Implementation of nanotechnology for increasing biohydrogen production from anaerobic digestion of biomass

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Abstract: Biohydrogen has significant feasibility since biological processes are much less energy intensive compared with electrolysis and thermo-chemical processes. Biological processes and bacterial fermentation are considered as the most environmentally friendly alternatives for satisfying future hydrogen demand. Biohydrogen production from biomass is considered profitable as biomass is abundant, cheap, and biodegradable. The combustion of H_2 with O_2 produces water as its only product: Unlike other fuels, the combustion of H_2 does not produce CO_2, CO, NO_x, or SO_2. Therefore, H_2 is an environmentally friendly fuel. The objective of this study is to increase biohydrogen production from biomass using nanotechnology. In this study, it is hypothesized that the biostimulation of hydrogen-producing purple non-sulfur (PNS) bacteria through the addition of nutrients in form of nanomaterials can enhance the bioresponses of such bacteria, where this leads to increase biohydrogen production from biomass. A biohydrogen production system and a model of photobioreactor were manufactured and installed. Food wastes were collected from kitchen leftovers of different fast-food suppliers and were used in this study as feedstocks for biohydrogen production. The production process was conducted as following: addition of 50 mg l^{-1} of nickel nanoparticles to the bacterial inoculum and then mixing them with biomass and water by a ratio of 0.5:1:2 which were then kept in the photobioreactor exposed to white light emitting diodes (LEDs) with a luminous flux of 3600 lumen and at 30°C for 14 days with mixing for 5 min every 30 min to produce biohydrogen. The results showed that the maximum biohydrogen yield was 40.7 mol H_2/mol sugar (2.68 times control) when Ni nanoparticles were added. Besides, during the active production period the H_2 percentages ranged from 48.0% to 51.7% when Ni nanoparticles were added which were higher by 15% than the control. It was concluded that the addition of nanomaterials leads to biostimulate the bacterial cells and enhance their activity and growth rate and, therefore, increase biohydrogen production from biomass.

Keywords: biohydrogen; nanomaterials; anaerobic treatment; biomass; biofuels; nanotechnology.

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1 Introduction

Energy has become a multi aspect problem all over the world. One aspect is the rising demand for energy as an outcome of population increase, modern life standards and the massive expansion in industry (Akia et al., 2014). On the other hand, fossil fuels –which is considered as one of our major sources of energy now, will be depleted in the next few years if the current consumption rate remains the same or increased (Höök and Tang, 2013). The environmental catastrophe is another aspect of this energy dilemma; fossil fuels are a main cause of hazardous emissions and greenhouse

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gases such as CO_2. The concentration of CO_2 in the atmosphere is 380 ppm now compared to 270 ppm before the industrial revolution between 1820 and 1840 (Huang et al., 2012; Kweku et al., 2017).

Finding alternative sources of energy became an urgent need to be used in parallel to fusel fuel soon and to completely replace fossil fuel eventually. These alternatives should be sustainable, clean and have no dangerous impact on the environment or at least have an impact that we can deal with or contain. Renewable energy such as solar energy, Biofuels, Geothermal energy, etc. are strong alternatives (Mohtasham, 2015).

Biohydrogen has significant feasibility since biological processes are much less energy intensive compared with electrolysis and thermo-chemical processes. It is widely recognized that considerable amounts of hydrogen (H_2) can be produced from renewable resources without using energy from fossil fuels. Biological processes and bacterial fermentation are considered as the most environmentally friendly alternatives for satisfying future hydrogen demand. Biohydrogen production from agricultural and agro-industrial solid waste and wastewater is considered as highly advantageous as materials of this kind are abundant, cheap, and biodegradable. The combustion of H_2 with oxygen produces water as its only product: Unlike other fuels, the combustion of H_2 does not produce carbon dioxide (CO_2), carbon monoxide (CO), nitrogen oxides (NO_x), sulfur dioxide (SO_2), hydrocarbons or particulate matter (PM). Therefore, hydrogen is an environmentally friendly fuel where endeavors focus on producing specially designed internal combustion engines that can use H_2 as fuel (Ladole et al., 2017; Oscar et al., 2015; Urbaniec and Bakker, 2015).

Hydrogen is a promising source of energy for the following reasons: (1) it is an environmentally friendly source of energy since the combustion of hydrogen results in a good amount of energy and H_2O ($2H_2+O_2 \rightarrow 2H_2O$). (2) The high energy density of 122-142 MJ Kg^{-1}. (3) can be directly used to generate electricity instead of direct combustion. Producing hydrogen through biological processes especially when we use the biological wastes such as agricultural wastes, food wastes, etc. can maximize the benefits of this rising alternative known as: Biohydrogen (Ladole et al., 2017; Oscar et al., 2015; Urbaniec and Bakker, 2015).

Biohydrogen can be produced using of these methods: (1) photo-fermentation by using photosynthetic bacteria, (2) dark fermentation method by using anaerobic bacteria, (3) Hybrid systems including dark and photo-fermentation processes, (4) bio-photolysis of water by using algae/ cyanobacteria, (5) microbial electrolysis cell (Das and Veziroğlu, 2001; Hay et al., 2013). Through the intensive research to find out the best method to produce biohydrogen from the previous methods, photo-fermentation is considered one of the best and more reliable methods for these reasons: (1) High substrate conversion ratio, (2) High biohydrogen yield, (3) wide spectrum of light can be used, and different types of biological wastes can be utilized (Zhang et al., 2008; Eroglu, and Melis, 2011).

Purple non-sulfur bacteria (PNSB) are a very interesting species. They are gram-negative photosynthetic bacteria which are gluttonous for light (Abdelqader et al., 2021). PNSB can grow heterotrophically and produce biohydrogen from simple organic acids such as volatile fatty acids such as (acetic acids, propionic acid, butyric acid, and valeric acid) under anaerobic conditions in the presence of light. However, biohydrogen production depends on specific conditions such as light intensities (3-10 klux) and wavelength (400-1000 nm), temperature (31℃ - 36℃) and optimum pH (6.8 -7.5) since the PNSB have multiple metabolic pathways. Purple non-sulfur bacteria (PNSB) can be found in wastewater, pig manure, alkaline lakes and earthworm drops. However, enrichment techniques are required after isolation to reach the needed concentration (Bryant and Frigaard, 2006; Chen et al., 2011; Budiman et al., 2014).

Several types of organic wastes can be used to produce biohydrogen such as agricultural waste, food waste, etc. Food wastes are organic solid wastes that account for 15%-63% of total municipal solid wastes worldwide. Additionally, according to the Food and Agriculture Organization (FAO), United Nation, more than 1.3 million tons of food is being wasted. Food waste is considered as a perfect alternative for Biohydrogen production since food wastes are rich in carbohydrates, proteins, fats, nitrogen, minerals, cellulose, and hemicelluloses (Kapdan and Kargi, 2006). Furthermore, agricultural wastes should be bioprocessed to produce environmentally friendly bioproducts which reduce the emissions (Samer et al., 2021a).

On the other hand, the main features of implementing nanotechnology are that the physical, chemical, electrical, and thermal properties of the synthesized nanomaterials differ intrinsically from the original material properties (Attia et al., 2018; Attia et al., 2022). As a beneficial approach, the supplementation of the growth media of PNS bacteria with nanomaterials can increase biological hydrogen production. For that purpose, different organic and inorganic nano-catalysts can be utilized (Abdelqader et al., 2021; Abdelsalam et al., 2021a).

The problem facing the biohydrogen production from biomass using hydrogen-producing bacteria can be identified as the unsteady light irradiation negatively affects biohydrogen production, where light saturation of photo-fermentation is the main significant factor that influences the fermentation efficiency and the biohydrogen yield, particularly in mass culture (Abdelqader et al., 2021; Abdelsalam et al., 2021a).

The addition of nanomaterials with large specific surface area and large band gap will enable the absorption of a large amount of the visible light (400-700 nm) which will create a rich light media around the photo bacteria responsible of biohydrogen production from organic matter. This will create a rich light media around the photosynthetic hydrogen-producing bacteria which will enhance the endurance ability of bacteria to unsteady light irradiation which in turn increases biohydrogen production from organic matter. This is generally hypothesized to overcome the challenge of light saturation and to provide more information on the light-to-H_2 energy conversion efficiency (Montano et al., 2009; Chen et al., 2011; Abdelsalam et al., 2019a).

The main objective of the present study is to increase biohydrogen production from food wastes through the implementation of nanotechnology by adding nanomaterials to the inoculum of hydrogen-producing bacteria.

2 Material and methods

2.1 Cultivation of purple non-sulfur bacterial (PNSB) mix consortium and preparation of inoculum

Purple non-sulfur bacteria were isolated from a stagnant water sample collected from both Teraat El-Marioutya (El-Marioutya Canal) in and a small water canal in a village called Shabramant (Giza, Egypt) in sterile containers. All samples were kept in the fridge (at 5 ℃) till use. The collected samples were aseptically inoculated in sterile Acetate Yeast Extract (AYE) basil medium (2 mL or g sample + 18 mL medium). AYE medium consisted of K_2HPO_4, 1.0 g; $MgSO_4$, 0.2 g; $CaCl_2$, 0.02 g; $Na_2S_2O_3$, 0.10 g; Na-Acetate, 2.2 g; Yeast Extract, 4.0 g; 1 L distilled water (Montano et al., 2009; Eroglu and Melis, 2011). The final pH was 7.0 ± 0.2. The medium ingredients were dissolved in distilled water, then distributed in screw cap tubes (18 mL) and autoclaved at 121 ℃ for 15 min at 1 atm pressure. Afterwards, the inoculated culture tubes were completely filled with the media and sealed with the screw cap. The tubes were then incubated under a 60 W incandescent bulb lamp at room temperature. The tubes were placed 25 cm from the light source. After four weeks of incubation, formation of red blooms will be noted, indicating growth of PNSB (Fig.1).

2.2 Installing and adjusting the biohydrogen production setup

A batch anaerobic system was designed and installed as shown in Figure 2. The experimental setup consists of: (1) High transparency glass flasks of volume 1 L with a well-sealed screw caps (photo bioreactors) and gas outlet connected to gas holder with 1/4″ connectors through polyurethane hose (4 mm internal diameter and 6 mm external diameter). (2) The temperature control; a thermostatic water bath

(Homemade, 90×60×25 cm, 135 L, 0 ℃ - 100 ℃). (3) Biohydrogen storage; the biohydrogen produced can be stored in ultra-clear polypropylene graduated cylinder (250±2 mL, Azlon, Staffordshire, UK) connected to gas outlet by 6 mm polyurethane hose at its base and placed upside down in another polypropylene cylinder (500 mL, Azlon, Staffordshire, UK) filled with water (Figure 2).

(a) Sterile medium and (b) Growth of PNS bacterial consortium

Figure 1 PNSB consortium enrichment in AYE medium

Figure 2 Biohydrogen production setup after installation.

2.3 Experimental design

The prepared PNSB mixed inoculum was injected in each photo bioreactor, 125 mL for each flask. The treatment was photo-bio stimulation of PNSB with He-Ne red laser -in triplicates- and the control.

2.4 Preparation of nickel nanoparticles

Nickel nanoparticles (Ni NPs) were synthesized by reduction method as follow: 5 mL (0.1 M) of $NiCl_2.6H_2O$ (crystallized 99.9%, Fluka) were added to 5 mL of CTAC (25 wt% in H_2O, 0.968 g mL^{-1} at 25 ℃, Aldrich) as a surfactant, then $NaBH_4$, 0.2M (powder 98%, Aldrich) was added dropwise until the salt solution color is totally disappeared and turned to black color that indicate the creation of Ni NPs. Transmission Electron Microscope (TEM) was implemented to characterize the morphology and distribution of NPs. The TEM images showed a homogeneously spherical shape of Ni NPs and were found to support the size reduction to nanometer range (7-10 nm). Figure 3 shows the resulting image from TEM of the prepared Ni NPs, where the specific surface area of Ni NPs is 40 m^2 g^{-1}.

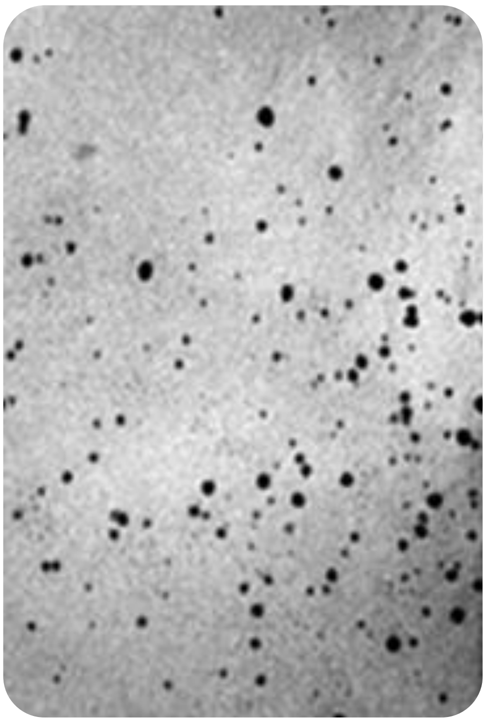


Figure 3 The TEM image of Ni nanoparticles.

2.5 Waste pretreatment

Food wastes were collected from many restaurants and mixed then chopped using a chopper (40544, TOUCH®, ElZenouki Electrical Appliances, El Obour City, Egypt) to a very fine size until it became in doughy form. Distilled water was mixed with the chopped wastes in ratio 2:1. Basic chemicals were used to adjust the initial pH of the mixture to 7.0. Each photobioreactor was filled with 900 mL (125 mL of PNSB mixed inoculum and 775 mL of food waste-distilled water mixture. A vacuum pump was used to create anaerobic conditions inside the photobioreactor. The properties of food wastes were listed in Table 1.

2.6 Experimental setup

The photobioreactors were perfectly sealed and so the rest of the setup components to avoid any leakage after running the experiment. The photobioreactors were inserted in the water bath to keep the temperate 35 ℃ as long as the experiment is running. a white Light Emitting Diode (LED) with a luminous flux of 3600 lumen was installed above the photobioreactors. The total biohydrogen yield was measured by water displacement method. The hydrogen percentage was measured using H_2 gas detector (XP-3140, New Cosmos Electric Co., Ltd., Tokyo, Japan). The components of the yielded gas were determined using a gas chromatography (GC, Shimadzu 2014, Japan) equipped with a thermal conductivity detector (TCD) included: hydrogen, carbon dioxide, methane, and nitrogen. Initial sugar (mol) was determined using HPLC (Shimadzu LC-10A, Japan) with a Shimadzu RID-10A differential refractive index detector.

Table 1 Chemical composition of mixed food wastes

Parameters	Mixed Food Waste (2:1)*
Total Solids [TS%]	6.42
Volatile Solids [VS%]	5.46
VS [% as TS]	85.04
Total Nitrogen [TN%]	1.49
C/N Ratio	33.1 : 1
Ash [%]	0.96
pH	4.50
Organic Carbon [OC%]	49.33

Note: * Water to fresh waste ration is 2:1

2.7 Calculations

The specific hydrogen production (mol H_2/mol sugar) was calculated as according to the following equations (Samer et al., 2022):

$$H_2\ (g) = H_2\ (mL) \times 0.0893 \times 10^{-3} \quad (1)$$

$$H_2\ (mol) = H_2\ (g) \times 0.4961 \quad (2)$$

$$\text{Specific } H_2 \text{ Prod.} = H_2\ (mol) \div 0.007 \quad (3)$$

The Cumulative Biohydrogen Production (mL) was simply calculated as the sum of today hydrogen yield with yesterday hydrogen yield. The Total gas is simply calculated as the sum of the daily gas production for all days of the experiments. The biohydrogen yield was calculated in the same way.

3 Results and discussion

The results show that the addition of nanomaterials to the inoculum of purple non-sulfur bacteria has a positive impact on biohydrogen percentage, daily biohydrogen production and cumulative biohydrogen production values. Additionally, the results show a positive impact on daily gas production values and cumulative gas production values, which means a positive impact on PNSB activity.

Figure 3 shows the effects of nanomaterials on total gas and biohydrogen yield. Figure 4 shows the effects of nanomaterials on specific hydrogen production. Figure 5 shows the effects of nanomaterials on daily (a) and cumulative gas production (b). Figure 6 shows the effects of nanomaterials on daily (a) and cumulative biohydrogen production (b). Figure 7 shows the effects of nanomaterials on hydrogen percentage.

The results showed that the maximum biohydrogen yield was 40.7 mol H_2/mol sugar (2.68 times control) when Ni nanoparticles were added (Figure 4). Besides, during the active production period the H_2 percentages were ranging from 48.0% to 51.7% when Ni nanoparticles were added which were higher by 15% than the control (Figure 7).

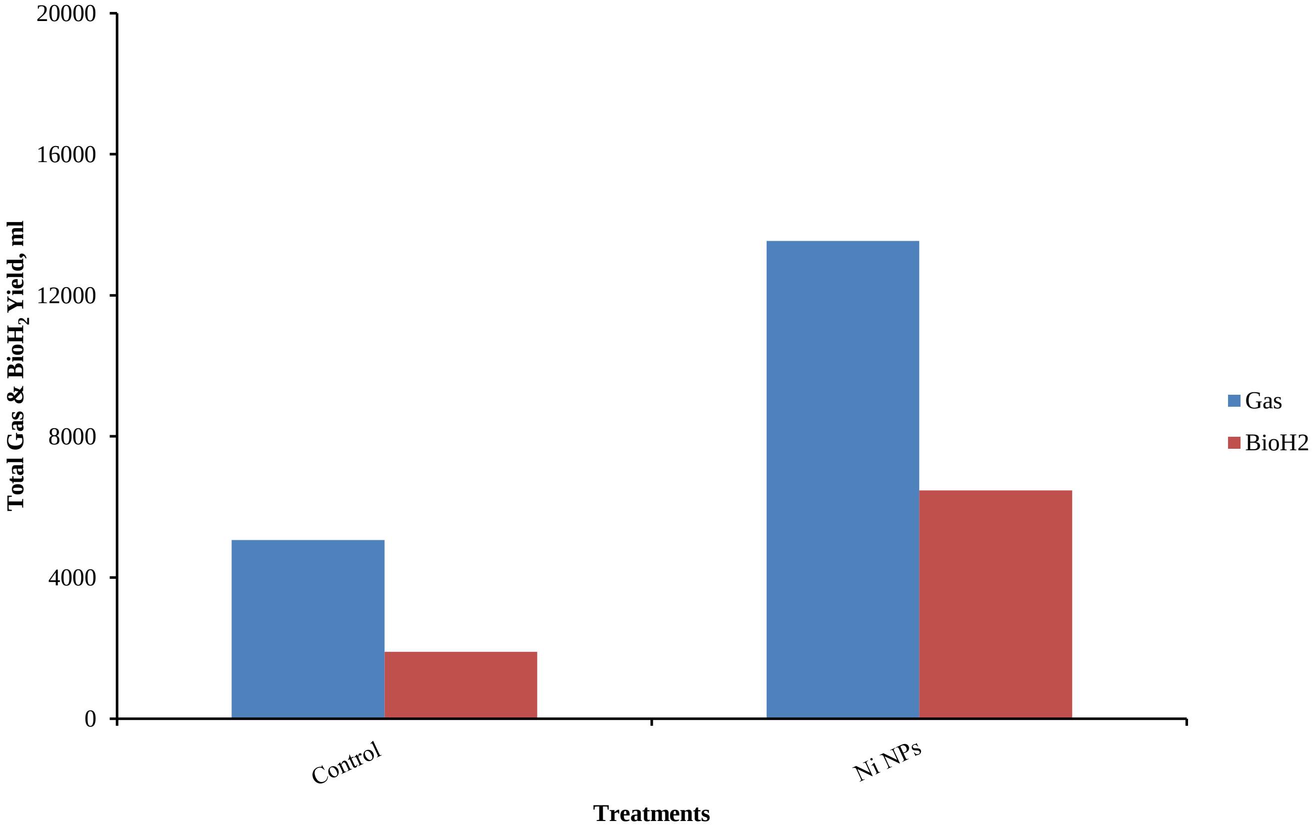


Figure 3 Effects of nanomaterials on total gas and biohydrogen yield

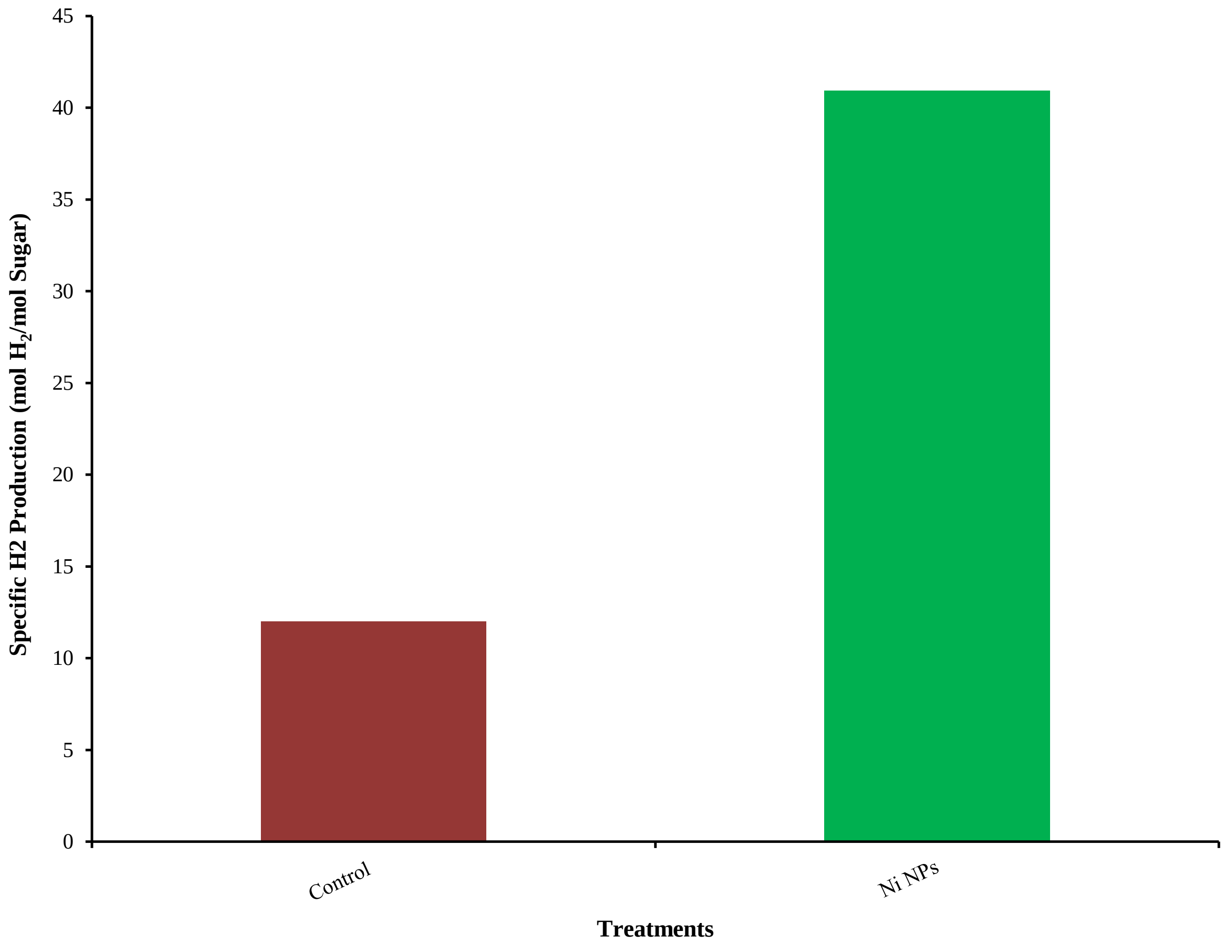


Figure 4 Effects of nanomaterials on specific hydrogen production.

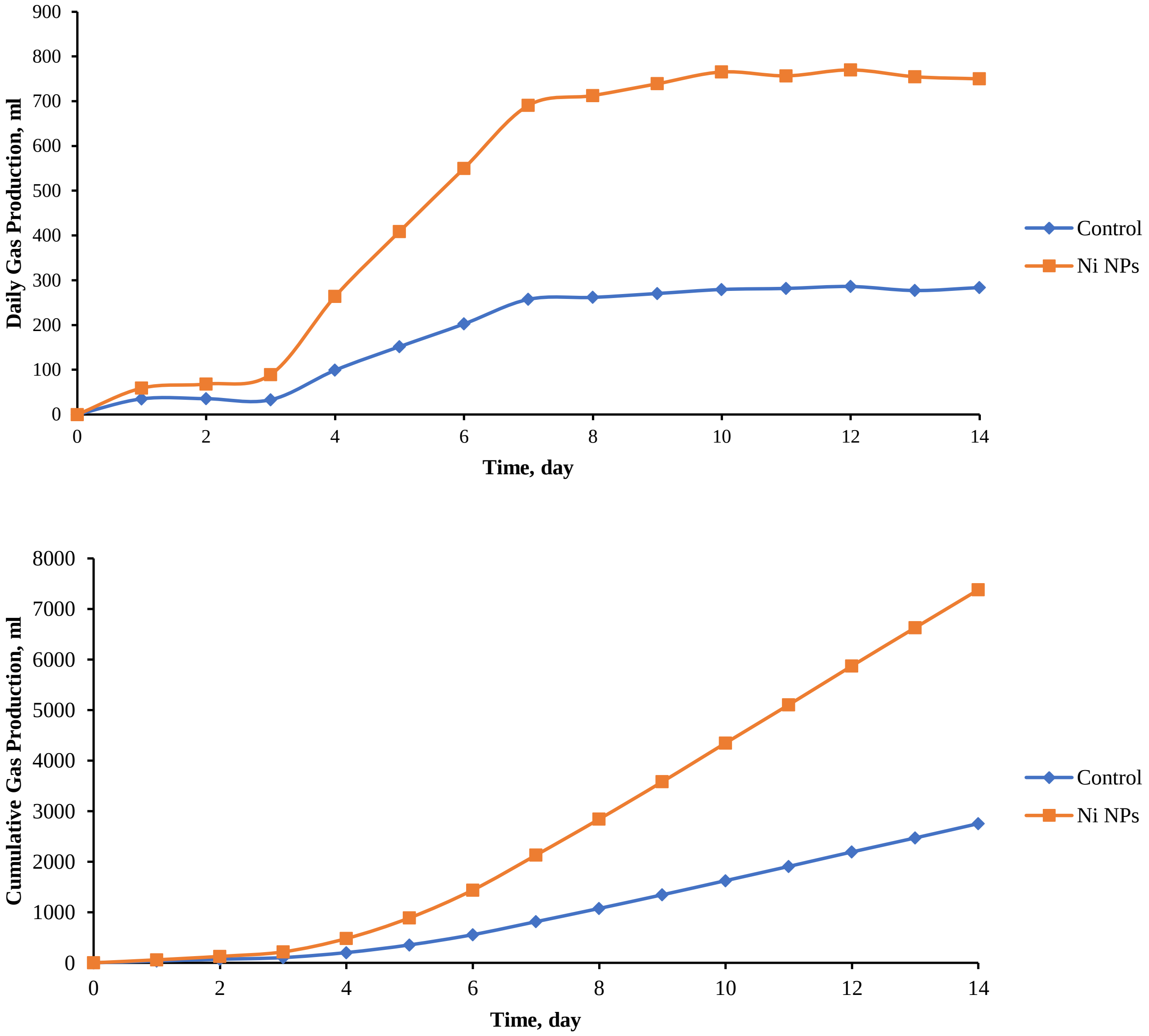
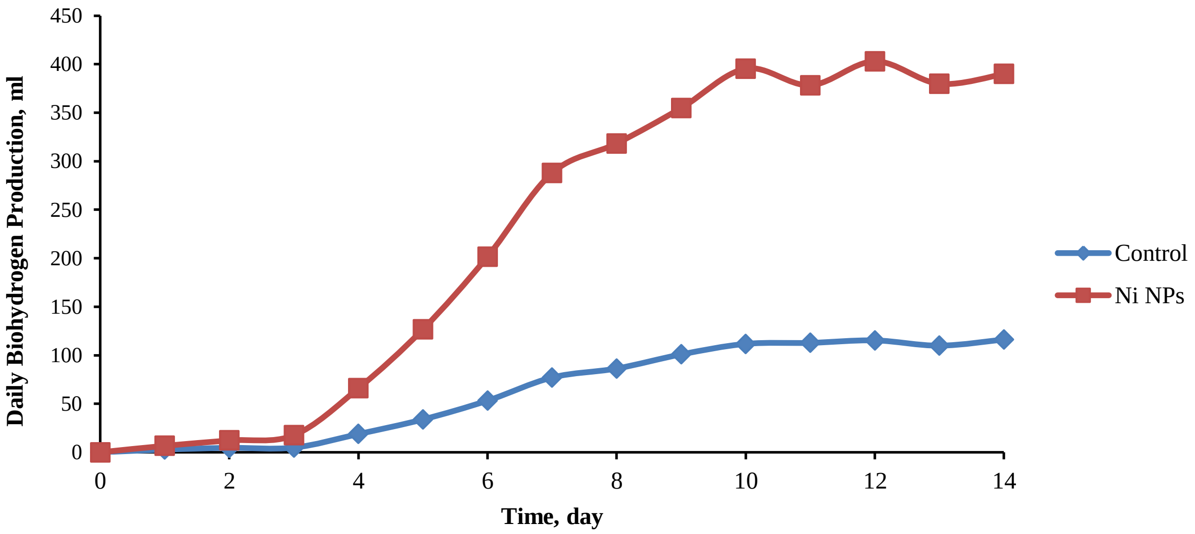


Figure 5 Effects of nanomaterials on daily (a) and cumulative gas production (b).



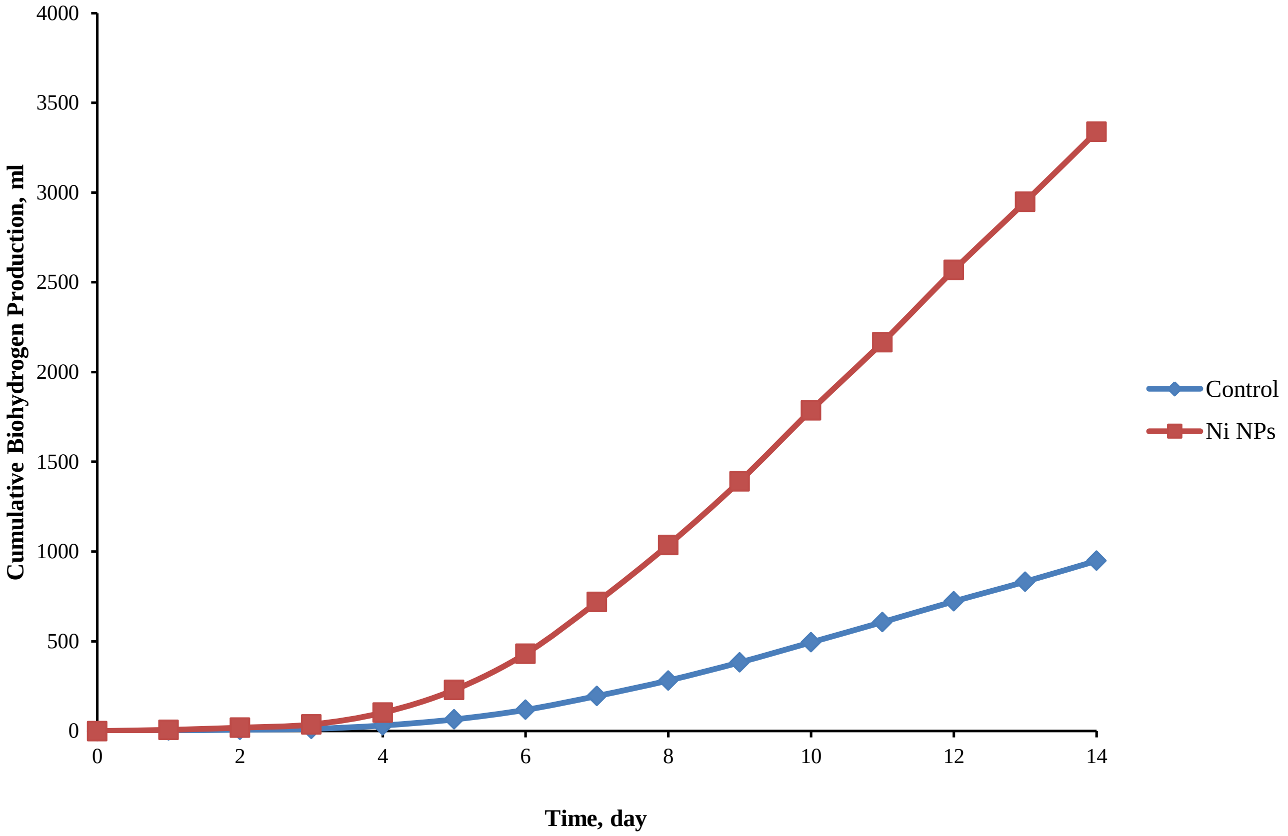


Figure 6 Effects of nanomaterials on daily (a) and cumulative biohydrogen production (b).

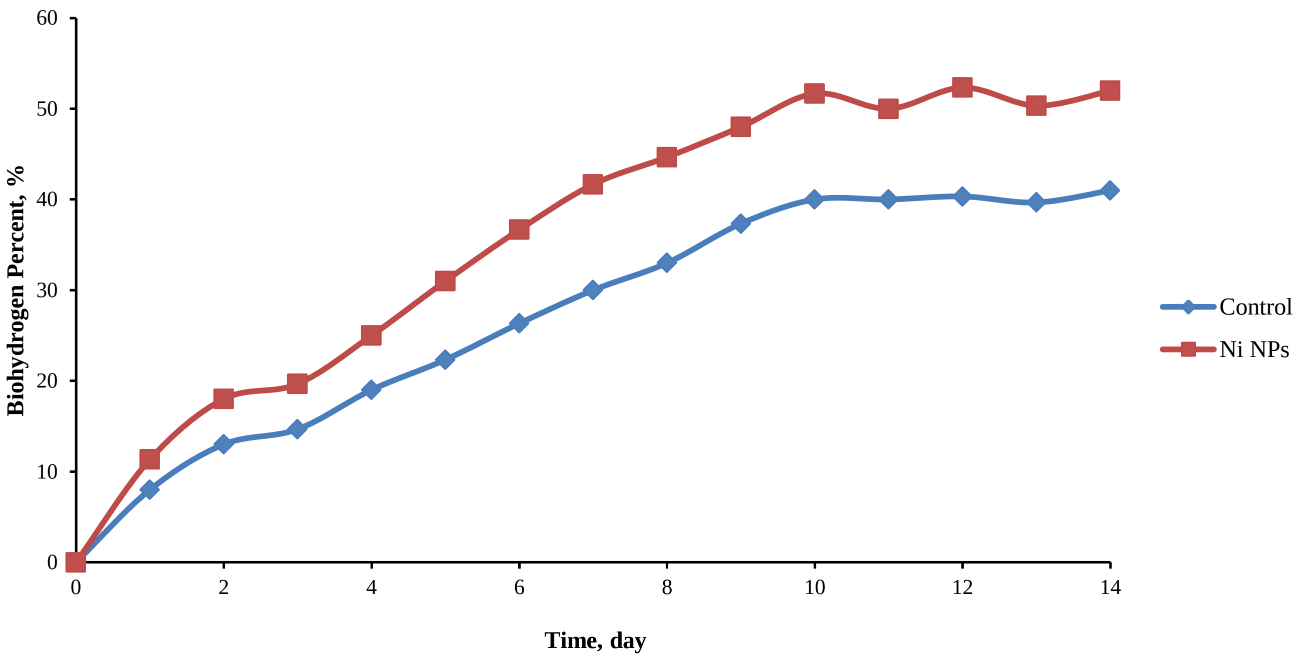


Figure 7 Effects of nanomaterials on hydrogen percentage.

This research represents one of the unique solutions of many problems facing the world these days. In this approach food wastes are being used as a substrate for biohydrogen production. The world produces millions of tons of food wastes and kitchen leftovers every year around the world which demands a radical solution to not only get rid of these wastes in an ecofriendly method but also to convert these wastes into a high-value product and source of energy, this will be positively reflected on the economy.

Purple non-sulfur bacteria (PNSB) are a strong alternative in fermentation process as bacteria, it has a good conversation ratio, that means PNSB can convert most of the organic acids from food wastes are rich in into biohydrogen (Attia et al., 2021).

Based on the results of this study, the addition of nanomaterials to the purple non-sulfur bacteria (PNSB) inoculum is an easy and affordable technique with a very good outcome. Maximizing biohydrogen production from food wastes using this environmental-friendly method will encourage to scale-up the process to an industrial scale to generate clean energy using an ecofriendly technique and solve the food wastes issue as well.

Some studies investigated the effects of nanomaterials irradiation using laser source on the biohydrogen production (Abdelsalam et al., 2018; Attia et al., 2021). However, the bacteria were not irradiated. Besides, several studies have investigated the effects of laser radiation on the methanogenic bacteria (Abdelsalam et al., 2019a,b; Abdelsalam et al., 2021a; Samer et al., 2021b,c). In contrast, the present study investigated the direct addition of nanomaterials bacterial inoculum without laser irradiation.

Similar to the current study, Attia et al. (2021) used nickel nanoparticles and found that the maximum biohydrogen yield was 41.0 mol H_2/mol sugar (2.42 times the control) which is comparable to the results of the current study where the maximum biohydrogen yield was 40.7 mol H_2/mol sugar (2.68 times control). Furthermore, Attia et al. (2021) found that during the active production period the hydrogen percentages were ranging from 48.7% to 52.3% for nickel nanoparticles. However, the control treatment gave less percentages of biohydrogen (approximately 15% lower) within the same fermentation period, which agree with the results of the current study where during the active production period the hydrogen percentages were ranging from 48.0% to 51.7% for nickel nanoparticles. However, the control treatment gave less percentages of biohydrogen (approximately 15% lower) within the same fermentation period. However, the current study disagrees with Attia et al. (2021) in the hydraulic retention time, where they have conducted the experiments over 26 days of hydraulic retention time. However, in the current study, the experiments were conducted over only 14 days of hydraulic retention time to get the comparable results.

In biology, PNS bacteria are considered to have metal-rich enzymatic pathways. PNS bacteria need several trace elements as micro-nutrients of which iron (Fe), cobalt (Co), and nickel (Ni) are the most important. Nanoparticles (NPs) can interact with biological systems by adsorption and desorption, either as released ions, transformed single nanoparticles, agglomerates or complexes with dissolved organic matter (DOM). DOM/NPs complexes are binding with metal binding receptors on the cell membrane to intracellular pathways. PNS bacteria actively transport Ni using ATP-dependent uptake systems in order to fulfil enzymatic requirements. The direct uptake of the single NPs via ATP-dependent uptake system depends on NPs size and speciation. NPs can be integrated in metalloenzyme, electron transfer and reduction pathways with higher binding affinity (Abdelsalam et al., 2019a,b; Abdelsalam et al., 2021a; Abdelsalam and Samer, 2019).

Future research will focus on the implementation of further types of trace metals, in form of laser photoactivated nanomaterials, to enhance the production of biohydrogen from biomass, where the laser radiation and nanotechnology were implemented in biogas production (Hijazi et al., 2020a,b; Samer et al., 2020; Abdelsalam et al., 2021b,c; Samer et al., 2021b,c; Ioannou-Ttofa et al, 2021) but a very few studies in biohydrogen production were conducted. Besides, the use of agricultural wastes, especially manure, in producing biohydrogen will minimize the recorded harmful gas emissions (Samer et al., 2011; Samer et al., 2013; Samer and Abuarab, 2014; Samer et al., 2014) from the agricultural sector. Generally, research is focusing on the use of agricultural wastes, manure, wastewater, agricultural crop residues, and different bioresources to produce several types bioproducts, bioenergy, and biofuels (Samer et al., 2019, 2021; Moustafa et al., 2021; Khalifa et al., 2022;

Faried et al., 2022; Saeed et al., 2022).

4 Conclusions

It can be concluded from this study that the nanomaterials have biostimulating effects on the biohydrogen-producing bacteria activity during the startup of the anaerobic digestion process of the substrate and through the HRT to the end of the experiments. Besides, the nickel nanoparticles yielded higher biohydrogen production compared to the control. The addition of nanomaterials with large specific surface area and large band gap enables the absorption of a large amount of the visible light (380-760 nm) which creates a rich light media around the photobacteria responsible of biohydrogen production from organic matter.

Conflicts of interest

There are no conflicts to declare.

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