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Research Article

Effect of Nanoparticle Addition on Biohydrogen Production

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Abstract

Rapid population growth and consumption lead to an increased demand for energy. Fossil fuels as the most dominant resources used for energy production are depleting, and more importantly, their combustion leads to the release of carbon dioxide (CO_2), triggering global warming and climate change. Therefore, recent studies have been focusing intensively on increasing the production of green hydrogen as a clean alternative [1-2]. Parallel to these efforts, bio-hydrogen has also been gaining significant attention.

In this study, high temperature (110°C for 10 minutes), acidic conditions (pH 2-5.5), and nanoparticle addition (magnetite nanoparticle; obtained from Ege University, Faculty of Science, Department of Biochemistry) methods were tested for enhancing the biohydrogen production. For this purpose, initially, the sludge was autoclaved at 110°C for 10 minutes. Following these pretreatment steps, four different trial sets (R1: pH 5.5, R2: pH 5.5 + 5mg/L Fe_3O_4 NP, R3: pH 7, R4: pH 7 + 5mg/L Fe_3O_4 NP) were established to investigate biohydrogen production. The reactors were operated under static conditions at 38°C with fed-batch using 2-4 g COD/day substrate load. The volumetric contents of the produced biogas were determined by sampling of headspace gases and analyzed using gas chromatography (GC).

According to the results, while reactors R1 and R2 produced a maximum volumetric percentage of biohydrogen of 10-15%, reactors R3 and R4 reached levels of 30% (on day 11) and 32% (on day 7), respectively. These results indicated that the acidification pretreatment did not increase hydrogen yield; however, the addition of nanoparticles under neutral conditions significantly improved biohydrogen production (compared to the control group) at earlier stages.

Keywords: Biohydrogen, Nanoparticles, Fe_3O_4, Gas chromatography, Magnetite

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Nanopartikül İlavesinin Biyohidrojen Üretimine Etkisi

Özet

Hızlı nüfus artışı ve tüketim, enerji talebinde artışa yol açmaktadır. Enerji üretiminde en yaygın kullanılan fosil yakıtlar tükenmektedir ve daha da önemlisi, bunların yanması karbondioksit (CO_2) salımına yol açarak küresel ısınma ve iklim değişikliğini tetiklemektedir. Bu nedenle, son zamanlardaki çalışmalar, yeşil hidrojen üretimini artırmaya yoğunlaşmıştır [1-2]. Bu çalışmalara paralel olarak biy0hidrojen çalışmalarına duyulan ilgi de önemli oranda artmaktadır.

Bu çalışmada, biyohidrojen üretimini artırmak için yüksek sıcaklık (110°C'de 10 dakika), asidik koşullar (pH 2-5.5) ve nanoparçacık ekleme (manyetit nanopartikül; Ege Üniversitesi, Fen Fakültesi, Biyokimya Bölümü'nden temin edilmiştir) yöntemleri test edilmiştir. Bu amaçla, öncelikle aşı çamur, 110°C'de 10 dakika boyunca otoklavlanmıştır. Bu ön işleme adımlarının ardından, biyohidrojen üretimini araştırmak için dört farklı deneme seti (R1: pH 5.5, R2: pH 5.5 + 5mg/L Fe3O4 NP, R3: pH 7, R4: pH 7 + 5mg/L Fe3O4 NP) oluşturulmuştur. Reaktörler, 38°C'de statik koşullarda, 2-4 g KOİ/gün günlük beslemeli olarak çalıştırılmıştır. Üretilen biyogazın hacimsel içerikleri, tepe gazlarının örneklenmesiyle belirlenmiş ve gaz kromatografisi (GC) kullanılarak analiz edilmiştir.

Sonuçlara göre, R1 ve R2 reaktörleri maksimum hacimsel biyohidrojen yüzdesi olarak % 10-15 üretirken, R3 ve R4 reaktörleri sırasıyla %30 (11.günde) ve %32 (7.günde) seviyelerine ulaşmıştır. Bu sonuçlar, asidifikasyon ön işleminin hidrojen verimini artırmadığını; ancak, nötr koşullar altında nanopartikül eklenmesinin (kontrol grubuna kıyasla) biyohidrojen üretimini erken aşamalarda başlattığını göstermektedir.

Anahtar Kelimeler: Biyohidrojen, Nanopartikül, Fe_3O_4, Gaz kromatografisi, Manyetik

1. Introduction

The rapidly increasing global population and the depletion of energy resources are leading to a greater demand for clean energy. Fossil fuels, the most used energy sources, are being exhausted, and more importantly, their carbon dioxide (CO_2) emissions are triggering global warming and climate change.

To prevent this situation, hydrogen, which has the highest energy content per unit mass and produces water as its combustion byproduct, has re-emerged as an important alternative. Hydrogen, classified among renewable energy sources, is the simplest and most abundant gas on Earth. It is 14 times lighter than air under standard pressure and temperature, colorless, odorless, tasteless, non-metallic, and flammable. Hydrogen, which has a diatomic structure, possesses an atomic mass of 1.00794 g/mol. Due to its high energy content, it finds applications in a wide range of fields.

Hydrogen gas is used in various industries for different purposes. For example, it is frequently used as an alternative fuel in automotive and transportation equipment, or as a fuel source in aerospace and aircraft industries, particularly in rocket engines. It also plays a significant role in the chemical, electronics, food and beverage, glass, metal production, pharmaceutical, and biotechnology industries. Hydrogen is a locally producible, easily and safely transportable energy system that can be utilized in every area from transportation to heating, from industry to our kitchens.

In our today, H_2 is typically produced from fossil fuels, biomass, and water. Biohydrogen is mainly produced through thermal, biological, and electrochemical pathways. Thermochemical and electrochemical production methods require high costs and are not always environmentally friendly processes. In contrast, the biological hydrogen production process has low energy requirements and, consequently, lower energy costs because it occurs at suitable pressures and temperatures. Since it can be obtained from waste materials, it also contributes to recycling. Biohydrogen, which is an environmentally friendly energy source, is produced from inorganic and organic materials using bacteria or microalgae, either in the presence or absence of light. It has several advantages: it does not cause environmental pollution, can be produced under appropriate temperature and pressure conditions, utilizes renewable energy sources, and most importantly, uses organic waste in its production and produces valuable by-products. Unlike the toxic gases released from the combustion of fossil fuels, the combustion of biohydrogen only produces water.

Biohydrogen production is carried out by photoheterotrophic and anaerobic microorganisms using carbohydrate-rich biomass wastes. The production of H_2 by microorganisms occurs via enzymes. The most commonly used enzyme, hydrogenase, either oxidizes H_2 into protons and electrons or reduces protons to release molecular hydrogen. The reduction of protons to hydrogen within the cell leads to the release of excess electrons, resulting in additional energy production steps in the metabolism. The most widely used and known enzymes in hydrogen production are nitrogenase, Fe-nitrogenase, and Ni-Fe hydrogenase. These enzymes catalyze the reaction: $2H + 2e^- \rightarrow H_2$

All biohydrogen production methods are highly advantageous in terms of sustainability, renewability, and energy efficiency.

a) H_2 Production via Dark Fermentation: This process is carried out by obligate anaerobic or facultative anaerobic bacteria under mesophilic and thermophilic temperatures and within a pH range of 5.0-6.0. Biomass such as low-cost, easily degradable, high-carbohydrate waste or simple sugars like glucose, sucrose, and lactose can be used. Dark fermentation can occur without the need for sunlight or artificial light. Fermentative bacteria involved in dark fermentation provide numerous benefits to the process. These include high hydrogen production rates, continuous production in the presence of organic matter both day and night, and high growth rates. Additionally, the amount of hydrogen produced in fermentation depends on hydraulic retention time (HRT), temperature, and pH value.

The equation for dark fermentation is: $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$

b) H_2 Production via Photofermentation is a process where biomass or organic matter is converted into hydrogen and carbon dioxide using solar energy as an energy source. Photofermentation is carried out under anaerobic conditions by photosynthetic bacteria. The optimum temperature range is 30-35°C, and the optimum pH is 7.0. In the photofermentation process, sunlight is used to convert water into protons, electrons, and oxygen. Nitrogenase is used as a catalyst in the process; protons and electrons react with nitrogen and ATP to produce ADP, H_2, and NH_3. In literature studies, the maximum hydrogen yield in photofermentation has been obtained as 80%. Despite providing high hydrogen yields, there are some factors that limit photofermentation. These include the nitrogenase enzyme, the presence and intensity of light, and production costs.

The equation for photofermentation is: $CH_3COOH + 2H_2O + \text{light} \rightarrow 4H_2 + 2CO_2$

c) Biophotolysis: This process involves the separation of molecular hydrogen and oxygen by green microalgae and cyanobacteria under specific conditions and in the presence of sunlight. It is divided into direct and indirect biophotolysis. In direct biophotolysis, hydrogen production uses solar energy and algae to convert water into chemical energy within a photosynthetic system. The most commonly used organisms are the green alga *Chlamydomonas reinhardtii* and the Cyanobacterium Synechocystis. The advantages of the biophotolysis method are its easy accessibility and low cost. Its disadvantage is the low efficiency (approximately 5%), which has been increased to 15% through further research. In indirect biophotolysis, biohydrogen can be produced using certain algae and cyanobacteria capable of directly producing hydrogen under specific conditions. Unlike microalgae, reactions in cyanobacteria can occur temporarily in separate light and dark cycles. This method takes place in two distinct stages. The O_2 evolution stage is separate from the H_2 production stage. In this way, the sensitivity of the hydrogenase enzyme to O_2 during the H_2 production stage is managed. In the first stage, cyanobacteria perform photosynthesis during the light cycle, producing organic matter and O_2 as a result of the reaction between CO_2 and H_2O. In the second stage, an O_2-independent cycle (dark cycle) follows, where organic matter is converted into soluble metabolites, H_2 and CO_2. *Anabaena cylindrica* is the best-known hydrogen-producing cyanobacterium.

d) Hybrid system; photofermentation and dark fermentation processes are mixed systems that combine directly or in series type configuration. The presence of light is necessary for the functioning of the hybrid system. Biohydrogen production is carried out by using fermentative bacteria, methanogenic bacteria and microalgae in series [14]. In recent studies, it has been observed that different types of nanoparticles have been added to the system to make biohydrogen production more efficient. Nanoparticle (NP) is defined as particles with dimensions of 100 nm and less. These particles constitute the foundations of nanotechnology [15]. In studies found in the literature, it has been proven that the use of inorganic nanoparticles such as silver, iron, titanium oxide and nickel increases the production of hydrogen. The physical and chemical properties of NP's have contributed to their application in dark fermentation. It is known that they increase the efficiency, production efficiency and efficiency rate of hydrogen-producing microorganisms. NP's have high specificity, higher catalytic activity and large surface area, which improves the efficiency of the dark biohydrogen fermentation process [16]. NP's increase the hydrogen efficiency and yield by directly targeting the hydrogenase enzyme and increasing the electron transfer efficiency in anaerobic microorganisms [17]. In a study conducted, *Bacillus sp*., *Bordetella*, *Enterobacter*, *Proteus* and *Pseudomonas sp*. their organisms have been used and it has been stated that NP's have a positive effect on hydrogen production [18]. As shown in Figure 1, during the production of biohydrogen, NP's enter through the bacterial wall due to their structural properties and change the electron transfer rate. Subsequently, this effect leads to an acceleration of hydrogen production during dark fermentation by increasing the activity of ferredoxin oxidoreductase due to increased quantum magnitudes and surface areas [19-20].

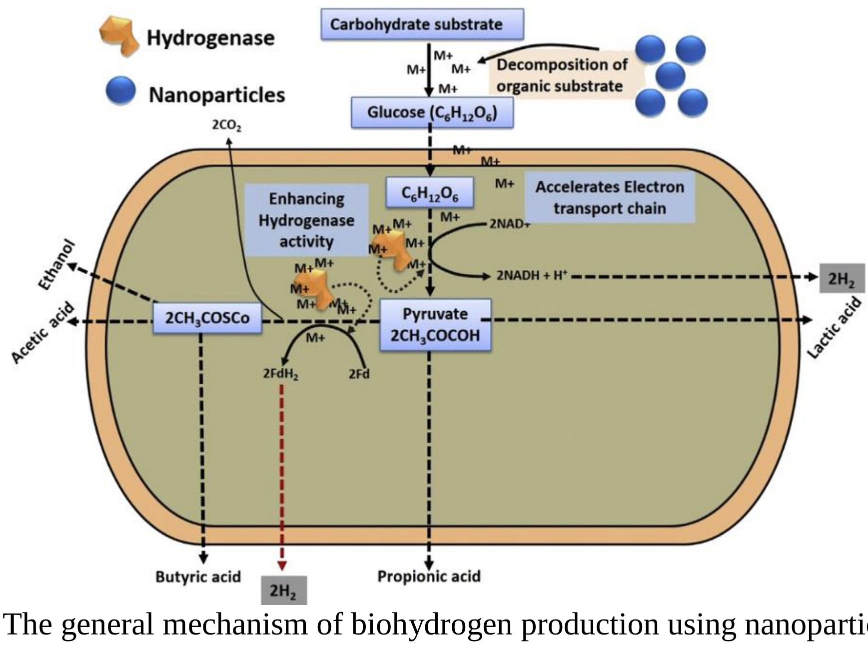

Figure 1. The general mechanism of biohydrogen production using nanoparticles [21]

A study conducted by Gadhe and Sonawane [22] showed that hydrogen production occurs (24%) by increasing the electron transfer rate by directly inducing ferredoxin reductase and hydrogenase enzyme activity by using nickel (NiO) and hematite oxide (Fe_2O_3) NP's separately. Later, hydrogen efficiency was increased by up to 27% by adding Fe_2O_3 and NiO together to milk waste. In another study, Fe_2O_3 and NiO NP's were used in dark fermentation, in which starch was used as a substrate. The experiment was performed on a laboratory scale using 0,20 g/L starch inoculated with 0,50 mg/L NP [17]. Both Fe and Ni NP's at a concentration of 37,5 mg/L produced about 149,8 mL/g-VS, which is about 200% higher than the control sample [23]. As a result of the study, it has been suggested that the efficient conductivity of FeO NP's may be the reason behind the increase in electron transfer rate and make it useful in hydrogen production [17].

Jiang et al. [24] reported that NP's helps electron transfer between two microbial cells decently. The optimal concentration of NP used in previous studies has been proven to be non-toxic or inhibitory for microorganisms and effective in the production of higher hydrogen concentrations through dark fermentation [25]. As a result, according to the literature review, the use of nanoparticles increases the production of biohydrogen by increasing the reaction rates and supporting hydrogen-producing organisms depending on the microbiota in the seed sludge.

2. Material and Methods

Anaerobic seed sludge, which was supplied from a biogas plant established in İzmir, was autoclaved for 10 minutes at 110 ^{o}C in order to inhibit the methanogenic bacteria contained in the sludge and support hydrogen-producing microorganisms. The autoclaved seed sludge was divided into two and trial sets A and B were established.

In the A trial set, the seed sludge was kept under room conditions for 24 hours by reducing the pH to 2-2.5 using 1M HCl. At the end of 24 hours, the seed sludge was increased to a pH 5-5.5 with 1M NaOH. In this test set of acid application, 2 different reactor assemblies (R1 and R2) were installed. The reactors prepared in 250 mL medium bottles, 5 mg/L Fe_3O_4 magnetic nanoparticles were added to the R1 reactor, while no additions were made to the R2 reactor.

In the B trial set, the seed sludge used was divided into two without acid application (pH 6-7) after the autoclave process and R3 and R4 reactor sets were installed in such a way that they had a volume of 250 mL. As in the other set of experiments, 5 mg/L Fe_3O_4 magnetic nanoparticles were added to the R4 reactor, while no additions were made to the R3 reactor.

Four different reactors were operated under static conditions in an incubation at 38°C for 10 days. The reactors were fed with molasses high in carbohydrate content of 2-4 grams per day per liter. Gas samples were periodically taken from the headspace of the reactors starting from the beginning of the experiment, and analyzed using gas chromatography (GC). The schematic representation of the study is provided in Figure 2.

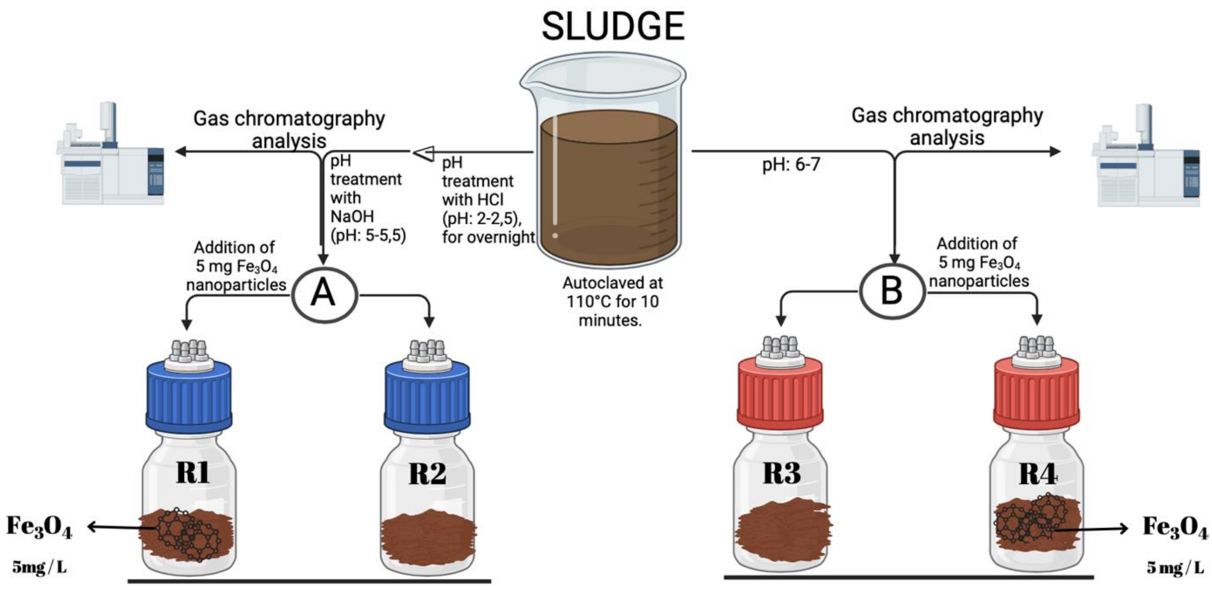

Figure 2. Experimental setup

3. Results and Discussion

The experimental setups were operated for 10 days. Biogas content was determined through regular GC analyses. According to the results obtained, reactors R1 and R2, inoculated with sludge left overnight at pH 2-2.5, were observed to produce lower levels of hydrogen compared to other experimental groups. In reactor R1, where acid pretreatment and nanoparticle addition were performed together, hydrogen production remained at around 2-3%, indicating no significant gas production. This is believed to be due to damage occurring to the structure of nanoparticles during acid pretreatment, which could also harm microorganisms producing hydrogen in addition to methanogenic groups. Similarly, in reactor R2 where nanoparticle addition was not performed (Figure 3), hydrogen production could reach a maximum of 10% by the end of the 10-day period.

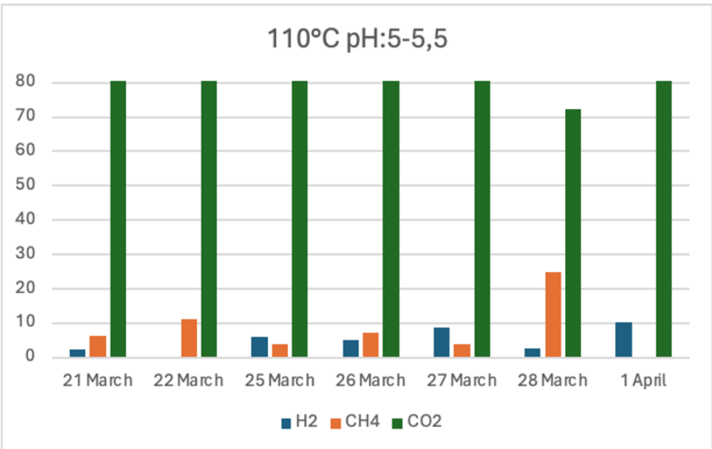

Figure 3. Data obtained from GC analysis of gas samples taken from reactor R2

The type of sugar used as a carbon source has been shown to affect hydrogen production in studies [26]. For instance, Mohanraj et al. [27] compared hydrogen production efficiencies with *E. cloacae* using different sugar types in the presence of Fe_2O_3 NP's (200 mg/l). The results indicated that glucose yielded 21.8% more H_2 production compared to sucrose. On the other hand, in another study using mixed cultures [28], it was reported that the highest H_2 yield (3.57 mol H_2 / mol sucrose) and hydrogen content (66%) were achieved with sucrose with the addition of the same amount of Fe_2O_3 NP's.

In our study, molasses used as the carbon source, obtained as a by-product from sugar beet production and containing 50% sugar (sucrose), 20% water, and 30% other sugars, is a valuable feedstock. According to our experimental results, comparing reactors R3 and R4 without acidic pretreatment (Figures 4 and 5), methane production in reactor R3 without nanoparticle addition is higher than hydrogen production and reached a maximum of 30% hydrogen level by the 12th day of the experiment. In reactor R4 with nanoparticle addition, hydrogen production started on the 5th day and reached 32% by the 7th day. The results indicate that nanoparticle addition enhances hydrogen production at earlier stages and suppresses methane production.

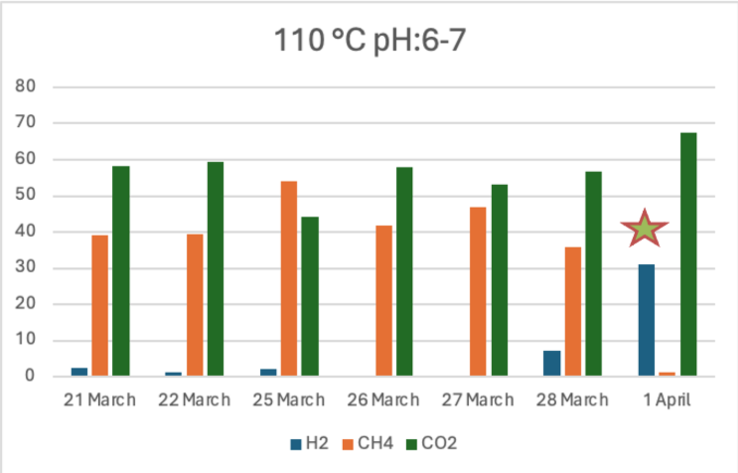

Figure 4. GC analysis of gas samples obtained from the R3 reactor

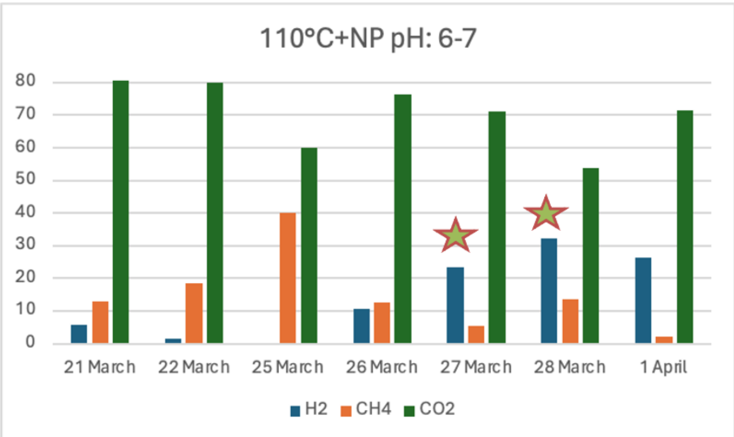

Figure 5. GC analysis of gas samples obtained from the R4 reactor

In microbial hydrogen production, it is known that physical conditions, nutrient type, daily feeding rate, operating temperature, and initial inoculum type have an impact [26]. For example, in a study by Zhang et al. (2021) [29], a pure culture of *Enterobacter cloacae* was used for biohydrogen production. The study investigated the effect of 0-50 mg/L Fe_3O_4 nanoparticles. Operating for 5 days in a self-designed fermenter containing 40 g/L of sugar as the nutrient medium, hydrogen production reached a cumulative level of 1625.2 ± 18.0 mL/L with the addition of 40 mg/L NP, resulting in 50.7% higher production compared to the control group.

In another study conducted by Masihi et al. [30], various concentrations of Fe_3O_4 NP's were used to enhance biohydrogen production from food waste. A single-stage hybrid reactor (dark/photofermentation) was used in the study. Fe_3O_4 can act as a scaffold for enzyme and substrate binding, thereby increasing the speed of enzyme reactions during hydrogen production. The study found that the fermentation reactor with added Fe_3O_4 nanoparticles increased hydrogen production efficiency by 2.1 times compared to the control experiment without NP addition.

In a study from 2011, Zhao et al. [31] first examined the catalytic effect of magnetic Fe_3O_4 nanoparticles on biohydrogen production. The mixed culture used in the study underwent an alkaline shock and lost hydrogen production activity. Fe_3O_4 nanoparticles and iron ions were used as activators to try to restore the bioactivity of the mixed culture. The results showed that mesoporous Fe_3O_4 nanoparticles were significantly more effective than iron ions in restoring bioactivity. The maximum cumulative hydrogen production efficiency was reported to be 26% higher in the reactor with added Fe_3O_4nanoparticles compared to the control group.

4. Conclusion

The use of materials such as magnetic nanoparticles or catalysts can enhance biohydrogen production by increasing reaction rates. Magnetic nanoparticles interact with hydrogenase enzymes to enhance enzyme activity and stability.

In this study, methods including high temperature (110°C), acidic pretreatment, and nanoparticle (Fe_3O_4) addition were tested to improve biohydrogen production. The results indicated that prolonged acidic conditions during inoculum preparation could damage both methanogenic and hydrogen-producing groups. Additionally, it was observed that the structure of nanoparticles could degrade under acidic conditions, and thus, higher hydrogen production occurred when near-neutral pH levels were preferred.

In the reactor where only thermal pretreatment was applied, hydrogen production reached about 30%, and it was observed that the onset of increased hydrogen production could be advanced by up to 5 days compared to the reactor with nanoparticle addition. Methane production occurred in both reactors; however, the amount of hydrogen produced in the nanoparticle-added reactor exceeded methane production by the 5th day. These results indicate suppression of methanogenic groups and dominance of hydrogen-producing microorganisms in the reactor.

Based on the data obtained and comparisons with other studies, the addition of nanoparticles appears to enhance hydrogen production by binding to and stimulating hydrogenase enzymes, thereby shortening the onset of hydrogen production within the reactor. These studies serve as preliminary investigations into the effects of different pretreatment steps and concentrations of various magnetite NP's.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

All authors involved in the study contributed to its design. Author 1 and Author 2 were responsible for the conception and execution of the study, data collection, and manuscript writing. Author 3 and Author 4 participated in data interpretation and guiding the study direction.

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