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The Effect of Magnetite Particle Size on Methane Production by Fresh and Degassed Anaerobic Sludge

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***Abstract*—**Anaerobic batch experiments were conducted to investigate the effect of magnetite-supplementation (7 mM) on methane production from digested sludge undergoing two different microbial growth phases, namely fresh sludge (exponential growth phase) and degassed sludge (endogenous decay phase). Three different particle sizes were assessed: small (50 - 150 nm), medium (168 – 490 nm) and large (800 nm - 4.5 µm) particles. Results show that, in the case of the fresh sludge, magnetite significantly enhanced the methane production rate (up to 32%) and reduced the lag phase (by 15% - 41%) as compared to the control, regardless of the particle size used. However, the cumulative methane produced at the end of the incubation was comparable in all treatment and control bottles. In the case of the degassed sludge, only the medium-sized magnetite particles increased significantly the methane production rate (12% higher) as compared to the control. Small and large particles had little effect on the methane production rate but did result in an extended lag phase which led to significantly lower cumulative methane production at the end of the incubation period. These results suggest that magnetite produces a clear and positive effect on methane production only when an active and balanced microbial community is present in the anaerobic digester. It is concluded that, (i) the effect of magnetite particle size on increasing the methane production rate and reducing lag phase duration is strongly influenced by the initial metabolic state of the microbial consortium, and (ii) the particle size would positively affect the methane production if it is provided within the nanometer size range.

***Keywords*—**Anaerobic digestion, iron oxide (Fe_3O_4), methanogenesis, nanoparticle.

I. Introduction

ANAEROBIC digestion (AD) is a biological process widely used to convert biodegradable organic matter into biogas in the absence of oxygen. Biogas containing methane (up to 60%) is a form of renewable energy with multiple economic and environmental benefits [1], [2]. In an attempt to enhance the microorganisms' activity and increase biogas production, the supplementation of AD with various chemical additives has been tried [3], [4]. One promising approach is the use of magnetite nanoparticles, since their superconductivity results in enhanced AD by increasing the maximum methane production rate and reducing the lag phase time [5], [6]. Methane production is increased because magnetite acts as an electrical conduit between electron-donating and electron-accepting organisms [7], [8]. Previous studies have shown that the stimulatory effect of magnetite nanoparticles on methane production is size dependent [9], [10]. In addition, the influence of magnetite on methanogenic activity has been examined using different types of fresh anaerobic microbial consortia (e.g. anaerobic sludge and paddy soil) [11], [12]. Considering these experiments, since magnetite has the ability to enhance methane production from fresh sludge, adding magnetite to aged (i.e. degassed) sludge may also produce positive results in terms of methane production. Methane production is based on a consortium of interdependent microorganisms including hydrolytic, acid forming, acetogenic, and methanogenic bacteria. In particular, it has been suggested that fresh sludge has a wide microbial diversity which ensures a sufficient level of hydrolytic and methanogenic activity [13]. Fresh sludge also has enough food to ensure a healthy food-microorganism (F/M) ratio which helps in achieving optimal production of biogas [14], [15]. When the sludge is aged (i.e. degassed) however, the F/M ratio becomes low, and as such, bacteria metabolism may be metabolically impaired [16]. During the degassing period, the anaerobic consortium undergoes endogenous decay and its metabolic state is expected to differ considerably from that of fresh sludge in which residual substrate is available to sustain microbial growth. Challenges still exist, however, as there is insufficient research reviewing the impact of adding magnetite nanoparticles on methane production on different ages of sludge. The effect of magnetite particle size on biogas production also warrants further research. Correspondingly, the aims of this research were (i) to identify whether magnetite particles have the same effect on methane production from degassed anaerobic sludge as with fresh sludge and (ii) to further study the effect of magnetite particle size on methane production.

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II. Materials and Methods

A. Synthesis of Magnetite Particles

Magnetite particles of different size ranges were prepared according to two methods. Firstly, large-sized magnetite particles were prepared via the co-precipitation method [17] by dribbling a solution of iron oxides ($FeCl_2/FeCl_3$ at a 0.5 molar ratio) into a 1.5 M NaOH solution. The obtained precipitate was washed by adding deoxygenated water and centrifuged at 4000 rpm. The average diameter of synthesized magnetite particles was found to be 800 nm - 4.5 µm. Secondly, medium-sized magnetite particles were prepared via the hydrothermal method [18]. Briefly, a solution of 8.1 g of $FeCl_3 \cdot 6H_2O$, 21.6 g of $NaCH_3COO$ and 240 mL ethylene glycol was transferred to a Teflon-lined stainless-steel autoclave and heated in an oven at 200 ^oC for 18 h. The

resultant black magnetite particles were washed with acetone and water several times. The average diameter of synthesized magnetite particles was 168 – 490 nm. Finally, small-sized magnetite was purchased from Sigma-Aldrich, China. These had a particle size of 50-100 nm and 97% purity.

B. Sludge Sample

Mesophilic digested sludge was collected from the wastewater treatment plant located in the Bromley suburb of Christchurch, New Zealand. The total volatile solid (VS) was measured according to standard methods [19] and found to be an average of 9 ± 1 g/L.

C. Batch Experiments

Batch experiments were conducted at 36 ± 1 °C in anaerobic 165 mL serum bottles with a working volume of 120 mL and 20 g of sludge. This corresponded to a final concentration at 1.5 g/L of VS. Anaerobic medium provided a source of nutrients and food was prepared as per [20]. Ten mL of propionate (pH-adjusted stock solution pH ~ 7) was added as a substrate at an initial concentration of 27 mM. Experiments were conducted in two groups. The first group used fresh digested sludge while the second group used degassed digested sludge. To degas the digested sludge, it was left to sit for one month at 36 ± 1 °C in an incubator. For both groups, magnetite-supplemented bottles were prepared by introducing small-size (commercial), medium-size (hydrothermal), and large-size (co-precipitation) magnetite particles, in separate bottles, to a specific final concentration of 7 mmole/L. Control bottles were prepared similarly except for the addition of magnetite particles. After preparing the serum bottles, they were directly flushed with N_2 gas and sealed with a rubber stopper. All bottles were continuously monitored and the headspace biogas was regularly collected for analysis.

D. Measurement of Methane

The volume of methane was determined by extracting 4 mL of biogas from the headspace of the serum bottle, using a gastight Hamilton syringe. The syringe was then connected to a water displacement device to measure the pressure. The fraction of methane in the biogas was determined by injecting the sample into a gas chromatograph (Agilent 19095P-Q04) fitted with a thermal conductivity detector (TCD) and a stainless-steel column with 30 m × 530 µm × 40 µm. Helium was used as carrier gas at 10 mL/min with pressure 10.6 psi. The temperature was set at 30°C for the oven; 70°C for the injector; and 155°C for the TCD. The methane produced from the experiment was plotted as a function of time. The modified Gompertz model (1) was fitted to the experimental cumulative methane production curves using SPSS software. The adjustable parameters were the lag phase duration (λ, d) and the maximum methane production rate (R mmole/g VS/d). Coefficients of determination (R^2) for the curve fitting were also calculated with SPSS.

$$M_p = CMP * EXP\left(-EXP\left(\frac{R*(\lambda-t)*2.7183}{CMP}+1\right)\right) \quad (1)$$

where: Mp is the predicted methane production (mmole/g VS), CMP is the cumulative methane at the end of incubation (mmole/g VS) and t is the time of methane production [21].

E. Statistical Analysis

The effect of magnetite nanoparticles on methane production with different supplement conditions (in terms of repeated measurements of lag phase, maximum methane production, cumulative methane production, and methane yield) was evaluated using a general linear model (GLM) procedure using SAS (2015). Differences between treatments were separated by the least significant difference (LSD) test. In addition, the p value was considered statistically significant at $p < 0.05$.

III. Results and Discussion

An example of the experimental cumulative methane production and the predicted methane value obtained by the simulation of the modified Gompertz model is shown in Fig. 1. It is well known that R^2 evaluates the accuracy of the model and how well it predicts future outcomes. The values of R^2 as shown in Tables I and II were between 0.97 – 0.99 indicating that fitting a modified Gompertz model was accurate.

The kinetic parameters for methane production from fresh and degassed digested sludge are also shown in Tables I and II.

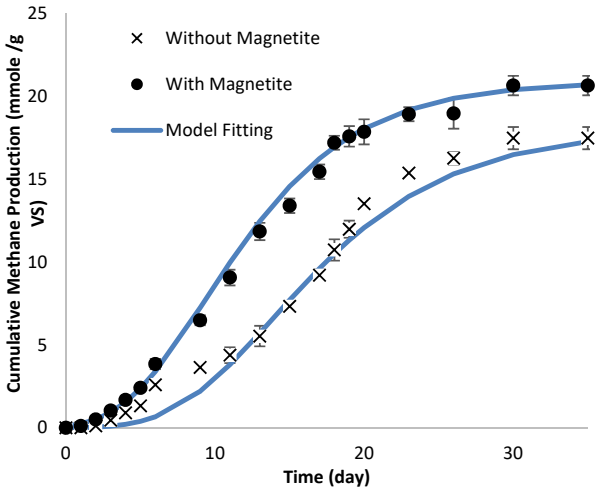

Fig. 1 Evolution of experimental and modelled methane production. Error bars represent the standard deviation of triplicate experiments

TABLE I
Influence of Magnetite Size on Methane Production Parameters from Fresh Sludge

Treatment	Rate (mmole/g VS/d)	Lag (day)	CMP (mL CH_4)	R^2
Control	0.98 ± 0.04[b]	6.48 ±0.7[a]	72.75 ± 3.38	0.97
Small	1.33 ± 0.03[a]	4.15 ± 0.2[c]	74.39 ± 1.05	0.99
Medium	1.32 ± 0.06[a]	3.81± 0.1[c]	73.04 ± 2.08	0.99
Large	1.20 ± 0.12[a]	5.48 ± 0.2[b]	71.11 ± 0.64	0.99
Standard Error	0.05	0.26	1.48	

[a,b,c] means in the same column with different superscripts are significantly different ($P < 0.05$). Standard error represents the dispersion of sample means around the population mean.

TABLE II
INFLUENCE OF MAGNETITE SIZE ON METHANE PRODUCTION PARAMETERS FROM DEGASSED SLUDGE

Treatment	Rate (mmole/g VS/d)	Lag (day)	CMP (mL CH_4)	R^2
Control	0.59 ± 0.04^{bc}	26.5 ± 1.6^{c}	76.64 ± 1.05^{ab}	0.98
Small	0.65 ± 0.00^{ab}	29.4 ± 1.9^{b}	71.42 ± 2.39^{c}	0.99
Medium	0.67 ± 0.02^{a}	25.0 ± 1.1^{c}	76.56 ± 1.46^{ab}	0.99
Large	0.56 ± 0.05^{c}	33.2 ± 1.1^{a}	59.81 ± 4.08^{d}	0.97
Standard Error	0.018	0.93	1.36	

[a,b,c,d] means in the same column with different superscripts are significantly different ($P < 0.05$). Standard error represents the dispersion of sample means around the population mean.

The addition of magnetite into fresh sludge resulted in an increase in methane production rate (by up to 32%) and a decrease in the lag phase (by 15% - 41%) as compared to the control ($P < 0.05$). There was, however, no significant difference in the CMP observed between the magnetite-supplemented and the control bottles. In contrast, only the medium-size magnetite significantly increased the maximum methane production rate from degassed sludge by 12%, as compared to the control. These results are in agreement with previous findings which demonstrate that magnetite-particle supplementation to a methanogenic sludge enhanced the rate of methane generation (when propionate was the substrate) up to 22%, 33% and 44%, respectively, as per [22], [7], and [23]. In addition, it has been reported that adding 25 mM of magnetite resulted in a 10% reduction in the lag-phase time as compared to their control [7]. These results suggest that electrically conductive magnetite could serve as electrical conduit between propionate-oxidizing acetogens and carbon dioxide-reducing methanogens.

Table I also shows that adding three magnetite size ranges (small, medium and large) did not significantly change the maximum methane production rate, nor lag phase or CMP; with the only exception being large magnetite, which significantly extended the lag phase as compared to the small and medium magnetite treatments. Table II shows that the maximum methane production rate was the highest and the lag phase was the shortest and the CMP was the highest in the medium size treatment. However, the results of CMP depend on the lag phase duration and the incubation period. These results indicate that magnetite size has a positive effect on methane production only when suitable particle sizes are used (small or medium but not large over 800 nm). It has been found that biogas production was 66.6% less when using 24 nm magnetite, than when using a size of 7 nm [24]. This suggests that magnetite size plays a significant role in methane production with very large sizes inhibiting the methane production process. This can be explained by nanoparticle aggregation; that is to say, an increase in magnetite size leads to a higher frequency of collision and particle-particle interaction, which increases the possibility of aggregation [25], [26]. Ultimately, excessive aggregation results in a decrease in surface area, and correspondingly, a decrease in reactivity, thereby undermining the performance of magnetite [27]. Overall, however, it is difficult to compare studies as many factors besides magnetite size play a role in methanogenesis. As such, within the parameters of any experiment, it is important to monitor magnetite size to identify the necessary level of aggregation that improves methane production.

The results also indicate that sludge age had a strong effect on the methane production rate and the lag phase in the presence of magnetite. The maximum methane production rate from fresh sludge (i.e. in the exponential growth phase) was significantly enhanced while the lag phase was significantly reduced, as compared to degassed sludge. The reason for the lower methane production rate and the extension to the lag phase in the degassed sludge may be that as the sludge ages in the incubator (i.e. degasses), microorganisms consume most of the available nutrients (i.e. food) and, after a period with no food, the microorganisms starve. As such, this may disturb the microbial growth rate sufficiently (i.e. in the decay phase) such that a high concentration of large-sized magnetite may have a negative impact on the lag phase, reflected in an increase in time that microbial communities need to adjust to a new environment. It has been reported that there is a relation between microbial activity and microbial community structure and this activity depends upon the type of biomass and the operational conditions that determine the growth of specific populations [28]. It has been suggested that the inoculum should be fresh and have an active microbial population (with an adequate balance between all the microbial communities) to ensure that the AD process does not face any limitations [29], [13]. In addition, when the sludge is fresh, the quick growth of microorganisms shortens the lag phase time [30], [31].

IV. CONCLUSIONS

Magnetite size plays a role in methane production. Overall, using large sizes (800 nm to 4.5 μm) does not enhance methane production, as compared to small (50-150 nm) and medium (168 to 490 nm) size ranges. In addition, adding magnetite to enhance the methane production is related to the growth phase of the microorganisms. That is, magnetite has a positive effect on methane production when the digested sludge is fresh (i.e. in the exponential growth phase), as compared to aged sludge (i.e. in the decay phase). Adding magnetite enhanced the methane production rate by up to 32% and 12% in the fresh and degassed sludge, respectively, as compared to the control. In addition, adding magnetite reduced the lag phase by up to 41% in the fresh sludge, as compared to the control.

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