Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-024-05354-2

ORIGINAL ARTICLE

Enhancing fermentation yield for biohydrogen production using eco-friendly nickel and cobalt ferrite nanoparticles

Oznur Yildirim[1] · **Bestami Ozkaya**[1]

Received: 20 November 2023 / Revised: 7 January 2024 / Accepted: 20 January 2024
© The Author(s) 2024

Abstract
In recent years, the sustainable metal oxide nanoparticle synthesis using various plant leaf extracts as reducing agents has gained significant attention in the field of renewable energy research. This study explores the biohydrogen yield enhancement potential of two types of nanoparticles derived from olive leaves. Synthesized nanoparticles are characterized by SEM, EDX, and FTIR spectrum analyses; these nanoparticles exhibit mean dimensions of 385 nm and 292 nm. Fermentation experiments were conducted using varying ratios of the two types of nanoparticles ranging from 50 to 500 mg/L to determine the impact of these nanoparticles on bio-hydrogen yield. Remarkably, with the usage of 200 mg/L of nickel ferrite nanoparticles boosted biohydrogen yield by 47%, while 200 mg/L of cobalt ferrite nanoparticles increased it by 41%. These findings highlight the potential of sustainably synthesized nanoparticles, especially those from olive leaves, as catalysts for enhancing biohydrogen production in dark fermentation processes. This research offers promising insights into eco-friendly bioenergy generation.

Keywords Nanoparticle synthesis · Bio-hydrogen · Olive leaf

1 Introduction

Due to the damage of fossil fuels to environmental ecosystems and the rapid depletion of fossil fuel resources, research in the field of sustainable and renewable energy generation technology has accelerated [1]. Biofuels, which emerged as alternative energy sources, not only reduce harmful emissions, but also provide sustainable waste management within the scope of circular bio-economy. Today, biofuels such as bioethanol, biodiesel, biogas, biomethane and biohydrogen are generated in large-scale facilities and are used in many fields. Among these, hydrogen draws attention as the most environmentally friendly fuel, thanks to its zero carbon emission [2]. It is a clean energy source that does not cause extra pollution during hydrogen production and during its use as fuel and can be produced easily. There are many different hydrogen production techniques and one of the most sustainable methods is bio-hydrogen production by biological means [3–5]. However, the efficiency of biological hydrogen production is generally lower compared to other methods [6]. Nanoparticles possess an extensive reactive surface area due to their fine particle size and exhibit altered electrical properties [7, 8]. Numerous studies on bio-energy production have explored the advantageous characteristics of metallic nanoparticles [9]. Specifically, during dark fermentation, metallic nanoparticles play a pivotal role in expediting e^- transfer by permeating cell walls of microorganisms and targeting hydrogenase enzymes [10, 11]. Acting as catalysts, metallic nanoparticles enhance enzyme activity, thereby directly augmenting hydrogen production [12]. Notably, iron oxide nanoparticles distinguish themselves among other metal nanoparticles for their ability to enhance hydrogen production by oxidizing reduced ferredoxin [13, 14]. In nanoparticle synthesis, several types of feedstock and classical methods such as sol-gel and precipitation are generally used [15]. Despite various methods for nanoparticle production, recent years have witnessed the emergence of plant-mediated sustainable nanoparticle production methods, which offer a more accessible, cost-effective, and environmentally friendly alternative compared to traditional approaches [16–18].

Green synthesis involves utilizing potentially pathogenic bacteria, fungi, various plants, and microalgae as biosynthesis agents. Microalgae, being non-pathogenic

✉ Oznur Yildirim
y.oznur2014@gmail.com; yldrmozn@yildiz.edu.tr

[1] Department of Environmental Engineering, Faculty of Civil Engineering, Yildiz Technical University, Davutpasa Campus, 34220 Istanbul, Turkey

Published online: 16 February 2024

and harnessing sunlight for energy, carbon dioxide for carbon, and ammonium salts for nitrogen, find extensive application in biotechnological processes like nanoparticle production [19]. The foremost advantage of employing plant extracts and microalgae for nanoparticle biosynthesis lies in the ready accessibility of these plant resources. Their abundant presence in nature makes this approach cost-effective and ideal for large-scale production [20]. A pivotal factor in the production of nanoparticles by green pathway is the antioxidant capacity of the chosen plant extract, which serves as a reducing agent. Some researches proved that plants with high antioxidant capacity possess strong reducing properties. Hence, the level of antioxidants directly correlates with the reducing potential [21].

In general, continuous efforts are being made in the scientific world to strengthen technological infrastructures in the processes in the field of bioenergy production, to discover new production methods or to increase the efficiency of classical bioenergy production methods. Developments in the field of nanotechnology, a wide variety of nanomaterials produced by different methods have begun to be used to contribute to developments in all areas of industry. There are many nanomaterial-based studies in the literature in the last 10 years. Studies using nanomaterials are not only in the field of increasing bioenergy production efficiency, but also include areas such as nanomaterial-based enzyme production, treatment of lignocellulosic wastes, and providing antibacterial properties to materials [22, 23]. However, there are limited studies published in the literature, especially in the field of NP production with the green synthesis method. Determining the optimum chemical and reducing agent ratio required for NP synthesis from different plant-derived materials will contribute to the use of similar materials in future studies. It is not possible to synthesize NP from every plant-based material, but as studies on this subject become widespread, the limits of the green synthesis method will be determined. Many plant extracts can be used in nanoparticle synthesis as well as olive leaves could be used in nanomaterials production systems because of having high anti-oxidant content [24–26]. In previous studies, our working group also synthesized different nanoparticles using olive leaf extract (OLE) for reduction reaction [27]. In this study, nickel ferrite and cobalt ferrite nanoparticles production by green route and their effects on dark fermentation yield was investigated. To the best of authors knowledge there is no study on the potential of nickel ferrite and cobalt ferrite nanoparticle produced from OLE with green synthesized method on bio-hydrogen fermentation in the literature. In this study, hydrogen yield increasing effect was observed in dark fermentation studies to control the functionality of the produced nanoparticles following the use of olive leaf in nanoparticle production. This study holds significant implications for advancing eco-friendly and efficient bioenergy generation methods.

2 Materials and methods

2.1 Nanoparticle synthesis

OLE was used as a reducing agent for NPs synthesis similar to our previous study [27]. Olive leaves samples underwent a cleansing process with DW and kept at 40°C for 24 h. For reducing agent preparation 10g of leaves were putted into100 ml DW for 20 minutes at 100°C. The boiled leaves solution filtered and the liquid phase was used for NPs production. For the nickel ferrite nanoparticles production Fe $(NO_3)_3{\cdot}9H_2O$ and $NiCl_2{\cdot}6H_2O$ with 2:1 (M) was dissolved in deionized water (25 mL) and mixing at 80 °C for metal solution preparation [28]. 25 ml of OLE was mixed with the prepared metal solution. Then the mixed solution autoclaved for 2 h at 200°C for the reaction. After the reaction solid phase was washed with DW and dried at 40°C for 24 h. For the cobalt ferrite nanoparticles same procedure was used except metal solution. The metal solution was prepared using $Fe(NO_3)_2{\bullet}9H_2O$ and $Co(NO_3)_2{\cdot}6H_2O$ with M ratio of 2:1 [29]. Finally, produced NPs were stored at room temperature until further usage.

2.2 Characterization of nanoparticles

The dimensions and shape of the nanoparticles were assessed through the application of scanning electron microscopy (SEM). Fourier Transform Infrared spectroscopy (FTIR), performed using equipment from Perkin Elmer Inc. in Wellesley, MA, was utilized to analyze the chemical composition of both the nanoparticles and the reducing agent. The EDX spectrum was generated through the application of energy-dispersive X-ray spectroscopy. The SEM and EDX analyses were conducted in Yildiz Technical University Central Laboratory.

2.3 Inoculum and experimental set-up

The experimental sets for fermentation were performed in serum bottles with a working volume of 50 mL. The preparation of fermentation media and *Clostridium sp.* was enriched in accordance with the methods detailed in our prior research [30]. Bottles were inoculated with 10% (v/v) inoculum. The medium was subjected to a 5-minute infusion of nitrogen gas that was free from oxygen. Nanoparticle concentrations in the range of 50 to 500 mg/L were applied for $NiFe_2O_4$ NPs containing reactors (N1, N2, N3, N4, N5, and N6) and $CoFe_2O_4$ containing reactors (C1, C2, C3, C4, C5, and C6). Reactors were stirred at 130 rpm at mesophilic conditions.

The generated biogas was measured by syringe. All experiments were performed repetitively.

2.4 Analytical procedures and kinetic analysis

During experiments the produced Volatile fatty acids (VFAs) were analyzed in Gas Chromatography (GC) (Shimadzu GC2010, Tokyo, Japan). Hydrogen generation analysis was conducted using also GC (Shimadzu GC2014, Tokyo, Japan). The detailed procedure for the analyses has been given in previous studies. [30]

The hydrogen production data was simulated by the Modified Gompertz model (Eq. (1)).

$$P(t) = P_m \times \exp\left[-\exp\left[\frac{R_m \times e}{P_m}(\lambda - t) + 1\right]\right] \quad (1)$$

where P is the cumulative bio-hydrogen production (mL), P_m is the maximum bio-hydrogen production potential (mL), R_m is the maximum HPR (mL h^{-1}), λ is the lag phase time(h), t is the incubation time (h).

3 Results and discussion

3.1 Examination of nanoparticles synthesized via green methods

The SEM technique was employed to investigate the morphology and structural characteristics of the $NiFe_2O_4$ nanoparticles that were synthesized. Figure 3a shows the formation of irregular cubic shape and presence of some large particles. The SEM results of synthesized $CoFe_2O_4$ NPs at are presented in Fig. 1b and the particles have uniform structure. Their average particle sizes were 385 and 292 nm for $NiFe_2O_4$ and $CoFe_2O_4$ nanoparticles, respectively. According to the EDX spectrum the the $NiFe_2O_4$ NPs sample includes nickel, iron and oxygen elements and iron, oxygen, and cobalt elements for $CoFe_2O_4$ nanoparticles. The EDX spectrum of samples indicating the purity of synthesized nanoparticles (Fig. 2a, b).

Bashir et al. [31] synthesized NiFe2O4 using Persa Americano seeds as a reducing agent and observed that the particle size varied in the range of 15-20 nm. Makofane et al. [32] They synthesized nickel ferrite using the extract of Monsonia burkeana plant and used the NPs they produced for bacterial degradation. The size of the NPs produced in the study ranged between 35-87 nm. Kushwaha et al. [33] produced $CoFe_2O_4$ with a particle size of 23.85 nm using hibiscus extract and examined its humudity sensing properties. In addition, there are studies that combine green synthesis methods using aloe vera with methods such as the classical sol-gel method to synthesize cobalt ferrite NPs and produce CoFe2O4 NPs with particle sizes in the range of 50-65 nm [34]. Compared to the studies here, the particle size of the NPs produced in our study is higher. While studies generally focus on NP synthesis and characterization of produced NPs, the number of studies examining their potential for use in different fields is rare.

The typical IR spectra of the $NiFe_2O_4$ and $CoFe_2O_4$ nanoparticles in the range of 400–4000 cm^{-1} were given in Figure 3. In the FTIR spectrum of the $NiFe_2O_4$ sample, a prominent absorption peak at 510 cm^{-1} is attributed to the presence of Ni-O bonds [35]. The band associated with the bending vibrations of H_2O molecules is situated at 1651 cm^{-1} on the spectrum [36]. A wide peak in the range of 3600–3000 cm^{-1} was identified as arising from the stretching vibrations of OH groups, found in both types of nanoparticles, possibly from hydroxyl or carboxyl groups. Furthermore, the presence of stretching vibrations in CH groups from either aromatic or aliphatic methyl groups was confirmed in both samples, as evidenced by the peak occurring

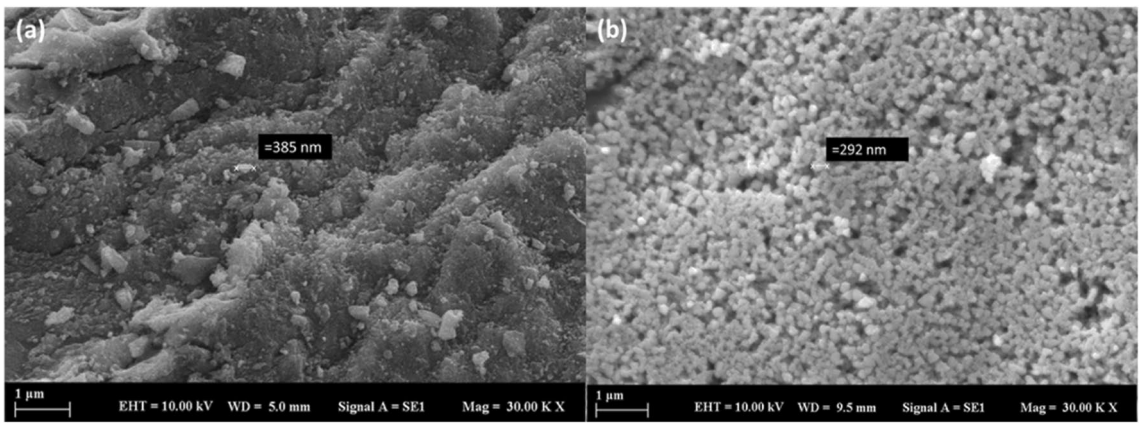


Fig. 1 SEM images of nanoparticles (**a**) $NiFe_2O_4$, (**b**) $CoFe_2O_4$

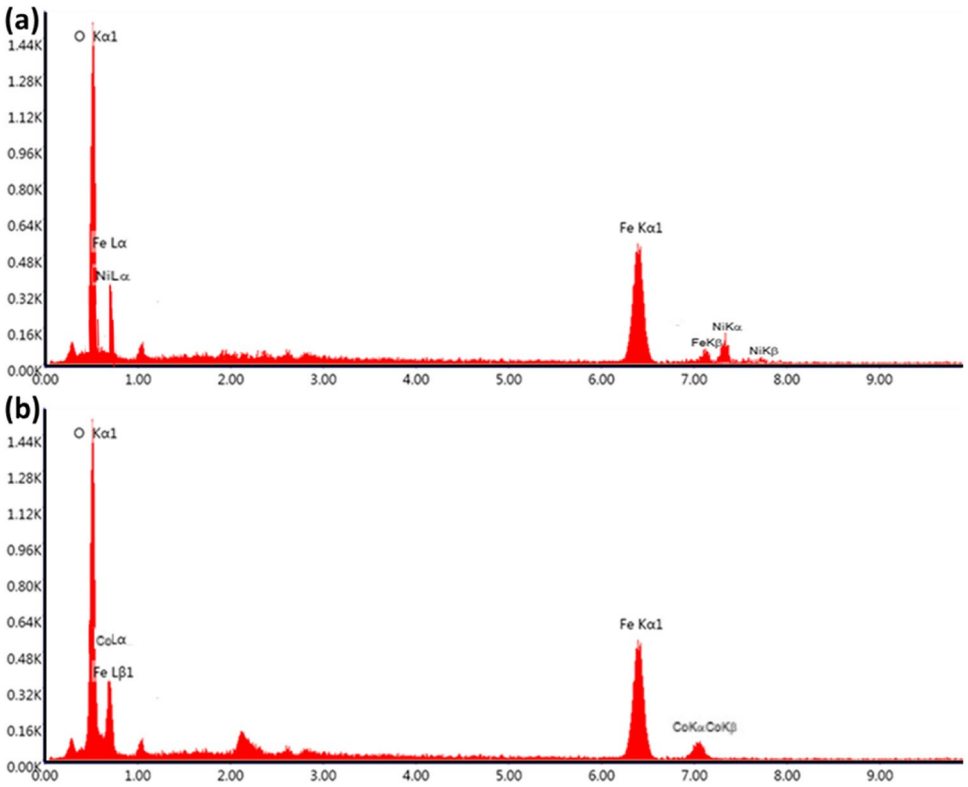


Fig. 2 EDX spectrum of green synthesized Nanoparticles (**a**) $NiFe_2O_4$, (**b**) $CoFe_2O_4$

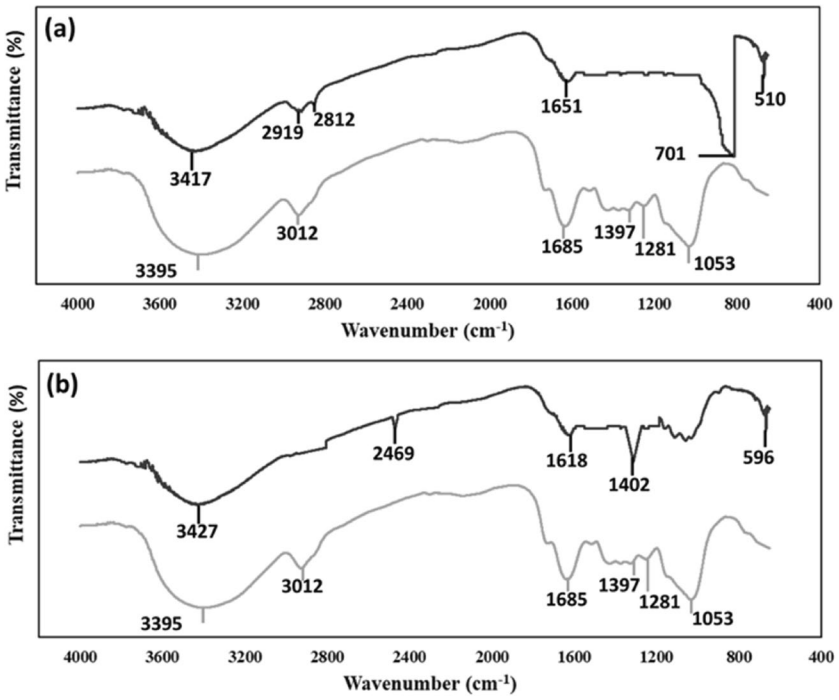


Fig. 3 FTIR images of nanoparticles (**a**) $NiFe_2O_4$, (**b**) $CoFe_2O_4$

at approximately 3000–2800 cm^{-1} [37]. The existence of aromatic compounds was indicated by the stretching vibration of the C=C group, which was observable in both samples with a peak occurring in the range of 1700–1500 cm^{-1}. Additionally, within the spectrum, two primary absorption peaks were observed between 530-580 cm^{-1}, corresponding to the stretching vibrations of metal-oxygen bonds [38]. The absorption peak observed at 510 cm^{-1} ($NiFe_2O_4$) and 596 cm^{-1} ($CoFe_2O_4$) was attributed to the inherent stretching vibrations of the metal ions [39]. We observed comparable

peaks in both the nanoparticles (NPs) and the OLE sample, suggesting that the NPs preserved certain functional groups from the OLE. These retained functional groups likely played a role in stabilizing the synthesized nanoparticles.

3.2 Cumulative bio-hydrogen production

Developments in biotechnological fields are increasing rapidly and the most popular studies in recent years are increasing the process efficiency by using nanomaterials in various bioenergy production studies [40, 41]. In this study, two types of NPs are used in fermentative bio-hydrogen generation experiments. In the studies performed, it was observed that the bio-hydrogen production potential increased with the use of both $NiFe_2O_4$ and $CoFe_2O_4$. Sets were established with 6 different NP concentrations. The agglomeration procedures frequently exert a substantial impact on the characteristics of particles [42]. Ligands and polymers can be used to prevent aggregation [43]. By employing plant extracts as reducing agents in eco-friendly synthesis techniques, a biopolymer-based coating is created around the nanoparticles (NPs) [44]. This research demonstrates that agglomeration was effectively minimized through the utilization of NPs derived from the eco-friendly synthesis process. A consistently mixed fermentation environment was established with a constant mixing speed throughout the fermentation process. Experimental data and model results are shown in Fig. 4.

The highest hydrogen yield was obtained with the generation of 292.12±1.21 mL H_2/g glucose and 265.74±1.89 mL H_2/g glucose, with the usage of 200 mg/L $NiFe_2O_4$ and $CoFe_2O_4$, respectively. The maximum hydrogen production yield was 2.02 mol H_2/mol glucose with the addition of $NiFe_2O_4$. Zhang et al. (2021b) conducted a research about the performance of $NiFe_2O_4$ NPs in the context of fermentation. Interestingly, their findings revealed that under mesophilic conditions, the optimal concentration of $NiFe_2O_4$ nanoparticles for achieving the highest hydrogen (H_2) yield was 100 mg/L, resulting in an impressive yield of 222 mL H_2/g glucose. However, when the experiment was conducted under thermophilic conditions, a different concentration, specifically 200 mg/L of $NiFe_2O_4$ nanoparticles, led to the highest H_2 yield, which amounted to 130 mL H_2/g glucose. In this study, higher hydrogen production efficiencies were obtained with $NiFe_2O_4$ NPs. This phenomenon could be caused by the NP production method and the characteristic properties of the obtained NPs. The NPs synthesized by Zhang et al. [45] had an average particle size of less than 50 nm. In this study, the average particle size was determined as 385 and 292. Higher performances are expected due to the increase in surface area at smaller nanoparticle sizes. But at the same time, the possibility of smaller nanoparticles passing into the cell and causing toxic effects increases. In this study, it was thought that achieving relatively higher bio-hydrogen production efficiencies may be due to the cells being exposed to less toxicity. Besides, NPs synthesized by green route are also known as microorganism friendly NPs which is the reason to get higher bio-hydrogen production yield [46].

It has been proven in many studies that the hydrogen production of iron-containing NPs is supported [47, 48]. Both NPs used in this study have iron content. Nanoparticles exhibit the capability to permeate the cellular membranes of fermentative bacteria, where they play a pivotal role in enhancing the rate of electron transfer within the cellular environment. This heightened electron transfer rate is integral to various cellular processes, thereby influencing the overall metabolic dynamics and performance of these

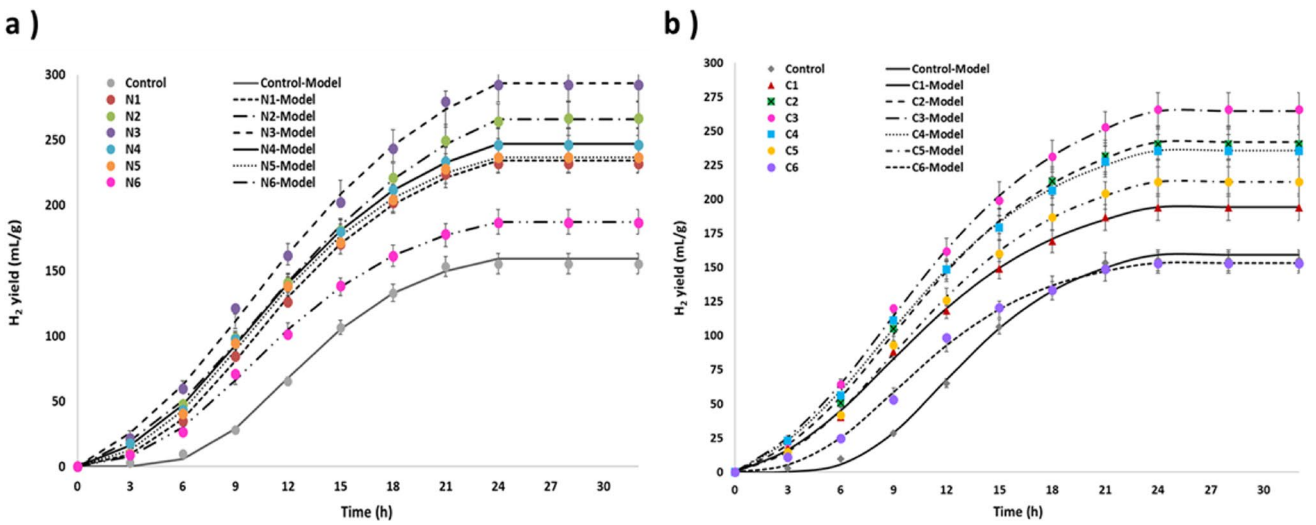


Fig. 4 Cumulative bio-hydrogen production (**a**) Nickel ferrite NPs addition (**b**) Cobalt ferrite NPs addition

microorganisms [49]. Iron addition increases the hydrogenase efficiency which indirectly increases hydrogen production. It has been observed how the NPs used in this study affect the hydrogen production mechanism as a result of the use of iron with cobalt and nickel. Obtaining higher efficiencies in nickel ferrite sets shows that nickel affects the electron transfer rate more positively than cobalt.

Cobalt (Co) assumes a pivotal role as the primary constituent of coenzyme B12 ($C_{72}H_{100}CoN_{18}O_{17}P$). Hence, judicious supplementation of cobalt significantly mitigates volatile fatty acid (VFA) accumulation during fermentation [50]. In a study by Zhang et al. [51], the utilization of nickel oxide nanoparticles (NiO NPs) derived from *Eichhornia crassipes* resulted in a notable 47.29% augmentation in dark fermentative hydrogen production rates. Conversely, [Ni–Fe] and [Fe–Fe] hydrogenase variants, distinguished by the central active metal, exhibit a higher prevalence in microbial organisms than the latter [52, 53]. The stimulatory influence of nickel on biohydrogen ($bioH_2$) production is attributed to its metal cofactor [54]. Noteworthy is the observation that nanoscale metal oxides exert diminished toxicity towards microbes compared to their pure metallic counterparts [13]. The inclusion of nanoparticles containing nickel and cobalt expedites the synthesis of hydrogenases. Nickel further contributes to the formation of [Fe-Ni] hydrogenase and acetyl-CoA synthase. Consequently, it not only facilitates the catalytic reduction of protons to molecular hydrogen (H_2) but also acts as a crucial intermediate in the generation of soluble microbial products (SMPs) by participating in acetyl CoA synthesis. Moreover, the liberation of Co and Ni ions from nanoparticles incorporating these elements optimizes bacterial community structure by supplying essential trace elements conducive to proliferation. These ions concurrently ameliorate the adverse impacts of inhibitory substances, such as humic acid.

The $NiFe_2O_4$ and $CoFe_2O_4$ boosted the hydrogen yield by 47% and 41%, respectively. For NPs concentrations higher than 200 mg/L, fermentation process was inhibited. This event was reported in another research paper [11]. This paper also reported that cell death occurs as a result of using large amounts of nanoparticles in dark fermentation. It was explained by Rittman and Herwig that the use of high amounts of iron and nickel negatively affects microbial metabolism [55]. However, some properties of metals, such as electrical conductivity, differ considerably at the nanoscale. For this reason, the effectiveness of metals in increasing the efficiency of microbial processes is frequently investigated. As in the micro dimension, NP additives above a certain concentration also created an inhibition effect at the nanoscale.

At the conclusion of the fermentation period, the control group achieved a peak hydrogen production rate, recording an impressive output of 155.28±2.71 mL of hydrogen per gram of glucose. This observation underscores the significant hydrogen-producing potential exhibited by the control conditions in our study. For the $NiFe_2O_4$ sets 231.74±1.41, 264.19±3.53, 292.12±1.21, 246.41±0.87, 236.5±1.34 and 186.89±3.76 mL H_2/g glucose achieved for N1, N2, N3, N4, N5 and N6 reactors, respectively. For the $CoFe_2O_4$ sets, bio-hydrogen production yields were 194.17±1.43, 240.74±2.32, 265.74±1.89, 235.55±1.13, 212.69±1.67 and 153.08±1.21 mL/g glucose for the C1, C2, C3, C4, C5 and C6 reactors, respectively. Higher hydrogen yields were achieved with $NiFe_2O_4$ NPs containing reactors. Zhang et al. [56], using cobalt ferrate in their study and maximum hydrogen yield of 205.24 mL/g glucose was achieved with the 400 mg/L NPs addition. In another study conducted by Zhang et al. [45], the highest hydrogen production of 222 mL/g glucose was obtained with the 100 mg/L $NiFe_2O_4$ NPs. Compared to similar studies, higher hydrogen yields were achieved in this study. Taherdanak et al. [57] has also proven that higher hydrogen production (149.8 mL/g VS) potentials can be achieved using nickel NPs. In addition, dark fermentation was done with iron NPs in the same study. The use of both NPs has increased hydrogen production. It has been proven that higher hydrogen yields can be achieved by using NPs formed by the combination of nickel ferrite NP production and metals contributing to hydrogen production with this research.

Kinetic variables for fermentation sets were calculated with the Modified Gompertz Equation (Table 1). R^2 values were calculated to validate the model data. According to kinetic variables and hydrogen yields, R^2 values were ≥ 0.95. Additionally, the p-values for H_2 and SMPs concentration calculated from the one-way ANOVA were not greater than 0.05 (p-H_2: 0.042 and p-SMPs: 0.048), which indicated that the dosing of NPs at different levels had an obvious influence on the concentrations of hydrogen and metabolites. This showed that the experimental results could be modeled accurately with the Modified Gompertz Equation. The model data and the experimental results fit well. The usage of 200 mg/L of $NiFe_2O_4$ and $CoFe_2O_4$ resulted in peak hydrogen production rates of 17.17 and 16.18 mL/g/d, respectively. According to the results of the kinetic study, while the lag phase was 7.48 h in the control set, the lag phase was considerably shortened in the reactors containing NPs. Gadhe et al. [58] reported that in addition to the increase in hydrogen yield, the lag phase of fermentation shortened as a result of his work using nickel NPs. He stated that the reason for this development is that depending on the wide surface area of NPs, they improve the certain enzymes activities such as ferredoxin, hydrogenase and ferredoxin oxidoreductase proteins. In addition, the quantum size properties of NPs also provide some additional benefits.

Both nanoparticles achieved maximum biohydrogen production efficiency when applied at a concentration of

Table 1 Modelling results for bio-hydrogen production sets

Set	NP	NPs Concentration (mg/L)	Bio-hydrogen yields (mL H_2/g)	Model parameters			
				P (mL/g VS)	R_m (mL/g/d)	λ(h)	R^2
Control	-	0	155.28±2.71	170.25	13.67	7.05	0.9529
N1	$NiFe_2O_4$	50	231.74±1.41	253.96	16.45	4.08	0.9768
N2		100	264.19±3.53	306.78	15.89	3.09	0.9889
N3		200	292.12±1.21	335.76	17.35	2.58	0.9886
N4		300	246.41±0.87	271.59	16.15	3.26	0.9815
N5		400	236.5±1.34	255.62	16.46	3.59	0.9765
N6		500	186.89±3.76	202.36	16.72	4.02	0.9761
C1	$CoFe_2O_4$	50	194.17±1.43	208.83	13.03	2.62	0.9746
C2		100	240.74±2.32	261.81	15.95	2.65	0.9771
C3		200	265.74±1.89	287.33	17.07	2.28	0.9771
C4		300	235.55±1.13	253.28	15.49	2.20	0.9736
C5		400	212.69±1.67	229.22	14.52	3.01	0.9755
C6		500	153.08±1.21	161.01	12.01	4.11	0.9649

200 mg/L. Considering this positive effect of nanoparticles, their combined effect is also a matter of curiosity. However, it is clearly seen in Table 1 that a decrease in process efficiency begins at concentrations above 200 mg/L. If both NPs are used together, adverse effects will probably occur even if lower concentrations are applied. It was reported by Zhang et al. [56] that the release of trace metals such as Co, Ni and Fe in NPs into the fermentation liquid may create inhibitory effects. However, in lower concentration applications, significant increases in biohydrogen yield can be observed due to the combined effect. The present investigation determined that the cobalt and nickel nanoparticles (NPs) employed in this study exhibited inhibitory effects beyond a concentration threshold of 200 mg/L. In a parallel investigation [59] utilizing NiCo2O4 NPs, it was reported that an inhibitory effect manifested, impeding microbial activity at concentrations exceeding 400 mg/L, ultimately resulting in microbial mortality.

3.3 Effects of nickel ferrite and cobalt ferrite NPs on fermentation metabolism

During the experiments, the produced soluble microbial products are shown in Fig. 5. For control, the content of 1.10 g/L ethanol, 1.49 g/L acetic acid, 0.53 g/L propionic acid and 1.84 g/L butyric acid was determined. It was determined that the soluble metabolic products (SMPs) content of the N3 reactor, where the maximum hydrogen yield was obtained, was higher than the other reactors. Higher production of soluble metabolic products also results in higher hydrogen production. Total SMPS content was 4.9 g/L for control set. While the total SMPs amount was higher than the control for all reactors. NPs generally increase the

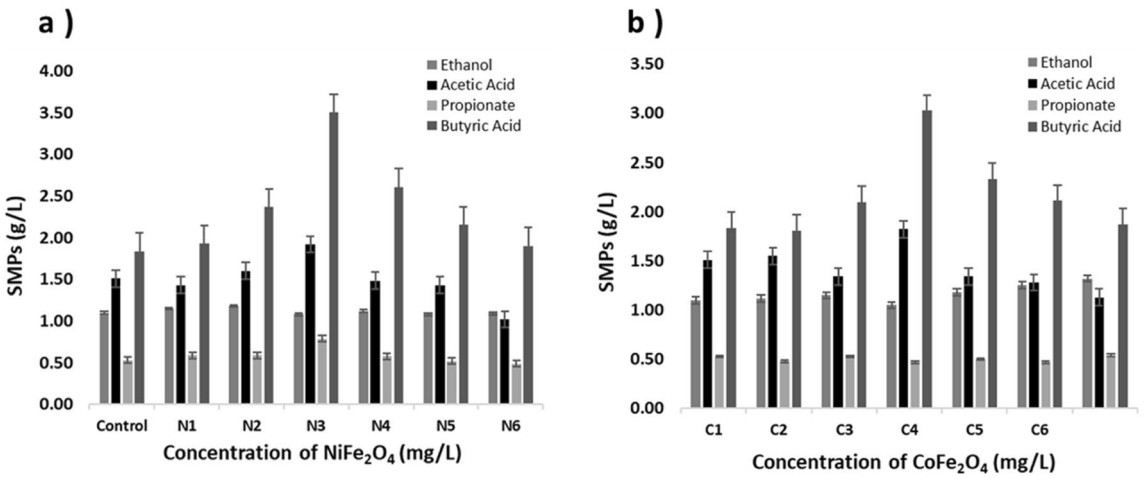


Fig. 5 The concentration of SMPs (**a**) $NiFe_2O_4$ containing reactors (**b**) $CoFe_2O_4$ containing reactors

substrate conversion rate according to results. Similar results were reported by researchers [45]. Within this context, it's important to note that glucose can undergo divergent metabolic pathways leading to distinct end products. Specifically, glucose can be enzymatically transformed into acetic acid, as described by Equation (2), or alternatively, it can follow a separate enzymatic route resulting in the production of butyric acid, as outlined in Equation (3). This dual metabolic capability highlights the versatility of glucose utilization within the biochemical processes under consideration. When the appropriate amount of NPs is used, the formation of SMPs increases in the study. Simultaneously, the notable improvement in hydrogen production efficiencies indicates a substantial connection between the generation of SMPs and the production of hydrogen. This suggests a meaningful correlation between SMPs production and the enhanced hydrogen production observed.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2)$$

$$C_6H_{12}O_6 \rightarrow C_3H_7COOH + 2CO_2 + 2H_2 \quad (3)$$

The use of $NiFe_2O_4$ NPs increased the production of SMPs more than $CoFe_2O_4$ NPs. SMPs results also reached its maximum in reactors where the highest hydrogen yield was obtained for both NPs. The maximum SMPs concentration for $CoFe_2O_4$ NP was 6.36 g/L (1.05 g/L ethanol, 1.82 g/L acetic acid, 0.47 g/L propionic acid and 3.03 g/L butyric acid) for the C3 reactor. In the N3 reactor, the SMP concentration was found to be 7.29 g/L (1.08 g/L ethanol, 1.92 g/L acetic acid, 0.79 g/L propionic acid and 3.50 g/L butyric acid). The reason why nanoparticles containing cobalt and ferrite show similar patterns is that both ions act to increase hydrogenase activity. Mishra et al. [60] reported that fermentative bacteria depend on nickel and cobalt ions for enzyme synthesis. Since both ions are necessary for enzyme synthesis and also play an active role in hydrogenase activity, adding NPs containing sufficient amounts of Ni and Co to the medium during fermentation will be beneficial in increasing fermentative hydrogen production efficiency. The superior performance of nickel ferrite compared to cobalt can be attributed to the direct integration of nickel ions within the hydrogenase structure. The inclusion of nickel in nickel ferrite enhances hydrogenase activity more significantly, leading to a more pronounced acceleration in catalytic performance when compared to cobalt-based counterparts.

Elevating the activity of hydrogenases directly contributes to the enhancement of hydrogen production [61, 62]. For this reason, the use of metallic substances containing Ni and Fe in the structure of hydrogenases in fermentation increases the hydrogenase efficiency. Metallic nanoparticles containing nickel and iron have been frequently used in studies in the literature [63–65]. According to SMP values, the use of metallic nanoparticles increases the ratio of butyric acid to acetic acid, allowing higher hydrogen production to be obtained. Similarly, an increase in the amount of butyric acid was observed in the study.

4 Conclusion

This study showed that olive leaf could be successfully used for nickel ferrite and cobalt ferrite nanoparticles production. Synthesized metalic nanoparticles by green route can increase the bio-hydrogen yield. It has been observed that the produced nanoparticles have an average size in the range of 200–400 nm. Both nanoparticles enhance the dark fermentation yield. Biohydrogen yield boosted 47% and 41% by nickel ferrite and cobalt ferrite nanoparticles, respectively. In prior researches, a variety of foliage has commonly been employed in the green synthesis approach. This research makes a valuable contribution to the existing body of literature by showcasing the potential for generating diverse nanoparticle types through the utilization of olive leaves, which could serve as a foundation for future investigations in this field.

Acknowledgements The authors express their gratitude to the Yildiz Technical University Central Laboratory for their assistance in conducting SEM and EDX analyses.

Data availability The authors confirm that the data supporting the findings of this study are available within the article.

Code availability All data generated or analysed during this study are included in this published article.

Author contributions Conceptualization, Oznur Yildirim and Bestami Ozkaya; methodology, Oznur validation, Oznur Yildirim; formal analysis, Oznur Yildirim; investigation, Oznur Yildirim; writing—original draft preparation, Oznur Yildirim; writing—review and editing, Bestami Ozkaya; supervision, Bestami Ozkaya. All authors have read and agreed to the published version of the manuscript.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK).

Declarations

Conflict of interest The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will

need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Arpia AA, Chen W-H, Lam SS et al (2021) Sustainable biofuel and bioenergy production from biomass waste residues using microwave-assisted heating: a comprehensive review. Chem Eng J 403:126233
2. Dincer I (2020) Covid-19 coronavirus : closing carbon age , but opening hydrogen age. Int J Energy Res 48(8):6093–6097. https://doi.org/10.1002/er.5569
3. Nikolaidis P, Poullikkas A (2017) A comparative overview of hydrogen production processes. Renew Sustain Energy Rev 67:597–611. https://doi.org/10.1016/j.rser.2016.09.044
4. Ishaq H, Dincer I (2021) Comparative assessment of renewable energy-based hydrogen production methods. Renew Sustain Energy Rev 135:110192
5. Martino M, Ruocco C, Meloni E et al (2021) Main hydrogen production processes: an overview. Catalysts 11:547
6. Tapia-Venegas E, Ramirez-Morales JE, Silva-Illanes F et al (2015) Biohydrogen production by dark fermentation: scaling-up and technologies integration for a sustainable system. Rev Environ Sci Bio/Technology 14:761–785
7. Sellers K, Mackay C, Bergeson LL et al (2008) Nanotechnology and the environment. CRC press
8. Fubini B, Ghiazza M, Fenoglio I (2010) Physico-chemical features of engineered nanoparticles relevant to their toxicity. Nanotoxicology 4:347–363
9. Singh YD, Panda MK, Sarma MK et al (2022) Nanotechnology for sustainable bioenergy production. Bio-Nano Interface. Springer, In, pp 339–355
10. Mohanraj S, Kodhaiyolii S (2014) ScienceDirect phytosynthesized iron oxide nanoparticles and ferrous iron on fermentative hydrogen production using Enterobacter cloacae : evaluation and comparison of the effects. Int J Hydrogen Energy 39:11920–11929. https://doi.org/10.1016/j.ijhydene.2014.06.027
11. Kumar G, Mathimani T, Rene ER, Pugazhendhi A (2019) Application of nanotechnology in dark fermentation for enhanced biohydrogen production using inorganic nanoparticles. Int J Hydrogen Energy 44:13106–13113
12. Xu S, Liu H, Fan Y et al (2012) Enhanced performance and mechanism study of microbial electrolysis cells using Fe nanoparticle-decorated anodes. Appl Microbiol Biotechnol 93:871–880
13. Beckers L, Hiligsmann S, Lambert SD et al (2013) Improving effect of metal and oxide nanoparticles encapsulated in porous silica on fermentative biohydrogen production by Clostridium butyricum. Bioresour Technol 133:109–117
14. Baeyens J, Zhang H, Nie J et al (2020) Reviewing the potential of bio-hydrogen production by fermentation. Renew Sustain Energy Rev 131:110023
15. Singh N, Dhanya BS, Verma ML (2020) Nano-immobilized biocatalysts and their potential biotechnological applications in bioenergy production. Mater Sci Energy Technol 3:808–824
16. Behzad F, Naghib SM, Tabatabaei SN et al (2021) An overview of the plant-mediated green synthesis of noble metal nanoparticles for antibacterial applications. J Ind Eng Chem 94:92–104
17. Paiva-Santos AC, Herdade AM, Guerra C et al (2021) Plant-mediated green synthesis of metal-based nanoparticles for dermopharmaceutical and cosmetic applications. Int J Pharm 597:120311
18. Lahiri D, Nag M, Ghosh S, Ray RR (2021) Green synthesis of nanoparticles and their applications in the area of bioenergy and biofuel production. In: Nanomaterials. Elsevier, pp 195–219
19. Ebrahiminezhad A, Bagheri M, Taghizadeh S-M et al (2016) Biomimetic synthesis of silver nanoparticles using microalgal secretory carbohydrates as a novel anticancer and antimicrobial. Adv Nat Sci Nanosci Nanotechnol 7:15018
20. Lee H-J, Lee G, Jang NR et al (2011) Biological synthesis of copper nanoparticles using plant extract. Nanotechnology 1:371–374
21. Kamath V, Chandra P, Jeppu GP (2020) Comparative study of using five different leaf extracts in the green synthesis of iron oxide nanoparticles for removal of arsenic from water. Int J Phytoremediation 22:1278–1294. https://doi.org/10.1080/15226514.2020.1765139
22. Verma ML, Puri M, Barrow CJ (2016) Recent trends in nanomaterials immobilised enzymes for biofuel production. Crit Rev Biotechnol 36:108–119
23. Arora A, Nandal P, Singh J, Verma ML (2020) Nanobiotechnological advancements in lignocellulosic biomass pretreatment. Mater Sci Energy Technol 3:308–318
24. Žuntar I, Putnik P, Bursać Kovačević D et al (2019) Phenolic and antioxidant analysis of olive leaves extracts (Olea europaea L.) obtained by high voltage electrical discharges (HVED). Foods 8:248
25. Moudache M, Colon M et al (2016) Phenolic content and antioxidant activity of olive by-products and antioxidant film containing olive leaf extract. Food Chem 212:521–527
26. Goldschmidt Lins P, Marina Piccoli Pugine S, Márcio Scatolini A, de Melo M (2020) Antioxidant actions of olive leaf extract (Olea europaea L.) on reactive species scavengers. J Anal Pharm Res 9:68–71
27. Yildirim O, Ozkaya B (2023) Effect of nanoparticles synthesized from green extracts on dark fermentative biohydrogen production. Biomass Bioenergy 170:106707
28. Amiri M, Pardakhti A, Ahmadi-Zeidabadi M et al (2018) Magnetic nickel ferrite nanoparticles: green synthesis by Urtica and therapeutic effect of frequency magnetic field on creating cytotoxic response in neural cell lines. Colloids Surf B Biointerfaces 172:244–253
29. Miri A, Sarani M, Najafidoust A et al (2022) Photocatalytic performance and cytotoxic activity of green-synthesized cobalt ferrite nanoparticles. Mater Res Bull 149:111706
30. Yildirim O, Tunay D, Ozkaya B, Demir A (2021) Effect of green synthesized silver oxide nanoparticle on biological hydrogen production. Int J Hydrogen Energy
31. Bashir AKH, Matinise N, Sackey J et al (2020) Investigation of electrochemical performance, optical and magnetic properties of NiFe2O4 nanoparticles prepared by a green chemistry method. Phys E Low-Dimension Syst Nanostruct 119:114002
32. Makofane A, Maake PJ, Mathipa MM et al (2022) Green synthesis of NiFe2O4 nanoparticles for the degradation of Methylene Blue, sulfisoxazole and bacterial strains. Inorg Chem Commun 139:109348
33. Kushwaha P, Chauhan P (2021) Facile green synthesis of CoFe2O4 nanoparticles using hibiscus extract and their application in humidity sensing properties. Inorg Nano-Metal Chem:1–8
34. Routray KL, Saha S, Behera D (2019) Green synthesis approach for nano sized CoFe2O4 through aloe vera mediated sol-gel auto combustion method for high frequency devices. Mater Chem Phys 224:29–35
35. Naseri MG, Saion EB, Ahangar HA, Shaari AH (2013) Fabrication, characterization, and magnetic properties of copper ferrite nanoparticles prepared by a simple, thermal-treatment method. Mater Res Bull 48:1439–1446
36. Wu Z, Duangmanee P, Zhao P, Ma C (2016) The effects of light, temperature, and nutrition on growth and pigment accumulation of three dunaliella salina strains isolated from saline soil. Jundishapur J Microbiol 9:1–9. https://doi.org/10.5812/jjm.26732

37. Punia P, Aggarwal RK, Kumar R et al (2022) Adsorption of Cd and Cr ions from industrial wastewater using Ca doped Ni--Zn nanoferrites: synthesis, characterization and isotherm analysis. Ceram Int 48:18048–18056
38. Dhanda N, Thakur P, Thakur A (2023) Green synthesis of cobalt ferrite: a study of structural and optical properties. Mater Today Proc 73:237–240
39. Mohammed FA, Khalaf MM, Mohamed IMA et al (2020) Synthesis of mesoporous nickel ferrite nanoparticles by use of citrate framework methodology and application for electrooxidation of glucose in alkaline media. Microchem J 153:104507
40. Khoo KS, Chia WY, Tang DYY et al (2020) Nanomaterials utilization in biomass for biofuel and bioenergy production. Energies 13:1–19. https://doi.org/10.3390/en13040892
41. Pandey MD (2022) Perspective of nanomaterials for sustainable biofuel and bioenergy production. Mater Lett 313:131686. https://doi.org/10.1016/j.matlet.2022.131686
42. Fraga Garcia P, von Roman M, Reinlein S et al (2014) Impact of nanoparticle aggregation on protein recovery through a pentadentate chelate ligand on magnetic carriers. ACS Appl Mater Interfaces 6:13607–13616
43. Gil-Sánchez I, Monge M, Miralles B et al (2019) Some new findings on the potential use of biocompatible silver nanoparticles in winemaking. Innov Food Sci Emerg Technol 51:64–72
44. Samuel MS, Ravikumar M, John JA et al (2022) A review on green synthesis of nanoparticles and their diverse biomedical and environmental applications. Catalysts 12:459
45. Zhang J, Zhao W, Yang J et al (2021) Comparison of mesophilic and thermophilic dark fermentation with nickel ferrite nanoparticles supplementation for biohydrogen production. Bioresour Technol 329:124853. https://doi.org/10.1016/j.biortech.2021.124853
46. Priya N, Kaur K, Sidhu AK (2021) Green synthesis: an eco-friendly route for the synthesis of iron oxide nanoparticles. Front Nanotechnol 3:655062
47. Yang G, Wang J (2018) Improving mechanisms of biohydrogen production from grass using zero-valent iron nanoparticles. Bioresour Technol 266:413–420. https://doi.org/10.1016/j.biortech.2018.07.004
48. Zhang L, Zhang L, Li D (2015) Enhanced dark fermentative hydrogen production by zero-valent iron activated carbon microelectrolysis. Int J Hydrogen Energy 40:12201–12208. https://doi.org/10.1016/j.ijhydene.2015.07.106
49. Harris D, McNicoll L, Epstein-Lubow G, Thomas KS, Lakhani CM, Tierney BT, Manrai AK, Yang J, Visscher PM, Chirag J, Patel Department (2017) Cellular uptake of nanoparticles: journey inside the cell. Physiol Behav 176:139–148. https://doi.org/10.1039/c6cs00636a.Cellular
50. Pobeheim H, Munk B, Lindorfer H, Guebitz GM (2011) Impact of nickel and cobalt on biogas production and process stability during semi-continuous anaerobic fermentation of a model substrate for maize silage. Water Res 45:781–787
51. Zhang Q, Xu S, Li Y et al (2021) Green-synthesized nickel oxide nanoparticles enhances biohydrogen production of Klebsiella sp. WL1316 using lignocellulosic hydrolysate and its regulatory mechanism. Fuel 305:121585
52. Mullai P, Yogeswari MK, Sridevi K (2013) Optimisation and enhancement of biohydrogen production using nickel nanoparticles--a novel approach. Bioresour Technol 141:212–219
53. Taherdanak M, Zilouei H, Karimi K (2016) The effects of Fe0 and Ni0 nanoparticles versus Fe2+ and Ni2+ ions on dark hydrogen fermentation. Int J Hydrogen Energy 41:167–173
54. Elreedy A, Ibrahim E, Hassan N et al (2017) Nickel-graphene nanocomposite as a novel supplement for enhancement of biohydrogen production from industrial wastewater containing monoethylene glycol. Energy Convers Manag 140:133–144
55. Rittmann S, Herwig C (2012) A comprehensive and quantitative review of dark fermentative biohydrogen production. Microb Cell Fact 11:20–25. https://doi.org/10.1186/1475-2859-11-115
56. Zhang J, Li W, Yang J et al (2021) Cobalt ferrate nanoparticles improved dark fermentation for hydrogen evolution. J Clean Prod 316:128275. https://doi.org/10.1016/j.jclepro.2021.128275
57. Taherdanak M, Zilouei H, Karimi K (2015) Investigating the effects of iron and nickel nanoparticles on dark hydrogen fermentation from starch using central composite design. Int J Hydrogen Energy 40:12956–12963. https://doi.org/10.1016/j.ijhydene.2015.08.004
58. Gadhe A, Sonawane SS, Varma MN (2015) Enhancement effect of hematite and nickel nanoparticles on biohydrogen production from dairy wastewater. Int J Hydrogen Energy 40:4502–4511. https://doi.org/10.1016/j.ijhydene.2015.02.046
59. Li Z, Wang J, Tian K et al (2022) Nickel--cobalt oxide nanoparticle-induced biohydrogen production. ACS omega 7:41594–41605
60. Mishra P, Thakur S, Mahapatra DM et al (2018) Impacts of nanometal oxides on hydrogen production in anaerobic digestion of palm oil mill effluent--a novel approach. Int J Hydrogen Energy 43:2666–2676
61. Hallenbeck PC (2009) Fermentative hydrogen production: principles, progress, and prognosis. Int J Hydrogen Energy 34:7379–7389
62. Vignais PM, Billoud B, Meyer J (2001) Classification and phylogeny of hydrogenases. FEMS Microbiol Rev 25:455–501
63. Rambabu K, Bharath G, Thanigaivelan A et al (2021) Augmented biohydrogen production from rice mill wastewater through nanometal oxides assisted dark fermentation. Bioresour Technol 319:124243
64. Rambabu K, Bharath G, Banat F et al (2021) Ferric oxide/date seed activated carbon nanocomposites mediated dark fermentation of date fruit wastes for enriched biohydrogen production. Int J Hydrogen Energy 46:16631–16643. https://doi.org/10.1016/j.ijhydene.2020.06.108
65. Cheng J, Li H, Ding L et al (2020) Improving hydrogen and methane co-generation in cascading dark fermentation and anaerobic digestion: the effect of magnetite nanoparticles on microbial electron transfer and syntrophism. Chem Eng J 397:125394

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.