Ilkogretim Online - Elementary Education Online, 2021; Vol 20 (Issue 4): pp. 4173-4182
http://ilkogretim-online.org
doi: 10.17051/ilkonline.2021.04.453

Potential Application Of Microbial Nanotechnology For Bioremediation Of Waste Water To Optimize Biohydrogen Production

Pallavi Singh[1]*, Navin Kumar[2], Bindu Naik[3], Hemaadri Singh Rana[4]

[1,2] Department of Biotechnology, Graphic Era Deemed to be University, Dehradun, Uttarakand-248002
[3] Department of Life Sciences, Graphic Era Deemed to be University, Dehradun, Uttarakand-248002
[4]Assistant Professor, School of Law, Graphic Era Hill University, Dehradun
Corresponding Author: ***Dr. Pallavi Singh**
E-mail: pallavisingh.22@gmail.com; pallavisingh.bt@geu.ac.in

ABSTRACT

Biofuels are a promising alternative to meet the ever increasing demand of energy and fuel worldwide. As there has been limitations in production of Bioethanol and Biobutanol by application of conventional fungal species like Saccahromyces cerevisae, the focus has shifted to production and application of Biohydrogen by utilizing waste water from various pharmaceutical and agricultural industries. Applications of Microbes and nanoparticles encapsulating enzymes like hydrogenases has accelerated the production of Biohydrogen to a greater extent. Biophotolysis and photo fermentation methodologies are conventional strategies optimized now to scale up and boost the yield of hydrogen from pilot scale to industrial scale. In a closed bioreactor or dark fermentative system, photosensitive microorganisms such as microalgae are grown in a photobioreactor for the generation of hydrogen. In this review, we have discussed latest strategies which are focused on utilization of nanotechnology and microbes by processing waste water from industries to generate Biohydrogen. Further, the challenges and promising avenues in the roadmap to enhance biohydrogen generation have been highlighted in this work.

Keywords: Biohydrogen, Biofuel, Nanoparticles, Bioremediation, Microbes, Enzymes, Nanotechnology, Microalgae, Energy.

INTRODUCTION

Hydrogen is an eco-friendly energy source because it releases water vapour as a byproduct into the environment on combustion. It is regarded as a carbon-free and environmentally friendly energy source. It has a twice the energy generation capability as compared to the energy content of hydrocarbon fuels. Hence, it is a better alternative energy source for power generation, transportation, and various industrial applications. The presence of excess organic materials increases the BOD and COD levels of the waste water. These organic materials act as nutrients for the better growth of the hydrogen producing microorganisms. Production of bio-hydrogen from waste water is an emerging technology in which the waste water is treated in an advanced way and the isolated organic waste is utilized to produce hydrogen with the help of microorganisms (1). Dark fermentation or light independent reaction (microbial electrohydrogenesis cells) is catalyzed by heterotrophic bacterial groups comprising facultative and obligate anaerobes, and photo fermentation or light dependent reaction (bio-photolysis) is catalyzed by photosynthetic bacteria. (2)

Agriculture residues and waste products from different industries are processed using microbial cells and nanoparticles having immobilized enzymes for production of various biofuels , especially biohydrogen. Yield of Hydrogen from various sources of waste water is shown in Table 1.

Source of waste water	Inoculums	Hydrogen yield	References
Rice mill	Enterobacter aerogenes, Citrobacter ferundii.	1.40-1.97 mol/mol	7
Cassava mill	Cow dung	1787 mL/L	5
Effluents from Distillery	Sludge (Anaerobic)	10.95 mmol/g COD	3,4
Condensed molasses soluble	Sludge (Anaerobic)	0.7-1.5 mol/mol	10
Beverage waste water	Enriched mixed culture from food waste compost	3.76 mol/mol	10
Sugar beet	Sludge (Anaerobic)	2.0 mol/mol glu	6,7
Dairy industry	Sludge (Anaerobic)	15.33 mmol/g COD	8
Cheese processing industry	Mixed culture Lactobacillu,s Clostridia, Lactobacillus	10.2 mM/g COD	9
Organic industry	Rhodobacter sphaeroides, R.	2.32 mol/mol165 mL/g COD	9

	capsulatus, Rhodovulum sulfidophilum, and Rhodopseudomonas palustris		
Brewery Industry	Klebsiella pneumoniae	10.80 mmol H2 h−1	11
Food and beverage processing waste water	Anaerobes from Sludge compost	0.33 LH2/L waste water	12
De-oiled Jatropha waste	Clostridium thermopalmarium, Bacillus ginsengihumi	0.307 L	13
Sugar cane distillery effluent	Citrobacter freundii , Enterobacter aerogenes, Rhodopseudomonas palustris P2	2.76 mol mol−1 glucose	13

Table 1: Yield of Hydrogen from various sources of waste water

Strategies for Production of biohydrogen from waste water

At high temperature and pressure, Hydrogen may be produced using the methods such as fossil - based hydrocarbons reformation, coal gasification, and partial oxidation. Bio-hydrogen production is divided into four categories: (i) photodecomposition of organic compounds (by photosynthetic bacteria) (ii) bio-photolysis of water (by algae/cyanophyta) (iii) dark fermentative hydrogen (by using facultative bacteria and anaerobes) (iv) microbial fuel cells. There are pros and cons associated with each biological process. During the Algal (green) and cyanobacterial photosynthesis the water molecule is broken down in to into gas (H2) and liquid (H2O), however, as the algal cells grow at a slow rate and activity of hydrogenase enzyme gets reduced in the presence of oxygen, this process becomes critically slow. In photosynthetic organisms and dark fermentation bacteria, the degradation of organic compounds for energy generation resonates similarity with each other. Organic acids are utilized as a substrate by photosynthetic bacteria, but they are hostile to ammonia and oxygen, making them unsuitable for commercial hydrogen production. Dark fermentation, on the other hand, breaks down a wide spectrum of organic wastes into simple sugars. (14)

The dark fermentation, on the other hand, has a lower COD removal effectiveness (33%). Furthermore, the dark fermentation's biomass growth rate and hydrogen generation rate are both higher than those of conventional hydrogen production techniques, making it an appealing contender for industrial and commercial bio-hydrogen production. (15)

(i) Bio-photolysis of water using algae/ Cyanophyta

Photosynthesis and hydrogen generation catalyzed by hydrogenases are the two processes in bio-photolysis or photo-biological hydrogen production.

$$\text{Photosynthesis:} \quad 2H_2O \rightarrow 4H^+ + 4e^- + O_2$$

$$\text{Hydrogen Production:} \quad 4H^+ + 4e^- \rightarrow 2H_2$$

Biophotolysis and photofermentation are light-catalyzed biological processes that use water to create hydrogen. In biological systems, it is a water-splitting mechanism that occurs in the presence of light. Water, light, and photosensitive microorganisms are therefore the three main components of biophtolysis. The molecules H2 and O2 are created by light energy (16). In a closed system, photosensitive microorganisms such as microalgae are grown in a photobioreactor for the generation of hydrogen. Biophotolysis-based hydrogen generation has been divided into three categories: direct, indirect, and photofermentation (17). In biophotolysis and photofermentation, the entire hydrogen generation processes may be described as

$$6H_2O + 6CO_2 \xrightarrow{h(\nu)} C_6H_{12}O_6 + 6O_2$$

$$C_6H_{12}O_6 + 6H_2O \xrightarrow{h(\nu)} 6CO_2 + 12H_2$$

The microalgae Chlamydomonas reinhardtii has been the best investigated for direct biophotolysis. Photosystems (both PSI and PSII) and hydrogenase are required. PSII (680 nm) and/or PSI (700 nm) absorb light in the form of photons, producing a powerful oxidant capable of oxidising water into protons, electrons/reducing equivalents, and O2. Electrons decrease protons to generate H2 (18,19).

Direct biophotolysis is the process of using algae/cyanobacteria to produce H2 through a series of processes. The coordinated action of the two photosystems utilised in plant-type photosynthesis mediates the overall response (PSI and PSII). Photon absorption divides water throughout the process, resulting in a drop in ferredoxin, which is used to enhance H2 production via proton reduction. The light-driven breakdown of water into H2 and O2 involves the nitrogenase and hydrogenase enzymatic activities (19). Chlamydomonas reinhardtii (20) and Anabaena cylindrical are green algae or cyanobacteria that mediate direct biophotolysis without or with respiratory O2 absorption (21).In both procedures, water is split into O_2 and H_2. Direct biophotolysis with respiratory O_2 uptake is a two-stage process involving PSII and PSI. Under aerobic conditions, electron equivalent from water splitting are used in the first step of direct biophotolysis to fix CO_2. In the second stage, water is split into H_2 and O_2, with O_2 being separated and routed to first phase. Several species and strains from at least 14 genera have been tested for H_2 production under a variety of growth conditions (22). The reported H_2 generation rates in these investigations are varied and vary on the species and environment. The capacity to create hydrogen from water in mild settings, such as at moderate temperatures and pressures, is a benefit of biophotolysis.

Disadvantages

Low photochemical efficiency and significant by-product (oxygen, O_2) inhibition of hydrogenase (H_2ase) are severe issues (23).H_2 is generated by nitrogenase in the absence of ammonium ions (NH_4) during photofermentation by anoxygenic photosynthetic bacteria. Because no O_2 is released in this scenario, O_2 inhibition is not a concern (24,25). However, nitrogenase's poor H_2 generation activity, NH_4-induced inhibition of nitrogenase expression, and low photochemical efficiency are all significant disadvantages.

Application of microbial nanotechnology for Bioremediation of waste water and production of bio-hydrogen

The nano-technological approach is viewed as a single, comprehensive package that incorporates various procedural aspects of traditional approaches while lowering the cost of wastewater treatment plants. Nanotechnology is now being used in a variety of fields, including food toxin detection, nanosensors, nanoneutraceuticals, targeted medication delivery, imaging, theranostics, and photodynamic microorganism inactivation (26,27,28). The usage of nanotechnology is more sustainable and eco-friendly when nanomaterials are biofabricated and microorganisms are used simultaneously. Chemically manufactured nanoparticles may have drawbacks in terms of chemical use and aqueous solution self-agglomeration. As a result, green nanoparticle manufacturing using plant extracts, fungal enzymes, and bacterial enzymes might be a viable option. They develop higher firmness in an aqueous system due to simultaneous precipitation. Aspergillus tubingensis (STSP 25) prefabricated iron oxide nanoparticles had been received from Avicennia officinalis in the Sundarbans, India (28,29). Synthesized nanoparticles were able to completely remove more than 85% of toxic metals, mainly pallidium, nickle, zinc ions from wastewater after five rounds of regeneration. Metal ions were chemically adsorbed on the nanoparticles' surfaces during endothermic processes (30). Another experiment used exopolysaccharides (EPS) from Chlorella vulgaris to co-precipitate iron oxide nanoparticles. Fourier-transform infrared spectroscopy was used to show that EPS functional groups may effectively modify nanoparticles (FTIR). The nanocomposite also removed 91 percent of PO_4^{3-} and 85 percent of NH_4^+. (31)

Application of various microbes to fabricate nanoparticles has shown to be an economical and sustainable environmentally suitable technique. Escherichia sp. SINT7, a copper-resistant bacterium, has been tremendously used in synthesis copper nanoparticles. It was discovered that biogenic nanoparticles degraded azo dye and textile wastewater. At a minute concentration of 25mg/l, it has been proved that Reactive black-5, congo red, direct blue-1, and malachite green were substantially reduced. 83.61, 97.07, 88.42, and 90.55 percent. However, at a increasing concentration of 100 mg/L, this was reduced to 76.84, 83.90, 62.32, and 31.08 percent, respectively. The industrial wastewater was also treated, and the suspended particles, chloride, and phosphate ions in treated samples were reduced. The performance of biogenic nanoparticles like these boosts industry's cost-effective and long-term manufacturing (32,33) used no additional Sulphur to make iron-sulfur nanoparticles. The extracellular transfer of electrons allowed these nanoparticles to degrade Napthol Green B dye. The utilization of Pseudo alteromonas sp. CF10-13 in nanoparticle manufacturing gives an environmentally acceptable biodegradation approach. The generation of hazardous gases and metal complexes was suppressed by the endogenous creation of nanoparticles. The utilization of biogenic particles in the cleanup of industrial effluents is a better method. Apart from directly producing nanoparticles from microbes, there are various additional ways in which microorganisms might aid in the advancement of nanotechnology. Microorganisms, for example, might supply catalytic enzymes that, in combination with nanoparticles, aid in wastewater cleanup.

Using technology to convert waste materials into usable goods is drawing the interest of researchers all around the world. We can decrease trash while also producing valuable things with this method. Adsorbents, clinker, biogas, biohydrogen, biomolecules, and a range of other products are all made using this approach (34). Nanotechnology has contributed in the enhancement of waste-to-resource conversion production rates. Kumar and colleagues released a study in 2019 on employing nanoparticles to promote biohydrogen production and improve dark fermentation processes (35). Nanoparticles added to fermentative bacteria have brought unprecedented possibilities for producing biohydrogen from wastewater. Many researchers have produced biohydrogen applying mixed culture bacteria and single, dual, and multiple nanoparticles. They observed that biohydrogen production was at its highest when a large number of nanoparticles were utilised (14 percent higher than when single nanoparticles were used). It was discovered that nanoparticles boosted hydrogenase and dehydrogenase activity, resulting in increased biohydrogen production. Similarly, combining nickel oxide and hematite nanoparticles increased biohydrogen production by 1.2–4.5 times as compared to employing just nanoparticles. The combination of nanoparticles produced the maximum hydrogen output of 7.75 mmol/g COD (9,10). As a result, nanotechnology may be employed to provide green energy for long-term industrial growth and environmentally responsible manufacturing.

Current challenges and future prospective in utilizing nanotechnology for biofuel production

Nanotechnology has attracted researchers' interest due to its numerous advantages, including a high available surface area, the capacity to serve several purposes, stability in difficult environments, quick and efficient material manipulations, greater interactivity, and so on. The combination of microbes and enzymes with nanotechnology has resulted in a more environmentally friendly approach to industrial wastewater control and biofuel production (36, 37). The employment of microorganisms can reduce the danger associated with chemically generated nanoparticles. The residues that remain are either biocompatible or readily separated using basic filtration/precipitation methods. The commercialization of these nanotechnological capabilities is the greater hurdle. So yet, just 1% of these nanotechnological features have been commercialized. As a result, companies will use these simple and effective microorganism-assisted nanotechnology procedures on a broad scale as a stepping stone. This needs ongoing support and confirmation from academics, as well as government financing, in order to cultivate nanotechnology's potential for sustainable and cost-effective manufacturing in industry Long-term basic and applied research is required in this field, but if successful, it will result in a long-term solution for sustainable hydrogen generation. Understanding the natural mechanisms and genetic rules that govern H2 generation is critical. In bigger bioreactors, metabolic and genetic engineering might be employed to show the process. Another alternative is to use artificial photosynthesis to duplicate the two phases.

References

1. Brentner, L. B., Peccia, J., & Zimmerman, J. B. (2010). Challenges in developing biohydrogen as a sustainable energy source: implications for a research agenda. Environmental science & technology, 44(7), 2243-2254.
2. Cai, J., Zhao, Y., Fan, J., Li, F., Feng, C., Guan, Y., & Tang, N. (2019). Photosynthetic bacteria improved hydrogen yield of combined dark and photo-fermentation. Journal of biotechnology, 302, 18-25.
3. Cappelletti, B. M., Reginatto, V., Amante, E. R., & Antônio, R. V. (2011). Fermentative production of hydrogen from cassava processing wastewater by Clostridium acetobutylicum. Renewable Energy, 36(12), 3367-3372.
4. Cheng, S., Li, N., Jiang, L., Li, Y., Xu, B., and Zhou, W. (2019). Biodegradation of metal complex Naphthol Green B and formation of iron–sulfur nanoparticles by marine bacterium Pseudoalteromonassp CF10-13. Bioresour. Technol. 273, 49–55. doi: 10.1016/j.biortech.2018.10.082
5. da Silva, A. N., Macêdo, W. V., Sakamoto, I. K., Pereyra, D. D. L. A. D., Mendes, C. O., Maintinguer, S. I., & de Amorim, E. L. C. (2019). Biohydrogen production from dairy

industry wastewater in an anaerobic fluidized-bed reactor. Biomass and Bioenergy, 120, 257-264.

6. Das, D., & Veziroglu, T. N. (2008). Advances in biological hydrogen production processes. International journal of hydrogen energy, 33(21), 6046-6057.
7. Devi, S. S., & Bhanumathi, V. (2021, June). A Novel Reversible Carry Look Ahead Adder for Bio-Medical Applications. In Journal of Physics: Conference Series (Vol. 1937, No. 1, p. 012027). IOP Publishing.
8. Dhar, B. R., Elbeshbishy, E., Hafez, H., & Lee, H. S. (2015). Hydrogen production from sugar beet juice using an integrated biohydrogen process of dark fermentation and microbial electrolysis cell. Bioresource Technology, 198, 223-230.
9. Elreedy, A., Fujii, M., Koyama, M., Nakasaki, K., and Tawfik, A. (2019). Enhanced fermentative hydrogen production from industrial wastewater using mixed culture bacteria incorporated with iron, nickel, and zinc-based nanoparticles. Water Res. 151, 349–361. doi: 10.1016/j.watres.2018.12.043
10. Enamala, M. K., Dixit, R., Tangellapally, A., Singh, M., Dinakarrao, S. M. P., Chavali, M., ... & Chandrasekhar, K. (2020). Photosynthetic microorganisms (Algae) mediated bioelectricity generation in microbial fuel cell: Concise review. Environmental Technology & Innovation, 19, 100959.
11. Fascetti, E., D'addario, E., Todini, O., & Robertiello, A. (1998). Photosynthetic hydrogen evolution with volatile organic acids derived from the fermentation of source selected municipal solid wastes. International Journal of Hydrogen Energy, 23(9), 753-760.
12. Gadhe, A., Sonawane, S. S., & Varma, M. N. (2014). Evaluation of ultrasonication as a treatment strategy for enhancement of biohydrogen production from complex distillery wastewater and process optimization. International journal of hydrogen energy, 39(19), 10041-10050.
13. Gadhe, A., Sonawane, S. S., and Varma, M. N. (2015). Influence of nickel and hematite nanoparticle powder on the production of biohydrogen from complex distillery wastewater in batch fermentation. Int. J. Hydrogen Energ. 40, 10734–10743. doi: 10.1016/j.ijhydene.2015.05.198
14. Ghiasian, M. (2019). Biophotolysis-based hydrogen production by cyanobacteria. In Prospects of Renewable Bioprocessing in Future Energy Systems (pp. 161-184). Springer, Cham.
15. Girigoswami, A., Mitra Ghosh, M., Pragya, P., Seenuvasan, R., & Girigoswami, K. (2021). Nanotechnology in Detection of Food Toxins–Focus on the Dairy Products. Biointerface. Res. Appl. Chem, 11, 14155-14172.
16. Govarthanan, M., Jeon, C. H., Jeon, Y. H., Kwon, J. H., Bae, H., and Kim, W. (2020). Non-toxic nano approach for wastewater treatment using Chlorella vulgaris exopolysaccharides immobilized in iron-magnetic nanoparticles. Int. J. Biol. Macromol. 162, 1241-1249. doi: 10.1016/j.ijbiomac.2020.06.227

17. Greenbaum, E. (1988). Energetic efficiency of hydrogen photoevolution by algal water splitting. Biophysical Journal, 54(2), 365-368.
18. Gupta, G. K., and Shukla, P. (2020). Insights into the resources generation from pulp and paper industry wastes: challenges, perspectives and innovations. Bioresour. Technol. 297:122496. doi: 10.1016/j.biortech.2019.122496
19. Hay, J. X. W., Wu, T. Y., Juan, J. C., & Md. Jahim, J. (2013). Biohydrogen production through photo fermentation or dark fermentation using waste as a substrate: overview, economics, and future prospects of hydrogen usage. Biofuels, Bioproducts and Biorefining, 7(3), 334-352.
20. Hsiao, C. L., Chang, J. J., Wu, J. H., Chin, W. C., Wen, F. S., Huang, C. C., ... & Lin, C. Y. (2009). Clostridium strain co-cultures for biohydrogen production enhancement from condensed molasses fermentation solubles. International Journal of Hydrogen Energy, 34(17), 7173-7181.
21. Kumar, G., Mathimani, T., Rene, E. R., and Pugazhendhi, A. (2019). Application of nanotechnology in dark fermentation for enhanced biohydrogen production using inorganic nanoparticles. Int. J. Hydrogen Energ. 44, 13106–13113. doi: 10.1016/j.ijhydene.2019.03.131
22. Kumar, G., Sivagurunathan, P., Kim, S. H., Bakonyi, P., & Lin, C. Y. (2015). Modeling and optimization of biohydrogen production from de-oiled Jatropha using the response surface method. Arabian Journal for Science and Engineering, 40(1), 15-22.
23. Laurinavichene, T., Tekucheva, D., Laurinavichius, K., & Tsygankov, A. (2018). Utilization of distillery wastewater for hydrogen production in one-stage and two-stage processes involving photofermentation. Enzyme and microbial technology, 110, 1-7.
24. Mahanty, S., Chatterjee, S., Ghosh, S., Tudu, P., Gaine, T., Bakshi, M., et al. (2020). Synergistic approach towards the sustainable management of heavy metals in wastewater using mycosynthesized iron oxide nanoparticles: Biofabrication, adsorptive dynamics and chemometric modeling study. J. Water Proces. Eng. 37:101426. doi: 10.1016/j.jwpe.2020.10142
25. Mishra, P., & Das, D. (2014). Biohydrogen production from Enterobacter cloacae IIT-BT 08 using distillery effluent. International Journal of Hydrogen Energy, 39(14), 7496-7507.
26. Noman, M., Shahid, M., Ahmed, T., Niazi, M. B. K., Hussain, S., Song, F., et al. (2020). Use of biogenic copper nanoparticles synthesized from a native Escherichia sp. as photocatalysts for azo dye degradation and treatment of textile effluents. Environ. Pollut. 257:113514. doi: 10.1016/j.envpol.2019.113514
27. Oh, Y. K., Raj, S. M., Jung, G. Y., & Park, S. (2013). Metabolic engineering of microorganisms for biohydrogen production. In Biohydrogen (pp. 45-65). Elsevier.

28. Pinto, F.A.L., Troshina, O., and Lindblad, P. (2002). A brief look at three decades of research on cyanobacterial hydrogen evolution. Int. J. Hydrogen Energy 27: 1209–1215.
29. Prabakar, D., Manimudi, V. T., Sampath, S., Mahapatra, D. M., Rajendran, K., & Pugazhendhi, A. (2018). Advanced biohydrogen production using pretreated industrial waste: outlook and prospects. Renewable and Sustainable Energy Reviews, 96, 306-324.
30. Ramprakash, B., & Muthukumar, K. (2014). Comparative study on the production of biohydrogen from rice mill wastewater. International Journal of Hydrogen Energy, 39(27), 14613-14621.
31. Ramprakash, B., & Muthukumar, K. (2015). Comparative study on the performance of various pretreatment and hydrolysis methods for the production of biohydrogen using Enterobacter aerogenes RM 08 from rice mill wastewater. International journal of Hydrogen energy, 40(30), 9106-9112.
32. Sivagurunathan, P., & Lin, C. Y. (2020). Biohydrogen production from beverage wastewater using selectively enriched mixed culture. Waste and Biomass Valorization, 11(3), 1049-1058.
33. Sivagurunathan, P., Sen, B., & Lin, C. Y. (2014). Batch fermentative hydrogen production by enriched mixed culture: combination strategy and their microbial composition. Journal of bioscience and bioengineering, 117(2), 222-228.
34. Vatsala, T. M., Raj, S. M., & Manimaran, A. (2008). A pilot-scale study of biohydrogen production from distillery effluent using defined bacterial co-culture. International journal of hydrogen energy, 33(20), 5404-5415.
35. Veeravalli, S. S., Shanmugam, S. R., Ray, S., Lalman, J. A., & Biswas, N. (2019). Biohydrogen production from renewable resources. In Advanced Bioprocessing for Alternative Fuels, Biobased Chemicals, and Bioproducts (pp. 289-312). Woodhead Publishing.
36. Yang, P., Zhang, R., McGarvey, J. A., & Benemann, J. R. (2007). Biohydrogen production from cheese processing wastewater by anaerobic fermentation using mixed microbial communities. International Journal of Hydrogen Energy, 32(18), 4761-4771.
37. Zhang, Z., Zhang, H., Li, Y., Lu, C., Zhu, S., He, C., ... & Zhang, Q. (2020). Investigation of the interaction between lighting and mixing applied during the photo-fermentation biohydrogen production process from agricultural waste. Bioresource Technology, 312, 122-145.