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IMPROVING THE ANAEROBIC TREATMENT OF SLUDGES AND HIGH-STRENGTH WASTEWATERS THROUGH ADDITION OF ELECTRICALLY-CONDUCTIVE PARTICLES

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Improving the Anaerobic Treatment of Sludge and High-Strength Wastewaters through Addition of Electrically-Conductive Particles

Abstract

The growing list of high-strength wastewaters across North Carolina (NC) are well suited for treatment and energy recovery using anaerobic digestion (AD). These include livestock wastes, food wastes, and wastewater-derived sludges that if left untreated, pose risks to NC's water resources and public health. AD, however, is largely underutilized to treat these wastes. This can be attributed to both economic and technology performance barriers. With respect to performance, one limitation is the operational instability that can arise when syntrophic relationships between key groups of microorganisms in the digester are disrupted; another is the incomplete conversion of organics to methane gas (CH_4). To overcome these limitations, we proposed augmenting digesters with electrically conductive microscale particles. These particles have proven effective as conduits for syntrophic microbe-to-microbe direct electron transfer, resulting in improved CH_4 generation and waste degradation rates using pure microbial strains in the lab. We hypothesized that supplementing digesters with these materials would result in a similar effect and could provide operators a means to stabilize and improve performance. Our objectives were to experimentally determine the impact of 1) material properties (type, conductivity, size) and 2) particle loading on AD performance. We assessed performance as CH_4 generation rates, CH_4 recoveries, and organic matter destruction [measured as chemical oxygen demand (COD) removal]. To fulfill our objectives, we supplemented lab-scale, serum bottle-based digesters with different conductive particle types (graphite, biochar, activated carbon) or non-conductive glass (a surface area control) and compared all particle amended bottles with controls lacking particles. We used swine wastewater collected from the NC State Swine Educational Unit as a representative high-strength wastewater.

Our results indicate that across all particle types tested, graphite was the most consistent in improving digester performance. Both CH_4 production rates and recoveries strongly correlated with the graphite loading rate, reaching a maximum production rate of 30 ± 2.5 mL-CH_4/(g-VS_{seed} day), which was $34 \pm 11\%$ higher than the no-particle control. The other material types did not show clear trends with loadings, and in most cases led to reduced performance relative to the no-particle control. Material electrical conductivity was not found to be a decisive factor for predicting CH_4 generation rates. This result was contrary to our expectations. A primary reason was that biochar, and more significantly, activated carbon, strongly adsorbed organic matter from the wastewater. Adsorption was found to have a negative impact on performance. Graphite exhibited little to no adsorptive behavior, resulting in a larger conversion of the initial COD into CH_4 gas. Under our conditions tested (single batch cycle; 19 day retention time), the additional CH_4 generated with graphite would not recuperate the cost of graphite. Our recommendations moving forward are threefold. First, fundamental investigations of microbial community structure and mechanisms in the presence of conductive versus non-conductive material are needed to determine if the improvements are associated with direct interspecies electron transfer (DIET). Second, alternative means of providing electrically conductive surfaces, such as graphitic brushes or cloths, should be explored, so as to avoid the costs associated with continuous particle amendments. Finally, a broader suite of high-strength wastewaters need to be studied to determine if similar responses as reported here are observed.

Acknowledgements

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1.0 Introduction

Across North Carolina there are a growing number of high-strength wastewaters that require effective treatment to protect the health of the environment and public. These sources include agricultural residuals, such as animal manure (dairy, swine, and poultry wastes), plant residuals, food waste, and industrial wastewater. Some wastes have been especially challenging in NC. For example, the 9 million-plus hogs in NC generate approximately 40 million gallons of swine manure every day (Price, 2012). Almost all of the roughly 2,500 hog farms flush this manure into open-air lagoons (**Fig. 1**). Odors, runoff, and spills from these lagoons are an ongoing problem that negatively impact NC's water resources (NRDC, 2013; Warrick, 1995).

Fig. 1. Open-air swine waste lagoon. (lenoirgreenelivestock.blogspot.com)

Another abundant high-strength waste stream in NC is waste activated sludge (WAS) generated at wastewater treatment facilities (WWTFs) (Tchobanoglous and Stensel, 2014). These sludges are formed when both organic and inorganic material settle and are collected at the bottom of large sedimentation basins. Since their water and organic content are too large for direct discharge to the environment, additional processing is required. Of the large (> 1 million gallon per day (MGD) capacity) WWTFs in NC, many of these sludges are treated using energy- and/or cost-intensive processes, such as aerobic digestion, lime addition, or drying (Tchobanoglous and Stensel, 2014).

One promising solution to achieve both stabilization and energy recovery from these waste streams is anaerobic digestion (AD) (**Fig. 2**). In an anaerobic digester, a suite of naturally occurring microorganisms degrades organic/inorganic material while simultaneously generating methane (CH_4) gas (Madigan et al., 2014). Advantages relative to conventional, energy-intensive aerobic processes include reduction of odors, destruction of pathogens, reduction in solids water content, and generation of nutrient-rich solids for land application (Tchobanoglous and Stensel, 2014). Despite the enormous potential of AD, underutilization is widespread across the US. The EPA AgSTAR program estimates that there are roughly 240 agricultural-based (swine, dairy, poultry and beef) anaerobic digesters in the US (EPA, 2014). A recent assessment concluded that an additional 8,000 livestock manure sites are well suited for AD (USDA, 2014). In NC, anaerobic digesters on livestock farms remain sparse. Only about 1% of all swine farms in NC currently use AD (EPA, 2014). Encouraging the implementation of this technology is a top priority at both the state and national level. There are several statewide mandates to generate electricity from swine waste and agreements with swine farmers to implement "Environmentally Superior Technology", such as AD (NCSU, 2014). At the federal level, there is also renewed interest in funding research and commercial ventures to make biogas from livestock waste a leading source of renewable energy (USDA, 2014).

Fig. 2. Anaerobic digesters (biocycle.net)

AD stabilization of WWTF-generated sludge is becoming more widespread, but there is additional room for growth across the country. More than 1,200 WWTFs with influent flow rates greater than 1 MGD in the US use AD to stabilize sludges. It is estimated that there are 3,500 additional locations where this technology could be implemented (USDA, 2014). Currently, only about 19% of WWTFs with influent flow rates greater than 5 MGD use biogas to offset onsite energy demand and/or generate electricity, as identified by the EPA (EPA, 2011). This number is expected to increase, and a more efficient way of producing CH_4 will be needed. In NC, several WWTFs are currently using ADs, while others are exploring their feasibility. The Neuse River water reclamation facility (WRF) in Raleigh is currently considering AD technology as part of the City of Raleigh's Climate/Energy Action Plan's goal to improve the city's energy and carbon budget (City of Raleigh, 2012).

Despite the great potential of AD technology for NC's water resources and energy sector, two major factors limit widespread adoption: (1) cost and (2) operational difficulties associated with unstable digesters. In terms of cost, the CH_4 generated can slowly pay back initial investments if it is utilized to generate onsite energy or return electricity back to the grid. Costs can also be reduced by receiving payments from external businesses as part of a cap-and-trade program, designed to reduce CH_4 emissions (as embodied in a future carbon tax). Greater implementation is thus dependent on the ability to produce more revenue-generating CH_4. As for digester instability, disruptions to the sensitive relationship among the microbial communities can lead to failed digesters, reduced CH_4 recovery, and contamination of receiving waterways. Strategies that both improve the robustness of microbial communities and their ability to generate CH_4 are thus essential to making AD a reality in NC. The work completed in this project examines such a strategy based on the latest understanding of electron transfer processes within microbial communities.

The overall objective of this project was to investigate the impact of electrically conductive, microscale particle addition on the performance of anaerobic digesters treating high-strength wastewaters. We hypothesized that the addition of these materials would enhance the rates of electron exchange within the digester microbial community and, in turn, the CH_4 production rates. The ability to stimulate CH_4 generation has been successfully shown in other studies when materials, such as activated carbon, biochar, and magnetite, were added to *defined* laboratory cultures, but it was unknown if the same effect would be obtained in the *undefined* cultures associated with real wastewaters. We aimed to fill this large knowledge gap by adding a suite of particle types, sizes, and loadings and analyzing their effect on digestion kinetics. To do this, we setup lab-scale, swine wastewater-fed anaerobic digesters and supplied them with three different conductive particle types (graphite, biochar, activated carbon). We compared their CH_4 production rates, CH4 recoveries, and organic matter removals with control digesters containing either non-conductive particles (glass) or no particles.

2.0 Methods

2.1. Particle properties

Three different electrically conductive materials were used in this study (**Table 1**). Graphite (Graphite Sales Inc., USA), biochar (Waste To Energy Inc., USA) and granular AC (Sigma-

Aldrich, USA) were ground with a mortar and pestle and sieved to give two size ranges: 2.0-2.4 mm (8-10 mesh) and 0.21-0.25 mm (60-70 mesh). Non-conductive glass beads (Corpuscular Inc., USA) with diameters of 2.0 mm and 0.21-0.25 mm were included as surface area controls.

Table 1. The size, conductivity, and surface area of particles tested in this study.

	Unit	Graphite	Biochar	AC	Glass
Size	mm	2.0 – 2.4 (G); 0.21 – 0.25 (P)			2.0 (G); 0.21 – 0.25 (P)
Conductivity[a]	S/cm	17 ± 2.6	0.22 ± 0.046	1.2 ± 0.25	Not conductive
Surface area[b]	m^2/g	0.38 ± 0.050 (G)	451 ± 11 (G)	530 ± 116 (G)	1.2E-3 (G)
		0.52 ± 0.23 (P)	531 ± 31 (P)	652 ± 13 (P)	1.0E-2 (P)

[a] Average ± standard deviation. n=10.
[b] Average ± range. n=2. Glass surface areas were calculated based on sizes.
G – granular; P – powdered

2.2 Swine wastewater properties

Our initial experiments using the primary/secondary solids from the South Durham Water Reclamation Facility did not yield appreciable differences between the treatments (particle addition) and no-particle controls. It is likely that the high solids content of this waste source limited the utilization of the particle surface area by the microorganisms. These findings suggest that similar waste streams may therefore not be suitable for accelerated digestion using conductive particles, but further work examining variable solid loadings is needed to verify this observation. After our unsuccessful initial experiments, we decided to test swine wastewater because of its lower solids content.

Swine sludge (seed) and wastewater (feed) were collected from NC State Swine Educational Farm on Apr 15, May 17 and Jun 13, 2016. Samples were stored at 4°C prior to use. The seed and feed were mixed to give a final ratio of 1:30 (seed:feed) and added to each lab-scale digester. The total chemical oxygen demand (TCOD), total solids (TS), volatile solids (VS), pH, and ammonia (NH_3-N) were recorded for the mixture (**Table 2**).

Table 2. Properties of the swine wastewater mixture (1:30, seed:feed) used in this study.

	Unit	Mixture
TCOD	g/L	4.7 ± 1.5
TS	g/L	7.1 ± 6.3
VS	g/L	4.7 ± 8.3
pH		7.4 ± 0.22
NH_3-N	mg/L	220±36

2.3 Anaerobic digester setup and operation

Sealed glass serum bottles (125 mL total volume) filled with 100 mL of wastewater were used to mimic anaerobic digesters. Three different particle loadings were added for each particle size tested. Granular (G) loadings and their abbreviation used in the manuscript were 2.2 (low; G-Low), 5.5 (medium; G-Med) and 11.1 (high; G-High) g-particles/g-VS seed. The powdered particles were 2.2 (low; P-Low), 4.8 (medium; P-Med) and 9.6 (high; P-High) g-particles/g-VS

seed. Each bottle was sealed with a butyl rubber stopper and aluminum cap, and the gas headspace was flushed with pure N_2 for 5 minutes to remove O_2. The bottles were placed on shakers (100 rpm) at 30 °C for 19 days. The gas production rate and total volume were recorded in real-time using a respirometer (BPA-800, Challenge Technology, USA), with the gas collected in an air bag (500 mL, Calibrated Instruments, Inc., USA) (**Fig. 3**). After the batch ended, the shaker was stopped and the solids were allowed to settle. Supernatant was collected (60 mL) and analyzed for volatile fatty acids (VFAs), pH and TCOD.

Fig. 3. Respirometer setup with lab-scale anaerobic digestion reactors (respirometer-sys.com).

2.4 *Analytical methods*

The electrical resistance of particles was determined with a digital multimeter (RadioShack, Fort Worth, TX, USA) using a two probe method (Singh, 2013). All the carbon materials were cut into cylinders with a knife before measurement, and it was assumed that the granular and powdered material had the same electrical properties. TS, VS were determined according to Standard Methods for the Examination of Water and Wastewater (Rice et al., 2012). TCOD was determined using Hach Method 8000, and NH_3-N was measured using Hach Method 10031 (DR/890 Portable Colorimeter, Hach, USA). Biogas composition was analyzed by gas chromatography (Model 8610C, SRI Instruments, USA) equipped with a thermal conductivity detector (TCD) and CTR I Column. pH was measured using an Orion 3-Star benchtop pH meter equipped with an Orion ROSS Ultra Refillable pH/ATC Triode (Thermo Fisher Scientific, USA). VFAs were analyzed using a Dionex ICS-5000+ HPIC system with a conductivity detector and Dionex IonPac AS11-HC column (Thermo Fisher Scientific, USA). Scanning electron microscopy (SEM) images were taken using a Hitachi S3200N variable pressure scanning electron microscope (VPSEM) with the Everhart-Thornley secondary electron detector (Hitachi, Japan).

2.5 *Methane production rates and recoveries*

Maximum CH_4 production rates (mL/day) for each batch were calculated as:

$$\text{Maximum } CH_4 \text{ production rate} = \max\left[\frac{\text{Total gas production (mL)} \times CH_4 \text{ concentration (\%)}}{\text{Time (d)}}\right] \quad (1)$$

CH_4 recoveries were calculated as:

$$CH_4 \text{ recovery} = \frac{\text{Real } CH_4 \text{ production (mL)}}{\text{Maximum } CH_4 \text{ production (mL) from COD removal}} \times 100\% \quad (2)$$

2.6 *Statistical methods*

The fitting and corresponding R-squared values and the CH_4 production models were determined using statistical software package R. P values less than 0.05 were considered to show significant difference.

3.0 Results

3.1. Methane production rates

The impacts of particle type, size, and loading were first assessed by measuring CH_4 generation rates from the swine wastewater-fed bottles. Since there was a high degree of variability in COD among the swine wastewater samples collected during the year, we normalized our results to the no-particle controls from each batch. G graphite- and biochar-amended reactors consistently obtained higher CH_4 production rates than the no-particle and glass control amended bottles (**Fig. 4A**; filled circles). The highest CH_4 production rate of 32 ± 1.8 mL-CH_4/(g-VS_{seed} d) (46 ± 8.0% higher than the no-particle control) was reached with the G-Low biochar treatment. Increasing the G biochar loading led to a strongly correlated (R^2 = 0.91) decrease in CH_4 production rate (**Fig. S1A**), possibly due to an overload of biochar that enhanced adsorption or increased pH, which is discussed later. Conversely, increasing the G graphite loading led to a strongly correlated (R^2 = 0.96) CH_4 production rate increase, reaching a maximum of 32 ± 11% higher than the no-particle control (**Fig. S1A**). Both the G AC and glass amended bottles did not show a clear trend with loading and generally underperformed relative to the no-particle control, with AC G-High being the only exception.

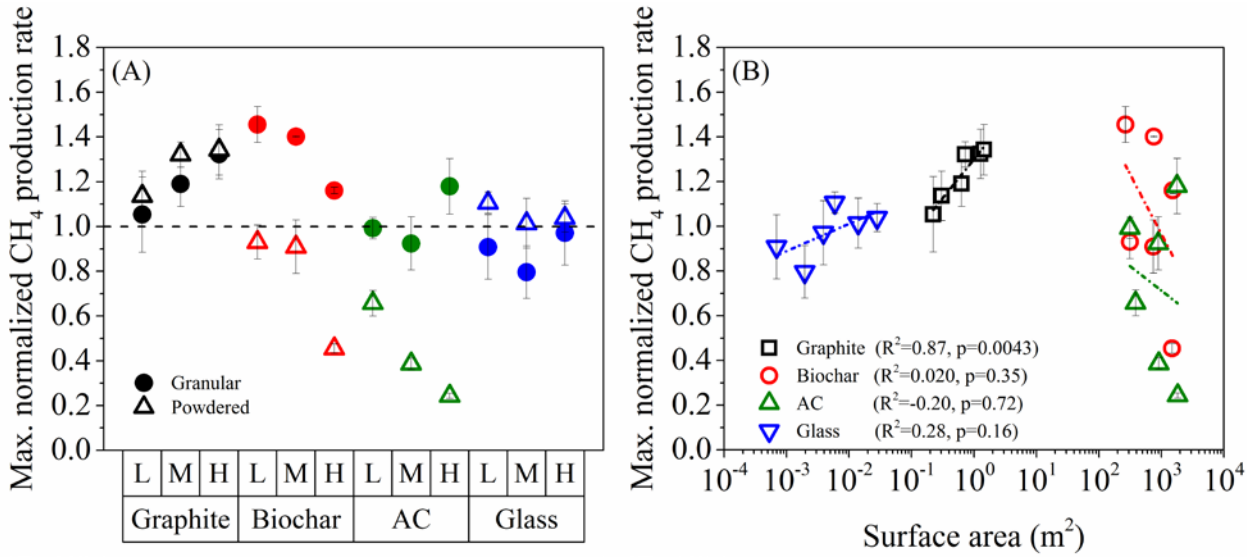


Fig. 4. Maximum CH_4 production rates normalized to each respective batch no-particle control average as a function of particle type and (A) loading or (B) surface area. The x-axis labels in (A) refer to the three loadings tested (L – Low; M – Medium; H – High). Error bars based on replicate reactors.

For the smaller, powdered size, graphite was the only material that led to CH_4 production rate increases with loading (**Fig. 4A**; open triangles). The production rate magnitudes of the P graphite were similar to the G graphite, indicating that the additional surface area provided by the smaller particle size afforded only an incremental improvement. Unlike G graphite, CH_4 generation rates did not correlate strongly with P graphite loading (**Fig. S1B**). Instead, a saturation type behavior was observed at higher loadings, indicating that no additional CH_4

production rate enhancements occurred at the highest loading. P glass was the only other material that obtained CH_4 generation rates at or slightly higher than the no-particle control. With P biochar and P AC, the rates decreased with increased loading. At the highest loading, P AC and P biochar resulted in CH_4 generation rates that were 76 ± 1.1% and 55 ± 1.2% lower, respectively, than the no-particle control. These were the lowest CH_4 production rates obtained across all particle types, sizes, and loadings.

The impact of particle surface area was determined by taking the product of loading and particle specific surface area (m^2/g). The data for both the G and P sizes were pooled and graphed to assess relationships between CH_4 production rates and surface area (**Fig. 4B**). The graphite particles produced the strongest positive correlation between CH_4 generation rates (R^2=0.87; p=0.004) and surface area. The glass particles were the only other material that showed a positive impact of surface area, although the correlation was weak (R^2 = 0.28). Even though higher particle surface areas were present in all the biochar and AC treatments, they did not follow the positive trends exhibited by the graphite and glass, and in fact had a negative relationship between CH_4 production rate and surface area.

3.2 *Methane recoveries*

We next examined the impact of particle type, size, and loading on CH_4 recoveries. The highest absolute CH_4 recovery of 63 ± 4.1% (an increase of 30 ± 8.4% relative to the no-particle control) was obtained with P-High graphite (**Fig. 5A**). Decreasing the graphite loading (for both G and P sizes) decreased CH_4 recoveries. G biochar yielded CH_4 recoveries larger than the no-particle control, although without a clear trend with loading (**Fig. S1C-D**). P biochar CH_4 recoveries were lower than the no-particle control and generally decreased as loading increased, reaching a minimum normalized recovery of −59 ± 3.1% at the highest loading. All AC sizes and loadings resulted in CH_4 recoveries below the no-particle control, and reached the lowest normalized CH_4 recovery of −78 ± 0.3%. CH_4 recoveries were impacted by particle surface area in a similar fashion as was observed for the CH_4 production rates (**Fig. 5B**). Graphite and glass again were the only materials that yielded positive correlations, with graphite yielding the strongest correlation (R^2 = 0.77).

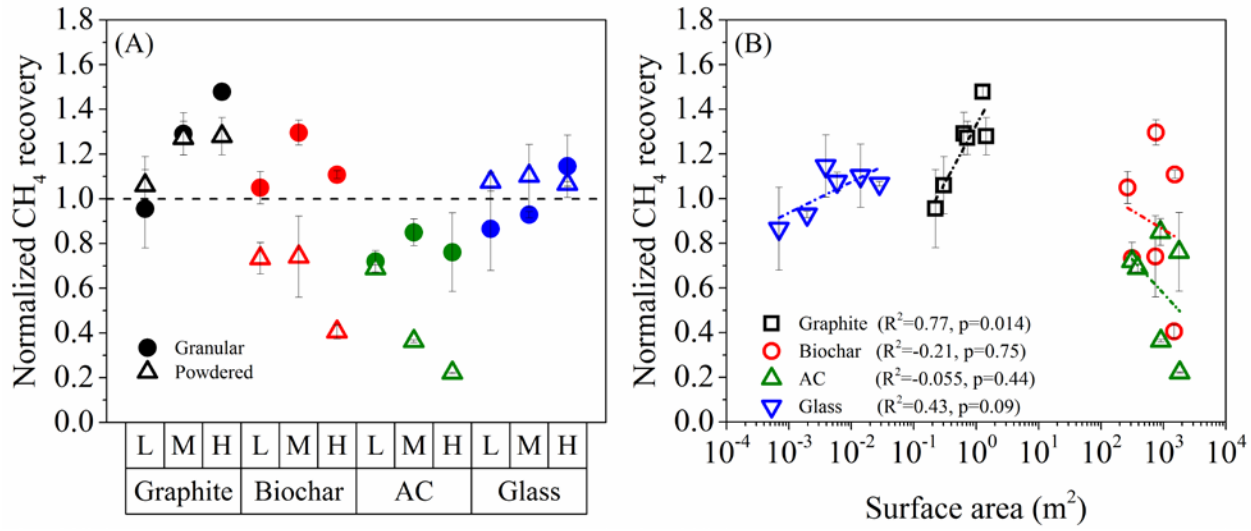


Fig. 5. CH_4 recoveries normalized to each respective batch no-particle control average as a function of particle type and (A) loading or (B) surface area. The x-axis labels in (A) refer to the three loadings tested (L – Low; M – Medium; H – High). Error bars based on replicate reactors.

3.3 Particle electrical conductivity

Since electron conduction through particles is a proposed mechanism of direct interspecies electron transfer (DIET) and has been implicated in increasing reaction rates (Chen et al., 2014b; Cruz Viggi et al., 2014; Zhao et al., 2015), we examined the relationship between particle conductivity and CH_4 generation rates. Particle conductivity was poorly correlated with the maximum CH_4 production rates except for the G-High treatment ($R^2 = 0.82$, **Fig. 6**). The G-Low and G-Med treatments did not correlate well with CH_4 production rates because biochar obtained the highest rates despite having an almost 80 fold lower conductivity relative to graphite. In the remaining treatments, CH_4 production rates with biochar and graphite followed a trend consistent with an increase in rate with conductivity. At all the P loadings, the AC CH_4 production rates dropped sharply relative to biochar. This resulted in the lack of a relationship between CH_4 production rates and conductivity under those treatments.

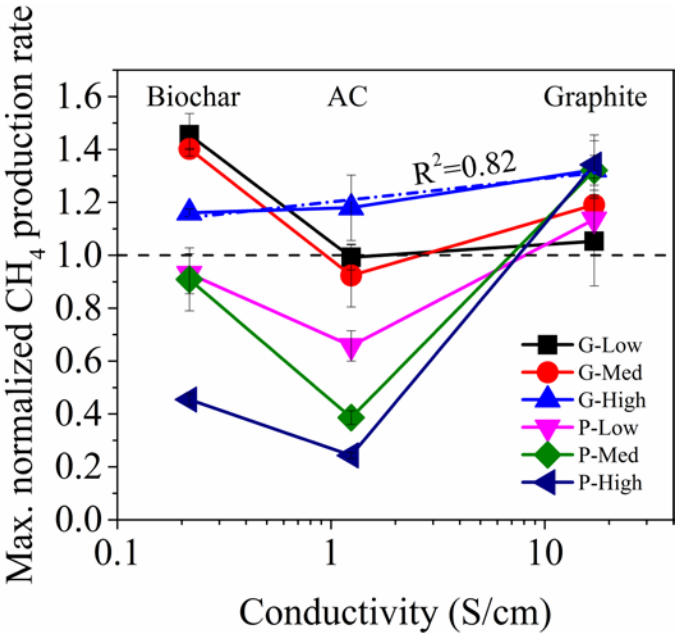


Fig. 6. Maximum CH_4 production rates and as a function of particle conductivity. Production rates are normalized to each respective batch no-particle control average. Error bars represent the range of replicate experiments.

3.4 Organic matter adsorption

Since biochar and AC are well known organic matter sorbents, we investigated how this property may have impacted our observed rates and recoveries (Ahmad et al., 2014; Bansode et al., 2004; Gupta et al., 2009). During our experiments, biochar and AC removed up to 20 ± 3.1% (G-High biochar) and 17 ± 1.0% (G-High AC) more COD compared with the no-particle controls (**Fig.**

7A). Despite these additional removals, CH_4 recoveries were generally lower than the controls, ranging from –15 ± 6.0% (G-Med AC) to –78 ± 0.3% (P-High AC) lower, except for G biochar (**Fig. 5A**). This finding indicates that the additional COD removed in the presence of these particles and loadings was not directed towards CH_4 generation. Abiotic tests, in which particles were added to sterile swine wastewater (at both G-Low and P-Low treatments), indicated that both biochar and AC could remove organics (measured as COD) from the wastewater in the absence of biological reactions (**Fig. 7B**). The highest control-normalized abiotic COD removal was associated with the P-Low AC (30 ± 0.2%) and the G-Low AC (26 ±2 .3%). G-Low and P-Low biochar removed a lower amount of COD, 12 ± 0.4% and 24 ± 0.8%, respectively, relative to the control. VFA analysis supported the abiotic COD sorption tests (**Fig. S2**). In particular, n-valerate was reduced by up to 29 ± 0.49% and hexanoate was reduced by more than 25%. It has been suggested by the Duclaux-Traube rule that aliphatic $–CH_2$ groups in VFAs contribute to the nonpolarity of adsorbates, and VFAs with longer chains are more likely to be adsorbed onto nonpolar AC surfaces (Freitas et al., 2007). Studies have shown that AC affinities are dependent on the VFA. Butyric acid, for example, was shown to exhibit a much higher affinity for AC than propionic and acetic acids (Freitas et al., 2007; Henrique et al., 2013). In this study, no significant adsorption of short chain fatty acids (C2-C4) were observed, but valerate (C5) and hexanoate (C6) were removed even though their concentrations were low.

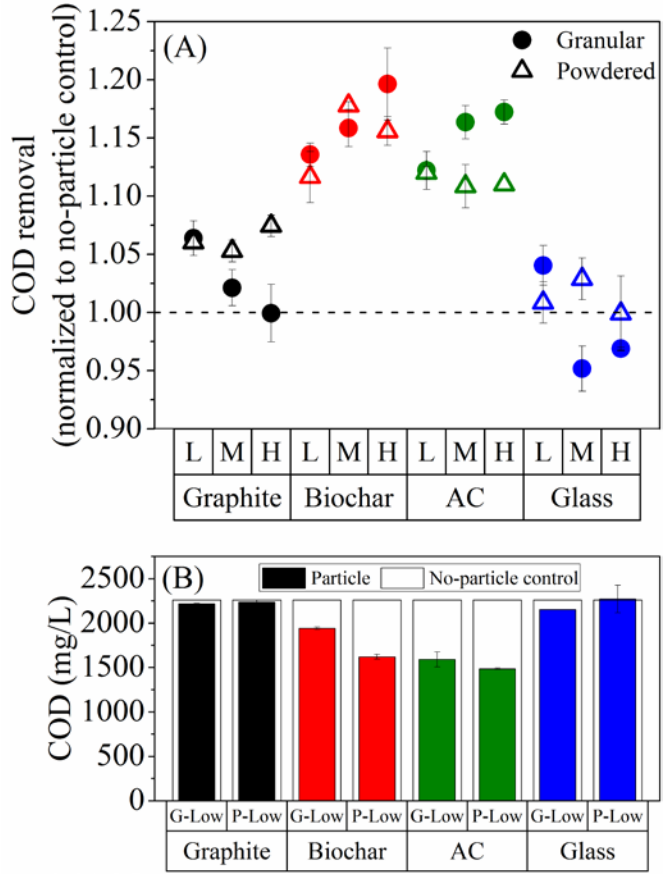


Fig. 7. (A) COD removal by particle amendments normalized to the no-particle control average removal, and (B) COD removal in sterile swine wastewater after a 19-day incubation. The open bars represent the initial COD at the start of the abiotic test. The particle loading was 2.2 g-particles/g-VS_{seed} (before autoclaving). Error bars represent the range of replicate experiments.

In contrast to biochar and AC, the graphite and glass particles showed little to no adsorptive behavior. The findings that P biochar and P AC removed more COD than the controls and graphite particles yet yielded lower CH_4 recoveries (**Fig. 5A**) strongly suggests that abiotic organic matter adsorption played an important role for these particle types. Noticeably, although the powdered particles were able to abiotically remove more COD, granules (especially biochar) enhanced biotic degradation and CH_4 generation rates (**Fig. 4A**), which explains the higher COD removal by granules in those bottles (**Fig. 7A**).

3.5 Electron balances

To determine the fate of electrons during each cycle, electron balances based on COD, VFA concentrations and CH_4 measurements were calculated. The electrons added as initial COD (COD_i) were categorized into the following product categories: 1) CH_4 gas ($COD_i \rightarrow CH_4$), 2) supernatant VFAs ($COD_i \rightarrow$ VFA), 3) settled solids and biomass ($COD_i \rightarrow$ solids), and 4) Other constituents in the final supernatant (e.g. proteins, polysaccharides) ($COD_i \rightarrow$ other). $COD_i \rightarrow$ solids was defined as the COD_i utilized for microbial biomass, adsorbed to the particle surface, or directly stored as electrons within the particles (Saquing et al., 2016; Simpson, 2008). The total number of electrons associated with each pathway is depicted as a percentage of the total COD_i added at the start of each batch normalized to the no-particle control electron balance (**Fig. 8**). With respect to $COD_i \rightarrow CH_4$, graphite was the only material that consistently converted higher percentages of COD_i to CH_4 than the no-particle control (**Fig. 8A**). The highest normalized $COD_i \rightarrow CH_4$ conversion reached 14.5 ± 3.0% greater than the control (P-High graphite). G-Low biochar resulted in a higher $COD_i \rightarrow CH_4$ conversion than graphite at the same loading, but subsequently decreased as the loading increased for both the G and P sizes (**Fig. 8B**). At the same time, $COD_i \rightarrow$ solids increased, suggesting that adsorption-based removal consumed a larger fraction of the initial electrons as more surface area was added. The lowest $COD_i \rightarrow CH_4$ conversion was observed with AC and was dependent on the particle size and loading (**Fig. 8C**). Roughly 29 ± 0.11% fewer electrons as CH_4 were obtained relative to the control for the P-High AC treatment. The percentage of $COD_i \rightarrow$ solids was generally much lower in the graphite reactors compared with biochar and AC reactors. These findings agree with the abiotic sorption tests that showed minimal COD sorption (~2%) and VFAs to the graphite particles. In all AC bottles, $COD_i \rightarrow$ solids in the settled portion of the digester was the primary fate of electrons. COD adsorption to the AC particles was a large driver of this observation, which is consistent with the results of the abiotic COD and VFA removal tests.

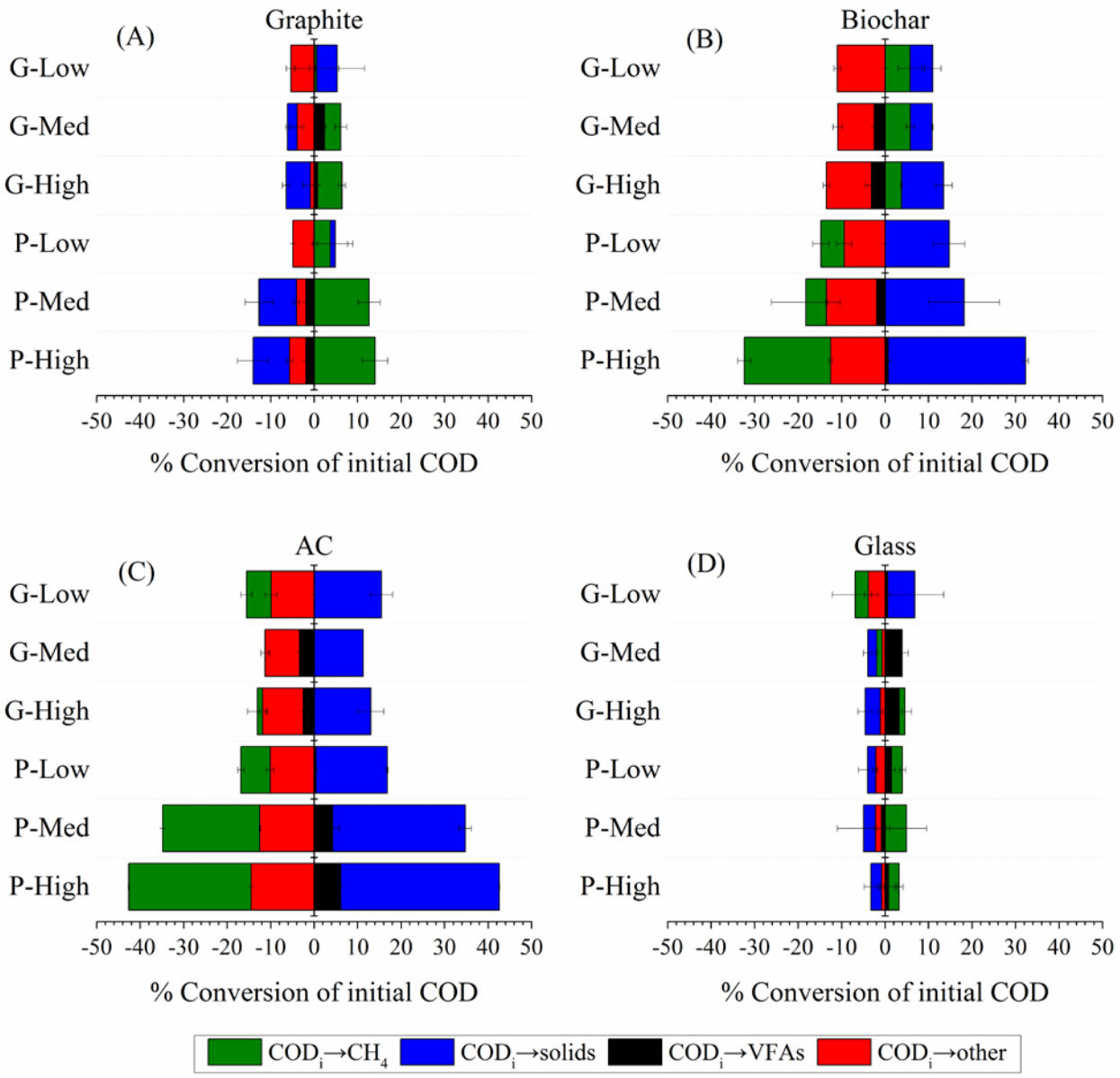


Fig. 8. Electron balances in reactors amended with (A) graphite, (B) biochar, (C) AC and (D) glass. Balances are based on the end of cycle COD distributions relative to the initial COD (COD_i). The arrows in the legend depict the fate of the COD_i. All values are normalized to the no-particle controls. Values to the right of zero show electron fate increases relative to the control, while those to the left indicate decreases. Error bars represent the range of replicate experiments.

To examine if the particles themselves contributed COD that would not have been accounted for in the initial COD measurement, an abiotic COD test of the particles alone was conducted. The COD values ranged from 8 to 23 mg COD/L, which was less than 2% of the total COD in the wastewater (**Table S1**) and thus could be ignored. We also explored the alternative hypothesis that CH_4 gas adsorption onto biochar and AC could explain the lower CH_4 recoveries. Abiotic sorption tests with either 100% or 10% CH_4 in the headspace yielded minimal decreases after 24

hours **(Fig. S3)**, likely due to the low solubility of CH_4 in water. These additional tests further confirm that organic matter adsorption was a key fate of COD in the presence of biochar and AC.

3.6 The influence of biochar and AC mediated pH changes

Carbon-based sorbents can affect solution pH depending on their functional group chemistry. A pH change may impact microbial growth rates. Biochar, in particular, has been extensively studied as a soil amendment and is known to increase soil pH and ameliorate acidification of agricultural lands (Hossain et al., 2011; Lehmann et al., 2003; Silber et al., 2010). In the presence of the highest P AC loading, the initial pH of the swine wastewater increased from 7.2 to 8.1 (**Table S2**). To determine if this pH change could explain some of the observed differences in CH_4 production rates among particle types, a separate test in the absence of any particles but with the initial pH adjusted from 7.2 to 8.1 by addition of sodium hydroxide was performed. The maximum CH_4 production rates and recoveries at the elevated pH were 20 ± 3.9% and 22 ± 2.1% lower, respectively, than the no-particle control at pH 7.2 (**Fig. 9**).

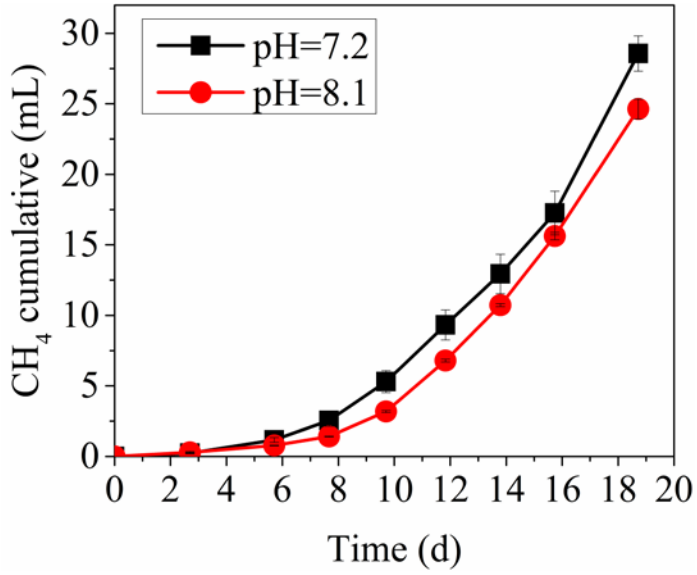


Fig. 9. Cumulative CH_4 production using a non-pH adjusted wastewater (pH 7.2) and an adjusted sample to pH 8.1. No particles were added. Error bars represent the range of replicate experiments.

Based on this result, the pH increase alone cannot explain why the CH_4 production rates and recoveries dropped by over 70% when the P-High AC particles were added to the bottles (**Fig. 4A; Fig. 5A**). Changes in pH are important because they can impact microbial activity and distribution of nitrogen-containing species. Methanogenesis is most efficient under neutral pH (Ward et al., 2008). Hydrolysis and acidogenesis have optima in slightly acidic environments (Ward et al., 2008). Theoretically, a pH increase from 7 to 8 results in a 8-fold increase of the free ammonia concentration when inorganic ammonium is present (Hansen et al., 1998). Ammonium levels typically range from 300 to over 4,000 mg/L in swine wastewater and at high concentrations (>1,500 mg/L) can negatively impact AD (Hansen et al., 1998; Ndegwa et al., 2005; Ward et al., 2008). In our study, the initial NH_3-N concentrations were only 220 ± 36 mg-

N/L, which was one fifth the free ammonia concentration that has been reported to inhibit methanogenesis at pH 8 (Hansen et al., 1998). To determine NH_3-N adsorption onto our different materials, an abiotic test was conducted (**Fig. 10**). The highest NH_3-N removal was 16 ± 0.0% in the G and P biochar (0.97 mg-NH_3-N/g-biochar) reactors and 18 ± 2.4% in the G-High AC (1.12 mg-NH_3-N/g-AC) bottles. These removals with biochar and AC via adsorption are consistent with results from other studies (Hale et al., 2013; Hollister, 2011; Takaya et al., 2016).

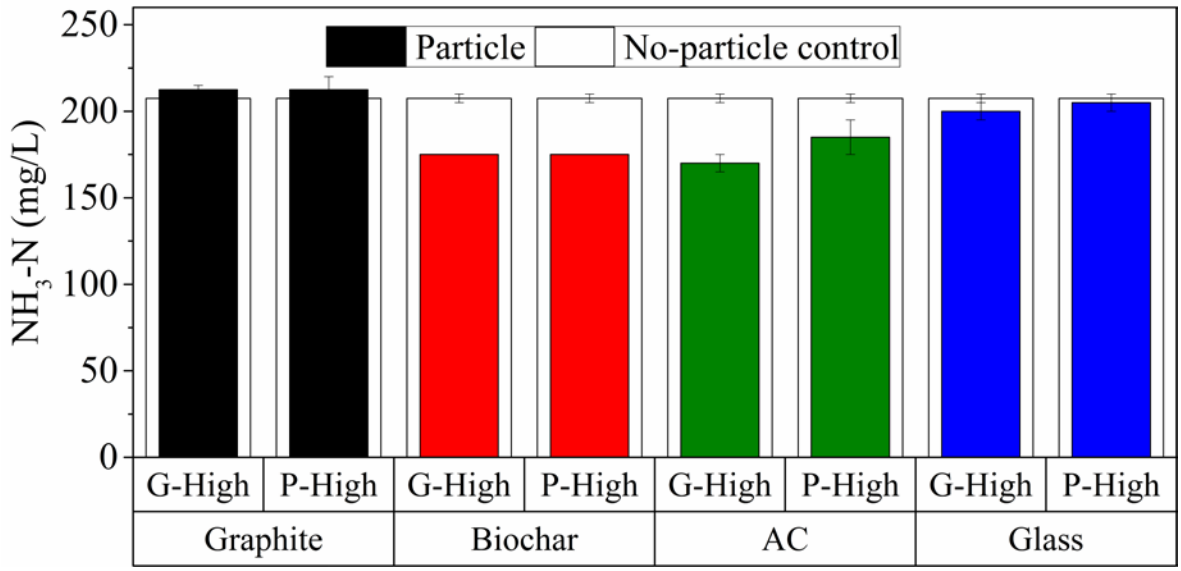
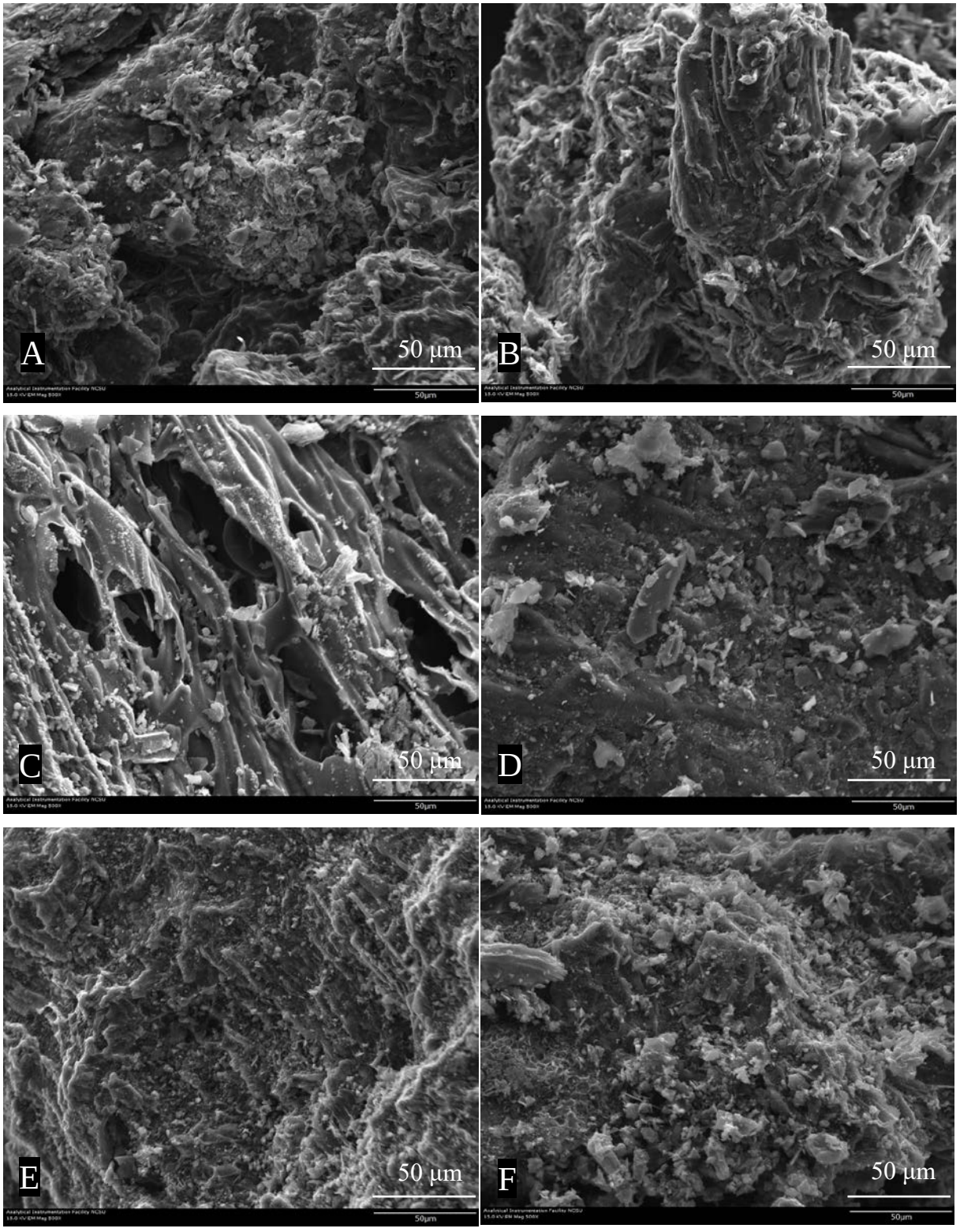


Fig. 10. NH_3-N adsorption using the highest particle loading under abiotic conditions. A 20 mL NH_4Cl solution (initial NH_3-N = 220 mg/L) was mixed with particles in 25 mL bottles and incubated on shakers at 30 °C for 19 days. The final NH_3-N concentrations in the supernatant were measured and shown as the shaded bars. Error bars represent the range of replicate experiments.

3.7 Particle surface properties

Surface properties, such as roughness and porosity, impact biofilm growth. We examined the surface structure and morphology of each particle type and size using SEM. Both G and P graphite consisted of sheet-like structures lacking noticeable pores (**Fig. 11A-B**). G biochar and AC had visible pore structures, with the G biochar pores being noticeably larger than those of the G AC (**Fig. 11C-F**). These pore structures were not visible on the P biochar and P AC, likely due to the destructive grinding and sieving when the powder was made in the lab. The glass particles were much smoother than the other materials and lacked any distinguishable surface structures (**Fig. 11G-H**).


A
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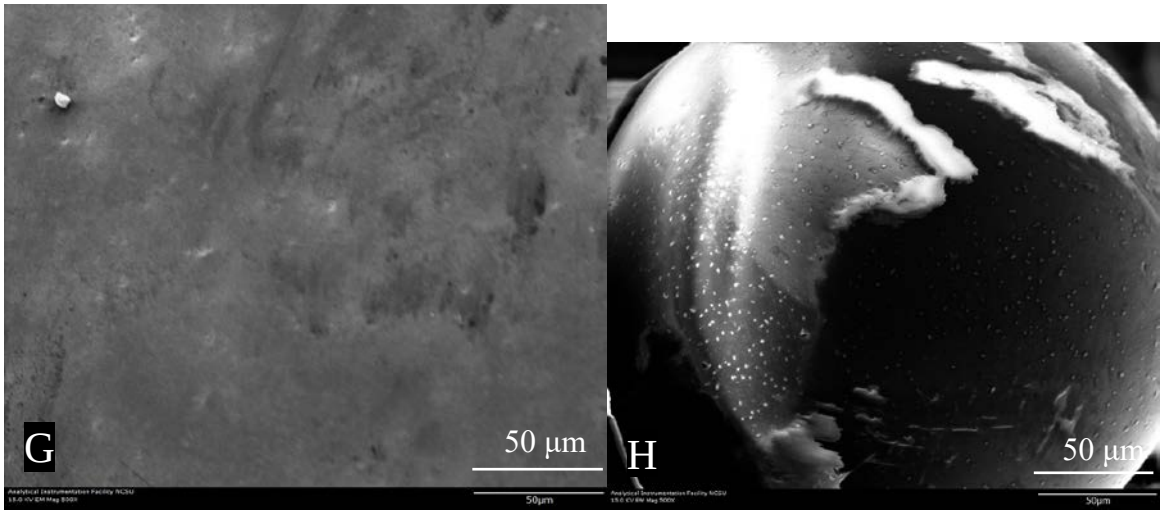

Fig. 11. Scanning electron micrographs of (A) G graphite, (B) P graphite, (C) G biochar, (D) P biochar, (E) G AC, (F) P AC, (G) G glass and (H) P glass. Images show new particles that were not added to the digesters.

4.0 Discussion

In this study, we systematically investigated the impact of three different electrically conductive particles on the anaerobic digestion of swine wastewater. We hypothesized that these particles would have a positive impact on CH_4 generation rates, CH_4 recoveries, and organic matter destruction relative to digesters without particles. Our results show that the effects are highly dependent on the material type and loading. Graphite was the only material tested which consistently improved digester performance across all loadings and sizes. Biochar and AC did not show clear trends with respect to size or loading, but the highest CH_4 production rate was obtained with granular biochar added at the lowest loading. Other studies have reported inconsistencies with respect to biochar as well. In some cases biochar improved CH_4 production rates upwards of 10-21%/g-biochar higher than the no-particle controls, but in others led to decreases up to –4.0%/g-biochar (Lü et al., 2016; Luo et al., 2015; Zhao et al., 2015). Reports with G AC typically show some of the lowest normalized improvements (Dang et al., 2016). Studies with graphite have yielded improvements between those of biochar and AC, up to 6.8%/g-graphite (Zhao et al., 2015). Comparing across different studies is not straightforward, however. Differences in substrate (ethanol, glucose, complex wastewaters), microbial communities, and particle surface properties, for example, introduce inherent variations that preclude direct comparisons. Our approach of comparing multiple particle types using the same wastewater, however, helps eliminate some of this variability. In this study, the increase in maximum CH_4 production rates reach upwards of 77%/g-biochar and 23%/g-graphite (powdered form; lowest loading). The increase in CH_4 recoveries reached as high as 19%/g-graphite and 18%/g-biochar (medium loading). Compared with other studies, the difference in percent increases could be due to the shapes, sizes, and loadings of particles as well as the types and loadings of organic matter in the wastewater.

The reasons for the consistent performance of the graphite cannot be attributed to surface area effects alone. Comparing the CH_4 production rate versus particle surface area slopes between the glass and graphite particles provides support for this conclusion. The slope for graphite was

almost three times larger than the slope of the glass particles. If the CH_4 production rate increase with graphite was solely attributable to the increase in surface area, we would have expected the graphite data to mirror the slope of the glass particle data. Since this was not the case, it suggests that another property of graphite contributed to the increase. Ruling out adsorption based on our abiotic tests, we hypothesize that the electrical conductivity played a role in increasing the rates. Whether or not stimulation of DIET occurred cannot be confirmed. Additional molecular scale analysis of the microorganisms and the electron transfer mechanisms they are using would be needed.

The explanation for some of the high CH_4 production rates with biochar are unclear, but may be due to its large pore sizes and possible nutrient availability on the surface. It has been reported that biochar favored the growth of methanogenic communities during the start-up period of AD and could act as pH buffer when acids accumulated during fermentation (Cooney et al., 2016; Lü et al., 2016; Luo et al., 2015; Sunyoto et al., 2016). Additionally, inorganic nutrients (e.g. phosphorus, potassium, calcium and magnesium) released into solution from biochar have been shown to provide additional benefits for microbial growth (Keiluweit et al., 2010; Silber et al., 2010; Sunyoto et al., 2016). It has been suggested that powdered biochar can facilitate the migration of bacteria to its internal surface and enhanced fermentation and acetogenesis (Lü et al., 2016; Luo et al., 2015). Sunyoto et al. observed that higher loadings of biochar in a digester led to a decrease in both CH_4 production rates and recoveries, with the recoveries dropping below those of the controls. One explanation for this observation was that biochar overloading led to propionic acid accumulation, slowing down methanogenesis. Our results with biochar also showed decreasing digester performance as the loading increased; however, further research as to why this trend was observed is needed.

One unexpected finding is that there was no relationship between CH_4 production rates and particle conductivity. Multiple studies have suggested that conductive materials may enhance methanogenesis through DIET, yet no evidence has directly linked the degree of conductivity to enhanced rates of electron exchange. For instance, a low conductivity biochar (2.1 μS/cm) performed as well as a more conductive one (4.3 μS/cm) in accelerating anaerobic degradation of ethanol (Chen et al., 2014a). GAC with a significantly higher conductivity (3,000 ± 327 μS/cm) than the biochar did not further increase the reaction rate (Chen et al., 2014a; Liu et al., 2012). However, applying a lower GAC loading generated a higher increase (60- and 76-fold/g GAC) in the endproduct production rates (succinate in this case). Coupling these studies with our findings indicates that conductivity alone cannot explain or predict DIET reaction rates. Other material properties may have therefore played a larger role or masked the contribution of conductivity.

Adsorption was found to be a determining property in the fate and conversion of organics within the digesters. Adsorption was most pronounced with biochar and AC and varied with particle size. The differences in COD conversion efficiencies to CH_4 between G and P biochar was likely due to mechanistic differences in organic matter adsorption. The higher COD to CH_4 conversion of the G biochar suggests that the adsorbed COD was readily bioavailable for microbial growth. Conversely, when powdered biochar and AC in both sizes were used, a larger proportion of the initial COD ended up in the solids fraction without being consumed biologically. As a result, lower conversions of COD to CH_4 occurred, which explains why reduced CH_4 recoveries for those particle treatments were observed. The bioavailability of adsorbates, such as organics, is

related to desorption efficiency (Aktaş and Çeçen, 2007). Usually the desorption process is not 100% efficient and a certain amount of organic matter remains inaccessible. For example, it has been suggested that thermally activated carbon has higher adsorption efficiency and lower desorption efficiency compared with chemically activated carbon (Aktaş and Çeçen, 2006). This was the type of AC used in our work. This implies that the adsorbates on our AC may not have been readily available to the microorganisms. However, the adsorptive and desorptive processes can be very complex and largely dependent on the inherent properties of carbon (e.g. wood source, pyrolysis temperature), the properties of adsorbates (e.g. functional groups) as well as surrounding conditions (e.g. pH, temperature) (Aktaş and Çeçen, 2007; Essandoh et al., 2015). Further studies are required to better understand the impact of adsorption and desorption and how they are related to CH_4 production.

Surface property variations can also explain some of our observed performance differences. Surface roughness, for example, is important because it can influence bacterial adhesion and biofilm formation (Pons et al., 2011). Surface pores and roughness also play a role in microbial access to both protection and nutrients. Rougher surfaces have been shown to provide biofilms with more resistance to bulk solution changes (Simpson, 2008). Biochar pores can harbor microbes and protect them from inhibitors (Lü et al., 2016). A similar effect may occur on AC surfaces, but relative to biochar, AC pores are smaller than biochar because of the different synthesis methods, rendering some of the measured surface area inaccessible (Huggins et al., 2016; Simpson, 2008).

5.0 Conclusions and Recommendations

AD is a wastewater treatment and energy recovery technology that can provide NC's utilities and farms with environmental and energy benefits. Underutilization of AD is largely driven by economic and performance limitations. Here we address a fundamental aspect related to performance: syntrophic relationships among microorganisms within ADs. Our objective was to determine if the addition of electrically conductive particles could increase the performance of ADs treating swine wastewater. The rationale for this approach is based on emerging research indicating that these materials can accelerate syntrophic reaction rates through a process called DIET.

Our findings show that of the three materials tested (graphite, biochar, AC), graphite was the only material that consistently improved CH_4 production rates and CH_4 recoveries. Digesters augmented with graphite exhibited strong relationships between these metrics and particle loading, surface area, and size. Biochar and AC particle addition improved performance relative to digesters without particles only under certain loadings or sizes. In general, the powdered form of these materials underperformed substantially. The high adsorption capacity of these carbon-based sorbents was a primary reason for their reduced performance. Adsorption was a key fate of the initial organic material in the wastewater (measured as COD). We found that materials that had higher adsorption yielded lower CH_4 recoveries. Graphite, which displayed little to no adsorption during abiotic tests, converted a larger proportion of initial COD to CH_4 than any other material. Several other factors may have contributed to the differences among materials. Most notably, surface properties, including surface roughness and porosity, likely contributed to

performance differences. Conductivity, however, was poorly correlated with CH_4 production rates.

With respect to the economic feasibility of particle addition to wastewater, we performed a preliminary assessment based on particle cost and the CH_4 generated from our tests. First, material cost varies considerably. Based on bulk pricing (www.alibaba.com) the costs for each material were approximately \$100 - \$2,000 / ton for graphite, \$40 - \$4,000 / ton for AC, and \$0.5 - \$800 / ton for biochar. Second, by taking into account the particle loadings and wastewater volumes used in our tests, graphite was the most expensive at \$3.36/m^3-wastewater, followed by AC (\$1.34/m^3) and biochar (\$0.02/m^3). Third, assuming the CH_4 gas sells at the industrial natural gas price of \$4.62/1,000 ft^3 we determined the net profit of the additional CH_4 generated beyond the no-particle controls. This analysis shows that in all cases graphite particle addition results in a net loss per cubic meter of treated wastewater. In order to break even, more than 100 m^3 of wastewater would need to be treated for each particle loading. The only material to generate a net profit, albeit below \$1/m^3, was the granular biochar at both the medium and low loadings. This analysis suggests that 1) financial feasibility is highly dependent on the material type and loading, and 2) a thorough analysis of particle retention and reusability is needed to estimate potential cost savings.

Our recommendations moving forward include investigations of both fundamental and applied topics related to DIET. Fundamentally, a more rigorous understanding of the microbial mechanisms associated with DIET is needed. Once the microorganisms and mechanisms are identified, this knowledge can be used for the informed selection and/or design of materials that effectively promote DIET. With respect to application, alternative means of providing high-surface area conductive surfaces within digesters should be explored. Our preliminary economic analysis indicates that continuous particle addition is likely not a cost-effective option. However, there is a variety of commercially available materials that are both conductive and can provide large surface area to volume ratios. Examples include graphite brushes, carbon cloths, and graphite felts. These “fixed” structures may provide a more cost-effective option. Rigorous explorations of these materials at the lab-scale using a variety of high-strength wastewaters are needed prior to determining their use for large-scale applications.

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Appendix 1: List of abbreviations and symbols

CH_4 – methane gas; a flammable gas produced during anaerobic digestion.

WWTF – wastewater treatment facility

WRF – water reclamation facility

MGD – million gallons per day; units of flow

EPA – Environmental Protection Agency

AC – activated carbon; a high surface area, carbon-based material synthesized from virtually any organic material; commonly used as a sorbent in drinking water treatment plants.

COD – chemical oxygen demand; a measure of inorganic and organic content of a water sample.

TCOD – total chemical oxygen demand; a measure of both the soluble and insoluble inorganic/organic content.

COD_i – initial chemical oxygen demand; the COD at the start of the batch.

VS – volatile solid; a measure of the volatile fraction of the wastewater solids.

TS – total solids; a measure of all solids in a sample, including both dissolved and suspended fractions.

VFA – volatile fatty acid; a group of fatty acids produced from microoganisms during fermentation of organic matter (ex – acetic acid).

AD – anaerobic digestion; an anaerobic process during which microorganisms degrade organic material, resulting in the generation of methane gas.

NH_3-N – ammonia; represents ammonia concentration in terms of nitrogen.

DIET – direct interspecies electron transfer; electron exchange between two syntrophic organisms through physical connection and conduction of electrons.

MIET – mediated interspecies electron transfer; electron exchange between two syntrophic organisms through the transfer of soluble metabolites, such as hydrogen gas or formate.

Appendix 2: Project outputs

Publications

1) Invited manuscript for special issue on emerging investigators: Cheng, Q.; Call, D. F. Hardwiring microbes via direct interspecies electron transfer: mechanisms and applications. *Environ. Sci. Process. Impacts* 2016, *18*(8), 968–980. Peer reviewed.
2) Cheng, Q.; de los Reyes, F. L.; Call, D. F. Influence of electrically conductive particle type, size, and loading on methane generation in swine wastewater-fed anaerobic digesters. In preparation.

Presentations

1) Cheng, Q; de Los Reyes, F.; Call, D.F. Influence of Electrically Conductive Particles on Methane Generation in Swine Wastewater Fed Anaerobic Digesters. 19th Annual Water Resources Research Institute (WRRI) Conference. Raleigh, NC. March 15-16, 2017. Poster.
2) Call, D. F.; Cheng, Q.; Murray, C. C.; Tavares, V. L.; de los Reyes, F. L. Accelerating methane generation rates in anaerobic digesters with electrically conductive materials. 89th Water Environment Federation Technical Exhibition and Conference (WEFTEC). New Orleans, LA. September 28, 2016. Oral.
3) Cheng, Q.; Murray, C. C.; Tavares, V. L.; de los Reyes, F. L.; Call, D. F. Electrically conductive particles supporting direct interspecies electron transfer (DIET) in anaerobic microbial communities. 252nd American Chemical Society (ACS) National Meeting and Exposition. Philadelphia, PA. August 23, 2016. Oral.

Appendix 3: Supplemental Figures and Tables

Table S1. COD (mg/L) of deionized water plus particle addition (3.4g/100mL)

	Graphite	Biochar	AC	Glass
COD (mg/L)	G: 13±7 P: 21±7	G: 8±2 P: 16±4	G: 11±5 P: 23±17	G: 13±1 P: 17±5

Table S2. pH in deionized water and swine wastewater with particle addition[a]

	Graphite	Biochar	AC	Glass	Blank
Deionized water	G: 5.2±0.030 P: 5.3±0.010	G: 8.5±0.020 P: 9.0±0.010	G: 9.3±0.020 P: 9.4±0.030	G: 9.1±0.020 P: 9.0±0.020	
Swine wastewater	G: 7.3±0.010 P: 7.4±0.020	G: 7.7±0.020 P: 7.8±0.010	G: 8.0±0.020 P: 8.1±0.030	G: 7.8±0.030 P: 7.7±0.030	7.6±0.040

[a] 20 mL deionized water or swine wastewater mixture was mixed with particles in 50 mL tubes, and the tubes were shaken by hand and then allowed to stand for 5 min. Supernatant pH was measured. The highest particle-to-working volume ratio was used.

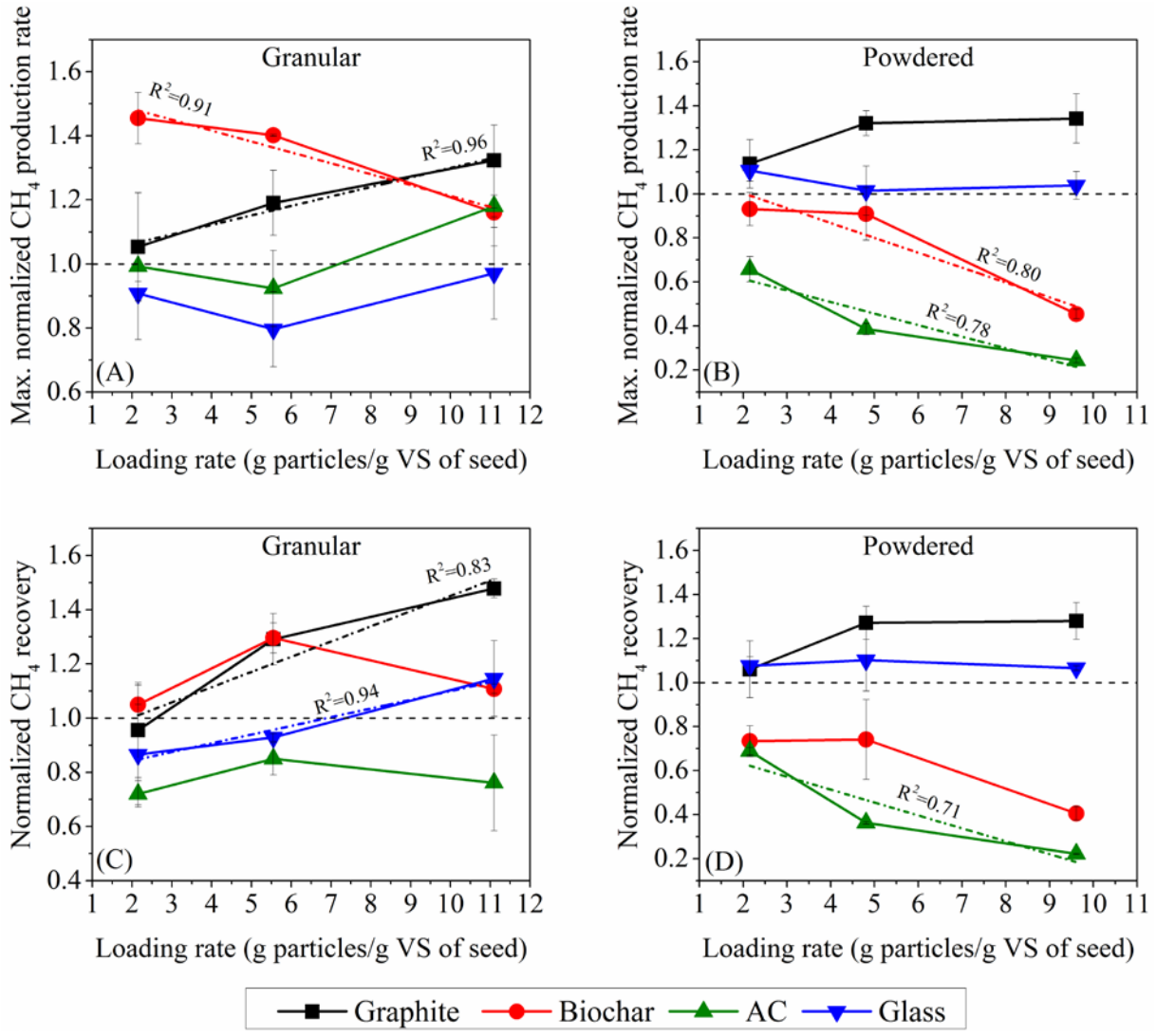

Fig. S1. Maximum CH_4 production rates as a function of particle loading from (A) granule-amended reactors [G] and (B) powder-amended reactors [P]. CH_4 recoveries as a function of particle loading from (C) granule-amended reactors [G] and (D) powder-amended reactors [P]. Error bars represent the range of replicate experiments. Only a correlation coefficient greater than 0.7 was shown on the graph.

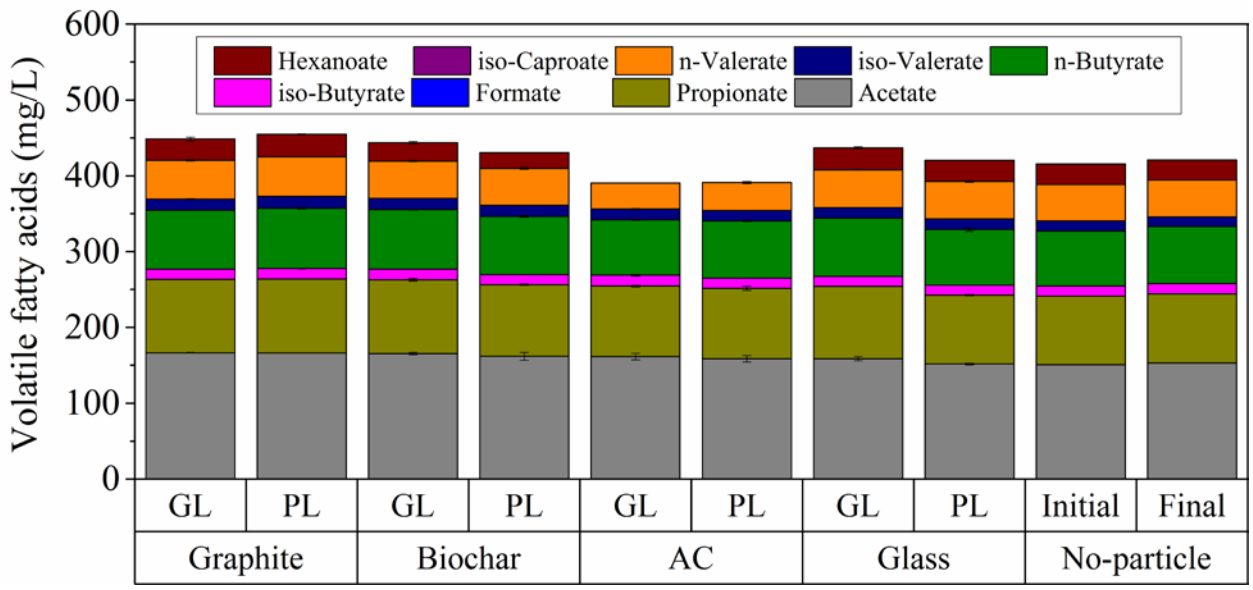

Fig. S2. Volatile fatty acids in sterile swine wastewater after 19-day incubation. The detection limit was 20 mg/L, and any concentration below this limit was not shown. The loading of particles to sterile swine wastewater was 2.2 g particles/g VS (before autoclaved). Error bars represent the range of replicate experiments.

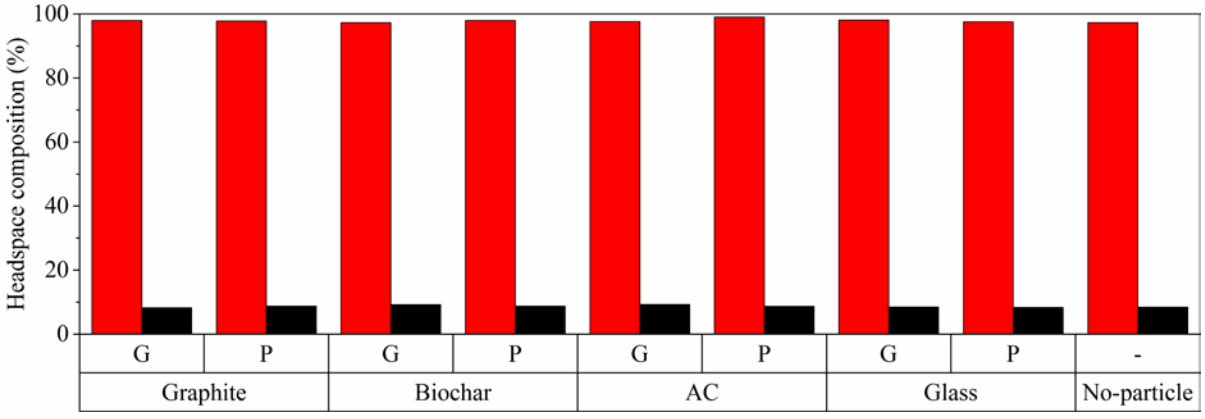

Fig. S3. Abiotic CH_4 absorption in deionized water amended with particles. 120 mL gas containing either 100% CH_4 (in red) or 10% CH_4 (in black) was directly injected into 20 mL of deionized water in 25 mL serum bottles amended with particles. The highest particle-to-working volume ratio was used. The bottles were incubated at 30 °C and the gas composition analyzed after 24 hours.