Nanoadditives for Enzymatic Biohydrogen Production

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Abstract: Using nanoadditives in biohydrogen production can be considered an optimal solution for existing challenges. Nanoparticles (NPs), affect the growth of microorganisms, intracellular electron transfers and the activity of metalloenzymes. The main research gap is to analyze and evaluate the effect of different types of NPs in several studies. The primary purpose of this study is to cover the research gap, by exploring the research databases, employing a complete list of search items followed by PRISMA guidelines. The taxonomy of the present study can cover these weaknesses and can successfully complement other studies. Evaluations have been conducted by the findings of the identified articles during the PRISMA guidelines. We also applied a feature selection technique to find the most important factor affecting the biohydrogen production yield. Accordingly, the additive values with the Relief feature selection score of 0.47±0.17 provided the highest impact on the biohydrogen production yield, followed by PH, with a score of 0.36±0.13. Finally, results showed that Fe-based additives boosted the catalytic mechanisms and metabolisms in all of the substrates. Conversely, Carbon-based additives enhanced glucose degradation in the substrates, and Biochar improved biological activity. The type of substrates and the number of NPs in each substrate are different. This study presents comparable results for each finding, based on the evidence. The main findings of this review can be of valuable help in the initial development of integrated additives, with different mechanisms of action.

Keywords: Biohydrogen; nano-additive; enzymatic biohydrogen production; bioenergy; nanoadditive; energy; sustainability; energy production; review; nanomaterials

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1 Introduction

Biohydrogen production offers a clean, renewable, and sustainable energy source that significantly reduces greenhouse gas emissions and air pollution, enhancing environmental protection and public health. Utilizing organic waste materials, it addresses waste management issues and improves energy security through diversification and decentralized production. This technology promotes economic growth by creating green jobs, particularly in rural areas, and drives technological innovation, fostering the integration of renewable energy systems. Aligning with global climate goals, biohydrogen reduces reliance on fossil fuels, lowers carbon footprints, and improves energy access for remote communities, thereby supporting a more resilient and sustainable future while providing broad social and economic benefits [1]. The reduction of fossil fuels, price instability and environmental concerns have driven interest in alternative fuels like biohydrogen. Biohydrogen offers a clean, renewable energy source by using organic waste, addressing waste management, and reducing greenhouse gas emissions and air pollution, thus protecting the environment and public health. This approach builds a robust, diverse energy infrastructure, aligns with global climate goals, and improves energy access in remote communities. By leveraging local biomass, biohydrogen promotes sustainability and offers broad social and economic benefits, fostering a more resilient and equitable energy future [2]. Biofuels benefit energy density close to fossil fuels, making them promising alternative resources to the existing fuels with reduced carbon footprint [3]. Hydrogen as a clean alternative energy source with a high energy potential (142 kJ/g) [4] and feasibility in production and transportation as well as direct use for the generation of electricity by fuel cells can be considered as the most suitable substitute compared to other biofuel resources [5]. Recently, several studies have been developed for assessing Biohydrogen as a sustainable energy resource for different aims. Levin et al. (2004) investigated the biological approaches for hydrogen production due to their advantages over the mechanism and bioreactor system [6]. Kotay and Das (2008) concluded that Biohydrogen is an excellent sustainable energy resource to cope with future renewable energy demands. Biohydrogen can be the critical turning point in response to the future energy supply. The production technique for Biohydrogen can be considered an ideal hydrogen production way among the range of renewable H_2 production technologies [7]. Brentner et al. (2010) researched developing hydrogen as an alternative energy resource to reduce Greenhouse Gas (GHG) emissions and other air pollutants and decrease the use of fossil fuels [8]. Consequently, the biohydrogen production can be considered as a sustainable energy production approach compared to the traditional hydrogen production technics. Nanoparticles have been employed as effective additives in biohydrogen production. Nanoparticles can augment the chemical structure of the biohydrogen production substrate and increase hydrogen efficiency and hydrogen evolution rate. But the effect of each type of additive is different. Accordingly, there is a need to conduct review studies in this field, to extract the strengths and weaknesses of various additives. Accordingly, this study presents a systematic state-of-the-art of the effects of nano-additive on enzymatic biohydrogen production. The research gaps for prospects are optimizing bioreactor conditions and metabolic engineering and investing in a strategic research plan to increase biohydrogen production as a practical system to sustainably meet future hydrogen demand [8]. In a study by Singh et al. (2015), the possible techniques for producing Biohydrogen from lignocellulosic biomass and the main technological challenges have been discussed. The study provided a proper discussion for comparing the promising biohydrogen production techniques. The results show that dark fermentation is a promising biohydrogen production technique. Similar studies can successfully discuss the research gaps and identify the technological improvement options [9]. According to the studies, the higher efficiency of biohydrogen and lower pollutants compared with other renewable energy sources and higher calorific value have highlighted Biohydrogen and attracted attention as energy carriers (Figure 1).

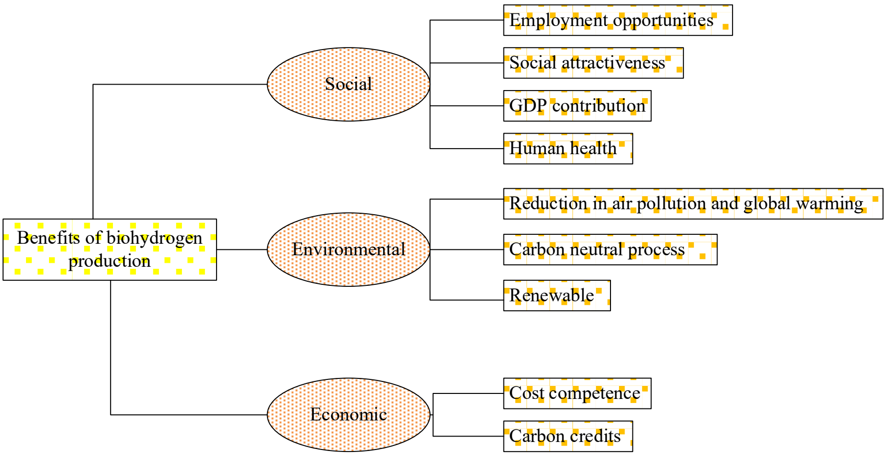

Figure 1
Benefits of biohydrogen production

Despite the various advantages of Biohydrogen, over other energy production methods, large-scale biohydrogen production is often limited and that affects its commercial applications [10]. Some methods are examined for intensifying biohydrogen production, such as improving the pretreatment process and optimizing the production process. However, the above methods are laborious and time-consuming, increasing production costs [11-13]. Therefore, more straightforward and cost-effective methods to achieve high-performance biohydrogen production seem necessary. Using nano-additives can be considered as a cost-effective and straightforward solution. Recent developments in nanotechnology have expanded nanoparticles (NPs) in agriculture, engineering, pharmacy, medicine, and energy [13] [14]. In addition, NPs are known to enhance various biological processes [15]. Accordingly, the application of NPs helps improve biohydrogen production because of their effects on the growth of microorganisms, intracellular electron transfers and the activity of metalloenzymes.

Moving toward the use of Biohydrogen and the demand for this alternative fuel is increasing. Such progress requires a detailed study of process improvement and biohydrogen production from different sources and methods. This review study was conducted to comprehensively and accurately investigate the use of various additives to improve the production performance of Biohydrogen. Recently, a limited number of studies have been conducted to review the application of additives in the hydrogen production process. Srivastava et al. (2020) developed a survey of approaches containing the effect of NPs on cellulosic biohydrogen production, pretreatment technology, enzyme and sugar production [16]. Soares et al. (2020) conducted a review paper on the dark-fermentation of lignocellulosic biomass for biohydrogen production. They highlighted the influential factors in dark fermentation, environmental factors, and economic analysis [17]. Kosei et al. (2016) developed a state-of-the-art to present the effect of enzymes in the biohydrogen production process from microalgae and to evaluate enzymatic reactions based on cell dynamics, metabolism, structure, function, and challenges regarding sustainable biohydrogen production [18]. An in-depth study shows that none of the references used a standard method for database collection and review orientation; hence, this is one of the disadvantages of the mentioned studies. Accordingly, a reliable survey about the effects of the additives in enzymatic biohydrogen production presents a comprehensive discussion of the existing solutions. It follows the PRISMA guideline (Mosavi, Faghan et al. 2020) currently missing from the literature. The present study contains three main sections, after the Introduction, they are as follows:

- **Methodology:** To describe the main procedure for collecting the database
- **Results and Discussion:** To investigate the main limitations, research gap, and possible solutions
- **Conclusions:** To point out the main findings, directions, and future perspectives

2 Methodology

This section presents the main procedure of the study for preparing the database. Preparing the database for the review phase adopts the PRISMA guideline [19] [20]. According to [19] [20], PRISMA guideline describes four main steps, e.g. (1) identification, (2) screening, (3) eligibility, and (4) inclusion for preparing an essential platform for a systematic review. The study primarily aimed to investigate the additives applied for enzymatic biohydrogen production. Due to the vast application range of additives in biohydrogen production, no specific period was considered for the study. We also removed studies that included limited applications of additives from the database so that we could understand the main applications in this area and the role of effective additives in enzymatic biohydrogen production. All additives were allowed to fall into this category. An extensive list of keywords for enzymatic biohydrogen production was provided. All records were independently supported by enzymatic biohydrogen production. Records were deleted if the incompetence was clearly stated, and in case of uncertainty or insufficient information was retained for full-text screening.

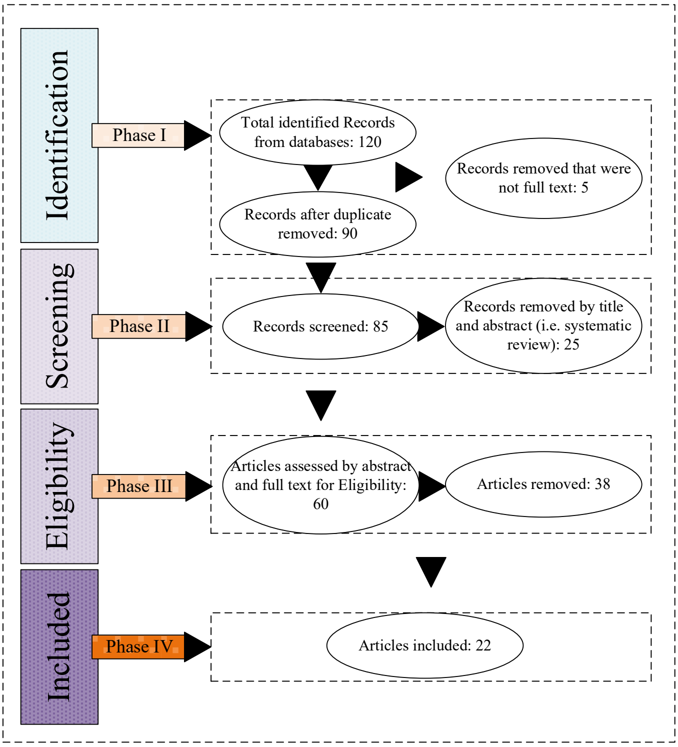

Figure 2

Flowchart of the research methodology

By searching on Thomson Reuters Web-of-Science (WoS) and Elsevier Scopus, 120 most relevant articles were selected. The next step was eliminating duplicate cases and categorizing the relevant articles. In this step, 30 (about 25%) articles were identified. Also, nearly ten articles were eliminated due to the lack of specific additive details for generating the study's architecture and structure. The next phase was eligibility to study the full-text papers by authors and choose the most relevant papers by monitoring eligibility for the final review step. 22 (about 18%) articles were chosen for the evaluation phase at this level. The last step of the PRISMA guideline was to create the research database for qualitative and quantitative evaluations. The database of the study included 22 papers for the required analyses. Figure 2 presents the flowchart of the PRISMA guideline for the present study.

The following section starts the review procedure and surveys the selected papers according to the taxonomy of the study.

2.1 Review Section

The review section includes the main procedure for investigating the selected papers. As previously mentioned, this study presents the state-of-the-art effects of additives in enzymatic biohydrogen production. Therefore, extracting features related to biohydrogen production in each study is a fundamental first step for further investigation. Accordingly, Table 1 presents the summarized sections of the selected papers. Table 1 has nine columns: the first column refers to the additive type; the second column describes the additive preparation procedure.; the third column shows the publication year; the fourth column refers to the substrate name; the fifth column presents the inoculum name; the sixth column mentions the hydrogen production procedure; the seventh column refers to the fermentation condition; the eighth column provides the bioreactor type; the last column presents the related reference.

Table 1

Studies on the effect of additives on enzymatic biohydrogen production

Additive	**Additive preparation**	**Year**	**Substrate**	**Inoculum**	**Production technique**	**Fermentation condition**	**Bioreactor type**	**Ref.**
Rhamnolipids (RLs) and Tea Saponin (TSn)	RLs @ 0.02, 0.05, 0.08, 0.11, and 0.14 g/L and TSn @ 0.50, 1.00, 1.50, 2.00, and 2.50 g/L	2021	Corncob	HAU-M1 mixed strain	Photo-fermentative	Temperature of 50±1 °C, @ 150 rpm for 48 h	200 mL conical flasks	[21]
Fe-modified zeolite	Containing A-3 zeolite powder (76%, Wako Pure Chemical Industries, Ltd.), starch (5%), $FeSO_4$ solution (9%), lignite (5%), and montmorillonite (5%)	2021	Glucose	Sewage sludge	Dark Fermentation	Temperature of 35 °C	250 mL Schott Duran bottle	[22]

Enzymatic hydrolysate	NA.	2021	Corn Stover as initial carbon source	NA.	Dark and photo-fermentation	Temperature of 30±1 °C, light intensity of 3000±200 lx, and	Continuous baffled bioreactors	[23]
Biochar	Pyrolysis	2021	Sugarcane bagasse	Sewage sludge	Dark fermentativ	Temperature of 37±1 °C @ 150 rpm	120 mL serum bottles	[24]
Combined carbohydrase enzymes, Termamyl SC, Dextrozyme GA, and Cellic CTec2	Termamyl SC, Dextrozyme GA, and Cellic CTec2 by 160-units/g VS, 320-units/g VS, and 40–filter paper units/g VS, respectively	2021	Chlorella sp. biomass	Wastewater treatment	Dark fermentative	Temperature of 35±1 °C @ 150 rpm	250 mL glass bottles	[25]
Fe_3O_4 nanoparticles during enzymatic hydrolysis	Synthesized through waste seeds of Syzygium cumini	2021	Sugarcane bagasse	Clostridium pasteurianum and bacterium strain Rhodobacter sp. For dark and photo-	Dark and photo-fermentation	Temperature of 37±2 °C @ 192 rpm	NA.	[26]
Magnetite nanoparticles	Pretreating @ 90 °C for 20 min through enriching spore-forming anaerobic bacteria	2021	Glucose as a sole carbon source	Wastewater treatment	Dark fermentation	Temperature of 35±1 °C	5.7 L cylindrical shaped	[27]
Sodium citrate	NA.	2019	Waste sludge	Activated seed sludge	Batch fermentation	Temperature of 37 °C @ 100 rpm	250 mL serum bottles	[28]
Ferrihydrite nanorods	Slowly adding Fe(II)/Fe(III) acidic solution (0.8 M $FeCl_3$ and 0.4 M $FeCl_2$ in 0.4 M HCl) into a vigorously mixed 1.5 M NaOH solution	2019	glucose	Clostridium pasteurianum	Dark fermentation	Temperature of 37 °C	25 mL serum vials	[29]
Graphene oxide	Hummer's method: 2.5 g of graphite mixed with 60 ml of concentrated H_2SO_4. 7.5 g of $KMnO_4$	2018	Cellulosic substrates including rice straw (RS) and orange peels (OP)	Clostridium pasteurianum	Dark fermentation	Temperature of 37 ± 2 °C @ 192 rpm	150 ml shaker flask	[30]

Fe°	N.A.	2018	Organic Market Waste	treated cow manure	Dark fermentation	Temperature of 30 ± 1 °C @ 120 rpm	500 mL serum bottles	[31]
Granular activated carbon	NA.	2017	Waste water including NH_4Cl 1 g, NaCl 2 g, $MgCl_2{\cdot}6H_2O$ 0.5 g, $CaCl_2{\cdot}2H_2O$ 0.05 g, $K_2HPO_4{\cdot}3H_2O$ 1.5 g, KH_2PO_4 0.75 g, $NaHCO_3$ 2.6 g, and	POME-sludge	Dark fermentation	Temperature of 60 °C	Lab-scale glass	[32]
TiO_2 and magnetic hematite nanoparticle	Magnetic nanoparticles, with a nominal size of 50–100 nm and 97% trace metal basis, and TiO_2 nanoparticles were provided by the Physicochemical Treatment Laboratory	2016	Wastewater containing xylose	C.pasteurianum CH5	Dark fermentation	Temperature of 35 °C @ 120 rpm	120 mL vials	[33]
Ferric citrate	NA.	2013	Sludge of dairy wastewater	Rhodopseudomonas palustris PT	Syngas/digestion	Temperature of 30 ± 1 °C @ 200 rpm	Serum bottles	[34]
Pd, Ag and Cu or Fe oxide (Fe_xO_y)	Pd/SiO_2, Ag/SiO_2, Cu/SiO_2 and Fe/SiO_2 cogel by porous silica matrix	2013	Glucose	Clostridium butyricum strain	Fermentation	Temperature of 30 ± 1 °C @	270 mL bottles	[35]

Based on Table 1, the three main factors are additive types, substrates, and fermentation conditions. Accordingly, Fan et al. (2021) employed Rhamnolipids (RLs) and Tea Saponin (TSn) as additives for the photo-fermentation of Corncob in the presence of HAU-M1 mixed strain as the inoculum of the fermentation. The fermentation was performed @ temperature of 50±1 °C and a mixing intensity of 150 rpm for 48 h [21]. Zhao et al. (2021) employed Fe-modified zeolite for fermentation of glucose in the presence of Sewage sludge @ temperature of 35 °C. In the study of Li et al. (2021), the enzymatic hydrolysate was used for the dark and photo-fermentation of Corn Stover as the initial carbon source @ temperature of 30±1 °C, the light intensity of 3000±200 lx, for 24 h [23]. Bu et al. (2021)

employed Biochar for dark fermentation of Sugarcane bagasse in the presence of Sewage sludge @ temperature of 37±1 °C and a mixing intensity of 150 rpm. Sriyod et al. (2021) employed combined carbohydrase enzymes, Termamyl SC, Dextrozyme GA, and Cellic CTec2 for enzymatic dark fermentation of Chlorella Sp. Biomass in the presence of wastewater treatment @ temperature of 35±1 °C and mixing intensity of 150 rpm. Srivastava et al. (2021) employed Fe_3O_4 nanoparticles (NPs) for dark and photo-fermentation of sugarcane bagasse @ temperature of 37±2 °C and mixing intensity of 192 rpm. Mostafa et al. (2021) employed Magnetite NP as an additive for dark fermentation of glucose as a sole carbon source in the presence of wastewater treatment @ temperature of 35±1 °C. Yang and Wang (2019) employed sodium citrate for batch fermentation of waste sludge @ temperature of 37 °C and mixing intensity of 100 rpm Zhang et al. (2019) employed Ferrihydrite nanorods for dark fermentation of glucose @ temperature of 37 °C. Srivastava et al. (2018) employed graphene oxide for dark fermentation of Cellulosic substrates including rice straw (RS) and orange peels (OP) @ temperature of 37 ± 2 °C and mixing intensity of 192 rpm. Jamali et al. (2017) added Fe° for the enzymatic dark fermentation of waste water @ temperature of 60 °C [32]. Hsieh et al. (2016) employed TiO_2 and magnetic hematite NP for dark fermentation of wastewater containing xylose @ temperature of 35 °C and mixing intensity of 120 rpm. Pakpour et al. (2013) used ferric citrate for the fermentation process of glucose @ temperature of 30 ± 1 °C and mixing intensity of 200 rpm [34]. Beckers et al. (2013) employed Pd, Ag and Cu or Fe oxide for the fermentation of glucose @ temperature of 30 ± 1 °C. Based on the operations performed, the stirring process is important in that it causes better mixing and increases the contact surface of the materials and improves the process via better reaction. Moreover, the type of additive can be effective in improving the fermentation process and production of Biohydrogen. The highest proportion of additives is related to metallic additives, which account for almost 50% [35].

3 Results and Discussion

This section presents the statistical analysis of the results. Figure 3 illustrates the allocation of the studied additives for enzymatic biohydrogen production each year. The trend started in 2006, but the highest percentage is related to 2023. This trend indicates the importance of the subject and the rising trend in upcoming years.

Figure 4 presents the allocation of the area of the studies using the keywords of the subject extracted from Scopus. Accordingly, the highest share is related to energy, followed by chemical engineering and environmental science.

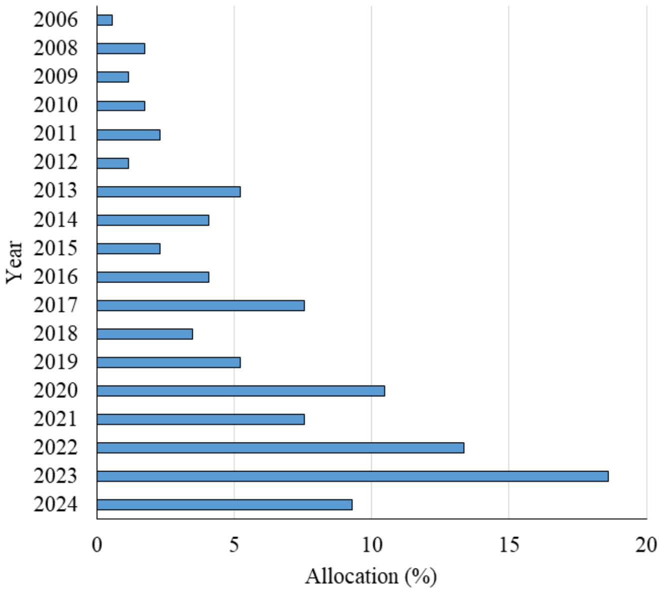

Figure 3
Trend of published papers for application of additives in enzymatic biohydrogen production

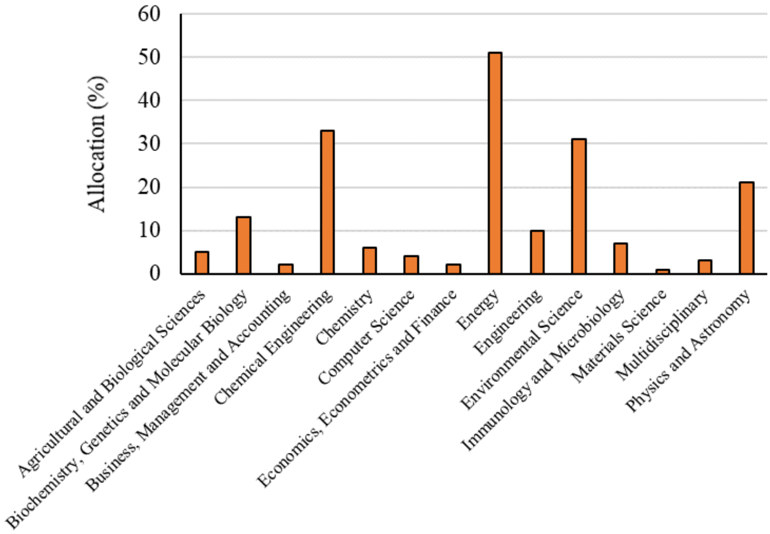

Figure 4
Allocation of published literature based on the area of the study

Figure 5 presents the allocation of keywords for biohydrogen production by an enzymatic process in the presence of additives. Accordingly, it can be noted that hydrogen production (~7.3%), Biohydrogen (~5.5%), and hydrogen (~5%) have the highest allocations. This section helps researchers properly search the related subject in scientific literature.

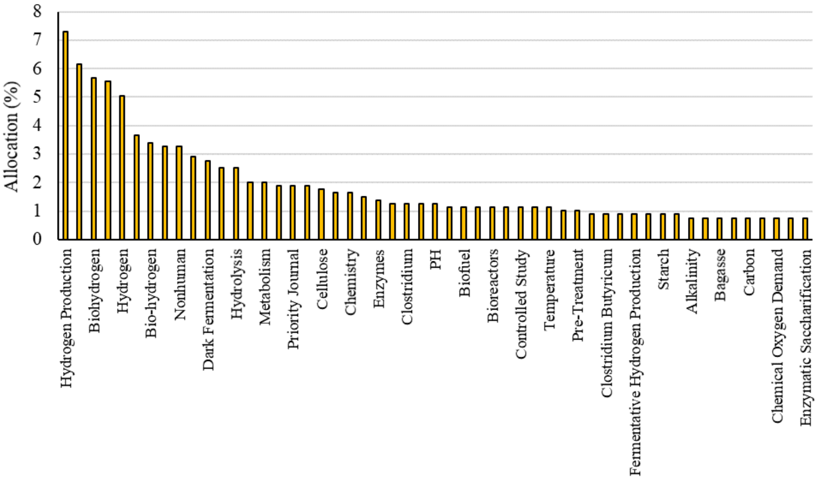

Figure 5

Allocation of the keywords for biohydrogen production through enzymatic process in the presence of additives

Table 2 presents this review's main results and findings according to the conducted studies.

Table 2

Main results and findings

Additive	**Results**	**Pros. and Cons.**	**Ref.**
Rhamnolipids (RLs) and Tea Saponin (TSn)	+ 67.85%	The proposed technique effectively disintegrated the substrates	[21]
Fe-modified zeolite	+ 310%	The hybrid-Fe process provided the practical potential for biohydrogen production	[22]
Enzymatic hydrolysate	+ 35.69%	The proposed enzymatic process improved the electron transferring for the effective utilization of substrates during the fermentation process	[23]
Biochar	+ 317.1%	Biochar provides better process stability and higher biological activity	[24]

Combined carbohydrase enzymes, Termamyl SC, Dextrozyme GA, and Cellic CTec2	+ 82.46%	The combined enzyme treatment provided the highest reducing sugar yield, and cell wall hydrolysis and facilitated biomass scarification by amylase enzymes	[25]
Fe_3O_4 NPs during enzymatic hydrolysis	3427 mL/L cumulative hydrogen for 408 h	The proposed additive extended thermal stability	[26]
Magnetite NPs	+ 50%	The solution improves conditions for bacterial metabolism	[27]
Sodium citrate	+ 411.1%	The proposed technique effectively disintegrated the substrates and induced the solubilization of substances	[28]
Ferrihydrite nanorods	+ 68.9%	The proposed technique improves glucose conversion efficiency and promotes cell growth	[29]
Graphene oxide	Cumulative 2870 mL/L could produce for 168 h	The proposed technique provided an increased metabolism	[30]
Fe°	+ 46%	The proposed approach increased the metabolism	[31]
Granular activated carbon	100.8±3.7 mmol H_2/l.d	The presence of activated carbon guaranteed cellulose degradation efficiency	[32]
TiO_2 and magnetic hematite NP	+ 24.9%	Magnetic NP metal did not stimulate the hydrogenase enzyme activity but showed potential in improving substrate degradation	[33]
Ferric citrate	+ 85%	pretreatment process is an important factor in biohydrogen production	[34]
Pd/SiO_2, Ag/SiO_2, Cu/SiO_2 and Fe/SiO_2	Pd/SiO_2= + 10.5% Ag/SiO_2= + 8.2% Fe/SiO_2 dissol= + 38% Fe/SiO_2 cogel= + 31% Cu/SiO_2= + 20%	The additives improve the hydrogen production through a catalytic mechanism involving extracellular mediated-molecules	[35]

According to the pros. and cons. column, it can be concluded that the mechanism of action of additives is more on substrate degradation, improving electron exchange, increasing stability and biological activity, or improving the overall metabolism of the system. Figure 6 presents the main findings, for better comparison.

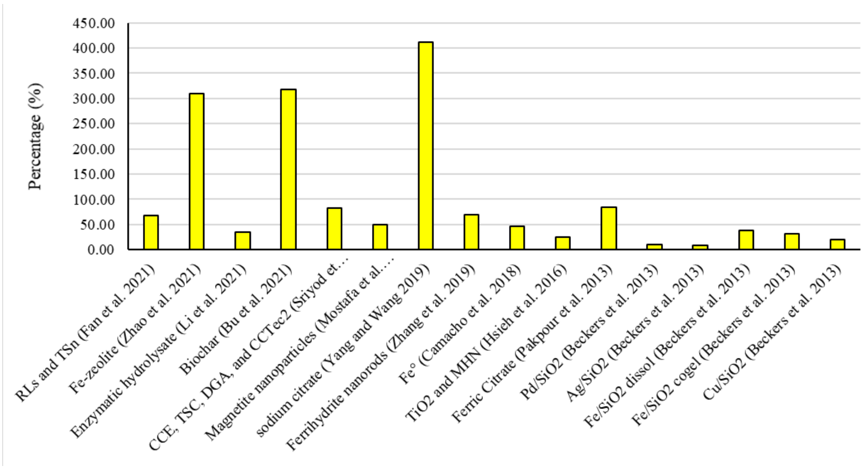

Figure 6

Trend of findings related to each additive

According to the results and findings, the mechanisms of action and the effects of each additive are different. It can be concluded that the effective points in each production stage can be easily identified and, based on the strengths, added to the substrate and inoculum to improve enzymatic activity. For example, Fe-based additives improve the catalytic mechanisms and metabolisms [26] [31]. Fe-based additives and photocatalysts enhance the substrate degradation [27] [33]. Carbon-based additives intensify the glucose degradation [32]. Citrate-based additives improve the solubilization [28] [34]. Biochar boosts biological activity [24].

3.1 Feature Selection

In the following, we employed the Relief feature selection technique to consider the effects of substrate content and additive values on biohydrogen production. In this analysis, the substrate content was called X, the additive value was called Y, and the pH of the substrate was called Z for each study separately. We emphasized that there are three classes, including X, Y, and Z, which affect the biohydrogen production in each study (Eq. 1):

$$BP = nX^{i} + mY^{j} + lZ^{k} \tag{1}$$

Where BP is biohydrogen production yield, n, m, and l refer to the weights of substrate content, additive value, and PH, and i, j, and k refer to the power of each feature. In this procedure, we want to extract the effects of each feature on the BP.

This method considers feature vectors from the Euclidean distance from the target (the amount of biohydrogen production). The nearest instance is also called the "near-tar." class, and the nearest instance of the different class is called "near-lo." [36]. Accordingly, the weight vector is updated with Eq. 2:

$$Weight_i = Weight_i - (X_i - near_{tar.})^2 + (X_i - near_{lo})^2 \tag{2}$$

Based on Eq. 2, the weight of the features proportional to the close specimens of the objective function increases, and if the attribute does not fit the samples relative to the objective function, its weight decreases. Results of the Relief feature selection technique have been presented in Table 3. Table 3 has five columns, including the additive type, weights of X, Y, and Z, and the related references.

Table 3
Weights values of relief feature selection technique

Additive	**X**	**Y**	**Z**	**Ref.**
Rhamnolipids (RLs) and Tea Saponin (TSn)	0.01	0.11	0.1	[21]
Fe-modified zeolite	0.50	0.55	0.32	[22]
Enzymatic hydrolysate	0.33	0.58	0.41	[23]
Biochar	0.24	0.41	0.39	[24]
Combined carbohydrase enzymes, Termamyl SC, Dextrozyme GA, and Cellic CTec2	0.29	0.58	0.51	[25]
Fe_3O_4 NPs during enzymatic hydrolysis	0.12	0.25	0.22	[26]
Magnetite NPs	0.15	0.29	0.28	[27]
Sodium citrate	0.44	0.68	N.A.	[28]
Ferrihydrite nanorods	0.39	0.71	0.53	[29]
Graphene oxide	0.26	0.6	0.50	[30]
Fe°	0.32	0.49	N.A.	[31]
Granular activated carbon	0.10	0.21	N.A.	[32]
TiO_2 and magnetic hematite NP	0.22	0.63	N.A.	[33]
Ferric citrate	0.11	0.34	N.A.	[34]
Pd/SiO_2, Ag/SiO_2, Cu/SiO_2 and Fe/SiO_2	0.18	0.57	N.A.	[35]
Average	0.24	0.47	0.36	All the references
Std. deviation	0.13	0.17	0.13	All the references

According to Table 3, the Additive value with the Relief feature selection score of 0.47±0.17 has the highest impact on the biohydrogen production yield followed by PH with a score of 0.36±0.13, and the substrate content with the Relief feature selection score of 0.24±0.13 has the lowest impact on the biohydrogen production yield. In general, it can be concluded that the additive value followed by the pH of the substrates are the two most influential parameters in biohydrogen production yield. This evidence helps us with proper policy-making in biohydrogen production.

According to the studies surveyed, the main limitations in this field are making and synthesizing additives as these methods are often time-consuming and economically limited. Further studies in this field can make significant progress in covering some of the limitations. Other limitations include environmental issues; hence, a minimal number of studies have studied the story from the viewpoint of environmental constraints. In this regard, conducting environmental studies, especially life cycle assessment methods and production process optimization, can be the main direction for future studies. Regarding optimization methods, machine learning has recently taken a practical step towards developing modeling and optimization by entering the discussion of biohydrogen production. These methods can perform the production process in a multivariate optimization. All methods are moving in the direction of sustainable production (Table 4). For sustainable production, there is a need for optimization in terms of energy, environment, and economy. These orientations lead to the sustainable production of Biohydrogen.

Table 4
Sustainability factors and opportunities for future studies

Additive	**Hydrogen yield**	**Hydraulic retention time**	**Environmental crisis**	**Economic analysis**	**Waste management**	**Energy efficiency**	**Ref.**
Rhamnolipids (RLs) and Tea Saponin (TSn)	☑	☑	☒	☒	☒	☑	[21]
Fe-modified zeolite	☑	☑	☒	☒	☒	☒	[22]
Enzymatic hydrolysate	☑	☑	☒	☒	☒	☑	[23]
Biochar	☑	☒	☒	☒	☑	☒	[24]
Combined carbohydrase enzymes, Termamyl SC, Dextrozyme GA, and Cellic CTec2	☑	☒	☒	☒	☑	☑	[25]
Fe3O4 NPs during Enzymatic hydrolysis	☑	☒	☒	☒	☒	☒	[26]
Magnetite NPs	☑	☒	☑	☒	☒	☒	[27]
Sodium citrate	☑	☒	☑	☒	☑	☑	[28]
Ferrihydrite nanorods	☑	☒	☑	☒	☑	☒	[29]
Graphene oxide	☑	☒	☒	☒	☑	☒	[30]
Fe°	☑	☒	☒	☒	☑	☑	[31]
Granular activated carbon	☑	☒	☒	☒	☑	☒	[32]
TiO_2 and magnetic hematite NP	☑	☑	☒	☒	☒	☒	[33]
Ferric citrate	☑	☒	☒	☒	☑	☒	[34]
Pd/SiO_2, Ag/SiO_2, Cu/SiO_2 and Fe/SiO_2	☑	☒	☒	☒	☒	☒	[35]

Conclusions

The enzymatic route is one of the most effective methods for biohydrogen production. However, it is less economically viable on a large scale, compared to other methods. To address this issue, researchers have explored various additives to enhance the efficiency of enzymatic biohydrogen production. This review examines the challenges and impacts of these additives, offering a comprehensive analysis that has been lacking. It identifies the strengths and weaknesses of different additives and provides practical insights for improving enzymatic activity, by incorporating specific additives into the substrate and inoculum.

The review also highlights important issues for future research. It emphasizes the need for environmental studies, such as life cycle assessments, to understand the broader impacts of enzymatic biohydrogen production.

Furthermore, it discusses the potential of machine learning for optimizing production processes. By integrating advanced techniques, the efficiency and economic viability of enzymatic biohydrogen production can be significantly improved, paving the way for more sustainable energy solutions.

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