

Article

Impact of Iron Oxide Nanoparticles on Anaerobic Co-Digestion of Cow Manure and Sewage Sludge

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Abstract: Supplementation with iron oxide nanoparticles has been suggested as a potential method for improving energy generation through anaerobic digestion, specifically by enhancing the rate of methane production. This investigation examined the effects of iron oxide (Fe₃O₄) nanoparticles (NPs) on anaerobic co-digestion of cow manure (CM) and sewage sludge (SS) through batch testing conducted under mesophilic conditions (35 °C) using a RESPIROMETRIC Sensor System 6 Maxi—BMP (RSS-BMP). The use of Fe₃O₄ nanoparticles at doses of 40, 80, 120, and 160 mg/L (batches M1, M2, M3, and M5) was studied. The use of 160 mg/L Fe₃O₄ nanoparticles in combination with mixtures of different ratios (M4, M5, and M6) was further investigated. The findings indicate that the addition of Fe₃O₄ nanoparticles at a concentration of 40 mg/L to anaerobic batches did not significantly impact the hydrolysis process and subsequent methane production. Exposing the samples to Fe₃O₄ NPs at concentrations of 80, 120, and 160 mg/L resulted in a similar positive effect, as evidenced by hydrolysis percentages of approximately 94%, compared to 60% for the control (C2). Furthermore, methane production also increased. The use of Fe₃O₄ nanoparticles at a concentration of 160 mg/L resulted in biodegradability of 97.3%, compared to 51.4% for the control incubation (C2). Moreover, the findings demonstrate that supplementing anaerobic batches with 160 mg/L Fe₃O₄ NPs at varying mixture ratios (M4, M5, and M6) had a significant impact on both hydrolysis and methane production. Specifically, hydrolysis percentages of 94.24, 98.74, and 96.78% were achieved for M4, M5, and M6, respectively, whereas the percentages for the control incubation (C1, C2, and C3) were only 56.78, 60.21, and 58.74%. Additionally, the use of 160 mg/L Fe₃O₄ NPs in mixtures M4, M5, and M6 resulted in biodegradability percentages of 78.4, 97.3, and 88.3%, respectively. In contrast, for the control incubation (C1, C2, and C3) biodegradability was only 44.24, 51.4, and 49.1%.

Keywords: co-digestion; cow manure; iron oxide nanoparticles; BMP system; sewage sludge



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1. Introduction

Increased population growth, improved living standards, and dietary preference have increased the demand for livestock products, thus resulting in increased generation of livestock manure, which, if not properly managed, can negatively affect air, soil, ground and surface water quality. Traditional approaches to manure management, such as high stack storage that is commonly practiced in developing countries, cannot provide regulated pathogen and nutrient control. This leads to adverse environmental impacts, in addition to a reduction in the manure's nutrient value.

The process of anaerobic digestion (AD) involves the concerted action of three distinct microbial groups that degrade complex organic matter: fermentative, acidogenic, and methanogenic microbes, provide an efficient, cost effective and sustainable management approach for livestock manure. However, the mono-digestion of manure leads to a low methane rate and yield owing to the significant presence of a greater concentration of

nitrogen (N) and relatively low C/N ratio, which renders it unsuitable for microorganisms and consequently restricts the AD process [1,2]. Therefore, co-digestion of livestock manure with other types of substrates such as the sewage sludge is more advantageous due to its ability to provide a balanced combination of macro- and micronutrients for anaerobic microorganisms, optimal moisture content, favorable microbial metabolism, buffering capacity, biodegradability, and the ability to dilute toxins [3–5]. Typically, utilizing a co-substrate such as sewage sludge significantly enhances biogas production by up to 1.27 to 3.46 times compared to mono-digestion of manure. This is attributed to the favorable synergistic effects within the digestion medium and the provision of essential nutrients [6–10]

In Jordan, the estimated annual production of cow manure is 1 million tons [11]. Currently, the most common cow manure management practices in Jordan include open-field storage (i.e., high tack storage), direct application to crops, and disposal to landfills. This considerable amount of manure is hazardous, as it is a major source of surface and ground-water pollution, nutrient leaching, ammonia and methane emissions, and pathogen release if it remains inadequately managed [12]. For Jordan, co-digestion with sewage sludge is highly feasible since the area with most intensive generation of manure (Dhaly1/Zarqa Governorate) is 20.4 km away from Kherbit As-Samara, the largest wastewater treatment plant in Jordan.

Additionally, several studies have shown that iron oxide nano particles (IONPs) such as magnetite (Fe_3O_4) and maghemite (Fe_2O_3) effectively enhance the quality and yield of biogas [13–18]. Attributing such enhancement to the facilitation of direct interspecies electron transfer (DIET) [19–21] revealed that 200 mg/L of magnetite promoted the activities of protease, cellulase, dehydrogenase, acetic kinase and coenzyme F420, by 3.8, 1.5, 1.2, 1.2 and 1.6 times, relative to the blank group, respectively [22,23].

The main objective of this work was to determine how to enhance the anaerobic co-digestion of cow manure and sewage sludge by focusing on IONP mixing ratios for biogas enhancement. This involved (i) assessing the biochemical methane potential (BMP) of anaerobic co-digestion of manure and sewage sludge, (ii) investigating the impact of iron oxide nanoparticles on the anaerobic co-digestion of cow manure and sewage sludge, specifically on hydrolysis, acidification, and methane production, and (iii) applying the Gompertz model to verify the experimental results.

2. Materials and Methods

2.1. Microstructure Characterization of the Nanoparticles

The iron (II,III) oxide (Fe_3O_4) nanopowder 50–100 nm, was sourced from (Sigma-Aldrich, Darmstadt, Germany) and the stock solution prepared at 1 g/L. The Dynamic Light Scattering measured the size of nanoparticles (Zetasizer ZS, Malvern Instruments, Worcestershire, UK).

During Dynamic Light Scattering

During Dynamic Light Scattering (DLS) measurement, peaks of Fe_3O_4 nanoparticles were observed in the charge transfer spectra, with recorded values of 68 and 72 nm. The current investigation utilized a laser diffraction approach incorporating multiple scattering techniques to ascertain the particle size distribution of nanoparticles composed of iron oxide. The findings suggest that the nanoparticles mentioned, possess a particle size distribution of roughly 70 nm, as shown in Figure 1.

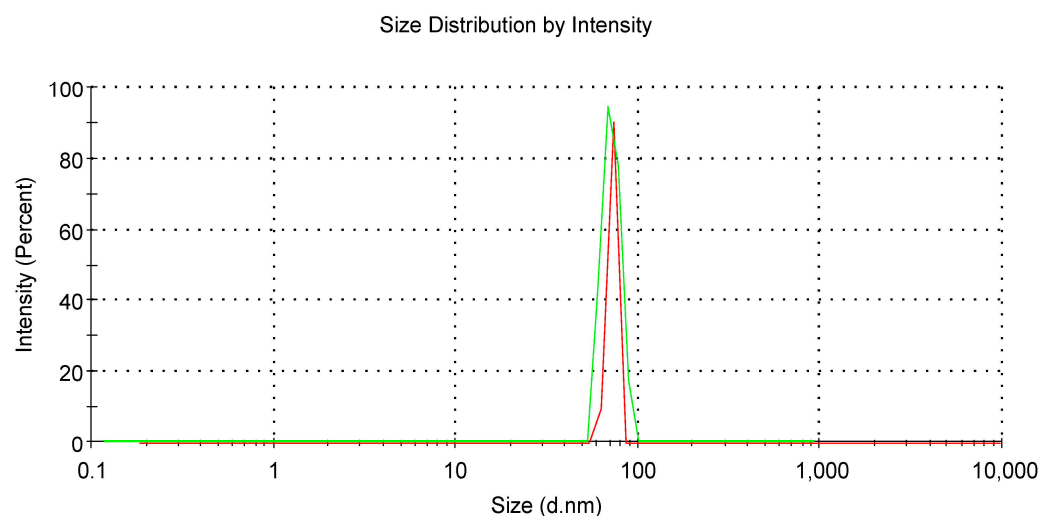


Figure 1. DLS particle size analysis curve for Fe_3O_4 nanoparticles.

2.2. Raw Materials for Co-Digestion

2.2.1. Substrates

Two kinds of substrate were employed in this research: sewage sludge (SS) and cow manure (CM). Fresh cow manure was collected from three farms in the Dhalyl area in (Zarqa, Jordan) with 3200, 3798, and 4526 head of cattle. A weighted average composite sample of cow manure obtained from the three farms was prepared. The mixture was then homogenized, and a representative 3 kg sample was analyzed. The sewage sludge sample was collected from Al Shallaleh Wastewater Treatment Plant in Irbid, Jordan. The treatment facility receives an average flow of 22,289 m³/d of municipal wastewater annually. The influent wastewater is characterized by total chemical oxygen demand (TCOD) concentration and total suspended solids (TSS) of 1891 and 722 mg/L, respectively.

2.2.2. Inoculum

As a source of inoculum, anaerobically digested waste activated sludge was obtained from the Al Shallaleh Wastewater Treatment Plant (Irbid, Jordan). The anaerobic digester is a thoroughly mixed reactor that operates at 37 °C, with a solids retention duration of 20 days. A total solids (TS) concentration of 23.32 gTS/L and a volatile solids (VS) concentration of 17.84 gVS/L were determined.

Prior to being used in the anaerobic digestion group experiments, the inoculum was preincubated for 4 days at 35 °C under anaerobic conditions to eradicate any residual biodegradable organic material.

2.3. Anaerobic Co-Digestion Batch Tests

The RESPIROMETRIC Sensor System 6 Maxi—BMP (RSS-BMP) was used to gather and store pressure data during anaerobic batch testing. The tests were carried out in triplicate using 1000 mL bottles. The necessary macro- and micronutrients were provided according to [24]. The CM and SS substrates were introduced at ratios of 30:70, 50:50, and 70:30 (CM:SS), defined by their VS content. The inoculum levels were calculated using an inoculum-to-substrate ratio of 1.0 g VS inoculum/g COD substrate. Following the addition of the inoculum, substrate, and medium solution and 200 mL distilled water, various aliquots of produced nanoparticle stock solution were added to achieve the necessary nanoparticle concentrations. Following that, distilled water was added to obtain a liquid volume of 300 mL, and the bottles were firmly sealed with RSS-BMP[®] measuring heads. To create anaerobic conditions, the headspace air was purged for 3 min using nitrogen gas. After that, the bottles were incubated at 37 °C under continuous mixing at 50 rpm for 30 days. It is worth noting that the pressure that is created in the first two hours was dissipated, since it is caused mostly by dissolving gases as the temperature rises. Only

the inoculum, substrates, medium solution, and distilled water were added to the control bottles. Increased pressure at constant volume was detected by the RSS-BMP measuring heads to determine methane gas output. The methane content of the gas was automatically evaluated until the test was finished, at which point the cumulative methane gas curve plateaued. Soluble COD and VFA concentrations were measured every 2 days using 2 mL liquid samples. To adjust for inoculum methane generation, 3 blank bottles containing all additions except substrates were employed. Table 1 gives the details of the mixes.

Table 1. Details of cow manure and sewage sludge ratios and Fe₃O₄ NP concentrations in mixtures.

Mix ID	Cow Manure and Sewage Sludge Ratio	Fe ₃ O ₄ NP Concentration (mg/L)
C1	30% CM:70% SS	0
C2	50% CM:50% SS	0
C3	70% CM:30% SS	0
M1	50% CM:50% SS	40
M2	50% CM:50% SS	80
M3	50% CM:50% SS	120
M4	30% CM:70% SS	160
M5	50% CM:50% SS	160
M6	70% CM:30% SS	160

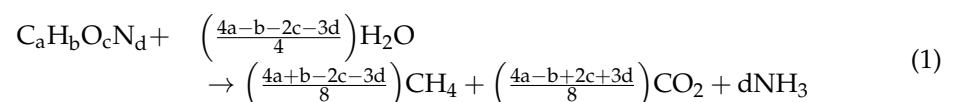
2.4. Analytical Methods

A pH meter (edge[®] Dedicated pH/ORP Meter Hanna-HI2002, Viale Delle Industrie, Italy) was used to measure pH and temperature, a salinity meter (edge[®] Dedicated Conductivity/TDS/Salinity Meter Hanna-HI2003, Viale Delle Industrie, Italy) was used to measure the electrical conductivity (EC) of the substrate, and a muffle furnace (Carbolite CWF 1100, Carbolite Gero Ltd., Hope, UK) was used to determine total solids (TS) and volatile solids (VS). The standard technique was used to compute total and volatile solids (EPA Method 1684, 2001) [25]. Total nitrogen (TN), total phosphorous (TP), chloride ion (Cl), and total ammonia nitrogen (TAN) were determined using a waste-to-distilled water ratio of 1:10. All specimens underwent analysis according to the methodology outlined by Radojevic and Bashkin (practical environmental analysis) [26]. We analyzed carbon, oxygen, hydrogen, and nitrogen using an elementary analyzer (Perkin-Elmer Vector 8910) following the manufacturer's instructions.

3. Calculations

3.1. Theoretical Biochemical Methane Potential

The theoretical biochemical methane potential (BMP_{Th}) can be determined based on the stoichiometry of the substrate's anaerobic degradation reaction, using the empirical mole composition of the CM and SS as computed from the elementary analysis [27]:



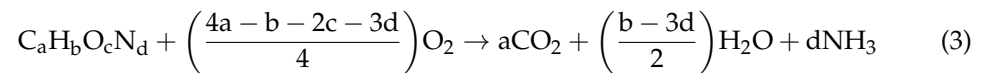
Therefore,

$$\text{BMP}_{\text{Th}}(\text{LCH}_4/\text{kgVS}) = \frac{22.4 \times \left(\frac{4a+b-2c-3d}{8}\right) \times 1000}{12a + b + 16c + 14d} \quad (2)$$

where 22.4 refers to the volume (L) that is occupied by an ideal gas under standard conditions, including temperature of 273 K and pressure of 101.3 kPa, and 1000 is the factor for converting volume measurements from liters (L) to milliliters (mL).

3.2. Theoretical Chemical Oxygen Demand

The theoretical COD (COD_{Th}) can be calculated using the stoichiometric substrate oxidation reaction:



$$COD_{Th} (gCOD/gVS) = \frac{32 \times \left(\frac{4a + b - 2c - 3d}{4} \right)}{12a + b + 16c + 14d} \quad (4)$$

3.3. Experimental Biochemical Methane Potential

In this study, we computed the experimental biochemical methane potential ($BMP_{experimental}$) by determining the highest methane production achieved in batch test bottles, adjusted by the maximum methane production of blank bottles and divided by the quantity of substrate added in VS units:

$$BMP_{experimental} (LCH_4/kgVS) = \frac{BMP_{Testbottles} - BMP_{Blankbottles}}{VS} \quad (5)$$

3.4. Biodegradability, Hydrolysis, and Acidification Percentage

3.4.1. Biodegradability Percentage

Anaerobic biodegradability was analyzed by determining the percentage of experimental biochemical methane potential (BMP) relative to the theoretical BMP:

$$Biodegradability\% = \frac{BMP_{experimental}}{BMP_{Th}} \quad (6)$$

3.4.2. Hydrolysis Percentage

Hydrolysis was analyzed based on the proportion of solubilized chemical oxygen demand (COD) relative to the initial particulate COD of the substrate:

$$Hydrolysis\% = \frac{COD_{CH_4,t} + COD_{s,t} - COD_{s,t=0}}{COD_{Th,initial} - COD_{s,t=0}} \quad (7)$$

where $COD_{CH_4,t}$ indicates the COD equivalent of methane generated at time t and $COD_{s,t}$ represents soluble COD at that time, $COD_{s,t=0}$ denotes soluble COD at time $t = 0$, and $COD_{Th,initial}$ refers to the initial theoretical COD.

3.4.3. Acidification Percentage

Acidification was analyzed by determining the percentage of acidified COD relative to the initial theoretical COD of the substrate:

$$Acidification\% = \frac{COD_{CH_4} + COD_{VFA,t} - COD_{VFA=0}}{COD_{Th,initial}} \times 100 \quad (8)$$

where $COD_{CH_4,t}$ represents the COD equivalent of methane produced at time t , $COD_{VFA,t}$ represents the VFA equivalent COD at time t , $COD_{VFA,t=0}$ represents the VFA equivalent COD at time $t = 0$, and $COD_{Th,initial}$ represents the initial theoretical COD.

3.5. Statistical Analysis

Statistical studies were conducted using IBM SPSS Statistics (version 26). The mean ($\bar{x}$), standard deviation (σ), and coefficient of variation (CV%) of CM and SS were calculated. ANOVA with Bonferroni correction and a 95% confidence interval was used to assess the influence of iron (II,III) oxide (Fe_3O_4) NPs on the anaerobic co-digestion process.

3.6. Modeling of Methane Production

IBM SPSS Statistics (version 26) was used to estimate the cumulative methane production observed over the 30-day duration of the AD process using a modified Gompertz model. Several previous studies also used this model [28–30].

$$Y(t) = Y_m \times \exp \left\{ -\exp \left[\frac{\mu_m \cdot e}{Y_m} \times (\lambda - t) + 1 \right] \right\} \quad (9)$$

where $Y(t)$ is cumulative methane yield at time t (L CH_4 /KgVS), Y_m is maximum methane production rate (L CH_4 /LgVS), m is maximum methane production rate (L CH_4 / KgVS.d), λ is lag phase duration (d), and t is the incubation period (d), $e = \exp(1) = 2.718$.

4. Results and Discussion

4.1. Characteristics of Substrates

Table 2 presents the CM and SS characteristics. The measured pH of CM of 7.16 is comparable and within the mean values documented in the literature that is in the range of 6.82 to 7.78.

Table 2. Characteristics of cow manure and sewage sludge.

Parameter	Unit	Cow Manure	Sewage Sludge
Total solids	gTS/kg wet weight	143.8	-
	gTS/L	-	20.88
Volatile solids	gVS/kg wet weight	120.7	-
	gVS/L	-	16.18
Total Kjeldahl nitrogen	gN/kg VS	2.42	132.35
Total phosphorous	gP/kg VS	0.670	24.7
Total ammonia nitrogen	gN/kg VS	0.233	16.83
Total organic carbon	%DM	50.2	53.2
Chloride ion	mg/kg DM	6990	-
Electrical conductivity	$\mu\text{S}/\text{cm}$	1226.5	337
C/N	%	20.74	6.50
pH	-	7.16	7.32
Elemental analysis			
Carbon	%DM	54.31	44.49
Hydrogen	%DM	5.85	5.45
Oxygen	%DM	37.04	43.37
Nitrogen	%DM	2.8	6.69

The total solid content of CM was 143.8 gTS/kg wet weight, a value that falls outside the range of 165–175 gTS/kg wet weight previously reported in the literature. Regarding the nutritional composition, the total Kjeldahl nitrogen of 2.42 gN/kgVS observed in this study falls within the range of 2–3 gN/kgVS previously reported in the literature. It was determined that the phosphorous concentration of 0.670 gP/kgVS was consistent with values reported in the literature, typically 0.5–1 gP/kgTS. Regarding ammo-

niacal nitrogen, the result obtained in this study, 0.233 gN/kgVS, falls within the range of 0.1–0.5 gN/kgVS previously reported in the literature. Adequate levels of ammonia concentrations result in increased buffering capacity for the anaerobic digestion process, according to Agyeman et al. [31].

The C/N ratio of CM was 20.74, which is comparable with the value previously reported in the literature (19.74), and within the range of 20–30 stated by the International technical standards for optimal anaerobic digestion [32]. Nevertheless, upon co-digestion with SS, which is generally characterized by a low C/N ratio of 6.5, the resulting C/N ratio will be reduced. However, several researchers have demonstrated that co-digestion of CM with SS can be achieved under C/N ratios < 15 [33–35].

In the context of nutrient supplementation, the measured C:N:P ratio (130:6.9:1.3), when compared with the ratio reported in the literature for successful and stable anaerobic digestion (130:5:1) [36], shows a slight intensification in nitrogen and phosphorous level compared to carbon content. However, it is essential to note that the observed increase only accounts for 38% and 30% of the recommended value for nitrogen and phosphorous levels, respectively.

Regarding anaerobic biodegradability, the BMP_{Th} values for CM and SS were determined to be 522.77 and 374.44 $LCH_4/kgVS$, respectively. These values were calculated based on empirical mole compositions of $C_{22.63}H_{28.98}O_{11.58}N$ for CM and $C_{7.76}H_{11.30}O_{5.68}N$ for SS, assuming complete conversion of COD. The contribution of sulfur was negligible and insignificant, as the elemental analysis results indicated a sulfur content below the detection limit. According to the empirical composition, the COD_{Th} values for CM and SS were found to be 1.49 and 1.07 gO_2/gVS , respectively.

4.2. Effects of Fe_3O_4 NPs on Hydrolysis and Acidification with 50% CM and 50% SS

The effect of Fe_3O_4 NPs on COD solubilization was evaluated because of the significance of hydrolysis in the kinetics of anaerobic digestion and the fact that it is often the rate-limiting stage. Anaerobic batch experiments were performed using Fe_3O_4 NP concentrations of 40, 80, 120, and 160 mg/L. According to the findings (Figure 2), the maximum soluble COD concentration of the control incubation (C2) was 655 mg/L, which was attained after 6 days of incubation. Maximum soluble COD concentrations for batches incubated with Fe_3O_4 NPs were 2225, 1738, 1532, and 1132 mg/L for M5, M3, M2, and M1, respectively. An increase in soluble chemical oxygen demand (sCOD) was found to be accompanied by a corresponding increase in methane production [37]. Paletta et al. reported observed that the inclusion of Fe_2O_3 nanoparticles (NPs) resulted in an augmentation of metabolic intermediate synthesis and an improvement in the activity of key enzymes within methanogenic Archaea [38–40]. Accordingly, the hydrolysis percentages attained after 6 days were calculated in order to determine whether the increased soluble COD in Fe_3O_4 NP amended batches was caused by stimulated hydrolysis or the accumulation of soluble COD as a result of decreased methanogen consumption. The findings demonstrate that incubation batches M5, M3, M2 and M1 achieved hydrolysis percentages of 91.68, 81.47, 76.78, and 72.85%, respectively, compared to C2, which achieved 44.65%, thus it is concluded that Fe_3O_4 NPs stimulated hydrolysis. In contrast to the 60.21% obtained in C2 in the final period, the final hydrolysis percentages reached in the Fe_3O_4 NP modified batches were 81.24, 88.98, 92.57, and 98.74% for M1, M2, M3, and M5, respectively [41]. The beneficial effects of Fe_3O_4 NPs on the hydrolysis process have been reported by Hassaan et al., Bharathiraja et al., and Montingelli et al. [42–44]. These studies highlight that the hydrolytic enzymes released during cell breakdown facilitate the acceleration of biomass hydrolysis. Moreover, the enhancing effect of magnetite was also verified in two phase anaerobic digestion system, wherein the supplementation of magnetite has enhanced the decomposition of complex organics and paved the way for subsequent methanogenesis in the methanogenic phase [39,41,45].

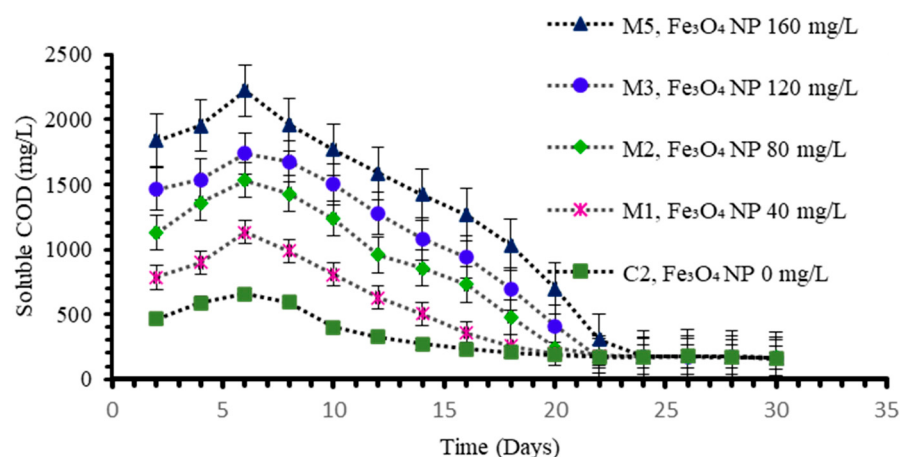


Figure 2. Effect of different Fe_3O_4 NP doses on soluble COD.

The impact of Fe_3O_4 NPs on the availability of VFAs for methanogenesis was evaluated based on the direct correlation between methane yield and VFA production resulting from substrate acidification. The findings indicate that introducing Fe_3O_4 nanoparticles resulted in a notable increase in acetate production. The highest acetate concentrations of 4489, 4507, 4664, and 4722 mg/L were observed after 10 days of incubation in M1, M2, M3, and M5, respectively. In contrast, the maximum acetate concentration in C2 was 3657 mg/L, which was achieved after one day of incubation. The findings shown in Figure 3 indicate that acetate was the primary volatile fatty acid (VFA) observed, with its generation appearing to be positively correlated with the quantity of Fe_3O_4 nanoparticles added. Kang et al. and Chen et al. [36,46] reported that the concentration of VFAs derived from cow manure was 1.5–2 times greater than that obtained from activated sludge. Following 10 days of incubation, decreased concentrations of VFAs were observed, corresponding to a significant increase in the rate of methane generation, as shown in Figure 4. The percentage of acidification, which accounts for both VFA generation and consumption due to methane production, was calculated following the 10-day incubation period to determine the net increase in VFA production caused by Fe_3O_4 NPs. The results indicate that Fe_3O_4 nanoparticles had a beneficial effect on the acidification process, with acidification of 61.14, 62.71, 68.51, and 76.65% in batches M1, M2, M3, and M5. By comparison, 47.44% acidification was achieved in C2. Mu et al. [47] suggested that the cellular uptake of NPs within methanogens may be responsible for their stimulating effect. This uptake may involve integration with metabolic intermediates and key enzyme activity associated with sludge hydrolysis, acidification, and methanization. The findings presented are consistent with the outcomes documented by Hao et al. [48].

4.3. Effects of Fe_3O_4 NPs on Methane Production with 50% CM and 50% SS

The processes of hydrolysis and acidification, as observed, are likely to have an impact on the subsequent methanogenesis. At the conclusion of the incubation period, the total methane production in the Fe_3O_4 NP treated groups (Figure 3a) reached 285.4, 328.8, 382.3, and 511.2 LCH₄/kgVS in batches M1, M2, M3, and M5, respectively. These findings suggest that the optimal concentration for enhancing biogas and methane production is 160 mg/L Fe_3O_4 magnetic nanoparticles. This result is consistent with the findings of Liu et al. [49], who observed that the release of iron ions resulting from the dissolution of magnetic nanoparticles may have contributed to the stimulation of bacterial activity. In addition, Fe_3O_4 magnetic nanoparticles facilitate the dispersion of iron ions within the slurry. Furthermore, the corrosion process of nanoparticles facilitates consistent release of iron ions within the bioreactor. The findings of Mu and Chen and Deppenmeier [50,51] indicated that higher amounts of nanoparticles have a significant influence on the hydrolysis of soluble protein and the electron donor transformation activity associated with redox-driven proton

translocation in methanogenic Archaea, as expressed by coenzyme F420. The activity of coenzyme F420 was found to be dependent on the dosage of nanoparticles. The enhanced methane production of batch M1 was compared to that of the control incubation, and the results indicated a 5.7% increase. It was found that M2, M3, and M5 exhibited statistically significant increases in methane production by 21.8, 41.6, and 89.4%, respectively. In studies by Abdelsalam et al., Hao et al., and Zhang et al. [52–54], the introduction of Fe_3O_4 resulted in significant increases in methane production ranging from 10 to 80%. The findings demonstrate biodegradability of 51.4% for C2 and 54.3, 62.6, 72.8, and 97.3% for M1, M2, M3, and M5, respectively. The findings unequivocally demonstrate that the incorporation of Fe_3O_4 nanoparticles resulted in augmented methane production efficiency during the anaerobic co-digestion of cow manure and sewage sludge.

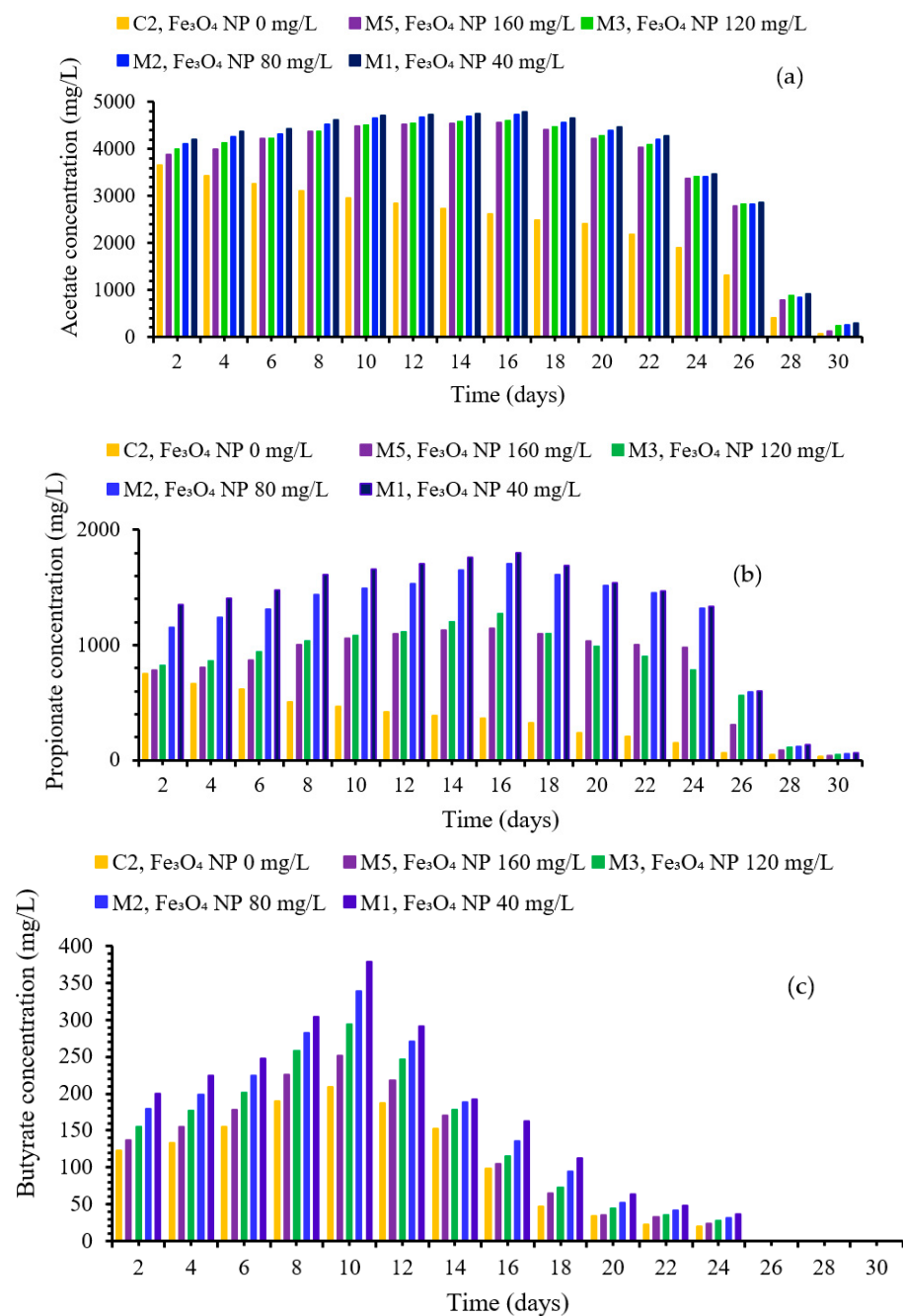


Figure 3. Effect of Fe_3O_4 magnetite NPs on VFA production: (a) acetate, (b) propionate, and (c) butyrate.

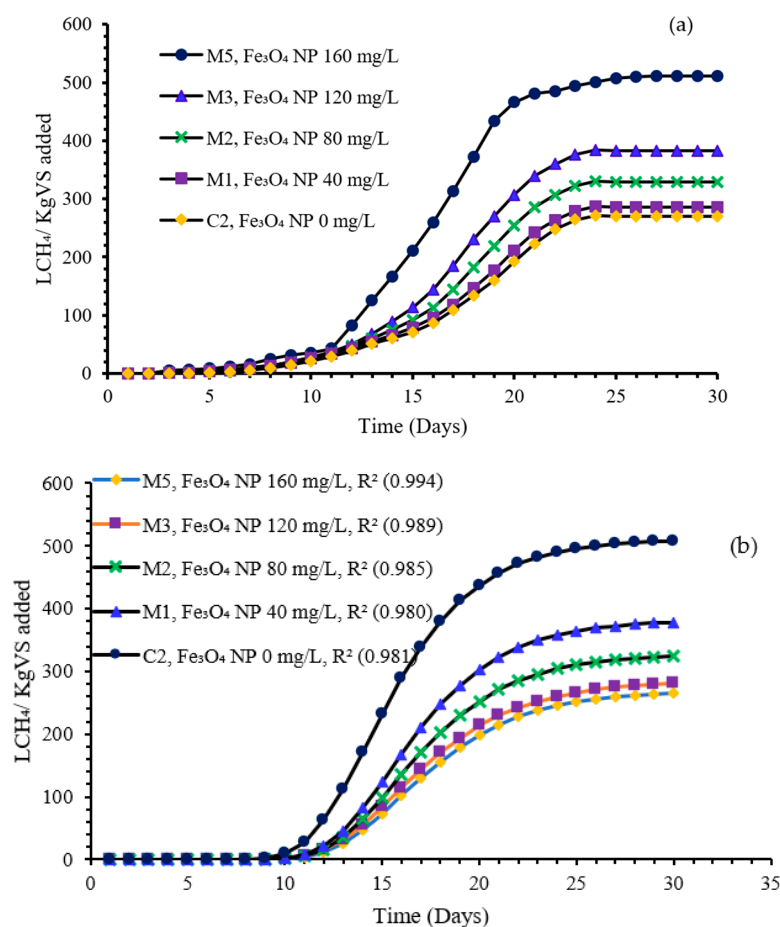


Figure 4. Cumulative methane production with different Fe₃O₄ NP doses: (a) experimental data, (b) modified Gompertz model fit.

Including Fe₃O₄ nanoparticles in the samples increased the methanogenesis of solubilized substrates, as indicated by the observed accumulation of VFAs (Figure 3) and the data presented in Figure 4. The modified Gompertz model was utilized to model experimental methane production data, as shown in Figure 4b. The results indicate that batches M1, M2, M3, and M5 exhibited lag phases of 12.32, 12.24, 12.20, and 11.19 days, respectively. According to Santos et al. and Zhao et al. [55,56], the typical lag phase is between 1.5 and 15.4 days. The inhibitory substances that are either present in the substrate or produced during fermentation have an impact on this parameter, as well as the maximum production rate. These substances include VFAs, long chain fatty acids, and ammonia, as noted by Sánchez et al., Kafle and Kim, and Kafle et al. [57–59], who conducted BMP tests and reported the presence of a lag phase in the tested substrates. They found that the modified Gompertz model was more effective at predicting BMP compared to the first-order kinetic model. Ajayi-Banji and Rahman [60], utilizing a solid-state system, conducted a study showing that incorporating magnetic nanoparticles (Fe₃O₄) in the batch digestion of pretreated corn stover and cow manure resulted in improved reactor stability by promoting acid conversion. This led to a reduction in the initial lag phase and an increase in the degradation rate of various substrate components. Consequently, greater quantities of methane were produced within a significantly reduced time frame. Subsequent to this interval, there was a notable increase in maximum methane production rate, particularly for batches M1, M2, M3, and M5, with increases of 5.8, 28.6, 55.2, and 98.6%, respectively, compared to the control incubation, as shown in Figure 5. The initial decrease could possibly be attributed to the prompt generation of acid, which leads to lower localized pH, thereby initially impeding the process of methanogenesis. The increased methane production observed

in batches supplemented with Fe_3O_4 NPs can be attributed to enhanced hydrolysis and acidification compared to the control incubation.

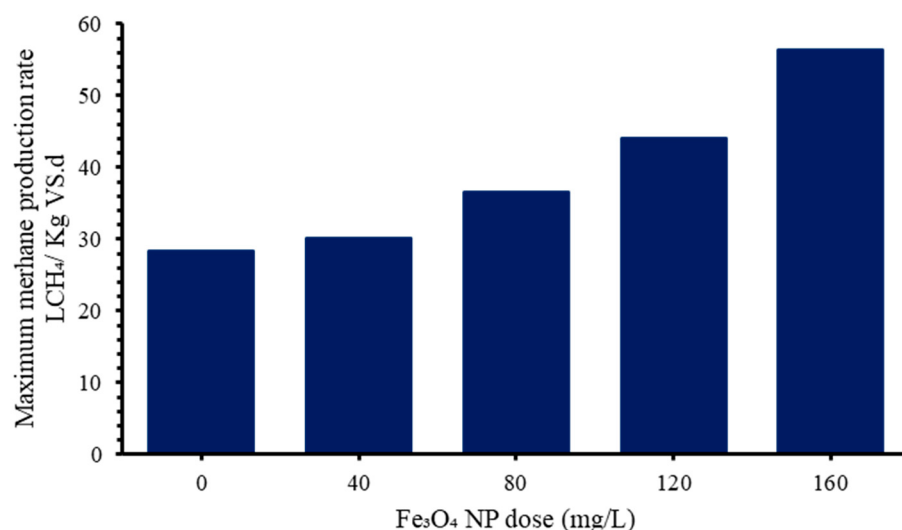


Figure 5. Effects of different Fe_3O_4 NP doses on maximum methane production, computed from a modified Gompertz model.

4.4. Effects of Fe_3O_4 NPs on Hydrolysis and Acidification for Different Ratios of CM and SS

The impact of Fe_3O_4 nanoparticles on the solubilization of chemical oxygen demand (COD) was assessed with various proportions of CM and SS utilizing 160 mg/L of Fe_3O_4 NPs in three mixtures (M4, M5, and M6). Based on the results presented in Figure 6, it can be observed that the highest concentrations of maximum soluble COD in the control batch were achieved after 6 days of incubation, with values of 1533, 655, and 1673 mg/L. The highest soluble COD concentrations observed in the batches incubated with 160 mg/L Fe_3O_4 NPs were 1941, 2225, and 1673 mg/L in M4, M5, and M6, respectively.

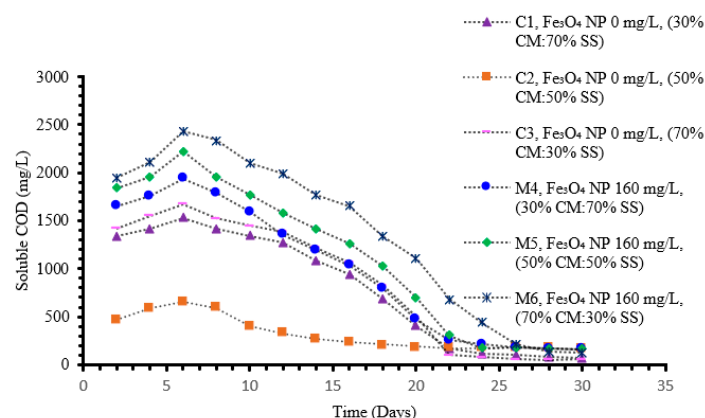


Figure 6. Effect of Fe_3O_4 NPs on soluble COD in mixtures C1, C2, C3, M4, M5, and M6.

Barua and Kalamdhad [36] noted a positive correlation between increased soluble chemical oxygen demand (sCOD) and methane generation. The hydrolysis percentages were calculated after 6 days to investigate whether the elevated sCOD in Fe_3O_4 NP treated batches was due to enhanced hydrolysis, or the accumulation of soluble COD resulting from reduced methanogen consumption. The results indicate that batches M4, M5, and M6 incubated with 160 mg/L Fe_3O_4 NPs had hydrolysis percentages of 84.45, 91.68, and 86.37%, respectively. In contrast, C1, C2, and C3 had hydrolysis percentages of 40.54, 44.65, and 43.21%. Therefore, it was concluded that hydrolysis was stimulated in M4, M5, and

M6. The results indicate that the hydrolysis percentages achieved in the modified batches were significantly higher than those obtained in C1, C2, and C3. Specifically, the final hydrolysis percentages were 94.24, 98.74, and 96.78% in M4, M5, and M6, and 56.78, 60.21, and 58.74% in C1, C2, and C3. Studies conducted by Hassaan et al., Bharathiraja et al., and Montingelli et al. [41–43] reported on the advantageous effects of Fe_3O_4 NPs on the hydrolysis process. These studies brought to light the fact that the hydrolytic enzymes that are discharged during cellular degradation expedite the process of biomass hydrolysis.

The study aimed to assess the influence of Fe_3O_4 nanoparticles on the availability of volatile fatty acids (VFAs) for methanogenesis. This was based on the established relationship between methane yield and VFA production, which is a consequence of substrate acidification. The results suggest that incorporating Fe_3O_4 nanoparticles led to significantly increased acetate production. We observed the highest acetate concentrations of 3300, 4722, and 3460 mg/L after 10 days of incubation with 160 mg/L Fe_3O_4 NPs in M4, M5, and M6 at 3300, 4722, and 3460, respectively. By comparison, incubation under controlled conditions resulted in peak acetate concentrations of 2868, 2644, and 3657 mg/L, respectively, which was achieved within a single day. The results illustrated in Figure 7 demonstrate that acetate was the predominant VFA detected, and its production seemed to be positively associated with the dosage of Fe_3O_4 nanoparticles. In studies by Chen et al. and Kang et al. [61,62], the VFA concentration obtained from cow manure was 1.5–2 times higher than that obtained from activated sludge. The production of methane increased concomitant with increased magnetite nanoparticle concentration. After 10 days of incubation, a decrease in VFA levels was noted, which was consistent with a notable rise in methane production, as illustrated in Figure 8.

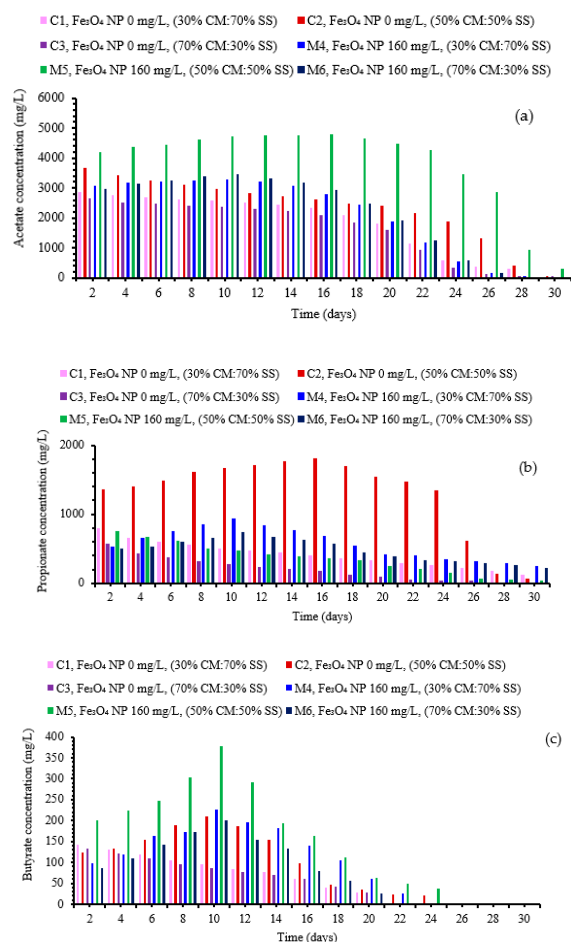


Figure 7. Effect of Fe_3O_4 NPs on VFA production in mixtures C1, C2, C3, M4, M5, and M6: (a) acetate, (b) propionate, and (c) butyrate.

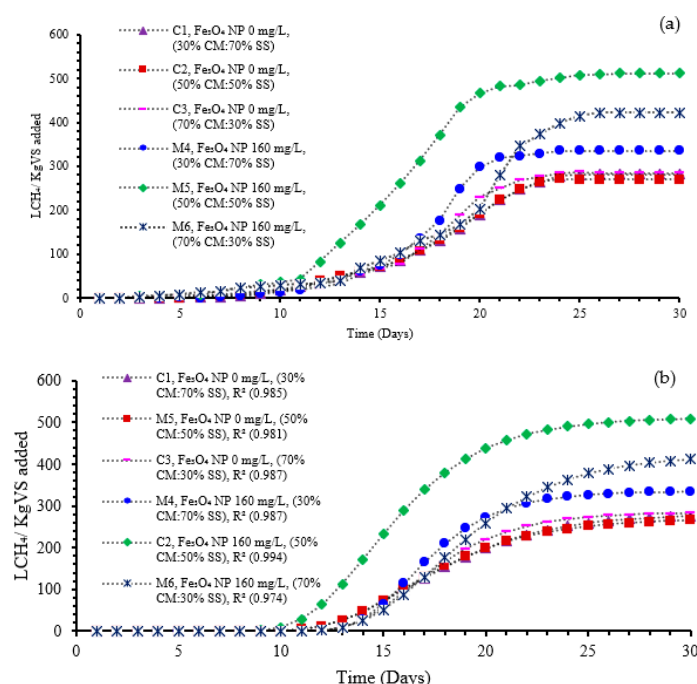


Figure 8. Cumulative methane production in mixtures C1, C2, C3, M4, M5, and M6 with 160 mg/L of Fe₃O₄ NPs: (a) experimental data, (b) modified Gompertz model fit.

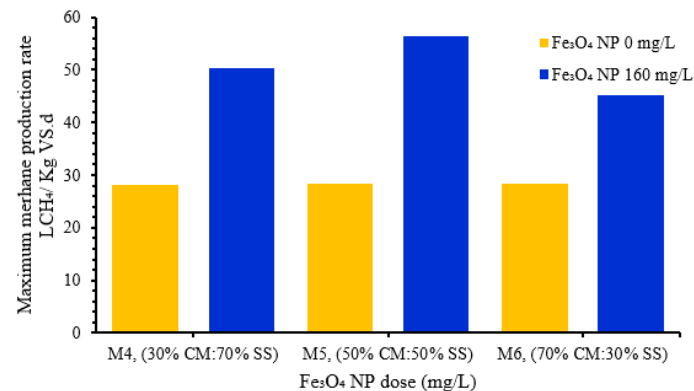
The acidification percentage was determined by considering the generation of VFAs and their consumption by methane production subsequent to a 10-day incubation period, in order to ascertain the net rise in VFA production resulting from the presence of Fe₃O₄ nanoparticles. The findings demonstrate that incorporating Fe₃O₄ nanoparticles has a favorable impact on the acidification process, with values of 74.18, 76.65, and 72.45% in batches M4, M5, and M6, respectively. The values of 45.21, 47.44, and 46.74% achieved in C1, C2, and C3 were used as a benchmark for comparison. The results presented are in line with the findings reported by Zhao et al. [63].

4.5. Effects of Fe₃O₄ NPs on Methane Production for Different Ratios of CM and SS

The observed hydrolysis and acidification processes were expected to have an effect on the subsequent methanogenesis. Upon the completion of incubation, the Fe₃O₄ NP treated groups (Figure 8a) exhibited cumulative methane production of 335, 511.2, and 422.5 LCH₄/kgVS in M4, M5, and M6, respectively, with a corresponding NP concentration of 160 mg/L. Comparing the enhancement in methane production between batches C1, C2, and C3 to that of the control incubation, the findings revealed an increase of 18.7%. The research findings indicate that the use of 160 mg/L Fe₃O₄ NPs in M5 and M6 resulted in significantly increased methane production by 89.4 and 48.6%, respectively. The biodegradability percentages for the control incubation were 44.24, 51.4, and 49.1%, while the values for M4, M5, and M6, containing 160 mg/L Fe₃O₄ NPs, were 78.4, 97.3, and 88.3%. Abdelsalam et al., Hao et al., and Zhao et al. [23,54,64] reported that incorporating Fe₃O₄ led to a notable enhancement in methane production, with an observed increase of 10–80%. The summary of the impact of different ratios of CM and SS for 160 mg/L Fe₃O₄ NPs on hydrolysis, acidification and methane production, is shown in Table 3 and Figure 9 is shown maximum methane production computed from a modified Gompertz model. Consequently, the best results, compared to the three other combinations, is achieved by blending a quantity of 50CM:50SS with 160 mg/L IONP (M5).

Table 3. Summary of the impact of different ratios of CM and SS for 160 mg/L Fe₃O₄ NPs on hydrolysis, acidification and methane production.

Mix ID	Cow Manure and Sewage Sludge Ratio	Hydrolysis %	Acidification %	Methane Production LCH ₄ /kgVS	Biodegradability %
M4	30% CM:70% SS	84.45	74.18	335	78.4
M5	50% CM:50% SS	91.68	76.65	511.2	97.3
M6	70% CM:30% SS	86.37	72.45	422.5	88.3

**Figure 9.** Effect of 160 mg/L Fe₃O₄ NPs on maximum methane production in mixtures C1, C2, C3, M4, M5, and M6 computed from a modified Gompertz model.

5. Conclusions

Supplementing an anaerobic co-digestion of cow manure and sewage sludge with Fe₃O₄ nanoparticles enhanced the hydrolysis, acidogenesis and methanogenesis process. Based on the batch-wise experimental results, the hydrolysis and acidification percentages were enhanced by 91.68% and 76.65% for the 50/50 co-digestion of CM and SS at the optimum Fe₃O₄ dose of 160 mg/L. The improved hydrolysis/acidification has facilitated methanogenesis, resulting in a 98.6% increased methane yield and maximum methane production rate. Results have also shown that the Gompertz model is suitable for fitting the measured biogas yields, and the kinetic parameters suggested that anaerobic digestion with Fe₃O₄ nanoparticles has a higher maximum biogas production rate and higher hydrolysis rate. This study investigated the impact of iron oxide nanoparticles on anaerobic co-digestion of cow manure and sewage sludge.

- The findings indicate that adding Fe₃O₄ NPs to anaerobic co-digestion batches M3 and M4 resulted in a notable enhancement in hydrolysis, reaching 92.57 and 98.78%, respectively. The value of 60.21% achieved in C2 was used for comparison.
- The acidification percentages were found to be 68.51 and 76.65% for batches M3 and M4, respectively. In contrast, the value for C2 was only 47.44%.
- Batches M3 and M4 achieved a cumulative methane yield of 382.3 and 511.2 LCH₄/kgVS, respectively. The aforementioned production yields exhibit a rise of 55.2% and 98.6% in relation to the yield achieved during the control incubation.
- The findings indicate that the addition of Fe₃O₄ NPs to anaerobic co-digestion batches M4, M5, and M6 resulted in a notable increase in hydrolysis, reaching 94.24, 98.78, and 96.78%, respectively.
- The acidification percentages for batches M4, M5, and M6 were 74.18, 76.65, and 72.45%, respectively. In contrast, the values for C1, C2, and C3 were only 45.21, 47.44, and 46.74%.

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