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Efficiency and Condition Optimization of Biohydrogen Production Using Marine Algae

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Abstract This study optimizes the efficiency and conditions for biohydrogen production using algae, with a focus on enhancing the efficiency of the hydrolysis step during dark fermentation and improving photobiological hydrogen production. The study identifies macroalgae as an efficient biomass source for biohydrogen production, emphasizing the importance of pretreatment to enhance hydrolysis. Detoxification techniques are crucial to control inhibitory substances formed during pretreatment. Additionally, the use of oxygen scavengers such as sodium sulfite, sodium metabisulfite, and sodium dithionite significantly improves photobiological hydrogen production in *Chlorococcum minutum*, with sodium sulfite showing the highest efficiency. The review also highlights the potential of green algae and cyanobacteria as sustainable sources for biohydrogen, bioethanol, and biodiesel, and discusses the economic and environmental benefits of these methods. The findings suggest that optimizing pretreatment methods and using effective oxygen scavengers can significantly enhance biohydrogen production from marine algae. This not only provides a sustainable and clean energy source but also supports the transition to a circular bioeconomy. Further research into the economic feasibility and commercialization potential of these methods is recommended.

Keywords Biohydrogen; Marine algae; Pretreatment; Oxygen scavengers; Sustainable energy; Circular bioeconomy

1 Introduction

Biohydrogen (BioH_2) is emerging as a promising alternative to conventional fossil fuels due to its high energy efficiency and environmentally friendly nature. Unlike fossil fuels, which contribute significantly to greenhouse gas emissions and global warming, biohydrogen combustion produces only water as a byproduct, making it a clean energy carrier (Goswami et al., 2020; Mona et al., 2020; Sharma et al., 2021). The increasing depletion of fossil fuel reserves and the urgent need to mitigate climate change have driven extensive research into renewable energy sources, with biohydrogen production being a focal point (Anto et al., 2020; Jiménez-Llanos et al., 2020). Various methods, including photofermentation, dark fermentation, and microbial electrolysis, have been explored for biohydrogen production, each offering unique advantages and challenges (Bhatia et al., 2020; Kanwal and Torriero, 2022).

Marine algae, including both microalgae and macroalgae, have gained attention as a viable feedstock for biohydrogen production. Algae are considered a third-generation biofuel feedstock due to their rapid growth rates, high biomass yields, and ability to capture carbon dioxide, thus contributing to carbon sequestration (Nagarajan et al., 2020; Kumar et al., 2021). Microalgae, such as *Chlorella* sp., and macroalgae, such as *Saccharina latissima*, have shown significant potential in biohydrogen production through various metabolic pathways, including direct and indirect photolysis, dark fermentation, and photofermentation (Lin et al., 2019; Jiménez-Llanos et al., 2020). The use of marine algae not only supports sustainable energy production but also aids in wastewater treatment and the reduction of environmental pollutants (Mona et al., 2020; Sharma et al., 2021).

Optimizing the conditions for biohydrogen production is crucial to enhance yield and make the process economically viable. Factors such as growth techniques, growth media, pretreatment methods, and operational parameters (e.g., temperature, light intensity, nutrient concentration) significantly impact biohydrogen productivity (Anto et al., 2020; Nagarajan et al., 2020; Sharma et al., 2021). Effective pretreatment of algal biomass is essential to break down complex carbohydrates into fermentable sugars, thereby improving the efficiency of subsequent fermentation processes (Lin et al., 2019; Kumar et al., 2021). Addressing technical and scientific obstacles, such as the formation of inhibitory substances during pretreatment and the recalcitrance of algal cell walls, is vital for advancing biohydrogen production technologies (Nagarajan et al., 2020; Kumar et al., 2021).

This study aims to explore the efficiency and condition optimization of biohydrogen production using algae, providing a comprehensive review of the advanced technologies and methods currently employed in algae-based biohydrogen production. Various metabolic pathways, pretreatment methods, and operational parameters influencing biohydrogen yield are discussed. The study also highlights the challenges that need to be addressed and the future research directions required to enhance the commercial feasibility of algae-based biohydrogen, with the hope of contributing to the development of a sustainable and efficient biohydrogen production system.

2 Marine Algae as a Feedstock for Biohydrogen Production

2.1 Characteristics of marine algae (biomass composition, growth rates, etc.)

Marine algae, including both microalgae and macroalgae, are characterized by their high photosynthetic efficiency and ability to accumulate significant quantities of biomass. The biochemical composition of marine algae typically includes carbohydrates, lipids, and proteins, which are essential for biohydrogen production. For instance, microalgae have a high lipid content, which is advantageous for biofuel production (Williams and Laurens, 2010; Adeniyi et al., 2018). Additionally, marine macroalgae such as *Ulva* sp. can accumulate high amounts of starch and cellulose, making them suitable for bioethanol and biohydrogen production (Qarri and Israel, 2020).

The growth rates of marine algae can vary significantly depending on environmental conditions. For example, *Ulva* sp. has shown specific growth rates (SGRs) ranging from 1.4% to 19.3% per day, depending on the season and cultivation conditions (Qarri and Israel, 2020). This high growth rate, coupled with the ability to grow in various water types, including seawater and wastewater, makes marine algae a versatile and sustainable feedstock for biohydrogen production (Wang and Yin, 2018) (Figure 1).

2.2 Advantages of marine algae over terrestrial biomass

Marine algae offer several advantages over terrestrial biomass for biohydrogen production:

Higher Growth Rates: Marine algae generally have higher growth rates compared to terrestrial plants, which allows for more rapid biomass accumulation (Wang and Yin, 2018; Qarri and Israel, 2020).

Non-Arable Land Use: Marine algae do not require arable land for cultivation, thereby avoiding competition with food crops and reducing the pressure on terrestrial ecosystems (Wang and Yin, 2018).

CO_2 Fixation: Marine algae have superior CO_2 fixation capabilities, which can help mitigate greenhouse gas emissions and contribute to environmental sustainability (Adeniyi et al., 2018; Wang and Yin, 2018).

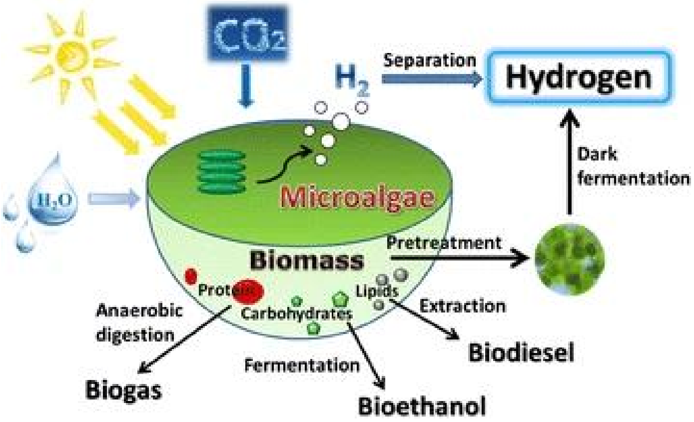

Figure 1 Potential pathways from microalgae to biofuels (Adopted from Wang and Yin, 2018)

No Lignin Content: Unlike terrestrial biomass, marine algae do not contain lignin, which simplifies the pretreatment process and enhances the efficiency of biohydrogen production (Wang and Yin, 2018).

Versatility in Water Use: Marine algae can be cultivated in seawater, freshwater, and even wastewater, making them highly adaptable and reducing the need for freshwater resources (Adeniyi et al., 2018; Wang and Yin, 2018).

2.3 Case studies of marine algae species used in biohydrogen production

Chlamydomonas reinhardtii is a well-studied microalga known for its high photosynthetic efficiency and ability to produce biohydrogen through dark fermentation. Pretreatment methods such as hydrothermal processing have been shown to enhance the hydrolysis of its biomass, leading to improved biohydrogen yields (Nagarajan et al., 2020).

Ulva lactuca, a type of green macroalga, has been investigated for its potential in biohydrogen production. Studies have demonstrated that hydrothermal pretreatment can significantly increase the solubilization of its biomass, thereby enhancing the production of biohydrogen and biomethane (Lin et al., 2019). Additionally, *Ulva lactuca* has shown promising growth rates and biomass yields in land-based cultivation systems, making it a viable feedstock for biohydrogen production (Qarri and Israel, 2020).

Saccharina latissima, a brown seaweed, has been explored for its biohydrogen production potential through dark fermentation. Hydrothermal pretreatment of *S. latissima* has been found to improve the solubilization of its biomass and increase the yield of fermentable sugars, which are crucial for biohydrogen production. The energy conversion efficiency of biohydrogen production from *S. latissima* can reach up to 72.8% under optimized conditions (Lin et al., 2019).

3 Biohydrogen Production Mechanisms

3.1 Biological pathways involved in biohydrogen production

Biohydrogen production involves several biological pathways, primarily categorized into photobiological and fermentative processes. Photobiological pathways include direct and indirect biophotolysis, where microalgae use sunlight to split water molecules, releasing hydrogen. Fermentative pathways, such as dark fermentation, involve the anaerobic breakdown of organic substrates by microorganisms to produce hydrogen. These pathways are influenced by various factors, including the type of feedstock, pretreatment methods, and the specific metabolic routes employed by the microorganisms (Nagarajan et al., 2017; Ahmed et al., 2021; Sharma et al., 2021).

3.2 Photobiological hydrogen production through algae photosynthesis

Photobiological hydrogen production leverages the photosynthetic capabilities of algae. In direct biophotolysis, algae directly convert solar energy into hydrogen by splitting water molecules using photosystem II. Indirect biophotolysis involves the production of hydrogen through intermediate metabolic processes, where algae first produce organic compounds that are subsequently converted to hydrogen. This method is highly efficient in terms of sunlight conversion but is sensitive to oxygen, which can inhibit the hydrogenase enzyme responsible for hydrogen production (Nagarajan et al., 2017; Ahmed et al., 2021; Sharma et al., 2021).

3.3 Dark fermentation and metabolic pathways

Dark fermentation is a process where anaerobic microorganisms decompose organic substrates, such as sugars and starches, to produce hydrogen. This method does not require light and can utilize a wide range of feedstocks, including lignocellulosic biomass and algal biomass. The metabolic pathways involved in dark fermentation include glycolysis, where glucose is broken down to pyruvate, which is then converted to hydrogen and organic acids through various enzymatic reactions. The efficiency of dark fermentation can be enhanced through pretreatment methods that increase the availability of fermentable sugars (Park et al., 2013; Ergal et al., 2018; Bhatia et al., 2020; Kumar et al., 2021; Sim et al., 2021).

3.4 Enzymes and cofactors involved in hydrogen production

Several key enzymes and cofactors play crucial roles in the biohydrogen production process. Hydrogenase and nitrogenase are the primary enzymes involved. Hydrogenase catalyzes the reversible oxidation of molecular hydrogen and is sensitive to oxygen, which can inhibit its activity. Nitrogenase, primarily involved in nitrogen fixation, can also produce hydrogen as a by-product. The activity of these enzymes is influenced by various factors, including the availability of cofactors such as ferredoxin and flavodoxin, which transfer electrons to the hydrogenase enzyme. Optimizing the expression and activity of these enzymes is critical for enhancing biohydrogen production (Nagarajan et al., 2017; Ding et al., 2020; Tiang et al., 2020; Sharma et al., 2021; Sim et al., 2021).

4 Optimization of Environmental Conditions for Biohydrogen Production

4.1 Light intensity and quality

Light intensity, duration, and spectrum play crucial roles in the photosynthetic efficiency of algae, which directly impacts biohydrogen production. High light intensity can lead to photoinhibition, reducing the activity of hydrogenase enzymes and thus decreasing hydrogen production. For instance, *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* showed optimal hydrogen production at lower light intensities, with a significant drop in efficiency at higher intensities due to photoinhibition (Hwang and Lee, 2021). Additionally, innovations in light management, such as spectral filtration and plasmonic waveguides, have been shown to enhance photosynthetic productivity by improving light quality and distribution, thereby reducing non-productive pathways like the production of reactive oxygen species (Nwoba et al., 2019).

Research has demonstrated that optimizing light conditions can significantly enhance hydrogen production in algae. For example, reducing the optical cross-section of the light-harvesting antenna by selectively decreasing chlorophyll b levels has been shown to improve photosynthetic efficiency and biomass yield. This approach allows algae to dynamically adjust their light-harvesting antenna sizes in response to varying light intensities, thereby maintaining high photosynthetic rates and biomass productivity (Sayre, 2020). Such strategies are essential for maximizing hydrogen production under different environmental conditions.

4.2 Temperature and pH

Temperature is a critical factor influencing the metabolic processes of algae, including those involved in biohydrogen production. Optimal temperature ranges are necessary to maintain enzyme activity and metabolic rates. Deviations from these optimal ranges can lead to reduced hydrogenase activity and lower hydrogen yields. Effective temperature control in photobioreactors can help maintain the metabolic balance required for sustained hydrogen production (Nwoba et al., 2019).

The pH level of the culture medium significantly affects the biohydrogen production process. Algae require specific pH conditions to optimize enzyme activity and metabolic functions. Maintaining an optimal pH range is crucial for maximizing hydrogen yield, as extreme pH levels can inhibit hydrogenase activity and disrupt cellular processes. Research indicates that fine-tuning pH levels in conjunction with other environmental factors can lead to improved hydrogen production efficiency (Nwoba et al., 2019).

4.3 Nutrient availability

Nutrient availability, particularly nitrogen and phosphorus, plays a vital role in the growth and metabolic activities of algae. Adequate levels of these nutrients are necessary for maintaining cellular functions and supporting biohydrogen production. However, an excess of nutrients can lead to suboptimal hydrogen yields due to the preferential use of resources for biomass growth rather than hydrogen production. Balancing nutrient levels is therefore essential for optimizing biohydrogen production (Nwoba et al., 2019).

Nutrient starvation, particularly nitrogen deprivation, has been shown to induce biohydrogen production in algae. Under nutrient-limited conditions, algae shift their metabolic pathways to favor hydrogen production as a survival mechanism. This process involves the downregulation of photosynthetic activity and the activation of hydrogenase enzymes. Studies have demonstrated that controlled nutrient starvation can significantly enhance hydrogen yields, making it a viable strategy for optimizing biohydrogen production (Hwang and Lee, 2021).

5 Biotechnological Strategies to Enhance Biohydrogen Production

5.1 Genetic Engineering of Algal Strains

Genetic engineering of algal strains has emerged as a promising strategy to enhance biohydrogen production. Techniques such as metabolic pathway engineering and the modification of hydrogenase and nitrogenase enzymes are pivotal in this context. By targeting specific genes and pathways, researchers have been able to increase the efficiency of hydrogen production (Figure 2). For instance, the engineering of hydrogenases to improve their activity under oxygenic conditions has shown significant promise (Dubini and Ghirardi, 2014; Anwar et al., 2019; Khan and Fu, 2020). Additionally, the introduction of genetic modifications that enhance the photosynthetic efficiency and biomass accumulation in algae can further boost hydrogen yields (Mathews and Wang, 2009; Vargas et al., 2016).

Several case studies have demonstrated the potential of genetically modified algae in enhancing biohydrogen production. For example, the D1 mutant strains of *Chlamydomonas reinhardtii* have shown increased hydrogen production under simulated outdoor conditions, with strain D239-40 being particularly effective (Oncel et al., 2015). Another study highlighted the use of a chloroplast-targeted genetic modification parameter, which significantly increased hydrogen production efficiency up to a certain threshold (Vargas et al., 2016). These case studies underscore the importance of genetic engineering in optimizing algal strains for biohydrogen production.

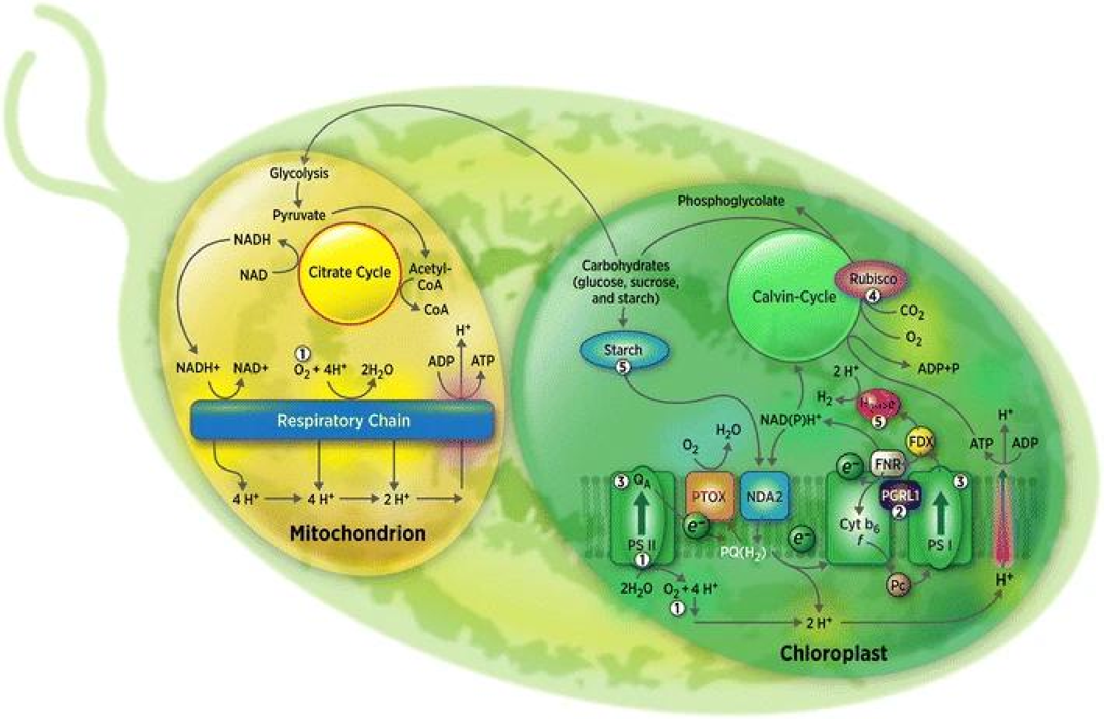

Figure 2 Representation of the hydrogen photoproduction-related pathways in *Chlamydomonas* (Adopted from Dubini and Ghirardi, 2014)

Image caption: Hydrogen production occurs in the chloroplast, where the photosynthetic chain and the hydrogenases are located (see text for more details). The respiratory chain is located in the mitochondrion, and there is an extensive communication between the two organelles that can impact the level of hydrogen production (adapted from Kruse et al. 2005). The circled numbers indicate where current genetic engineering efforts have impacted H_2 photoproduction, as described in the text. The barriers overcome by these modifications are: (1) O_2 sensitivity, addressed by PSII inactivation and/or increased O_2 consumption; (2) proton gradient dissipation, addressed by the pgrl1 knockout mutation (decreased CEF); (3) photosynthetic efficiency, addressed by knockdown of light-harvesting antennae or truncating antenna proteins; (4) competition for electron, addressed by *Rubisco mutagenesis*; (5) low reductant flux and hydrogenase expression, addressed by impacting starch accumulation/degradation, FDX-HYD fusion, and overexpressing hydrogenase, respectively. It must be noted that, for clarity, not all the genetic engineering approaches mentioned in the text are represented in the figure (Adopted from Dubini and Ghirardi, 2014)

5.2 Co-culturing and Symbiotic Systems

Co-culturing algae with bacteria has been explored as a strategy to enhance biohydrogen production. Bacterial-algal systems can create a symbiotic environment where bacteria provide essential nutrients or remove inhibitory by-products, thereby improving the overall efficiency of hydrogen production. For instance, the use of purple non-sulphur bacteria (PNSB) in photo-fermentative systems has shown to enhance hydrogen yields through various biotechnological approaches, including medium optimization and genetic engineering (Tiang et al., 2020).

Successful examples of co-culture systems include the dynamic membrane bioreactor (DMBR) used for continuous biohydrogen production from red algal biomass. This system achieved a high hydrogen production rate by leveraging the metabolic activities of both algae and bacteria, with *Clostridium* sp., *Anaerostipes* sp., and *Caproiciproducens* sp. playing significant roles in the process (Sim et al., 2021). Another example is the integration of dark- and photo-fermentation processes, which has been suggested to further enhance hydrogen production in co-culture systems (Tiang et al., 2020).

5.3 Reactor Design and Process Engineering

Optimizing bioreactor designs is crucial for maximizing biohydrogen production from marine algae. Photobioreactors, in particular, have been the focus of many studies due to their ability to provide controlled light conditions and enhance photosynthetic efficiency. Innovations in photobioreactor design, such as improved light distribution and mixing, have been shown to significantly increase hydrogen production rates (Vargas et al., 2016; Anwar et al., 2019). Additionally, the use of advanced materials and coatings can further optimize light absorption and reduce energy losses (Tiang et al., 2020).

Scaling up biohydrogen production systems from laboratory to commercial scale presents several challenges, including maintaining consistent light and nutrient distribution, managing oxygen levels, and ensuring economic feasibility. Solutions to these challenges include the development of robust bioreactor designs that can operate efficiently under varying environmental conditions and the optimization of process parameters to reduce costs. For instance, the economic assessment of biohydrogen production from macroalgae has highlighted the need for cost-effective pretreatment methods and detoxification techniques to enhance the hydrolytic process during dark fermentation (Ahmed et al., 2021; Kumar et al., 2021). Additionally, integrating biotechnological advancements with process engineering can help overcome scale-up challenges and achieve sustainable biohydrogen production (Mathews and Wang, 2009; Dubini and Ghirardi, 2014).

6 Challenges in Biohydrogen Production Using Marine Algae

6.1 Energy balance and economic feasibility

One of the primary challenges in biohydrogen production using marine algae is achieving a favorable energy balance and economic feasibility. The production processes, including pretreatment and fermentation, often require significant energy inputs, which can offset the benefits of the biohydrogen produced. For instance, hydrothermal pretreatment of marine macroalgae like *Saccharina latissima* has shown that while biohydrogen yields can be increased, the overall process energy efficiency can drop significantly when considering the energy input for pretreatment, fermentation, and digestion (Lin et al., 2019). Additionally, the high costs associated with cultivation, harvesting, and extraction of algal biomass further complicate the economic viability of biohydrogen production (Anto et al., 2020). Strategies such as integrating advanced pretreatment methods and optimizing bioreactor conditions are essential to improve energy efficiency and reduce costs (Shankaran et al., 2022).

6.2 Environmental impacts and sustainability considerations

Biohydrogen production from marine algae presents several environmental and sustainability challenges. While biohydrogen is a clean fuel, the processes involved in its production can have environmental impacts. For example, the pretreatment of algae can generate inhibitory substances that need to be managed to prevent environmental contamination (Kumar et al., 2021). Moreover, the sustainability of biohydrogen production is closely linked to the lifecycle assessment of the entire process, including the energy and resources required for algal cultivation and processing. The use of marine algae also raises concerns about the potential impacts on marine ecosystems, particularly if large-scale harvesting is implemented. Therefore, it is crucial to develop sustainable practices that minimize environmental impacts and ensure the long-term viability of biohydrogen production (Jiménez-Llanos et al., 2020; Sharma et al., 2021).

6.3 Technological bottlenecks in scaling biohydrogen production

Scaling up biohydrogen production from marine algae faces several technological bottlenecks. One significant challenge is the low hydrogen yield and productivity in current systems, which hinders commercial-scale operations (Goswami et al., 2020). The efficiency of biohydrogen production is influenced by various factors, including the metabolic pathways of algae, reactor design, and operational conditions (Show et al., 2019). For instance, the optimization of bioreactor configurations and the manipulation of light and nutrient conditions are critical for enhancing biohydrogen yields (Rajesh Banu et al., 2021). Additionally, the development of continuous bioreactor systems, as opposed to batch systems, is necessary for industrial-scale implementation. Addressing these technological challenges requires ongoing research and innovation to improve the efficiency and scalability of biohydrogen production processes (Show et al., 2019).

7 Commercialization and Industrial Scale-Up

7.1 Status of commercial biohydrogen production using marine algae

The current status of commercial biohydrogen production using marine algae is still in its nascent stages (Figure 3). While there have been significant advancements in the laboratory and pilot-scale studies, large-scale commercial operations are limited. The primary focus has been on optimizing the biological fermentation processes, which are considered more eco-friendly and economically viable compared to thermochemical processes (Goswami et al., 2020). Despite the potential of marine algae as a sustainable and renewable feedstock for biohydrogen production, the transition to commercial-scale operations faces several challenges, including low hydrogen yield and high production costs (Jiménez-Llanos et al., 2020; Sharma et al., 2021).

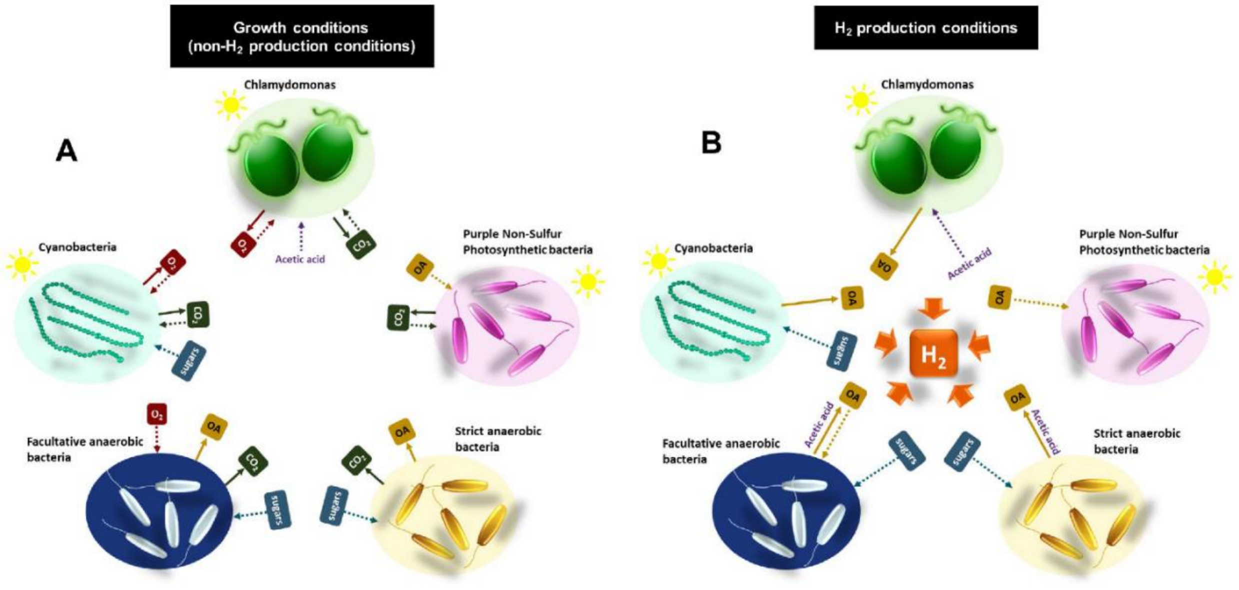

Figure 3 Potential metabolites exchanged among different H2-producing microorganisms during growth conditions (A) and H_2-producing conditions (B). The secretion and uptake of metabolites are indicated with plain and dotted arrows, respectively. Depending on the specific culture conditions the same metabolites can be secreted or accumulated. Organic Acids (OAs) mainly include ethanol, glycerol, formate, acetic acid, lactate, succinate and butyrate. When predominant, the specific OA is indicated next to the arrow (Adopted from Fakhimi et al., 2020)

7.2 Case studies of pilot projects and commercial ventures

Several pilot projects have been initiated to explore the feasibility of biohydrogen production from marine algae. For instance, an 11 m^3 pilot-scale bioreactor was used to investigate biohydrogen production via dark- and photo-fermentation, utilizing solar energy to reduce production costs and carbon emissions. This project demonstrated promising results with gas production rates of 96.30 mol/m^3-d and 224.68 mol/m^3-d for dark- and photo-fermentation, respectively (Lu et al., 2020). Another study focused on the hydrothermal pretreatment of the brown seaweed *Saccharina latissima*, which significantly improved the solubilization and subsequent production of biohydrogen and biomethane, achieving a maximum energy conversion efficiency of 72.8% (Lin et al., 2019). These pilot projects highlight the potential for scaling up biohydrogen production, although they also underscore the need for further optimization and cost reduction.

7.3 Barriers to scaling up production and reducing costs

Several barriers hinder the scaling up of biohydrogen production from marine algae. One of the primary challenges is the high cost associated with the cultivation, harvesting, and extraction processes of algal biomass (Anto et al., 2020). Additionally, the low hydrogen yield from current biological processes remains a significant bottleneck (Goswami et al., 2020; Sharma et al., 2021). Technical and scientific obstacles, such as the need for efficient pretreatment methods to enhance hydrolysis and the control of inhibitory substances formed during pretreatment, also pose challenges (Lin et al., 2019; Kumar et al., 2021). Furthermore, the complexity of integrating upstream and downstream processes to achieve economic viability and the need for advanced strategies to improve biohydrogen production efficiency are critical areas that require attention (Jiménez-Llanos et al., 2020; Sharma et al., 2021). Addressing these barriers through technological innovations and process optimizations is essential for the successful commercialization and industrial-scale production of biohydrogen from marine algae.

8 Environmental Sustainability of Marine Algal Biohydrogen Production

8.1 Ecological impacts of large-scale algae cultivation

Large-scale cultivation of marine algae for biohydrogen production can have significant ecological impacts. The cultivation process can enhance biomass production and nutrient recycling, especially when using polycultures, which have been shown to improve energy return on investment (EROI) and reduce greenhouse gas emissions (GHGs) compared to monocultures (Carruthers et al., 2019). However, the environmental sustainability of these systems is highly dependent on the specific cultivation methods and geographic locations. For instance, open raceway ponds (ORP) and tubular photobioreactors have different environmental impacts, with ORPs being more feasible under favorable climatic conditions (Pérez-López et al., 2017). Additionally, the use of marginal lands and industrial flue gases for CO_2 can further mitigate the ecological footprint of algae cultivation (Zaimes and Khanna, 2013).

8.2 Life cycle analysis (LCA) of biohydrogen from marine algae

Life cycle analysis (LCA) is a crucial tool for evaluating the environmental sustainability of biohydrogen production from marine algae. Various studies have highlighted the importance of LCA in identifying the most sustainable pathways for algal biofuel production. For example, a comparative LCA of different algal species and cultivation methods showed that certain bicultures could significantly improve sustainability metrics (Carruthers et al., 2019). Another study demonstrated that using advanced cultivation and processing methods could reduce the environmental impacts of macroalgae-based biofuels (Aitken et al., 2014). Furthermore, the LCA of microalgae production for aquaculture purposes revealed that upscaling could improve resource efficiency and reduce the carbon footprint (Taelman et al., 2013). These findings underscore the importance of optimizing cultivation and processing methods to enhance the environmental sustainability of marine algal biohydrogen production.

8.3 Strategies for minimizing environmental footprint

Several strategies can be employed to minimize the environmental footprint of marine algal biohydrogen production. One effective approach is the use of advanced metabolic engineering to enhance the productivity and efficiency of algal strains. For instance, genetically engineered cyanobacteria with higher photon conversion efficiency can significantly reduce the environmental impacts of biofuel production (Villacreses-Freire et al., 2021). Additionally, optimizing the cultivation and harvesting processes, such as using flat panel enclosed photobioreactors and direct transesterification, can lead to substantial energy savings and reductions in water consumption and GHG emissions (Brentner et al., 2011). Another strategy involves selecting appropriate geographic locations and reactor designs to minimize energy consumption for temperature regulation and other environmental burdens (Pérez-López et al., 2017). By implementing these strategies, the environmental footprint of marine algal biohydrogen production can be significantly reduced, making it a more sustainable alternative to conventional energy sources.

9 Future Prospects and Innovations

9.1 Emerging technologies and their potential to enhance biohydrogen efficiency

Emerging technologies in the field of biohydrogen production from marine algae are showing significant promise in enhancing efficiency and scalability. One such technology is the integration of advanced pretreatment methods, such as hydrothermal and chemo-sonic pretreatments, which have been shown to improve the solubilization of algal biomass and increase biohydrogen yields. For instance, hydrothermal pretreatment of the brown seaweed Saccharina latissima resulted in a significant increase in biohydrogen yield, demonstrating the potential of this method to enhance energy conversion efficiency (Lin et al., 2019). Similarly, the combination of alkaline and sonication pretreatment techniques has been found to be energetically favorable, reducing energy consumption while increasing biohydrogen production (Shankaran et al., 2022).

Moreover, advancements in genetic engineering of microalgae are paving the way for optimized biohydrogen production (Li, 2024). By identifying and manipulating bioenergy genes and pathways, researchers are able to enhance the photosynthetic efficiency and hydrogen production capabilities of certain algal strains (Beer et al., 2009). Additionally, the development of integrated microalgae-based H2 production processes, which combine various biological fermentation routes, offers a promising approach for commercial-scale operations (Goswami et al., 2020).

9.2 Future research directions in marine algae biotechnology

Future research in marine algae biotechnology should focus on several key areas to further optimize biohydrogen production. One critical area is the exploration of different marine algal species and their specific metabolic pathways for hydrogen production. Understanding the unique properties and potential of various species can lead to the identification of the most efficient biohydrogen producers (Kumar et al., 2021; Sharma et al., 2021).

Another important direction is the improvement of pretreatment and fermentation processes. Research should aim to develop more efficient and cost-effective methods for breaking down algal biomass and converting it into hydrogen. This includes investigating the effects of different pretreatment conditions on the yield and quality of biohydrogen, as well as optimizing fermentation parameters to maximize production (Lin et al., 2019; Nagarajan et al., 2020).

Additionally, there is a need for comprehensive techno-economic assessments to evaluate the feasibility and sustainability of biohydrogen production from marine algae. These assessments should consider the entire life cycle of the production process, from biomass cultivation to hydrogen extraction, to identify potential bottlenecks and areas for improvement (Kumar et al., 2021).

9.3 Policy and market implications for biohydrogen as a green energy solution

The adoption of biohydrogen as a green energy solution has significant policy and market implications. Policymakers need to create supportive frameworks that encourage research and development in biohydrogen technologies, as well as provide incentives for the commercialization and adoption of these technologies. This includes funding for pilot projects, subsidies for biohydrogen production facilities, and the establishment of standards and regulations to ensure the quality and safety of biohydrogen as a fuel (Goswami et al., 2020; Zhang et al., 2021).

From a market perspective, the development of a robust supply chain for biohydrogen is crucial. This involves not only the production of biohydrogen but also its storage, transportation, and distribution. Investments in infrastructure, such as hydrogen refueling stations and pipelines, are necessary to facilitate the widespread use of biohydrogen in various sectors, including transportation, industry, and power generation (Jiménez-Llanos et al., 2020; Sharma et al., 2021).

Furthermore, public awareness and acceptance of biohydrogen as a clean and sustainable energy source are essential for its market success. Educational campaigns and demonstrations of biohydrogen technologies can help build consumer confidence and drive demand for biohydrogen-powered products and services (Al-saari et al., 2019).

10 Concluding Remarks

The research on biohydrogen production using marine algae has demonstrated significant advancements in optimizing conditions to enhance efficiency. Various studies have highlighted the importance of pretreatment methods to improve the hydrolytic process during dark fermentation. For instance, thermal pretreatment of *Laminaria japonica* at 170 ℃ for 20 minutes maximized hydrogen yield to 109.6 mL H_2/g COD (added). Similarly, batch dilute-acid hydrolysis of Gelidium amansii optimized at 161 ℃~164 ℃, 12.7-14.1% S/L ratio, and 0.50% H_2SO_4 resulted in a maximum hydrogen production of 37.0 mL H_2/g dry biomass.

Moreover, the combination of microwave and hydrogen peroxide pretreatment under alkaline conditions significantly improved COD solubilization and hydrogen yield, achieving 87.5 mL H_2/g COD. The use of marine bacterium *Vibrio tritonius* under saline conditions also showed promising results, with optimal hydrogen production at 10 g/L NaCl. These findings underscore the critical role of pretreatment and optimization of environmental conditions in enhancing biohydrogen production efficiency from marine algae.

Marine algae hold immense potential for the future of sustainable hydrogen production due to their high biomass yield, rapid growth rates, and ability to capture CO_2 efficiently. Unlike terrestrial biomass, marine algae require less energy and water for cultivation and have negligible lignin content, making them a superior feedstock for biohydrogen production. The ability of green algae to produce hydrogen under both light anoxic and dark anoxic conditions further enhances their viability as a renewable energy source.

The integration of marine algae into biohydrogen production systems aligns with the principles of a circular bioeconomy, promoting the use of renewable resources and reducing reliance on fossil fuels. Advances in genetic and metabolic engineering are expected to overcome current limitations, such as the rigid nature of algal cell walls and the cost-effectiveness of production processes. As research continues to optimize pretreatment methods and fermentation conditions, marine algae are poised to play a crucial role in the transition to a sustainable and clean energy future.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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