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Nanoparticle-microbe interactions in biofuel fermentation: current understanding and prospective applications

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The unique properties of nanomaterials enable them to unexpectedly interact with biological systems, allowing them to play a critical role in the biodegradation of organic waste and accelerating the metabolic activities of biofuel-producing microorganisms. The present perspective highlights the studies conducted on fermentative systems for biofuel production (FSBP) promoted by the acquired catalytic activities of various nanomaterials. It discusses the recent nanoparticle-engineering-based developments, including the development of nanomaterials exhibiting biocompatibility, conductivity, a balanced zeta potential etc., which substantially determines the nanomaterial-microorganism interactions. Furthermore, it highlights functionalized nanoparticles applicable for FSBP, where a wide variety of nanomaterials, including metal oxides, composite metal oxides, carbon-based nanomaterials, etc., have been explored to improve biofuel production. The applicability of functionalized nanoparticles in the production of biohydrogen, biomethane, bioethanol, and other hydrocarbon fuels is presented. Different types of nanoparticles and their possible functional mechanisms are highlighted. Finally, future perspectives for functionalized nanoparticles in FSBP are also discussed.

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1. Introduction

Fermentative systems for biofuel production (FSBP) are globally popular ways to catalyze the transformation of biomass into chemical energy (H₂, CH₄, and ethanol); they can generate environmentally benign energy even from pollutants sourced from organic wastes and wastewaters.¹ FSBP has the potential to produce biofuels from almost any organic source, including complex polysaccharides, lignocellulosic materials, and complex proteins.² Although the performance of FSBP has been significantly improved by providing optimal physicochemical conditions and the application of genetically engineered microbial strains, the overall system productivity at industrial levels remains a major constraint.³ Integrating nanostructure-based catalytic systems with anaerobic microbiomes is a promising approach for providing sustainable bioenergy conversion from organic-rich waste biomasses.⁴ In particular, technologies harvesting stored chemical energy in the form of complex organic-rich biomass into biofuel in the form of hydrogen, methane, ethanol or other hydrocarbons are paving the way towards the potential realization of replacing the existing fossil-

based fuels, showing the recent milestone of being attempted at the industrial level.⁵ The intrinsic properties of these nanostructured metal ions and oxides with peculiar electro-chemical properties offer a promising tool to enhance the metabolic activities of a particular bacteria or bacterial consortium through electrostatic interactions between the cell surface and nanoparticles.⁶ Indeed, the size of the nanoparticles establishes whether the particles attach to the microbial cell surface or internalize into the cells, depending upon the nature of the biofuel-producing microorganism.⁷

The application of nanocatalyst technology has had a growing impact on FSBP in recent years. Indeed, the design of a highly functionalized nanocatalyst as a nanoscale particle that has a high electrical conductivity, oxidation-reduction potential and magnetic properties offers a promising new way to generate economically viable biofuels.⁸ Nanomaterials empower the FSBP by promoting the activity of the microorganisms of microbial communities, which have elite physicochemical properties.⁹ These nanomaterials promote the FSBP by intervening at different levels, including 'hydrolysis of complex matters'¹⁰ or 'stimulation of the microbial membrane transport system'¹¹ through the 'microbial nanoparticle interaction system' or directly disseminating the electron transport system¹² in microbial metabolic systems. Nanomaterials have been used either as a matrix to immobilize the functionalized biofuel-based microorganism or as an additive to the FSBP, as depicted in Fig. 1. Numerous functionalized nanocatalysts have

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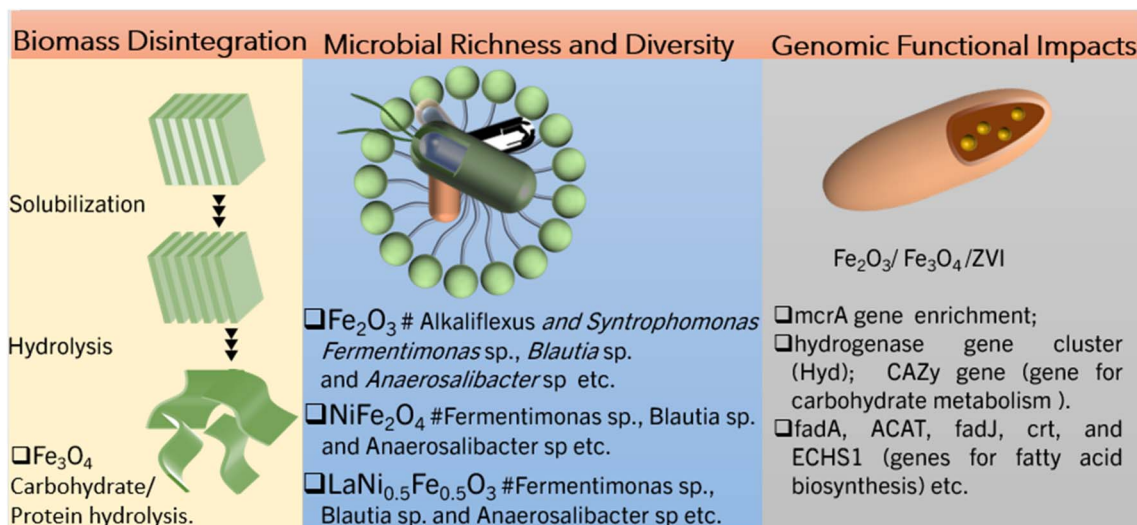


Fig. 1 Schematic representation of the mechanistic pathways for nanomaterials in enhancing fermentative biofuel production, as derived from the literature (inspired by ref. 24–26 and 27–30).

been developed to improve biofuel production in lab-scale examinations. For example, zero-valent iron and its oxides have been synthesized as catalysts to enhance microbial metabolic activities and subsequently increase hydrogen productivity using organic waste as a substrate.¹³ Indeed, catalysts based on metals, such as Ag, Au, Ti, Pd, Cu, Mn, and Mg, are known to exhibit improved bio-catalytic activities.¹⁴ Morphology and concentration influence the catalytic potential of nanocatalysts, which is further determined by the presence of active sites as well as low-power electronic fluctuations.¹⁵ Transition-metal-based nanocatalysts accompanied by metallic non-magnetic and loosely magnetic atoms have caused electronic oscillations to be reported for high-coordination statistics.¹⁶ These electronic fluctuations inside the nanoparticles are responsible for the catalytic reactions in FSBP. Therefore, the design and synthesis of these nanocatalysts are important aspects, and following the recent advances in nanotechnology research, various methods have been explored to synthesize them using a wide range of materials (refer to ref. 17). Hydrothermal processes, liquid-phase separation, and liquid-precipitation methods are the major routes to prepare catalysts for biofuel (particularly H_2) production.^{4,18–21} As the synthesis of nanocatalysts is dynamic and complex, approaches such as micro-emulsification, flame syntheses, Stober's approach, and sol-gel methods have inspired researchers to develop catalysts for biofuel-based reactions, in particular, the synthesis of biologically produced ethanol.^{22,23}

A wide range of literature reports support the supplementation of nanoparticles for improved biofuel production. Nanoparticles have a significant impact on biological metabolic processes, including biocompatibility, conductivity, zeta potential and so on.³¹ The biocompatibility of nanomaterials is an important feature that protects microbial cells and enzyme production from environmental stress during microbial growth and during the course of FSBP.³² For example, graphene oxides and other nanoparticles help anaerobes to grow owing to their

biocompatible features at a certain concentration. The interactions of nanoparticles and microbial enzymes form a structure known as a 'corona' during continuous interaction due to the absorption features of nanoparticles, and the surface properties of this complex change the formation of this structure and subsequently improve the enzyme stability (depending upon the type and concentrations of the nanoparticles applied to the system).³³ Besides, designing a nanocatalyst with high 'conductivity' (σ) for robust catalytic activity in biohydrogen production during FSBP is another aspect that needs to be considered. Supplementation of conductive nanomaterials is known to be stimulated through 'direct interspecies electron transfer system' promoters and impacts acetogenesis/methanogenesis.³⁴ As hydrogen production catalyzes the hydrogenase enzyme reduction process, nanoparticles such as Fe^{2+} and Ni^{2+} can augment the ferredoxin oxidoreductase and hydrogenase enzymes involved in FSBP, evidenced by increased hydrogen yield by a proportional increase in NAD^+/NADH .³⁵ The impacts of highly conductive magnetic nanocatalysts ($\text{Fe}^{2+/3+}$) on the metabolite flux balance of the system and the metagenomics levels during homoacetogenic activity illustrate the key role of these conductive nanocatalysts.³⁶ 'Zeta potential' (ζ -potential), a measurement of the overall surface charge of all particles, including the nanocatalyst and fermentative microorganism ($-20/-30$ mV), is also an important aspect in FSBP, as it provides an insight into the interaction of nanomaterials with microorganisms. Depending upon the specific membrane and nanomaterial structure, membrane interaction with nanoparticle matter results in blebbing, and tubule formation or the creation and enlargement of membrane defects.³⁷ In FSBP systems, a nanoparticle with a negative zeta potential tends to be electrically stabilized in the culture medium during the incubation of a microorganism. Interestingly, negatively charged nanoparticles have both high compatibility and a higher cellular uptake rate.³⁸ Therefore, nanoparticles with significant characteristics of biocompatibility, conductivity,



zeta potential, surface area, and morphological structures and others can exhibit accelerative effects on microbial metabolic pathways in FSBP systems for optimal performance. With the advancement of material design and synthesis approaches, the characteristics of nanoparticles can be tailored and can unravel the complexity associated with FSBP. The application of nanoparticles has significantly alleviated the uncertainty of low bi-fuel productivity and the overall performance of microbial cells.

2. Choosing functionalized nanomaterials for FSBP

2.1 Biohydrogen

Among many other valuable hydrocarbon biofuels, H_2 is considered as a high-calorific-value biofuel and a promising future fuel, the combustion of which is environmentally friendly as it does not result in the production of harmful gases.³⁹ Adding nanoparticles to the FSBP system is a promising approach, where these nanoparticles interact with hydrogen-producing anaerobes and enable more efficient synthesis of microbial metabolites.^{4,40} Nanomaterials are positioned to play key roles in improving complex carbohydrate metabolism mediated by glucose hydrogenase.⁴¹ Enhanced substrate degradation, increasing overall microbial activity by facilitating electron transfer and ultimately leading to increased biohydrogen yields, has been reported in a wide range of literature reports.^{42,43} The catalytic activity of nanoparticles in boosting FSBP is based on microbial metabolic activities, in particular the acceleration of *in vivo*-interspecies electron transfer processes by facilitating electron shuttling (the transfer of electrons from an organic substrate to biohydrogen-producing anaerobes).⁶ In support of this, in 2007, Zang and Shen reported pioneering work demonstrating the use of gold nanoparticles (AuNPs) to produce hydrogen through fermentation, achieving a 56% increase in hydrogen yield from wastewater compared to the control. The fermentative system was carried out by coupling the polysaccharides, bacterial culture and hydrogen production using AuNPs.⁴⁴ Hydrogenases are the key

enzymes in glucose-mediated dark fermentative hydrogen production (Fig. 2). They are mainly classified into [Ni-Fe] and [Fe-Fe] hydrogenases depending on the central active metals, with the former being more frequently found than the latter. These enzymes convert the NADH produced during the decomposition of glucose into hydrogen and NAD.⁴⁵

It has been reported that alkali-based magnetic nanosheets in FSBP under mesophilic and thermophilic conditions increase the hydrogenase activity by 10.5 and 42.8% and their hydrogen yield by 65.4 and 43.3%, respectively.⁴⁶ Nanoparticles and nanocomposite materials are less toxic to microbes than pure metal NPs. Besides, other examples have also suggested that the presence of Ni^{2+} , Fe0, and Fe^{2+} increased the hydrogen yield by 55, 37 and 15%, in contrast to the control yield.⁴⁷ Cobalt, a vital element of coenzyme B12 ($C_{72}H_{100}CoN_{18}O_{17}P$), at an adequate dosage can evidently alleviate the VFAs in FSBP, indicating that appropriate 'Co'-induced FSBP reactions favor bacterial metabolic activities.^{47,48} Furthermore, the presence of Co_3O_4 -ACNPs was reported to increase the hydrogen production rate up to 70 mL h^{-1} , when the medium is supplemented with 0.02 g L^{-1} to 0.10 g L^{-1} . The increment in H_2 production was attributed to an increased number of photocatalytically active sites. The high textural porosity and electrical conductivity of the Co_3O_4 -ACNPs increased H_2 production, but it then decreased when the dosage increased up to 0.12 g L^{-1} . This decrease in hydrogen production was attributed to an excess of species, leading to a more turbid mixing solution and the potential for light scattering within the solution.⁴⁹ Other reports from related studies have indicated an increment in hydrogen yield as well as production rate, as exemplarily highlighted by the diverse variety of nanoparticles supplemented into fermentative media shown in Table 1, signifying the important aspects of nanocatalysts for improving overall H_2 productivity.

Several modes of interactions have been proposed where the nanomaterials interact with anaerobic H_2 -producing bacteria. Direct experimental evidence from transmission electron microscopy (TEM) has validated the physical attachment of single-walled carbon nanotubes by adsorbing them at a lower ionic strength.⁶⁵ When they reach the exterior membrane of

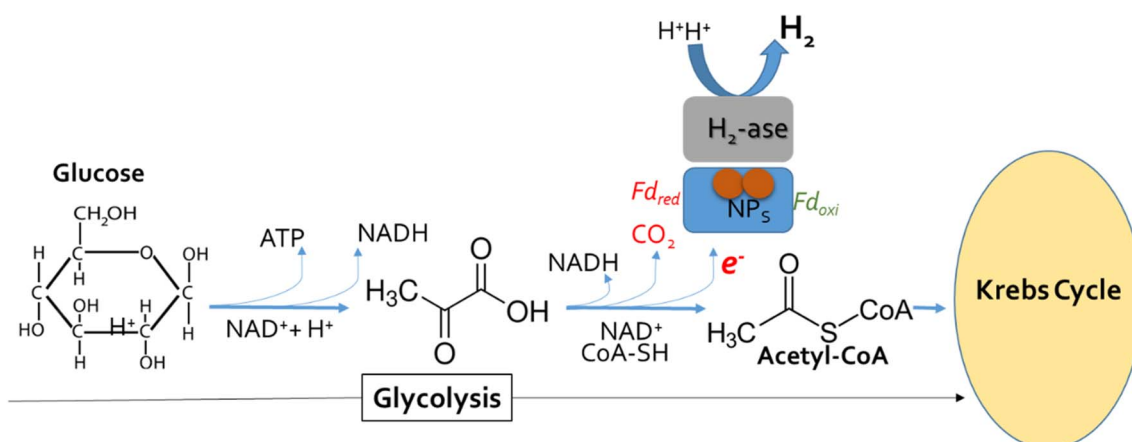


Fig. 2 Representation of microbial metabolism in glucose-mediated dark fermentative hydrogen production and the role of nanomaterials (NPs).



Table 1 A diversity of nanomaterials and application conditions, and their impact on biofuel productivity

Nanomaterial	Reaction conditions	Product type & yield	Remarks	Ref.
Biohydrogen				
α -Fe ₂ O ₃	NPs 100 mg per L per glucose/ anaerobic sludge *	2.45 mol H ₂ per mol substrate	1. Type and dose of nanomaterials significantly affect H ₂ -yield	50
α -Fe ₂ O ₃	NPs 200 mg per L per Glucose/ <i>C. species</i> *	213 ml H ₂ per g Glucose	2. Low level doses increased the overall H ₂ -yield (hormetic effects)	51
NiO ₂ /Fe ₂ O ₃	NPs 100 mg per L per glucose/ <i>Lactobacillus delbreuckii</i> *	1.94 mol H ₂ per mol per glucose	3. Composite and immobilization improve the catalytic behavior of nanoparticles	42
Sulfur-doped carbon nanoparticles	NPs 0.4 g per L per glucose-sucrose/ <i>Clostridium butyricum</i>	4354.99 ± 43.96 μmol H ₂ per g per glucose		38
Ca _{0.5} Mg _{0.5} Fe ₂ O ₄	NPs 400 mg per L per glucose/ anaerobic sludge*	171.9 ± 2.5 ml H ₂ per g per glucose		52
LaMn _{0.7} Ni _{0.3} O ₃	NPs 100 mg per L per complex organic/anaerobic sludge*	246.10 mL H ₂ per g per glucose		53
LaMn _{0.7} Fe _{0.3} O ₃	NPs 100 mg per L per complex organic/anaerobic sludge*	223.08 mL g glucose per g per glucose		53
Biomethane				
(1.5%) Fe ₃ O ₄ -granular activated carbon	Not available/glucose/anaerobic sludge**	388.1 ml CH ₄ per g per COD	1. Nanomaterials alter the functional and structural dynamics of anaerobic microbes	54
AgNPs	NPs 50 mg kg ⁻¹ paddy soils/paddy soil-cambisols/microcosm paddy soil-ultisols/microcosm**	*0.097 μg kg ⁻¹ h ⁻¹ /*0.17 μg kg ⁻¹ h ⁻¹	2. Pairing metallic oxides with other metallic oxides influences the electroactive methanogens	55
Sn-Mn-Fe	NPs 100 mg per L per animal waste/ microbial consortium**	231.2% increment CH ₄	3. Microbial-material interfaces and their dynamics are dependent on nanomaterial dose	56
AlFe ₂ O ₄	NPs 1.5 g per L per refinery wastewater/activated sludge**	135 mL CH ₄ per g per COD		57
MgFe ₂ O ₄	NPs 1.5 g per L per refinery wastewater/activated sludge**	80 mL CH ₄ per g per COD		57
Fe ₃ O ₄ @CNTs	NPs 500 mg per L per complex organic biomass/microbial consortium**	5.07% increment in CH ₄		58
Bioethanol				
CoFe ₂ O ₄ @SiO ₂ -CH ₃	Magnetic composite nanoparticles/ synthetic medium/ <i>Clostridium ljungdahlii</i> *	C ₂ H ₅ OH/213.3% increment	1. Nanomaterial augmentation has been shown to be effective in bioethanol production	59
NiO	0.05 wt%/synthetic medium/ <i>Saccharomyces cerevisiae</i> BY4743/***	1.19-fold increment in C ₂ H ₅ OH	2. Nanomaterial characteristics such as nature, concentrations, type of substrate, inoculum <i>etc.</i> are determining factors of bioethanol productivity	60
AuNPs	Enzyme-NPs composite/aquatic weeds/ <i>G. arilaitensis</i> *	50.1% increment in C ₂ H ₅ OH		61
Pt-Ru/RGO NPs	NPs 1.0 mg L ⁻¹ /algal biomass/ <i>Saccharomyces cerevisiae</i> *	1.46-fold increment in C ₂ H ₅ OH		62
g-C ₃ N ₃	NPs 150 mg L ⁻¹ /potato peel waste/ <i>Saccharomyces cerevisiae</i> *	22.61% increment in C ₂ H ₅ OH		63
Ca-Fe NPs	Immobilized magnetic nanoparticles/ wheat straw/immobilized <i>Saccharomyces cerevisiae</i> ***	36.11% increment in C ₂ H ₅ OH		64

microbial cells, they interact with the components of the plasma membrane or extracellular matrix and enter the cell, through endocytosis. The engulfment of nanoparticles occurs by membrane invagination, followed by budding and pinching off to form an endocytic vesicle, which is later sorted/trafficked to particular cellular organelles.⁶⁶ Beyond physical attachment to the microbial cell surface, several other mechanisms have been inferred from indirect evidence or proposed based on theoretical grounds. For example, *C. butyricum* is known for its ability to transfer electrons out of the cell, with the help of c-type cytochromes on the outer cell surfaces, which are later

channelized by the nanoparticles supplemented in the fermentative system.⁶⁷ Stimulations of enzymes participating in hydrogen production systems are often hypothesized with direct and indirect evidence. For instance, iron oxides prompt the electric conductivity of electron transport systems and intracellular electron transfer in mixed microbial systems, thereby improving the overall dark fermentative system (DF-system) performance.⁶⁸ The quantum size is hypothetically correlated with the rate of electron transfer between the nanomaterials and hydrogenase enzymes catalyzing the conversion of H₂ to protons and *vice versa*, either acting as electron sinks or



Table 2 Established and proposed mechanisms, and the various nanoparticle-microbe interactions

Microbes-nanomaterials	Proposed mechanism	Level of evidence	Key supporting evidence/technique	Ref.
Membrane interactions	Physical disruption of the membrane by nanoparticle adhesion	Directly observed	TEM, AFM imaging showing nanoparticle-membrane contact	65
	Induction of lipid peroxidation <i>via</i> ROS	Strangely inferred	Assay of malondialdehyde (MDA); gene expression of oxidative stress markers	70
Enzyme stimulation	Allosteric activation of extracellular enzymes	Hypothesized	Molecular docking simulations; some preliminary kinetic data	71
	Co-factor mimicry	Not cleared	Conflicting reports on whether nanoparticles can substitute for natural co-factors	72
Microbial immobilization	Aggregation of cells <i>via</i> nanoparticle bridging	Directly observed	SEM imaging, light microscopy showing large cell-nanoparticle aggregates	73
Electron transport	Acting as an electron conduit between cells and the electrode	Strongly inferred	Cyclic voltammetry, chronoamperometry showing enhanced current	74, 75
	Direct electron donation/acceptance	Hypothesized	Theoretical calculations: Require further spectroscopic validation	76

by delivering reducing power from H₂ oxidation (H₂ ↔ 2H⁺ + 2e⁻).⁶⁹ Both the direct and indirect mechanisms of nanoparticles are plausible, and direct proof for either remains elusive due to the DF-system's microbial metabolic complexity. The interaction modes based on the available literature are categorized into membrane interactions, enzyme stimulations, microbial immobilizations and electron transport. To clarify the distinction between established and proposed mechanisms, the various microbe interaction modes based on the available literature are summarized in Table 2.

2.2 Biomethane

Fig. 3 illustrates some possible mechanistic approaches by which these nanomaterials can influence the metabolic activities associated with methane (CH₄) production. The biochemical pathways to reduce CO₂ to CH₄ within the 'hydrogenotrophic-methanogenic pathway' involve several co-enzymes and metallic cofactors that serve as redox carriers in the form of co-substrates or prosthetic groups. Targeting these redox carriers (coenzyme₄₂₀, FAD, molybdopterin, iron-sulfur clusters, cobalamin and other methanogenic enzymes), nanocatalyst development could be useful to increase methane productivity.⁷⁷ The occurrence of direct interspecies electron transfer (DIET) between the microbial communities and interference caused by conductive nanoparticles have been explored in recent years in anaerobic digestion systems (AD systems) to improve methane production. Notwithstanding this fact, many studies have reported improved methane production in AD systems achieved with nanoconductive materials, such as metal oxides, carbon nanotubes, *etc.* These materials are highly stable, exhibiting large surface area, better adsorption capacity, high electric conductivity, and so on.⁷⁸ Following these options in the literature, metal oxides and zero-valence nanoparticles are the most common additives in AD systems to improve CH₄ production. In recent studies, ZnO, CuO, SiO₂, Al₂O₃, Fe₃O₄, TiO₂, and Fe⁰ have been widely applied in AD systems.⁷⁹ For

example, the metabolic activity of methanogens in AD systems can be enhanced through the addition of magnetic nanoparticles. It is evidenced that these materials act as a source of essential trace metals, thereby boosting key metabolic pathways. For instance, metallic nanomaterials containing Fe, Co, and Ni are known to improve the yield and stability of AD-system. This is demonstrated by the increased biogas production observed when AD systems are supplemented with Ni-ferrite or Ni-Co-ferrite.⁸⁰ In another study, Ni and Co NP supplements resulted in improved decomposition of total solids and subsequently improved biogas production.⁸¹ Iron-based nanoparticles are effective in regulating H₂S toxicity and facilitating the solubilization of substrates in AD systems, and hence improve biogas productivity.⁸²

Studies suggest that the inclusion of nanoparticles in AD systems promotes the synthesis of short-chain fatty acids (acetate, butyrate, formate, valeric acid, *etc.*) that are necessary during the production of biomethane.⁸⁴ Eleven different nanoparticles, namely CeO₂, CuO, Mn₂O₃, FeO, TiO₂, AgO, Al₂O₃, CuO, ZnO, Fe₂O₃, and SiO₂, were investigated for their effect on methanogenesis in an AD-system by Gonzalez-Estrella *et al.* (2013). They reported that, except for CuO and ZnO, all of the remaining metal oxides showed influencing effects on methanogenesis.⁸⁵ Notable influences of metallic nanoparticles and their derivatives on the performance of AD are summarized in Table 1. The improvement in CH₄ productivity in the AD system is ascribed to different aspects. The significant impact of metal ions, such as Fe²⁺ and Fe³⁺, on the growth of anaerobic microbial populations, as well as their role in reducing the lag phase during AD, contributes to enhanced methane productivity. Meanwhile, metal oxides at optimal concentrations, such as Cr, Cd and Zn, exert positive effects but have also been reported to interfere with biogas production, signifying the negative effects of nanoparticles on biogas production.⁸⁶ A report from Rana *et al.*, analyzed the impact of iron oxides with an emphasis on α-Fe₂O₄ on microbial growth and biogas



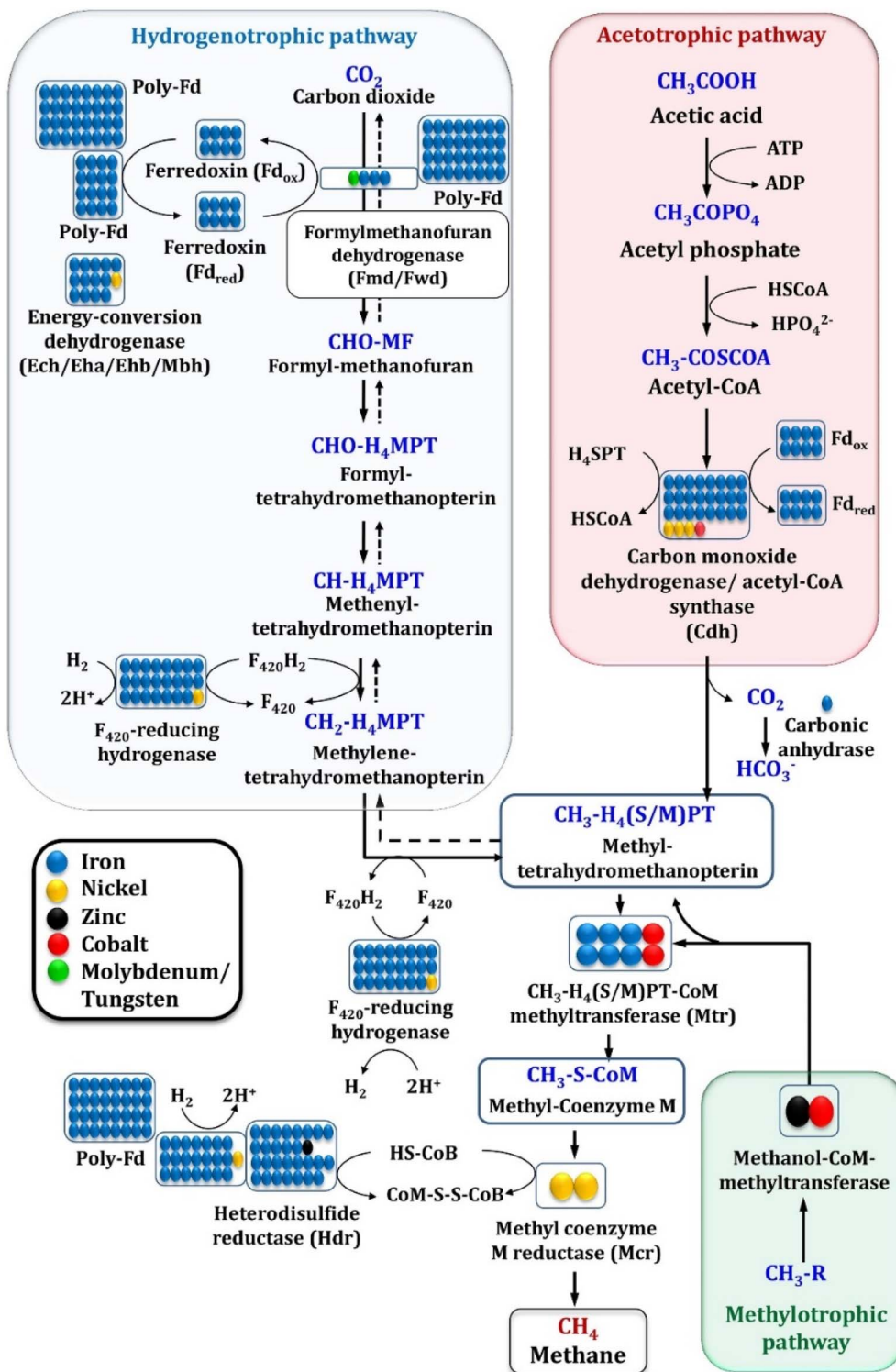


Fig. 3 Nanomaterial-mediated metalloenzymes and metabolic pathways involved in methanogenesis [This figure has been adapted from ref. 83 with the permission of Elsevier, copyright ©2022 Elsevier Ltd].

production, and elucidated that the addition of 30 mg L⁻¹ of FeNPs raises the methane yield by 70%.⁸⁷ Other studies of bio-stimulating impacts on methanogenesis have been reported for additives of aluminum ferrite (AlFe₂O₄) and magnesium ferrite (MgFe₂O₄). When AD was supplemented with AlFe₂O₄ and

MgFe₂O₄, the methane yield was observed to increase by 88% with MgFe₂O₄.⁸⁷ This implies that nanosized catalysts have a significant influence on the AD process compared to bulk-size additives, and it is reported that they can stick to/penetrate the surface of microorganisms due to their negative surface



charges, break down complex organics, increase biomass for microorganisms, and thus facilitate metabolism in different phases of AD.^{9,37} Incorporating nanomaterials into CH₄-producing AD systems appears to be essential to overcome the slow rate of microbial direct interspecies electron transfer. However, a nuanced and accurate understanding of the nanotechnological role in microbial communities often overshadows the significant research highlighting their limitations and even detrimental effects.^{88,89} The supplementation of nanomaterials into AD systems has been proposed to enhance DIET in a syntrophic microbial community, but their practical application is still complex and operational condition-dependent.⁹⁰ Studies have also reported the inhibitory effects and acknowledged their crucial limitations.⁸⁸ For instance, the antagonistic effect of zinc oxide nanoparticles (1.0 mM) on AD systems was evidenced by Zhu *et al.* (2020), where the positive zeta potential of ZnO was observed to be 18 ± 0.64 mV and it effected the stability of the nanoparticles (Zhu *et al.*, 2020), causing the release of Zn²⁺ into the aqueous phase and thereby inducing oxidative stress and antibacterial properties in the AD system.⁹¹ In another study, stronger inhibition by Ag NPs and CuO NPs (30 mg g⁻¹ TS) was reported by Abdulsada *et al.* (2025), revealing the potent effects on bacterial diversity and inhibition of dominant bacterial species during anaerobic digestion of sludge. The increase in relative abundance of the microbial community, particularly responsible for hydrolysis and acidogenesis, and the simultaneous decrease in the relative abundance of the microbial community responsible for the acetogenic process of the AD system, signified their antagonistic effects at higher concentration.⁹² The potential of NPs mediating DIET and their characteristics are important and could be based on several key factors. For instance,

(a) Nanotoxicity and cellular stress: high surface-area-to-volume ratio and quantum confinement affect the reactivity of NPs, which can be toxic to microbial communities in AD systems. nZVI/AgNPs/TiO₂ produce reactive oxygen species (ROS) in aqueous media and induce oxidative stress, ultimately leading to the inhibition of metabolic activities.⁹³⁻⁹⁵ Besides, the NPs are not perfectly stable materials and can leach metal ions into the AD system, and a sudden release of a higher concentration of these ions may lead to metal overload toxicity and disrupt enzymatic function.

(b) Aggregation and loss of functionality: the NPs applied to the AD system are theoretically electron conduits and depend on the dispersion and maximized interactions with the microbial cells/microbial community; however, the complexity of the AD system makes them prone to aggregation.⁹⁶ The aggregation reduces the bioavailability, causes physical encumbrance, and negatively influences the AD system physicochemical parameters (pH, ionic strength *etc.*), thereby inhibiting DIET rather than promoting it.^{88,97}

(c) Context dependency of NPs: the performance dependency of the AD system and NP application is not universal, and highly dependent on the delicate balance of NP characteristics (composition, size-shape, surface coating), microbial community and operational conditions.

While the positive effects of conductive nano-additives on methane production in anaerobic digestion systems are well-documented, their ideal application remains a challenge.⁹⁸ Realizing their full potential for enhancing DIET and overall system performance requires further exploration into key factors. These include optimizing material characteristics to ensure both high conductivity and biocompatibility, and defining operational conditions based on the precise interplay between the nanoparticles and the specific microbial community.

2.3 Bioethanol

Nanomaterial integration offers a promising way to overcome the challenges associated with fermentative bioethanol (C₂H₅OH) production. Nanosized catalysts, such as metals, metal oxides, nanorods, CNTs, and nanowires, are widely reported in the literature to ease and improve bioethanol production processes using a diverse range of cellulosic and lignocellulosic biomasses.^{99,100} The pretreatment and enzymatic hydrolysis of complex organic biomasses before fermentation are considered as key steps. Therefore, various approaches to accelerate these pathways and reduce toxic products (having an inhibitory effect), by applying a nanocatalyst have been successfully reported in the literature.¹⁰¹ The unique physical and chemical properties of these nanoparticles have diversified their applications in bioethanol generation and some of them are summarized in Table 1. These nanosized catalysts have ability to increase the enzymatic hydrolysis process during fermentation by improving enzyme activity or the gas fluid mass transfer rate.¹⁷ For example, Kim *et al.* reported that supplementing CoFe₂O₄@SiO₂-CH₃ and methyl-functionalized silica nanoparticles during syngas fermentation increases water mass transfer to increase bioethanol production.⁵⁹ Another study by Sanushi *et al.* reported that the supplementation of NiO nanoparticles increases bioethanol production by 1.60 fold with a simultaneous 2.10 fold decrease in acetic acid concentration.⁶⁰ Bioethanol production using microorganisms immobilized in nanoparticles is documented in the literature. Cherian *et al.* studied bioethanol production by cellulose immobilization onto MnO₂ nanoparticles. The microbial culture of *Aspergillus fumigatus* immobilized on MnO₂ provides support and increases the catalytic activity of the cellulase enzyme for the hydrolysis process.¹⁰² The use of nanoparticles for better bioethanol production in syngas fermentation using *Clostridium ljundahlit* was reported by Kim *et al.*, who examined six different types of nanoparticles, *i.e.* palladium on alumina, palladium on carbon, hydroxyl-functionalized SWCNTs, silica, iron(III) oxide, and alumina, where the presence of silica nanoparticles resulted in better mass transfer in the gas-liquid system. SiO₂ with methyl functionalization enhanced the concentrations of CO₂, CO, and H₂ by 200.2%, 272.9%, and 156.1%, respectively, and provided better activity than silica nanoparticles that were unmodified or iso-propyl-functionalized for mass transfer enhancement.¹⁰³ Beniwal *et al.* immobilized B-galactosidase in SiO₂ nanoparticles for the hydrolysis of whey and the culture of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*. This



approach resulted in a bioethanol yield of 63.9 g L^{-1} , and the nanomaterial could be reused up to 15 times without losing enzymatic catalytic activity.¹⁰⁴ Lee *et al.* demonstrated that it is possible to immobilize β -glucosidase enzymes using polymer magnetic nanofibers to improve bioethanol production using cellulosic biomass. They suggested that the attachment of β -glucosidase on these polymeric nanomaterials stabilizes the enzymatic activity and also improves its reusability.¹⁰⁵ Song *et al.* reported the co-immobilization of two cellulose enzymes, *i.e.* β -glucosidase and cello-biohydrolase, using super-magnetic nanoparticles, which exhibited improved activity of 67.1% and 41.5%, respectively, for bioethanol production.¹⁰⁶ Desai and Pawar (2020) also investigated and reported improved thermal stability and operational temperature ranges of cellulase with bacteria-originated nanomaterials bearing hematite.¹⁰⁷ In general, nanoparticles have the ability to stabilize enzymes and enhance microbial activity to improve bioethanol production. In this context, the nature of nanomaterials is an important factor that affects the hydrolysis and conversion process of complex organic matter, which subsequently improves productivity. Therefore, techniques to develop effective, economically viable nanocatalysts are important and need to be understood for bioethanol production.

2.4 Biodiesel

Biodiesel is known for its strong potential as a cleaner green fuel from energy efficacy and feasible economic perspectives compared with other bioenergy resources. The incorporation of nanoparticles due to their intrinsic properties of a larger surface-area-to-volume ratio to achieve a higher yield of biodiesel is an emerging technology, particularly in the transesterification process. Biodiesel (a methyl or ethyl ester of fatty acids), from feedstocks such as Jatropha, Pongamia, Mahua, and edible and non-edible vegetable oils, is produced *via* an esterification process, where the triglycerides react with alcohol in the presence of the desired catalyst.¹⁰⁸ These nanomaterials improve biodiesel production through various means, which include reducing the activation energy (in the case of the transesterification process) or improving the reaction kinetics (microalgal metabolism that leads to increased biomass, heterogeneous solid, and enzymatic-based reactions).¹⁰⁹ The applications of nanoparticles to grow algal cultures and improve the ultimate yield in lipid extraction for biodiesel production have been widely reported. For biodiesel production, Jeon *et al.* (2017) reported that, in a fermentative system, the use of silica oxides (SiO_2) and methyl-functionalization can increase the growth of microalgal species of *Chlorella vulgaris* for higher lipid extraction, with the value being 3 times higher than the control one.¹¹⁰ A similar approach has also been reported by Sakai *et al.* (2010) during the production of butyl-biodiesel. Here, electrospun polyacrylonitrile fibers were used to immobilize *Pseudomonas cepacian* lipase and an 80% conversion rate was observed at 2.4 mg mL^{-1} of nanocatalyst.¹¹¹ The use of functionalized Fe_3O_4 @silica core-shell nanoparticles in a one-pot microalgae conversion to biodiesel has also been explored, where dried algae, algae oil and

concentrated algae were investigated, and a 97.5% biodiesel yield was achieved in the presence of the nanocatalyst from algae oil.¹¹² $\text{Fe}_3\text{O}_4/\text{ZnMg}(\text{Al})\text{O}$ magnetic nanoparticles have been evaluated for their effect on biodiesel production and have been reported to increase the yield up to 94% production using microalgae oil.¹¹³ The usefulness of iron oxide nanoparticles lies in the immobilization of the lipase enzyme (Lipase: triacylglycerol hydrolases). Eversa Transform as a catalyst has been explored and 88.1% conversion of biodiesel was reported when oleic acid and ethanol were used as the substrate.¹¹⁴ The immobilization of the lipase enzyme to increase transesterification in biodiesel production systems has been reported in a recent study. In the study by Lish *et al.*, (2025), silica-coated magnetic nanoparticles were functionalized with epoxy and amine groups and covalently immobilized *Candida antarctica* lipase B (CALB), and 20 mg of CALB loaded on 1 g of support resulted in 92% biodiesel conversion from rapeseed oil.¹¹⁵ Nowadays, a wide range of nanocatalysts have been synthesized and investigated for biodiesel production from algal and other feedstocks. In particular, various approaches have been innovated to emphasize enzyme-immobilized nanoparticle hybrid nanoparticles, magnetic and supra-magnetic nanoparticles, carbon dots, and others to facilitate the selective and efficient conversion of biodiesel. While challenges remain for large-scale use, the effectiveness of nanoparticle-catalyzed biodiesel production has been successfully demonstrated in laboratory studies. The selectivity, versatility, and reusability of these nanocatalysts are key attributes that help overcome existing technological barriers.

3. Interactive prospects of nanomaterials in anaerobic digestion

The interaction between nanomaterials and AD systems or fermentation underpins many of the challenges and is considered not as a linear relationship in biological systems.⁵² The indispensable role of nanomaterials in AD systems and their interactive effects could be comprehended in the following aspects. (a) Fermentative microbes (acidogens and acetogens) are the biological catalysts that are responsible for the hydrolysis and biotransformation of complex organic matter, and (b) the balance of dose-dependent biocidal effects is a critical factor that governs their applicability in AD systems. Typically, Gram-positive anaerobes have been found to be more sensitive to potentially toxic nanomaterials at higher concentration, and this increased sensitivity is likely to be attributed to differences in bacterial cell membrane composition and cell wall structure.¹¹⁶ For instance, in an investigation on the toxicity of AgNPs and CuONPs on AD systems, the introduction of 30 mg g^{-1} TS concentration to the AD system resulted in a sharp inhibition of the bacterial community responsible for hydrolysis and acidogenesis.⁹² In another assessment on CeO_2 , 200 mg g^{-1} of TSS was added to the AD system, which prompted dispersibility and Ce^{4+} dissolution, and later intensified the toxicity to the microbial community, which distorted the AD process.¹¹⁷ In contrast, introducing a lower dose of nanomaterial is an



efficient way to exert a significant biological effect, with the microbial metabolic activity remaining largely unchanged.¹¹⁸ However, introducing a moderate concentration of nanomaterials to the system, or within a narrow-concentration range, boosts the metabolic activity due to slow release of essential metal ions that are co-factors for the key metabolic enzymes,¹¹⁹ thereby improving the fermentation/AD system. Exceeding the threshold concentration of nanomaterials exerts a negative effect, leading to a sharp decline in microbial activity and eventually, microbial cell death.¹²⁰ This toxicity can be exerted in different manners, *e.g.* (a) excess doses cause nanotoxicity depending on the type of nanomaterials used in the AD system, leading to the formation of reactive oxygen species like hydroxyl ions (OH⁻). (b) Physical membrane disruption caused by the sharp edges or high surface energy of the nanomaterials leads to destabilization of the microbial cell wall and membrane, and ultimately leakage of the intracellular content.¹²¹ Therefore, a careful system-specific optimization to identify the optimal range is necessary to get the benefits from precise dosing in AD systems.

Long-term exposure of mixed microbial consortia enables the entire consortium to behave more efficiently. However, in AD systems where the microbial community represents a complex collective behavior, the presence of nanomaterials triggers a chronic shift towards the adaptive and selective pressure in response to nanomaterials.^{122,123} The microbial community shifting in response to nanomaterials can be modulated in different ways. For example, (a) selective elimination: some anaerobic species are sensitive to specific metal ions and exhibit oxidative stress; for example, a decline in methanogenic bacteria in microbial communities has been reported when the AD system is supplemented with 0.46 mM or 1 mg g⁻¹ VSS of cadmium (Cd).¹²⁴ (b) Dominancy of resistant bacteria: many bacterial species possess robust antioxidant defense systems (*e.g.* a high level of catalase and superoxidase) or possess the ability to produce protective extracellular polymeric substances (EPS).^{125,126} From a nano-ecotoxicology perspective, when the nanomaterials encounter the AD system's microbial community, the surrounding matrix, such as pH,¹²⁷ ionic strength of the medium,¹²⁸ presence of sulfides¹²⁹ and organic matters,¹³⁰ significantly influence the nanoparticles' behavior. The interaction between nanomaterials in anaerobic environments (AD systems) can affect the nanomaterials' surface chemistry and physical state as well.¹³¹ For example, nanomaterials tend to remain dispersed in a liquid medium rather than agglomerating, which can affect the surface charge and dissolution, and thereby their bioavailability. Variation in the pH of the aqueous medium in the system can trigger the surface charge modification of the respective surface functional groups. *e.g.* metal oxides TiO₂, CuO and ZnO have functional groups (-OH) that possess the ability of protonation and deprotonation depending on the system pH.¹³² Microbial cell surfaces are typically negatively charged at neutral pH, and the changes in the nanomaterials (+ve charged) will mean that they are electro-statistically attracted to the microbes, increasing their interaction and potential toxicity. Besides, the ionic strength of AD systems,

which exhibits the potential of dissolved salts (such as Na²⁺, Ca²⁺, SO₄²⁻ *etc.*), can create an 'electrical double layer' and mediate a repulsive force between the particles.¹³³ This can weaken the repulsive forces between the nanomaterials and lead to rapid aggregation and sedimentation of the nanomaterials in the AD system. These aggregations can further lead to reduced bioavailability and thereby overall microbial performance in the AD system. The presence of sulfides in anaerobic environments, particularly in AD systems, is common in particular wastewater treatment plants.¹³⁴ On the integration of nanomaterials into AD systems, in the presence of sulfides, nanomaterials such as ZnO exhibit a higher affinity to sulfide ions and form a highly insoluble metal sulfide. This transformation in the nanomaterials prevents the release of the required metal ions that modulate the metabolic pathways in the AD system.

Besides, organic matter, such as humic acids and fulvic acids, can also be observed in AD systems.¹³⁵ These bulky organic molecules prevent nanomaterial aggregation and induce steric hindrance. Organic matter readily absorbs onto the surface of nanomaterials and forms biological envelopes, which further exert electrostatic repulsion and thereby prevent direct contact between the nanomaterials and the microbial cell membrane.¹³⁶ The trade-off of better microbial metabolic performance at lower doses of nanomaterial exposure and inhibition of performance at higher doses, known as the 'hormesis effect',^{137,138} is considered as a critical concept to understand the real-world impact of nanomaterial integration into fermentative/AD systems.¹³⁹ In AD systems, the microbial chain reaction is performed by a diverse range of microbial consortia and is independent of the consortium. The entire process relies on a fragile balance and efficient electron transfer between the intermediates. Therefore, exposure of nanomaterials to the AD system modulates the overall system either with 'stimulation' or 'inhibition' depending on the type of nanomaterial and concentration, and their environmental transformations.¹⁴⁰ Considering the 'stimulation' side of the trade-off, trace metal oxides are essential for enzyme growth, maturation and functionality, *e.g.* hydrogenases, co-enzymes, co-factors *etc.*, reflecting their dose-dependent stimulatory effect. For example, exposure of AD systems to iron nanomaterials (FeO/Fe₂O₃) with a mild dose increases the overall methane yield; the optimal dose of Fe₃O₄ nanomaterials enhances iron bioavailability, supporting long-term CH₄ production in the AD system. Iron-based nanocomposites enhance the system stability and reduce metal toxicity, and offer a sustainable environmental efficiency at minimal environmental impact.¹⁴¹ The inhibition side of the trade-off (high dose effect) concerns the positive effects of nanomaterials on AD systems quickly being overshadowed by overwhelming toxicity. For example, a higher dose on TiO₂ triggers oxidative stress by generating increased production of ROS, thereby inhibiting the growth of sensitive methanogens in AD systems.¹⁴² Physical disruption and adsorption due to higher nanomaterial concentration lead to adsorption onto microbial cell surfaces. This alters the membrane transport channels essential for nutrient uptake and waste excretion. It also deviates from the



syntrophic relationship within the microbial community. Although the 'hormesis' concept is essential for the practical application of nanoparticles into AD systems, the available literature remains limited, and the hormesis-oriented approach to explore nanoparticle-assisted AD remains fascinating for transformative industrial technology. The impacts of these environmental factors in response to nanomaterials are not independent because they exert their effect in a concerted manner. Exposure to nanomaterials is complex and can restructure the microbial community structure, while the critical physicochemical matrix results in changes in the nanomaterial behaviors. Therefore, understanding the transformation of nanomaterials after exposure to the AD system is absolutely essential to predict long-term fate and effects in anaerobic microbial eco-systems.

4. Bridging the gap in practical considerations in nanomaterial-assisted AD systems

The use of NPs in anaerobic digestion/fermentation has exhibited significant promise, with studies reporting an enhanced H_2/CH_4 yield, AD system and process stability, and accelerated microbial metabolism (hydrolysis, acidogenesis, acetogenesis, methanogenesis). However, a substantial research gap still exists between these promising bench-scale observations and industrial-scale implementation. Before nanoparticle-assisted AD systems can be considered practically approachable, there are some critical considerations that are related to scalability, economic feasibility, long-term stability, and environmental impact that need to be explored.

(a) Scalability and homogenous application: the scaling up of nanoparticle-assisted anaerobic digestion to the industrial level from milliliters to thousands of cubic meters presents a formidable challenge regarding the cost of synthesis *vs.* scale.¹⁴³ The synthesis of functionalized high-purity nanomaterials is complex and expensive, and economically prohibitive in comparison to conventional additives like trace metals.

(b) Techno-economic perspectives: in considering the integration and adaptation of nanotechnology to AD systems, a compelling return on investment is an important aspect. Retaining or recycling the nanomaterial for continuous redosing may reduce operational expenditure, and therefore, cost-benefit analysis could favor this integrative technology.¹⁴⁴

(c) Long-term stability: due to the continuing operations of industrial AD plants, timescales are rarely replicated in lab-scale observations/studies. Over time, the active sites of nanomaterials become fouled by biomass and extracellular polymeric substance (EPS), leading to mineral precipitation.¹⁴⁵ In addition, the highly reductive and chemically complex AD system environment can lead to an alteration in the nanoparticle characteristics. For instance, Fe_3O_4 or zerovalent ions are rapidly corroded and lose their activity during long-term exposure to the AD system.¹⁴⁶

(d) Environmental prospects and regulatory policies: the introduction of nanomaterials into anaerobic digestion systems

raises significant safety and environmental concerns. For example, if nanomaterial-augmented AD sludge (digested) is used as an agricultural fertilizer, the accumulated nanomaterials may enter the food chain, impacting soil health.¹⁴⁷ This further leads to a potential risk of soil and water contamination. Therefore, a regulatory landscape for the use of nanomaterials in AD systems and clear guidelines and potential classifications of nano-contaminated digests¹⁴⁸ as a special waste category are still required for the assessment of environmental fate and associated risk.

5. Future perspectives of functionalized nanoparticles for FSBP

Biofuels produced from waste biomass through biological metabolic systems represent a sustainable energy source as an alternative to fossil-based hydrocarbons. However, the challenge in producing them from an economic perspective still remains. The integration of emerging advanced technological systems, such as nano-sized catalysts, into biologically controlled processes has advantages, including higher production rates and yields. The fabrication of nanosized metal oxides and their derivatives has been successfully achieved *via* a variety of methods, which have shown great potential for biofuel production. These fabricated nanosized metal catalysts have exhibited a significant improvement in electron transport systems of bacterial metabolism, which subsequently improves the biofuel production in FSBP. The current research trends (Table 1) show the potential of nanosized material catalysts for improving biohydrogen yield and process efficiency. These examples identify various types of nanoparticles that have been investigated in the context of H_2 production (above section: biohydrogen), in which metal nanocatalysts, bimetallic nanocatalysts, zero-valent ions, immobilized nanomaterials with bacteria, and carbon-based nanoparticles have emerged as prominent categories. Despite the progress that has been made to accelerate the production rate and yield of biohydrogen, methane, ethanol *etc.*, there are several issues that still need to be addressed by future research. In particular, when nanosized metal oxides exhibit the ability to increase hydrogen production *via* FSBP, factors that need to be considered are the 'low-cost production and facile synthesis of nanomaterials', 'nanoparticle aggregation' and 'microbial toxicity', which hinder their world-wide application at industrial levels. As discussed in the aforementioned section, anaerobic digestion is composed of hydrolysis, acidogenesis, acetogenesis and methanogenesis, and each stage can be affected by the presence of metal NPs. Iron-based nanoparticles reduce the ORP of the AD system, increase the cell wall permeability and enhance the DIET, and subsequently stabilize acetogenesis and methanogenesis, resulting in a higher yield of CH_4 . The addition of nanoparticles has been claimed to improve biogas production in most of the studies, but apparently, the performance of these nanoparticles depends upon size, shape, quantity, toxicity, nanoparticle-bacterial interactions and other unexplored factors. The integration of nanomaterials, particularly in AD systems,



demonstrates a clear process for overall process enhancement through hormetic stimulation. However, unpredictable interactions with the bacterium or microbial community as well as system physiological conditions such as pH, ionic strength, presence of salts and sulfites and organic matter constituents, have hindered the widespread application of this technology. The bioavailability and the nature of nanomaterials once exposed to complicated AD systems should be quantified, and their downstream impact on agricultural land posing an environmental risk under oxygenated conditions should be considered. Therefore, understanding the material complexity and its transformation in the complex media of anaerobic systems remains an area requiring further exploration. Most of the abovementioned literature measures the process-level impact and respective increment in biofuel production and (H₂, CH₄, C₂H₅OH, biodiesel) yield; therefore, community-level tracking using 16 s rRNA gene sequencing could help to understand the metagenomic shift and molecular level mechanism that drives the specific biological effects. Fundamental studies on assessing the interaction between nanoparticles and microorganisms with detailed techno-economic analysis will provide further understanding of the entire AD system. Research based on integrative analysis, considering the development of specific nanoparticles modulating the performance of AD systems, strengthens the feasibility to operate at a large-scale industrial level. Exhibiting the ability to increase the fermentative microbial metabolic rate, various nanoparticles (aforementioned section: bioethanol) have been reported for successful improvement in bioethanol production. The approach of using enzymes immobilized in nanoparticles and the contribution of engineered nanoparticles favors improved catalytic activities of cellulase, lipases and other biocatalysts, and favors the reuse of the enzymes, allowing continuous use of the enzymes in waste hydrolysis and bioethanol production. Therefore, the development of economic and efficient nano-supports for biocatalyst nanoparticles is imperative for the economic feasibility of the FSBP system to produce bioethanol. Despite the advances in nanomaterials for biofuel production, there are concerns about excessive and uncontrolled use, which could adversely affect the health of our ecosystem, which can be stimulated due to the negative consequences of disposing of the used nanoparticles, due to unlimited exposure of the nanoparticles to the native environment.

6. Conclusion

The unique physicochemical properties of nanomaterials make them highly promising for biotechnological applications, particularly in fermentation technology for enhancing biofuel production. This review has documented a wide spectrum of nanomaterials with the potential to improve the yield of bi-hydrogen, biomethane, bioethanol, and related biofuels. Collectively, the evidence indicates that nanomaterials, at optimal concentrations, can effectively redirect microbial metabolism to favor biofuel synthesis. These materials influence FSBP systems through three primary mechanisms: a physicochemical mechanism, where nanomaterials interact

directly with biomolecules in biological fluids; a structural mechanism, which involves modifying cellular integrity or enhancing system interfaces; and a catalytic mechanism, leveraging active ions on nanoparticle surfaces to drive excessive metabolite production. Despite this potential, integrating nanoparticles into FSBP systems faces significant challenges, including the inefficient bioconversion of complex feedstocks into simple sugars, overall low process efficiency, and constrained metabolic activity of key microbial consortia such as hydrolytic bacteria, acidogens, acetogens (H₂ producers), and methanogens (CH₄ producers). To facilitate the broader application of nanomaterials in FSBP systems, future research should prioritize two key areas: first, more studies are needed to elucidate the fate of nanomaterials—including their migration, aggregation, dissolution, transformation, and interactions with organic matter—within complex anaerobic environments, as current understanding remains limited; second, given the potential for unexpected biological interactions, transcriptional response analyses are essential to unravel the molecular mechanisms by which nanomaterials improve organic waste biodegradability and biofuel production. Addressing these knowledge gaps will be crucial for advancing the efficient and sustainable application of nanotechnology in biofuel fermentation.

Conflicts of interest

The authors declare that they have no competing interests.

Data availability

No new data were produced in this review article.

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