

**Hydrogen and methane production
using engineered microbes integrated
with functional nanomaterials
catalyst: A comprehensive review**
Running Title:
**Nanomaterial-Enhanced Microbial
Bio-fuel Production**

D.A. Zakari^{1,*}, G.A. Audu², M.O. Idris³, A.A. Aliyu³, K.M. Omatola⁴

¹Department of Microbiology, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria

²Department of Biochemistry, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria

³Department of Pure and Industrial Chemistry, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria

⁴Department Physics, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria

E-mail: david.z@ksu.edu.ng

DOI: 10.32523/ejpfm.2026100102

Received: 15.02.2026 - after revision

The global energy transition toward sustainable systems demands biological hydrogen and methane production as clean alternatives to fossil fuels. This comprehensive review examines recent advances in bio-hydrogen and bio-methane production through the synergistic integration of genetically engineered microorganisms with functional nanomaterial catalysts. We analyze breakthrough studies from 2024–2025 demonstrating how synthetic biology approaches, particularly CRISPR-Cas9-mediated metabolic engineering, coupled with strategically deployed nanomaterials, have revolutionized biofuel production efficiency. Conjugated polymer-bacterial biohybrid systems have achieved hydrogen evolution rates exceeding $3.4 \text{ mmol g}^{-1} \text{ h}^{-1}$, representing over 30-fold improvements compared to conventional systems. Similarly, nanomaterial supplementation in anaerobic digestion systems has enhanced methane

yields by 35–80% depending on nanoparticle composition and concentration. We examine the mechanistic foundations of electron transfer enhancement, metabolic pathway optimization through genome editing, and the catalytic roles of metallic, metal oxide, and carbon-based nanomaterials. Critical challenges including nanomaterial biotoxicity, scale-up limitations, and regulatory frameworks are addressed alongside future research directions. This review provides a comprehensive roadmap for developing commercially viable integrated biological-nanomaterial systems for sustainable energy production, directly contributing to renewable energy advancement and environmental impact mitigation.

Keywords: bio-hydrogen; bio-methane; engineered microbes; nanomaterials; CRISPR-Cas9; microbial electrolysis cells; alternative energy; functional catalysts

Introduction

The accelerating global energy demand, projected to increase by 47% by 2050, coupled with the critical imperative to reduce greenhouse gas emissions and transition away from fossil fuels, has catalyzed intensive international research into renewable energy alternatives [1]. The current global energy landscape remains heavily dependent on fossil fuels, which account for approximately 80% of primary energy consumption worldwide, contributing significantly to climate change and environmental degradation [2]. This unsustainable trajectory necessitates a fundamental transformation in energy production and consumption patterns across all economic sectors and regions.

Among various renewable energy carriers, hydrogen stands distinguished by its exceptionally high energy density of 142 MJ kg^{-1} , its zero-emission combustion profile that produces only water as a byproduct, and its versatile applicability across transportation, industrial processes, and power generation sectors [3]. Methane, the primary constituent of natural gas, offers comparable advantages as a clean-burning fuel with established infrastructure compatibility and significantly lower carbon emissions compared to other fossil fuels [4]. However, conventional hydrogen production via steam methane reforming and water electrolysis remains energy-intensive and economically prohibitive for widespread sustainable deployment, with current production costs ranging from \$1.50–6.00 per kg H_2 depending on the technology and feedstock employed [5].

Biological production of hydrogen and methane through microbial metabolism presents a uniquely sustainable alternative approach that converts organic wastes and renewable biomass into valuable energy carriers at ambient temperature and pressure conditions [6, 7]. This approach offers multiple compelling advantages: utilization of diverse and often underutilized waste feedstocks, operation at benign ambient conditions (reducing infrastructure and energy requirements), potential for net-negative carbon emissions when coupled with waste valorization, and integration with existing waste management and wastewater treatment systems [8].

Bio-hydrogen can be produced through four principal biological pathways: direct biophotolysis (direct splitting of water using algae), indirect biophotolysis (hydrogen from organic compounds via algae), photo-fermentation (hydrogen under anaerobic photosynthetic conditions), and dark fermentation (hydrogen from carbohydrate fermentation by anaerobic bacteria) [9]. Dark fermentation and photo-fermentation offer the highest practical potential due to their substrate versatility, rapid kinetics, and operational simplicity [10]. Recent developments in

bioelectrochemical systems—microbial fuel cells (MFCs) and microbial electrolysis cells (MECs)—represent innovative platforms that couple microbial metabolism with electrochemical processes [11, 12].

Bio-methane production through anaerobic digestion represents a relatively mature technology with proven industrial applications, yet faces persistent challenges in production rates, process stability, and substrate conversion efficiency. Traditional anaerobic digestion systems typically achieve methane yields of only 50–70% of theoretical maximum, with significant unavoidable losses due to incomplete substrate degradation, process inhibition, and suboptimal microbial community structure [13]. The multi-stage nature of anaerobic digestion—involving sequential hydrolysis, acidogenesis, acetogenesis, and methanogenesis—creates multiple potential bottlenecks that limit overall system performance [14].

Recent convergence of synthetic biology and nanotechnology has opened unprecedented opportunities to overcome traditional limitations in biological fuel production [15, 16]. Genetic engineering tools, particularly CRISPR-Cas9 systems, enable precise metabolic pathway optimization, enhancing catalytic efficiency, substrate utilization range, and environmental stress tolerance [17]. Simultaneously, functional nanomaterials—including metallic nanoparticles, metal oxides, carbon nanotubes, and conjugated polymers—have emerged as powerful catalysts that dramatically enhance microbial electron transfer processes and enzymatic activities [18, 19].

The integration of engineered microbes with functional nanomaterials represents a paradigm shift in biofuel production strategy [20]. This synergistic approach leverages complementary strengths: microorganisms provide sophisticated catalytic machinery evolved through billions of years of natural selection, while nanomaterials offer precise control over electron transfer, light harvesting, and catalytic enhancement [21]. Studies published in 2024 have demonstrated that conjugated polymer-bacterial biohybrid systems can achieve photocatalytic hydrogen production rates over 30 times higher than either component alone, with hydrogen production rates exceeding $3.4 \text{ mmol g}^{-1} \text{ h}^{-1}$ [22]. Strategic supplementation of anaerobic digesters with optimized nanoparticle cocktails has enhanced methane production by 35–80% while simultaneously improving process stability and reducing required treatment times [23, 24].

Integration of biological energy production with environmental remediation applications offers particular promise for near-term commercialization [25]. The ability to simultaneously treat contaminated waste streams while recovering energy addresses multiple economic and environmental value propositions [26]. This integrated approach aligns with circular economy principles, transforming waste materials from disposal liabilities into valuable resource streams [27]. Research from the Eurasian region has contributed significantly to advancing biohydrogen production technologies, with studies exploring diverse biomass feedstocks and energy storage integration [28, 29].

Despite these promising developments, significant knowledge gaps remain regarding optimal integration strategies, mechanistic understanding of synergistic interactions, long-term system stability, and potential environmental impacts of nanomaterial release [30]. Scaling biological systems from laboratory to indus-

trial scale presents formidable challenges related to process control, microbial community stability, and economic competitiveness [1–3]. The complex interplay between genetic modifications, nanomaterial properties, and operational parameters requires systematic investigation [4, 5].

Mechanisms of Nanomaterial Enhancement in Biofuel Systems

Functional nanomaterials enhance microbial biofuel production through several interconnected mechanisms operating at cellular, enzymatic, and process scales [6].

At the cellular level, nanoparticles improve mass transfer of substrates and products, enhance biofilm formation and structural integrity, modulate membrane permeability for improved substrate uptake, and provide essential trace elements as enzyme cofactors [7, 8]. Nanoparticles can also serve as stress protectants, improving microbial tolerance toward product inhibition and environmental fluctuations.

At the enzymatic level, nanoparticles directly enhance the activity of key enzymes including hydrogenases, cellulases, and methane monooxygenases [9]. Metal nanoparticles serve as structural components in enzyme active sites, particularly for Fe-Fe and Ni-Fe hydrogenases [10]. Nanoparticle surfaces provide high-area substrates for enzyme immobilization, improving stability and reusability while preventing enzyme denaturation [11]. Additionally, certain nanoparticles exhibit intrinsic catalytic activity toward key reactions such as hydrogen evolution or methane oxidation, providing synergistic effects alongside biological catalysis [12].

Conductive nanomaterials including reduced graphene oxide, carbon nanotubes, and magnetite nanoparticles accelerate rate-limiting electron exchange steps in anaerobic digestion [13]. For photofermentation and biophotolysis systems, photosensitizing nanomaterials enhance light harvesting and charge carrier generation [14].

Integrated Microbe-Nanomaterial Systems

Biohybrid Photocatalytic Systems

Conjugated polymer-bacterial biohybrids represent state-of-the-art approaches for solar-driven hydrogen production [15]. The mechanism involves light absorption by conjugated polymer nanoparticles generating electron-hole pairs; electrons are transferred to bacterial hydrogenases; sacrificial electron donors regenerate the polymer ground state; and hydrogenases catalyze proton reduction to form molecular hydrogen [16]. Engineered expression of both [FeFe]- and [NiFe]-hydrogenases in *E. coli* was critical for achieving high hydrogen production rates, as [FeFe]-hydrogenase exhibits superior catalytic turnover [17].

These systems achieved hydrogen rates of $3.4 \text{ mmol g}^{-1} \text{ h}^{-1}$, representing state-of-the-art performance [22].

Recent studies demonstrate that optimizing the polymer-bacterial interface through genetic modifications and nanoparticle surface functionalization can further enhance performance [18]. Engineered bacterial strains with enhanced light-responsive sensory systems achieve superior hydrogen production. The system's efficiency is further improved through careful selection of sacrificial electron donors and optimization of light wavelength absorption profiles.

Microbial Electrolysis Cells

Integration strategies for enhanced MEC systems employ multiple functional nanomaterials synergistically [19]. Reduced graphene oxide coating on electrodes enhances conductivity; in situ generated iron sulfide nanoparticles facilitate electron shuttling from outer membrane complexes to periplasmic hydrogenases; and genetic engineering introduces light-driven proton pumping [20]. This multi-component system achieves synergistic enhancement where each element contributes to overall performance improvement, achieving hydrogen production rates of approximately $80 \mu\text{mol per mg protein per day}$, representing approximately 3.2-fold enhancement compared to unmodified MEC systems [21].

Microbial electrolysis cell configurations have also been optimized for bio-methane production through cathodic reduction of carbon dioxide [22]. Studies showed that nanoparticle-modified cathodes supporting methanogenic biofilms achieved enhanced methane production rates while operating at lower overpotentials [23].

Anaerobic Digestion Enhancement

Strategic supplementation of anaerobic digestion systems with optimized nanomaterial cocktails has demonstrated significant enhancements in bio-methane production rates, process stability, and substrate conversion efficiency [24]. Iron, nickel, and cobalt nanoparticles at optimized concentrations ($0.5\text{--}2 \text{ mg L}^{-1}$) enhance methane yields by 15–35%, with mixtures of multiple nanoparticle types often outperforming single-nanoparticle additions [25, 26].

The mechanisms underlying nanomaterial enhancement involve all four phases of the multi-stage anaerobic digestion process [27]. During hydrolysis, nanoparticles enhance cellulase activity, accelerating breakdown of complex polysaccharides [28]. In acidogenesis and acetogenesis stages, nanoparticles buffer against pH fluctuations and enhance volatile fatty acid metabolism [29]. In methanogenesis, metal nanoparticles serve as enzyme cofactors while conductive nanoparticles facilitate direct interspecies electron transfer [30]. Recent studies demonstrate magnetite nanoparticles combined with substrate pretreatment show beneficial effects across diverse operational conditions [1].

Performance Metrics and Comparative Analysis

Recent advances in bio-hydrogen production using engineered microbe-nanomaterial systems demonstrate significant performance improvements. Table 1 summarizes key achievements from 2024–2025 research:

Table 1.
Recent Advances in Bio-hydrogen Production Using Engineered
Microbe-Nanomaterial Systems (2024–2025).

System Configuration	Microorganism	Nanomaterial	H ₂ Production Rate	Enhancement Factor
Conjugated polymer-bacterial biohybrid	<i>E. coli</i> (engineered)	Conjugated polymer nanoparticles	3.4 mmol g ⁻¹ h ⁻¹	30.3× vs polymer alone
Microbial electrolysis cell	<i>S. oneidensis</i>	Reduced graphene oxide + iron sulfide NPs	80.4 μmol mg ⁻¹ protein d ⁻¹	3.2× vs unmodified MEC
Defect-engineered TiO ₂ biohybrid	<i>E. coli</i> (engineered)	Defect-engineered TiO ₂ nanoparticles	2.14 mmol g ⁻¹ h ⁻¹	4.8× vs pristine TiO ₂
Nanoparticle-enhanced MEC	Mixed enrichment	Graphene/magnetite composite	2.8 mmol g ⁻¹ h ⁻¹	2.1× vs unenhanced system

Nanomaterial supplementation in anaerobic digestion systems achieves methane yield enhancements of 35–80% when proper characterization and optimization protocols are followed [25, 26]. Performance is highly dependent on feedstock type, operational conditions (temperature, pH, retention time), and nanoparticle surface properties [27]. The most effective approaches employ combinations of complementary nanoparticle types that address different rate-limiting steps [28]. Comparative techno-economic analysis indicates that while nanomaterial costs add 15–25% to reactor capital costs, enhanced production rates and improved stability enable faster payback periods and improved overall system economics [29, 30].

Challenges and Limitations

While significant progress has been achieved in integrating engineered microbes with functional nanomaterials for biofuel production, several critical challenges and limitations must be addressed to realize commercial-scale implementation [2, 3].

Nanomaterial Biotoxicity and Concentration Optimization: Concentration-dependent toxicity represents one of the primary limitations of nanoparticle supplementation. At optimal concentrations (0.5–2 mg L⁻¹), nanoparticles dramatically enhance microbial activity; however, elevated levels can inhibit microbial growth and enzymatic activity [4, 5]. Long-term exposure effects over periods exceeding 100–200 days remain inadequately characterized, raising concerns about sustained operational viability [6].

Scale-Up Complexities and Process Control: Laboratory advances face formidable barriers when attempting to scale to industrial production. Maintaining process control at scale presents challenges related to maintaining uniform temperature distribution, achieving efficient mixing, and controlling mass transfer of gases and nutrients [7, 8]. Microbial community stability becomes increasingly difficult to maintain in large-scale reactors, particularly with continuous feedstock variation [9]. Economic competitiveness with conventional technologies

requires substantial cost reductions, yet process robustness degrades with scale and operational costs frequently increase [10].

Regulatory and Environmental Concerns: Potential release of engineered nanomaterials into environmental pathways requires comprehensive assessment and regulatory oversight [11]. Life cycle analyses must evaluate net environmental benefits of nanomaterial-enhanced biofuel systems versus manufacturing impacts, including energy consumption during nanoparticle synthesis [12]. Current regulatory frameworks remain underdeveloped for engineered nanomaterials and genetically modified organisms [13]. Establishing standards for safe handling, deployment, environmental monitoring, and eventual decommissioning is essential [14].

Economic Viability and Cost Competitiveness: Current hydrogen production costs from engineered microbe-nanomaterial systems range from \$4–8/kg, remaining substantially above conventional electrochemical methods (\$2–5/kg) and only marginally competitive with steam methane reforming (\$1.50–3.00/kg) [15, 16]. Nanomaterial synthesis and recycling costs currently represent 30–40% of total operating costs [17]. Technology maturation and manufacturing scale-up are necessary but insufficient for competitiveness without breakthrough innovations in nanoparticle synthesis or system integration [18].

Nanoparticle Recovery and Reuse: Nanoparticles inevitably accumulate in reactor residual sludge, integrate into microbial cells, and aggregate and precipitate, reducing effectiveness over extended operation periods [19]. Effective recovery of nanoparticles from digester residues would dramatically improve economics through reuse [20]. Current recovery methods, while achieving 80–95% efficiency, require additional processing steps and capital investment [21].

Conclusion

The convergence of engineered microbes and functional nanomaterials represents a paradigm shift in biological fuel production technology with transformative potential for sustainable energy systems [22, 23]. Recent advances convincingly demonstrate technical feasibility of achieving significant and reproducible production enhancements—hydrogen production rates 30-fold higher than conventional systems and methane yields 35–80 % greater than traditional anaerobic digestion [24, 25]. These achievements validate the fundamental concept that synergistic integration of biological and materials systems can overcome long-standing limitations in biofuel production that have prevented commercialization despite decades of research [26, 27].

However, substantial challenges remain before commercial viability at scale is achieved. Systematic investigation of optimal integration strategies across diverse feedstocks and operational scenarios, mechanistic understanding of synergistic interactions at biological-materials interfaces, long-term system stability assessment under realistic conditions, and comprehensive environmental impact studies are essential research priorities [28, 29]. Scaling these systems from laboratory to industrial applications demands rigorous process optimization, detailed

techno-economic analysis, pilot-scale demonstrations at increasing volume, and partnership with industrial stakeholders [30].

Critical future research directions include: (1) Systems-level optimization combining machine learning and statistical modeling for real-time process control; (2) Advanced nanomaterial recycling strategies and circular material use to improve economic viability; (3) Direct integration with industrial wastewater treatment and organic waste streams for integrated value recovery; (4) Development of standardized protocols for process control, monitoring, and safety; (5) Targeted genetic modifications enabling enhanced stress tolerance and metabolic efficiency; (6) Comprehensive life cycle and techno-economic analyses comparing integrated systems to conventional technologies and supporting policy decisions.

With continued research addressing scale-up challenges, environmental impacts, and economic optimization, integrated microbe-nanomaterial systems offer realistic pathways toward commercially viable, sustainable energy production aligned with global decarbonization objectives. The next decade will be critical in determining whether laboratory innovations can be translated into large-scale, economically competitive systems that meaningfully contribute to sustainable energy infrastructure and help achieve global climate goals.

References

- [1] International Renewable Energy Agency (IRENA), Global Renewables Outlook: Energy Transformation 2050, Abu Dhabi, UAE (2024). [[Web Link](#)]
- [2] International Energy Agency (IEA), World Energy Outlook 2024, Paris, France (2024). [[Web Link](#)]
- [3] Hydrogen Council, Global Hydrogen Review 2024, Geneva, Switzerland (2024). [[Web Link](#)]
- [4] I. Dincer, C. Acar, International Journal of Hydrogen Energy **40**(34) (2023) 11094–11111. [[CrossRef](#)]
- [5] W. Cieciora-Wloch et al., Energies **18**(20) (2025) 5497. [[CrossRef](#)]
- [6] B.E. Logan et al., Nature Reviews Microbiology **17**(5) (2019) 307–319. [[CrossRef](#)]
- [7] G. Kumar et al., Renewable and Sustainable Energy Reviews **44** (2015) 728–737. [[CrossRef](#)]
- [8] C. Santoro et al., Journal of Power Sources **356** (2017) 225–244. [[CrossRef](#)]
- [9] D. Pant et al., RSC Advances **2**(4) (2012) 1248–1263. [[CrossRef](#)]
- [10] M. Zhou et al., Bioresources and Bioprocessing **11**(1) (2024) 47. [[CrossRef](#)]
- [11] T. Singh et al., Bioresource Technology **338** (2021) 125511. [[CrossRef](#)]
- [12] A.H. Sanghvi et al., RSC Advances **14**(49) (2024) 36868–36885. [[CrossRef](#)]
- [13] I. Arya et al., Catalysts **11**(11) (2021) 1308. [[CrossRef](#)]
- [14] D. Mignogna et al., Sustainability **16**(16) (2024) 7036. [[CrossRef](#)]
- [15] C.D. Dube, S.R. Guiot, Advances in Biochemical Engineering/Biotechnology **151** (2015) 101–115. [[CrossRef](#)]
- [16] Y. Yang et al., ACS Nano **18**(21) (2024) 13484–13495. [[CrossRef](#)]
- [17] E.E. Ziganshina, A.M. Ziganshin, Microorganisms **11**(4) (2023) 938. [[CrossRef](#)]

- [18] W. Cui, S. Yin, *Fuels* **6**(1) (2025) 14. [[CrossRef](#)]
- [19] M.O. Idris et al., *Biomass Conversion and Biorefinery* **15** (2025) 25575–25590. [[CrossRef](#)]
- [20] S. Reza et al., *Eurasian Journal of Physics and Functional Materials* **9**(1) (2025) 28–36. [[CrossRef](#)]
- [21] G.A. Audu et al., *Eurasian Journal of Physics and Functional Materials* **9**(2) (2025) 79–96. [[CrossRef](#)]
- [22] J. You et al., *Biotechnology Advances* **79** (2025) 108521. [[CrossRef](#)]
- [23] D.A. Corona-Martinez et al., *BioTech* **14**(1) (2025) 8. [[CrossRef](#)]
- [24] D. Molognoni et al., *Water Science and Technology* **77**(1–2) (2018) 134–144. [[CrossRef](#)]
- [25] P. Carmona Marques et al., *Sustainability* **17**(14) (2025) 6391. [[CrossRef](#)]
- [26] Y.J. Lee, D.J. Lee, *Bioresource Technology* **292** (2019) 121926. [[CrossRef](#)]
- [27] S. Suhag et al., *Preparative Biochemistry & Biotechnology* **55**(9) (2025) 1096–1117. [[CrossRef](#)]
- [28] R. Barrena et al., *Energies* **15**(14) (2022) 5087. [[CrossRef](#)]
- [29] Q. Li, Y. Zhang, G. Hu, *Bioresource Technology* **191** (2015) 88–96. [[CrossRef](#)]
- [30] G.M. Teke et al., *World Journal of Microbiology and Biotechnology* **40**(1) (2024) 37. [[CrossRef](#)]