Accelerating and Stabilizing Anaerobic Digestion by Promoting Direct Interspecies Electron Transfer

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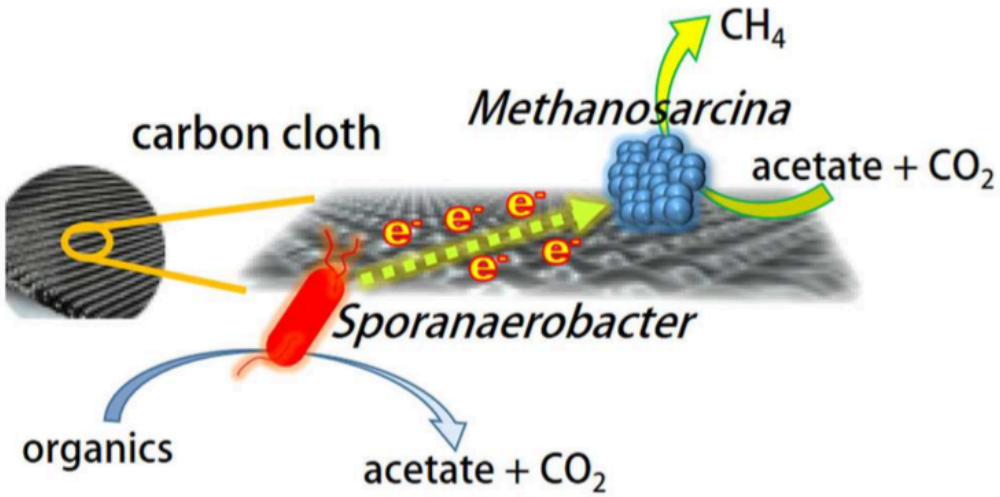
KEYWORDS: Food waste, solid waste, methane, methanogens, carbon cloth, granular activated carbon, fermentation, syntrophy



FINAL REPORT

Prepared for the
Environmental Research and Education Foundation

2017

EXECUTIVE SUMMARY

The purpose of this research was to determine whether the anaerobic digestion of wastes can be improved by promoting direct interspecies electron transfer (DIET) between the bacteria responsible for degrading components of the solid waste and methane-producing microorganisms (methanogens). Previous studies had demonstrated DIET in anaerobic digesters degrading ethanol in brewery wastes and had found that electrically conductive carbon materials such as carbon cloth, biochar, and granular activated carbon could accelerate rates of DIET. Our EREF-supported studies demonstrated that a broad range of organic wastes can be metabolized by bacteria and methanogens cooperating via DIET. These include short-chain fatty acids such as acetate, propionate, and butyrate; as well as other alcohols, and possibly fermentable sugars and amino acids. Stimulating DIET with conductive materials, such as carbon cloth, accelerated the conversion of wastes to methane and permitted higher rates of organic loading. The impact of conductive materials was particularly noticeable in studies with solid waste, in which methane was only produced in the presence of conductive materials. Surprisingly, in food waste digesters carbon cloth even appeared to promote DIET between fermentative bacteria and methanogens, decreasing the production of reduced short-chain fatty acids that can lead to digester souring. These results suggest that incorporation of carbon cloth or some other permanent conductive material in digesters may facilitate faster conversion of organic waste to methane and reduce the potential for digester souring. Future studies should focus on optimizing the deployment of conductive materials within digesters and consider complimentary strategies that may further enhance DIET.

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INTRODUCTION

The purpose of this project was to determine whether the anaerobic digestion of complex organic wastes could be improved by enhancing the electrical connections between methane-producing microorganisms (i.e. methanogens) and the microorganisms that provide the methanogens with the electrons they require to convert carbon dioxide to methane. The experimental approach was based on the discovery of a microbial strategy for electrical communication known as direct interspecies electron transfer (DIET). DIET is an alternative to the well-known process of interspecies hydrogen transfer in which hydrogen is the electron carrier between the electron-donating microorganisms and the methane-producing microorganisms. Details on the discovery of DIET and the microbiology, biochemistry, and physics of DIET can be found in two recent literature reviews (1, 2).

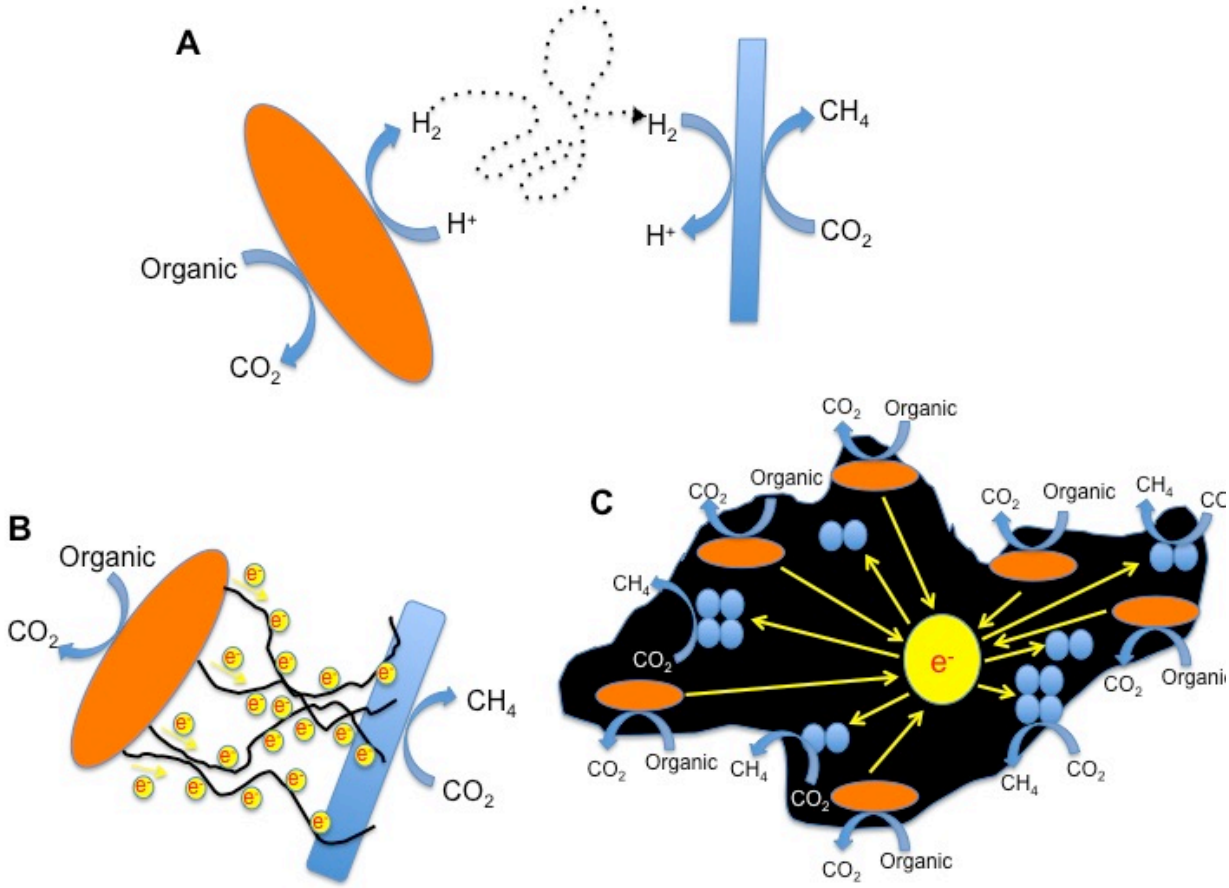

Figure 1. Mechanisms for electron transfer to methane-producing microorganisms (methanogens) during anaerobic digestions. (A) Interspecies hydrogen transfer in which slow, diffusive exchange of hydrogen is the mechanism for electron exchange between electron-donating microorganisms (orange) and methanogens (blue). (B) Rapid, direct interspecies electron transfer (DIET) to methanogens (blue) via electrically conductive protein filaments (black) produced by the electron-donating bacteria (orange). (C) Accelerating DIET with electrically conductive material (black) that serves as a support for electron-donating microorganisms (orange) and methanogens (blue). Electrons flow into the conductive material from the electron-donating microorganisms and methanogens extract electrons from the conductive material for the reduction of carbon dioxide to methane.

As summarized in those recent literature reviews, previous studies demonstrated that electrically conductive carbon materials such as granular activated carbon, biochar, and carbon cloth promoted DIET in defined laboratory co-cultures. The purpose of this project was to determine whether conductive materials would have a similar impact on more complex microbial communities and enhance the conversion of food waste to methane.

RESULTS AND DISCUSSION

The primary aims of the research were to: (1) Determine an optimal strategy for promoting DIET in anaerobic solid waste and (2) Determine whether anaerobic solid waste digestion via DIET is faster and more resilient to upsets than traditional anaerobic solid waste digestion.

Metabolism of Short-Chain Fatty Acids and Alcohols with DIET

An important step to achieving the overall goals was to determine which substrates could be metabolized to methane with DIET and to determine which electrically conductive materials might best promote DIET. Previous studies focused only on ethanol. However, butyrate and propionate are also important intermediates in the conversion of complex organic wastes to methane. Defined co-culture studies (3) and mixed communities of microorganisms in an inoculum from an anaerobic digester (4), both indicated that propionate and butyrate could be metabolized with DIET. This information suggested that the technically more complex studies with food waste should be pursued.

Best Conductive Carbon Materials to Support DIET

Prior to embarking on the technically complex studies with food wastes, some preliminary studies were conducted to help define what conductive carbon materials were likely to best promote DIET in mixed microbial communities. Studies were conducted in reactors converting ethanol to methane. Graphite, biochar, and carbon cloth stimulated methane production with carbon cloth promoting the highest methane production rates (5). The COD removal efficiencies in the three reactors supplemented with conductive materials were all higher than 93%, whereas the COD removal in the control reactor averaged only 83%.

As the hydraulic retention time of the reactors was decreased from 24 h to 8 h (organic load rate [OLR] from 4.11 kg COD/m^3/d to 12.33 kg COD/ m^3/d) over the next 42 days the gap of methane production between the control and supplemented reactors increased further. Methane production rates that were 30-45% higher with the conductive materials and there was no decline in COD removal rates. In contrast, reactors without conductive carbon materials performed poorly with an accumulation of acetate and a drop in pH (5). Removing conductive materials resulted in poor reactor performance, comparable to the control reactors. These results suggested that promoting DIET with conductive materials could enhance electron exchange between the electron-donating microorganisms and the methanogens and that conductive material, such as carbon cloth, that could be designed as a permanent component of the reactors helped accelerate and stabilize anaerobic digestion.

Promoting DIET Enhances Conversion of Food Waste to Methane

With the information summarized above it was then possible to rationally design studies to evaluate whether promoting DIET would enhance the conversion of solid waste to methane. As is common in such studies dry dog food was used as a consistent source of organic waste (6, 7).

In reactors that were semi-continuously fed simulated food waste, carbon cloth and other conductive materials maintained stable conversion of waste to methane up to organic loading rates of approximately 10 kg COD/(m^3 day) (6). In contrast, control reactors with non-conductive polyester cloth only maintained a balanced digestion up to loading rates of 6.7 kg COD/(m^3 day) (6). These studies demonstrated that promoting DIET with conductive carbon materials accelerated and stabilized methane production from complex organic wastes, permitting higher organic loading rates.

The enhanced conversion of wastes to methane in the presence of conductive carbon cloth, but not with equivalent amounts of non-conductive polyester cloth suggests that DIET was responsible for improving the methane production. In order to further evaluate what microorganisms were involved in DIET the composition of the microbial community attached to carbon or polyester cloth, as well as in the bulk sludge was determined (6). Bacterial and methanogenic sequences enriched on the surface of the carbon cloth differed significantly from the other samples. For example, bacteria in the genus *Sporanaerobacter* and *Enterococcus* were much more abundant on the surface of the carbon cloth than on the surface of the polyester or in the bulk sludge (Figure 2). The specific enrichment of *Sporanaerobacter* and *Enterococcus* species on the carbon cloth is consistent with the concept that members of these genera are likely to be capable of extracellular electron transfer and thus could be expected to donate electrons to other species through a conductive conduit such as carbon cloth (6).

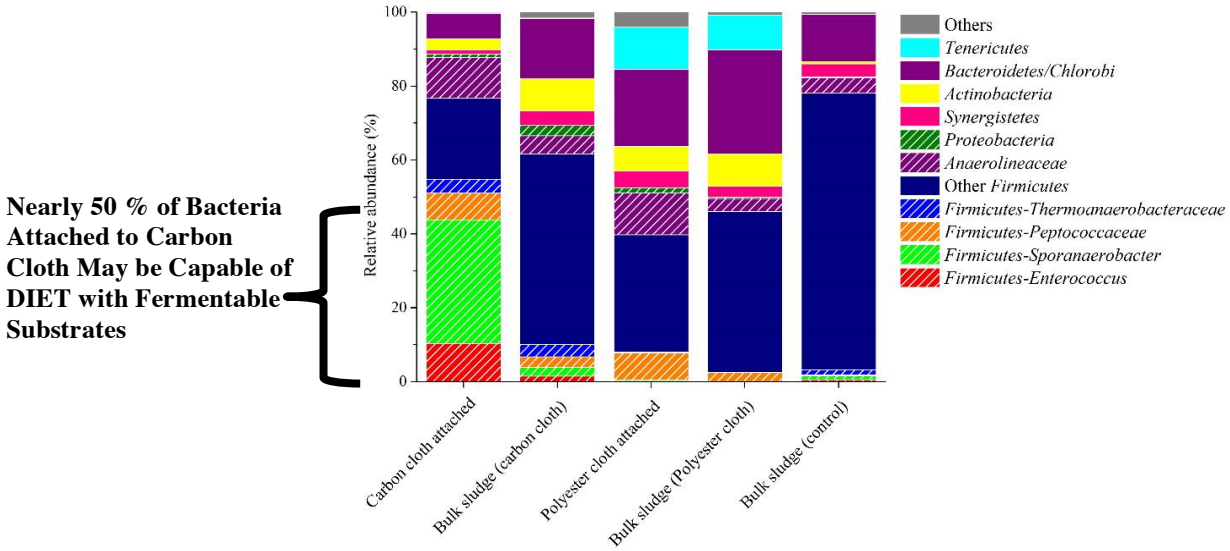

Figure 2. Composition of the bacterial community in the food-waste reactors.

Further evidence for DIET was a specific enrichment of *Methanosarcina*, which are known to participate in DIET, on the carbon cloth (Figure 3). This was accompanied by a dramatic decrease in H_2-utilizing methanogens within the bulk sludge of the carbon cloth reactors (Figure 3). Whereas H_2-utilizing methanogens, such as *Methanomicrobiales*, *Methanomassiliicoccales,* and *Methanobacteriales*, accounted for more than 50 % of the methanogens in the bulk sludge of the control reactor, they accounted for less than 10 % of the methanogens in the reactors with carbon cloth (Figure 3). These results suggest that there was very little H_2 production in the

presence of carbon cloth. This is consistent with electron transfer to methanogens proceeding through DIET.

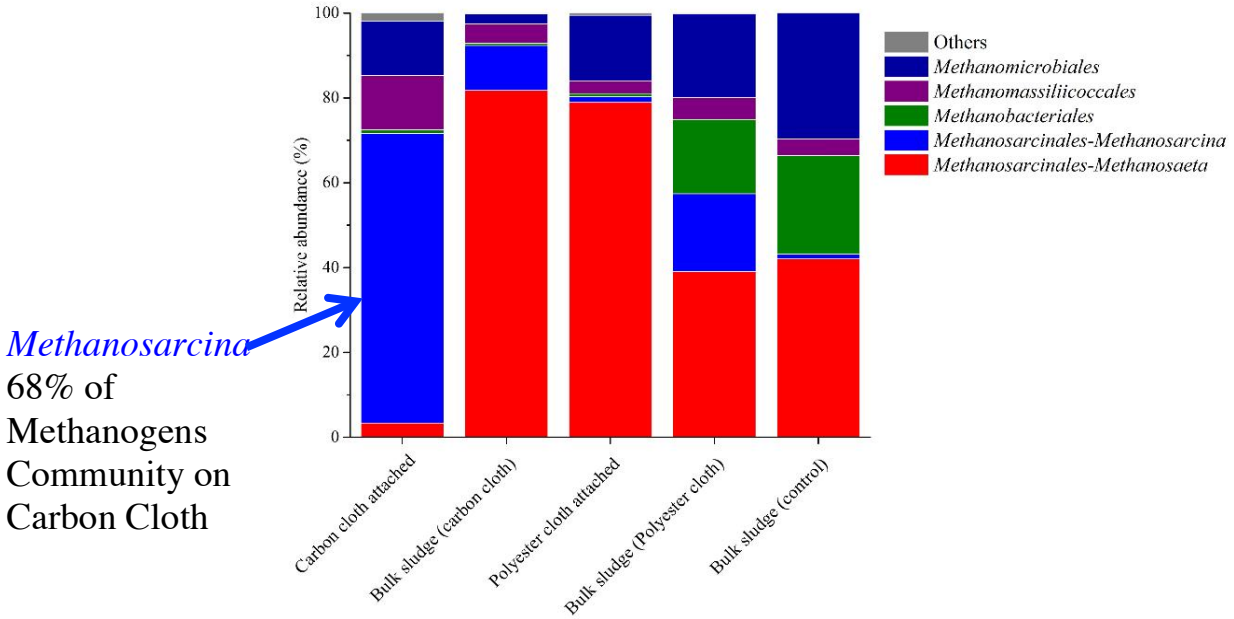

Figure 3. Composition of the methanogenic community in the food-waste reactors.

These results suggested that conductive carbon cloth promoted DIET in the reactors degrading food waste. It appeared that carbon cloth was promoting the growth and metabolism of microorganisms that can metabolize fermentable substrates, such as sugars and amino acids (6).

In order to evaluate the potential for conductive carbon materials to promote the conversion of solid waste to methane a new set of reactors were initiated with 137g dog food to yield an initial total solid (TS) content of 11% (7). There was very little methane production in control reactors or reactors amended with non-conductive polyester cloth (Figures 4 and 5). However, both granular activated carbon (GAC) and carbon cloth promoted methane production.

For the first 6 days of the experiment, it was necessary to add 10 ml of 7N NaOH to the reactors daily to keep the pH from dropping below a pH of 4.5 and souring the reactors. After that, the pH of the GAC- and carbon cloth-amended reactors stabilized at approximately 8.2 whereas the pH of the polyester cloth and non-amended control reactors stabilized around 7 (Figure 6).

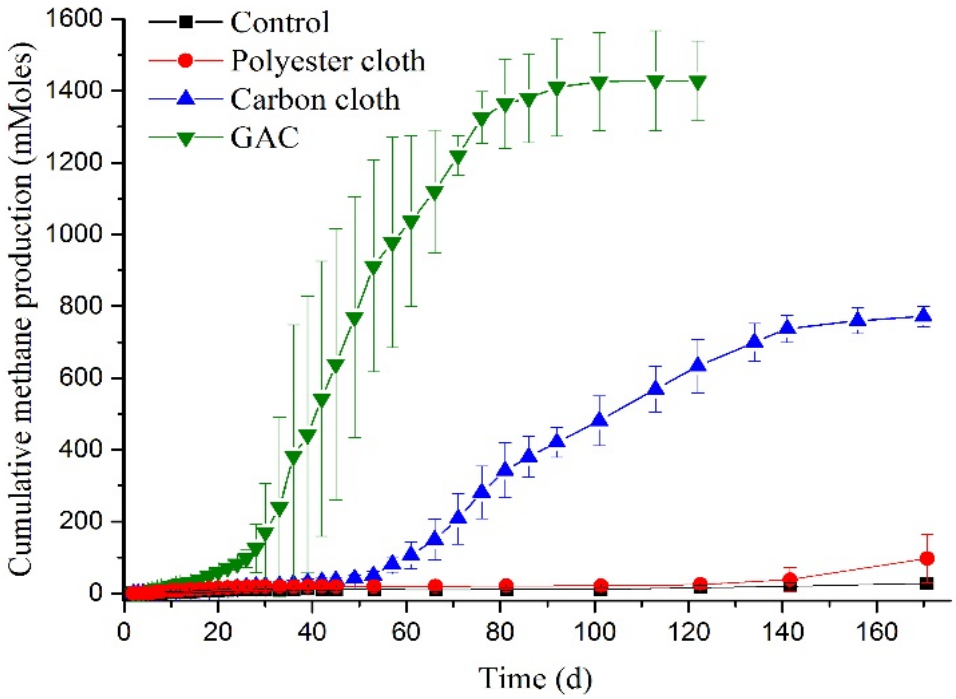

Figure 4. Methane production over time in the solid waste reactors. The results are the mean and standard deviation of triplicate reactors for each treatment

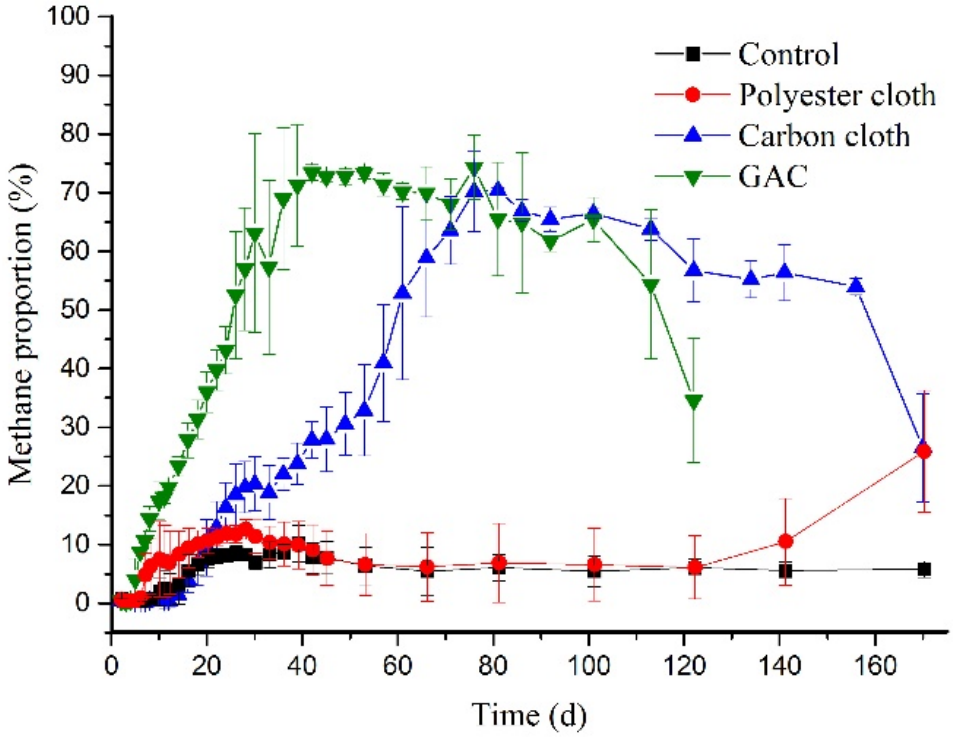

Figure 5. Methane content in biogas produced over time in the solid waste digesters. The results are the mean and standard deviation of triplicate reactors for each treatment.

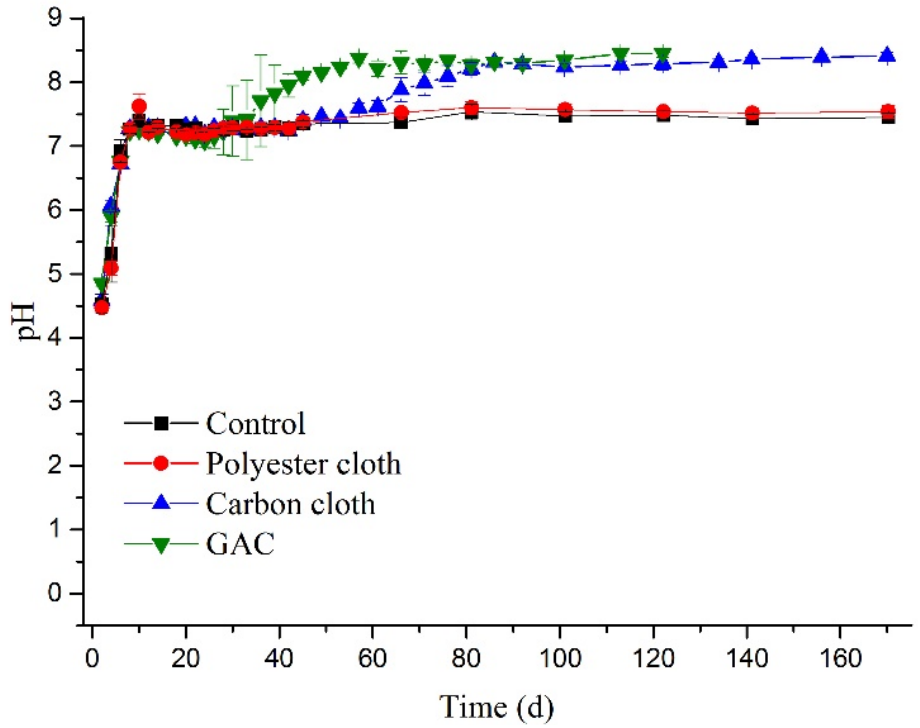

Figure 6. pH over time in the solid waste digesters. The results are the mean and standard deviation of triplicate reactors for each treatment.

The enhanced methane production in the presence of conductive materials was associated with the removal of acetate and butyrate, which accumulated in the control reactors (Figure 7). However, propionate accumulated even in the presence of the conductive materials (Figure 7). The conductive materials significantly increased the removal of COD over that observed in the controls (Figure 8).

These results demonstrated that conductive materials that facilitate DIET can greatly enhance the conversion of solid waste to methane (7). However, even in the presence of the conductive materials the conversion of the waste was incomplete, primarily due to the accumulation of propionate. Propionate is the short-chain fatty acid that is typically metabolized the slowest. One strategy for increasing propionate removal might be to add more conductive material to the solid wastes. Other potential strategies are discussed in the Conclusions section.

Unlike the previous studies, the composition of the microbial community in digesters with solid waste did not yield clear evidence for DIET (6). One reason is that with such high concentrations of organic matter a substantial amount of the interspecies electron transfer may have been through H_2 produced by fermentative microorganisms rapidly metabolizing fermentable substrates. This result also suggests that addition of more conductive material or other approaches may be necessary to fully optimize solid waste digestion.

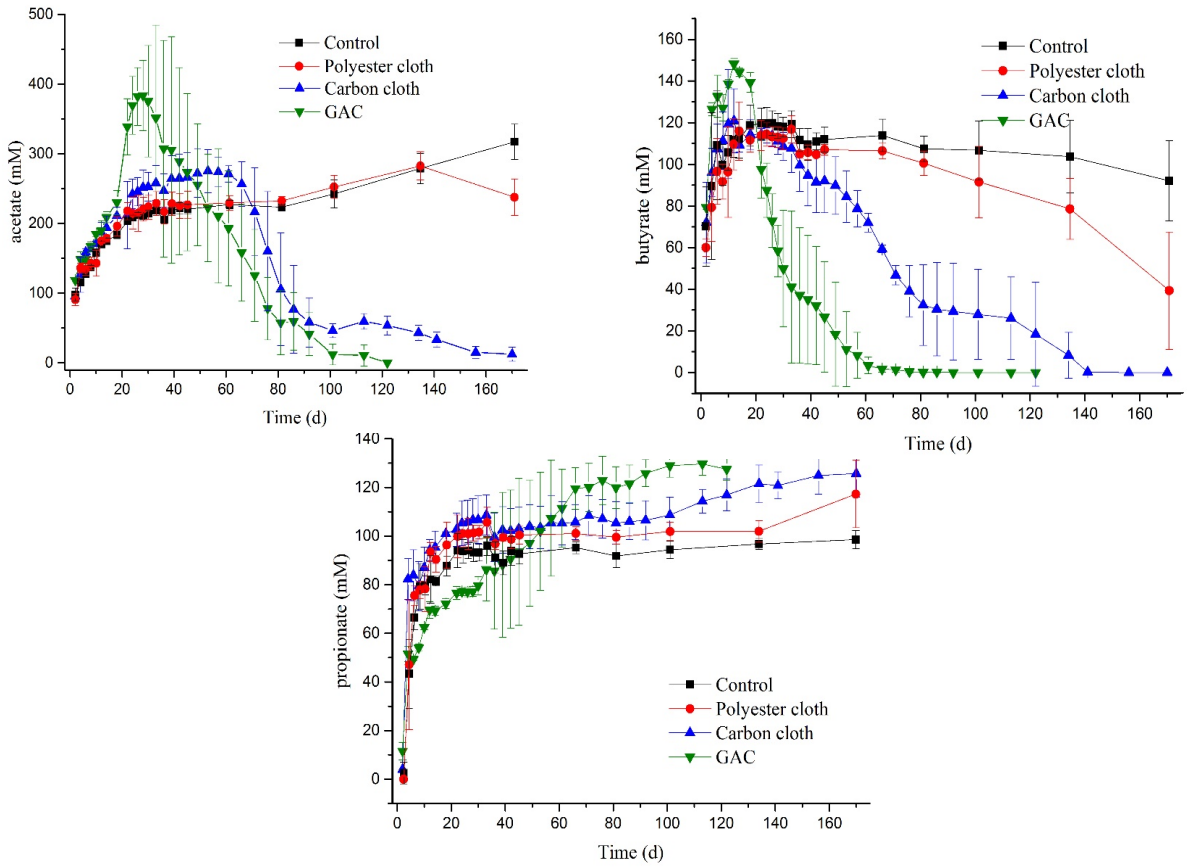

Figure 7. Concentration of acetate, butyrate, and propionate over time in the solid waste reactors. The results are the mean and standard deviation of triplicate reactors for each treatment.

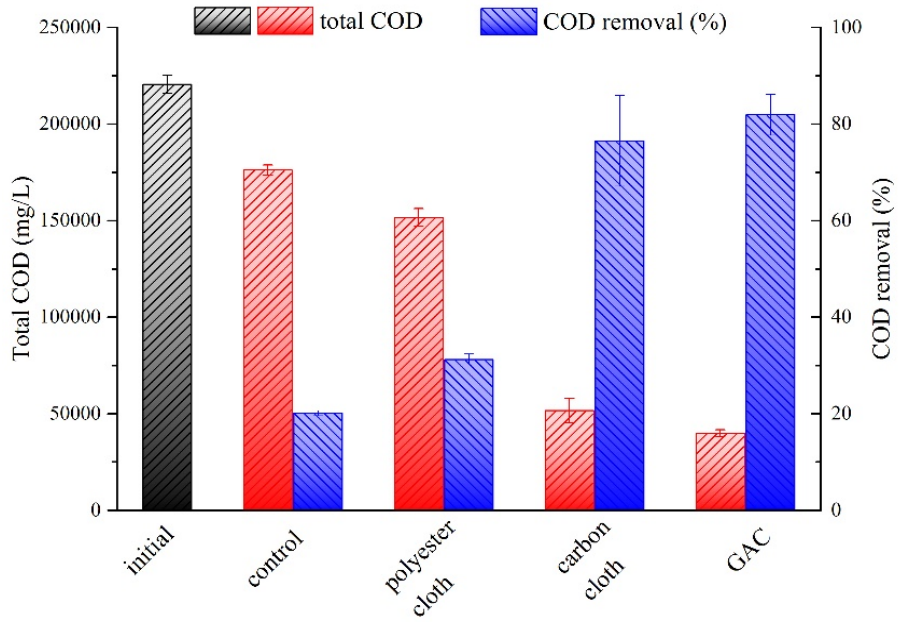

Figure 8. COD removal in the solid waste reactors. The results are the mean and standard deviation of triplicate reactors for each treatment.

CONCLUSIONS

The results demonstrate that it is possible to accelerate and stabilize the conversion of solid waste to methane by promoting direct interspecies electron transfer (DIET). This can be accomplished with an inexpensive yet durable material such as carbon cloth that can readily be incorporated as a permanent component of solid waste digesters. Additional engineering studies will be required to determine the optimum strategy for deploying the conductive material in full-scale systems. Also, the results suggested that solid waste digestion will require a higher density of conductive material than was deployed in the study reported here in order to optimize DIET.

Surprisingly, an expanded role of DIET in the degradation of complex wastes was revealed. In the presence of carbon cloth some fermentative bacteria appear to use the conductive material as a conduit for electron transfer to methanogens. This form of DIET has the potential to reduce the production of reduced intermediates that often inhibit anaerobic digestion and lead to souring. Further study of this form of DIET is warranted in order to better understand the role of DIET in the conversion of solid waste to methane.

Our studies suggest that the conversion of solid waste to methane could still be further optimized with additional study of DIET in such systems. It is important to know whether simply increasing the amount of conductive material in digesters would promote even faster conversion of solid wastes to methane and prevent unwanted accumulation of propionate. Another complimentary approach might be employ graphite electrodes as a temporary electron acceptor during periods of rapid propionate formation, to further promote microbial degradation of this intermediate. Another possibility is inoculation with DIET-based communities that are optimized for propionate metabolism at the time of reactor start-up. We hope to evaluate these possibilities in future research.

MATERIALS AND METHODS

Reactor Design

Reactors for food waste studies were built from glass jars with a working volume of 2 liters outfitted with sampling ports and a gas outlet connected to a gas collection bag. All reactors were incubated at 37 °C in the dark and shaken at 100 rpm continuously. For more details see (6, 7). Upflow reactors were constructed from glass tubes fitted with an inlet at the bottom and outlet and gas sampling bag at the top. For more details see (4, 5). The dry organic fraction of municipal waste was simulated with a commercial dog food (The Honest Kitchen; CA). The dog food was initially dried (moisture content<7.26%) and mainly consisted of protein (>26%), fat (>19%) and fiber (<6.3%), which is similar to contents of actual municipal solid waste. Sludge obtained from an anaerobic digester at a municipal wastewater treatment plant located in Pittsfield, Massachusetts was used as the seed sludge for all reactors.

Conductive Materials and Controls

Two different materials with similar geometric surface areas of ca. 1000 cm^2 were added to triplicate reactors. The carbon cloth additions consisted of three pieces of carbon cloth (Zoroflex; buyactivatedcharcoal.com) that were each 8 x 20 x 0.1 cm. This comprised a volume of 48 cm^3 and geometric surface area of 977 cm^2. Granular activated carbon was added as 100 g of 8–20 mesh particles (Sigma, Inc.) to yield a volume of ca. 120 cm^3 and geometric surface area of ca. 900 cm^2. As a control that provided a surface for microbial attachment, but was non-

conductive, three pieces of polyester cloth (Heavy Duty Fabric and CORDURA; http://heavydutyfabric.com) that were 8 x 20 x 0.1 cm were added. This gave the same volume and surface area as the carbon cloth. One set of triplicate reactors received no materials.

Analytical Methods

Analyses of TS, volatile solid (VS), TSS and VSS were conducted in accordance with Standard Methods for the Examination of Water and Wastewater (8). Chemical oxygen demand (COD) was determined with Hach's method 8000 (Hach DR/890 colorimeter procedures manual). VFAs were measured with a high-performance liquid chromatograph (Bio-Rad; Hercules; California), and pH was monitored with a pH analyzer (UB-10; Denver Instrument; Denver). The gas volume in the 10 L-gas-sampling bag was measured every 24 h by a digital mass flow meter (FMA4000; Omega; USA). The composition of biogas was analyzed with a gas chromatograph with a flame ionization detector (GC-8A; Shimadzu; Japan).

Microbial Community Composition

All samples were immediately frozen with liquid nitrogen prior to DNA extraction and stored at -80°C. For extraction, frozen samples were ground into a fine powder with a mortar and pestle before processing with BIO101 FastDNA soil kits (MP Biomedicals, Ohio) according to the manufacturer's instructions. DNA was quantified with a NanoDrop spectrophotometer (Thermo, Fisher Scientific, Delaware) and sent to the MR DNA sequencing facility (Shallowater, Texas) for 16S rRNA bacterial and archaeal sequence analysis. Bacterial and archaeal 16S rRNA genes were amplified and sequenced on a 454 platform (Roche). Sequence data has been deposited in the Genbank database. Accession numbers are available in the relevant publications (4-7), which also provide additional technical details.

Defined Co-Culture Studies

All microbial cultures were grown under strict anaerobic conditions as previously described (3). Analytical procedures for measuring the consumption of substrates and the production of endproducts with high performance liquid chromatography and gas chromatography followed previously published methods (3).

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APPENDIX I-LIST OF EREF-SUPPORTED PUBLICATIONS

Zhao, Z., Y. Zhang, T. L. Woodard, K. P. Nevin, and D. R. Lovley. 2015. Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials. Bioresource Technology. 191:140-145.

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