofuel and Bioproducts	30
<i>Porphyra</i>	Aquaculture Tanks	Salinity 25–35 ppt	Edible Seaweed	31
<i>Botryococcus</i>	Closed Photo-bioreactor	Light Intensity: 200–300 $\mu$ mol/m <sup>2</sup> /s	Biofuel and Bioproducts	27
<i>Chlamydomonas</i>	Flat Panel Photo-bioreactor	Temperature 20–25 °C	BH Production	28
<i>Arthrospira</i>	Open Raceway Pond	pH 10–11	Food Colorant	29
<i>Gracilaria</i>	Integrated Multi-trophic Aquaculture	Temperature 20–30 °C	Seaweed Cultivation	30
<i>Euglena</i>	Closed Photo-bioreactor	Light Intensity 2000–5000 lux	Nutraceuticals	32
<i>Amphora</i>	Vertical Tubular Photo-bioreactor	Temperature 15–20 °C	Bioactive Compound Production	33

**Table 1.** (continued)

Types of algae	Cultivation method	Operating conditions	Applications	References
<i>Phaeodactylum</i>	Bubble Column	pH 7.5–8.5	Diatomaceous Earth	34
<i>Navicula</i>	Land-Based Tank	Temperature 15–25 °C	Biofouling Control	35
<i>Cyclotella</i>	Flat Panel Photo-bioreactor	Light Intensity 100–500 $\mu\text{mol/m}^2/\text{s}$	Biofiltration	36
<i>Merismopedia</i>	Open Pond	pH 7–9	Water Treatment	37
<i>Microcystis</i>	Horizontal Tubular Photo-bioreactor	Temperature 25–30 °C	Toxin Analysis	38
<i>Oscillatoria</i>	Raceway Pond	pH 7–8	Wastewater Treatment	39

sedimentation, flotation, or filtration.<sup>52</sup> The choice of a separation technique is influenced by factors, such as floc thickness, density, and the specific requirements of subsequent processing stages. Flocculation plays a crucial role in generating larger, denser aggregates, essential for algae bio-harvesting. Effective flocculation methods can enhance the profitability of algae-based systems across various applications, including biofuels, bioplastics, and wastewater treatment. They can also improve harvesting efficiency while reducing energy consumption and costs.<sup>40</sup>

Centrifugation involves separating algal cells from the culture media based on density differences. Subsequent steps include drying and processing to eliminate excess water from the harvested algae biomass, making it suitable for use in other processes.<sup>14</sup> Centrifugation primarily serves to separate algae spores from the liquid culture media in bio-harvesting. This process effectively separates materials by leveraging the gravity differences between the denser algal cells and the lighter culture medium.<sup>53</sup>

Harvesting the algal culture, usually cultivated in open ponds, closed bioreactors, or other systems, reaching a specific cell population or growth stage before harvesting. If the cell density is low, concentration methods like settling or filtration may be necessary to increase biomass quantity and reduce overall culture volume. Concentrated algae culture is placed in centrifuge tubes or jars and then rapidly spun in a centrifuge to generate centrifugal force.<sup>54</sup>

Centrifugal force pushes denser algal biomass to the vessel or tube's outer layer, while the lighter culture medium remains at the center. After centrifugation, the purified medium can be decanted or removed from the top, while the denser algal biomass settles as a pellet at the bottom.<sup>55</sup>

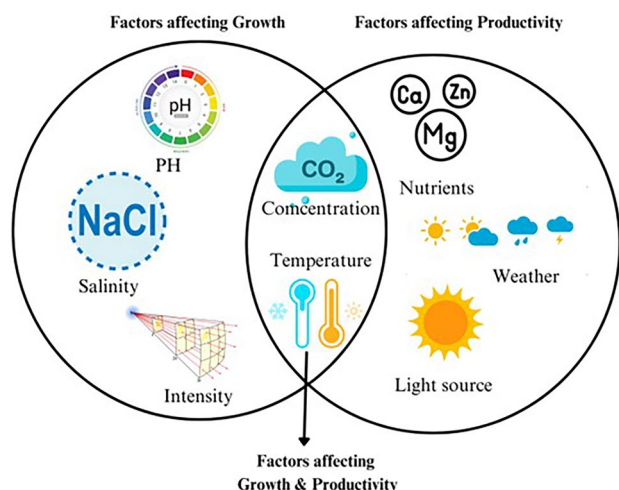
Several factors can enhance centrifugation's efficiency in bio-harvesting. The speed or rotating velocity of the centrifuge influences the separation effectiveness. Higher speeds create

stronger centrifugal forces, but careful selection is required to prevent damage to algae cells.<sup>56</sup> Duration affects separation efficiency, varying based on algae type, cell density, and desired biomass concentration. Longer durations may be necessary for higher biomass recovery.<sup>57</sup> The centrifuge's design and vessel type impact separation efficiency. Factors like vessel shape, presence of baffles or concave bottoms, and automated control systems affect centrifuge performance. After centrifugation, the algal biomass may undergo further processes, such as washing, drying, cell rupturing for lipid extraction, or downstream processing for biofuels or other applications.<sup>58</sup> Centrifugation proves to be a useful and widely used technique in bio-harvesting algae due to its high separation efficiency. It can complement other harvesting methods like flocculation or filtration, especially beneficial for large-scale operations requiring higher biomass concentrations.<sup>59</sup>

### Factors affecting bio-algae growth and productivity

Depending on the environment and cultivation methods, various factors significantly influence the growth and productivity of algae. In Fig. 1, we illustrate the factors affecting bio-algae growth and productivity. Below are concise descriptions of several important elements that impact the growth and production of algae.<sup>15</sup>

Light intensity represents the total luminescence that permeates the green algae crop within a specific space and time. Metabolic rates, development, and algae production are directly influenced by light intensity. Consider the following aspects. Different species of algae have distinct optimal light intensity requirements. Some species thrive in low-light conditions, while others perform best in brighter environments. Identifying the specific light intensity range that facilitates maximum growth for a chosen species of algae is crucial.<sup>60</sup> As the brightness of light increases, the rate of photosynthesis reaches its peak at the



**Figure 1.** Factors affecting growth and productivity.

light saturation point. Further increases in light intensity beyond this point do not significantly enhance photosynthetic activity. Excessive light may even inhibit algal cells, causing damage. Hence, maintaining control over the saturation point is crucial for ensuring ideal developmental conditions.<sup>61</sup> Light attenuation refers to the diminishing intensity of light as it penetrates a specific cultural medium. Algae cells nearer to the culture's surface receive more intense light compared to those deeper within the culture. Therefore, accounting for the culture's depth is essential to ensure adequate light distribution across the algae population.<sup>62</sup> Algae's ability to convert ultraviolet radiation into biomass or valuable molecules is termed light efficiency. Improving light efficiency can notably enhance algae cultivation productivity.<sup>63</sup> Algal development responds differently to various light wavelengths. Chlorophyll pigments in algae predominantly absorb light in the blue and red spectral ranges. Optimizing the light spectrum, either through artificial lighting or maximizing natural sunshine, can significantly increase development efficiency.<sup>64</sup>

The duration of light exposure, commonly referred to as the photoperiod, significantly impacts algae growth and production. For certain species, the most effective light-dark cycles are those that maximize growth. Ensuring the ideal photoperiod while maintaining consistent lighting conditions is crucial to prevent disruption to the growth cycles of targeted algae species.<sup>65</sup>

*Even Lighting* uniform light distribution within the culture ensures that all algae cell types receive sufficient light for photosynthesis. Inconsistencies in development rates may arise due to shading effects or uneven light dispersion. Stirring or mixing the culture can help distribute light uniformly and prevent localized shadowing.<sup>66</sup> Efficient light management techniques are pivotal for enhancing algae development. These techniques encompass optimal configuration and design of growing systems. This involves strategic placement of algae colonies to reduce shade, employing bioreactors, using reflective or transparent materials to maximize light penetration, among other methods.

*Light Integration* consistent and controlled lighting conditions can be achieved by integrating artificial lighting sources with natural sunlight. Supplementary lighting systems like LEDs can be employed when natural sunlight is insufficient. These systems can either supplement the light or modify the light spectrum to optimize growth.<sup>67</sup> Monitoring and tracking light quantity and quality in the culture can significantly enhance lighting conditions. Employing light detectors and monitoring devices can adjust lighting factors to attain optimal growing conditions.<sup>68</sup> Maximizing biomass yield and desired chemical output necessitates the optimization of light levels and efficiency in algal development. Understanding the specific lighting requirements of the algae species being cultivated and implementing effective light management techniques is imperative for both development and output.

*Temperature Impact* temperature plays a pivotal role in algae growth as different algae species thrive within specific temperature ranges.<sup>69</sup> Each species has an optimal temperature range for effective growth, typically falling between 20 and 35 °C. Maintaining this range is crucial to maximize development and output. Temperature significantly influences algae metabolic rates.<sup>70</sup> Within the ideal range, higher temperatures accelerate algal cells' metabolic processes, resulting in faster growth rates. Conversely, temperatures outside this range can impede or halt growth. Temperature directly affects the rate at which algae produce oxygen through photosynthesis. Warmer temperatures generally boost photosynthetic activity, increasing biomass output. However, excessively high temperatures can induce heat stress, damaging the photosynthetic system and consequently hampering growth.<sup>71</sup>

*Nutrient Uptake and Bioavailability* temperature significantly influences nutrient uptake and digestion in algae cells. Higher temperatures often stimulate faster growth by accelerating nutrient intake and digestion rates. However, temperature changes can also affect the accessibility and bioavailability of nutrients in the growth medium, potentially impacting algae development.<sup>72</sup>

*Metabolic Activity and Enzyme Function* temperature affects the activity of genes involved in various metabolic activities in algal cells. Enzymes catalyze crucial biological reactions, such as photosynthesis and food metabolism. Enzyme activity peaks at optimal temperatures, facilitating efficient metabolism and growth.<sup>44</sup>

*Thermal Stress* algae are susceptible to thermal stress when exposed to extreme temperatures outside their preferred range. High temperatures can damage cells, inhibit photosynthesis, and slow growth rates. Conversely, low temperatures can impede growth by slowing metabolic processes. Sudden temperature changes or prolonged exposure to unfavorable conditions can reduce efficiency or even lead to cell death. Certain algae species can adapt to temperature variations by adjusting physiological and metabolic processes. Understanding these temperature demands and adaptability can enhance cultivation and production of specific algae species.<sup>73</sup>

*Temperature Control* effective algae production necessitates precise temperature control. Various techniques, including

cooling or heating systems, insulation, water movement, and thermal sensors, can manage temperature based on the cultivation system. Maintaining stable growth conditions is crucial for maximum productivity. Different algae species and specific cultivation goals might require varying temperature ranges, hence understanding thermal preferences is vital for optimal growth and productivity.<sup>74</sup>

*Carbon Dioxide (CO<sub>2</sub>) Impact* (CO<sub>2</sub>) concentration significantly affects algae growth and production. Algae utilize (CO<sub>2</sub>) in photosynthesis to convert light into chemical energy. Higher (CO<sub>2</sub>) concentrations accelerate growth by enhancing photosynthesis.<sup>75</sup>

*Carbon Accessibility* (CO<sub>2</sub>) is a vital nutrient for algae growth. Elevated (CO<sub>2</sub>) levels in the culture medium provide an abundant carbon supply essential for metabolic processes like lipid synthesis and cell division. Inadequate (CO<sub>2</sub>) availability can limit algal development and productivity.<sup>76</sup>

*(CO<sub>2</sub>) Limitation* in closed cultivation systems or natural environments, (CO<sub>2</sub>) levels may gradually decrease as algae use it for photosynthesis. Insufficient (CO<sub>2</sub>) slows algal growth and reduces productivity. Thus, maintaining a consistent and adequate (CO<sub>2</sub>) supply is crucial for sustaining optimal growth rates.<sup>77</sup>

*Algae Cultivation for Carbon Capture and Utilization (CCU)* algae cultivation serves as an effective technique for carbon capture and utilization. It involves growing algae in environments with high (CO<sub>2</sub>) concentrations, like corporate flue gas emissions. Algae use these (CO<sub>2</sub>) emissions to generate biomass and biofuels, thereby reducing environmental impact. (CO<sub>2</sub>) enrichment procedures, achieved through introducing pure (CO<sub>2</sub>) gas or utilizing devices cycling (CO<sub>2</sub>)-rich gases, significantly enhance algal growth and productivity.<sup>78</sup>

*pH Control* (CO<sub>2</sub>) absorption during photosynthesis elevates the pH of the growth medium. Proper pH levels are crucial for algal growth, as extremely high pH can have adverse effects. Managing pH through (CO<sub>2</sub>) detection and supplementation maintains a favorable atmosphere for optimal growth.<sup>79</sup>

*(CO<sub>2</sub>) Sources* (CO<sub>2</sub>) can be sourced from various places such as biogas, (CO<sub>2</sub>)-rich waste gases, and industrial flue exhausts. Effective utilization of these (CO<sub>2</sub>) sources not only reduces greenhouse gases but also provides an affordable and sustainable carbon source for algae development. Understanding and managing (CO<sub>2</sub>) concentration is vital, as ideal levels vary based on algae species, cultivation methods, and growth conditions.<sup>3</sup>

*Utilizing (CO<sub>2</sub>) for Algae Cultivation* algae cultivation effectively harnesses (CO<sub>2</sub>) for carbon sequestration and the production of essential biomass and natural goods. Aeration and mixing play pivotal roles in promoting the growth and productivity of algae.<sup>80</sup>

*Nutrient Distribution* mixing ensures even distribution of nutrients within the culture medium, providing all algae cells with essential components necessary for proper growth and maximum productivity.<sup>81</sup> *(CO<sub>2</sub>) Dispersion*: effective mixing facilitates uniform dispersion of (CO<sub>2</sub>) throughout the medium. This aids algal cells in utilizing (CO<sub>2</sub>) for biomass production,

crucial for photosynthesis. Uniform (CO<sub>2</sub>) concentration is maintained, and limitations are avoided through adequate mixing.<sup>82</sup> *Sun Penetration*: mixing prevents stagnant areas within the growth medium, ensuring continuous movement that allows light penetration. Stagnation can hinder light entry, reducing available light energy for photosynthesis.<sup>83</sup>

*The Role of Effective Mixing and Aeration in Algae Cultivation*. *Light Dispersion* effective mixing ensures uniform light dispersion, ensuring each algal cell receives sufficient light for development. *Preventing Algae Settlement*: algae cells typically float in the growth medium, but settling reduces the effective population density. Mixing prevents settling, enhancing algal access to nutrients, light, and (CO<sub>2</sub>).<sup>84</sup> *Balancing Shear Stress*: while mixing is crucial, excessive shear stress from agitation can harm algal cells and decrease output. Striking a balance between minimizing shear stress and increasing mixing intensity is vital for healthy growth.<sup>85</sup> *Gas Exchange*: aeration is vital for maintaining aerobic conditions and involves supplying oxygen and expelling (CO<sub>2</sub>). Enhanced dissolved oxygen levels support algal respiration and metabolic activities. Additionally, it helps remove excess (CO<sub>2</sub>) to prevent potential harm to algae.<sup>86</sup>

*Algal Cell Motility* some algae species can move within their growing media, enhancing access to light, nutrients, and (CO<sub>2</sub>). Mixing and aeration prevent cell agglomeration and promote uniform distribution, aiding motile algae in their efficient utilization of resources and growth.<sup>85</sup> *Biofilm Prevention*: proper mixing and aeration prevent the formation of biofilms on the growth medium's surface or vessel walls. Biofilms can impede light penetration, nutrient availability, and gas exchange, adversely affecting algal growth. Maintaining a clean growth environment helps prevent biofilm formation.<sup>87</sup>

The ideal mixing and aeration settings vary based on the algal species, system layout, and operating conditions. Balancing intensity and duration is crucial to prevent excessive energy use and cell damage, fine-tuning these parameters according to the specific demands of the algae strain can significantly enhance growth and yield.<sup>88</sup>

*Microbial Contamination* unwanted microbes like bacteria, fungi, and protozoa can contaminate algae cultures, reducing productivity by competing for resources. To prevent microbial contamination, stringent hygiene measures including maintaining sterile conditions and employing proper sterilization procedures are necessary.<sup>89</sup> *Algal Contamination*: coexistence of various algal species or strains can occur, with some strains potentially outcompeting others. Unwanted algal species might reduce overall output by depleting shared resources or hindering the establishment of desired algae strains.<sup>90</sup>

*Impact of Environmental Contaminants* heavy metals, pesticides, and other pollutants negatively affect algal growth and productivity by accumulating in their biomass. Employing clean water sources and safeguarding the growth system against external pollutants are essential for maintaining a regulated growth environment.<sup>91,92</sup> *Cross-Contamination and Ecological Effects*: shifting or handling algal cultures between different systems risks cross-contamination. Containment measures, like barriers

or closed cultivation systems, prevent escaped algal cultures from disturbing local ecosystems. Regular surveillance helps detect contamination, enabling timely intervention.<sup>93,94</sup>

*Salinity and Algal Adaptation* salinity levels significantly impact algal growth. Algal species have varying salinity needs based on their habitat. Matching algal strains with suitable salinity conditions is crucial for optimal growth.<sup>95</sup> Osmotic Stress and Algal Metabolism: exposure to salinity extremes leads to osmotic stress in algae. High salinity may dehydrate algal cells, while low salinity can cause excessive water intake, impacting metabolism and growth. Maintaining salinity within the ideal range reduces osmotic stress and enhances productivity.<sup>96,97</sup>

*Nutrient Availability and Adaptation* salinity influences nutrient availability in the growth medium. Coastal or brackish water habitats often have higher concentrations of essential nutrients like phosphorous, nitrogen, and trace elements. Algae adapted to such environments might have specific nutritional requirements or uptake methods.<sup>98</sup> Maintaining adequate nutritional intake and monitoring is crucial for optimal development under varying salinity conditions. Salt Toxicity and Algal Response: excessive salinity can poison algal cells, disrupting cellular functions, photosynthesis, and causing oxidative stress. While freshwater algae are more vulnerable, algae strains adapted to high salt conditions may resist or regulate salt concentrations. To ensure healthy growth, salinity levels must remain within the tolerance range of the specific algal strain.<sup>99</sup>

*Species Selection and Salinity Tolerance* when selecting algal strains for cultivation, considering their salinity requirements is vital. Matching the salt tolerance of the chosen algal strain with the growth system's salinity conditions can enhance productivity. Different strains within a species may demonstrate varying salinity tolerances, impacting growth and productivity.<sup>19</sup> Adaptation to Growth Stage: salinity needs might vary at different growth stages. Adjusting salinity levels accordingly can potentially enhance productivity in algae-growing systems. Understanding and modifying salinity requirements based on growth stages can significantly impact algal growth and yield.<sup>100</sup>

*Nutrient Availability and Algal Growth* algae require a balanced supply of essential nutrients like nitrogen, phosphorous, potassium, and trace elements for growth and metabolic functions. Adjusting the growth substrate's composition to meet specific nutrient needs is essential. Supplementing nutrients, such as fertilizers or organic matter, might be necessary to ensure adequate nutrient availability.<sup>50,101</sup>

Algae utilize atmospheric carbon dioxide (CO<sub>2</sub>) for photosynthesis. A sufficient and accessible (CO<sub>2</sub>) source must be provided in the growth medium to enable effective photosynthetic activity. (CO<sub>2</sub>) can be offered in various forms, such as carbonates (CO<sub>3</sub><sup>2-</sup>), dissolved (CO<sub>2</sub>) gas, or bicarbonate ions (HCO<sub>3</sub><sup>-</sup>). Certain cultivation systems employ carbon dioxide enrichment methods or the utilization of carbon-rich flue gases to enhance algal growth and productivity. Salinity refers to the salt concentration in the growth medium. Different algal species exhibit varying salinity preferences; some thrive in freshwater while others prefer brackish or saline environments. Adjusting the growth medium's salinity to correspond with the

ideal salt range for the particular algal strain being cultivated is crucial for optimal growth.<sup>102</sup>

Heat and light, though not directly part of the growth medium's composition, significantly influence algal growth and production. Maintaining an adequate temperature and providing sufficient, appropriate light exposure are essential for optimal growth.<sup>103</sup> Preventing contamination is vital for uninterrupted algal development. Ensuring the growth medium is created and maintained in a contamination-resistant manner by following proper sterilization procedures and routinely checking its microbiological quality is essential.<sup>9,104</sup> The growth medium's composition needs careful planning and optimization to provide the necessary nutrients, carbon sources, pH balance, salinity levels, and other conditions required for the specific algal types or strains being cultivated.<sup>105</sup> Regular monitoring and adjustments to the growth medium's characteristics and composition are vital for ensuring optimal development and production in algal cultivation systems.<sup>106</sup>

## Bio-hydrogen production from bio-algae

Bio-hydrogen (BH) production, a technique involving the generation of hydrogen dioxide (H<sub>2</sub>) from diverse biological sources, holds promise as a sustainable and clean fuel, making it an attractive renewable energy technology.<sup>107</sup> The pathway for BH production typically involves several phases, which can vary depending on the specific technique employed. Figure 2 represents the bio-hydrogen production pathway illustration. The choice of feedstock plays a critical role in the BH production pathway, impacting process availability, substance, and overall efficiency.<sup>108</sup> Several variables, including feedstock availability, cost, environmental sustainability, and compatibility with the chosen BH production process, influence the decision about the type of feedstock.<sup>107</sup> Table 2 gives the comparative analysis of Hydrogen Production Techniques from Algae.

Biomass refers to organic materials derived from plants or similar biological sources. It encompasses specific energy crops like switchgrass and miscanthus, agricultural residues like maize stover and wheat straw, forestry residues like wood chips and sawdust, and even organic waste streams like food scraps and animal manure.<sup>120</sup> Biomass feedstocks are appealing due to their abundance and renewability. Microalgae, small plants and algae capable of photosynthesis, convert carbon dioxide and solar energy into biomass. Algae have rapid growth rates and can produce substantial biomass whether cultivated in closed photo bioreactors or open ponds. Certain types of algae are suitable for BH production as they also generate hydrogen through photo-fermentation processes.<sup>121</sup>

### Overview of BH production pathway

Various organic waste streams like food waste, agricultural residues, and wastewater serve as viable feedstocks for bio-hydrogen production. These organic materials can undergo anaerobic fermentation to generate hydrogen. Utilizing organic waste not



only offers a sustainable energy source but also benefits the environment and aids in waste management.<sup>122</sup> Lignocellulosic biomass, consisting of complex sugars like hemicellulose and cellulose found in agricultural and forestry residues, can be converted into hydrogen through biochemical or thermal processes. However, pretreatment processes are often necessary to simplify the structures and enhance accessibility to microorganisms for efficient hydrogen production.<sup>123</sup> Volatile substrates like syngas (a mix of carbon monoxide, carbon dioxide, and hydrogen) and methane (CH<sub>4</sub>) can serve as feedstocks for bio-hydrogen production. Biogas from organic waste decomposition produces methane, while syngas can be derived from the combustion of carbon-rich materials like wood.<sup>30</sup>

Choosing a feedstock for bio-hydrogen production involves several considerations, such as availability, cost, long-term sustainability, compatibility with the chosen production method, and overall energy balance. An ideal feedstock would contain ample carbohydrates or organic matter easily convertible into hydrogen by selected microbes or conversion technologies. Additionally, environmental friendliness in the material's sourcing is crucial for sustainable BH production.<sup>29</sup>

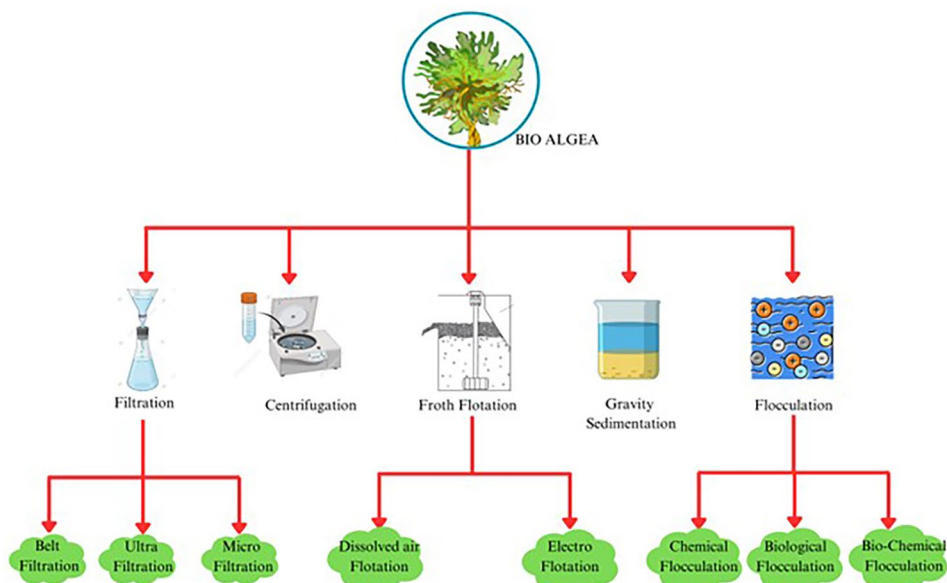
Pretreatment is a critical step in BH production, enhancing feedstock accessibility and bioavailability for subsequent hydrogen generation processes. The choice of pretreatment method is dictated by the feedstock properties and the specific biological hydrogen production process being employed. Physical pretreatment methods involve physical actions to weaken the feedstock's structure and increase accessibility to enzymes or microorganisms. Techniques like milling, grinding, or shredding are employed to reduce particle size and enhance surface area. Heat-based methods, such as steam explosion or microwave heating, can also be utilized to disrupt cellulose structures and facilitate enzymatic or microbiological access to the feedstock.<sup>7,49</sup>

Chemical pretreatment techniques involve using chemicals to alter the composition or structure of the feedstock. Common

methods include acid hydrolysis, alkaline treatment, and oxidative delignification. Acid hydrolysis, using mild acids like sulfuric acid or hydrogen peroxide, breaks down complex carbohydrates into simpler sugars.<sup>124</sup> Alkaline treatment aids in lignin removal and hemicellulose solubilization, while oxidative delignification breaks down lignin using oxidizing agents like hydrogen peroxide or ozone.<sup>16</sup> These processes employ bacteria or enzymes to break down complex feedstock structures. Utilizing lignocellulolytic organisms or bacteria that produce enzymes capable of degrading cellulose, hemicellulose, and lignin is one such method. Biological pretreatment is often combined with other pre-processing techniques to enhance biomass conversion efficiency. The primary objectives of pretreatment in bio-hydrogen (BH) production are to facilitate better enzymatic and microbial access to the feedstock, increase carbohydrate accessibility, reduce inhibitory substances, and enhance overall process efficiency.<sup>125</sup> To optimize pretreatment techniques, a balance between biomass disintegration efficiency and overall process demands must be achieved. The choice of pretreatment method depends on various factors, including feedstock composition, preferred hydrogen production technology (e.g., dark fermentation or photofermentation), and specific BH system requirements. Figure 3 represents the bio-hydrogen production by photobiological way. Optimization of pretreatment conditions is crucial to maximize feedstock bioconversion, yield, and efficiency in BH production.<sup>126</sup>

The conversion of organic material into hydrogen gas through microorganism metabolic processes during fermentation is a vital stage in BH manufacturing. Dark fermentation and photofermentation are the two primary fermentation techniques used. Dark fermentation occurs in the absence of light and involves a consortium of hydrogen-producing microbes breaking down organic substrates, such as complex organic compounds or carbohydrates, anaerobically (Fig. 4). Typically, this fermentation process occurs in a bioreactor under

**Figure 2.** Different harvesting ways of Bio Algae.



**Table 2.** Optimizing algal cultivation for sustainable biofuel production.

Types of algae	Chemicals present	Biomass content	Lipids content	Production pathway	Hydrogen production rate	References
<i>Chlorella</i>	Chlorophyll, Carotenoids, Proteins, Polysaccharides	40–60%	15–25%	Dark Fermentation	0.5–1.5 mmol H <sub>2</sub> /g biomass/h	109
<i>Spirulina</i>	Phycocyanin, Carotenoids, Proteins, Polysaccharides	50–70%	10–20%	Anaerobic Fermentation	1–2 mmol H <sub>2</sub> /g biomass/h	110
<i>Nannochloropsis</i>	Chlorophyll, Fucoxanthin, Proteins, Lipids	30–50%	20–30%	Photobiological Process	0.8–1.2 mmol H <sub>2</sub> /g biomass/h	4
<i>Dunaliella</i>	Beta-carotene, Glycerol Digalactoside, Lipids	30–50%	15–25%	Mixotrophic Fermentation	0.5–1 mmol H <sub>2</sub> /g biomass/h	85
<i>Porphyridium</i>	Phycobiliproteins, Polysaccharides, Lipids	40–60%	10–20%	Heterotrophic Fermentation	0.8–1.5 mmol H <sub>2</sub> /g biomass/h	111
<i>Isochrysis</i>	Fatty Acids, Lipids, Carbohydrates, Proteins	30–50%	15–25%	Mixotrophic Fermentation	0.5–1.2 mmol H <sub>2</sub> /g biomass/h	17
<i>Tetraselmis</i>	Chlorophyll, Carotenoids, Proteins, Lipids	20–40%	10–20%	Anaerobic Fermentation	0.3–0.8 mmol H <sub>2</sub> /g biomass/h	18
<i>Botryococcus</i>	Botryococcenes, Lipids, Polysaccharides	40–60%	20–30%	Green Algal Fermentation	0.8–1.5 mmol H <sub>2</sub> /g biomass/h	112
<i>Ulva</i>	Chlorophyll, Carotenoids, Polysaccharides, Lipids	50–70%	15–25%	Photobiological Process	1–2 mmol H <sub>2</sub> /g biomass/h	113
<i>Scenedesmus</i>	Chlorophyll, Carotenoids, Proteins, Lipids	30–50%	10–20%	Anaerobic Fermentation	0.5–1 mmol H <sub>2</sub> /g biomass/h	4
<i>Dictyochloropsis</i>	Chlorophyll, Carotenoids, Polysaccharides, Lipids	40–60%	20–30%	Heterotrophic Fermentation	0.8–1.5 mmol H <sub>2</sub> /g biomass/h	114
<i>Porphyra</i>	Phycobiliproteins, Carotenoids, Polysaccharides, Lipids	50–70%	15–25%	Mixotrophic Fermentation	1–2 mmol H <sub>2</sub> /g biomass/h	115

**Table 2.** (continued)

Types of algae	Chemicals present	Biomass content	Lipids content	Production pathway	Hydrogen production rate	References
<i>Botryococcus</i>	Botryococenes, Lipids, Polysaccharides	30–50%	10–20%	Green Algal Fermentation	0.5–1 mmol H <sub>2</sub> /g biomass/h	116
<i>Chlamydomonas</i>	Chlorophyll, Carotenoids, Proteins, Lipids	40–60%	20–30%	Anaerobic Fermentation	0.8–1.5 mmol H <sub>2</sub> /g biomass/h	117
<i>Arthrospira</i>	Phycocyanin, Carotenoids, Proteins, Polysaccharides	20–40%	5–10%	Photobiological Process	0.3–0.8 mmol H <sub>2</sub> /g biomass/h	118
<i>Gracilaria</i>	Phycobiliproteins, Polysaccharides, Lipids	30–50%	15–25%	Heterotrophic Fermentation	0.5–1.2 mmol H <sub>2</sub> /g biomass/h	119
<i>Porphyra</i>	Chlorophyll, Phycobiliproteins, Polysaccharides, Lipids	40–60%	10–20%	Anaerobic Fermentation	0.8–1.5 mmol H <sub>2</sub> /g biomass/h	66
<i>Chlorella</i>	Chlorophyll, Carotenoids, Proteins, Lipids	50–70%	15–25%	Green Algal Fermentation	1–2 mmol H <sub>2</sub> /g biomass/h	109
<i>Scenedesmus</i>	Phycocyanin, Carotenoids, Proteins, Polysaccharides	30–50%	10–20%	Mixotrophic Fermentation	0.5–1 mmol H <sub>2</sub> /g biomass/h	77
<i>Dictyota</i>	Chlorophyll, Carotenoids, Polysaccharides, Lipids	40–60%	20–30%	Anaerobic Fermentation	0.8–1.5 mmol H <sub>2</sub> /g biomass/h	84
<i>Tetraselmis</i>	Chlorophyll, Carotenoids, Proteins, Lipids	20–40%	10–20%	Photobiological Process	0.3–0.8 mmol H <sub>2</sub> /g biomass/h	18
<i>Scenedesmus</i>	Phycocyanin, Carotenoids, Proteins, Polysaccharides	30–50%	10–20%	Anaerobic Fermentation	0.5–1 mmol H <sub>2</sub> /g biomass/h	77
<i>Isochrysis</i>	Fatty Acids, Lipids, Carbohydrates, Proteins	30–50%	15–25%	Mixotrophic Fermentation	0.5–1.2 mmol H <sub>2</sub> /g biomass/h	85

Table 2. (continued)

Types of algae	Chemicals present	Biomass content	Lipids content	Production pathway	Hydrogen production rate	References
<i>Gracilaria</i>	Phycobiliproteins, Polysaccharides, Lipids	30–50%	15–25%	Green Algal Fermentation	0.5–1.2 mmol H <sub>2</sub> /g biomass/h	115

carefully controlled conditions.<sup>47</sup> Initially, the organic matter substrate undergoes hydrolysis and is transformed into simpler molecules, primarily sugars, before dark fermentation begins. Subsequently, fermentative bacteria, such as facultative anaerobic microbes or obligate anaerobes, metabolize these sugars, resulting in the production of hydrogen as a metabolic by-product.<sup>9</sup>

This metabolic pathway involves various enzymatic processes, including fermentation and glycolysis, leading to the formation of organic acids, hydrogen, carbon dioxide, and other metabolites. Dark fermentation offers advantages such as high hydrogen yields, rapid fermentation rates, and the ability to use diverse organic substrates for BH production. Challenges include the generation of metabolic by-products (e.g., organic acids) that may hinder hydrogen production and competition for substrates among different microbial species in a mixed population. Photofermentation (Fig. 5), on the other hand, is a process where photosynthetic bacteria use light to convert organic substrates into hydrogen.<sup>80</sup> Photofermentation, in contrast to dark fermentation, employs specific photosynthetic microbes like purple non-sulfur bacteria (such as *Rhodobacter* spp., *Rhodospseudomonas* spp.), algae, or green sulfur bacteria. These photosynthetic bacteria utilize light energy during photofermentation to break down organic substances such as sugars or gaseous fatty acids into protons and other by-products. Various light-driven reactions, including sunlight and hydrogenase-mediated hydrogen generation, are involved in this process.<sup>127</sup> Photofermentation allows the BH manufacturing process to be powered by solar energy, which is advantageous for sustainable and renewable hydrogen generation. Both dark fermentation and

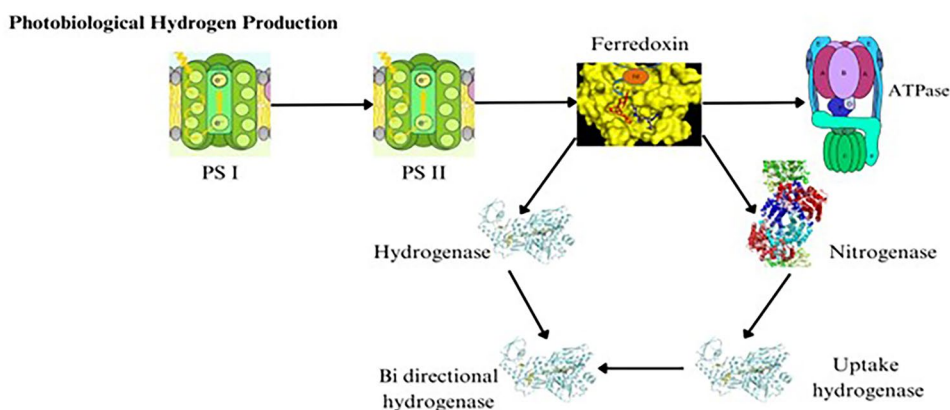
photofermentation have undergone extensive research and optimization for bio-hydrogen production. Factors like pH, moisture content, substrate concentration, and the composition of the microbial community or culture significantly influence the efficiency and productivity of the fermentation process. Additional techniques, such as consortium selection, process optimization, and vessel design, are used to enhance the yield and speed of BH production.<sup>12</sup>

Ultimately, fermentation plays a critical role in BH production and presents promising prospects for ecologically sound and renewable hydrogen synthesis from various organic substrates. Ongoing research and development activities are focused on improving fermentation procedures, optimizing microbial consortia, and exploring novel substrates to enhance the efficiency of BH generation and make it a viable alternative energy source.<sup>128</sup>

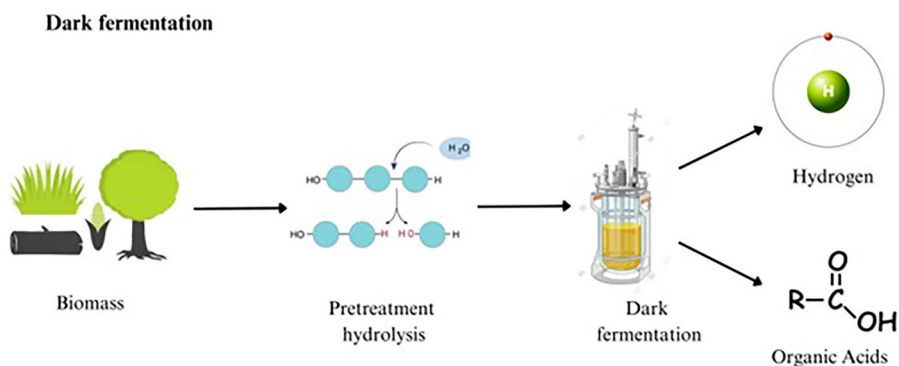
*Hydrogen Segregation and Recovery* the isolation and purification of hydrogen gas produced during the fermentation process are essential steps in creating the BH pathway. These procedures aim to generate high-purity hydrogen gas applicable for various purposes, including energy production. The selection of a method for hydrogen separation and recovery depends on several factors, such as production volume, required purity, and cost-effectiveness.<sup>123</sup>

Here are several frequently used methods. Pressure Swing Adsorption (PSA) is a popular technique for separating and recovering hydrogen. It relies on the gas adsorption capacity of specific adsorbents at varying pressures. The hydrogen-containing fermentation gas is passed through a bed of adsorbent material (typically activated carbon or zeolites) under high pressure.

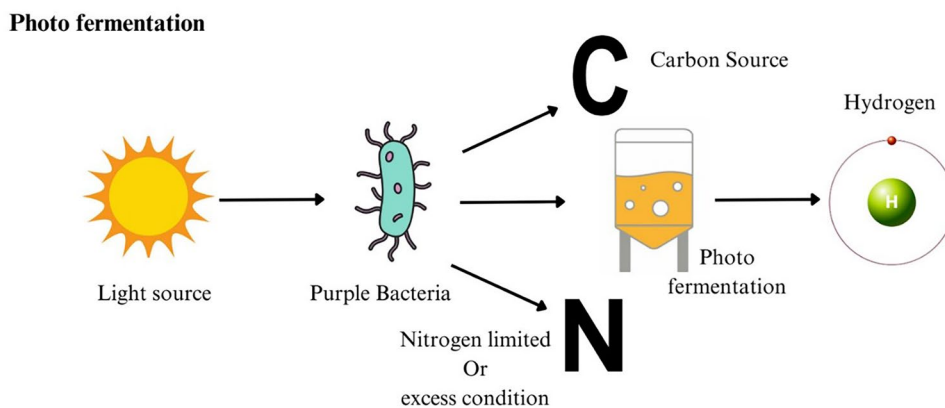
Figure 3. Bio-hydrogen by Photobiological way.



**Figure 4.** Bio-hydrogen Production by Dark Fermentation Process.



**Figure 5.** Bio-hydrogen production by Photofermentation process.



Hydrogen atoms primarily adhere to the adsorbent material, unlike other gases like carbon dioxide and methane. Subsequently, the adsorbed hydrogen is recovered after the adsorbent bed is depressurized and the liberated gases are purged.<sup>129</sup>

This technique uses selective membranes to separate gas within a fermentation gas mixture. The membranes' pores or channels allow small hydrogen molecules to pass through, while larger molecules like atmospheric carbon dioxide or methane cannot. Applying pressure to the gas mixture across the membrane enables preferential flow and accumulation of hydrogen on the other side.<sup>130</sup> Membrane separation offers advantages like simplicity, scalability, and minimal energy usage. Cryogenic Separation method utilizes differences in gas boiling points. By cooling the fermentation gas to cryogenic levels ( $-253\text{ }^{\circ}\text{C}$  or lower), gases with varying boiling points can be separated. Hydrogen, having a lower boiling point, precipitates out, while other gases remain in the gaseous state. However, this technique demands substantial energy for chilling and is commonly employed in large-scale hydrogen production facilities.<sup>131</sup>

Chemical solvents, such as water-soluble amine solutions, selectively absorb hydrogen from the fermentation gas, while other gases remain in the gas phase. Adjusting temperature or pressure allows the solvent to release the absorbed hydrogen for recovery.<sup>132</sup> Electrochemical cells or membranes enable the selective movement of hydrogen ions ( $H^+$ ) while excluding other gas molecules. Applying an electric potential across the membrane

facilitates the separation of hydrogen from other gases.<sup>27</sup> This method is energy efficient and yields highly pure hydrogen.

Hydrogen gas, once separated and recovered, undergoes additional purification steps, such as cooling or contaminant removal, before being used for various applications. Efficient hydrogen separation and recovery are essential for eco-friendly bio-hydrogen production systems, utilizing electricity as a renewable energy source.<sup>133</sup> Ongoing research aims to enhance separation methods, reduce energy requirements, and boost the overall effectiveness of bio-hydrogen production pathways.

Hydrogen gas fuels electrochemical reactors in hydrogen fuel cells, generating electricity. This electricity powers diverse applications, from appliances to automobiles and even grid-scale systems, producing only water as a by-product.<sup>134</sup> Hydrogen serves as a critical fuel source in industrial processes, including methanol and ammonia synthesis, and in applications requiring high temperatures or reducing atmospheres, such as metal treatment and petroleum refining.<sup>102</sup> Fuel cell vehicles (FCVs) utilize hydrogen gas as a fuel source, converting it and airborne oxygen into electricity to power the vehicle, emitting only water vapor. The infrastructure for FCVs, including hydrogen refueling stations, is still under development in many areas. Storage options such as metal hydrides, cryogenic liquid storage, and high-pressure gas cylinders are used to store hydrogen gas for future use. Advances in storage systems aim to improve space, safety, and usability.<sup>54</sup>

In power-to-gas applications, surplus renewable electricity, such as that generated from wind or solar sources, can be harnessed to electrolyze water, producing hydrogen gas. This hydrogen can then be stored underground in caverns or injected into natural gas pipelines. This process facilitates the storage of renewable energy in the form of hydrogen for future use. Biogas upgrading involves combining bio-hydrogen with biogas obtained from organic waste. By adding hydrogen to biogas, which mainly comprises methane, its energy content is increased, enhancing its combustion properties.<sup>62</sup> The resulting bio-methane, enriched with hydrogen, serves as a sustainable alternative for natural gas, suitable for heating, electricity generation, or as a vehicle fuel.

The choice of utilization or storage methods is influenced by various factors, including infrastructure availability, energy requirements, environmental considerations, and specific project needs.<sup>135</sup> Ongoing advancements in hydrogen storage and utilization technologies aim to enhance efficiency, cost-effectiveness, and overall effectiveness, supporting the transition to a green hydrogen-based economy. It is essential to note that the specific procedures and technologies employed may vary across different bio-hydrogen production processes. Continuous research and technological innovations strive to explore new pathways and improve the sustainability and efficiency of bio-hydrogen generation.<sup>113</sup>

### *Factors affecting bio-hydrogen production from bio-algae*

Bio-hydrogen production from algae is influenced by numerous factors that impact algae growth, photosynthesis efficiency, and the metabolic processes for hydrogen manufacture. Optimizing these factors is vital for maximizing bio-hydrogen production. Photosynthesis, the process converting light energy into chemical energy, is light dependent. While increased light levels promote photosynthesis and biomass production, excessive intensity may cause photo-inhibition, reducing hydrogen synthesis. Moreover, the light spectrum influences respiration effectiveness and hydrogen generation.<sup>136</sup> Algae require specific temperature ranges for optimal hydrogen production and growth. Different algae species thrive in varying temperature conditions. Higher temperatures can accelerate metabolic processes but may stress algae or impede enzymes crucial for hydrogen production. Maintaining ideal temperatures is crucial for maximizing bio-hydrogen synthesis.<sup>137</sup>

Adequate (CO<sub>2</sub>) levels are essential for algae photosynthesis. Elevated (CO<sub>2</sub>) concentrations enhance algal growth, boosting biomass and hydrogen production. Various sources like direct injection, biogas, or industrial exhaust emissions significantly affect bio-hydrogen production based on (CO<sub>2</sub>) availability and concentration.<sup>138</sup> Efficient mixing and aeration in the growth medium ensure uniform nutrient distribution, homogeneous culture, and proper gas exchange. Mixing prevents algal sedimentation, encourages light penetration, and enhances gas movement, crucial for nutrient and oxygen delivery to algae cells, thus increasing bio-hydrogen production.<sup>139</sup>

Undesirable microbe contamination significantly hampers bio-hydrogen production. Competition for nutrients, release of inhibitory substances, and changes in microbial communities can negatively impact algae growth and hydrogen production. Employing closed systems, strict hygiene protocols, and proper sterilization minimizes contamination, improving bio-hydrogen output. Algal growth and metabolism are affected by the salt level in the growth medium. Different algae species exhibit varying salinity preferences, influencing their metabolism, nutrient intake, and osmotic balance. Optimal bio-hydrogen production entails selecting suitable algae species for specific salinity settings and optimizing salinity levels.<sup>140</sup>

Maximizing bio-hydrogen generation from algae necessitates balancing and optimizing these variables, including selecting appropriate algae strains and culture methods. Creating conducive conditions for effective bio-hydrogen production requires careful consideration and control of the interdependent components.<sup>141</sup>

### *Reactor designs and configurations for bio-hydrogen production from bio-algae*

The design and setup of a reactor play a crucial role in bio-hydrogen production from bio-algae. Factors such as the type of algae, growing conditions, hydrogen production methods, and operational scale significantly influence the choice of reactor design. Here are several commonly used reactor types for bio-hydrogen production from algae.<sup>142</sup> Photo-bioreactors (PBRs) provide controlled environments for algae growth and hydrogen production. PBRs, available in columnar, flat-panel, or tubular shapes, optimize gas exchange, mixing, and light penetration. They often utilize transparent materials like glass or plastic for efficient light utilization. PBRs offer precise control over culture conditions, nutrient availability, and (CO<sub>2</sub>) delivery, thereby enhancing biomass growth and hydrogen production.<sup>143</sup>

Sealed systems designed to offer a controlled environment for algae growth. These reactors, which can be flat-panel, airlifts, or cylindrical, provide advantages, such as increased biomass productivity, better control over growth conditions, reduced contamination risk, and enhanced hydrogen production efficiency. However, they require higher construction costs and energy inputs compared to open pond systems. Economical and straightforward, open pond systems are utilized for large-scale bio-algae cultivation. These ponds, which can be shallow or deep, utilize solar energy for photosynthesis. Benefits include minimal setup and running costs, ease of maintenance, and scalability. However, susceptibility to contamination, land requirement, and lack of environmental control can hinder hydrogen generation effectiveness.<sup>144</sup>

These reactors integrate membrane filtration technology with algae cultivation. Offering continuous operation and the ability to separate algal biomass and culture media, they enable high biomass concentration and improved hydrogen production.

Membrane bioreactors reduce contamination risks and streamline biomass harvesting processes. Combining various reactor designs or culture techniques, hybrid systems aim to increase bio-hydrogen production. For instance, integrating open ponds with closed photo-bioreactors harnesses the benefits of both systems, allowing extensive cultivation in ponds while maintaining controlled conditions for enhanced biomass output and hydrogen production.

Splitting hydrogen production and cultivation processes, these systems first optimize algae growth conditions and then expose the residue to specific stimuli for hydrogen synthesis. They enhance process efficiency and offer better control over hydrogen generation conditions. Maximizing biomass yield and hydrogen output per unit area, stacked systems vertically stack reactors or layers. This design optimizes light accessibility and surface area for algae growth, benefiting both open pond and closed photo-bioreactor layouts. The choice of reactor design depends on operational size, financial considerations, control of growth conditions, hydrogen generation efficiency, and downstream processing needs. Selection should align with the specific goals and limitations of the bio-hydrogen extraction method from algae. Each reactor design has its own set of advantages and limitations.<sup>145</sup>

## Sustainability considerations

The production of bio-hydrogen from algae requires environmentally conscious techniques at every stage of the process. Algae farming involves cultivating microorganisms through photosynthesis to produce biomass from sunlight and carbon dioxide. Sustainable algae farming aims to enhance biomass production while minimizing water and energy consumption. A fundamental aspect of sustainability involves using reused or treated water.<sup>69</sup> Algae farming methods often utilize sewage or alternative sources of filtered water instead of scarce freshwater supplies. This practice reduces the overall environmental impact of production processes and conserves water resources. Algae require essential nutrients such as nitrogen, phosphorous, and trace elements to thrive. In sustainable cultivation methods, nutrient levels in the culture medium are carefully managed and optimized. This ensures that algae receive the necessary nutrients for growth while minimizing the excess release of nutrients into the environment. Excessive nutrient discharge can lead to water contamination and the proliferation of harmful algal blooms.<sup>146</sup>

### Environmental sustainability

Sustainable techniques in algae cultivation often integrate organic and eco-friendly substitutes to reduce dependence on synthetic fertilizers and pesticides. Examples include using organic waste or compost as renewable nutrient sources in the growing medium. Integrated pest management methods are employed for pest and disease control without resorting to chemical pesticides. Furthermore, sustainable algae farming explores

energy-saving approaches, such as utilizing renewable energy sources like solar or wind power to fuel cultivation processes.<sup>147</sup> Technologies like bioreactors or raceway ponds may also be employed to maximize solar exposure and enhance biomass productivity. The overarching goal of sustainable algae farming is to minimize the environmental impact of production processes. This involves maximizing biomass productivity while conserving water resources, reducing energy consumption, optimizing nutrient utilization, and minimizing reliance on synthetic fertilizers and pesticides. Biomass production involves using living organisms such as plants or algae to generate energy or valuable products. Algae's photosynthetic capabilities are typically utilized in biomass production.<sup>148</sup>

During photosynthesis, algae convert light energy and carbon dioxide, along with nutrients, into organic compounds. The primary outcome of this process is biomass, consisting of complex organic compounds like carbohydrates, lipids, and proteins. These macromolecules serve as energy stores and structural components within algal cells.<sup>146</sup> Controlled environments such as ponds, bioreactors, or photo-bioreactors are commonly used to facilitate algae growth and biomass production. These systems provide the necessary conditions, including light, nutrients, and carbon dioxide. Algae can be grown in either open water bodies with natural sunlight or confined systems with artificial light. As algae perform photosynthesis, they absorb carbon dioxide, converting it into biomass. The harvested biomass can be separated from the growth medium using techniques like centrifugation, filtration, or sedimentation.<sup>61</sup>

Post-harvest, the biomass can undergo various processes depending on the intended final product. Techniques like transesterification or fermentation can convert it into biofuels, such as biodiesel or bioethanol. Alternatively, the biomass can be utilized to produce valuable substances like pigments, nutraceuticals, or pharmaceuticals. The production of biomass from algae offers numerous advantages.<sup>12</sup> Algae exhibit a rapid growth rate and higher biomass production per unit area compared to traditional crops. They can be cultivated on non-arable land and utilize resources like carbon dioxide and wastewater, potentially reducing environmental impacts. Additionally, algae's capacity for carbon dioxide absorption during photosynthesis aids in carbon capture, contributing to mitigating greenhouse gas emissions.<sup>8</sup> The generation of biomass from algae through photosynthesis holds great promise for sustainable energy production and the creation of valuable products, offering multifaceted benefits for the environment and various industries.

Algae are utilized in biophotolysis to produce hydrogen gas, leveraging water and sunlight. This process involves feeding algae with water and exposing them to light to promote photosynthesis.<sup>149</sup> During photosynthesis, algae convert carbon dioxide and water into oxygen and carbohydrates, thereby converting sunlight into chemical energy.<sup>51</sup> Some algae species possess hydrogenase enzymes that facilitate the production of hydrogen gas as a by-product of photosynthesis during biophotolysis. These enzymes aid in splitting water molecules ( $H_2O$ ) into oxygen ( $O_2$ ) and hydrogen gas ( $H_2$ ).

The collected hydrogen gas from algae can serve various purposes. As a versatile energy source, hydrogen can power fuel cells to generate electricity, fuel vehicles, and participate in chemical reactions. It is considered an environmentally friendly energy source as it does not emit harmful pollutants or greenhouse gases during combustion or utilization.<sup>56</sup> Algae-based hydrogen production presents several advantages, including the ability to thrive in diverse conditions, effective utilization of sunlight for biomass production, and carbon dioxide sequestration during photosynthesis.<sup>42</sup> Despite the potential of algae-based biophotolysis, widespread application faces challenges. These include enhancing hydrogen production rates, improving hydrogenase enzyme efficiency, expanding algal cultivation systems, and developing cost-effective harvesting and processing techniques. To transform algae-based hydrogen production into a feasible and sustainable energy alternative, continuous research and technological advancements are necessary.

Algae need specific environmental conditions to limit oxygen access to produce hydrogen.<sup>94</sup> Algae typically require a carbon source like carbon dioxide for photosynthesis and growth. Restricting the readily available carbon source forces algae to utilize alternative metabolic routes, such as hydrogen synthesis. Algae use sulfur as a nutrient for metabolism and growth. Decreasing sulfur content alters algae's metabolic processes, favoring hydrogen synthesis.

*Oxygen Deprivation* restricting aeration or sealing the culture to limit oxygen exchange induces anaerobic conditions, leading algae to shift from aerobic metabolism to anaerobic fermentation, potentially resulting in hydrogen production. Once captured, hydrogen produced from algae can serve as a clean and renewable energy source. The process of separating and collecting hydrogen gas generated during fermentation or photofermentation is known as hydrogen recovery. Various separation techniques can be employed to obtain pure hydrogen gas.<sup>4</sup>

Membrane separation represents a common technique used to isolate hydrogen gas from other gases within a system by employing a selectively permeable membrane. This barrier allows hydrogen molecules to pass through while blocking other molecules like carbon monoxide or methane. Another method for hydrogen recovery is Pressure Swing Adsorption (PSA). In PSA, certain gases are selectively adsorbed, while hydrogen passes through an array of adsorbents, such as carbon dioxide or zeolites.<sup>82</sup> Utilizing these separation techniques to recover and utilize the hydrogen produced through fermentation or photofermentation procedures can lead to a more environmentally friendly and sustainable power system.

However, there are still several challenges to address before achieving commercial-scale production of bio-hydrogen (BH) from algae. These hurdles include developing effective and cost-efficient farming structures, selecting strains and implementing genetic modifications to enhance hydrogen production, and optimizing processes to increase yields.<sup>150</sup> To overcome these challenges and enhance the economic viability and scalability of BH production from algae, continuous research and technological advancements are underway.<sup>100</sup>

## Social sustainability

Producing bio-hydrogen (BH) from algae with social sustainability requires a process that benefits society as a whole, considering both local and broader societal impacts. Socially sustainable algal farming requires careful management. Algae are cultivated and harvested for various purposes, such as food production, animal feed, biofuel production, pharmaceutical research, and environmental applications. Choosing the appropriate algae strain for growth is the initial stage. Different strains can be tailored for diverse end uses, each with its unique growth requirements.<sup>135</sup> There are various systems for growing algae, including open ponds or specialized photo-bioreactors. Closed systems like photo-bioreactors regulate environmental factors such as temperature, light, and nutrient supply. Open ponds, larger outdoor basins, may require additional nutritional management and depend on natural sunlight. Algae require essential nutrients like nitrogen, phosphorus, and micronutrients for growth. Light exposure and temperature control are crucial for optimizing photosynthesis and growth.<sup>151</sup>

Once algae reach the desired biomass, different harvesting techniques are employed based on the growth system and intended usage. Common methods include filtration, centrifugation, flocculation, and sedimentation.<sup>129</sup> Involving and collaborating with local communities is integral to the decision-making and project implementation process. Community engagement ensures addressing community concerns, securing their participation, and leveraging the project's benefits in developing algae cultivation and BH production facilities. Various approaches facilitate community involvement. Public consultations allow community members to express their ideas, concerns, and suggestions about the project. These consultations, conducted through open forums, workshops, or public meetings, seek community participation in project specifics.<sup>92</sup>

Sharing information plays a crucial role in fostering community involvement. Clear, accessible information about the project's goals, potential benefits, and associated risks should be disseminated. Various channels such as newsletters, pamphlets, websites, or local presentations are used to keep the community informed and address any concerns or queries they may have, ensuring transparent communication channels.

Community engagement in the establishment of algae culture and bio-hydrogen (BH) production facilities involves several essential components. Encouraging locals to join groups or advisory panels that design projects allows active participation in the decision-making process.<sup>25</sup> Involving community members in project monitoring or assessment procedures fosters a sense of responsibility and control. Ensuring the initiative benefits the community is pivotal. This could involve providing employment opportunities, offering training courses, or supporting local businesses through procurement practices.<sup>94</sup> Establishing mutually beneficial partnerships with regional institutions or organizations creates a collaborative atmosphere, ensuring continuous consultation and active participation of community members in the decision-making process. The overall goal is to garner local support and contribute to community sustainability



by addressing local concerns and ensuring their active involvement and benefits.<sup>9</sup>

Social sustainability of BH, as an energy source, encompasses elements, such as price and accessibility. For a sustainable society, it is crucial to ensure the economic feasibility and cost-effectiveness of producing and utilizing BH, ensuring access for a wide consumer base.<sup>152</sup> Minimizing the cost of BH production involves discovering effective and affordable production methods, like utilizing advanced bioreactors or efficient electrolysis technologies. Lowering production costs contributes to making BH more affordable and accessible to a broader population.

The infrastructure for distributing and utilizing BH should be designed with inclusivity in mind, emphasizing accessibility. This involves expanding the number of refueling stations and integrating BH into existing energy distribution systems. Creating a ubiquitous and accessible infrastructure enables people and communities, especially those in underdeveloped nations, to readily acquire and utilize BH as a sustainable energy source.<sup>153</sup> Addressing the accessibility and affordability of BH becomes crucial in underdeveloped regions dealing with energy poverty and limited access to clean energy sources. Facilitating access to clean and affordable energy sources like bio-hydrogen contributes to social responsibility, supporting economic development, and improving overall living standards in these communities.<sup>134</sup>

“Accessible technology” in the context of producing bio-hydrogen (BH) focuses on designing technical solutions that are usable and affordable for a diverse range of users, including those with impairments or limited resources. The technology should employ machinery that is both cost-effective and easily producible, minimizing barriers to entry and enabling a broader range of stakeholders to participate. Scalability is also essential, allowing the technology to adapt to varying production capacities and meet diverse community needs.<sup>14</sup> BH production procedures should be user-friendly, accommodating individuals with varying technical expertise. Simplifying complex processes, providing clear instructions, and incorporating automation or user-friendly interfaces can streamline the production process.<sup>154</sup>

Providing stakeholders with adequate training and support is essential for effective technology implementation. Conducting workshops, distributing instructional materials, and offering technical support can enhance acceptance and enable broader participation in BH production.<sup>154</sup> Focusing on these accessibility criteria in BH production promotes inclusivity, enabling underserved groups or regions to participate and benefit from clean energy solutions, thereby fostering social sustainability. Economic viability is fundamental for ensuring the social sustainability of BH production from algae. Commercial viability of algal production is critical, considering the balance between potential benefits, generated income, and associated production costs. This viability relies on sufficient funding, proper resource allocation, and cost-effective technology.<sup>155</sup>

Moreover, BH generation should support regional economic growth by creating employment opportunities within the community. Establishing BH plants can generate jobs, thereby reducing unemployment rates and contributing to economic

development.<sup>73</sup> Encouraging local businesses and entrepreneurship in related operations, such as algae farming, processing, and distribution, diversifies the economy and fosters innovation. Adding value across the BH supply chain is vital for economic viability. Converting algae into biofuels or high-value compounds derived from algae processing by-products enhances the economic potential of BH production. This practice adds value and ensures the long-term viability of the regional economy.<sup>90</sup>

In summary, the social sustainability of BH production requires economic viability as a critical component. Factors such as job creation, local entrepreneurship, value addition across the supply chain, and financial feasibility contribute to the process’s financial sustainability and positive impact on the regional economy and community. Integrating these considerations into BH production ensures a more comprehensive and responsible approach, addressing environmental, economic, and social concerns while safeguarding the welfare of stakeholders and communities involved in the process.<sup>79</sup>

### *Economic feasibility*

The economic viability of producing BH from algal biomass hinges on several cost factors that need careful consideration. The choice of culture system significantly impacts expenses. Open ponds often have lower construction costs but may incur higher operational expenses due to water loss, evaporation, and contamination risks. Closed photo-bioreactors, while providing controlled conditions, typically involve higher initial investments. The required infrastructure—such as ponds, tanks, or photo-bioreactors—is determined by the production scale, influencing overall cultivation costs. Maintaining optimal growth conditions for algae requires energy inputs for temperature control, lighting, and mixing. Energy costs, whether from electricity, sunlight, or other sources, affect overall maintenance expenses. Essential tasks like sanitation, monitoring, and nutrient replenishment incur costs related to labor, equipment, and materials.

Establishing cultivation facilities demands capital investments, including land acquisition, infrastructure construction, and equipment purchases. These upfront expenses significantly influence overall cultivation costs. Algae need various nutrients like nitrogen and phosphate for growth. The cost of nitrogen components obtained from sources such as organic waste or synthetic fertilizers contributes to the total production cost. Harvested algal biomass requires processing for conversion into valuable products like biofuels or chemicals. Equipment, energy, and time for procedures like centrifugation, filtration, and drying add to production expenses. It is important to note that the cost of cultivating algal biomass varies based on specific conditions, location, technological advancements, and market dynamics. Ongoing research aims to reduce costs and enhance the economic viability of the algae-growing industry for various applications.

Biomass harvesting involves gathering materials from various sources, such as agricultural waste, energy-specific crops, forestry residue, or algae. The harvesting technique used depends

on the type and properties of the material. Mechanical Harvesting method utilizes specialized equipment like combines, forage harvesting tools, or balers to cut, collect, and transport biomass. It is commonly used for materials like switchgrass, wheat straw, or corn stover.<sup>84</sup>

Biomass harvesting techniques vary based on the source material. In specialized or small-scale applications, manual gathering, albeit labor intensive, is utilized for specific crops like fruits, nuts, and specialty produce. For biomass sourced from aquatic environments, techniques such as filtration, centrifugation, or skimming are employed to gather materials from water bodies, notably from algae or aquatic plants.<sup>11</sup> Once harvested, biomass typically undergoes pretreatment before transformation into biofuels, bioproducts, or bioenergy. Pretreatment aims to dismantle complex biomass structures, reducing inhibitors that might impede subsequent processes and enhancing material accessibility and reactivity.<sup>156</sup>

Hydrogen production efficiency measures the amount of hydrogen gas generated per unit of input or resource, particularly concerning algal biomass. Ongoing research focuses on enhancing hydrogen production efficiency from algal biomass, concentrating on various aspects. Researchers aim to identify optimal conditions for algae growth that promote increased hydrogen production. Factors like pH levels, carbon dioxide concentration, light intensity, temperature, and nutrient availability significantly impact algae's capacity to convert sunlight and nutrients into cellulose and hydrogen gas. Genetic engineering techniques are employed to modify algae strains, either enhancing their inherent hydrogen production capability or incorporating genes from other organisms. Genetic engineering techniques are employed to modify algae strains, either enhancing their inherent hydrogen production capability or incorporating genes from other organisms. These alterations target specific enzymes or metabolic pathways to boost hydrogen yields.<sup>157</sup>

Improving hydrogen recovery methods, upgrading biomass pretreatment techniques, and optimizing extraction and conversion processes are part of ongoing efforts to enhance hydrogen production efficiency. Integrating multiple phases of the hydrogen generation process aims to reduce energy losses and boost overall effectiveness. Continuous enhancements in cultivation conditions, genetic engineering, and process optimizations aim to increase hydrogen output per unit of algal biomass. The ultimate goal is to decrease the overall cost of hydrogen production, rendering it economically viable as a renewable energy source.<sup>156</sup>

Algal biomass, a versatile resource, offers various pathways for sustainable and economically viable products. Algal biomass serves as a precursor for biofuels like biodiesel and bioethanol, offering eco-friendly alternatives to fossil fuels, thus promoting environmental sustainability. Proteins, carbohydrates, and lipids in algae biomass are utilized for producing bioplastics and eco-friendly substitutes to traditional fossil fuel-based plastics, significantly reducing reliance on non-renewable resources and addressing environmental concerns related to plastic waste.

Algal biomass can be converted into premium animal feed, high in vitamins, minerals, and proteins, fostering sustainable

growth in aquaculture and agriculture industries. Extracting pigments, antioxidants, and bioactive substances from algae biomass adds value across various industries like food, cosmetics, pharmaceuticals, and agriculture.<sup>142</sup> Maximizing biomass utilization generates multiple revenue streams, enhances economic viability, and encourages a more sustainable approach by minimizing waste.

Large-scale bio-hydrogen production requires cutting-edge and efficient technologies. Economies of scale, where average production costs decrease as production volume increases, play a crucial role in rendering bio-hydrogen economically competitive compared to other energy sources.<sup>158</sup> Market demand for bio-hydrogen significantly impacts its commercial viability. Factors such as energy policies, market trends, and environmental concerns influence market demand. Government policies supporting renewable energy sources and hydrogen technologies can enhance market demand for bio-hydrogen.<sup>21</sup>

Continuous technological advancements in agriculture seek cost savings and long-term economic viability through Utilizing technologies like drones and sensors to optimize resource use, improve decision-making for irrigation, fertilization, and pest control, and enhance yields.<sup>148</sup> Innovative farming techniques use vertical stacking in controlled environments, reducing land and water use while enabling year-round production, leveraging advancements in hydroponics and automated systems.<sup>159</sup> Developing crops with enhanced traits such as increased yield, nutritional value, and resistance to pests and environmental stressors through genetic modification and biotechnology. Use of machines for planting, harvesting, and weed control, which can increase productivity, reduce labor costs, and minimize chemical inputs.<sup>160</sup> Leveraging data analysis and machine learning for predictive models to improve planting schedules, identify diseases, and suggest remedies for better yields and resource management.<sup>161</sup>

The economic viability of bio-hydrogen production from algal biomass is influenced by several factors, including cultivation costs, hydrogen production efficiency, co-product utilization, market demand, and technological advancements. As technology advances and processes are optimized, the cost of algal-based generation is expected to decrease, increasing its viability as a sustainable energy source.<sup>126</sup>

Firms such as Algenol get financial rewards by manufacturing a variety of goods (e.g., ethanol and hydrogen). As demonstrated by Algenol's model, using non-arable land and seawater lowers the costs related to feedstock competitiveness and freshwater utilization. Studies conducted by the University of California, Berkeley and the Research Institute of Innovative Technology for the Earth (RITE) show that advances in genetic modification and bioreactor design can dramatically lower the cost of production. The use of industrial CO<sub>2</sub> emissions by Seabiotic for algae culture demonstrates the possibility of both ecological and commercial benefits. These case studies demonstrate how resource effectiveness, technical innovation, legislative assistance, and the incorporation of bio-hydrogen generation with current manufacturing procedures may all improve financial viability.

## Policy and regulatory framework for sustainable bio-hydrogen production from bio-algae

Different nations or regions employ various policies and frameworks to encourage the generation of sustainable bio-hydrogen from algal biomass. Governments implement renewable energy policies aiming to combat climate change, reduce greenhouse gas emissions, enhance energy security, and transition toward a low-carbon economy.<sup>42</sup> Governments set targets or mandates specifying the percentage of total energy production or consumption derived from renewable sources, encouraging the adoption of renewable energy. Feed-in Tariffs (FiTs) guarantee premium prices for the electricity generated from renewable sources, incentivizing investments in renewable energy projects.<sup>52</sup>

Under the Renewable Energy Directive (RED II), which encourages the use of environmentally friendly bioenergy, BH derived from algae is categorized as an advanced biofuel. The generation of BH must satisfy sustainability and reductions in emissions of greenhouse gases in order to be eligible. Facilities that produce BH may be eligible for carbon credits if they can show a decrease in emissions. A financial incentive to switch to greener manufacturing technology is provided by the trading of carbon credits. Algal-derived bio-hydrogen is recognized as an advanced biofuel and is assigned Renewable Identification Numbers (RINs). Producers of BH can profit monetarily by trading RINs. The Strategic Energy Plan encourages the advancement and application of technology for producing BH, particularly those derived from algae, financial assistance and government support for pilot programs, and large-scale commercial enterprises. The Clean Energy Finance Corporation (CEFC) provides investments and loans for projects aimed at producing bio-hydrogen.

Tax refunds or deductions are provided to individuals, companies, or organizations investing in bio-hydrogen and other renewable energy technologies. These incentives lower the overall cost of implementing renewable energy systems, increasing their economic viability.<sup>42</sup> Governments offer financial support through grants or subsidies to fund research, development, and deployment of renewable energy technologies, assisting in overcoming initial cost barriers and fostering the marketplace for bio-hydrogen technology. Renewable Portfolio Standards (RPS) laws require electricity providers to procure a certain percentage of their electricity from sustainable sources, encouraging investment in renewable energy generation.

Net metering laws allow homeowners or businesses with renewable energy systems to reduce their electricity bills by supplying excess power to the grid, receiving compensation for the electricity they provide.<sup>151</sup> Governments can enact green procurement laws prioritizing purchases of goods and services related to renewable energy. Direct assistance via partnerships or funding can further support renewable energy projects.

Feed-in tariffs ensure fair compensation for renewable energy producers, stimulating investments in bio-hydrogen production. Tax credits also encourage engagement in bio-hydrogen production by reducing infrastructure or R & D costs.<sup>51</sup> Governments use grants and subsidies to support infrastructure development, pilot programs, demonstration facilities, and research in

bio-hydrogen production, reducing initial costs and risks associated with investments.<sup>162</sup> In essence, these policies and incentives aim to expedite the transition to sustainable energy, promote investment, lower financial barriers, and increase demand for bio-hydrogen and other renewable energy technologies.

Governments frequently set renewable energy targets, specifying the proportion of electricity that must originate from renewable sources, including bio-hydrogen, by a specific date. Such targets convey a clear message to the sector, encourage market competitiveness, and stimulate the development of new BH technology.<sup>52</sup> These goals play a pivotal role in advancing the transition toward cleaner and more sustainable energy sources. Laws and incentives for renewable energy, including bio-hydrogen, are crucial tools for governments to promote the adoption of renewable energy sources. Financial support, goal setting, and the creation of a supportive regulatory environment can accelerate the transition to a cleaner, more environmentally friendly energy future.<sup>115</sup>

Environmental regulations are laws enacted by governments to protect the environment and ensure sustainable practices across various industries. Compliance with these regulations is vital for the sustainable production of bio-hydrogen. Environmental laws often set limits on emissions of pollutants into the air. Industries engaged in BH production must implement activities that reduce air pollution and adhere to prescribed emission limits. This might involve deploying advanced pollution control techniques or transitioning to cleaner energy sources.<sup>115</sup> Laws concerning water pollution control aim to maintain the cleanliness of water bodies. Throughout the cultivation, harvesting, and processing of bio-algae for bio-hydrogen synthesis, preventing the release of hazardous compounds into water sources is crucial. To comply with these regulations, efficient water management techniques such as proper wastewater treatment and avoidance of chemical run-off are essential.<sup>163</sup>

Businesses involved in BH synthesis must manage waste appropriately in accordance with environmental rules. Waste in the bio-hydrogen production process may include feedstock residues, contaminants, or other by-products. These waste materials need to be handled in compliance with established regulations, which may involve recycling, proper disposal methods, or other environmentally friendly procedures. Authorities may impose specific regulations regarding land usage, particularly concerning activities like bio-algae farming. These laws are designed to prevent environmental degradation and ensure responsible land management. BH production facilities are required to adhere to these regulations, which could include guidelines on land protection, reclamation, and biodiversity preservation.<sup>70</sup> Adhering to and complying with these environmental regulations is critical for sustainable and environmentally friendly bio-hydrogen production, ensuring that the process minimizes its environmental impact and maintains a commitment to ecological preservation.

Governments establish specific guidelines and criteria for the cultivation, harvesting, and processing of bio-algae to ensure compliance with environmental legislation. These guidelines

emphasize minimizing adverse environmental effects, maximizing resource utilization, and promoting sustainable practices throughout the manufacturing process.<sup>86</sup> Businesses engaged in BH production must understand and adhere to these rules to conduct operations in an environmentally friendly manner. Governmental financial support aimed at promoting and advancing R &D activities in specific areas of interest, such as BH production, is referred to as “research and development funding.”<sup>56</sup> Governments allocate funds to promote scientific research, technological development, and efficiency enhancements in the bio-hydrogen sector.

Grants are a common form of R &D financing wherein researchers and organizations can apply for financial aid to conduct studies, experiments, and technological advancements related to BH production. These funds may cover labor, equipment, and material costs associated with research.<sup>60</sup> Subsidies provided by governments to commercial entities or research institutes participating in BH R &D can come in the form of tax benefits, reduced regulations, or funding for infrastructure improvements. Moreover, collaborative research programs can be established, bringing together academic institutions, governmental bodies, and commercial enterprises to collaborate on R &D initiatives. These collaborative efforts, through information sharing, interdisciplinary research, and pooled resources, aim to advance BH production from bio-algae in a synergistic manner.<sup>53</sup>

Governments implement market support efforts to create an environment conducive to the acceptance and advancement of bio-hydrogen as an energy source. Components of market support include subsidies, renewable energy goals, and carbon pricing mechanisms.<sup>164</sup> Subsidies or financial incentives offered by governments can enhance the affordability and competitiveness of BH compared to other energy sources, consequently boosting demand and making BH a more attractive alternative energy source.<sup>165</sup> Additionally, setting specific goals for renewable energy generation, including bio-hydrogen, provides predictability and long-term demand signals, fostering investor confidence and encouraging BH infrastructure growth. Governments may also introduce carbon pricing mechanisms like carbon taxes or cap-and-trade programs to incentivize industries to reduce their carbon footprint. By putting a price on carbon emissions, these mechanisms encourage a shift toward lower-carbon technologies.<sup>166</sup>

Governments can position BH as an environmentally friendly or zero-carbon energy source, giving it a competitive edge in the market. This encourages businesses to adopt BH technology, as it helps lower their carbon emissions and comply with legal requirements.<sup>66</sup> The implementation of subsidies, renewable energy goals, and carbon pricing mechanisms accelerates the transition to a more environmentally friendly and low-carbon energy industry by stimulating demand and encouraging investment in BH infrastructure. It is crucial to note that different jurisdictions may have varying laws and regulations governing sustainable BH generation. These norms and regulations may change over time as advancements in BH production occur. Staying informed and adhering to applicable rules and regulations is essential for sustainable BH production. Consulting with local or national authorities can provide access to the most recent and

accurate data regarding the specific laws and rules pertaining to BH production in a particular area.<sup>15</sup>

Governmental financial support, often termed “research and development (R &D) funding,” plays a pivotal role in advancing research initiatives and technological innovations within the bio-hydrogen production sector. These funds are allocated to foster scholarly investigations, technological advancements, and productivity improvements aimed at enhancing BH production processes using bio-algae as a primary feedstock.<sup>97</sup> R &D funding encompasses various forms, including grants, loans, or subsidies, directed toward academia, organizations, and businesses involved in bio-hydrogen from bio-algae R &D projects.<sup>142</sup>

Funding supports fundamental research efforts focused on optimizing growth conditions, enhancing conversion efficiency, and exploring the hydrogen production potential of diverse bio-algae species. These initiatives drive the development of new technologies, such as advanced cultivation systems, genetically modified bio-algae strains with heightened hydrogen production capabilities, and innovative harvesting and extraction methods.<sup>9</sup> Government funding facilitates the establishment of research infrastructure, experimental facilities, and pilot-scale BH production units. These resources enable researchers and engineers to conduct experiments, test cutting-edge equipment, and scale up viable BH production techniques.<sup>9</sup>

R &D funding encourages collaborations among academic institutions, research organizations, and industrial stakeholders. By promoting knowledge transfer, technology exchange, and the commercialization of BH-related innovations, governments foster collaborations and cooperative projects within the bio-hydrogen domain.<sup>64</sup> Financial support extends to educational programs, workshops, and training efforts focusing on BH production from bio-algae. These initiatives facilitate information dissemination within the sector and contribute to the development of a skilled workforce, essential for advancing BH production technologies.<sup>167</sup>

Government incentives are a major factor in determining how the BH sector operates. They stimulate technical advancement, boost competitiveness in the market, lower manufacturing costs, and support environmental sustainability. Governments can ensure that the BH sector grows quickly and remains an important component of the global renewable energy landscape by offering financial assistance and a stable legislative framework. These incentives support business expansion while simultaneously advancing more general environmental and energy security objectives. Table 3 outlines the significance of Algal Biomass and its Growth-Boosting Properties for Sustainable Biomass Production, underscoring the potential impact of funding on research outcomes and industry growth.<sup>167</sup> In conclusion, governmental support and R &D funding are instrumental in fostering innovation, encouraging collaborative endeavors, and nurturing educational initiatives to advance BH production from bio-algae. This support contributes significantly to the evolution of a more sustainable and environmentally friendly energy landscape. For the production of bio-hydrogen from algae to become profitable, rapid technological improvements are needed. However, innovation can happen at an erratic rate.

## Conclusion and future perspectives

The discovery of microalgae capable of photosynthetic hydrogen production has paved the way for bio-hydrogen (BH) generation. These microalgae employ photosynthesis using water and sunlight, yielding hydrogen gas as a by-product. Research indicates that specific conditions such as ideal temperatures, light levels, and nutrient availability can enhance BH synthesis in algae. Moreover, modifying growth conditions and genetically editing algae strains can further increase hydrogen production. The potential for BH production from algae as a sustainable energy source is promising. Algae's adaptability across various settings, including open lakes, closed photo-bioreactors, and wastewater treatment plants, makes it a versatile option for large-scale production. Furthermore, algae cultivation for BH production offers environmental advantages, such as wastewater treatment and carbon dioxide sequestration. However, before widespread commercialization, several challenges need addressing. These include enhancing hydrogen production efficiency, developing cost-effective growth systems, and ensuring production scalability. Despite these challenges, optimistic research findings and ongoing technological advancements suggest that BH from algae could be a pivotal element in future energy landscape.

Identifying or genetically modifying algae strains with high hydrogen generation potential and environmental stress tolerance is crucial. Optimizing farming practices, light utilization, nutrient availability, and overall biomass yield is essential to maximize hydrogen production. Designing efficient and scalable systems for hydrogen production in algae requires improvements in mass transfer, light dispersion, and overall productivity in reactor designs. Efforts aimed at addressing these research gaps and challenges are vital for realizing the potential of BH from algae as a future renewable energy source. Techniques such as genetic engineering, reactor design optimization, and maximizing resource utilization will play a key role in harnessing algae's capability for clean and sustainable energy production.

Absolutely, the sustainable utilization of resources, effective harvesting and processing techniques, managing oxygen sensitivity, and ensuring techno-economic viability are pivotal aspects in unlocking algae's potential as a viable alternative energy source, particularly in producing bio-hydrogen (BH). Algae require various nutrients like nitrogen and phosphate for growth and hydrogen production. To maintain a continuous supply without harming the ecosystem, research must focus on recycling nutrients, exploring waste utilization methods, and establishing sustainable procurement approaches. Developing efficient and cost-effective methods for harvesting biomass, extracting hydrogen, dewatering, and separating biomass on a large scale is crucial. These processes need to be both energy efficient and scalable for commercial production.

Algae are often sensitive to oxygen, hindering hydrogen production. Altering algae metabolism pathways or creating anaerobic microenvironments could mitigate the inhibiting effects of oxygen on hydrogen production. Evaluating both

technological and financial feasibility is essential for large-scale BH production from algae. Understanding production costs, infrastructure requirements, and hydrogen storage expenses is crucial for attracting investments and developing sustainable business models. Addressing these challenges will be critical to realize algae's potential as a future alternative energy source. Overcoming these hurdles will lead to the development of effective, cost-efficient, and sustainable techniques for BH production from algae, positioning it as a viable and environmentally friendly energy solution.

BH generated from algae using water and sunlight is considered renewable and clean. It has the potential to replace fossil fuels, reduce greenhouse gas emissions, and foster the development of more environmentally friendly energy sources. Algae can thrive in various water sources like freshwater, seawater, and wastewater. They grow rapidly and produce abundant biomass per unit area, making them an efficient and plentiful feedstock for BH production compared to conventional energy crops. BH produced from algae is considered carbon neutral as the carbon dioxide released upon combustion equals the amount absorbed during the algae's growth phase. This characteristic makes BH an effective option for mitigating carbon emissions and combating climate change. Algae can utilize nutrients from waste to clean industrial effluents and wastewater. This dual-purpose strategy allows simultaneous waste treatment and BH production, offering a comprehensive and sustainable solution.

To fully realize the potential of BH production from algae, certain challenges need to be addressed. With conventional hydrogen production techniques and alternative sustainable hydrogen sources (such as electrolysis utilizing renewable power), competitive pricing might be challenging to achieve. Overcoming market resistance and doubt is necessary to get acceptance for bio-hydrogen as a feasible alternative energy source from companies and consumers. It is important to provide steady and enduring policy backing. Regular shifts in the direction of policy may discourage investment and innovation. Although it can be difficult, creating standards across the industry for the production, transport, and storage of BH is crucial. Enhancing algal strains with high hydrogen generation capacity through genetic engineering is crucial to boost efficiency. Designing efficient and scalable photo-bioreactor systems is essential for large-scale BH production and optimal algae growth. Ideal conditions including light intensity, temperature, nutrient availability, and pH levels need to be established to maximize hydrogen generation. Developing cost-effective methods to collect and purify hydrogen using algal biomass through various technological approaches is necessary. To commercialize BH production from algae, efforts are directed toward reducing production costs and enhancing overall efficiency to ensure competitiveness with other energy sources. Overall, the production of BH from algae presents significant potential as a clean and renewable energy source. Addressing these challenges and optimizing the process will pave the way for its real-world applications in future.

**Table 3.** Algal biomass: unveiling growth-boosting properties for sustainable biomass production.

Types of algae	Storage properties	Organisms	Application	References
<i>Chlorella</i>	Starch	Single celled	Biofuel production	168
<i>Spirulina</i>	Glycogen	Filamentous, multicellular	Nutritional supplement	169
<i>Dunaliella</i>	Lipids	Halotolerant	Biofuel, food coloring	143
<i>Haematococcus</i>	Astaxanthin	Freshwater, single celled	Nutraceutical, aquaculture	170
<i>Nannochloropsis</i>	Triacylglycerols	Eukaryotic microalgae	Biodiesel production	144
<i>Scenedesmus</i>	Starch, lipids	Filamentous, colonial	Biofuel, wastewater treatment	171
<i>Isochrysis</i>	Lipids	Marine, single celled	Biodiesel, aquaculture feed	172
<i>Botryococcus</i>	Hydrocarbons	Colonial, green microalgae	Biofuel production	173
<i>Tetraselmis</i>	Lipids, starch	Eukaryotic microalgae	Biodiesel, aquaculture feed	174
<i>Porphyridium</i>	Phycocerythrin	Single celled, red algae	Biopharmaceuticals, cosmetics	175
<i>Schizochytrium</i>	Lipids	Heterotrophic microalgae	Omega-3 supplements	145
<i>Phaeodactylum</i>	Lipids	Diatoms, single celled	Biodiesel, aquaculture feed	176
<i>Prymnesium</i>	Starch, Lipids	Golden algae	Biofuel, bioremediation	177
<i>Chlamydomonas</i>	Starch, lipids	Single celled, green algae	Bio-hydrogen production	137
<i>Dictyostelium</i>	Glycogen	Cellular slime mold	Bioremediation, research model	136
<i>Euglena</i>	Paramylon	Flagellate, single celled	Biofuel, bioremediation	139
<i>Prymnesiophyceae</i>	Lipids, starch	Planktonic, marine algae	Biodiesel, food source	178
<i>Ulva</i>	Starch, lipids	Macroalgae	Bioremediation, animal feed	140
<i>Porphyra</i>	Starch	Red macroalgae	Human food, bioremediation	141
<i>Gracilaria</i>	Agar, carrageenan	Red macroalgae	Food industry, bioplastics	179

### Author contributions

B. Senthil Rathi contributed to Conceptualization, Methodology, Formal Analysis, Investigation, Supervision, and Writing–Original draft preparation. V. Dinesh Aravind contributed

to Investigation, Data Curation, and Writing–Original draft preparation. G. Ranjith contributed to Investigation, Data Curation, and Writing–Original draft preparation. V. Kishore contributed to Investigation, Resources, and Writing–Original

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## Data availability

No new data were created or analyzed in this study.

## Declarations

### Conflict of interest

The authors declare that they have no competing interests.

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