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Environmental Control and Optimization Strategies for Hydrogen Production Systems Using Chlorella

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Abstract: Chlorella, with its large biomass and ability to produce hydrogen directly or indirectly, has become a potential green hydrogen resource. However, its hydrogen production efficiency is significantly affected by environmental conditions and system design. This paper systematically analyzes the environmental factors affecting chlorella hydrogen production, elucidates the toxicity mechanisms of pollutants such as microplastics and heavy metals, and examines the stress response mechanisms of chlorella. By integrating strategies for constructing hydrogen production systems, the paper proposes solutions to enhance chlorella hydrogen production efficiency from both environmental regulation and technological optimization perspectives, providing theoretical support for the practical application of chlorella hydrogen production.

1. Introduction

Against the backdrop of the global energy transition toward a low-carbon future, the importance of hydrogen as a zero-carbon energy source has become increasingly evident. Chlorella, with its unique hydrogen-producing potential, has emerged as a focal point of research in the field of biological hydrogen production. It can produce hydrogen directly through photosynthesis and indirectly through biomass fermentation, combining environmental benefits with sustainability advantages. However, in practical applications, pollutants in the natural environment can interfere with its metabolism and inhibit the activity of key hydrogen-producing enzymes. Traditional system designs also face efficiency bottlenecks. Additionally, the stress-tolerance mechanisms of microalgae have not been fully utilized. Therefore, conducting in-depth research into environmental impacts and system optimization is crucial for breaking through the efficiency bottlenecks in microalgae hydrogen production and advancing its industrialization.

2. Environmental factors affecting hydrogen production by chlorella

2.1 The toxicity of microplastics and heavy metals

Today, terms such as “white pollution” and “water pollution” have become global hot topics.

Industrial and domestic wastewater have caused excessive levels of nitrogen (N) and phosphorus (P) elements, as well as heavy metals [1], in water bodies. The non-biodegradable nature of plastic waste [2] has also made microplastics one of the most common pollutants in water bodies [3]. Let us take heavy metal zinc and microplastics (PVC [4], PE [5]) as examples . In water bodies, it is not merely individual microplastic particles and heavy metal ions. Microplastics often adsorb divalent zinc ions, forming aggregates of microplastics and zinc ions [6]. Once these aggregates adhere to the cell wall structure [7] of chlorella, they create a barrier effect, This shielding effect not only impairs the efficiency of nutrient and gas exchange between the algae and the external environment but also blocks light, affecting photosynthesis and reducing the production of photoelectrons. Exposure to heavy metals in water can damage algal cells [8], disrupting cellular structure, reducing membrane integrity, and impairing material exchange between cells and the external environment. Additionally, besides inducing oxidative stress in diatoms [9], more severe consequences include triggering programmed cell death in diatom cells [10, 11], leading to the loss of transmembrane phospholipid asymmetry in the plasma membrane, causing cells to lose normal plasma membrane function and disrupting the body's internal balance.

In summary, the toxicity of microplastics and heavy metals to algae can be summarized in three points. The first point is the shielding effect formed by the aggregation of microplastics and heavy metals, the second point is the damage to the integrity of the cell membrane, and the third point is the oxidative stress or even programmed cell death that may be caused by the stimulation of algae cells.

3. Stress resistance mechanisms of chlorella under environmental stress

3.1 Significance of research on the stress resistance of chlorella and extracellular stress resistance mechanisms

Chlorella species are highly diverse and widely distributed in nature, with chlorella being the dominant species in many water bodies in China [12]. Research into the mechanisms underlying chlorella's stress resistance under environmental stress conditions can aid in designing hydrogen-producing chlorella solutions through environmental optimization. The stress resistance of chlorella to environmental stress [13] can be explored from both extracellular and intracellular perspectives. Extracellular polymers (EPS) are complex high-molecular-weight polymers secreted by microorganisms and algae [14], primarily composed of proteins and polysaccharides. They act as a barrier on the outermost layer to isolate cells from toxic substances and can to some extent inhibit the toxic effects of conventional pollutants such as heavy metals and microplastics.

3.2 Cellular characteristics of chlorella and intracellular stress response regulation mechanisms

Based on the characteristics of chlorella at the cellular level, there are three key features: first, a smaller cell volume [15]; second, a highly reduced genome [16]; and third, physiological regulation that relies more on RNA than on proteins [17]. These three characteristics enable chlorella to regulate its internal processes when exposed to environmental stress. First, the smaller cell volume enhances the algae's light capture efficiency, providing greater regulatory flexibility for its photosynthetic electron transport system [18] when exposed to changes in light intensity. Second, the highly reduced genome significantly reduces the time required for transcription, enabling the algae to mobilize RNA for rapid responses when faced with environmental stress. Finally, chlorella rely more on RNA rather than protein regulatory factors for physiological regulation, which effectively reduces dependence on certain nutrients under environmental stress conditions such as low salinity.

4. Optimization strategies for hydrogen production systems using chlorella

4.1 Optimization of hydrogen production environmental parameters

Traditional methods of hydrogen production using algae have not placed a high priority on the hydrogen production environment, as conventional approaches involve drying algae into powder form before fermentation for hydrogen production [19]. During fermentation, the fermented material loses its biological activity, so the requirements for the hydrogen production environment are not particularly stringent. In modern methods, in addition to using chlorella biomass solution fermentation for hydrogen production, there is also the direct hydrogen production method using chlorella. Chlorella has a high biomass content [20] and is rich in cellulose [21]. During biomass solution fermentation for hydrogen production, enzyme preparations are typically added to decompose cellulose and other substances to assist in catalytic fermentation. The amount of biological hydrogen produced by mixed enzyme preparations is often greater than that produced by single enzyme preparations. When the initial pH is 7.0 [22], the enzyme preparation with the best effect is a mixture of cellulase: protease = 3:2 [23]. When using chlorella for direct hydrogen production, since it involves the photobioreaction pathway in chlorella live cells, protecting the biological activity of key enzymes during the reaction process is crucial. Therefore, modern chlorella direct hydrogen production places high demands on environmental suitability.

4.2 Construction and innovation of hydrogen production systems

The key to hydrogen production in chlorella lies in their electron transport chain, which originates from photosynthesis [24]. The construction of a hydrogen production system based on chlorella revolves around how to utilize their electrons. Within this framework, the hydrogen production system of chlorella can be divided into two types based on how electrons are utilized: indirect electron utilization and direct electron utilization. Under anaerobic and light-exposed conditions, chlorella can decompose water to produce oxygen and hydrogen. However, hydrogen production is limited under these conditions because oxygen inactivates the hydrogenase enzymes in chlorella [25]. Therefore, if oxygen and hydrogen production can be separated in time or space, hydrogen production can be significantly increased, and production costs can be reduced. Sulfur deprivation of chlorella [26] can achieve temporal separation of hydrogen and oxygen production.

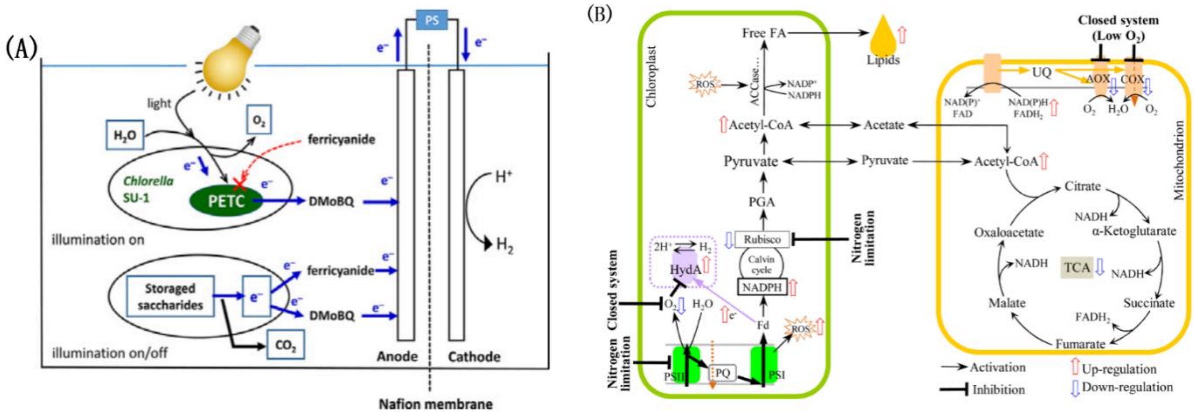

Fig. 1. (A) Schematic diagram of the bioelectrochemical system [33] (B) Mechanism of simultaneous induction of photosynthetic hydrogen production and lipid accumulation in algae [34].

This production system [27] is divided into two stages: in the first stage, chlorella undergo normal photosynthesis without sulfur deprivation, fixing carbon and allowing oxygen production, In the second stage, sulfur deprivation is applied to the algae under anaerobic conditions to produce hydrogen. Sulfur deprivation inhibits protein synthesis in the algae, thereby reversibly suppressing oxygen-containing photosynthesis and protecting hydrogenase from inactivation by oxygen. Recent studies have shown that nitrogen restriction of the algae can also promote hydrogen production, as shown in Figure 1(B). All of the above are hydrogen production systems that indirectly utilize electrons generated by photosynthesis in the algae. A bioelectrochemical system refers to a technology that converts chemical energy into electrical energy or chemical energy through extracellular electron transfer mechanisms [28]. This technology enables the transfer of electrons generated by photosynthesis in the algae to the extracellular environment, thereby achieving direct utilization of photoelectrons. As shown in Figure 1(A), combining a bioelectrochemical system with an electrolytic cell can establish a chlorella hydrogen production system. Under light conditions, chlorella generate electrons, which are transferred from the intracellular to the extracellular space via the photosynthetic electron transport chain. Through the conduction of an extracellular acidic solution medium [29], the electrons reach the anode of the electrolytic cell [30] and then flow to the cathode, where they combine with hydrogen ions to produce hydrogen gas. This system is highly efficient, simple in steps, and has great potential [31], representing a typical example of a direct utilization of photoelectrons and an environmentally friendly [32] microalgae hydrogen production system.

5. Summary

The study systematically reviewed the mechanisms by which environmental factors influence the hydrogen production process in chlorella, elucidating the toxic effects of microplastics and heavy metals through pathways such as aggregation shielding, cell membrane damage, and oxidative stress. It also revealed the intrinsic mechanisms by which chlorella responds to environmental stress, including the use of extracellular polymeric barriers and genomic streamlining. Additionally, the study identified technical pathways to enhance hydrogen production efficiency, including the optimization of mixed enzyme preparations, sulfur deprivation strategies, and the construction of bioelectrochemical systems. Future research should focus on elucidating the combined toxic effects of composite pollutants, leveraging multi-omics technologies to deepen the exploration of stress-resistant molecular regulatory networks, and advancing the large-scale integration of hydrogen production systems. This includes enhancing the activity of key enzymes through gene editing technology, optimizing electrode materials and reactor configurations, and conducting long-term operational stability tests to reduce industrialization costs. These efforts aim to facilitate the transition of microalgae hydrogen production technology from laboratory research to practical applications, providing theoretical and technological support for the sustainable production of clean energy.

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