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Review article

A review on sustainable approach for biohydrogen production from agricultural waste

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ABSTRACT

The global rise in population is leading to higher consumption of energy and food.Increasing the reliance on fossil fuel which are generating ample amount of organic waste. Dependency on fossil fuel is continuously increasing greenhouse gas (GHG) emission and global carbon footprint. In current scenario we need alternative of fossil fuel for energy security, which is eco-friendly, cost effective and scalable. Biohydrogen production from organic waste will be the best alternative. The process utilizes organic waste as substrate, which is fermented by microorganism to produce biohydrogen gas. Although biohydrogen is a promising alternative as fuel, it hasn't reached its theoretical maximum production limit. This review explains the existingdrawbacks unique (accumulation of by-products, oxygen sensitivity of the enzymes, high energy demand, low light conversion efficiency, pH imbalance and acidification, scaling up and reactor design) to each method. Along with various microbial strains and species applied across the different biological methods for hydrogen production.The yield is also showing a variation from species to species and substrate to substrate. The aim of the study was to compare the existing data collected from various studies such as review and research paper to understand each drawback in order to overcome them, which will help in industrialization of biohydrogen.

1. Introduction

With rapid increase in population, the demand for agricultural produce is increasing at an incredible pace globally. To meet the global demand farming is being done at a step that has been never seen before. This process of balancing the demand and yield is generating a copious amount of agricultural waste. According to projections, the global agricultural waste market would be valued at USD 20128.44 million in 2025 and grow at a compound annual growth rate (CAGR) of 7.2% to reach USD 37626.69 million by 2034. The recycling and reuse of the more than 998 million tons of agricultural leftovers produced each year is greatly aided by the worldwide agricultural waste. market. Approximately 54% of crop residues such as paddy, husk, straw, bran, corn stalk, corn cob, sugarcane bagasse and molasses are recycled through the processes of composting, biogas generation, and biofuel production worldwide. Utilizing agricultural waste, lowers methane emissions by 18% yearly and contributes to 32% of the world's biomass energy generation. Over 160 nations have laws governing the recycling of agricultural waste, and 38% of them require waste-to-energy or composting on farms. Sustainable waste management increases soil productivity by 22-27%, according to the Agricultural Waste Market Analysis, which propels the growth of the rural economy and circular agriculture programs ((*Agricultural Waste Market Trends | Report [2034]*, n.d.).

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India alone generates more than 500 million tons of crop residues yearly (Reddy et al., 2025). In few states of India, the agricultural waste (crop residue) is disposed by burning, this practice is commonly known as stubble burning. Stubble burning effects north Indian states like Punjab, Haryana, Rajasthan, Uttar Pradesh, Bihar and National capital Delhi Fig 1. Stubble burning causes air pollution which can be measured by monitoring the increase in the levels of PM 2.5 and PM 10, oxides of nitrogen, oxides of sulfur, CO, CO2, NH3 from the month of October to January. Due to onset of winter (from November to February) the problem of air pollution becomes much serious as a thick layer of smog is formed in the atmosphere by the combination of various factors like stubble burning, vehicular emission, fire crackers and construction activities and these factors are leading to health problems like Asthma, Cancer, Chronic Obstructive Pulmonary Disease (COPD), heart disease and non-chronic issues like breathing difficulty, increase in allergic response in children's and elderly (Gatkal et al., 2024). Stubble burning possesses a significant threat to microbial biodiversity of soil, nearby native flora and fauna, human health and wellbeing. It leads to soil erosion, decreased soil fertility. The issue exists due to the lack of awareness among farmers, lack of efficient technology for proper disposal of agricultural waste, ineffective policy making, existing methods (dumping, open burning, animal feeding, mulching, and composting) are time consuming and less effective (Reddy et al., 2025).

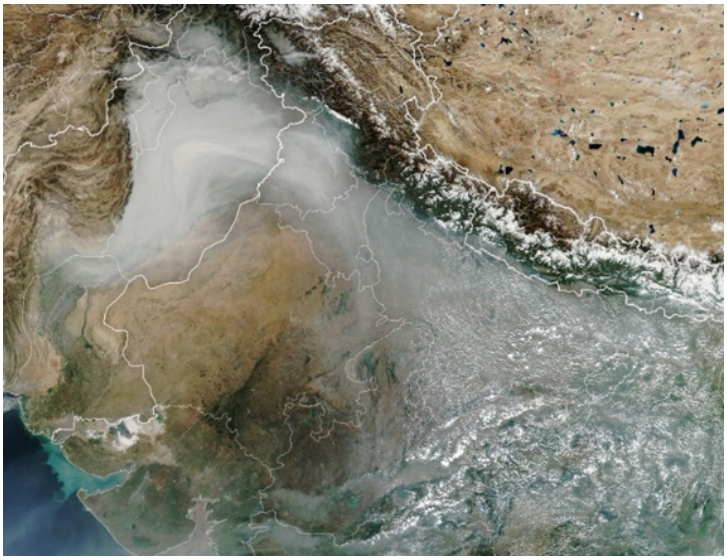

Fig. 1 Smoke produced from stubble burning in the month of November 2024

A non-traditional method of agricultural waste disposal is conversion of waste to energy, which is sustainable, reduces greenhouse gas emissions and carbon footprint. Biologically produced hydrogen is termed as Biohydrogen. It exhibit the potential to be recognized as fuel of the future due to its exceptional energy density of 122 kJ/g (Dowaidar, 2025). Biohydrogen will be pivotal to meet the growing energy demand in future, as it will reduce the dependency on fossil fuels contributing to the significant reduction of carbon footprint caused by burning fossil fuels. It has several advantages like clean energy source when burnt it produces water vapour and heat as byproduct, biomass has highest heating value of 142 kJ/g per unit mass, viable and safer alternative to use when compared to methane (Vidal et al., 2025). Biohydrogen can be carbon neutral, clean energy carrier when produced from renewable source of organic wastes such as agricultural waste, food waste, animal waste, municipal waste and wastewater.

The research in the direction of biohydrogen production from lignocellulosic (agricultural) waste directly aligns with the United Nations Sustainable Development Goals (SGDs). It contributes to SDG 7 (Affordable and Clean Energy), SDG 13 (Climate Action) by reducing carbon footprint and GHG generated during open burning. It also incorporates well in SDG 12 (Responsible Consumption and Production) by utilizing waste as a raw material while maximizing resource efficiency and supports circular economy approach (*THE 17 GOALS | Sustainable Development*, n.d.). On national level, it comes in line with India's National Green Hydrogen Mission which seeks to generate 5 million metric tonnes of biohydrogen per year by 2030 (*National Green Hydrogen Mission | MINISTRY OF NEW AND RENEWABLE ENERGY | India*, n.d.).

This study will review existing biological methods of biohydrogen production from agricultural waste along with their unique advantages and drawbacks.

2. Different biological methods of biohydrogen production

India is an agriculture-based country which increases the availability of biomass increasing the feasibility of biohydrogen production. The process includes microorganisms that act on complex organic substrate (agricultural and other waste) breaking them into simpler compounds such as alcohol and organic acids along with the production of byproducts like water, hydrogen, methane and carbon dioxide. Biological

production of hydrogen is achieved by hydrogenase enzyme. Hydrogenases catalyse the rescindable oxidation of hydrogen into its basic essentialconstituents: two electrons (e^-) and two protons (H^+). Based on the metal in their active site hydrogenase can be subdivided into three groups: Iron (Fe) hydrogenase, Iron-Iron (FeFe) hydrogenase, Nickle-Iron (NiFe) hydrogenase. Iron hydrogenase is vital for biophotolysis and FeFe hydrogenase is vital for dark fermentation process (Dowaidar, 2025).

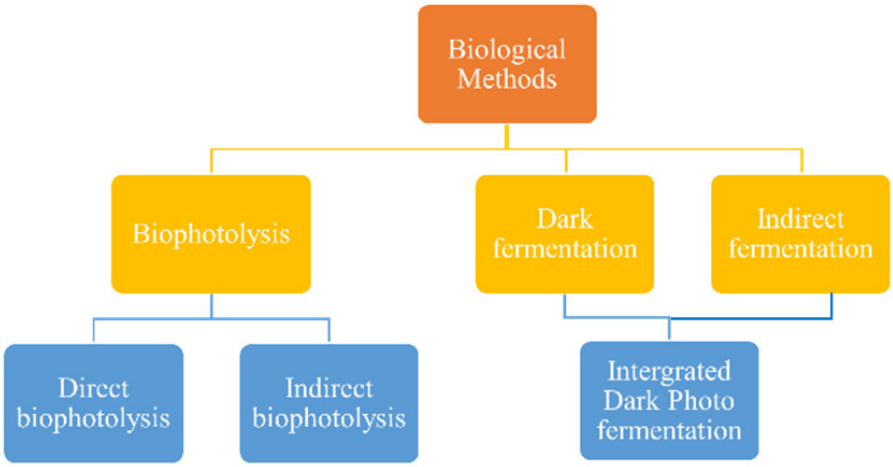

Fig. 2: Classification of biological methods for biohydrogen production

Table 1 Different microalgae and cyanobacteria with their yield and operational parameters

	Microalgae		
Algae	**Temperature**	**Yield**	**Reference**
*Closteriummoniliferum*AARL G041	25 ˚C	0.38 mmol/h/mg	Faraloni et al.(2025)
Chlamydomonas reinhardtii CC-1036	27-29 ˚C	9.2 mL/L/h	Melitos et al.(2021)
Chlorella pyrenoidosa	28 ˚C	93.86 mL/L	Faraloni et al.(2025)
Platymonassubcordiformis	NA	2.7 mL/L/h	Melitos et al.(2021)
Scenedesmus obliquus	25 ± 1 ˚C	8.53 mL/L/h	Faraloni et al.(2025)
Tetraspora sp. CU2551	36 ˚C	0.182 ± 0.020 mmol/h/mg	Faraloni et al.(2025)

	Cyanobacteria			
Cyanobacteria	**pH**	**Temperature**	**Yield**	**Reference**
Synechocystis sp. PCC 6803	NA	28 ˚C	3-5 ml/g/day	
Fischerellamuscicola	7.5	25 ˚C	0.35 ± 0.08 mmol/g/h	
Nostoccalcicola	7.5	25 ˚C	0.09 ± 0.01 mmol/g/h	Faraloni et al. (2025)
Scytonemabohneri	7.5	25 ˚C	0.09 ± 0.01 mmol/mg/h	
Tolypothrixdistorta	7.5	25 ˚C	0.21 ± 0.05 mmol/mg/h	
Geitlerinema sp. RMK-SH10	NA	NA	0.271 mmol/h/mg	
Anabaena sp. PCC 7120	8.2	22 ˚C	0.86 mL/h/L	

2.1 Biophotolysis

Biophotolysis include splitting of water molecule into H_2 and O_2 using the photon energy (sunlight) equation 1. Table 1 lists few microorganisms capable of biophotolysis for biohydrogen production.

$$2H_2O \rightarrow 2H_2 + O_2 \qquad eq.1$$

Biophotolysis can be subdivided into two types: Direct and Indirect biophotolysis.

2.1.1 Direct biophotolysis

The process is usually carried out by photoautotrophic microorganisms like green algae and cyanobacteria (Kundu et al., 2025). These organisms produce biohydrogen through pigments like chlorophyll that utilizes photons of wavelength of less than 680 nm as shown in equations 2 and 3 (Samrot et al., 2023). Under dark condition green algae can also produce hydrogen using carbon dioxide fixation in anaerobic condition. In recent years *Chlamydomonas reinhardtii* grabbed a lot of attention as a potent hydrogen producing single celled green algae(Dowaidar, 2025).

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2 \qquad eq.2$$

$$4H^+ + 4e^- \rightarrow 2H_2 \qquad eq.3$$

2.1.2 Indirect biophotolysis

Indirect biophotolysis employs cyanobacteria for the production of biohydrogen using carbon dioxide fixation. In this process H_2 and O_2 are produced at two separate steps. Cyanobacteria utilize carbon dioxide to make their own atoms, left over carbon dioxide is used as a carbon source in presence of sunlight. It is a two-stage procedure that includes photosynthesis and reduction of glucose to produce biohydrogen, where carbon dioxide acts as an electron carrier between photosynthesis and hydrogen production as shown in equation 4 and 5 (Samrot et al., 2023).

$$12H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + CO_2 \qquad eq.4$$

$$C_6H_{12}O_6 + 12H_2O \rightarrow 12H_2 + 6CO_2 \qquad eq.5$$

Table 2 Drawbacks of Bio photolysis

Drawback	Description	References
Low H_2production rates and yields	• Hydrogen output is typically low, e.g., 0.05 mL H_2/L/h in outdoor photobioreactors (PBRs) or instantaneous rates of 100-200 µmolH_2/mg chlorophyll/h sustained only briefly. • Theoretical yields are rarely achieved due to inefficiencies. • Restricts scalability; overall productivity is 10-100 times lower than chemical methods like electrolysis.	Faraloni et al. (2025)
Oxygen sensitivity of hydrogenase enzymes	• O_2 produced during photosynthesis inhibits [FeFe]hydrogenase (highly active but O_2sensitive) and reduces [NiFe]hydrogenase efficiency, requiring strict anaerobic conditions (O_2 below ~0.1%) or separation strategies. • Leads to H_2 inhibition; necessitates energy-intensive O_2 removal (e.g., via absorbers) or nutrient starvation to downregulate O_2 evolution.	Faraloni et al. (2025); Teke et al. (2023)
High energy and ATP demand	• Processes require substantial ATP (e.g., 16 ATP per H_2 via nitrogenase in indirect biophotolysis) and continuous high-intensity light, with poor light-to-H_2 conversion (e.g., needing 8 quanta per H_2 molecule). • Increases operational costs; solar to H_2 efficiency is only 1% (vs. theoretical 13.4%), far below photovoltaic electrolysis (10-12%).	Faraloni et al. (2025)
Scaling- Up and reactor design challenges	• Inefficient light utilization (e.g., outdoor saturation at 10x needed irradiance causes energy loss via quenching); difficult to maintain symbiotic conditions or prevent contamination in large systems. • Low technological readiness (TRL 4-5); ethical/regulatory hurdles in genetic engineering for O_2tolerant strains; variable wastewater substrates add inconsistency.	Faraloni et al. (2025); Teke et al. (2023)
Other limitations	• Formation of by-products reduces substrate yield; high thermodynamic/ kinetic barriers; need for specialized conditions like sulfur/ potassium deprivation, which stress cells and lower biomass. • Long-term economic risks; limited substrate availability in some setups.	Teke et al. (2023)

These challenges highlight why biophotolysis remains largely experimental, though ongoing research in genetic engineering, hybrid systems, and optimized photo bioreactors aims to address them (Teke et al., 2023).

2.2 Dark fermentation

Dark fermentation is a biological process that employs facultative (E. coli, Enterobacter, Citrobacter) and obligate (Clostridium sp., archae) anaerobic microorganism for the production of Biohydrogen. It utilizes organic wastessuch as food waste, wastewater,

Table 3 below present different substrate along with microbial inoculum used for biohydrogen productionvia dark fermentation. Dark fermentation offers higher yield when compared to other biological methods but has some drawbacks. Table 4 discusses the key drawbacks of dark fermentation process.

agricultural waste (cellulose, hemicellulose and lignin) as raw material, which on hydrolysis releases fermentable sugars. In absence of light and oxygen these sugars are converted to hydrogen through specific metabolic pathways Fig. 3. Few evidently established pathways are (Kumar Sharma et al., 2022):

Acetate pathway: $C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 4H^+ + 2HCO_3^-$

Butyrate pathway: $C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COO^- + 3H^+ + 2HCO_3^-$

Ethanol pathway: $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2H^+ + 2HCO_3^-$

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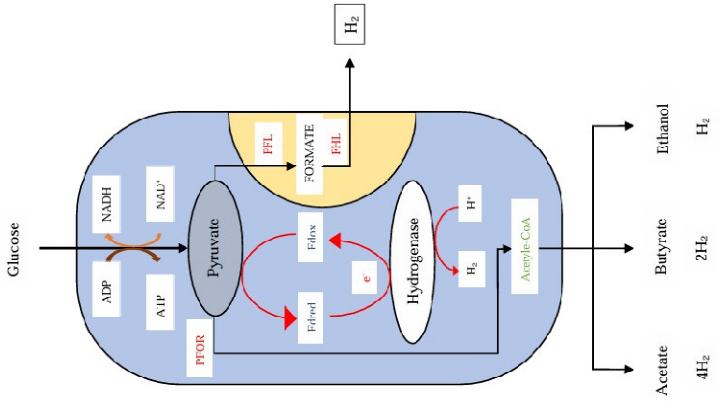

Fig. 3 Metabolic pathway of dark fermentation

Table 3 Different substrate along with yield, inoculum and operation parameters

Substrate	Inoculum	pH	Temperature	Yield	Reference
Rice straw	*Thermotoganeapolitana*	7.5	75 ˚C	50.85 mL/g	
Rice husk	Anaerobic granular sludge	7-7.5	35 ˚C	320.6 mL/g	
Wheat straw	H_2 producing microflora	NA	35 ˚C	4.68 mL/g	Deka et al. (2025)
Maize stalk	*Clostridium thermosaccharolyticum* DSM 869, *Clostridium thermocellum* DSM 7072	NA	55 ˚C	68.2 mL/g	
Maize cob	Municipal sewage sludge	5.5	40 ˚C	93.10 mL/g	
Sugarcane bagasse	*Bacillus subtilis* AuChE413	7	37 ˚C	39.6 mL/g	
Willow	*Shewanellaoneidensis* MR- 1	NA	NA	787.6 ± 69.3 mL H_2/L	Vidal et al.(2025)
Hay	*Sorangiumcellulosum* So ce27	NA	NA	851 ± 20.8 mL H_2/L	Vidal et al.(2025)

2.3 Photo fermentation

Nitrogenase producing purple nonsulfur bacteria use photon energy and organic acids as substrate for the production of hydrogen as shown in equation 6. Purple non sulphur bacteria are able to grow as photoautotrophs, photoheterotrophs, and chemoheterotrophs, upon conditional accessibility of carbon, oxygen, and light sources. Some PNS bacteria capable of photo fermentation are *Rhodobacter capsulatus, Rhodopseudomonas palustris, Rhodobacter sphaeroides* and *Rhodovulum sulfidophilum*as shown in Table 5 (Samrot et al., 2023)**.**

$$CH_3COOH + 2H_2O \rightarrow 4H_2 + 2CO_2 \qquad eq.6$$

The Table 6 discusses key drawbacks of Photo fermentation process based on the result of different studiesconducted.

Table 4 Drawbacks of dark fermentation

Drawbacks	Description	References
Low hydrogen yield	Dark fermentation typically achieves a maximum yield of about 4 mol H_2 per mol glucose, far below the theoretical 12 mol, primarily due to incomplete substrate oxidation and the production of by-products like acetic, propionic, and butyric acids that divert metabolic pathways.	Osman et al. (2020a)
Accumulation of byproducts	The production and buildup of volatile fatty acids (e.g., acetic and butyric acids) compete with hydrogen formation, leads to incomplete oxidation of organic matter, and requires ongoing monitoring to maintain process efficiency.	Albuquerque et al. (2024)
pH imbalance and acidification	Acid accumulation from volatile fatty acids causes a drop in pH, which inhibits hydrogen-producing microorganisms and alters metabolic pathways, necessitating precise pH control for optimal activity.	Osman et al. (2020a)
Presence of hydrogen-consuming methanogens	Methanogenic bacteria in inoculum or reactors consume produced H_2 to form methane, significantly reducing net yields; heat or chemical pretreatments are often needed to suppress them.	Albuquerque et al. (2024)
Substrate pretreatment	Complex substrates like lignocellulosic biomass are hindered by lignin barriers and slow cellulose hydrolysis, demanding energy-intensive pretreatments (e.g., chemical or physical) to make them accessible, which adds costs and complexity.	Osman et al. (2020a)
Substrate inhibition and osmotic stress	High organic loading rates increase osmotic pressure, inhibiting bacterial growth and shifting fermentation toward alcohol or acid production rather than H_2.	Albuquerque et al. (2024)
Hydrogen partial pressure	As H_2 accumulates in the reactor, its elevated partial pressure inhibits further production by affecting hydrogenase enzymes.	Osman et al. (2020a)
Operational instability and parameter sensitivity	Factors like hydraulic retention time (HRT), temperature, and microbial contamination require precise control; deviations lead to instability, especially in continuous systems or during scale-up.	Albuquerque et al. (2024)
Low production rates and scaling challenges	The process is inherently slow compared to alternatives like electrolysis, with additional hurdles in transferring lab-scale efficiencies to industrial levels due to downtime and contamination risks.	Mokhtarani et al. (2025)

Table 5: Different substrate along with microbial species employed for biohydrogen production (Teke et al., 2023)

Substrate	Microorganism	Yield
Acetic acid	*Rhodopseudomonassphaeroides* strain A7	31.54 mL/L/h
Butyric acid	*Rhodopseudomonaspalustris* NCIMB 11774	20 mL/L/h
Lactic acid	*Rhodopseudomonaspalustris* DSM 127	8.4 mL/L/h
Malic acid	*Rhodopseudomonaspalustris* 420L	21.8 mL/L/h

2.4 Two-stage approach (Integration of dark and photo fermentation)

Developed recently to improve the yield of biohydrogen production. It incorporates advantages of both dark and photo fermentation while eliminating negatives of both.In first step (dark fermentation) organic waste is fermented to produce hydrogen and byproduct such as organic acids as shown in equations 7. Second step (photo fermentation) involves utilizing organic acid as substrate to produce hydrogen equation 8.

$C_6H_{12}O_2 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2CH_3COOH$ $eq.7$ $CH_3COOH + 2H_2O \rightarrow 2H_2 + 2CO_2$ $eq.8$

Table 6 Drawbacks of photo fermentation

Drawbacks	Description	Reference
Low hydrogen yield and production Rate	One of the key challenges is the inherently low rate of hydrogen production, often due to inefficient metabolic pathways and substrate utilization. For example, the maximum reported yields in some studies are around 642-1287 mL H_2 per unit of substrate, but these are far from theoretical maximum limit of 12 mol H_2/mol glucose. This low output occurs from factors like incomplete substrate conversion and competition from other metabolic by products.	Osman et al. (2020b)
Low light conversion efficiency	Photosynthetic bacteria require specific light wavelengths (typically 400-950 nm) for optimal activity, but light penetration in bioreactors is often poor due to cell shadowing and self-shading at high cell densities. This results in low light to hydrogen conversion efficiencies, typically below 10%, necessitating advanced reactor designs or artificial lighting, which increases energy inputs.	Cheng et al. (2022)
High energy requirements for enzymes	Hydrogen production relies on nitrogenase enzymes, which have high energy demands (requiring ATP) and are sensitive to oxygen and ammonium ions. This enzymatic inefficiency limits overall process performance, especially under non-ideal conditions.	Cheng et al. (2022)
Substrate pretreatment	Effective use of lignocellulosic biomass or wastes requires pretreatment to break down complex organics, which can be costly and energy-intensive. Additionally, optimizing the carbon-to-nitrogen (C/N) ratio is crucial, as imbalances can reduce yields or favor biomass growth over hydrogen production.	Melitos et al. (2021); Putatunda et al.(2023)
Photobioreactor design and costs	Scaling up requires customized photobioreactors to ensure uniform light distribution, mixing, and anaerobic conditions, but these are expensive to build and operate. Techno-economic analyses highlight high capital costs and operational expenses as major barriers to commercialization.	Ahmed et al. (2021); Putatunda et al. (2023)
pH	The process is sensitive to pH fluctuations, with optimal ranges around 6-8, deviations can inhibit bacterial growth and enzyme activity. Buffers like phosphate can help stabilize pH, but maintaining ideal growth conditions (temperature, anaerobiosis) adds complexity.	Guo et al. (2020)
Hydrogen separation and purification	Produced hydrogen is mixed with CO_2 and other gases, requiring energy-intensive separation techniques to achieve purity for applications like fuel cells.	Putatunda et al. (2023)
economic and scalability barriers	Overall, the process struggles with high production costs compared to fossil-based hydrogen, limiting industrial adoption. Integration with dark fermentation or nanotechnology shows promise for enhancement, but these add further R&D challenges.	Ahmed et al. (2021); Cheng et al. (2022)

3 Conclusion

Biohydrogen can be a clean, green source of energy when produced from organic waste while utilizing electricity produce by solar and wind energy. Can help in meeting global energy demand while reducing the dependency on fossil fuels. Biological methods of hydrogen production are eco-friendly and have a potential to reduce organic wastes, while promoting circular economy. It includes biophotolysis, photo fermentation, dark fermentation and combination of dark and photo fermentation. The drawbacks of each method are discussed in Tables 2, 4 and 6. Each method has its own unique challenge, which can be mitigated through further research and development, continuous data collection is needed to understand technological advancement. Inclusion of digital system can effectively measure and regulate the parameters like pH, temperature, hydrogen partial pressure, dissolved oxygen and hydrogen concentration, CO_2 level, CH_4 levels in headspace. These factors are essential in controlling the yield of biohydrogen.

4 Future recommendation

To commercialize biohydrogen production at industrial scale more research and development is needed: to design a cost-effective, efficient photo bioreactors and fermenters that require low to none maintenance. Process must be optimized to achieve the theoretical limit of 12 mole of H_2 per 1 mole of sugar and to ease the separation of byproducts from bioreactor. A much-needed step is development of specific pilot scale bioreactors for H_2 production. Integration of mass spectrometry to improve the existing gas measurement methods.Instead of mono-culturing, co-culturing can attain significant increase of up to 46%. Application of genetic engineering for strain improvements of existing microorganisms enabling them to use a wide variety of substrate, increasing the yield and rate of biohydrogen production while reducing the formation of byproducts. There is a diverse microbial community from which identification, isolation and application of novel microbial species for the production of biohydrogen is needed. Another most common factor is pretreatment of lignocellulosic waste, existing methods use chemicals which are not eco-friendly, physical methods are energy demanding while biological methods are time consuming but sustainable. In depth research is needed to improve biological pretreatment method utilizing enzymes and microbial consortium.

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Author contribution

Saurabh Verma (Research scholar) has conceptualized, prepared a draft of the article and formal analysis. Deepika Mishra had tabulated the data. Jiwan Singh (Supervisor) has reviewed, edited and validated the manuscript.

Conflicts of Interest

There are no conflicts of interest declared by the authors.

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