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Impact of Iron Oxide Nanoparticles on Anaerobic Co-digestion of Chicken Manure and Sewage Sludge Substrates

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Abstract

This investigation examined the influence of iron oxide nanoparticles (IONP) on the anaerobic co-digestion of chicken manure (CM) and sewage sludge (SS). The experiment was conducted in many batches, with the temperature maintained at 35 °C, using a RESPIROMETRIC Sensor System 6 Maxi—BMP (RSS-BMP). The utilisation of IONP at dosages of 40, 80, 120, and 160 mg/L in batches M1, M2, M3, and M4. The results showed that the introduction of IONP at a concentration of 40 mg/L into anaerobic batches had no significant effect on the methanogenesis process and the consequent generation of methane. Exposing the samples to IONP at doses of 80, 120, and 160 mg/L had a comparable beneficial impact. In addition, there was also an increase in methane production. The concentration of CH_4 was increased by 32.5%, 50.5%, and 62.7% when employing 80, 120, and 160 mg/L IONP, respectively, compared to the control test. IONP, when present at a concentration of 160 mg/L, achieved a biodegradability of 96.7%, but the control incubation (C4) only achieved a biodegradability of 56.1%.

Keywords: Chicken manure; Sewage sludge; Kinetic analysis; Anaerobic co-digestion; Iron oxide nanoparticles; Methane production.
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1. Introduction

The chicken farming industry is expanding rapidly due to population expansion and rising human consumption. As a result, a considerable amount of chicken manure is generated, requiring effective management and treatment. Inadequate handling of chicken manure may result in several negative consequences, such as the production of unpleasant smells, the attraction of mice, insects, and other pests, the spread of animal illnesses, and the contamination of groundwater.[1,2] The emission of ammonia NH_3, methane CH_4, and carbon dioxide CO_2 from open storage practices contributes to air pollution issues associated with greenhouse gases.[3] The process of AD is often used for the treatment of animal waste, and there is extensive research and practical application in the field of biogas generation from animal wastes.[4–7]

The anaerobic digestion (AD) process involves the concerted action of three distinct microbial groups, namely the fermentative, acidogenic, and methanogenic microbes, to degrade complex organic matter cost-effectively.[8] The various assemblages of microorganisms are accountable for transforming intricate organic compounds into biogas through four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.[9] The anaerobic mono-digestion process encounters several obstacles, such as inadequate biodegradation and biogas production, unbalanced nutrients, and a deficiency of anaerobic bacteria in the AD system.[10,11] Due to these factors, several researchers have enhanced AD performance by expediting the biological conversion rate of organic matter within the AD system by implementing co-digestion techniques.[12–14] Co-digestion is widely recognized as a more favorable approach than mono-digestion, primarily because it offers a well-rounded blend of macro- and micronutrients for anaerobic microorganisms.

Additionally, co-digestion provides optimal moisture content, promotes favorable microbial metabolism, enhances buffer capacity, improves biodegradability, and reduces toxic substances.[15–17] Utilizing a co-substrate typically significantly enhances biogas production, ranging from 1.27 to 3.46 times

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higher than the identical substrate's mono-digestion. This is attributed to the favorable synergistic effects within the digestion medium and the provision of essential nutrients.[13,18] The chicken manure (CM) is often regarded as a challenging substrate for AD.[19,20] The CM has a substantial nitrogen composition, mainly consisting of two principal constituents: uric acid and undigested proteins. Uric acid accounts for about 70% of the organic nitrogen content, while undigested proteins comprise 30%.[21,22] The generation of inhibitory concentrations of unionized NH^{3+} and NH^{4+} ions during anaerobic digestion is associated with the presence of these components.[23,24] These substances inhibit fermentation when increased total solids (TS) loadings are applied.

The literature suggests a range of values for inhibitory concentrations, typically between 70 and 250 gNH_3/m^3.[25–28] Nevertheless, other research has shown that anaerobic digestion systems need high free ammonia concentrations, typically 600 to 1100 gNH_3/m^3, to impede its functionality. These trials were carried out in the chicken manure supplementation.[29–31]

The issue of ammonia buildup during the fermentation of CM may be addressed by appropriately diluting the substrate to have a dry matter concentration ranging from 0.5% to 3%. However, the economic viability of this approach is compromised by the substantial water requirements.[32–35] A widely used alternative approach for mitigating ammonia buildup consists of the co-digestion of chicken digestate, known for its high carbon content. This strategy enables the maintenance of an optimal carbon-to-nitrogen ratio within the fermentation substrate. Co-digestion involves the use of various substrates, such as grass, different types of straws,[36] maize,[37] and agricultural or municipal wastes.[38,39]Sewage sludge has been identified as a very lucrative substrate in biogas generation by anaerobic digestion.[40–42] Nevertheless, previous studies have shown that when combined with additional residues that possess complementary and balancing properties, more favorable outcomes in terms of methane production are achieved upon completion of the process.

According to the findings of Sillero *et al.*,[43] it can be inferred that the anaerobic co-digestion of sewage sludge and chicken manure substrates yielded more benefits than mono-digestion of sewage sludge. In addition, the Borowski *et al.*[44] proposed the introduction of chicken manure at a concentration of 30% to sewage sludge, resulting in a notable enhancement of 50% in the overall gas output. Furthermore, the concurrent digestion of chicken manure with sludge yielded a greater efficiency in eliminating volatile solids (43.16–49.35%) compared to the digestion of sludge in isolation (33.85-36.33%). This observation has been corroborated by several other studies.[45–48]

Jordan's annual chicken manure production is expected to be around 3,402 million kg/year.[49] The predominant methods used in Jordan for managing chicken manure are open-field storage, direct application to crops, and disposal in landfills. The significant volume of manure is a potential danger due to its substantial contribution to the pollution of surface and groundwater, erosion of nutrients, emissions of ammonia and methane, and release of pathogens without appropriate management practices.[50] Co-digestion with sewage sludge is highly feasible in Jordan, as the region with the manure production (Al salt/Al Balqa Governorate) is only 10.2 kilometers from the Wadi Shuaib wastewater treatment facility. This study presents a resolution to the problem of collecting chicken manure while simultaneously promoting environmental preservation and energy production.

Prior studies have assessed and suggested innovative approaches to enhance the efficiency of anaerobic co-digestion. Supplemental co-digestion is a method that is included in the pretreatment process. Nanoparticulate iron compounds (Fe) are a potential solution to reduce ammonia inhibition and enhance biogas production.[51–53] The inclusion of IONP in AD systems has been linked to the enhancement of hydrolysis, acidogenesis, and CH_4 generation, as well as the prevention of excessive acidification. Moreover, it has been shown to improve COD removal and facilitate the conversion of propionate to acetate.[54–56] Previous investigations have demonstrated that IONP is necessary as a cofactor in several enzymatic activities.[57] This promotes fermentation and creates more favorable circumstances for AD by reducing the oxidative-reductive potential of the digestive medium.[58] The study conducted by Alkhrissat *et al*.[59] examined the effects of IONP on anaerobic co-digestion, including cow dung and sewage sludge. The researchers discovered that including IONP at concentrations ranging from 40 to 160 mg/L enhanced hydrolysis and methane generation. Moreover, it was shown that greater concentrations of IONP correlated with increased biodegradability percentages.

The main objective of this study was to investigate the impact of IONP on varying concentrations of 40, 80, 120, and 160 mg/L mixing ratios using the anaerobic co-digestion of chicken manure and sewage sludge with different ratios of CM and sewage sludge (SS). The study aimed to achieve two main objectives: (i) to assess the BMP by conducting anaerobic co-digestion of chicken manure and sewage sludge, with a specific focus on hydrolysis and methane production, and (ii) to validate the results obtained from the experimental investigation using the Gompertz model. Table 1 provides a comprehensive overview of the recent advancements in the

Table 1. Summary of the recent studies conducted in the period 2020-2024.

Authors	Study objectives	Methodology	Main findings	Limitations
Bkoor Alrawashdeh *et al.* [60]	Anaerobic Co-Digestion of Olive Mill Waste and Chicken Manure • Investigates potential for reducing bio-waste. • Determines optimal organic loading rate and pre-treatment technique. • Investigates effects of IONPs on co-digestion. • Assesses impact of IONP doses on biogas, methane production, TS, VS., COD, color, turbidity, and process stability.	The methodology for analyzing materials like CM and OMW substrates, inoculum, and their characteristics like C/N ratio and Fe concentration.	Study Findings: • Significant impact of IONPs supplementation on inhibitor removal efficiency. • Significant increase in hydrolysis percentages with IONP doses (20-35 mg/g VS.) compared to control test.	The limitations of the study are not explicitly stated in the paper.
Aguilar-Moreno *et al.* [61]	Fe_3O_4 Nanoparticles Impact on Methane Production • Highest methane production rate and cumulative yield with 20mg/L Fe_3O_4 nanoparticles. • Biostimulates methanogenic activity. • Volatile fatty acid concentrations consistent across treatments.	The study synthesized Fe_3O_4 nanoparticles through co-precipitation, analyzed their size and zeta potential, confirmed their structure using TEM and XRD, and evaluated their impact on chicken litter digestion.	NPs Application Impact on Methane Production: • Highest rate of methane production (2.55 mL $CH_4 \cdot gvsf^{-1} \cdot d^{-1}$) and overall methane yield (137.23 mL $CH_4 \cdot gvsf^{-1} \cdot d^{-1}$). • 73.9% increase in methane yield compared to control. • Consistent concentration of volatile fatty acids across treatments.	Study Limitations: • Mesophilic evaluation only. • Lack of Fe_3O_4 NPs effect evaluation. • Requires further research under thermophilic conditions.
Wei *et al.* [62]	Study Objectives: • Investigate KOH and LFD modification of CS. • Assess impact on co-digestion efficiency. • Measure biomethane production increase. • Evaluate reduction in digestion times.	The study improved corn stover biodegradability using potassium hydroxide and digestate liquid fraction, followed by co-digestion with chicken manure, aiming to enhance efficiency.	Anaerobic Digestion Technology for Bioenergy Conversion • Widely used for biowaste conversion. • Improved biodegradability of corn stover boosts co-digestion efficiency. • Increases biomethane production and reduces digestion times..	Study Limitations: • Limited consideration of biowaste combinations. • Lack of discussion on modification drawbacks. • Absence of consideration for environmental or economic implications.
Li *et al.* [63]	Study Objectives: • Investigate phosphorus transformation in batch anaerobic digestion with chicken, pig, and dairy manures. • Understand changes in Labile-P of total phosphorus in digestates. • Explore metal ions' influence on phosphorus transformation and plant fertilizer production.	The study examined phosphorus transformation in batch anaerobic digestion processes, analyzing changes in Labile-P, metal ion influence, and phosphate species formation using X-ray diffraction.	Anaerobic Digestion Study Findings • Significant reduction in Labile-P of total phosphorus in manure digestates. • Production of satisfactory fertilizer for plants. • Influence of metal ions on phosphorus transformation. • Formation of various phosphate species.	Study Limitations: • Focus on specific manure types and treatment methods. • Lack of long-term soil/crop health research. • Absence of economic feasibility/scalability discussion.
Schommer *et al.* [64]	Study Objectives: • Analyze methane production from swine manures and feathers. • Focus on manure types, co-	The study utilized mesophilic anaerobic co-digestion of swine manures with untreated/pretreated	Study Findings: • Modified Gompertz model best fits anaerobic co-digestion experiments kinetic data. • Microbial pretreatment	Study Limitations: • Feather recalcitrance challenge. • Potential ammonia inhibition impact on

substrates, and total solids.	feathers, using diluted fresh and matured manures as Co-AD inoculums and feathers as co-substrates.	enhances methane production by improving feather biodegradability. • Feather hydrolysates identified as nitrogen-rich substrate for co-digestion with nitrogen-deficient biomasses.	yields. • Delays in biodegradation suggested by FF. • Need for microbial pretreatment exploration for increased methane production.

impact of iron oxide nanoparticles on the anaerobic co-digestion of chicken manure and sewage sludge substrates. It compiles research results from a selected group of articles published over the last four years, providing a systematic summary of the main research, Limitations and contributions in this field.

2. Methodology

2.1 Characterization of the nanoparticles

The IONPs, with a particle size of 50-100 nm, were obtained from (Sigma-Aldrich, Darmstadt, Germany). A stock solution was produced at a concentration of 1 g/L.

2.2 Substrates

This study used two types of substrates, namely SS and CM. Fresh chicken manure was obtained from the farm located in Al-Balqa, Jordan. The farm had 7241 chickens, and their ages are close among them. A composite sample of chicken dung was created using a weighted average methodology, which included combining farm samples. Afterward, the mixture was subjected to homogenization. Following this, a representative sample weighing 5 kg was taken for examination. The sewage sludge used in this study was acquired from the Al Shallaleh Wastewater Treatment Plant, which is situated in Irbid, Jordan. The treatment facility receives an average daily intake of 22,289 m^3 of municipal wastewater annually. The discharged wastewater has significant attributes regarding its total chemical oxygen demand (TCOD) levels and total suspended solids (TSS), quantified at 1891 and 722 mg/L.

2.3 Inoculum

The inoculum used in this investigation was obtained from anaerobically digested waste-activated sludge. The sludge was taken from the Al Shallaleh Wastewater Treatment Plant in Irbid, Jordan. The anaerobic digester is a thoroughly mixed reactor operating at 37 °C, with a solids retention duration of 20 days. The examination indicated that the total solids (TS) content was detected at 23.30 gTS/L, while the volatile solids (VS) concentration was 17.72 gVS/L.

Before being used in the anaerobic digestion batch studies, the inoculum underwent a preincubation period of four days at a temperature of 35 °C, during which it was subjected to anaerobic conditions. The preincubation technique was performed to eliminate any remaining biodegradable organic materials thoroughly.

2.4 Anaerobic co-digestion batch tests

The RESPIROMETRIC Sensor System 6 Maxi-BMP (RSS-BMP) collected and preserved pressure data during the test. The studies were performed in triplicate using a bottle with a volume of 1000 mL. The necessary macro and micronutrients were provided according to Angelidaki *et al*..[65] The CM and SS substrates were introduced in various ratios, namely 0:100, 30:70, 50:50, 70:30, and 0:100 (CM:SS) in batches (C1, C2, C3, C4 and C5) and application of IONP at concentrations of 40, 80, 120, and 160 mg/L in batches (M1, M2, M3, and M4), according to their respective volatile solids concentrations. Based on the information provided in Table 2. Inoculum ratio was determined using an inoculum-to-substrate ratio of 0.5 gVS inoculum/gCOD substrate. Following the administration of the inoculum, substrate, and medium solution, distilled water was added to the combination to attain a liquid volume of 300 mL, and the bottles were securely sealed using RSS-BMP® measuring heads. The air in the headspace was eliminated for 3 minutes by introducing nitrogen gas to provide an environment devoid of oxygen. Subsequently, the bottles were incubated at 37 °C with continuous agitation at a speed of 50 rpm for 30 days. Notably, the pressure generated during the first two-hour period undergoes dissipation primarily due to the dissolution of gases as the temperature increases. The control bottles (mono digestion of CM and SS) were supplemented with the inoculum, substrates, medium solution, and distilled water. The RSS-BMP measuring heads were used to detect increases in pressure under constant volume conditions to quantify the release of methane gas. Throughout the experiment, the methane concentration of the gas was consistently evaluated, and in conclusion, the cumulative methane gas curve was in stable condition.

2.5 Methods of analytics

The pH and temperature were measured using a pH meter (edge® Dedicated pH/ORP Metre Hanna-HI2002, Viale Delle Industrie, Italy). The substrate's electrical conductivity (EC) was measured using a salinity meter (edge® Dedicated Conductivity/TDS/Salinity Metre Hanna-HI2003, Viale Delle Industrie, Italy). The determination of total solids (TS) and volatile solids (VS) was done using a muffle furnace (Carbolite CWF 1100, Carbolite Gero Ltd., Hope, UK). Total and volatile solids were computed using the standard approach outlined in.[66] The concentrations of total nitrogen (TN), total phosphorous (TP), chloride ion (Cl), and total ammonia nitrogen (TAN) were measured using a waste-to-distilled water ratio of 1:10. The examination of all specimens was conducted using the approach described by Radojevic and Bashkin in their book "Practical Environmental Analysis".[67] We analyzed carbon, oxygen, hydrogen, and nitrogen using an elementary analyzer (Perkin-Elmer Vector 8910) per the instructions provided by the manufacturer.

Table 2. Details on chicken manure and sewage sludge ratios and the concentrations of IONP in mixes.

Mix ID	Chicken manure and sewage sludge ratio	IONP concentration (mg/L)
C1	100% SS	0
C2	30% CM: 70% SS	0
C3	50% CM: 50% SS	0
C4	70% CM: 30% SS	0
C5	100% CM	0
M1	70% CM: 30% SS	40
M2	70% CM: 30% SS	80
M3	70% CM: 30% SS	120
M4	70% CM: 30% SS	160

3. Calculations

3.1 Theoretical potential for biochemical methane production

To determine the theoretical methane potential of a substrate, one may analyze the element compositions (C, H, O, and N) based on its chemical composition, as described by Buswell *et al*.[68] Subsequently, the proportions of these components were used in a stoichiometric equation. The stoichiometric Equation took into account the inclusion of components., as shown in (1) and (2) [69]

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c-3d}{4}\right)H_2O \rightarrow \left(\frac{4a+b-2c-3d}{8}\right)CH_4 + \left(\frac{4a-b+2c+3d}{8}\right)CO_2 + dNH_3 \tag{1}$$

Therefore,

$$BMP_{Th}(LCH_4/kgVS) = \frac{22.4\times\left(\frac{4a+b-2c-3d}{8}\right)*1000}{12a+b+16c+14d} \tag{2}$$

The value 22.4 represents the volume (in liters) filled by an ideal gas under normal circumstances, which consist of a temperature of 273 K and a pressure of 101.3 kPa. The factor of 1000 is used to convert the volume measurement from liters (L) to milliliters (mL).

3.2 Conceptual estimation of chemical oxygen demand

The theoretical chemical oxygen demand (COD_{Th}) content of the substrate was determined using equations (3) and (4). The Equation applies to all substrates under laboratory conditions:

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c-3d}{4}\right)O_2 \rightarrow aCO_2 + \left(\frac{b-3d}{2}\right)H_2O + dNH_3 \tag{3}$$

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

3.3 Experimental biochemical methane potential

To assess the reliability of the procedures described below, the experimental biochemical methane potential ($BMP_{experimental}$) is determined by adjusting the $BMP_{experimental}$ value obtained from volatile solids (VS) using Equation (5).

$$BMP_{experimental}\,(LCH_4/kgVS) = \frac{BMP_{\,Test\ bottles} - BMP_{Blank\ bottles}}{VS} \tag{5}$$

3.4 Biodegradability percentage

Efficiency calculation for biodegradation percentage The assessment of anaerobic biodegradability included measuring the BMP as a proportion of the $BMP_{theoretical}$ using Equation (6).

$$Biodegradability\% = \frac{BMP_{experimental}}{BMP_{Th}} \tag{6}$$

3.5 Analytical examination of statistical data

The statistical analyses were performed using IBM SPSS Statistics (version 26). The study used ANOVA with Bonferroni correction and a 95% confidence interval to evaluate the anaerobic co-digestion process. The study examined the standard forms of mixed models and best-fit regression equations to optimize the response variables. This analysis was conducted using ANOVA with the assistance of software OriginPro 2018.

3.6 Simulation of methane generation

This model is specifically designed for batch digesters since the growth of bacteria directly influences methane output. The Gompertz equation, as shown in equation (7), elucidates the correlation between the cumulative methane production and the digestion duration, considering the potential methane production, the peak rate of methane production, and the length of the delay phase.[70] This equation is often used in simulating the buildup of methane and has been recognized as a reliable experimental model for nonlinear regression.[69,71]

$$Y(t) = Y_m \times exp\left\{-\,exp\left[\frac{\mu_m\cdot e}{Y_m}\times(\lambda - t) + 1\right]\right\} \tag{7}$$

In Equation (9), Y(t) is the cumulative methane production (LCH_4/KgVS) at a given time (t), Y_m is the cumulative LCH_4/ KgVS.d production (LCH_4/LgVS), λ is the stagnation period (d), μ_m is the maximum methane yield (LCH_4/ KgVS.d), t is the digestion time (d), and e is Euler's number (e = 2.7183).

4. Results and discussion

4.1 Characteristics of Substrates

Table 3 displays the properties of CM and SS. The recorded pH value of 9.72 in the CM is consistent with the average values reported in the existing literature, which typically fall within the range of 8.51 to 10.24.

Table 3 displays the characteristics of two substrates, namely CM and SS. The VS content of dried CM and SS was 201.79 gVS/kg wet weight and 16.18 gVS/L, respectively.

Table 3. Characteristics of chicken manure and sewage sludge.

Parameter	Unit	Chicken manure	Sewage sludge [59]
Total solids	gTS/kg wet weight	262.14	-
	gTS/L	-	20.88
Volatile solids	gVS/kg wet weight	201.79	-
	gVS/L	-	16.18
Total kjeldahl nitrogen	gN/kg VS	11.75	132.35
Total phosphorous	gP/kg VS	4.23	24.70
Total organic carbon	%DM	31.45	53.20
Electrical conductivity	µS/cm	3412.50	337.00
C/N	%	10.24	6.50
pH	–	9.72	7.32
BMP_{Th}	$LCH_4/kgVS$	496.15	374.44
Elemental analysis			
Carbon	%DM	41.54	44.49
Hydrogen	%DM	5.51	5.45
Oxygen	%DM	32.70	43.37
Nitrogen	%DM	4.01	6.69

This suggests that organic matter is the main constituent of dry CM and SS. The elemental makeup of CM and SS was described using the molecular formulae $C_{17.31}H_{27.30}O_{10.22}N$ and $C_{7.76}H_{11.30}O_{5.68}N$, respectively, as shown in Table 3. The BMP_{Th} of CM and SS were calculated to be 496.15 and 374.44 $LCH_4/kgVS$, respectively, using Equations (1) and (2). The BMP_{Th} approach demonstrates remarkable dependability in accurately estimating methane output, with a small percentage error compared to the BMP_{exp} method. This discovery aligns with the findings of the investigations by Yasim *et al.* and Nielfa *et al.*[72,73] The impact of sulfur was minimal and inconsequential, as shown by the elemental analysis findings, which revealed a sulfur concentration below the detectable threshold. Based on the empirical analysis, the theoretical COD values for CM and SS were determined to be 1.32 and 1.07 (gO_2/gVS), respectively, using Equations (3) and (4).[74] found that low concentrations of TAN ranging from 50 to 200 mg/L stimulate the development of anaerobic bacteria.

Conversely, high TAN levels over 1200 mg/L inhibited the AD process. Insufficient nitrogen generally restricted the methane synthesis of SS, whereas excessive nitrogen hindered the anaerobic digestion of chicken manure.[24,75]

4.2 Effects of CM and SS different ratios on methane production

Upon reaching the end of the incubation period, the varying ratios of CM and SS groups exhibited different levels of methane production. Specifically, in batches C1, C2, C5, C3, and C4, the total methane production reached 278.8, 325.3, 341.1, 367.6, and 389.7 $LCH_4/kgVS$, respectively (Fig. 1a). This outcome aligns with the discoveries made by Rahman *et al.*[78] The results indicate that C1, C2, C5, C3 and C4 reveal biodegradability rates of 47.8%, 41.5%, 53.1%, 61.2%, and 68.1%, respectively, using Equation (6). The results indicate that including different proportions of chicken manure and sewage sludge increased methane production efficiency during the anaerobic co-digestion process. The experimental methane production data was modeled using the modified Gompertz model in Fig. 1b, using Equation (7). The findings suggest that batches C1, C2, C5, C3, and C4 had lag periods lasting 12.9, 12.6, 12.3, 11.5, and 11.1 days, respectively. According to the research conducted by Shapovalov *et al.* and Yin *et al.*[79,80] using chicken manure as a substrate, it has been shown that the mean length of the lag phase spans from 2.1 to 15.1 days. As a result, larger amounts of methane were generated within a somewhat shorter period.

4.3 Model fitting and regression analysis

The investigation used a mixture design technique to examine the interactions among the components in a mixture, explicitly focusing on optimizing the response. The mixed-design experiment subjected the independent variables to fitting procedures using linear, quadratic, and complete cubic models. The equation for the model is shown in equation (8). The standard error of regression (S) was used as a metric to assess the adequacy of the model in both regression and analysis of variance (ANOVA) analyses. Consequently, a more robust predictive capacity of the equation is associated with a decrease in the value of S. An additional parameter that was taken into account for the evaluation of the model was the R^2 coefficient of regression since it signifies the model's association with one or more predictor variables. In addition, the regression models used the proportion of the CM:SS of each combination as independent variables, while the cumulative methane production was considered the response variable. The best model (linear, quadratic, and cubic models) was chosen based on minimizing the S and maximizing the R^2. Upon evaluating the criteria, it was determined that the

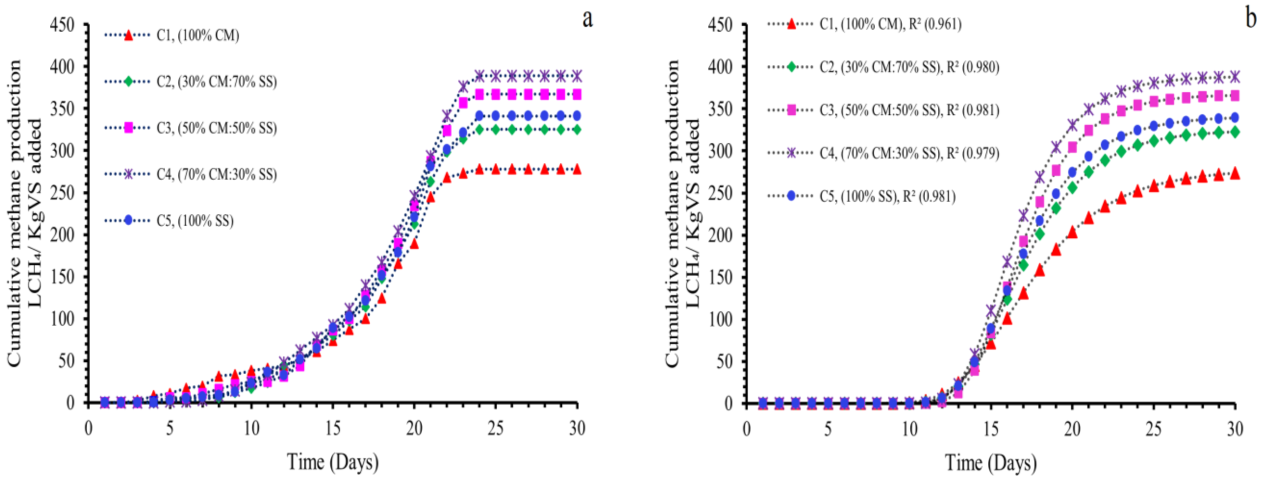

Fig. 1 Cumulative methane production with varying ratios of CM and SS with standard deviation range (0.02-0.24): (a) experimental data, (b) modified Gompertz model fit.

complete cubic model was the most suitable for testing. The R^2 value, which quantifies the degree of fit of regression equations for the cubic model, is 99.9%. Upon evaluating the criteria, it was determined that the complete cubic model is the most appropriate choice for the CM:SS ratio.

$$Cubic\ modele\ y = -0.0005x3 + 0.0477x2 + 0.5571x + 278.74 \tag{8}$$

4.4 Model validation

The experimental findings strongly correlated with the anticipated values described in Table 4. The experimental data reveals that the highest values for cumulative methane production are 389.7 LCH_4/kgVS. These values are seen when the mixture consists of 70% CM and 30% SS. Experiments were undertaken using the optimal compositions found to validate the model. The outcomes derived from using a 73 % CM and 27% SS composition yielded a 390.5 LCH_4/kgVS value for BMP, as shown in Fig. 2. The little deviation in the experimental findings compared to the expected data was afterward validated by the congruity between the acquired and predicted data.

4.5 Effects of IONP on Hydrolysis with 70% CM and 30% SS

The impact of IONP on COD solubilization was assessed due to the importance of hydrolysis in the kinetics of anaerobic digestion and its frequent role as the limiting factor. IONP concentrations of 40, 80, 120, and 160 mg/L were used in anaerobic batch studies. Based on the results (Fig. 3), the lowest concentration of soluble COD in the control incubation (C4) was 576 mg/L, achieved after six days of incubation. The

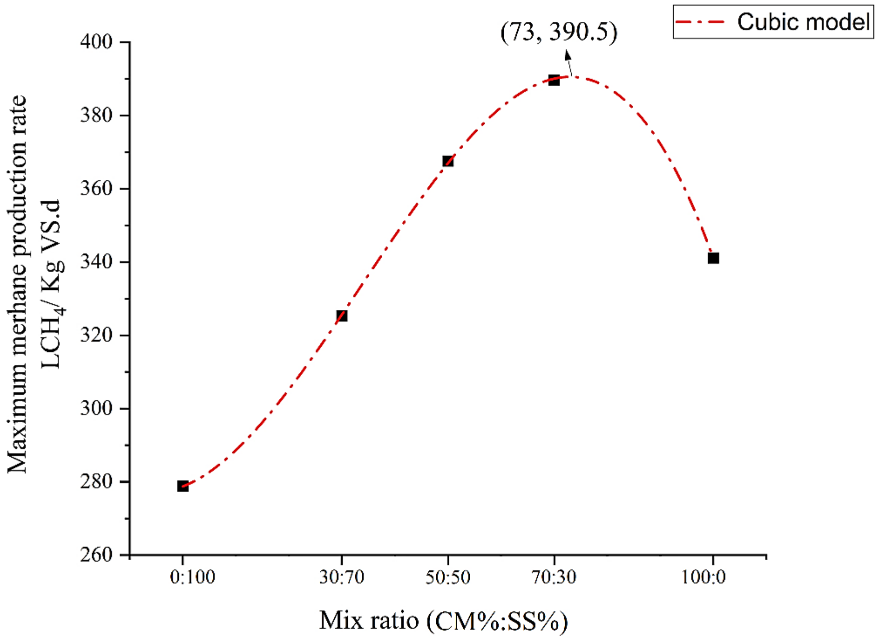

Fig. 2 Optimization plot for BMP depending on mixing ratios of CM:SS.

Table 4. Actual and expected values of cumulative methane production at different mixing ratios for CM:SS.

Mixing ratio CM/SS	Cumulative methane production $LCH_4/kgVS$	
	Actual value	Expected value
0:100	278.8	278.7
30:70	325.3	324.9
50:50	367.6	363.3
70:30	389.7	380.0
100:0	341.1	311.4

highest amounts of soluble COD that could be dissolved for batches treated with IONP were 1974, 1614, 1429, and 1081 mg/L for M4, M3, M2, and M1, respectively. The study revealed a direct correlation between the rise in sCOD and the increase in methane generation.[81–83] Discovered that adding IONP increased the production of metabolic intermediates and enhanced the performance of important enzymes in methanogenic Archaea.[81–83] The hydrolysis percentages after six days were computed to identify the reason for the increased soluble COD in IONP-amended batches. This was done to ascertain whether the increase was due to enhanced hydrolysis or the buildup of sCOD caused by reduced methanogen consumption. The results indicate that the incubation batches M4, M3, M2, and M1 reached hydrolysis percentages of 90.27%, 80.12%, 74.41%, and 70.23%, respectively. In comparison, batch C4 achieved a hydrolysis percentage of 40.47%. Therefore, it can be inferred that IONP enhanced the hydrolysis process. The ultimate hydrolysis percentages achieved in the IONP-modified batches were much higher than the 61.34% obtained in C4 during the last period. Specifically, the percentages were 80.01%, 86.78%, 90.98%, and 96.17% for M1, M2, M3, and M4, respectively.[84] The positive impacts of IONP on the hydrolysis process have been documented by Hassaan *et al.*, Bharathiraja *et al.* and Montingelli *et al.*[85–87] These findings emphasize that the hydrolytic enzymes released during cell breakdown promote the speeding up of biomass hydrolysis. Furthermore, the positive impact of magnetite was also confirmed in a two-phase anaerobic digestion system. The addition of magnetite improved the breakdown of complex organic compounds and facilitated the subsequent production of methane during the methanogenic phase.[82,84,88]

4.6 Effects of IONP on methane production with 70% CM and 30% SS

The observed mechanisms of hydrolysis and acidification are expected to influence the following methanogenesis. After the incubation period, the IONP-treated groups (shown in Fig. 4a) produced a total methane of 421.9, 556.3, 518.4, and 560.6 $LCH_4/kgVS$ in batches M1, M2, M3, and M4, respectively. These data indicate that the most effective concentration for improving biogas and methane production is 160 mg/L of IONP. This result aligns with the discoveries made by Liu *et al.*,[89] who noted that the liberation of iron ions due to the breakdown of magnetic nanoparticles could have played a role in stimulating bacterial activity. Furthermore, IONP aids in even distributing iron ions throughout the slurry. Moreover, the corrosion mechanism of nanoparticles enables a continuous and controlled release of iron ions inside the bioreactor. The research conducted by[90] demonstrated that increased quantities of nanoparticles substantially impact the breakdown of soluble protein and the conversion of electron donors in methanogenic Archaea. This activity is closely linked to the movement of protons driven by redox reactions, as indicated by the presence of coenzyme F420. The efficacy of coenzyme F420 was shown to be contingent upon the nanoparticle dose. The methane enhanced of batch M1 was compared to that of the control incubation, and the findings showed a 22.5% increase. The study revealed that M2, M3,

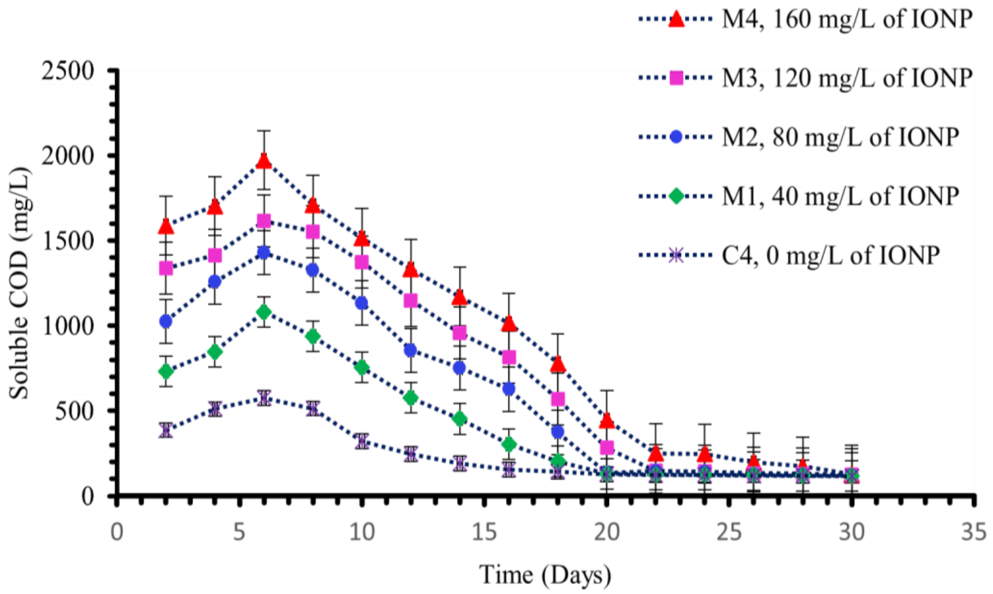

Fig. 3 The effect of 70% CM and 30% SS with IONP dosages on soluble COD.

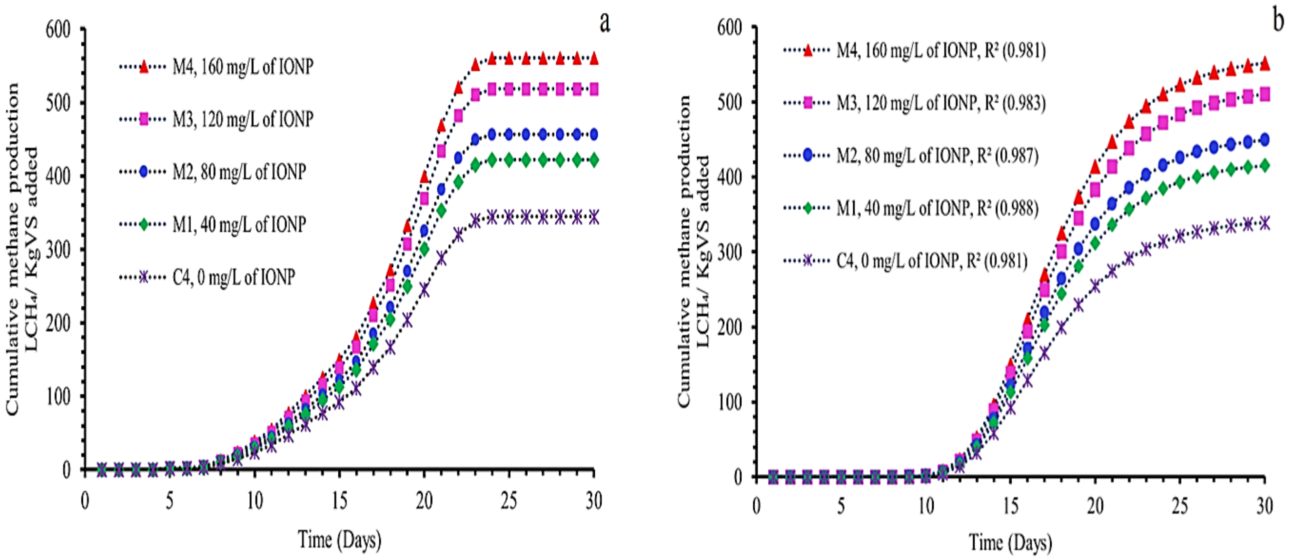

Fig. 4 Cumulative methane production with different IONP doses and standard deviation range (0.022-0.15): (a) experimental data, (b) modified Gompertz model fit.

and M4 had increases in methane production, with corresponding increments of 32.5%, 50.5%, and 62.7%. The research[91–93] showed that adding IONP led to substantial enhancements in methane generation, with increases ranging from 10 to 80%.[91–93] The results indicate that C4 has a biodegradability rate of 56.1%, whereas M1, M2, M3, and M4 have biodegradability rates of 60.4%, 74.7%, 87.2%, and 96.9%, respectively. The results clearly show that adding IONP increased methane generation efficiency when chicken manure and sewage sludge were digested together without oxygen. Following this period, there was a substantial increase in the maximum rate of methane production for batches M1, M2, M3, and M4, with increases of 22.5%, 32.5%, 50.5%, and 62.7%, respectively, compared to the control incubation. This is shown in Fig. 5.

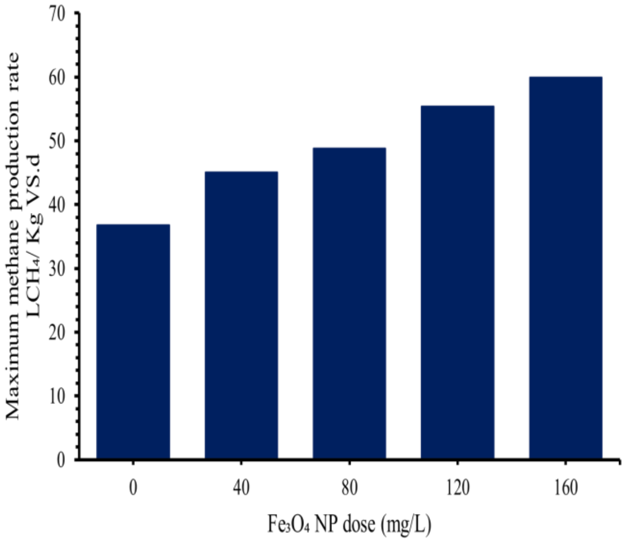

Fig. 5 Effects of different IONP doses on maximum methane production, computed from a modified Gompertz model.

5. Conclusions

This study focused directly on the impact of the concentration of the IONP on anaerobic co-digestion of CM and SS. IONP enhanced anaerobic co-digestion performance. Adding 160 mg/L of the IONP improved the methane volume 1.4-fold over digestion without IONP. The CH_4 content was enhanced by 32.5%, 50.5%, and 62.7% using 80, 120, and 160 mg/L IONPs, respectively, compared to the control test. Adding 160 mg/L of the IONP resulted in the maximum methane content and biodegradability. A modified Gompertz model represented the methane production well and quantified that adding 160 mg/L of the IONP increased the rate and extent of methanogenesis without altering the lag time.

Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

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