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The Role of Iron-Based Nanoparticles in Enhancing the Efficiency of Biohydrogen and Biobutanol Production by Clostridial Strains

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Abstract:

The rapid depletion of non-renewable fossil fuels such as coal, petroleum, and gas has led to a global energy crisis and environmental pollution. Hydrogen and biobutanol have emerged as vital renewable energy sources due to their cleanliness and high energy potential (122 kj/g). The production of renewable energy from these sources involves the utilization of nanoparticles in conjunction with microorganisms from the Clostridial bacteria family. The active sites of hydrogenase metal atoms, namely NiFe, FeFe, and Fe-hydrogenase, play a significant role in this process. Iron (Fe) metals particularly influence hydrogen production, as they enhance the activity of the hydrogenase enzyme. Fe is an essential component of cytochromes found in anaerobic microbes. Consequently, introducing Fe-based nanoparticles is assumed to promote microbial cell growth and hydrogenase activity. Various factors, such as nanoparticle size, concentration, operational conditions, operational mode, and substrate, impact the production of biohydrogen and biobutanol. Furthermore, a protein sequence analysis reveals the presence of a multi-active site for amylase activity in Clostridium. This discovery suggests that this enzyme can translate and augment biofuel production rates. By exploring and harnessing these avenues, the world can advance toward a more sustainable future by capitalizing on the potential of hydrogen and biobutanol as renewable energy sources while mitigating the negative consequences of the depletion of non-renewable fossil fuels.

Keywords: Iron-based Nanoparticle, Biohydrogen, Biobutanol, Clostridial strains,

1. Introduction:

Across the globe, the use of fossil fuels has a significant contribution to energy, causing harm to both the environment and human health. In order to address this issue and meet the world's energy demands, a new energy source is necessary. By producing biofuels, we can decrease our reliance on fossil fuels and, when possible, replace them to mitigate the limitation of their finite resources. The major worry is that within the next 150-200 years, the world can face a lack of coal and

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petroleum energy. In the near future, there are possibilities for the production of biofuels, which aim to decrease environmental pollution. Biofuels, including bio methanol, biohydrogen (H) biomethane, and biodiesel, contain much more energy than bio fossil [1]. Hydrogen and biobutanol are renewable energy sources considered essential and the most pristine and high-potential energy sources about (122 kj/g)[2][3]. Butanol is a crucial biological liquid fuel, which can be produced from renewable resources by the anaerobic acetone-butanol-ethanol (ABE) fermentation process conducted by solventogenic Clostridium strains, such as Clostridium acetobutylicum and Clostridium beijerinckii [4][5]. The world is facing an energy crisis and environmental pollution due to the rapid depletion of non-renewable fossil fuels such as coal, petroleum, and gas. There is a push toward developing new technologies and chemical materials to address this issue to create a new form of clean energy.

For renewable energy production, some chemical particles or nanoparticles were involved in inducing the production of hydrogen and biobutanol with the assistance of microorganisms. The utilization of nanoparticles (NPs) has been a significant rise in various applications such as immobilizing proteins, production of biosensors, and generating biofuels [6][7][8][9][10]. The considerable advancement In nanotechnology has enhanced its capability to augment biological processes [9]. Hydrogen production by microorganisms catalyzes the reactions by hydrogenase enzymes (Hydrogenases are critical enzymes in the energy metabolism of many organisms). Enzymes play a crucial role in anoxic environments, particularly where molecular hydrogen H2 serves as a critical intermediate. Hydrogenase enzymes are classified into different groups based on the type of metal atom present in their active site, which are thus categorized as [NiFe]-, [FeFe]- and [Fe]-hydrogenases[11]. Hydrogen production is influenced by the presence nanoparticles of the iron (Fe) and nickel (Ni) metal and Ions, which enhance the hydrogenase enzymatic activity[12][13]. Size of Fe-based, Ni-based and ions including Mg, Cu, Na, NH4, and K nanoparticles have their efficiency in production rate. These element are essential for microbes to initiate their metabolism, also have different responses to hydrogen yield (HY) and hydrogen evolution rate (HER)[14][15][16][17]. The modification and adjustment of nanoparticle size and concentration have led to improvement in hydrogen production yield for Fe-based NPs but for Ni-based NPs or lone, the smaller (less than 42 nm) NPs improved the hydrogen yield, whereas, bigger size of NPs (40-50 nm) seemed to increase the hydrogen HER (hydrogen evolution rate). The reason behind high production with large size NPs is its stability for maintaining its frame for a long time[18].

1.1.Impact size and concentration of Fe-based NP production rate

The concentration, and size of Fe-ion/Fe effect in biohydrogen production. Statistically analyzed results from ANN-RSM showed that size and concentration of Fe based NPs strongly affect the production rate. Fe is the essential element to form the metal content at the active sites of hydrogenases such as ([FeFe], [FeNi], and [Fe]), thus catalyzing the reduction reaction of H+ to H2 [1]. Furthermore, fe-based NPs have shown to enhanced the activity of ferredoxin oxidoreductase by decreasing the level of dissolved oxygen (DO) and improving electron transfer via their surface quantum properties [1][19]. In addition, Fe-based components could participate in enriching the microbial community and enhancing the growth of H2-producing bacteria[20]. Iron is an important trace element in the formation of hydrogenases and other enzymes. A commonly used technique to increase BioH2 production during dark fermentation is the pre-

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addition of iron to the culture medium.[21]. over all size, concentration of nanopartical, and substrate has considerable effects on biohydrogen production (Table 1)

Table 1- BioH2 production with the addition of Fe-based nanoparticles

Nanoparticles	Opt/mgL^{-1}	Substrate	Siz/nm	HY/mmolL^{-1}	HER/mmolL^{-1}g^{-1}	Reference
Fe2O3 (NPs)	50	Glucose	50	1.92	2.5	[22]
Fe2O3 (NPs)	50	CDW	33	16.75	102.5	[23]
Fe2O3 (NPs)	200	DW	23	7.85	62.4	[24]
Fe2O3 (NPs)	50	Wastwater	6.5	1.9	49.4	[25]
Fe2O3 (NPs)	200	MEG	100	8.4	0.6	[26]
Fe2O3 (NPs)	300	CAS	20	3.875	1.9	[27]
Fe2O3 (NPs)	200	Glucose	20	9.2	3.1	[27]
Fe2O3 (NPs)	60	Glucose	60	1.92	2.5	[22]
Fe(NPs)	400	Grass	50	2.9	5.4	[28]
Fe(NPs)	25	Starch	35	3	0.4	[29]
Fe(NPs)	300	Malate	16	20	0.4	[30]
Fe(NPs)	50	Xylose	75	13.3	2	[13]
Fe(NPs)	200	MSJ	50	0.9	2.4	[31]
Fe(NPs)	200	Sucrose	50	15.9	10.1	[12]
Fe(NPs)	175	Glucose	59	12.9	5.69	[32]
Fe(NPs)	250	Malate	12	24.2	0.8	[33]
Fe3O4(A-C-NPs)	250	Glucose	30	11.656	3.2	[34]
Magnetite (NPs)	200	SJ	50	6.7	0.23	[35]
Hematite (NPs)	200	Sucrose	55	10.4	6	[36]

1.2.The impact of nanoparticles on the production of biohydrogen.

NPS (Nanoparticle) application has been increasing significantly for protein immobilization, biosensors, and biofuel production [6][7][8][9][10]. NPs can influence microbial metabolic activity for biohydrogen production under aerobic conditions by efficiently transferring electrons[37]. Various NPs, such as silver (Ag), Fe, Ni, Copper (Cu), gold (Au), and palladium (PD), stimulate BHP (biohydrogen production) by their surface [6][38][38][37]. The large size of NPS enables a solid ability to adsorb and transfer the electron better [39]. Effective microorganisms catalyze the production of biohydrogen and biobutanol from various substrates, including glucose, sucrose, lignocellulose, and rice mill wastewater. These substrates, in conjunction with the action of microorganisms, enhance both the production rate and the quality of hydrogen.

Clostridium bacteria can produce hydrogen gas, but not all strains are equally efficient. The type of substrate they use, such as complex carbohydrates or simple sugars, significantly affects hydrogen production. Different themes also have varying hydrogen production rates and yields

depending on the substrate they consume. Selecting an appropriate strain and substrate is pivotal in achieving efficient hydrogen production, thus contributing to sustainable energy development (see Table 2)—for instance, the amylolytic Clostridium sp. Strain BOH3 demonstrates the ability to produce both butanol and hydrogen from food waste, eliminating the requirement for enzymatic pretreatment. According to protein sequence analysis, amylase in Clostridium sp. strain BOH3 may contain a multi-active site for activity and has high ability of translation than Clostridium beijerinckii NCIMB 8052 [40]. As a solvent, butanol is a vital chemical to produce drugs, antibiotics, and vitamins used in considerable industrial interest. In general, the sugars present in food waste are complex macromolecules that require initial breakdown into monosaccharides prior to microbial fermentation. To enhance the hydrolysis efficiency of food waste, a range of pretreatment methods, such as physical, thermochemical, and enzymatic hydrolysis, can be employed. These methods facilitate the effective breakdown of complex sugars, allowing for subsequent microbial fermentation. Among these methods, the addition of commercial enzymes like amylase and xylanase has proven effective in enhancing the breakdown of starch in food waste. Although efficient enzymatic pretreatment can enhance butanol and biohydrogen production from food waste, The economic viability of this process is hindered by the high costs associated with commercial enzymes. These expenses can significantly reduce the overall economic value of the process [41]. To boost production rates, we aim to develop a streamlined process for one-step butanol and hydrogen production from food waste, utilizing an amylolytic Clostridium sp. strain BOH3 that can produce its own amylase. Our primary goal is to enhance butanol production and yield by actively promoting amylase activities. Ni and CO-based metal nanoparticles reduce the toxicity of microorganisms and improve their role in biohydrogen production [41]. Achieving the optimal concentration and temperature for each element is crucial for maximizing biofuel production. For instance, the concentration of NiFe2O4 nanoparticles (NPs) within the 50-200 mg/l range has been found to enhance hydrogen generation. However, when the concentration of NiFe2O4 NPs exceeds 400 mg/l, it reduces hydrogen productivity. Interestingly, at a concentration of 100 mg/l and a temperature of 37 °C, there was a significant 38.6% increase in hydrogen production compared to the control. Similarly, at a concentration of 200 mg/l and a temperature of 55 °C, there was a notable increase of 28.3% compared to the control [42]. According to metabolic mentoring, NiFe2O4 NPs enhanced the butyrate pathway corresponding to the increasing abundance of clostridium barium in mesophilic fermentation[42] [43]. Nanoparticles are commonly employed to address potential obstacles that can impede the activity of hydrogen-producing microbes. These obstacles may include accumulating volatile fatty acids (VFAs), organic overload, and other inhibitory factors[44]. To surpass these limitations, researchers have explored the utilization of nanoparticles[43]. Some metal nanoparticles (e.g., FeO3 and TiO2) accumulate inside the microorganism cells and interact with intercellular comportment via chemical, physical, or biological mechanisms[18].

Nanoparticles could be single-metal and two single-metal, like the spinal ferrite NPs, such as nickel ferrite (NiFe2O4), copper ferrite (CuFe2O4), and manganese ferrite (MnFe2O4). These nanomaterials are used in magnetic drug delivery, electronic device, and information storage [43]. Iron, which affects hydrogen and butanol production, is considerable to understand; iron is

essential in producing biohydrogen and biobutanol[45][46]. Butanol production could be enhanced with suitable iron content. The iron cluster in hydrogenase Is a distinctive form of the fe-s center known as the hydrogen cluster. According to many research reports, hydrogen evolution or butanol production are individuals, But few reported simultaneous hydrogen and butanol production. The production of hydrogen and butanol is a competitive process that relies on the balance between NADH and NAD, crucial components of metabolic electron transfer. Researchers have taken a keen interest in utilizing various nanoparticles due to their unique physical and chemical properties, particularly in dark hydrogen fermentation. The objective is to enhance the efficiency of hydrogen-producing microbes, reduce costs, and achieve higher hydrogen production rates. By leveraging the benefits of nanoparticles, the aim is to optimize the production process for improved outcomes.

Table 2: - Biohydrogen generation from different types of carbohydrates using strains of Clostridium butyricum

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Microbial strains	Substrates	Substrate Concentration	Operational Conditions	Operation Mode (working volume)	Hydrogen Production Rate	Hydrogen Yield molH2/ Hexose	Reference
C. butyricum	Glucose	3 g/L	37 _C, initial pH 6.5	Batch (250 mL)	/	2.09	[47]
C. butyricum KCCM 35433	Glucose	5 g/L	35 _C	Batch (100 mL)	/	1.23e1.42 mol/mol glucose	[48]
C. butyricum IFO 3847	Glucose	9 g/L	37 _C, initial pH 7.0	Batch (1 L)	/	1.26	[49]
C. butyricum IAM 19002	Glucose	9 g/L	37 _C, initial pH 7.0	Batch (1 L)	/	1.04	[49]
C. butyricum IMA 19003	Glucose	9 g/L	37 _C, initial pH 7.0	Batch (1 L)	/	1.2	[49]
C. butyricum CWBI1009	Glucose	1-10 g/L	30 _C, initial pH 7.3	Batch (200 mL)	/	0.23e2.4	[50] [51]
C. butyricum TM-9A	Glucose	10 g/L	37 _C, initial pH 8	Batch (9 L)	/	3.34 mol/mol glucose	[52]
C. butyricum W5	Glucose	10 g/L	39 _C, initial pH 6.5	Batch (1.5 L)	Maximum 7.61 mmol/L/h	0.82	[53]
C. butyricum IFO 384	Glucose	10 g/L	37 _C, initial pH 7.0	Batch	/	0.9	[54]
C. butyricum TM-9A	Glucose	10 g/L	37 _C, initial pH 7.2	Batch (20 mL)	/	2.67e3.1	[55]
C. butyricum A1	Glucose	10 g/L	37 _C, initial pH 6.5	Batch (100 mL)	/	1.9	[56]
C. butyricum DSM 10702	Glucose	10 g/L	37 _C, initial pH 6.8	Batch (30 mL)	/	2.4e3.1	[57]
C. butyricum RAK25832	Glucose	10 g/L	30 _C, initial pH 8.0	Batch (30 mL)	/	0.91e2.23	[58]
C. butyricum ST5	Glucose	10 g/L	37 _C, initial pH 6.5	Batch (10 mL)	/	0.7	[59]
C. butyricum INET1	Glucose	10 g COD/L	35 _C, initial pH 7.0	Batch (100 mL)	/	2.24	[51]
C. butyricum EB6	Glucose	15.7 g/L	37 _C, initial pH 5.6	Batch (1 L)	/	2.2	[54]
C. butyricum RAK25832	Fructose	10 g/L	30 _C, initial pH 8.0	Batch (30 mL)	/	0.86	[58]
C. butyricum TM-9A	Xylose	10 g/L	37 _C, initial pH 7.2	Batch (20 mL)	/	0.6	[55]
C. butyricum INET1	Xylose	10 g COD/L	35 _C, initial pH 7.0	Batch (100 mL)	/	1.2	[51]
C. butyricum RAK25832	Xylose	10 g/L	30 _C, initial pH 8.0	Batch (30 mL)	/	1.27	[58]
C. butyricum CWBI 100	Sucrose	4.3 g COD/L	30 _C, initial pH 7.3	Batch (200 mL)	/	0.44	[54]
C. butyricum RAK25832	Sucrose (10 g/L)	10 g/L	30 _C, initial pH 8.0	Batch (30 mL)	/	0.41	[58]
C. butyricum CGS2	Sucrose	20 g COD/L	37 _C, initial pH 7.1	Batch (300 mL)	/	0.95	[60]
C. butyricum CGS2	Sucrose	10e40 g COD/L	37 _C, feed flow pH 9.3	Continuous (460 mL)	63e292 mL/h/g COD	0.31e0.92	[60]
C. butyricum CGS5	Sucrose (COD ¼ 20 g/L)	20 g COD/L	37 _C, initial pH 7.5	Batch (200 mL)	/	1.39	[58]
C. butyricum TISTR 1032	Sucrose (COD ¼ 25 g/L)	25 g COD/L	37 _C, initial pH 6.5	Batch (70 mL)	Maximum 3.5 L/L/d	1.52	[51]
C. butyricum INET1	Sucrose (COD ¼ 10 g/L)	10 g COD/L	35 _C, initial pH 7.0	Batch (100 mL)	/	0.72	[51]
C. butyricum CWBI 1009	Lactose (COD ¼ 4.3 g/L)	4.3 g COD/L	30 _C, initial pH 7.3	Batch (200 mL)	/	0.34	[54]
C. butyricum INET1	Lactose (COD ¼ 10 g/L)	10 g COD/L	35 _C, initial pH 7.0	Batch (100 mL)	/	1.83	[51]
C. butyricum RAK25832	Lactose (10 g/L)	10 g/L	30 _C, initial pH 8.0	Batch (30 mL)	/	0.56	[58]
C. butyricum TM-9A	Molasses	25 g/L	37 _C, initial pH 8	Batch (9 L)	/	8.11 mmol/g molasses	[52]
C. butyricum DSM 10702	Starch	1 g/L	37 _C, maintained at pH 7.0	Batch (100 mL)	/	3.2	[61]
C. butyricum CWBI 1009	Starch	4.3 g COD/L	30 _C, initial pH 7.3	Batch (200 mL)	/	0.73	[58]
C. butyricum NCIB 9576	Starch	10 g/L	37 _C, initial pH 7.0	Batch (100 mL)	/	2.58	[62]
C. butyricum INET1	Starch	10 g COD/L	35 _C, initial pH 7.0	Batch (100 mL)	/	2.17	[51]
C. butyricum	Glycerol	5 g/L	37 _C, initial pH 7.4	Batch (170 mL)	/	3.57	[63]
C. butyricum INET1	Glycerol	10 g COD/L	35 _C, initial pH 7.0	Batch (100 mL)	/	0.66	[51]

C. butyricum DSM 2578	Glycerol	10-50 g/L	37 _C, initial pH 7.0	Batch (30 mL)	/	0.58-0.73	[64]

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1.3.The impact of FONPs on biohydrogen fermentation and the production of hydrogen from glucose and pre-treated cassava starch

Different concentrations of FONPs (Fluorinated Oxide Nanoparticles) have been found to impact the hydrogen yield from glucose substrate production. The hydrogen yields gradually increased from 164.5 to 171, 183, and 192 ml/g with varying concentrations of FONPs ranging from 0 to 200 mg/l. Notably, a concentration of 200 mg/l of FONPs resulted in the highest hydrogen production compared to the absence of FONPs nanoparticles. This increase can be attributed to the enhanced hydrogenase activity and improved electron transfer efficiency in E. erogenous cells facilitated by FONPs. Adding FONPs to the fermentation process likely bolstered the electron transfer process due to their excellent conductive properties[36]. Clostridium is used to be an efficient biohydrogen and biobutanol producer among all other microorganisms. Enterobacter strains are considered to be more promising for industrial-scale for hydrogen production. The reason was the rapid growth rate of this microorganism and its ability to utilize a wide range of substrates and have strong adaptability to dissolved oxygen, hydrogen pressure, and PH [65].

2. Result:

The use of fossil fuels worldwide has a substantial impact on both the environment and human health, making it imperative to find alternative energy sources. To meet the global energy demands and tackle this issue, the development of a new energy source is crucial. Producing biofuels offers a promising solution as it allows us to reduce our dependence on fossil fuels and, whenever feasible, replace them. This transition to biofuels helps address the limitations of finite fossil fuel resources while working towards mitigating their harmful effects. The major worry is within the next 150-200 years will face a lack of coal and petroleum energy.to gain this aim, Nanoparticles significantly impact the output, helpful for increasing the production of hydrogen, and biobutanol enhances the action of hydrogenase as a cofactor. **Fe-based** Nanoparticles have substantial effects on metabolic pathway, are the essential element to form the metal content at the active sites of hydrogenase ([FeFe], [FeNi], and [Fe]), and enhance the activity of ferredoxin oxidoreductase by decreasing the level of dissolved oxygen (DO) and improving electron transfer via their surface quantum properties and speed up the production of biofuel. Considering the Parameters such as concentrations, sizes, temperatures, and substrate are essential for the maximum output of biohydrogen and biobutanol. hydrogen and biobutanol are renewable energy sources and the cleanest and high potential energy sources (122 kj/g). Fe-based NPS can influence the microbial metabolic activity for the production For the output of biohydrogen and biobutanol different substrates are used, such as glucose, sucrose, lignocellulose, and rice mill wastewater, catalyzing by microorganismlaction of effective microorganism help the production rate and quality of hydrogen. Such as an amylolytic Clostridium sp. Strain BOH3 can produce butanol and hydrogen from food waste without the need for enzymatic pretreatment. According to protein sequence analysis has been shown that amylase in Clostridium sp. strain BOH3 may contain a multi-active

site for activity, has a high ability of translation, can produce 14.1 g/L butanol and 16.2 mmol hydrogens from 180 g/L food waste. In general, sugars stored in food waste are macromolecules (such as starch), which are usually hydrolyzed to monosaccharides before microbial fermentation. Collection the waste food from around to produce biofuel energy is an effective method for maintaining a clean environment.

Conclusion

In conclusion, using fossil fuels harms the environment and human health, and a new energy source is necessary to meet the world's energy demands. Biofuels are a viable alternative, as they can decrease our reliance on fossil fuels and mitigate the limitation of their finite resources. Nanoparticles, particularly Fe-based ones, have been shown to significantly impact the production of biofuels such as hydrogen and biobutanol by enhancing microbial metabolic activity. Different substrates, such as food waste, can be use to produce biofuels, and effective microorganisms like Clostridium sp. strain BOH3 can increase the production rate and quality. Overall, collecting waste food to produce biofuel energy is an effective method for maintaining a clean environment.

3. Future perspective

Extensive research has been conducted to identify nanoparticles that are compatible with microorganisms and do not impede their metabolic activity. These nanoparticles play a crucial role in preventing and modifying any adverse effects on microbial functions. At the pilot scale level, the production of biobutanol and biohydrogen using Clostridium sp. strain BOH3 has demonstrated promising outcomes. This particular strain exhibits the remarkable ability to produce biobutanol and biohydrogen from food waste without the need for enzymatic pretreatment, which significantly lowers production costs. Leveraging the capabilities of Clostridium sp. strain BOH3 at the pilot scale presents immense potential for generating biobutanol and biohydrogen from food waste. This approach not only helps in waste reduction but also offers a sustainable and efficient production process that is economically viable for the future.

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