

Hydrogen production with a newly discovered cyanobacteria strain

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Abstract. The escalating environmental challenges precipitated by the uncontrolled use of fossil fuels, notably the rise in global average temperatures, underscore the urgent need for sustainable energy solutions. This context has heightened the significance of exploring renewable energy sources, particularly through the lens of biological research aimed at uncovering viable alternatives to conventional energy forms. Against this backdrop, our study embarked on the isolation and characterization of the cyanobacterium *Synechocystis* sp. strain PSU 1262, sourced from the Badam River in the Turkestan region, employing 16S rRNA sequencing for its identification. The primary objective of our research was to assess the hydrogen-producing potential of PSU 1262 under a variety of environmental conditions. Our findings reveal that this strain achieves peak hydrogen production in slightly alkaline conditions and exhibits a marked decline in productivity when subjected to elevated sodium nitrate levels. Furthermore, we determined that PSU 1262 optimally generates hydrogen within a specific temperature range (27-30°C) and under certain light intensity conditions. These insights are pivotal, indicating that precise environmental management is essential for maximizing the biohydrogen output of this strain. The study conclusively highlights the capability of PSU 1262 as a promising agent for biohydrogen production, offering a significant contribution to the field of renewable energy and presenting a sustainable alternative to mitigate the environmental impact of traditional energy sources.

1 Introduction

Today, the challenge of protecting our environment has escalated to unprecedented levels, with the preservation of natural values increasingly sidelined by the demands of economic growth. The Earth faces a critical juncture, marred by irreparable ecological issues primarily driven by the unchecked combustion of fossil fuels. This legacy of human activity, relying on resources accumulated through millennia of microbial action, now

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results in the relentless release of carbon gases into the atmosphere. Such emissions disrupt the Earth's natural cycles, inflict damage on aquatic and terrestrial life, and compromise human health by exacerbating chronic and malignant diseases. The pressing nature of these challenges underscores the urgent need for sustainable, renewable energy sources, among which hydrogen stands out as a promising candidate [1].

In this urgent quest for environmental sustainability, the exploration of cyanobacteria as a viable source of clean hydrogen energy emerges as a beacon of hope. Cyanobacteria, with their vast physiological and morphological diversity, offer a natural pathway to hydrogen production through both direct and indirect biophotolysis. Research highlights how strains like *Oscillatoria* sp. [2] and *Anabaena* sp. [3] harness these processes for hydrogen generation, demonstrating the potential of these microorganisms in contributing to a green energy future. Significant studies, such as those by Allahverdiyeva et al. [4] have delved into the capabilities of numerous cyanobacterial species, revealing a substantial capacity for hydrogen production. Furthermore, investigations into the effects of photosystem II inhibitors on hydrogen production by *Aphanothece halophytica* under varying light conditions have opened new avenues for optimizing this biological process [5].

Centered on these promising developments, this study introduces the newly isolated PSU 1262, focusing on its ability to produce hydrogen under diverse environmental conditions. By integrating the environmental imperative with the innovative potential of cyanobacteria, we aim to contribute to the foundational knowledge required for advancing towards a sustainable "hydrogen economy." This research not only seeks to understand the specific conditions that optimize hydrogen production in *Synechocystis* sp. PSU 1262 but also to underline the broader implications of harnessing cyanobacteria for clean energy production in the face of pressing environmental challenges.

2 Materials and Methods

Cells of *Synechocystis* sp. strain PSU 1262 were cultivated in BG-11 nutrient medium, with each experiment beginning with cells in the logarithmic phase [6]. These cells were then adapted to fresh BG0-11 medium under optimal light and temperature conditions to suit the specific requirements of the subsequent experimental treatments. This preparatory step ensured that the cells were in a physiological state conducive to responding to the experimental variables being tested. Hydrogen production across all studies was quantitatively assessed using gas chromatography, with measurements taken at regular intervals to determine the effects of varying environmental conditions on H₂ output. In assessing the effect of pH, cells were incubated in BG-11 medium adjusted to different pH levels (4-12) using universal buffers. Following a 72-hour incubation period under continuous light exposure, hydrogen production was monitored every 24 hours to evaluate the impact of pH on H₂ generation [7]. To explore the effect of temperature, the experiment involved incubating the cells at a range of temperatures from 20°C to 35°C. After a 62-hour period of anaerobic incubation under constant light intensity, hydrogen production was measured to ascertain the optimal temperature for maximizing H₂ production. The impact of nitrogen sources was investigated by adding varying concentrations of sodium nitrate (NaNO₃) to the growth medium. Following a three-day incubation period under light, hydrogen production rates were measured every 24 hours, highlighting the influence of nitrogen availability on biohydrogen output. For the effect of light intensity, cells adapted to the new BG0-11 medium were subjected to various light intensities (0, 60, 120, 240, and 360 μmol photons/m²/sec). After a 24-h adaptation period, the cells were placed under anaerobic conditions and continuously incubated at the designated light intensities for 62

hours. Hydrogen production was then measured at 24-h intervals to elucidate the relationship between light intensity and H₂ production efficiency [8].

3 Results and discussion

Isolation and identification. The *Synechocystis* sp. strain PSU 1262 was isolated from the Badam River in the Turkestan region (Geo-coordinates: 42.265652, 69.687269). The identification of the cyanobacterial cultures was conducted using universal primers for 16S rRNA, with sequence similarity searches performed in the BLAST online database, leading to the identification of closely related species. Phylogenetic analysis revealed that strain PSU 1262 shares 98.52% similarity with the *Synechocystis* sp. CACIAM 05 strain. This genetically identified cyanobacterium, a non-heterocystous type, was further utilized in studies aimed at biological hydrogen production.

Effect of pH. The study focused on the growth dynamics of PSU 1262 cultured in BG-11 nutrient media, which was modified using universal buffers to span a pH range from 4 to 10. Preliminary investigations indicated that the cells did not grow at pH levels below 4. Our experiments further explored the cellular adaptation and growth across a broader pH spectrum, particularly noting the growth responses to acidic and alkaline conditions.

Fig. 1 A illustrates the growth pattern of PSU 1262 cells in BG-11 media that was adjusted to various pH levels using universal buffering systems. The optical density of the cell cultures, measured at 730 nm as a proxy for cell concentration, was recorded every three days to monitor growth. The findings reveal that PSU 1262 exhibits a remarkable capacity to adapt to alkaline environments, with optimal growth observed in conditions that approach neutrality and extend into slight alkalinity. In contrast, acidic environments were found to hinder cell growth significantly. The initial concentration of cells was standardized at an optical density of 0.1 at 730 nm, facilitating a controlled comparison across all pH levels tested.

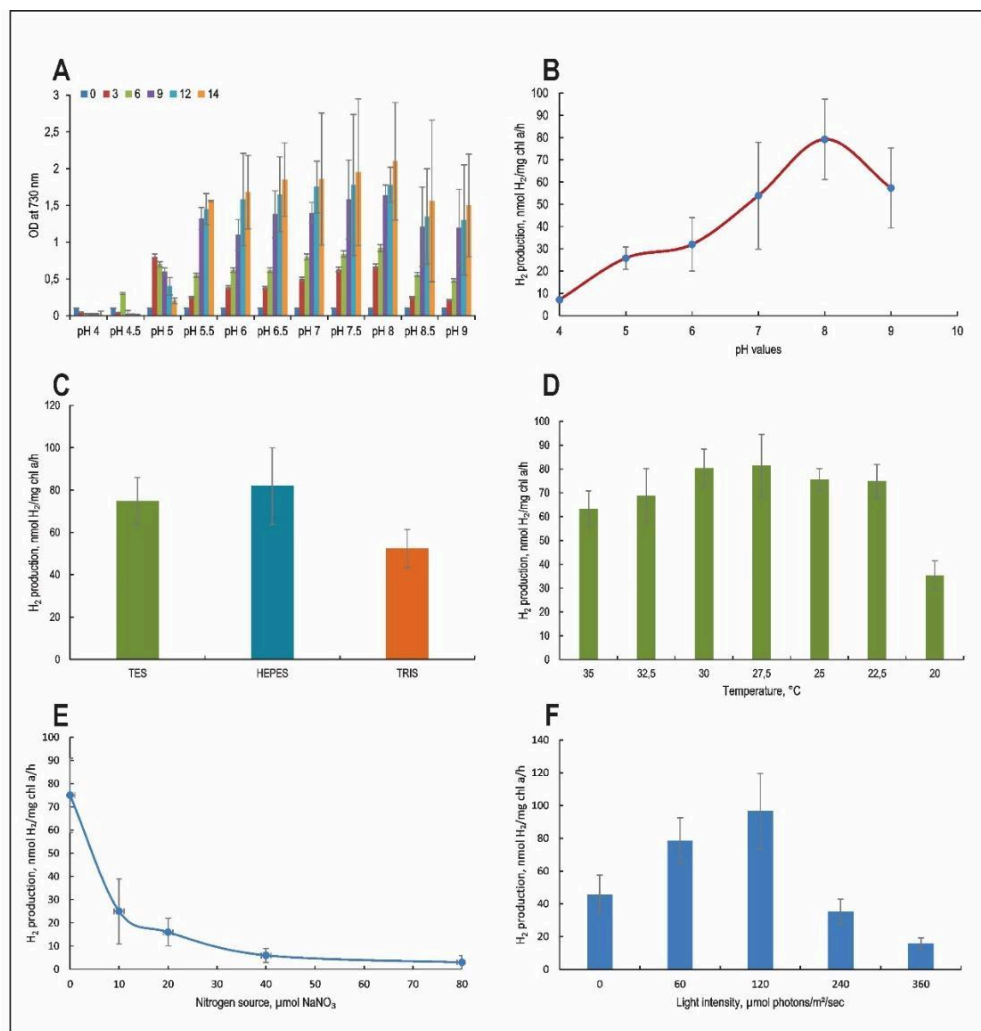


Fig. 1. Optimizing environmental conditions for enhanced biohydrogen production in *Synechocystis* sp. strain PSU 1262. Note: A – Growth dynamics in varied pH; B – pH impact on hydrogen production; C – Optimal pH enhancement; D – Temperature influence on H₂ output; E – Sodium nitrate's effects; F – Light intensity relationship.

Through this experiment, it became evident that the growth of PSU 1262 is profoundly influenced by the pH of the culture medium. At lower pH levels, notably around "pH 4" and "pH 4.5", the negative growth rates (-0.018 and -0.016, respectively) suggest a decline in cell concentration over time, reflecting adverse conditions for cell proliferation. As the pH increases, the growth rates improve significantly, peaking in the range of "pH 7" to "pH 8", which is consistent with the earlier observation that PSU 1262 prefers neutral to slightly alkaline conditions. The highest growth rates are observed at higher pH levels (pH 8) with values reaching up to 2.1. This indicates that the cells exhibit a marked increase in proliferation as the environment becomes less acidic and more alkaline.

Next, our investigation into the effects of pH on hydrogen production by PSU 1262 has elucidated key insights into optimizing environmental conditions for biohydrogen production (Fig. 1 B). The experimental data revealed a pronounced influence of pH on

hydrogen output, with production rates increasing alongside the pH scale, reaching an apex at pH 8 (79.2 nmol H₂/mg chl a/h). This peak signifies the optimal condition for hydrogen generation, beyond which a decrease at pH 9 suggests a limit to the pH-induced enhancement of production efficiency.

This pH-dependent trend in hydrogen production is indicative of the physiological and metabolic adaptability of PSU 1262 to environmental conditions. The optimal production observed at slightly alkaline conditions could reflect the enzyme dynamics within the cyanobacterium, particularly those enzymes involved in the hydrogen production pathway, such as hydrogenase. The decline in production at higher pH levels may be attributed to potential inhibitory effects on these enzymes or other metabolic shifts that unfavorably impact the biohydrogen generation process.

Effect of Buffer Optimization. Our observations, as visually represented in Fig. 1 C, indicated that a pH of 8 significantly enhanced the strain's hydrogen splitting activity. Conversely, pH values of 4 and 9 were found to negatively impact hydrogen productivity. This led to further investigations into the effect of pH 8 on hydrogen yield, utilizing concentrations of 50 μmol for TES, HEPES, and TRIS buffers. Notably, the HEPES buffer at pH 8 demonstrated a positive effect on hydrogen production.

In this study, the impact of buffer systems on hydrogen production at an optimal pH of 8 was meticulously analyzed. Among the buffers tested, HEPES notably outperformed the others, yielding 81.9 nmol H₂/mg chl a/h, surpassing the production rates observed with TES (74.9 nmol H₂/mg chl a/h) and TRIS (52.4 nmol H₂/mg chl a/h). This differential effect underscores the pivotal role of buffer composition in the biohydrogen production process, likely due to the stabilization of pH levels conducive to optimal enzymatic activity and metabolic conditions.

The superior efficacy of the HEPES buffer aligns with its known chemical stability and minimal interference with cellular processes, thus providing an environment that supports enhanced hydrogen production. The observed decrease in productivity with TRIS and the moderate performance of TES suggest that not all buffers equally support the biochemical pathways involved in hydrogen generation.

Effect of temperature on H₂ production. A pivotal aspect of optimizing biohydrogen production processes involves understanding the environmental factors that influence the efficiency of hydrogen-producing organisms [9]. In this context, our study examined the effect of temperature on hydrogen production by PSU 1262, a cyanobacterium known for its potential in renewable energy applications (Fig. 1 D).

Our results delineated a clear temperature-dependent pattern in hydrogen production. While the strain's growth was optimal at 25°C, the hydrogen production peaked between 27°C and 30°C, with the highest efficiency observed at 27.5°C (81.3 nmol H₂/mg chl a/h). This temperature was identified as the most conducive to hydrogen production, indicating a specific thermal preference that deviates slightly from the optimal growth temperature.

Temperatures below and above this optimal window resulted in diminished hydrogen output, highlighting the sensitivity of PSU 1262 to thermal variations. Notably, at 20°C, hydrogen production significantly dropped to 35.3 nmol H₂/mg chl a/h, while a temperature increase to 35°C reduced the production rate to 63.2 nmol H₂/mg chl a/h. This thermal response curve underscores the critical role of maintaining appropriate temperature conditions to maximize hydrogen production efficiency.

Effect of N-source. The findings revealed a pronounced negative effect of NaNO₃ on hydrogen production. In the control setup, hydrogen production was measured at 75 nmol H₂/mg chl a/h, establishing a baseline for the experiment. However, upon the introduction of NaNO₃, a significant reduction in hydrogen output was observed. Notably, at a concentration of 10 μmol NaNO₃, hydrogen production dropped to 25 nmol H₂/mg chl a/h.

This decreasing trend persisted with increasing NaNO_3 concentrations, culminating in a minimal production rate of 3 nmol H_2 /mg chl a/h at 80 $\mu\text{mol NaNO}_3$ (Fig. 1 E).

The data underscores the inhibitory role of sodium nitrate in the biohydrogen production pathway of PSU 1262. The addition of NaNO_3 , even in modest amounts, led to a threefold decrease in hydrogen output, with further increases in concentration exacerbating the decline. These results suggest that the presence of nitrate ions may interfere with the metabolic processes conducive to hydrogen production, possibly diverting electron flow away from hydrogenase enzymes or otherwise impairing the photosynthetic machinery responsible for H_2 generation [10].

Effect of light intensity and duration of light exposure. The experiment revealed a distinct relationship between light intensity and hydrogen production efficiency. Initially, in complete darkness, hydrogen production was recorded at 45.6 nmol H_2 /mg chl a/h. Introduction of light significantly enhanced production rates, peaking at 120 $\mu\text{mol photons/m}^2/\text{sec}$ with an output of 96.6 nmol H_2 /mg chl a/h. This optimal light intensity highlights the balance between sufficient energy provision for photosynthetic activity and the avoidance of photoinhibitory effects. However, at higher intensities (240 and 360 $\mu\text{mol photons/m}^2/\text{sec}$), a decline in hydrogen production was observed, alongside evidence of cell lysis, indicating detrimental effects of excessive light exposure (Fig. 1 F).

The findings underscore the pivotal role of light intensity in modulating biohydrogen production in PSU 1262. While moderate light intensities stimulate hydrogen production by enhancing photosynthetic electron flow towards hydrogenase enzymes, excessive light can induce stress responses, including photoinhibition and cell lysis, thereby reducing hydrogen output. The observed peak in hydrogen production at 120 $\mu\text{mol photons/m}^2/\text{sec}$ provides a critical insight into the optimal light conditions for maximizing biohydrogen production while minimizing adverse effects on cell viability [11].

4 Conclusion

The isolation and detailed examination of PSU 1262 hydrogen production under various environmental conditions underscore its potential for renewable energy applications. The strain's optimal biohydrogen production occurs in slightly alkaline conditions, with environmental factors such as temperature, light intensity, and nitrogen source concentration playing significant roles in its metabolic output. These findings not only provide a foundation for further optimization of cyanobacterial hydrogen production systems but also highlight the importance of environmental condition management in biohydrogen production processes. Future research will aim to enhance the efficiency and scalability of PSU 1262 for practical applications in sustainable energy generation.

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