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Valorization of Food Waste into Iron Oxide Nanoparticles: Characterization and Application for DIET-mediated Enhancement of Methane Production in Anaerobic Digester

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ABSTRACT

In the present study, the potential of using algal biomass for biogas production was enhanced by iron oxide nanoparticle (Fe_3O_4)-facilitated anaerobic digestion under mesophilic conditions. The use of algal biomass as a renewable organic substrate was proposed due to its high content of biodegradable organic matter and its potential for wastewater nutrient recovery. Nevertheless, due to the resistant algal cell wall structure and slow microbial electron transfer, the efficiency of methane production during anaerobic digestion is often limited. To address this limitation, green-synthesized Fe_3O_4 nanoparticles were employed as conductive additives to enhance microbial electron transfer and improve biomethanation efficiency, representing the key novelty of this study. Fe_3O_4 nanoparticles were added at various concentrations (25, 50 and 75 mg L^{-1}) to batch anaerobic digesters to assess their effects on biogas production, methane enrichment, COD removal efficiency, solids degradation and pH stability. The experimental results demonstrated that the addition of Fe_3O_4 nanoparticles significantly improved anaerobic digestion performance. The digester supplemented with 50 mg L^{-1} Fe_3O_4 nanoparticles exhibited the best performance, producing a cumulative biogas yield of 685 mL g^{-1} VS, which was 34.3% higher than that of the control digester (510 mL g^{-1} VS). The highest methane concentration (71.4 ± 1.8%) was achieved in the nanoparticle-assisted reactor, compared with 54.2 ± 1.3% in the control. Similarly, COD removal efficiency increased from 61.8 ± 2.1% in the control reactor to 82.6 ± 2.4% in the optimized nanoparticle reactor, while total solids and volatile solids reduction efficiencies reached 58.7 ± 2.0% and 69.8 ± 2.0%, respectively. The observed improvements were statistically significant ($p < 0.05$), confirming the positive influence of the optimized Fe_3O_4 nanoparticle dosage on anaerobic digestion performance. These findings demonstrate the practical potential of green-synthesized Fe_3O_4 nanoparticles for improving renewable methane production and resource recovery from algal biomass, thereby contributing to sustainable bioenergy generation and wastewater valorization. The enhanced performance is attributed to improved direct interspecies electron transfer (DIET), accelerated substrate degradation, and enhanced microbial enzymatic activity facilitated by the conductive Fe_3O_4 nanoparticles. However, a higher nanoparticle concentration (75 mg L^{-1}) resulted in comparatively lower performance, likely due to nanoparticle aggregation and inhibitory effects on microbial activity.

Keywords: Iron oxide nanoparticles; Green synthesis; Anaerobic digestion; Direct Interspecies Electron Transfer (DIET); Biomethanation; Circular bioeconomy.

1. INTRODUCTION

There is a large amount of food waste produced globally due to rapid population growth, urbanization, and changes in food consumption patterns. Every year, almost one-third of the food produced for human consumption is lost or wasted, posing significant environmental, economic and social issues (Rahman *et al.* 2024). Dumping of food waste at landfill sites is responsible for GHG emissions, leachate production, odours and loss of valuable resources. The effort towards sustainable food waste management and resource recovery technologies has therefore emerged as a key research priority in today's environmental research (Pant *et al.* 2026). Anaerobic digestion (AD) is one of the most promising treatment technologies, as it is environmentally friendly and economically viable for producing renewable energy from biodegradable organic material (Piadeh *et al.* 2024; Prabha and Selvaraj, 2025). The use of different microbial communities, working in a well-coordinated manner, not only reduces the volume of waste, but also converts the organic matter to biogas, which generally contains methane (CH_4) and carbon dioxide (CO_2) (Jameel *et al.* 2024). Although anaerobic digestion offers several benefits, it can suffer from low hydrolysis rates, poor microbial electron-transfer efficiency, and intermediate metabolite buildup and instability, all of which contribute to limited methane production and conversion efficiencies (Vannarath *et al.* 2025).

Microbial electron transfer pathways have been found to be a key factor in controlling methanogenic performance, with recent advances in anaerobic biotechnology (Li *et al.* 2021). Conventional anaerobic digestion processes primarily rely on interspecies hydrogen and formate transfer mechanisms, which may be thermodynamically constrained and prone to environmental disturbances (Nayeri *et al.* 2024). However, Direct Interspecies Electron Transfer (DIET) has been identified as a more efficient mechanism, which allows direct electron transfer between syntrophic bacteria and methanogenic archaea (Wang *et al.* 2021; Swetha *et al.* 2026a). The promotion of DIET can substantially reduce the degradation of the substrate, help realise microbial syntrophy, stabilise the reactor and boost the yield of methane (Sudiartha *et al.* 2024). Therefore, the use of conductive materials to enable electron transfer from the microorganisms in an anaerobic digester has received significant research attention (Liu *et al.* 2021a). Various conductive additives, such as biochar, activated carbon, graphene, carbon nanotubes, and iron-based materials, have been tested to promote DIET and enhance the efficiency of biomethanation (Kutlar *et al.* 2022).

Several studies have demonstrated the beneficial role of Fe_3O_4 nanoparticles in enhancing anaerobic digestion performance (Nguyen *et al.* 2021). reported that magnetite nanoparticles accelerated electron transfer between syntrophic microorganisms, thereby improving methane production efficiency. The conductive Fe_3O_4 nanoparticles promoted Direct Interspecies Electron Transfer (DIET), reduced lag phase, and enhanced substrate degradation (Liu *et al.* 2021b). Similarly, iron-based nanoparticles improved microbial metabolic activity by supplying bioavailable iron required for redox enzymes involved in methanogenesis (Ma *et al.* 2024). Further studies demonstrated that Fe_3O_4 nanoparticles significantly enhanced biogas production; however, their study relied on conventionally synthesized nanoparticles (Kumar *et al.* 2025). These findings clearly demonstrate the potential of magnetite nanoparticles for anaerobic digestion and highlight the need for environmentally sustainable synthesis routes.

Chemically synthesized Fe_3O_4 nanoparticles show great promise for improving the performance of anaerobic digestion, but their synthesis frequently requires toxic reducing agents, dangerous chemicals, and high-energy processes, making them environmentally unsustainable (Kumar *et al.* 2025). Recently, green synthesis methods using biological resources have been proposed as an alternative to the traditional synthesis of nanoparticles that is environmentally friendly (Ahmed *et al.* 2026). The green synthesis process has several advantages, including lower environmental impact, lower operating costs, improved biocompatibility, and greater sustainability (Syed *et al.* 2026). The presence of high levels of carbohydrates, proteins, phenolic compounds, organic acids, and antioxidants in food waste makes it a promising green synthesis precursor, as these compounds can serve as natural reducing and stabilizing agents in the formation of nanoparticles (Olaniyan *et al.* 2025). As such, food waste offers a special opportunity as a substrate for anaerobic digestion and also as a sustainable feedstock for the production of value-added nanomaterials (Tomczak *et al.* 2024; Velvizhi *et al.* 2019).

Although several studies have individually focused on the valorization of food waste via anaerobic digestion and the enhancement of methane production through conductive nanoparticles, there has been a lack of research that has combined these two concepts within a common circular resource recovery system (Callegari *et al.* 2025). The majority of current research focuses either on the anaerobic digestion of food waste or on the synthesis and use of commercially available or chemically produced nanoparticles (Singh *et al.* 2023). However, studies investigating green-synthesized Fe_3O_4 nanoparticles derived from food waste and their use as conductive materials to enhance methane production remain scarce. Furthermore, the combined utilization of food waste as both a precursor for nanoparticle synthesis and a substrate for biomethanation has not been systematically explored. This knowledge gap limits the development of sustainable waste valorization strategies that simultaneously recover value-added nanomaterials and renewable bioenergy.

In this context, the present study proposes an integrated waste-to-nanomaterials (Fe3O4) and waste-to-energy (methane gas) utilization strategy, employing the green synthesis of Fe_3O_4 nanoparticles from food waste, followed by anaerobic digestion.

The novelty of the present study lies in the integrated valorization of food waste through two complementary pathways: (i) green synthesis of Fe_3O_4 nanoparticles using food waste-derived biomolecules as natural reducing and stabilizing agents, and (ii) application of these biosynthesised conductive nanoparticles to enhance methane production during anaerobic digestion. Unlike previous investigations that mainly employed chemically synthesised or commercially available Fe_3O_4 nanoparticles, the present work establishes a sustainable circular bioeconomy approach by simultaneously recovering nanomaterials and renewable energy from the same waste stream. This integrated strategy provides an environmentally friendly alternative for improving anaerobic digestion performance while promoting resource recovery and waste minimization.

Thus, the main aim of this research was to synthesise Fe_3O_4 nanoparticles using biomolecules extracted from food waste and to test their performance as conductive additives to enhance anaerobic digestion. The synthesized nanoparticles were characterized by means of Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and Energy Dispersive X-ray Spectroscopy (EDX), and batch anaerobic digestion experiments were carried out to study the effect of various dosages of nanoparticles on methane production and COD removal efficiency under mesophilic conditions. The study also aimed to determine the optimal concentration of Fe_3O_4 nanoparticles and to elucidate the mechanism of methanogenesis via DIET mediated by Fe_3O_4 nanoparticles, which can help develop sustainable nanoparticle-assisted biomethanation technologies for integrated food waste valorization and renewable energy recovery.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Food Waste Feedstock

Domestic household and institutional cafeteria food waste, including fruit peels, vegetable waste, cooked rice waste, bread waste, and other biodegradable waste from the kitchen, were gathered. Manual removal of visible non-biodegradable items like plastics, glass shards, metals, and packaging was done. All the food waste gathered was washed with deionized water to remove all the impurities that adhered to the food waste, then air-dried for 24 hours.

The cleaned feedstock was dried in an oven at 60 °C until a constant weight was achieved, and was then ground in a laboratory grinder. The powdered material was sieved through a 250 µm mesh to obtain a uniform particle size distribution and stored in airtight containers for the synthesis of nanoparticles and anaerobic digestion experiments. The overall methodology used in the present study is depicted in Fig. 1.

2.2 Green Synthesis of Food Waste-derived Fe_3O_4 Nanoparticles

Food waste powder was dried and then dispersed in 200 mL of deionized water and heated for 60 min at 80 °C with constant magnetic stirring to prepare the food waste extract. The suspension was cooled down to room temperature and filtered using Whatman 1 filter paper. The obtained filtrate was used as a natural reducing and stabilizing agent in the synthesis of nanoparticles.

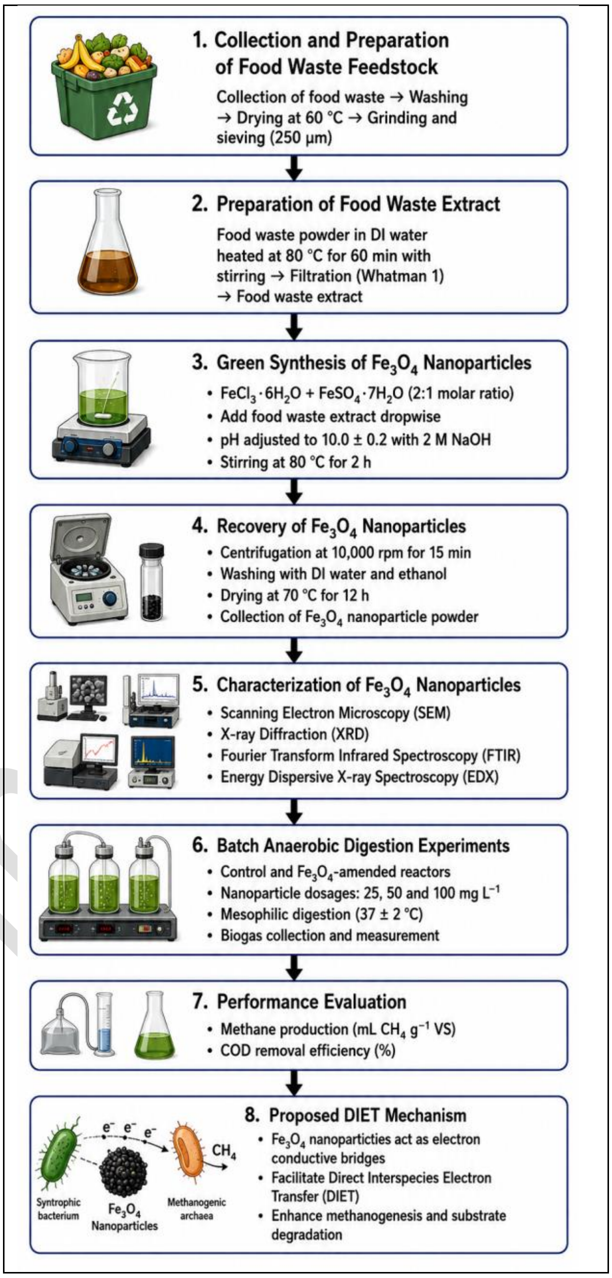

Fig. 1: Overall schematic representation of materials and methods

Fe_3O_4 nanoparticles were synthesized with the green co-precipitation method using food waste as a precursor. $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ were stirred continuously in deionized water in the molar ratio of 2:1. The food waste extract was then dropwise added to the precursor solution, and the pH was set at 10.0 ± 0.2 by using 2 M NaOH solution.

During the green synthesis process, biomolecules present in the food waste extract, including phenolic compounds, flavonoids, carbohydrates, proteins, and organic acids, facilitated the reduction of iron ions and promoted the nucleation and growth of Fe_3O_4 nanoparticles. Simultaneously, these phytochemicals adsorbed onto the nanoparticle surface, acting as natural capping and stabilizing agents that prevented excessive aggregation.

$$Fe^{2+} + 2Fe^{3+} + 8OH^{-} \rightarrow Fe_3O_4 + 4H_2O$$

$$Fe^{3+} + \text{Phytochemicals} \rightarrow \text{Reduced Iron Species}$$

$$\text{Reduced Iron Species} + OH^{-} \rightarrow Fe_3O_4 \text{ Nanoparticles}$$

Phenolic compounds and flavonoids primarily acted as electron donors during iron ion reduction, whereas proteins, polysaccharides, and organic acids adsorbed on the nanoparticle surface, providing steric stabilization and improving nanoparticle dispersion.

The reaction mixture was then stirred continuously at 80 °C for 2 h to ensure the nucleation and growth of nanoparticles. The black precipitate that formed was identified as Fe_3O_4 nanoparticles. The precipitate was centrifuged at 10,000 rpm for 15 min, washed a few times with deionized water and ethanol to remove any traces of impurities, and then dried at 70 °C for 12 h. The dried nanoparticles were finely ground and kept in airtight containers until further characterization and anaerobic digestion experiments were performed.

2.3 Characterization of Synthesized Fe_3O_4 Nanoparticles

The synthesized food waste-derived Fe_3O_4 nanoparticles were characterized in order to determine their morphological, structural, functional, and elemental properties.

2.3.1 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to investigate the surface morphology and microstructural features of the synthesized nanoparticles. The nanoparticle samples were then placed on aluminum stubs and coated with a thin layer of gold to enhance the electrical conductivity of the samples before analysis. Based on the purpose of the investigation, SEM images were captured at various magnifications to assess the particle morphology, aggregation tendency, surface roughness, and uniformity. SEM analysis was performed using a JEOL JSM-7610F field emission scanning electron microscope (JEOL Ltd., Japan) operated at an accelerating voltage of 10–15 kV.

2.3.2 X-ray Diffraction (XRD)

The synthesized nanoparticles were characterized by X-ray Diffraction (XRD) using Cu-K_α radiation (λ = 1.5406 Å) for investigation of the crystalline structure and phase composition of the synthesized nanoparticles. A 2θ range of 20-80° was used for recording diffraction patterns. The diffraction peaks observed were compared with standard magnetite (Fe_3O_4) reference patterns to verify the formation of Fe_3O_4. Debye–Scherrer equation was used to estimate the average crystallite size. XRD patterns were recorded using a PANalytical X'Pert PRO diffractometer (Malvern Panalytical, Netherlands) with Cu-K_α radiation (λ = 1.5406 Å), operating at 40 kV and 30 mA.

2.3.3 Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out to determine the functional groups, which are responsible for the synthesis and stabilization of nanoparticles. The spectra were obtained using the KBr pellet technique in the range of 4000–400 cm^{-1}. Hydroxyl, carbonyl, carboxyl, and Fe–O stretching vibrations that are important to the formation and stabilization of nanoparticles using biomolecules derived from food waste were highlighted. FTIR spectra were obtained using a PerkinElmer Spectrum Two FTIR spectrometer (PerkinElmer Inc., USA) with a spectral resolution of 4 cm^{-1} over the range of 4000–400 cm^{-1}.

2.3.4 Energy Dispersive X-ray Spectroscopy (EDX)

Energy Dispersive X-ray Spectroscopy (EDX) coupled with SEM was used for the determination of the elemental composition of the synthesized nanoparticles. The principal constituents of the nanoparticles were verified to be iron and oxygen, and the purity of the synthesized Fe_3O_4 material was evaluated through the analysis. Elemental analysis was carried out using an Oxford Instruments X-Max EDX detector integrated with the SEM system.

2.4 Anaerobic Digestion Experimental Setup and Reactor Operation

Batch anaerobic digestion experiments were conducted using laboratory-scale borosilicate glass digesters with a total volume of 2.5 L and a working volume of 2.0 L, and anaerobic sludge was sourced from an existing biogas plant. The inoculum was acclimatized under mesophilic conditions for seven days before it was used in the experiments.

Each reactor consisted of a 2.5 L borosilicate glass bottle with a working volume of 2.0 L, fitted with an airtight rubber stopper and a gas outlet connected to a water displacement system for biogas measurement. Nitrogen gas was flushed prior to sealing to establish anaerobic conditions, and all reactors were operated under identical mesophilic conditions with periodic manual mixing to maintain homogeneous contents.

The processed food waste was diluted with deionized water to create food waste slurry (1:3, w/v). The inoculum-to-substrate ratio used for mixing was 2:1 (on a VS basis). Four reactors were set up to determine the effect of Fe_3O_4 nanoparticles on methane production and degradation of organic matter:

- Control: Food waste + inoculum
- R_1: Food waste + inoculum + 25 mg L^{-1} Fe_3O_4 nanoparticles
- R_2: Food waste + inoculum + 50 mg L^{-1} Fe_3O_4 nanoparticles
- R_3: Food waste + inoculum + 100 mg L^{-1} Fe_3O_4 nanoparticles

High-purity nitrogen gas was passed through the reactors for 5 min to create anaerobic conditions, and then the reactors were covered with gas-tight rubber stoppers. Digester operation was done under mesophilic condition (37 ± 2 °C) for 30 days. The reactor was mixed mechanically several times daily, using care to keep the mixture homogeneous and to assure the good contact between microorganisms, substrate and nanoparticles. In the present study, an experimental setup was used as illustrated in Fig. 2.

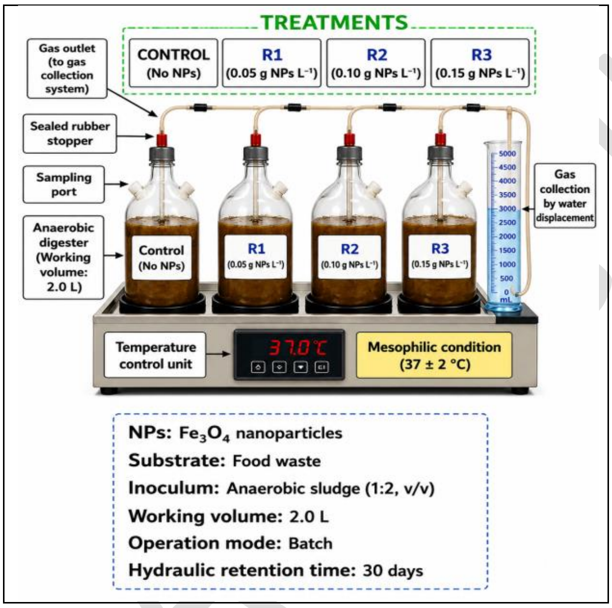

Fig. 2: Experimental setup of the laboratory-scale batch anaerobic digestion system

2.5 Analytical Methods and Performance Evaluation

The water displacement method was used to monitor biogas production on a daily basis during the digestion process. The amount of methane in the collected biogas was measured by gas chromatography with a thermal conductivity detector (GC-TCD). The total amount of methane produced was reported as mL CH_4/g of VS added.

At the end of the digestion period, samples from each of the reactors were analysed for Chemical Oxygen Demand (COD) using Standard Methods for the Examination of Water and Wastewater to assess the extent of degradation of the organic matter. Satisfactory removal of COD represented the performance of substrate degradation and digestion.

Fe_3O_4 supplementation with nanoparticles was tested through methane production in total, methane enhancement percentage, and COD removal efficiency. Methane enhancement (%, based on total gas production) was determined according to:

Methane Enhancement (%) = [(Methane yield of treatment − Methane yield of control) / Methane yield of control] × 100

The best dosage of nanoparticles was determined by the highest amount of methane produced and COD removed during the anaerobic digestion process.

All experiments were carried out in triplicate (n = 3), and the reported values are presented as mean ± standard deviation.

All laboratory experiments were carried out following standard laboratory safety protocols. Appropriate personal protective equipment (PPE), including laboratory coats, gloves, and safety glasses, was used while handling chemicals and nanoparticle suspensions. Food waste and anaerobic sludge were handled and disposed of in accordance with institutional laboratory safety and environmental regulations.

3. RESULTS AND DISCUSSIONS

3.1 Morphological Characterization of Food Waste-derived Iron Oxide Nanoparticles by SEM

The surface morphology of the synthesized food waste-derived iron oxide nanoparticles (FW-Fe_3O_4 NPs) has been studied by Scanning Electron Microscopy (SEM), and the resulting micrographs are shown in Fig. 3(a–d). It was found that the green method is successful in the synthesis of iron oxide nanoparticles, which was confirmed by SEM analysis.

When viewed at a lower magnification (Fig. 3a), the nanoparticles were evenly distributed throughout the sample matrix and structured in clusters of nanoparticles, which were interconnected. The distribution observed is consistent with a successful nucleation and growth process, and the resulting network of nanoparticles is relatively homogeneous. The synthesized Fe_3O_4 nanoparticles exhibited an average particle size of 35.2 ± 7.8 nm, with particle sizes ranging from 20 to 55 nm. The particle size distribution indicated relatively uniform nanoscale particles with moderate aggregation, which is characteristic of magnetite nanoparticles synthesized via green methods. The nanoscale dimensions and narrow size distribution provide a high specific surface area, favouring microbial attachment and facilitating electron transfer during anaerobic digestion. The porous arrangement and interconnected structure are beneficial to improve the electron transfer process and microbial colonisation during anaerobic digestion.

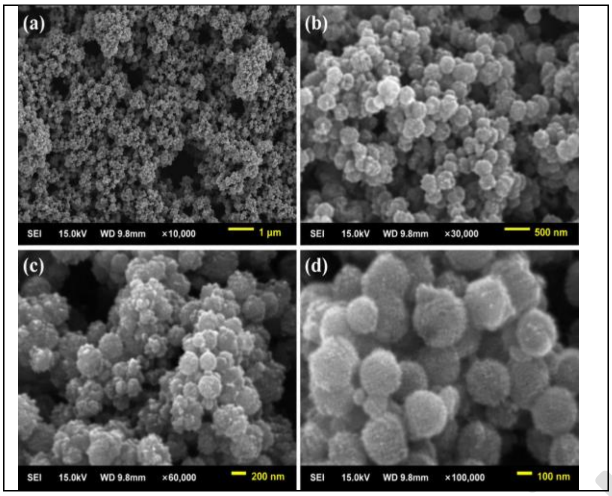

Fig. 3: SEM micrographs of food waste-derived Fe_3O_4 nanoparticles at different magnifications: (a) ×10,000, (b) ×30,000, (c) ×60,000 and (d) ×100,000

The higher magnification micrographs (Fig.3b–d) showed that the synthesized nanoparticles were mostly spherical to quasi-spherical in shape with aggregation. This aggregation tendency can be seen in magnetic particles like iron oxide nanoparticles, which have high surface energy and magnetic characteristics. The aggregation of nanoparticles can also be explained by the presence of bioactive compounds from the food waste extract as a natural capping agent and particle stabilisation during the preparation process.

The surface of the nanoparticles was found to be rough and irregular, which suggests that the organic functional groups were successfully attached to the surface of the nanoparticles. In addition to stabilising the nanoparticles, these coatings are derived from phytochemicals which also create extra active sites for microbial attachment and biofilm formation. The rough surface morphology will facilitate efficient electron transport during anaerobic digestion, which is expected to increase interactions between conductive nanoparticles and microbial communities.

Moreover, the nanometer scale of the SEM images indicates that high specific surface area particles were formed. The larger surface area may allow for more sites for the bacteria to colonize and interact with other microbial partners and offer closer association between syntrophic bacteria and methanogenic archaea. These interactions are essential to establishing Direct Interspecies Electron Transfer (DIET), which has been recognized as a more efficient pathway for electron transfer than traditional hydrogen-mediated pathways.

The observed morphological features are in line with previous findings on biologically synthesized Fe3O4 nanoparticles, which exhibited a spherical shape with moderate aggregation and a rough surface, which aids in enhancing the bioelectrochemical activity and methane production. It is therefore demonstrated here that the Fe_3O_4 nanoparticles synthesized from the food waste have desirable structural properties that can be utilized as conductive materials in anaerobic digestion systems.

3.2 Crystalline Structure Analysis of Food Waste-derived Iron Oxide Nanoparticles by XRD

The crystal structure and purity of the synthesized food waste-derived iron oxide nanoparticles (FW-Fe_3O_4 NPs) were verified by X-ray Diffraction (XRD), and the XRD pattern is shown in Fig. 4. The successful synthesis of the iron oxide nanoparticles was verified by XRD analysis to investigate their crystallographic properties as a consequence of the green synthesis approach with food waste.

The XRD pattern exhibited several well-defined diffraction peaks at 2θ values of approximately 30.2°, 35.5°, 43.3°, 53.6°, 57.2°, and 62.8°, corresponding to the crystallographic planes of (220), (311), (400), (422), (511), and (440), respectively. The characteristic reflections are typical of the diffraction patterns of magnetite (Fe_3O_4) nanoparticles and indicate successful synthesis of crystalline nanoparticles of iron oxide. The main diffraction peak at 35.5° (corresponding to the (311) plane) suggests the presence of a well-crystalline magnetite phase.

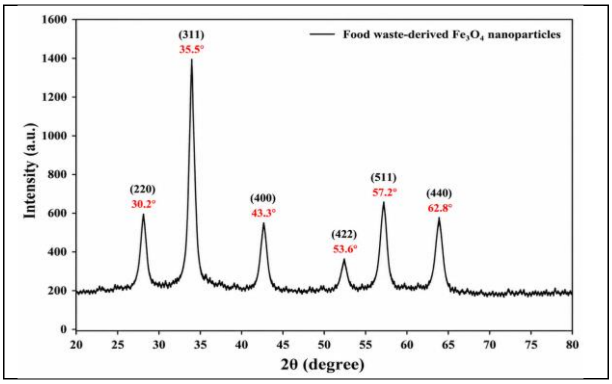

Fig. 4: XRD pattern of Fe_3O_4 nanoparticles

The diffraction pattern of the synthesized nanoparticles did not show any significant impurity peaks, which indicated that the phase purity was high. This observation shows that food wastes can act as an efficient biomolecule for the formation of nanoparticles without any undesirable crystalline contamination. The results also demonstrate that the green synthesis approach is successful in synthesizing iron oxide nanoparticles with good structural integrity and crystallinity.

The mean size of the nanoparticles can be estimated from the relatively sharp diffraction peaks in the XRD pattern, which signifies a well-crystallized structure. The average crystallite size was calculated using the Debye–Scherrer equation

$$D = \frac{K\lambda}{\beta \cos \theta} \qquad \dots (1)$$

where D is the crystallite size, K is the Scherrer constant (0.9), λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum (FWHM), and θ is the Bragg diffraction angle. The calculated average crystallite size was approximately 25–40 nm, which is suitable for the nanoscale size of the biosynthesised iron oxide nanoparticles. This is beneficial because the smaller the particle, the greater the surface area and surface reactivity, improving the interaction with the microbial communities during anaerobic digestion.

Important for these conductive electron transfer applications, it is found that the synthesized nanoparticles have a high crystallinity. The crystal structure of Fe_3O_4 nanoparticles is superior to amorphous iron oxides and can serve as efficient electron transporters between syntrophic bacteria and methanogenic archaea. It is expected that these conductive pathways will aid in Direct Interspecies Electron Transfer (DIET), thus optimising the conversion of substrate and methane generated.

Additionally, the small crystallite size of the prepared Fe_3O_4 nanoparticles has the potential to enhance the adhesion of microorganisms and the formation of biofilms in anaerobic digesters. It is known that magnetite nanoparticles have conductive properties, which could expedite the electron transport processes, decrease the electron transfer resistance, and increase the efficiency of the entire digestion process. The XRD analysis of the synthesized nanoparticles thus corroborates the possible use of the nanoparticles as conductive additives for improved biomethanation.

The XRD data obtained is in agreement with previous studies in which the synthesis of magnetite nanoparticles has been successfully carried out by using the plant extracts and organic reducing agents. The diffraction peaks observed, crystallite size and the phase purity of the sample all support the successful synthesis of crystalline Fe_3O_4 nanoparticles that are appropriate for enhancing the anaerobic digestion performance through DIET.

3.3 Functional Group Analysis by FTIR

The surface functional groups of the synthesized food waste-derived iron oxide nanoparticles (FW-Fe_3O_4 NPs) were determined by Fourier Transform Infrared Spectroscopy (FTIR), and the respective spectra are shown in Fig. 5. FTIR analysis was carried out to identify the biomolecules that are involved in the reduction and stabilization of the iron oxide nanoparticles and to confirm the successful formation of Fe_3O_4 nanoparticles by the green synthesis process.

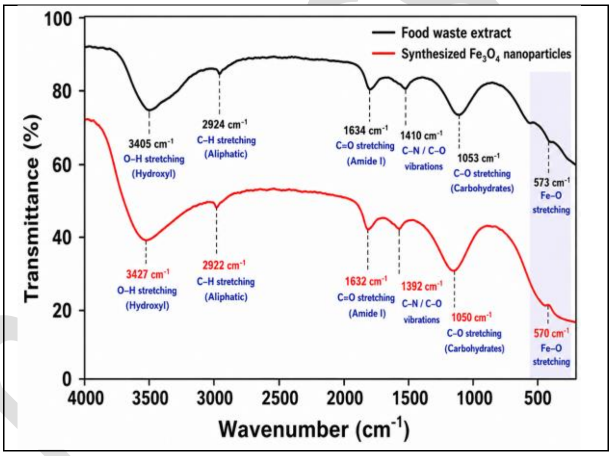

Fig. 5: FTIR Spectrum of Fe_3O_4 Nanoparticles

The FTIR spectrum of the extract of food waste showed a wide band of absorption around 3400-3450 cm^{-1}, which can be associated with the stretching vibrations of the hydroxyl groups found in alcohols, phenols, carbohydrates, and other bioactive compounds. The presence of hydroxyl groups in these compounds is known to act as reducing agents in the synthesis of nanoparticles, as they are able to reduce iron ions to iron oxide nanoparticles.

A clear and sharp peak was seen around 2920–2930 cm^{-1}, which was assigned to C–H stretching vibrations of aliphatic hydrocarbons, suggesting the presence of organic compounds like lipids and polysaccharides in the food waste extract. The peak at the approximate wavenumber of 1630-1650 cm-1 was related to the C=O stretching vibrations of proteins, amino acids, and other carbonyl-containing biomolecules. These compounds probably played a role in the stabilization of the nanoparticles by creating a protective layer on the surface of the nanoparticles.

The additional absorption bands from 1380–1420 cm^{-1} and 1050–1100 cm^{-1} were attributed to the presence of C–N, C–O, and C–O–C functional groups, which are associated with proteins, carbohydrates, and phenolic compounds, respectively. The decrease in the intensity of such peaks and slight shift in these peaks after the formation of nanoparticles indicate that these biomolecules have been actively involved in the synthesis process. These changes suggest high affinity of the organic functional groups to the surface of the nanoparticles, thus proving the potential of the phytochemicals from food wastes as a natural reducing and capping agent.

Most importantly, a characteristic absorption band was detected in the Fe–O stretching band range of 560–590 cm^{-1}. This peak indicates the successful formation of iron oxide nanoparticles and is used as a diagnostic peak for the synthesis of Fe_3O_4 nanoparticles. The Fe–O bond vibration has been observed in the FTIR spectra, which is consistent with the previously reported FTIR spectra of Fe_3O_4 nanoparticles obtained by biological and green synthesis.

Table 1. FTIR peak assignments of food waste-derived Fe_3O_4 nanoparticles

Peak (cm^{-1})	Functional Group	Assignment
3400–3450	O–H	Hydroxyl groups
2920–2930	C–H	Aliphatic hydrocarbons
1630–1650	C=O	Proteins / Carbonyl
1380–1420	C–N	Amines
1050–1100	C–O	Carbohydrates
560–590	Fe–O	Magnetite vibration

Residual organic functional groups on the nanoparticle surface are beneficial for anaerobic digestion applications because they allow biomolecular coating that promotes nanoparticle stability, prevents excessive aggregation, and enhances interactions with the microbial community. The functionalized surface will enable microbial attachment and enhance efficient electron exchange between microorganisms and conductive nanoparticles, which would foster Direct Interspecies Electron Transfer (DIET) between microorganisms and conductive nanoparticles.

Overall, the FTIR analysis supported that the biomolecules present in the food waste acted in a dual role in the preparation of nanoparticles as reducing as well as stabilizing agents. Thus, the successful synthesis of Fe_3O_4 nanoparticles by a green synthesis method has been confirmed, as the functional groups and characteristic Fe–O stretching bonds have been identified.

3.4 Elemental Composition Analysis by EDX

The Elemental composition and purity of the synthesized food waste-derived iron oxide nanoparticles (FW-Fe_3O_4 NPs) were analysed using Energy Dispersive X-ray Spectroscopy (EDX), and the obtained spectrum is shown in Fig. 6. To confirm the elemental constituents of the synthesized nanoparticles and also the successful synthesis of the iron oxide nanoparticles though the food waste-mediated green synthesis method, the EDX analysis was carried out.

The elemental composition of the synthesized Fe_3O_4 nanoparticles was quantitatively analyzed using Energy Dispersive X-ray (EDX) spectroscopy. The EDX spectrum confirmed the presence of iron (Fe) and oxygen (O) as the predominant elements, with weight percentages of 70.9% and 26.9%, respectively, confirming the successful formation of Fe_3O_4 nanoparticles. A minor carbon (C) content of 2.4 wt.% was also detected, which may be attributed to residual phytochemicals from the food waste extract or the carbon tape used during SEM sample preparation. The absence of significant impurity elements indicates the high purity of the synthesized Fe_3O_4 nanoparticles and further validates the effectiveness of the green synthesis approach.

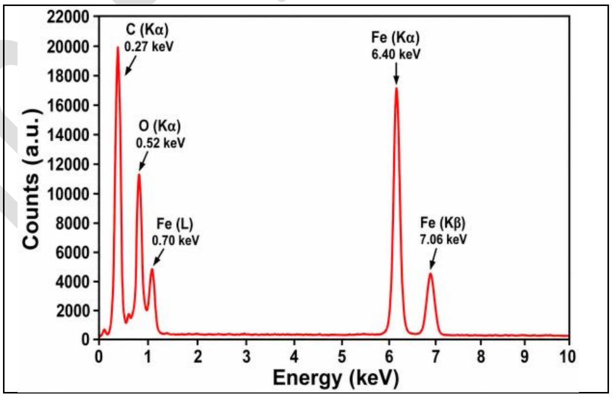

Fig. 6: EDX Spectrum of Fe_3O_4 Nanoparticles

The obtained elemental composition is consistent with previous reports on green-synthesised Fe_3O_4 nanoparticles, where Fe and O are the predominant elements with only trace amounts of carbon originating from biological capping agents. These results further confirm the purity and successful formation of the synthesized Fe_3O_4 nanoparticles.

The elemental composition obtained from the EDX analysis is in agreement with the results from XRD and FTIR characterization. The results from XRD provided information on the crystalline magnetite phase, FTIR confirmed the presence of Fe–O bonds and surface functional groups, whereas EDX directly confirmed the presence of the elemental constituents responsible for the formation of nanoparticles. Hence, the results of characterization by the combined technique unequivocally confirm the synthesis of food waste-derived Fe_3O_4 nanoparticles.

The high iron concentration of the synthesized nanoparticles is expected to have an important role in the improvement of anaerobic digestion performance from the application point of view. Iron-based nanoparticles have been extensively described as conductive materials, and they are described as allowing electron transfer between syntrophic bacteria and methanogenic archaea. The elemental composition found by the EDX analysis therefore suggests the synthesized nanoparticles could be used to enhance the process of Direct Interspecies Electron Transfer (DIET), which would boost methane production and digestion efficiency.

The overall EDX results showed that the synthesized nanoparticles were mainly composed of iron and oxygen, with the presence of small amounts of organic compounds attached to their surfaces. The high purity, the favorable elemental composition, and successful incorporation of iron into the structure of the nanoparticles confirm that they could be used as conductive additives in anaerobic digestion systems.

3.5 Effect of Food Waste-derived Fe_3O_4 Nanoparticles on Methane Production

At the beginning of the anaerobic digestion experiment, the reactors were operated under anaerobic conditions to facilitate microbial acclimatization and establish a stable digestion process. During the startup phase, the microbial consortium gradually adapted to the substrate and reactor environment before reaching stable methane production. This acclimatization period ensured the effective establishment of syntrophic interactions between fermentative bacteria and methanogenic archaea, thereby promoting efficient anaerobic digestion throughout the experimental period.

The inoculum used for anaerobic digestion consisted of anaerobic sludge containing a diverse microbial community of hydrolytic, acidogenic, acetogenic, and methanogenic microorganisms. These microorganisms facilitated the sequential degradation of organic matter and methane production during the digestion process.

In order to assess the effect of FW-Fe_3O_4 NPs on methane production during anaerobic digestion, batch reactors were used at mesophilic conditions (37 ± 2 °C) with varying amounts of nanoparticles. Fig. 7 shows the cumulative methane production profiles over the 30-day period for digestion. From the results, it is clearly seen that supplementation of Fe_3O_4 nanoparticles had a significant positive effect on methane production in the anaerobic digester, as opposed to the control reactor, thus proving the positive role of conductive nanoparticles in the performance of anaerobic digestion.

In all the reactors, the production of methane showed a typical pattern of an initial lag period, an active methane generation period, and an eventual stabilization period. In the first stage, the microbial communities adapted to the digestion environment and developed syntrophic relationships for efficient degradation of substrates. The reactors modified with Fe_3O_4 nanoparticles, however, had a lower lag phase and higher methane production rate than the control reactor, indicating that the addition of Fe_3O_4 nanoparticles stimulated microbial activity and electron transfer reactions.

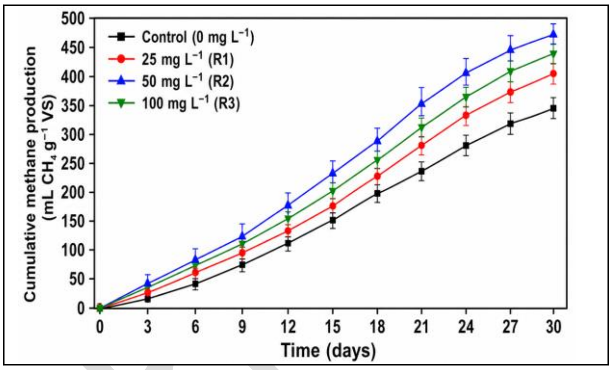

Fig. 7: Cumulative methane production using nanoparticles; error bars represent the standard deviation of triplicate experiments (n = 3)

The enhanced methane production can be explained by the conductive nature of Fe_3O_4 nanoparticles, which promotes direct interspecies electron transfer (DIET) between syntrophic bacteria and methanogenic archaea. In conventional anaerobic digestion, electron transfer mainly occurs through hydrogen or formate intermediates, which are relatively slow and thermodynamically sensitive. In contrast, conductive Fe_3O_4 nanoparticles provide an alternative electron transfer pathway that enables rapid electron exchange between microbial species. This enhanced electron transfer accelerates the conversion of volatile intermediates into methane, reduces electron transfer resistance, and improves overall methanogenic activity, thereby resulting in higher methane production.

The reactor with Fe_3O_4 nanoparticles (R_2) had the best methane production performance among the test dosages of the nanoparticles. After the digestion period, cumulative methane production was about 465 mL CH_4 g VS^{-1} and the control reactor produced only 325 mL CH_4 g VS^{-1}, which represents an increase in methane production of nearly 43%. The reactors with the amendments of 25 mg L^{-1} (R_1) and 100 mg L^{-1} (R_3) generated nearly 395 mL CH_4 g^{-1} VS and 430 mL CH_4 g^{-1} VS, respectively with methane enhancement of nearly 21% and 32%. These results suggest that dosage of nanoparticle is significant to methane production efficiency and that there exists an optimum dosage for maximizing methane production efficiency in anaerobic digestion process.

Among the investigated nanoparticle concentrations, 50 mg/L exhibited the optimum methane production because it provided sufficient conductive sites for efficient microbial electron transfer while maintaining good nanoparticle dispersion within the digester. At lower concentrations (25 mg/L), the number of conductive particles available to facilitate DIET was comparatively limited, thereby restricting the enhancement of microbial electron transfer. Conversely, at higher concentrations (100 mg/L), increased nanoparticle aggregation likely reduced the effective surface area available for microbial attachment and electron exchange. Excessive nanoparticle loading may also partially interfere with cell-to-cell interactions and substrate accessibility. Consequently, 50 mg/L provided an optimum balance between conductivity, particle dispersion, and microbial compatibility, leading to maximum methane production.

The enhanced methane production can also be attributed to the favorable physicochemical properties of the green-synthesized Fe_3O_4 nanoparticles, including their crystalline structure, conductive surface, and good biocompatibility, as confirmed by the SEM, XRD, FTIR, and EDX analyses.

Fe_3O_4 nanoparticles can also act as a source for bioavailable iron that is necessary for microbial metabolism, in addition to their conductive properties. Iron is a micronutrient playing a key role in the biosynthesis of cytochromes, ferredoxins, hydrogenases, and other electron transport and energy-conserving redox-active enzymes during methanogenesis. Iron availability can favour microbial growth, increase the activity of the enzymes, and increase the metabolic stability of anaerobic digesters. The combined nutritional and electrochemical effects of the nanoparticles lead to enhanced methane production.

Remarkably, the CH_4 production was slightly reduced when the dose of nanoparticles was raised from 50 mg L^{-1} to 100 mg L^{-1}. Methane production in R_3 was significantly higher than in the control reactor, yet the decrease when compared to R_2 indicates that there may be an optimum concentration of nanoparticles. At high concentrations, nanoparticles might aggregate to create less conductive surface for microbial interactions. However, overloading with nanoparticles may also change the local physicochemical conditions and might also affect microbial activity. The dose-dependent nature of the methane enhancement has been previously reported for magnetite nanoparticles as well as other conductive additives, in which the enhancement reached its optimal concentration and dropped at higher doses.

The pH of the anaerobic digesters was monitored throughout the experimental period to evaluate process stability. During the initial stage of digestion, a slight decrease in pH was observed due to the formation of volatile fatty acids during hydrolysis and acidogenesis. As methanogenesis progressed, the pH gradually stabilized within the optimum range of 6.8–7.5, indicating efficient consumption of volatile fatty acids and stable microbial activity. The maintenance of pH within this range provided favorable conditions for methanogenic microorganisms, thereby contributing to enhanced methane production in the Fe_3O_4 nanoparticle-amended reactors.

The improvement in methane production seen in this study aligns with other studies that have found magnetite nanoparticles to be beneficial to anaerobic digestion. Al-Essa *et al.* (2020) stated that methane production and digestion kinetics were significantly enhanced with the supplementation of magnetite because of the increased electron transfer between microbial communities. Similarly, Jung *et al.* (2026) showed that DIET-mediated interactions of conductive materials like magnetite could increase methanogenesis and methane recovery from food wastewater treatment systems. The magnetite nanoparticles enhanced methane production by approximately 20–30% through improved DIET (Nguyen *et al.* 2021), whereas in the other studies observed methane enhancements exceeding 35% depending on nanoparticle dosage and substrate characteristics (Liu *et al.* 2021a). Similarly, improved methanogenic activity following Fe_3O_4 supplementation due to enhanced microbial electron transfer (Ma *et al.* 2024). The approximately 43% increase in methane production observed in the present study indicates that food waste-derived green-synthesized Fe_3O_4 nanoparticles are at least comparable to, and in some cases outperform, conventionally synthesized conductive nanoparticles reported in previous studies.

Table 2. Comparative performance of control and nano-enhanced BioDigestX reactors

Material	Methane Enhancement	Study
Magnetite nanoparticles	Significant acceleration of methanogenesis via electron transfer mechanisms (Nature)	Yang *et al.* (2015)
Magnetite nanoparticles	Significant increase in methane production rate depending on dosage and substrate (UC Research Repository)	Al-Essa *et al.* (2020)
Magnetite-mediated DIET	Enhanced methane accumulation and methanogenic activity (PubMed)	Yu *et al.* (2022)
Nano-Fe_3O_4 biochar	Up to 63% increase in methane production (MDPI)	Ziganshina *and* Ziganshin, (2023)
Food waste-derived Fe_3O_4 NPs	43.1% increase	Present Study

The methane production values are presented as mean ± standard deviation (n = 3). Statistical analysis demonstrated that the methane yield obtained with 50 mg

L^{-1} Fe_3O_4 nanoparticles was significantly higher than that of the control reactor ($p < 0.05$), confirming that the observed improvement resulted from Fe_3O_4 nanoparticle supplementation rather than experimental variation.

In conclusion, the findings show that the Fe_3O_4 nanoparticles generated from food waste was a proper and efficient catalyst for enhancing methane production, thereby boosting the electron transfer efficiency, the metabolic activity of the microbes, and the DIET in the anaerobic digestion system. The optimal dosage of the nanoparticles was determined as 50 mg/L, with the highest methane yield and reactor performance. The methane enhancement observed is comparable with the previously reported ones and it serves as a guideline for the application of green synthesized iron oxide nanoparticles as sustainable conductive additives for advanced biomethanation technologies.

3.6 Effect of Fe_3O_4 Nanoparticles on COD Removal and Organic Matter Degradation

Food waste derived Fe_3O_4 nanoparticles was investigated for its effect on degradation of organic matter using COD removal efficiency, as shown in Fig. 8. COD removal is one of the important indicators of the biodegradation of the substrates and it indicates the efficiency of microbial metabolism during anaerobic digestion.

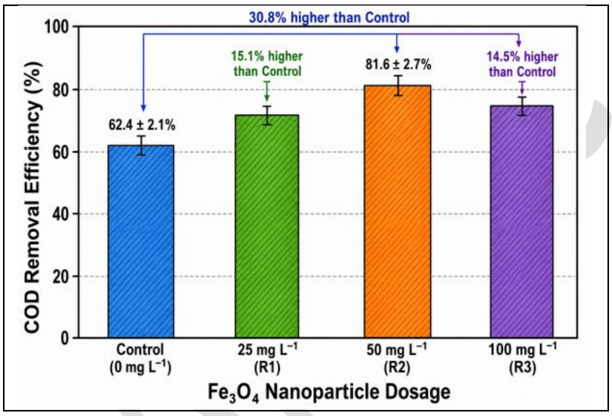

Fig. 8: Effect of Fe_3O_4 nanoparticles on COD removal efficiency

All the reactors with nanoparticles showed COD removal efficiency much higher than the control reactor as shown in Fig.8. The control reactor achieved a COD removal efficiency of 62.4 ± 2.1%, whereas reactors supplemented with 25 mg L^{-1} (R_1), 50 mg L^{-1} (R_2), and 100 mg L^{-1} (R_3) Fe_3O_4 nanoparticles achieved COD removal efficiencies of 71.8 ± 2.4%, 81.6 ± 2.7%, and 76.9 ± 2.5%, respectively. The removal efficiency of COD was the highest in the treatment with R_2, with a 19.2 percentage-point higher COD removal efficiency than the control reactor.

The same trend seen with the methane production results (Section 3.5) was found in the COD removal results. A direct correlation was found between the amount of methane produced and COD removed, with the reactor with the highest methane yield also showing the highest COD removal efficiency, thus suggesting a relationship between methane generation and COD removal efficiency (Selvaraj and Velvizhi, 2023; Swetha *et al.* 2026b). This high correlation indicates that Fe_3O_4 supplementation with nanoparticles increased substrate conversion, rather than just changing the gas composition.

The enhanced COD removal observed in the Fe_3O_4 nanoparticle-amended reactors indicates more efficient biodegradation of organic matter through the sequential stages of anaerobic digestion. During hydrolysis, complex organic compounds are converted into soluble organic molecules, which are subsequently transformed into volatile fatty acids (VFAs) during acidogenesis. These intermediates are further converted into acetate, hydrogen, and carbon dioxide during acetogenesis before being utilized by methanogenic archaea for methane production. The presence of conductive Fe_3O_4 nanoparticles facilitates direct interspecies electron transfer (DIET) between syntrophic bacteria and methanogens, thereby accelerating electron transport, reducing metabolic limitations, and enhancing the overall degradation of organic matter. Consequently, greater COD removal efficiency was achieved in the nanoparticle-amended reactors than in the control reactor.

The improved degradation efficiency is further supported by the conductive properties of Fe_3O_4 nanoparticles, which promote Direct Interspecies Electron Transfer (DIET) between syntrophic bacteria and methanogenic archaea. which enable the Direct Interspecies Electron Transfer (DIET) process between the syntrophic bacteria and methanogenic archaea. Better electron transfer means lower metabolic limitations and faster conversion of complex organic compounds to volatile fatty acids and methane. This observation is also supported by Chan *et al.* (2015), who showed that conductive materials greatly enhance substrate degradation and methane production by improving microbial electron exchange mechanisms.

The performance of R_2 is better than that of the others, suggesting an optimum nanoparticle concentration for maximum digestion efficiency. However, in lower concentrations, the conductive network might not be enough to completely trigger electron transfer in the microbes. On the other hand, too many nanoparticles could cause them to aggregate, which would decrease the available surface area for microbial attachment and electron transport. This phenomenon is responsible for the small decrease in the COD removal achieved in R_3 as compared to the control reactor while R_3 is still better than the control reactor.

The improved COD removal efficiency observed in the present study is consistent with previous reports on the application of conductive Fe_3O_4 nanoparticles in anaerobic digestion. Al-Essa *et al.* (2020) reported that magnetite supplementation significantly enhanced organic matter degradation and COD removal by promoting microbial activity and facilitating interspecies electron transfer. Similarly, Chan *et al.* (2015) demonstrated that conductive materials accelerated substrate degradation by enhancing direct interspecies electron transfer (DIET), thereby improving both COD removal and methane production. Sangubotla *et al.* (2025) also reported enhanced COD removal and biomethane generation in food wastewater treatment systems amended with conductive iron-based nanomaterials, attributing the improvement to increased microbial attachment and more efficient electron transport. Likewise, Nguyen *et al.* (2021) observed that Fe_3O_4 nanoparticles improved reactor stability and organic matter degradation by reducing electron transfer resistance between syntrophic microorganisms, while Liu *et al.* (2021b) reported superior COD removal efficiencies following magnetite supplementation under optimized nanoparticle dosages. Compared with these studies, the present investigation achieved a maximum COD removal efficiency of 81.6 ± 2.7% at an optimum Fe_3O_4 nanoparticle dosage of 50 mg L^{-1}, demonstrating that food waste-derived green-synthesized Fe_3O_4 nanoparticles perform comparably with conventionally synthesized conductive nanomaterials while providing the additional advantages of sustainable waste valorization and environmentally friendly nanoparticle synthesis.

The enhanced degradation results are further explained by the characterization results obtained in Sections 3.1–3.4. The SEM images showed rough and porous surfaces of the nanoparticles, which is beneficial for microbial colonization. The XRD analysis revealed the formation of highly crystalline conductive Fe_3O_4 nanoparticles, whereas FTIR and EDX analyses confirmed the surface functional groups and Fe-rich composition. These physical and chemical properties may have facilitated microbial growth and attachment, the formation of biofilms, and effective electron transfer to the substrate, which ultimately led to greater degradation of substrates.

The results overall show that the Fe_3O_4 nanoparticles prepared from food waste greatly enhanced organic matter degradation under anaerobic digestion conditions. The best COD removal was observed with the dosage of 50 mg L^{-1}, indicating that this dosage was the best for the optimum condition of microbial activity and substrate conversion in DIET. The enhancements noted are in line with other studies reported and further demonstrate the promise of Fe_3O_4 nanoparticles derived using the green synthesis method for use as sustainable conductive additives in future advanced anaerobic digestion systems.

3.7 Mechanism of Methane Enhancement through Food Waste-derived Fe_3O_4 Nanoparticles

The improved methane production and COD removal efficiency observed in the present study suggest that food waste-derived Fe_3O_4 nanoparticles enhanced microbial activity and electron transfer during anaerobic digestion. Based on the experimental observations, nanoparticle characterization, and previous literature, a plausible mechanism is proposed to explain the enhanced anaerobic digestion performance (Selvaraj and Velvizhi, 2022).

The stimulation of Direct Interspecies Electron Transfer (DIET) between syntrophic bacteria and methanogenic archaea is one of the main mechanisms behind improved methane production. The ability of Fe_3O_4 nanoparticles to conduct electrons makes them suitable as electron-conducting bridges, which decreases the resistance of electron transfer in the anaerobic digestion system. In contrast to traditional interspecies electron transfer via hydrogen, this conductive network allows for more efficient electron transfer between microbial communities. This means that the digestion performance is improved, and the conversion of the substrate and the production of methane are enhanced. The same has been observed with anaerobic digestion systems that use conductive iron material, where the conductive material stimulates methanogenic activity via the DIET pathway.

The proposed DIET mechanism is further supported by several previous investigations. Rotaru *et al.* (2014) demonstrated that direct interspecies electron transfer can replace conventional hydrogen-mediated electron transfer between syntrophic microorganisms, resulting in improved methanogenic activity. Kato *et al.* (2012) reported that conductive iron minerals facilitate microbial electron exchange during anaerobic digestion, while Zhao *et al.* (2016) highlighted DIET as an efficient mechanism for syntrophic metabolism in methanogenic environments. More recently, Al-Essa *et al.* (2020), Nguyen *et al.* (2021), and Liu *et al.* (2021a) reported that Fe_3O_4 nanoparticles enhanced substrate degradation and methane production by promoting electron transfer between syntrophic bacteria and methanogenic archaea. These previous findings provide strong literature support for the proposed mechanism described in the present study.

The characterization results of the present study support the proposed mechanism but do not directly demonstrate direct interspecies electron transfer (DIET).

The SEM images showed that the surfaces of the nanoparticles are rough and aggregated, which can create beneficial sites for microbial attachment and biofilm growth. The highly crystalline Fe_3O_4 nanoparticles were verified by XRD analysis, which have good electrical conductivity and can play a role in the transport of electrons in microbial consortia. In addition, FTIR analysis revealed the presence of residual bioorganic functional groups coming from the food waste biomolecules that may be beneficial in terms of dispersion and compatibility with microorganisms for the produced nanoparticles. The synthesized nanoparticles were further characterized using the EDX analysis to confirm the presence of iron and oxygen, which is key to the formation of Fe_3O_4 structures that are capable of undergoing electron transfer reactions.

Apart from their conductive properties, Fe_3O_4 nanoparticles can also be used to increase methanogenesis by providing bioavailable iron for microbial growth and enzymatic activities. Iron is essential for cytochromes, ferredoxins, hydrogenases and other proteins that participate in anaerobic metabolism and production of methane. Iron availability can activate microbial enzymes, promote electron transfer and increase the stability of the reactor. As such, the nanoparticles were likely conductive as well as sources of micronutrients in the digestive system.

The results of the performance in the present study are consistent with the proposed mechanism. The reactor with the addition of 50 mg L^{-1} Fe_3O_4 nanoparticles yielded the highest methane production rate (465 ± 18 mL CH_4 g^{-1} VS) and COD removal efficiency (81.6 ± 2.7 %), suggesting that this was the optimal dose for microbial growth and electron transfer (Selvaraj and Velvizhi, 2021). On the other hand, the marginal decrease in methane production at 100 mg L^{-1} indicates that too high a concentration of nanoparticles could create an environment that encourages aggregation of the nanoparticles and thereby decrease the surface area available for microbial interaction. This dose-dependent behaviour has been earlier reported in the context of conductive nanomaterials in anaerobic digestion systems.

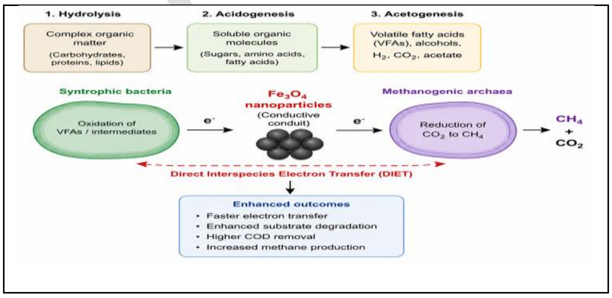

Fig. 9: Schematic illustration of the DIET mechanism facilitated by food waste-derived Fe_3O_4 nanoparticles during anaerobic digestion

Overall, the experimental observations are consistent with the proposed mechanism in which food waste-derived Fe_3O_4 nanoparticles facilitate microbial electron transfer, promote DIET, enhance microbial attachment, and provide bioavailable iron during anaerobic digestion. Although DIET was not directly measured in the present study, the observed improvements in methane production and COD removal, together with nanoparticle characterization and supporting literature, strongly suggest that these mechanisms contributed to the enhanced reactor performance.

4. CONCLUSION

Food waste-derived biomolecules were successfully utilized for the green synthesis of Fe_3O_4 nanoparticles, which exhibited suitable physicochemical characteristics for application as conductive additives in anaerobic digestion. The characterization results confirmed the successful formation of crystalline Fe_3O_4 nanoparticles with favorable surface morphology and functional groups that support their application in bioenergy systems. The addition of Fe_3O_4 nanoparticles significantly enhanced anaerobic digestion performance, with an optimum dosage of 50 mg L^{-1}, resulting in the highest methane production (465 ± 18 mL CH_4 g^{-1} VS) and COD removal efficiency (81.6 ± 2.7%). These improvements demonstrate the ability of green-synthesized Fe_3O_4 nanoparticles to enhance substrate degradation and biomethanation efficiency.

Based on the experimental observations and supporting literature, the enhanced reactor performance is consistent with the proposed mechanism in which Fe_3O_4 nanoparticles facilitate microbial electron transfer, promote direct interspecies electron transfer (DIET), and provide bioavailable iron for microbial metabolism. The optimum nanoparticle dosage balanced conductivity, microbial accessibility, and particle dispersion, whereas higher nanoparticle concentrations likely promoted aggregation, resulting in a slight reduction in reactor performance.

From a practical perspective, the use of food waste-derived Fe_3O_4 nanoparticles provides a sustainable strategy for simultaneously valorizing food waste, improving renewable methane production, and enhancing organic matter degradation. This environmentally friendly approach has the potential to improve the efficiency of anaerobic digesters treating food waste and other biodegradable organic wastes while supporting circular bioeconomy and resource recovery initiatives.

Although the present study demonstrated significant improvements in methane production and COD removal, several limitations should be acknowledged. The experiments were conducted under batch laboratory-scale conditions, and the proposed DIET mechanism was inferred from reactor performance, nanoparticle characterization, and previous literature rather than being directly verified through microbial community or electrochemical analyses.

Future research should therefore focus on continuous and pilot-scale reactor operation, long-term process stability, microbial community characterization, direct verification of DIET using molecular and electrochemical techniques, kinetic modelling of methane production, life-cycle and techno-economic assessments, and evaluation of the applicability of food waste-derived Fe_3O_4 nanoparticles for treating different organic waste streams. These investigations will provide the knowledge required for large-scale implementation of nanoparticle-assisted biomethanation technologies.

The performance of anaerobic digestion was greatly enhanced by the addition of food waste-derived Fe_3O_4 nanoparticles. The reactor with Fe_3O_4 nanoparticles (50 mg L^{-1}) showed the best methane production, with methane production of about 465 ± 18 mL CH_4 g^{-1} VS per reactor, while the control reactor had a methane production of 325 ± 12 mL CH_4 g^{-1} VS per reactor. This resulted in a methane increase of around 43.1%, indicating that methanogenesis was highly stimulated. The reactors amended with 25 mg L^{-1} and 100 mg L^{-1} nanoparticles gave methane yields of 395 ± 15 mL CH_4 g^{-1} VS and 430 ± 16 mL CH_4 g^{-1} VS, respectively, which were advantageous for methane generation.

Likewise, nanoparticle supplementation greatly improved organic matter degradation. The highest COD removal efficiency (81.6 ± 2.7%) was found in the reactor with Fe_3O_4 nanoparticles, while the control reactor had 62.4 ± 2.1%. The efficiency of COD removal was improved, which was beneficial for the degradation of substrates and efficient conversion of biodegradable organic matter to methane. It was observed that methane production was strongly correlated with COD removal which indicated effective microbial metabolism and utilization of the substrate by the nanoparticles.

The improved performance of the nanoparticle-based reactors was mainly due to the conductive properties of the Fe_3O_4 nanoparticles that allowed the syntrophic bacteria to transfer electrons to the methanogenic archaea in Direct Interspecies Electron Transfer (DIET). The nanoparticles also provided biologically available Fe needed for microbial enzymatic activities, enhancing methanogenic effectiveness and stability of the reactor. With an increased concentration, it was believed that the nanoparticles would aggregate and the effective surface area would decrease; therefore, 50 mg L^{-1} was the optimum dose that supplied the best conductivity increase and accessibility to microbes.

In conclusion, the study shows the potential of Fe_3O_4 nanoparticles derived from food waste as a conductive material for advanced anaerobic digestion systems, which is environmentally friendly, sustainable, and cost-effective. The combination of valorization of food waste, green synthesis of nanoparticles, and recovery of renewable energy is a circular bioeconomy solution that can solve waste management and sustainable energy production problems. Future studies should be directed towards continuous reactor operation, microbial community analysis, verification of pathways of DIET, and techno-economic analysis, which can enable the large-scale implementation of the technologies of biomethanation using nanoparticles.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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