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Residence Time Reduction in Anaerobic Reactors: Investigating the Economic Benefits of Magnetite-Induced Direct Interspecies Electron Transfer Mechanism

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Abstract: Existing research on direct interspecies electron transfer (DIET) has predominantly focused on the types and concentrations of conductive materials across diverse anaerobic digestion. However, insufficient understanding of the impact of residence time, a critical economic factor, prompted this investigation. Magnetite, a conductive material, was introduced into the anaerobic digestion of food wastewater, leading to a significant increase in ultimate methane production (B_u) with 25 mM-Fe_3O_4 ($p < 0.05$). Despite a subsequent decline in methane production efficiency from 388.9% to 7.1% over the 15- to 65-day anaerobic digestion period, the initial impact of increased methane production due to magnetite addition was evident. Control's maximum methane production rate (R_m) was 27.5 mL/day, reaching its highest point at 37.4 mL/day with 15 mM-Fe_3O_4, accompanied by a noteworthy 56.6% reduction in the attainment day of R_m ($R_{m\text{-}day}$), shortened to 8.2 days. Even with 100 mM-Fe_3O_4, while B_u showed no significant difference, $R_{m\text{-}day}$ exhibited a substantial reduction of 22.8. Despite the lower overall anaerobic digestion efficiency under some magnetite input conditions, this study confirmed a substantial shortening of $R_{m\text{-}day}$, suggesting that the DIET mechanism induced by conductive materials such as magnetite could reduce the residence time in continuous-type anaerobic reactors, contributing to improved economic feasibility.

Keywords: magnetite; anaerobic digestion; direct interspecies electron transfer; maximum methane production rate; residence time

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Citation: Kim, T.-B.; Lee, J.-H.; Yoon, Y.-M. Residence Time Reduction in Anaerobic Reactors: Investigating the Economic Benefits of Magnetite-Induced Direct Interspecies Electron Transfer Mechanism. *Energies* **2024**, *17*, 358. https://doi.org/10.3390/en17020358

Academic Editors: Alok Kumar Patel and Eliseu Monteiro

Received: 5 December 2023
Revised: 30 December 2023
Accepted: 8 January 2024
Published: 10 January 2024

1. Introduction

The transferable energy potential of food waste in Korea for 2020 has been quantified as 419,116 TOE/year, constituting 55.2% of the total energy potential derived from organic biomass generated in urban areas [1]. The annual generation of food waste in Korea is 5,160,000 tons, and over 95% of this volume is recycled through methods such as conversion into animal feed or compost [2]. Notably, a substantial volume of food wastewater is produced during recycling processes associated with food waste. In Korea, food wastewater is directed to anaerobic digesters, where it serves as a raw material for biogas production.

In 2021, the operational count of biogas production facilities in Korea is 110 [3]. Food wastewater, distinguished by its heightened organic matter content and accelerated decomposition rate compared to livestock manure and sewage sludge, has emerged as a commendable feedstock for anaerobic digestion. The anaerobic digestion of food wastewater offers advantages in odor control over alternative physical, chemical, and biological treatment methods. Moreover, the co-digestion of food wastewater with livestock manure facilitates the utilization of the resultant digestate as a fertilizer for farmland without requiring supplementary wastewater treatment. Consequently, this integrated approach is acknowledged for its rationality and economic viability in renewable energy production and the circular utilization of resources [4].

Anaerobic digestion is a biological treatment method in which anaerobic microorganisms facilitate the conversion of organic substrates into methane. This process occurs through a sequence of hydrolysis, acidogenesis, acetogenesis, and methanogenesis reactions under anaerobic conditions devoid of oxygen [5]. The anaerobic microorganisms engaged in this intricate process operate in a syntrophic (cross-feeding) manner, wherein the product of one microbial reaction serves as a substrate for the subsequent microbial reaction. Notably, symbiotic relationships, especially in terms of nutritional interactions between acetic acid-producing and methanogenic microorganisms, play an important role in enhancing the operational stability of anaerobic digesters. This symbiosis elevates the activity of anaerobic microorganisms while simultaneously preserving the equilibrium of reaction rates among methane-producing microorganisms [6]. Within the context of acetogenesis, the electrons liberated during the generation of H_2 and formate serve as crucial components of the syntrophic mechanism of methane production. Indirect interspecies electron transfer (IIET) facilitates methane production during anaerobic digestion process [7]. The effectiveness of IIET within an anaerobic digester is notably influenced by shifts in environmental conditions, including variations in the pH, temperature, organic loading rate (OLR), and volatile fatty acids (VFAs) concentrations. This sensitivity is particularly evident when utilizing food wastewater as a substrate for anaerobic digestion, given its distinctive characteristics, such as low pH, elevated organic loading, heightened VFAs concentration, and increased salt content (Na^+, Ca^{2+}, K^+, Mg^{2+}) [8]. The physicochemical properties of food wastewater can induce rapid environmental fluctuations in anaerobic digesters. These abrupt changes in the anaerobic digester environment, arising from the unique properties of food wastewater, have given rise to challenges in the IIET mechanism. An imbalance in the reaction rates among anaerobic microorganisms results in the accumulation of VFAs within the digester [9]. Additionally, the decrease in pH adversely affects the efficiency of methane conversion to organic matter.

Consequently, the intricate interplay between these physicochemical characteristics poses challenges in maintaining optimal conditions for the IIET mechanism, thereby compromising the overall methane conversion efficiency of organic matter in the anaerobic digestion process [10–12]. Recent studies have focused on direct interspecies electron transfer (DIET) to enhance the stability of anaerobic digesters and boost the methane production efficiency. This involves introducing conductive materials into the digester to facilitate electron transfer between acetic acid-producing (electron donor) species and methane-producing (electron accepting) species with the aim of improving the overall operational efficiency [13,14]. Magnetite, known for its high conductivity, has been reported to improve the efficiency of DIET among microorganisms engaged in syntrophic relationships when introduced into anaerobic digester. This is attributed to the promotion of syntrophic interactions between microorganisms, resulting in enhanced electron transfer efficiency.

The conductive materials that facilitate DIET include iron-based (magnetite, hematite, ferrihydrite, etc.) and carbon-based (GAC, biochar, graphite, etc.) substances. Numerous studies have indicated that their incorporation promotes electron transfer among anaerobic microorganisms, mitigates VFAs accumulation, and augments the decomposition rate of organic matter within anaerobic digesters [15–18]. Furthermore, conductive materials contribute to the stability of anaerobic digestion by providing a reaction surface for biofilm formation by anaerobic microorganisms [19]. Materials such as magnetite have been reported to enhance the anaerobic digestion environment, mitigate the inhibitory impact of salt on food wastewater through adsorption reactions, and alleviate the inhibitory effects of hydrogen sulfide [20,21].

Studies on enhancing anaerobic digestion efficiency through DIET have exhibited varied outcomes influenced by factors such as the conductive material type, input concentration, and raw materials. While some studies indicate improved efficiencies, others suggest reductions [22–25].

Thus far, DIET research has predominantly focused on assessing methane production efficiency with respect to the addition of conductive materials. However, anaerobic digesters typically require extended residence times that frequently exceed 30 days. Studies on DIET using magnetite have reported research aimed at reducing lag growth times, improving methane production rates, and increasing methane yields. These findings suggest a significant enhancement of conventional anaerobic digestion through the supplementation of conductive substances. This suggests a potential for substantial improvement in anaerobic digestion through the addition of conductive substances [26]. From an economic standpoint, assessing the efficiency of anaerobic digestion is imperative, considering not only the impact on methane production enhancement, but also the potential benefits derived from shortening residence time. In the anaerobic digestion process, residence time is an important design factor that affects the economic feasibility of the initial installation cost. Additionally, shortening the residence time has the advantage of reducing the volume of the anaerobic digester and significantly improving its operating stability of the anaerobic digester. Nevertheless, the assessment of the impact of anaerobic digestion following the inclusion of conductive materials has predominantly focused on the biochemical methane potential (B_u) and maximum methane production rate (R_m). However, research on the potential impact of reduced residence time in this context is lacking. Therefore, this study aimed to analyze the influence of shortened residence time, a critical factor affecting the economic feasibility and operational stability of anaerobic digestion digesters. Concurrently, we investigated the impact of introducing conductive materials on methane production efficiency. Specifically, an assessment index was developed, focusing on the time to attain the maximum methane production rate during batch anaerobic digestion of food wastewater with varying inputs of conductive material (magnetite, Fe_3O_4).

2. Materials and Methods

2.1. Materials

The food wastewater, which used as a raw material for the batch anaerobic reactor in this study, was collected from the liquid food wastewater generated during the first solid–liquid separation process at a food waste composting facility located in Icheon City, South Korea. The analysis results of the chemical properties of the collected food wastewater are shown in Table 1; the SCOD in the food wastewater accounted for 85.3% of TCOD.

Table 1. Chemical composition of food wastewater and inoculum used in the biochemical methane potential assay.

Parameters	pH	TS [1]	VS [2]	TKN [3]	NH_4^+-N [4]	TCOD [5]	SCOD [6]	Alkalinity (as $CaCO_3$)	TVFAs [7] (as Acetate)
	(-)	(mg/L)							
Food wastewater	3.73	112,467	99,311	4393	613	179,540	153,127	n.a. [8]	11,513
Inoculum	7.93	45,333	24,567	5018	3763	29,727	9727	29,208	407

All data correspond to the average of three replications. [1] Total solid, [2] volatile solid, [3] total kjeldahl nitrogen, [4] ammonium nitrogen, [5] total chemical oxygen demand, [6] soluble chemical oxygen demand, [7] total volatile fatty acids, [8] not analyzed.

2.2. Biochemical Methane Potential (BMP) Assay

The study utilized inoculum sourced from a 20 m^3/day mesophilic (38 °C) anaerobic digester in Icheon, South Korea. The digester processed a mixture of pig slurry and food wastewater (7:3, *w*/*w*). After two weeks of anaerobic storage at 38 °C, the inoculum was used in a biochemical methane potential (BMP) assay, ensuring the removal of any residual biodegradable fraction. Table 1 presents the chemical analysis of inoculum. Employing a 160 mL serum bottle as a batch-type anaerobic reactor, the BMP assay incorporated 75 mL of inoculum. Food wastewater was utilized as the substrate in the anaerobic batch reactors, maintaining a consistent substrate to inoculum ratio of 0.5 (g-$VS_{substrate}$/g-$VS_{inoculum}$). The volume of the food wastewater used in the experiment was 9.28 mL. Magnetite (Fe_3O_4) powder (diameter < 5 μm) (Samchun, Seoul, Republic of Korea, CAS No. 1317-61-9) was used to facilitate the DIET. Various concentrations of magnetite (5, 15, 25, 40, 70, and 100 mM-Fe_3O_4) were introduced, equivalent to 1.2, 3.5, 5.8, 9.3, 16.2, and 23.2 g-Fe_3O_4/L concerning the reactor's effective volume. This approach allowed for the systematic exploration of DIET efficiency in the different concentration of magnetite. The control group was prepared without magnetite addition. Additionally, a blank test was conducted in the batch anaerobic reactor with 75 mL of inoculum, excluding food wastewater, to account for the inoculum-generated biogas. Both the sample and blank anaerobic batch reactors were replicated three times to ensure robust experimental consistency. Operating under mesophilic conditions at 38 °C, the batch-type anaerobic reactor facilitated anaerobic digestion of 65 days. The reactor was sealed with a butyl rubber septum stopper and aluminum crimp seal. Daily measurements of biogas generation and gas concentration were conducted. The measurement intervals were flexibly adjusted to accommodate the varying biogas production rates.

The biogas was quantified using a water-column-type gas meter. The cumulative methane production curve was derived by converting the generated biogas to dry gas volume under standard conditions (0 °C, 1 atm), as expressed in Equation (1). In Equation (1), $V_{dry\ gas}$ denotes gas volume at standard conditions, with T as the anaerobic reactor's operating temperature (38 °C). $V_{wet\ gas}$ represents the volume of wet gas at reactor temperature. P stands for atmospheric pressure during gas measurement (assumed as 760 mmHg), and P_T is the saturated water vapor pressure (calculated for 38 °C in this study) [27].

$$V_{dry\ \ gas} = V_{wet\ \ gas\ at\,T\ ^\circ C} \times \frac{273}{(273+T)} \times \frac{(P-P_T)}{760} \tag{1}$$

2.3. Analysis of Reaction Kinetics

This investigation scrutinized the methane production kinetics concerning magnetite input using the cumulative methane production [P(t)] curve derived from the BMP assay. The methane production rate [P'(t)] curve, obtained through the initial differentiation of the cumulative methane production curve, revealed the peak value as the maximum methane production rate (R_m). To evaluate the reduction efficiency of residence time, we determined the time ($R_{m\text{-}day}$) to achieve the maximum methane production rate (R_m) from the methane production acceleration [P'(t)] curve generated by the second differentiation of the methane production rate [P"(t)]. The point of zero methane production acceleration is defined as $R_{m\text{-}day}$ and serves as a key index for residence time reduction [28].

2.4. Chemical Analysis

Chemical properties and organic composition evaluation involved analyses of pH, alkalinity, total solid (TS), volatile solid (VS), total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4^+-N), and total volatile fatty acids (TVFAs) [29]. The gas samples were subjected to CH_4 and CO_2 concentration determination using a Clarus 680 gas chromatograph (PerkinElmer, Inc., Waltham, MA, USA) equipped with a thermal conductivity detector (TCD) and a HayeSepQ packed column (CRS, Inc., Louisville, KY, USA). Helium was used as the carrier gas at a flow rate of 5 mL/min. The injector, oven, and detector were set to 150, 90, and 150 °C, respectively [30].

2.5. Statistical Analysis

The results were statistically analyzed using the general linear model (GLM) procedure in SAS® (version 9.4; SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test revealed significant differences ($p < 0.05$) in the means between treatments, providing a robust assessment of the experimental outcomes [31].

3. Results and Discussion

3.1. Biochemical Methane Potential Assay

The biochemical methane potential (B_u) of food wastewater by magnetite input concentration is shown in Table 2, and the cumulative methane production curve [P(t)] during the operation period of the batch anaerobic reactor is shown in Figure 1. During the first 15 days of batch anaerobic reactor operation, the control exhibited a methane production efficiency of 0.036 Nm^3/kg-COD_{added}. The introduction of magnetite significantly enhanced the methane production efficiency, reaching its peak at 0.180 Nm^3/kg-COD_{added} with a magnetite input of 15 mM-Fe_3O_4. However, beyond this concentration, the methane production efficiency declined, reaching 0.124 Nm^3/kg-COD_{added} with a magnetite input of 100 mM-Fe_3O_4. After extending the operation period to 20 days, the control yielded a methane production efficiency of 0.109 Nm^3/kg-COD_{added}. Notably, the application of magnetite further optimized the efficiency, with a peak observed at 0.210 Nm^3/kg-COD_{added} with a magnetite input of 25 mM-Fe_3O_4. However, similar to the 15-day period, the efficiency gradually decreased with increasing magnetite input, stabilizing at 0.191 Nm^3/kg-COD_{added} to 100 mM-Fe_3O_4. The methane production efficiency trends observed during the 30–65-day period after batch reactor operation showed consistent patterns. This period demonstrates that the impact of magnetite input on methane production efficiency remained stable. Finally, over the 65-day batch anaerobic reactor operation period, the B_u of the control was 0.255 Nm^3/kg-COD_{added}. As the concentration of magnetite increased, there was a significant increase in B_u, reaching 0.273 Nm^3/kg-COD_{added} ($p < 0.05$) at 25 mM-Fe_3O_4. However, B_u subsequently decreased with higher magnetite inputs, reaching 0.255 Nm^3/kg-COD_{added} ($p < 0.05$) with 100 mM-Fe_3O_4. For a 25 mM-Fe_3O_4 magnetite input yielding the highest B_u, the escalating impact on methane production diminished over time, decreasing by 388.9%, 92.7%, 26.3%, 17.6%, and 7.1% at 15, 20, 30, 40, and 65 days of anaerobic digestion, respectively. This underscores the declining efficacy of the magnetite input in enhancing methane production with prolonged residence time in the reactor.

Table 2. Biochemical methane potential by the addition of magnetite during the anaerobic digestion of food wastewater.

Parameter	Residence Time (Days)	Control	Magnetite Concentration (mM-Fe_3O_4)					
			5	15	25	40	70	100
Methane potential (Nm^3/kg-COD_{added})	15	0.036 [e 2]	0.165 [ab]	0.180 [a]	0.176 [a]	0.151 [bc]	0.133 [cd]	0.124 [d]
	20	0.109 [c]	0.197 [ab]	0.206 [ab]	0.210 [a]	0.195 [ab]	0.195 [ab]	0.191 [b]
	30	0.205 [e]	0.253 [ab]	0.253 [ab]	0.259 [a]	0.240 [bc]	0.228 [cd]	0.217 [de]
	40	0.227 [b]	0.259 [a]	0.264 [a]	0.267 [a]	0.258 [a]	0.254 [a]	0.233 [b]
	65 [1]	0.255 [b]	0.264 [ab]	0.272 [a]	0.273 [a]	0.262 [ab]	0.259 [ab]	0.255 [b]

All data correspond to the average of three replicates. [1] Methane potential obtained at 65-day means of the biochemical methane potential (B_u), [2] Means with no common letter in a row are significantly different at the 5% level by DMRT multiple range test.

The study concluded that in the batch anaerobic digestion of food wastewater, the primary influence of magnetite lies in amplifying the initial methane production rather than sustaining elevated levels throughout the digestion period. This temporal analysis emphasizes the dynamic nature of the impact of magnetite on methane production efficiency. Lee et al. [32] conducted a batch reactor study using food wastewater and observed that magnetite additions of 0.25%, 0.50%, 1.00%, and 1.50%-Fe_3O_4 resulted in B_u values of 0.411, 0.412, 0.431, and 0.419 Nm^3/kg-VS_{added}, respectively. Notably, all magnetite treatment groups exhibited a significant increase in B_u compared to the control (0.380 Nm^3/kg-VS_{added}), with a 1.00%-Fe_3O_4 concentration (approximately 45 mM-Fe_3O_4), demonstrating the highest methane production efficiency. Although this aligns with our findings, the concentrations representing the maximum methane potential differed. The experimental substrate of Lee et al. had a TCOD of 166,333 mg/L, which is similar to the food wastewater TCOD of 179,540 mg/L in our study. This suggests that the impact of magnetite on methane production efficiency remains consistent across varying organic matter concentrations. The positive impact of magnetite on the methane production efficiency was evident across diverse substrates. In the anaerobic digestion of pig slurry, Zhang et al. [33] demonstrated an escalating B_u with increasing magnetite concentrations (5, 75, 150, and 350 mM-Fe_3O_4), resulting in B_u values of 0.303, 0.291, 0.318, and 0.332 $Nm^3/kg\text{-}VS_{added}$. Remarkably, at 350 mM-$Fe_3O_4$, B_u exhibited a substantial 16.1% increase compared to the control (0.286 $Nm^3/kg\text{-}VS_{added}$). Similarly, in the anaerobic digestion of sewage sludge, Wang et al. [34] observed augmented methane production upon the addition of magnetite (10, 50, and 100 mg-Fe_3O_4/g-TS) for 24 days of operation. In contrast, the influence of magnetite addition on the anaerobic digestion efficiency of food wastewater was variable. Although studies by Lee et al. [32] and Zhang et al. [33] showed increased methane production efficiency with magnetite, this phenomenon was not consistently replicated across all scenarios, underscoring the substrate-specific nature of the effects of magnetite in anaerobic processes. The efficacy of improving the anaerobic digestion of food wastewater through magnetite addition appears to be contingent on specific conditions. Akturk and Demier [35] revealed that in the control, the B_u was 0.409 $Nm^3/kg\text{-}VS_{added}$. However, with the introduction of magnetite at concentrations of 2.0, 5.0, and 10.0 g-Fe_3O_4/L, B_u values were 0.272, 0.370, and 0.413 $Nm^3/kg\text{-}VS_{added}$, respectively. Notably, in the 2.0 and 5.0 g-Fe_3O_4/L treatments, B_u decreased by 33.6% and 9.7%, respectively, compared to the control, indicating a reduction in methane production efficiency.

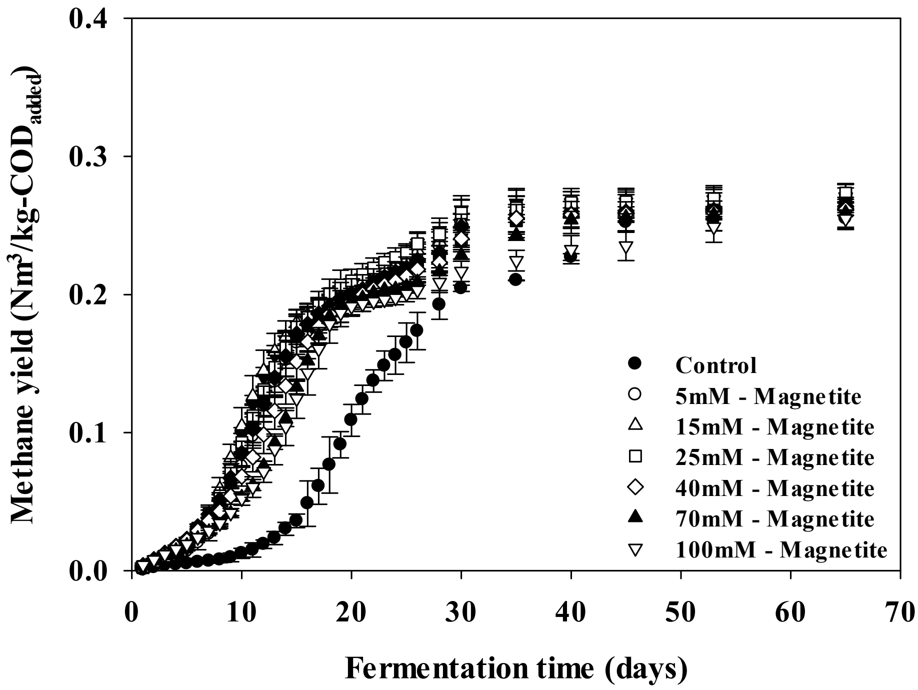

Figure 1. Methane yield curve by the addition of magnetite.

The variability in responses underscores the necessity for a tailored approach that considers specific conditions and optimal magnetite concentrations in food wastewater anaerobic digestion systems. Such nuanced insights are crucial for maximizing the benefits of magnetite while avoiding its potential drawbacks in different operational scenarios.

3.2. Reaction Kinetics of Methane Production

This study examined methane production kinetics in a batch anaerobic reactor for food wastewater. The cumulative methane production curves were differentially analyzed to derive the methane production rate [P′(t)] and acceleration [P″(t)] curves. Figure 2 shows the control curves, establishing a baseline, and Figure 3 illustrates the effects of magnetite supplementation. The control group exhibited an R_m of 27.5 mL/day, with an $R_{m\text{-}day}$ of 18.9 days. In the magnetite treatment group, R_m significantly increased to 35.1 mL/day at 5 mM-Fe_3O_4, further reaching 37.4 mL/day at 15 mM-Fe_3O_4, marking a substantial 36.0% increase compared to the control. However, with higher magnetite input (25–70 mM-Fe_3O_4), R_m gradually declined to its lowest level of 28.8 mL/day at 40 mM-Fe_3O_4, followed by a subsequent increase to 33.9 mL/day at 70 mM-Fe_3O_4. The $R_{m\text{-}day}$ values showed significant variation. In the presence of 5 mM-Fe_3O_4, $R_{m\text{-}day}$ decreased by 47.6%, reaching 9.9 days compared to the control. At 15 mM-Fe_3O_4, the shortest $R_{m\text{-}day}$ of 8.2 days was observed, representing a 56.6% reduction compared to the control. Additionally, at higher magnetite concentrations (25–100 mM-Fe_3O_4), $R_{m\text{-}day}$ exhibited a subsequent increase, with 14.6 days at 100 mM-Fe_3O_4, reflecting a 22.8% reduction compared with the control. Lee et al. conducted a study assessing the enhancement of methane production with magnetite in food wastewater, similar to our study's chemical composition (TCOD 166,333 mg/L). Injecting 1.00%-Fe_3O_4 increased R_m to 18.44 mL/day, a 17.2% improvement over the control (15.73 mL/day). This aligns with our findings and reinforces the positive impact of magnetite on methane production in the anaerobic digestion of food wastewater. Lee et al. [32] further analyzed the lag growth phase time (λ) during initial anaerobic digestion, a factor influencing residence time. They reported a shortening of λ from 0.541 days in the control to 0.469 days with 0.25%-Fe_3O_4. The positive effect of magnetite in enhancing the R_m extends beyond food wastewater to other raw materials. Zhang et al. [36] investigated magnetite supplementation in a batch anaerobic digester for pig slurry by adding 5, 75, 150, and 350 mM-Fe_3O_4 with 20–30 nm particle size. R_m increased to 11.4, 13.3, 13.4, and 13.4 mL/day, respectively, showing a significant increase of 48.9% compared to the control (9.0 mL/day) above 150 mM-Fe_3O_4. However, Zhang et al. focused on the enhancement efficiency of R_m without reporting $R_{m\text{-}day}$ values. The cumulative methane production curve in the study by Zhang et al. indicated a shortening effect on $R_{m\text{-}day}$ with increasing magnetite input. However, a detailed analysis of $R_{m\text{-}day}$, specifically its values and variations, was not performed. This trend underscores the need for a more nuanced exploration of the temporal dynamics of methane production, especially considering $R_{m\text{-}day}$, to optimize magnetite supplementation for diverse anaerobic digestion scenarios.

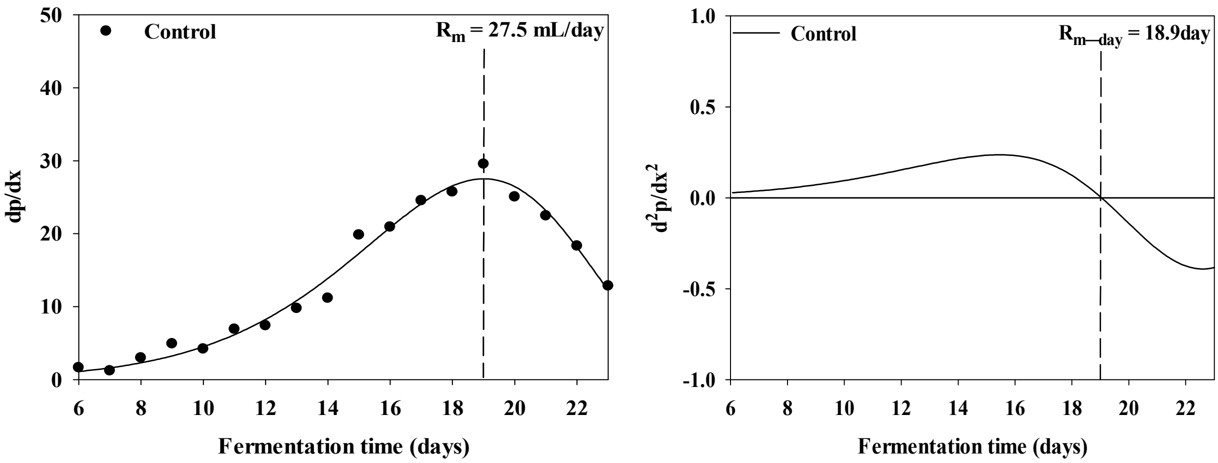

Figure 2. Analysis of maximum methane production rate and attainment day by the 1st and 2nd derivative from the cumulative methane production curve of control.

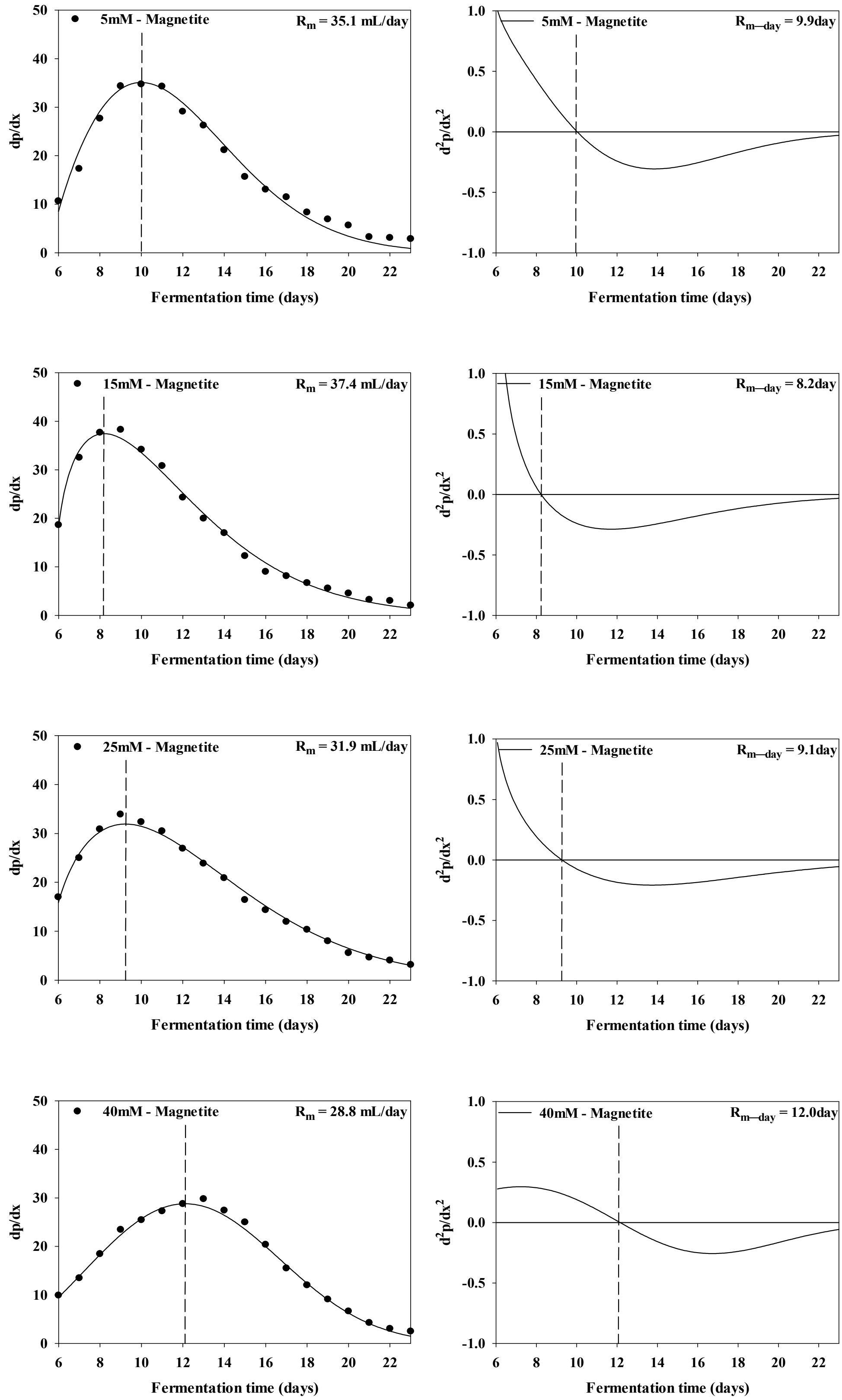

Figure 3. *Cont.*

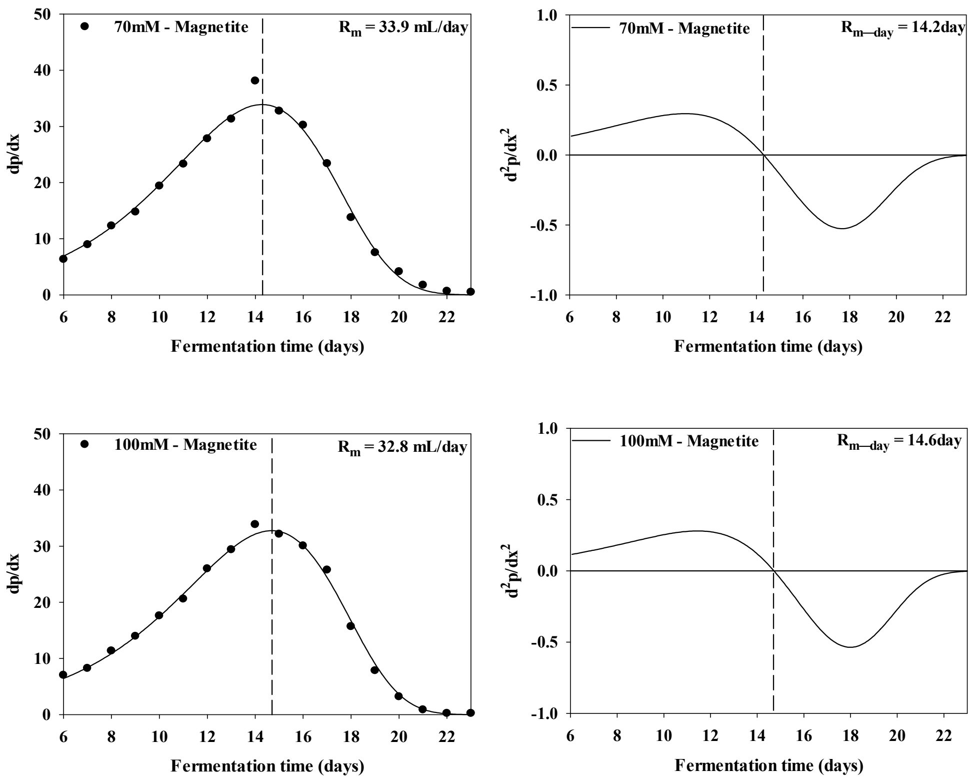

Figure 3. Analysis of maximum methane production rate and attainment day by the 1st and 2nd derivative from the cumulative methane production curve of Fe_3O_4 addition.

Specifically, Lee et al. [37] investigated the impact of magnetite addition in a batch anaerobic reactor using VFAs, specifically acetate, propionate, and butyrate, as substrates. In the absence of magnetite, the R_m values for acetate, propionate, and butyrate were 20.2, 8.7, and 19.6 mL/g-VSS/day, respectively. Introducing 20 mM-Fe_3O_4 magnetite resulted in R_m values of 19.9, 12.6, and 19.8 mL/g-VSS/day, with a notable 46% increase in propionate compared to the control. Examining these results in conjunction with the methanogenesis reaction of acetate ($CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$; $\Delta G^0 - 32.5$ kJ/mole), it was observed that magnetite had a marginal effect on increasing the reaction rate. This observation aligns with thermodynamic considerations, as acetate's negative standard Gibbs free energy change (ΔG^0) suggests a less substantial impact on reaction rate enhancement with magnetite. Further supporting this inference, Yamada et al. [38] demonstrated in a batch anaerobic reactor experiment that acetate was completely decomposed within 15 days with the addition of magnetite (5 and 10 mM-Fe_3O_4). Propionate and butyrate, which are substrates for acetogenesis, showed varied responses to the addition of magnetite, with propionate displaying a significant increase in R_m. The relatively larger ΔG^0 of propionate ($CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + 3H_2 + HCO_3^-$; ΔG^0 + 76.1 kJ/mole) compared to butyrate ($CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$; ΔG^0 + 48.1 kJ/mole) suggests that the favorable electron transfer facilitated by magnetite predominantly influences propionate's acetogenesis reaction [39,40]. This effect is particularly crucial because propionate accumulation in anaerobic digesters, which is indicative of low methanogen activity, poses operational challenges that inhibit methane production. Thus, the introduction of magnetite is implicated not only in enhancing overall methane production rates, but also in ameliorating issues associated with propionate accumulation during the anaerobic digestion of organic matter. The validity of this inference was substantiated by Jing et al. [41], who investigated methane production efficiency through magnetite introduction into a batch anaerobic reactor employing propionate as a substrate. The control group exhibited an R_m of 3.0 mL/day, whereas magnetite injections at 10, 100, and 1000 mg-Fe_3O_4/L resulted in respective R_m values of 4.11, 4.33, and 4.06 mL/day.

Notably, the discernible enhancement in anaerobic digestion efficiency with the addition of 10 mg-Fe_3O_4/L. Despite the divergence in findings across various studies on DIET, a recurring trend in the literature is the predominant focus on the augmentation of B_u and R_m following the addition of magnetite. Notably, there is a paucity of detailed analyses concerning the impact of residence time on anaerobic digestion, exemplified by the insufficient exploration of $R_{m\text{-}day}$. Figures 4 and 5 depict graphical representations of B_u and $R_{m\text{-}day}$, and R_m and $R_{m\text{-}day}$, respectively, based on varying magnetite inputs. In the anaerobic digestion of food wastewater, the results of the study revealed a distinct trend in the change in anaerobic digestion efficiency (B_u and R_m) relative to magnetite input compared to $R_{m\text{-}day}$. Notably, the addition of 100 mM-Fe_3O_4 demonstrated no statistically significant difference in B_u compared with the control (0.255 Nm^3/kg-COD_{added}). However, $R_{m\text{-}day}$ exhibits a substantial reduction of approximately 22.8%, as illustrated in Figure 4. Furthermore, the introduction of 40 mM-Fe_3O_4 led to a 5.1% improvement in R_m compared to that of the control (27.5 mL/day). Intriguingly, the $R_{m\text{-}day}$ experienced a more substantial reduction of approximately 36.5%, as shown in Figure 5.

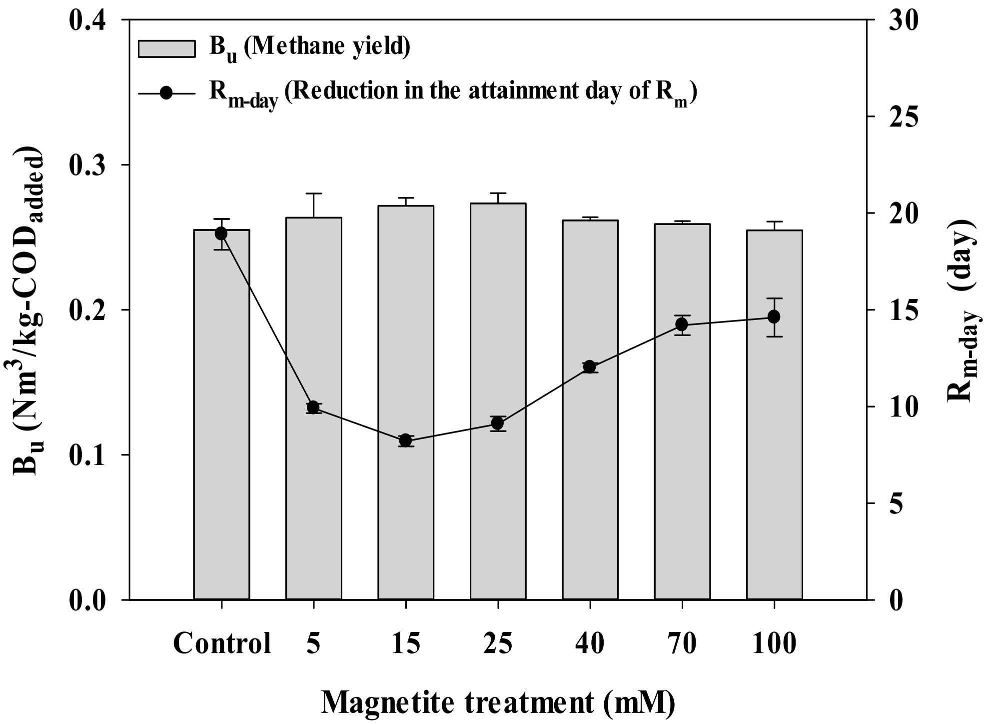

Figure 4. B_u and $R_{m\text{-}day}$ by concentration according to the addition of magnetite (B_u mean of methane yield and $R_{m\text{-}day}$ mean of reduction in the attainment day of R_m).

The addition of a conductive substance can enhance the efficiency of anaerobic digestion by promoting the decomposition rate of organic materials. In this study, the reaction rates (R_m and $R_{m\text{-}day}$) of food wastewater treated with magnetite were analyzed. The results showed that the highest efficiency was observed at a magnetite concentration of 15 mM, with R_m increasing by 36.0% and $R_{m\text{-}day}$ decreasing by 56.6% compared to the control. The enhanced anaerobic digestion reaction rate can shorten the HRT, providing the advantage of improving the economic feasibility of the anaerobic digestion system.

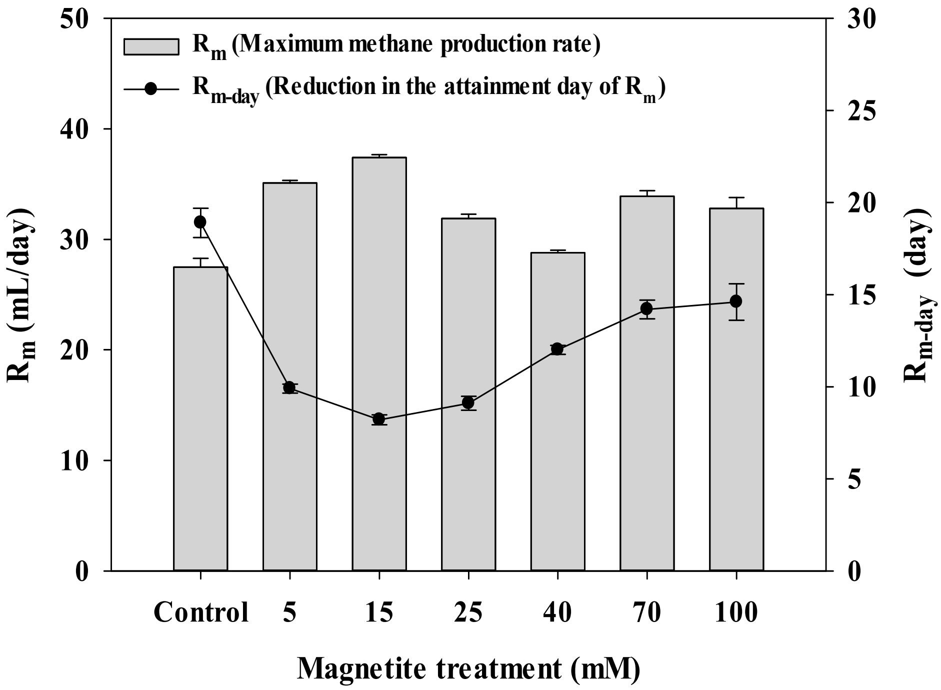

Figure 5. R_m and $R_{m\text{-}day}$ by concentration according to the addition of magnetite (B_u mean of maximum methane production rate and $R_{m\text{-}day}$ mean of reduction in the attainment day of R_m).

4. Conclusions

The direct interspecies electron transfer (DIET) mechanism, which facilitates electron transfer between acetic acid and methane-producing microorganisms, holds promise for enhancing anaerobic digester stability and methane production efficiency. While existing research on DIET has explored conductive materials under diverse anaerobic digestion conditions, comprehensive assessments of the residence time, which is crucial for economic feasibility, are lacking. In this study, magnetite, a conductive material, was introduced into food wastewater by anaerobic digestion, and the reduction in residence time and methane production efficiency were concurrently analyzed. Control's B_u was 0.255 $Nm^3/kg\text{-}COD_{added}$, which significantly increased to 0.273 $Nm^3/kg\text{-}COD_{added}$ ($p < 0.05$) with 25 mM-Fe_3O_4, showing the highest B_u. However, from 15 to 65 days, the methane production efficiency decreased, ranging from 388.9% to 7.1%. Initially, magnetite addition increased methane production, which diminished with prolonged reactor operation. R_m of control was 27.5 mL/day ($R_{m\text{-}day}$ of 18.9 days), peaking at 37.4 mL/day with 15 mM-Fe_3O_4, accompanied by a 56.6% reduction in $R_{m\text{-}day}$ (8.2 days). Even with 100 mM-Fe_3O_4, B_u remained comparable to the control, yet $R_{m\text{-}day}$ was shortened by approximately 22.8%. At 40 mM-Fe_3O_4, R_m improved by 5.1%, but $R_{m\text{-}day}$ decreased by approximately 36.5%. Despite the lower anaerobic digestion efficiency, $R_{m\text{-}day}$ was significantly shortened, suggesting the potential benefits of residence time reduction in a continuous anaerobic reactor. Introducing conductive materials via the DIET mechanism may enhance economic feasibility by optimizing the retention time of the anaerobic digestion facility. Continuous anaerobic digester research is essential to confirm these economic implications and to provide a holistic understanding of the impact of the DIET mechanism.

Author Contributions: Conceptualization, Y.-M.Y.; methodology, T.-B.K. and Y.-M.Y.; software, T.-B.K. and J.-H.L.; validation, T.-B.K. and Y.-M.Y.; formal analysis, T.-B.K. and J.-H.L.; investigation, T.-B.K., J.-H.L. and Y.-M.Y.; resources, Y.-M.Y.; data curation, Y.-M.Y.; writing—original draft preparation, T.-B.K.; writing—review and editing, Y.-M.Y.; visualization, T.-B.K. and J.-H.L.; supervision, Y.-M.Y.; project administration, Y.-M.Y.; funding acquisition, Y.-M.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the MAFRA and IPET for industrialization technology development in response to current livestock issues (Project NO. 321091-03).

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: Tae-Bong Kim has received research grants from KEC System Co., Ltd. Other authors have no conflict of interest to declare.

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