



## Article

# Experimental Investigation of Methane Generation in the Presence of Surface and Un-Surface Nanoparticles of Iron Oxide

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**Abstract:** The exploitation and harnessing of renewable energies are becoming increasingly important throughout the world. This study presents a method of methane (CH<sub>4</sub>) generation using biological disintegration of food waste (FW) by anaerobic digestion (AD). The CH<sub>4</sub> production was enhanced by the addition of three different types of iron oxide (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles (NPs) (Cetyltrime-thylbromide (CTAB), urea-capped Fe<sub>3</sub>O<sub>4</sub> NPs and Fe<sub>3</sub>O<sub>4</sub> NPs without capping). The bio generation of CH<sub>4</sub> and biodegradation of volatile solids (VS) were carried out in an AD treatment at mesophilic conditions (35–37 °C) for more than 50 days in batch mode. The concentration of all three types of NPs was kept constant at 75 mg/L. It was noticed that urea-capped NPs produced the maximum CH<sub>4</sub> (5.386 L), followed by Fe<sub>3</sub>O<sub>4</sub> NPs (5.212 L). Methane production in the control bioreactor was 2.143 L. The experimental results of CH<sub>4</sub> generation (a dependent variable) were analyzed against the concentrations of NPs used (as independent variables) in multiple regression analysis (MRA). The overall model for the experiments resulted in R<sup>2</sup> and R-adjusted values of 0.995 and 0.993, respectively.

**Keywords:** anaerobic digestion; methane generation; multiple regression analysis; food waste



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## 1. Introduction

Global warming and rapid urbanization have become big problems that must be talked about and managed. An increasing population and mass migration of people from rural to urban areas have added extra pressure to the energy demand in developed and developing nations [1]. On the other hand, in a report by the WWF, the burning of fossil fuels is reported as the primary contributor to global warming. With this in mind, renewable energy sources have been cited as the best alternative to decrease environmental pollution [2,3].

Alternative renewable energy sources include wind, solar, tidal, and biomass, and their use is planned to add energy production on a priority basis. The generation of biogas (which consists of 50–60% of methane with an upper calorific value of 6.0 kWh/m<sup>3</sup> at standard temperature and pressure (STP)) is achieved from the oxygen-free bio-disintegration of biomass such as animal manure, crop residue, food waste, etc. [4,5]. Anaerobic digestion (AD) has been reported as a well-established and well-proven technology to treat organic waste (OW) [6]. AD treatment depends on multiple factors such as pH, temperature, organic loading, supplements, and pretreatment types and extents. Nanotechnology has shown excellent compatibility and promising results in AD in recent years. In the literature, it is reported that the addition of nanoparticles (NPs) enhanced methane generation (CH<sub>4</sub>) from 21.5% (size of NPs lies between 50 to 100 nm) to greater than 150% by offering a greater surface area, a high reaction rate, and accelerated enzymatic activities [7,8].

Anaerobic digestion (AD) of organic matter (OM) has always been questioned due to the time-consuming bio-disintegration process. NPs have been called biodegradation accelerators due to their unique physicochemical properties. Therefore, urea-capped  $\text{Fe}_3\text{O}_4$  nanoparticles (U- $\text{Fe}_3\text{O}_4$  NPs) are attractive materials for enhancing methane gas production by improving polycyclic aromatic hydrocarbons (PAHs) [9–11]. The mathematical demonstration of experimental results is as important as novel research. It enhances our understanding of physical processes by establishing models to design and upscale future lab work [12]. In this regard, Nweke et al. [13] and Nwabanne et al. [14] applied a first-order kinetic model to determine the substrate consumption rate. Furthermore, the Monod model and modified Gompertz model were reported to predict microbial or biomass growth and methane production, respectively [15–18]. Moreover, Sung and Liu conducted a linear regression analysis to describe ammonia inhibition in anaerobic digestion. In another study, multiple regression analysis (MRA) was applied to compare the biomethane potential (BMP) of various feedstocks [19,20].

Hence, this study was designed to carry out a MRA of anaerobically produced methane in the presence of NPs. During the mathematical analysis, the cumulative  $\text{CH}_4$  production from the control bioreactor ( $R_c$ ) is established as the dependent variable and the bioreactors supplemented with NPs ( $\text{Fe}_3\text{O}_4$ , CTAB-capped, urea-capped) as the independent variables. This study provides data from a statistical analysis of the AD process when augmented with NPs.

## 2. Materials and Methods

### 2.1. Materials and Chemicals

Iron chloride tetra and hexahydrate (Fe-II and Fe-III) were used as fundamental salts to synthesize the NPs. The synthesis process was the same as in [8], the results of which have been published in our previous studies [4,18]. Non-homogeneous leftover food waste (FW) was used as a substrate and was collected from various on-campus locations.

### 2.2. Substrate and Nanoparticles Preparation

Before adding NPs to the bio digesters, the dried NPs were sonicated using a Sonicator (IK/UTSB/01), IMehran University, Jamshoro, Pakistan at 40 °C for 15 min to remove impurities and liquefy the NPs as shown in Figure 1. Then, the impurities were slowly removed from the surface.



**Figure 1.** Removal of impurities from NPs by sonication.

The leftover FW contained various wastes, which included mainly cooked and some uncooked portions. Initially, the biodegradables were separated from the non-biodegradables manually. The size was reduced using a mechanical grinder as shown in Figure 2; thus, the homogeneity of the fractions was ensured.



**Figure 2.** Preparation of substrate for anaerobic treatment.

### 2.3. Experimental Setup

The AD of FW was set to mesophilic conditions (35–37 °C) in a batch mode for 50 days. We used 500 mL culture bottles as the bioreactors with a 300 mL working volume, as presented in Figure 3. Organic loading and NP concentration (except control,  $R_c$ ) in each bioreactor were set to 6.653 gVS and 75 mg/L, respectively. The bioreactors were labeled  $R_{ct}$ ,  $R_u$ ,  $R_i$ , CTAB capped loaded, urea added, and  $Fe_3O_4$  supplemented.



**Figure 3.** Lab scale batch mode anaerobic digester setup and bioreactors.

Each bioreactor was run in duplicate to improve the accuracy of the reading. Before the bioreactors were placed into the thermally coupled water bath, nitrogen gas ( $N_2$ ) was used to purge the bioreactors for 5–10 min to confirm the anaerobic conditions. Furthermore, DC motors were installed for mixing the substrate for 30 s every 30 min. An alkali solution of 5M NaOH was used to measure  $CH_4$  by adsorbing the other gases, as reported in [21]. Note that methane generation was measured every day. All the pre-and post-parameters were analyzed during batch assays [4,18,22].

### 2.4. Regression Model

This study aimed to find an equation that describes or summarizes the relationships in a set of data [23]. The data set is based on the impact of different types of NPs on methane generation and was studied using linear regression (single factor/variable) and multiple regression models (MRM) in MS Excel 2016 (Microsoft, Redmond, WA, USA). Kafle and Chen performed the analysis using an earlier version of MS Excel (2007) [20]. Furthermore, this model was created in order to analyze the straight-line relationships between the four variables. The general multiple regression estimates the  $\lambda$ s in the equation as [20]:

$$Y = \lambda_0 + \lambda_1 X_1 + \lambda_2 X_2 + \lambda_3 X_3 + \dots + \lambda_n X_n \quad (1)$$

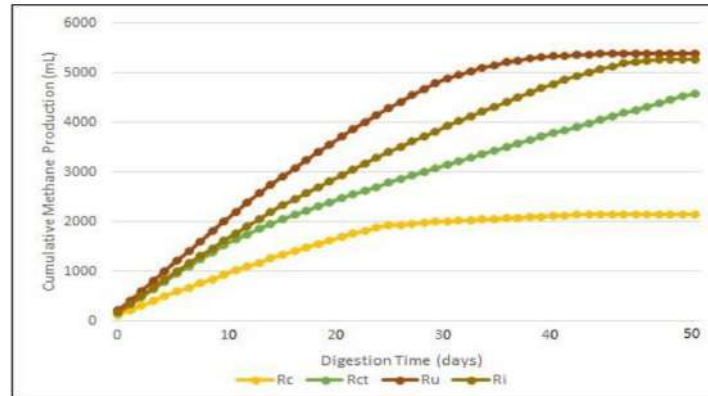
where  $X_n$  represents an independent variable (IV's) and  $Y$  is the dependent variable. The  $\lambda$ s are the unknown regression coefficients. The significance of the results was determined by assuming a confidence interval ( $\alpha = 0.05$ ) of 95%.

## 3. Results and Discussion

### 3.1. Nanoparticles vs. Methane Generation

The impact of the addition of NPs was experimentally studied in the batch mode of AD of FW to enhance bio-methane generation. Figure 4 shows that the bioreactors loaded with NPs ( $R_u$ ,  $R_{ct}$ , and  $R_i$ ) exhibit high methane production. Abdelsalam et al. [8] reported a 1.6-fold enhancement of methane when 20 mg/L of  $Fe_3O_4$  NPs were added to bioreactors. In our study,  $Fe_2O_4$ , CTAB, and urea-capped NPs enhanced  $CH_4$  generation by 1.45, 1.14, and 1.52 times at 75 mg/L concentration of NPs. Cumulatively, the methane production was 2.14 L, 4.584 L, 5.386 L, and 5.255 L in  $R_c$ ,  $R_{ct}$ ,  $R_u$ , and  $R_i$ , respectively. Khalid et al. [24] observed a 129% increase in methane yield while treating rice straw in the presence of magnetite and alkali pretreatment. Moreover, Zhang et al. [25] reported that methanogens, which are the prime bacteria involved in methane generation, could be improved by adding nano zero-valent iron NPs [26]. This difference in  $CH_4$  production might be caused by the composition of substrate irrespective of NP concentration. FW contains various components, and each component has its own biodegradability capacity. Apart from the bio methane increment, the addition of NPs increased biocompatibility and

improved process stability. The effect of NPs on pH, VS, TS, and other improvements in kinetics has been discussed and reported in our previous studies [4,18]. Furthermore, the supplementation of NPs expanded methane generation, accelerated the hydrolysis rate and reduced the lag phase.

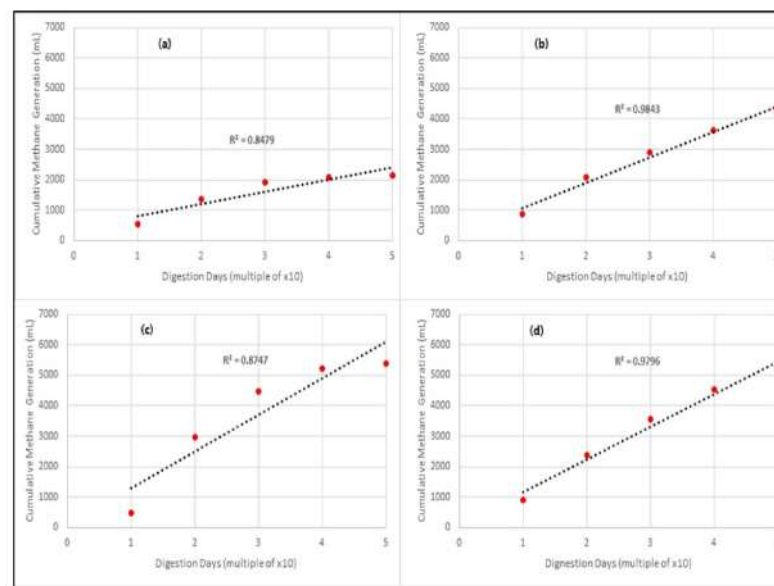


**Figure 4.** The impact of NPs on cumulative methane generation in  $R_u$ ,  $R_i$ , and  $R_{ct}$  bioreactors with respect to  $R_c$  (control bioreactor).

The addition of NPs endured the highest removal of volatile and total solids, which reached more than 92% and 90%, respectively [18]. Meanwhile, 20 mg/L of  $Fe_3O_4$  achieved 74% removal [8]. The results of our study conflict with the findings of Abdelsalam, who used a two-stage system involving a thermophilic liquefaction reactor and a mesophilic anaerobic filter to convert over 95% of volatile solids to methane [27].

### 3.2. Linear Regression Analysis (LRA)

The impact of NP addition on cumulative  $CH_4$  generation was analyzed by considering single-variable data. Figure 5 illustrates the scattered nature of the linear model of experimentally generated  $CH_4$ . In Figure 5a–d, the  $R^2$  lies in the range of 0.848 to 0.984. This shows that the linear regression model fits well with the experimental data.



**Figure 5.** Simple linear regression plots; (a)  $R_c$ , (b)  $R_{ct}$ , (c)  $R_u$ , and (d)  $R_i$ .

### 3.3. Multiple Regression Analysis (MAR)

All four variables ( $R_c$ ,  $R_{ct}$ ,  $R_u$ , and  $R_i$ ) were considered together during our analysis. The effect of different variables has been studied in the literature [28,29]. The MAR fit

the data well and produced a 0.993 coefficient of determination ( $R^2$ ). The R-adjusted was found to be 0.993, but with the linear regression model  $R^2$  was 0.848 to 0.984 (Table 1). The results in this study are closer to the effects of multiple regression analyses presented by [29]. More than 99% invariance in methane generation in the control bioreactor was observed by adding NPs to the bioreactors.

**Table 1.** Analysis of variance (ANOVA).

Model		Sum of Squares	df	Mean Square	F	Sig-F
1	Regression	19,412,872.36	3	6,470,957.453	3448.952147	0.00 <sup>a</sup>
	Residual	86,305.64594	46	1876.209694		
	Total	19,499,178.01	49			

<sup>a</sup> Predictors: (constant),  $CH_4$  generation from  $R_{ct}$ ,  $R_u$ , and  $R_i$ ; Dependent variable:  $CH_4$  generation from  $R_c$ .

The details of this analysis in MS Excel 2016 are given in the Supplementary Materials at the end of this article. The experimental data show that the maximum production of  $CH_4$  was achieved at 50 days of digestion. The R-adjusted value also confirms that 50 days of digestion is sufficient to measure the methane potential. In Table 1, the Sig-F value, which is lower than  $\alpha = 0.05$ , confirms that the methane production was significantly improved when NPs were added. The general multiple regression models developed based on the nonstandard coefficients is given in Equation (2):

$$Y_{CH_4} = 44.485 + 0.384R_{ct} + 0.588R_u - 0.525R_i \quad (2)$$

where  $Y_{CH_4}$  is the cumulative methane yield from  $R_c$ , which was set as a dependent variable. To test the validity of MRM, a global test must be used, which determines whether all the independent variables have regression coefficients equal to zero. The samples of regression coefficients correspond to the regressions as reported by [29,30]. In another study, the multiple regression model was estimated by considering three independent variables as given in Equation (3):

$$G_0 = -163.8 + 12.85CP - 13.06LIG + 7.85ADF \quad (3)$$

where  $G_0$  is the methane potential,  $CP$  is the crude protein,  $LIG$  is lignin, and  $ADF$  is acid detergent fiber. This model was found to be the best fit to predict the experimental results when  $R^2$  and R-adjusted were both 0.999. Therefore, the results of this study are entirely in agreement with the results of [20]. The summary of the complete regression analysis is given in the Supplementary Materials (Table S1).

#### 4. Conclusions

The addition of NPs to AD boosted methane generation significantly with good process compatibility. Three of the performance indicators, namely the methane generation from  $R_{ct}$ ,  $R_u$ , and  $R_i$ , are significant predictors of the dependent variable  $CH_4$  production from  $R_c$ . Our regression analysis showed that the multiple regression model (MRM) best fits the experimental data rather than the single factor/variable regression model. The  $R^2$  and R-adjusted coefficients of the MRM were found to be good. Thus, we mathematically demonstrated that adding NPs significantly improved  $CH_4$  generation.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/agriengineering4010009/s1>, Figure S1: Data for regression analysis, Table S1: Data in trend format for regression analysis.

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