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Publication Date

2020-03-01

DOI

10.1016/j.wasman.2020.02.007

Peer reviewed

Waste Management 105 (2020) 299–308

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Boosting landfill gas production from lignin-containing wastes via termite hindgut microorganism

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ARTICLE INFO

Article history:
Received 31 July 2019
Revised 30 December 2019
Accepted 9 February 2020

Keywords:
Landfill gas
Methane
Municipal solid waste
Greenhouse gases
Renewable energy
Termite microorganism
Lignocellulose

ABSTRACT

Lignocellulose comprises a significant portion of municipal solid waste (MSW) – 40–70% in developed countries, including paper, wood, and yard waste. Cellulose and hemicellulose are often shielded by lignin, posing a barrier to waste decomposition and landfill gas generation. Unfortunately, lignin is resistant to microbial degradation under low-oxygen conditions that normally occur in MSW landfills.

The bacterium strain TAV5, microaerophilic and member of phylum *Verrucomicrobia*, isolated from the hindgut of the *Reticulitermes flavipes* termite, the most widely distributed subterranean termite in North America. Its genome contains genes associated with methylotrophic competency which code for enzymes that structurally modify lignin. The overall goal of this research was to use TAV5 to modify lignin and boost methane production from MSW.

Batch-scale reactors (125 mL) were filled with paper, yard, or wood waste, and four ratios of mixed of waste. Reactors were seeded with different ratios of TAV5 to anaerobic digester (AD) microorganisms (representing landfill anaerobic microorganisms). Based on batch tests, optimal ratios of TAV5 to AD microorganisms were used to seed wastes (mixed, yard, and wood) in 6-gallon reactors.

Addition of TAV5 increased methane production from mixed waste, yard waste, and wood, by 49%, 34%, and 297%, respectively. TAV5 decreased acid soluble lignin by 7–39%, depending on waste type. TAV5 grown under aerobic conditions and room temperature (not requiring a heated anaerobic chamber) was found to remain viable and increase methane production under low-level oxygen conditions (1–2%). This finding will potentially lessen costs for growing large volumes of it for seeding landfills.

1. Introduction

Cellulose, hemicelluloses, and lignin are major components of plant biomass. Unfortunately, lignin cannot be degraded easily under anoxic conditions that are typical of municipal solid waste (MSW) landfills. Lignin often shields cellulose and hemicellulose, preventing their degradation as well. Without an effective method of decomposing lignin, at least 10–35% of lignocellulose will likely remain undegraded in landfills, taking up valuable landfill space and lowering the potential for producing renewable energy such as methane (CH_4) (De la Cruz, 2014).

Traditional physical/chemical methods for destroying lignin are costly due to energy and chemical requirements and can create toxic intermediates (Jang et al., 2013; Lim and Wang, 2013; Isroi et al., 2011; Mills et al., 2009; Ren et al., 2009; Sindhu et al., 2016; Yuan et al., 2014; Zuroff and Curtis, 2012). Biological treatments generally fall into 2 categories: use of microorganisms and use of enzymes (Zabed et al., 2019). Most microorganisms (fungi and bacteria) known to degrade lignin are aerobic (Seidl, 2009; Xu et al., 2018; Zabed et al., 2019); this limits their use in landfills, which have oxygen levels below the surface of 1–2% (Barlaz et al., 2016; Chun, 2014; Eklund et al., 1998). Use of a separate aerobic pre-treatment step for landfill waste would be costly.

One strategy for increasing lignin decomposition anaerobically is enzyme addition. Jayasinghe et al. (2011) evaluated lignin peroxidase, manganese peroxidase (MnP), and soybean peroxidase in various doses for increasing methane yield from mixed municipal solid waste. MnP was the most effective but costs $6500/gram (Infinite Enzymes. 2019).

An alternative to enzymes is to utilize the few microorganisms that are known to degrade lignin in low-oxygen environments. Among these, termite gut symbionts have been found to degrade lignin faster and more efficiently than microorganisms in ruminants and soil (Brune, 2014; Watanabe and Tokuda, 2010; Wong et al., 2014). The bacterium strain TAV5, of the family *Opitutaceae*,

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https://doi.org/10.1016/j.wasman.2020.02.007
0956-053X/

is a Termite-Associated Verrucomicrobium isolated from the hindgut of *Reticulitermes flavipes*, the most common subterranean termite in North America (Kotak et al., 2015; University of Florida, 2019). TAV5 can grow in low oxygen levels (1–2%), like those in landfills. The TAV5 genome contains genes coding for enzymes that can structurally modify lignin (Kotak et al., 2015). In addition, the genome sequencing of TAV5 revealed the presence of enzymes for formate metabolism, such as formate dehydrogenase (dehydrogenases are reported for cleavage of ether bond linkage to lignin); enzymes of the serine pathway, like serine hydroxy methyl transferase, involved in demethylation or rearrangement of the methyl group during lignin degradation; genes for the enzymes 3-carboxymuconate cyclase (EC 5.5.1.5) and 4-carboxymuconolactone decarboxylase (EC 4.1.1.44), which are involved in the degradation of protocatechuate that is derived from lignin; as well as genes coding for dioxygenases and dienelactone hydrolase (EC 3.1.1.45), known for ring cleavage of aromatic compounds. The TAV5 genome contains the *cbb*3-type cytochrome oxidase gene, implying the role of TAV5 in oxygen removal. The complete genome sequence of the *Opitutaceae* bacterium TAV5 was deposited in GeneBank under the accession numbers CP007053.1 (chromosome) and CP007054.1 (plasmid). These enzymes can potentially structurally modify lignin, improving the accessibility of polysaccharides to glycoside hydrolases (Kotak et al., 2015).

To date only one study has evaluated termite gut microorganisms for producing biogas from waste (6-month study of garden waste, which used crushed and ground termites of an unspecified species) (Gupta et al., 2012). The overall goal of our research was thus to increase methane generation from MSW via use of TAV5 cells (Rahimi, 2019). Our study had the following specific objectives:

(1) To determine the optimal addition of TAV5 (mass TAV5/-mass of waste) to enhance methane production, using batch reactor tests for paper, yard, and wood waste,
(2) To compare the effectiveness of TAV5 cultured at 2% O_2 versus 20% O_2 in breaking down lignin-containing waste and producing methane under low-oxygen conditions in batch reactors, and
(3) To test the effectiveness of the optimal addition of TAV5, determined in Objective 1, in decreasing lignin and generating methane in larger 6-gallon landfill reactors, representing landfill conditions.

Regarding Objective 2, in previous studies TAV5 has been grown only at 2% oxygen, which requires an anaerobic chamber. If TAV5 were able to be grown at 20% oxygen and room temperature, and remain viable at low-oxygen conditions and higher temperatures present in landfills, this would lessen the costs for growing large volumes of it for seeding landfills: it could be grown in open lab space rather than in heated anaerobic chambers.

Results of this study provide a basis for the next step in practical implementation of TAV5 solid waste treatment: pilot-scale field testing using small cells constructed adjacent to a landfill, with duplicate cells operated with and without seeding with TAV5.

2. Materials and methods

2.1. Overall experimental approach

Microorganisms typically found in landfills were represented by anaerobic digester (AD) microorganisms in the batch (125 mL) and 6-gallon reactors. AD microorganisms have been used in previous research to seed lab-reactor landfills, representing the mix of hydrolytic, acidogenic, acetogenic, and methanogenic microorganisms that break down organic wastes and generate methane in landfills (Karanjekar et al., 2015). The microorganisms in a digester are more concentrated and more easily used as seed than the microorganisms distributed among wastes in a landfill.

To accomplish Objective 1, batch scale reactors were prepared with TAV5 cells grown at 2% oxygen and 40 °C. Ratios of TAV5 to AD microorganisms (0–90%, in 10% increments) were tested using reactors filled with 3 types of separated waste (paper, yard, or wood). Additional batch scale reactors were tested using TAV5 to AD microorganism ratios of 0%, 10%, and 40% (10% and 40% gave the best performance for separated waste), for 4 different ratios of mixed waste (paper: wood: yard: 2:1:1, 1:1:1, 1:2:1, 1:1:2).

To accomplish Objective 2, batch scale reactors were prepared with TAV5 grown at 20% oxygen and room temperature. Ratios of TAV5 to anaerobic digester (AD) microorganisms (0–50%, in 10% increments) were tested using reactors filled with 3 types of separated waste (paper, yard, or wood). Additional batch scale reactors were tested using TAV5 to AD microorganism ratios of 0%, 10%, and 40%, for 4 different ratios of mixed waste (paper: wood: yard 2:1:1, 1:1:1, 1:2:1, 1:1:2).

To accomplish Objective 3, five 6-gallon laboratory reactors with yard, wood, or mixed waste were seeded with the optimum TAV5 to AD microorganism ratio, based on the batch scale tests (10% for paper and mixed waste, 40% for yard and wood waste; 40% was used for wood waste because it gave the highest methane production for the first 60 days of reactor operation, although 70% eventually surpassed it). TAV5 which had been grown at 2% oxygen and 40 °C was added to the reactors, because TAV5 was not grown at 20% oxygen and room temperature until later.

All reactors were sealed to enable development of low-level oxygen conditions necessary for generating methane; microorganisms utilized existing oxygen during the initial aerobic phase. Reactors were then incubated at 40 °C. The optimum temperature for methanogenesis is 30–40 °C, with maximum production at the upper end of the range (Hartz et al., 1982). In addition, actual temperatures in landfills often reach 40 °C. Hence, we wanted to test TAV5's ability to function at 40 °C.

2.2. TAV5 culturing and growth

The strain TAV5 was originally cultivated from the hindgut of wood-feeding termites using an integrative approach developed by Stevenson et al. (2004). Highlights of the cultivation procedure included the use of agar media with little or no added nutrients and relatively long periods of incubation (over 30 days). The isolation procedure relied on growth under low oxygen concentration (2%) and addition of catalase to the medium for protection of cells from exogenous peroxides.

All cell growth work for this project was carried out under sterile conditions by using a laminar air flow bench (LABCONCO) for inoculation of TAV5 cell on solid medium or in flasks containing liquid media. TAV5 was streaked into the Petri dishes containing R2A medium and after three weeks, TAV5 cells were inoculated into flasks containing R2Broth, which were placed on a rotary shaker with 200 rpm speed for 7 or 8 days. The optical density of cells was then measured using a Nano Drop 2000c Spectrophotometer. When the cells reached an optical density (OD_{600}) of 0.3 (approximately 8.7×10^7 cells per ml), 2-ml cultures were harvested by centrifugation in glycerol, and stored at −80 °C.

2.3. Waste collection

Solid waste components for the batch (125 mL) and 6-gallon reactors were paper, yard, and wood. The ratio of paper, yard, and wood (2:1:1) was chosen to represent the approximate

percent of waste that goes into landfills according to the US EPA (2014): 14.3, 7.9, and 8.1% for paper and card board, yard waste and wood, respectively. This represents an approximate ratio of (2:1:1) for paper, yard, and wood, respectively. Three other ratios (1:1:1). (1:2:1), (1:1:2) of paper, yard, and wood were tested for comparison.

Paper waste was obtained from UTA recycling bins (office paper, old newspaper, card board, glossy papers, newspapers, mail, magazines). Large pieces of waste were cut in order to fit into the reactors. Paper was not cut into finer pieces or shredded, because shredding can lead to faster degradation (Ress et al., 1998). Hence, the coarse structure of waste was maintained in the reactors as much as possible to try to replicate the actual conditions in landfills.

A mixture of grass, leaves, and tree/bush trimmings was obtained from UTA's Environmental Health and Safety Office to be representative of the variety particularly found in Texas. The species of trees found in North Texas are mostly Live Oak, Post Oak, Red Oak, American Elm, Pecan, Bald Cypress, and Crepe Myrtle. Wood waste was obtained from the wood chips located at Environmental Health and Safety Office at UTA.

2.4. Reactor assembly, filling and operation

2.4.1. Batch-scale reactors

Wheaton Serum bottles (125 mL) with rubber septa and aluminum crimp seals were used as a batch system. A total of ten bottles were filled with 5 g of mixed of paper (office paper, old newspaper, magazine, and card board) with equal ratios by weight, which was cut to 5 mm by 5 mm size; 10 bottles were filled with 5 g each of yard waste, and 10 were filled with wood waste.

AD seed was obtained from a continuously-stirred anaerobic sludge digester operated at a hydraulic residence time of 19 days at 20 °C from Village Creek Wastewater Treatment Plant and added to each serum bottle to achieve 15% by weight. Although methane production from the seed alone was not measured, the same mass of seed was added to batch reactors of each type (e.g. paper) and would thus have generated approximately the same amount of methane. The lowest cumulative methane generation from the reactors of each type thus represents an upper bound on methane generated by the AD seed (assuming no methane generation from the waste itself). These values were 0.64, 8.8×10^{-5}, and 5.9×10^{-3} L/kg, for paper, yard, and wood waste, respectively, and can be seen as the lowest curves on Fig. 1(a), (c), and (f). The values for yard and wood waste in particular are not significant compared to methane generation from the other reactors.

Following addition of the AD seed, TAV5 cells cultured at 2% oxygen and 40 °C (Objective 1) or 20% oxygen and room temperature (Objective 2) were added to achieve the desired ratio with AD microorganisms. Methane production from TAV5 microorganisms by themselves was not measured because they are not methanogens. Water was added to each bottle to achieve 45–55% moisture content. Finally, the bottles were sealed with an aluminum crimp with rubber septum and were incubated at 40 °C for 110–150 days, depending on when methane production ceased.

Similar batch reactors were prepared to determine the effectiveness of TAV5 on mixed waste. Two different ratios of TAV5, 10% and 40%, which were the best results from the batch tests for separated waste, were used, while the amount of AD microorganisms remained constant.

The volume of gas produced by the batch reactors was measured by equilibrating pressure using a 5 mL ground glass syringe, to ensure that all the excess pressure was removed. The gas composition was measured using a GC (SRI Instruments, Torrance, CA, Model 8610) equipped with FID detector. Gas content was measured weekly or as needed depending on gas production.

2.4.2. Six-gallon laboratory reactors

Experiments to better simulate waste decomposition in an actual landfill were conducted in 6-gallon HDPE wide-mouth plastic buckets (United States Plastic Corporation, OH) modified for gas and leachate collection and for water addition (Rahimi, 2015). As mentioned previously, TAV5 which had been grown at 2% oxygen and 40 °C was added to the reactors, because TAV5 was not grown at 20% oxygen and room temperature until later. Mixed waste reactor (paper: wood: yard ratios of 1:1:1), yard waste, and wood waste reactors were tested.

One liter of leachate from the collection bags was manually recirculated back to each reactor twice a week, with make-up water added as needed. Our previous work (Rijal, 2014) found that twice per week recirculation generated the most methane. The pH of the generated leachate was measured using a bench top Oakton pH meter. During the first 30 days, pH was measured every few days and potassium hydroxide base was added as needed to maintain the pH above 6, as methanogens cannot tolerate pH values below 6. After the first 30 days, pH was measured once per week.

Composition of the collected gases was measured using a Landtec GEM 5000, which measures methane (CH_4), carbon dioxide (CO_2), oxygen (O_2), and hydrogen sulfide (H_2S). In previous research, it was observed that methane concentrations measured using a Landtec were within ±7% of those measured with a gas chromatograph (Karanjekar, 2015). The volume of collected gas was measured using an air sampling pump (Universal XR Pump Model 44XR) and Defender 330. In the initial stages of gas production, gas composition and volume were measured daily or every few days. Once gas production stabilized, gas composition and volume were measured once per week. After the initial aerobic phase, oxygen concentrations averaged 1%, representative of the 1–2% low level oxygen conditions found in landfills.

2.5. Analytical methods

2.5.1. Acid soluble lignin

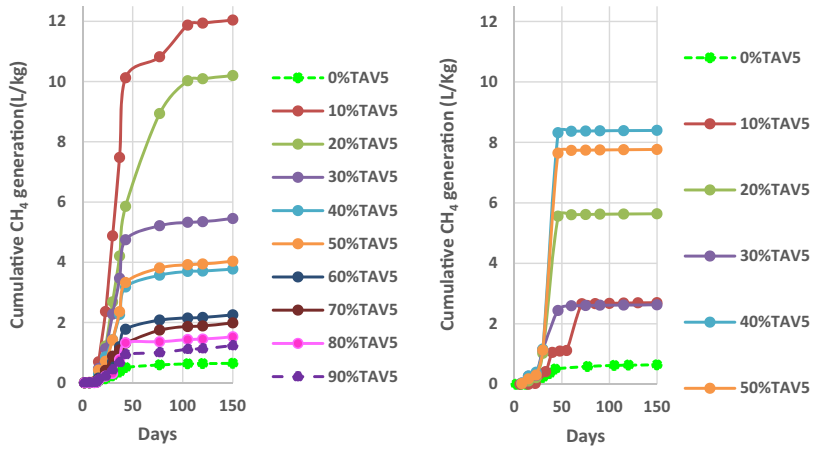
Acid soluble lignin and CHN analysis was done on non-degraded samples as well as samples obtained from batch scale tests and 6-gallon laboratory reactors at the end of reactor operations. The differences in these values were used to calculate the percent reductions in lignin shown in the results section. To analyze the solids for acid soluble lignin, a known weight (0.3 g) of ground sample was subjected to two-stage acid hydrolysis as follows. In the first stage, the sample was hydrolyzed in 3 mL of 72% sulfuric acid for 1 h at 30 °C. This was followed by a secondary hydrolysis after the addition of 83 mL of deionized water and a Fucose internal standard. The secondary hydrolysis was conducted in an autoclave (121 °C) for 1 h.

Acid soluble lignin was determined from UV absorbance at 215 nm of the filtered (0.45 μm) acid hydrolysate from the second stage digestion. For this purpose, a Shimadzu UV–VIS spectrophotometer (UV-2550, serial No. A108446) was used. Acid Soluble lignin was measured at 280 nm and 215 nm, and the lignin content was calculated by the following formula:

$$S = \frac{(4.53A215 - A280)}{300} \quad (1)$$

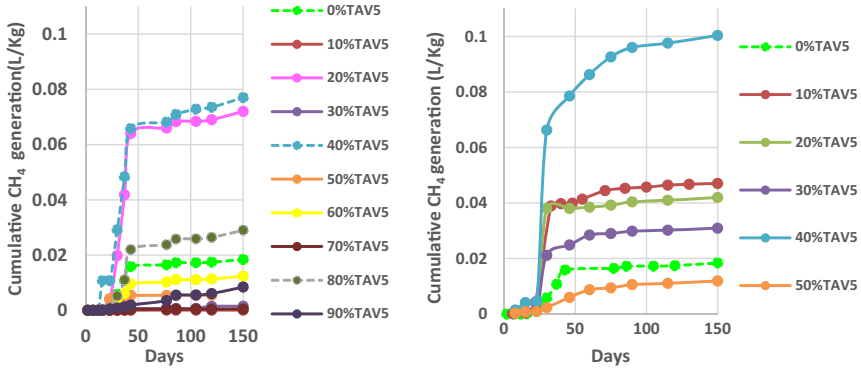
2.5.2. Total organic carbon

Total Organic Carbon was measured for samples at the beginning and end of batch-scale and 6-gallon reactor operation with a CHN analyzer (Perkin-Elmer PE 2400). All samples were acid



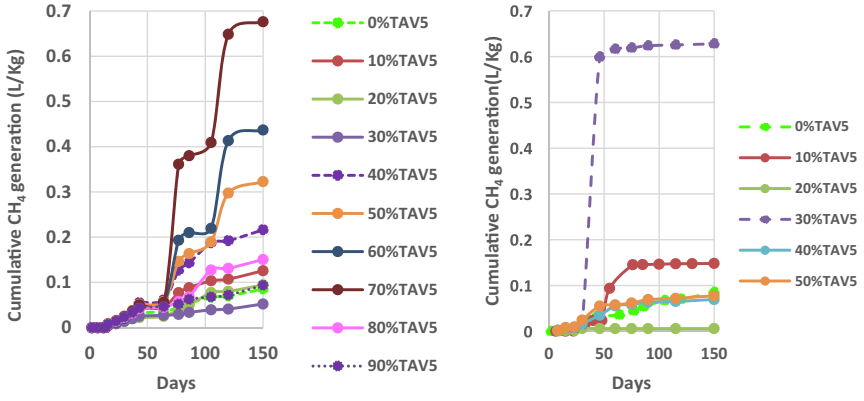
a) Paper, TAV5 grown under microaerophilic conditions

b) Paper, TAV5 grown under aerobic conditions



c) Yard waste, TAV5 grown under microaerophilic aerobic conditions

d) Yard waste, TAV5 grown under aerobic conditions



e) Wood waste, TAV5 grown under microaerophilic aerobic conditions

f) Wood waste, TAV5 grown conditions

Fig. 1. Cumulative methane generation for batch test reactors containing different percents of TAV5, grown under microaerophilic and aerobic conditions. 10% TAV5 indicates that the ratio of weights of TAV5 to anaerobic digester microorganisms was 0.1. Values reported per kg dry solids.

washed (1 M HCL) to eliminate inorganic carbon prior to analysis (Ryba et al., 2002).

2.5.3. Genetic identification of bacterial and fungal isolates

After TAV5 was grown at different conditions (2% O_2/40 °C and 20% O_2/room temperature), the strain was re-isolated and identified to ensure that it was still the same strain. In addition, bacterial composition analysis via 16S rRNA Next Generation Sequencing (Illumina MiSeq platform) was conducted on the TAV5 and AD seed at the beginning of reactor operation, as well as on solid residual samples extracted from batch and 6-gallon reactors, and leachate from 6-gallon reactors, at the end of operation. TAV5 was

re-isolated and identified at the end of reactor operation, to confirm that it still existed.

To confirm that TAV5 cultured at different conditions were from the original strain, bacterial colonies were isolated from liquid media and plated on agar. Bacterial identification of individual colonies was performed via Sanger sequencing of a 526 bp region of the 16S rRNA gene segment containing variable regions 1–3, as defined by primer 8F (5′AGAGTTTGATCCTGGCTCAC) (Gray, 2005), and 534R (5′WTTACCGCGGCTGCTGG) (Lee, 2010). Bacterial composition analysis via 16s rRNA Next Generation Sequencing was done to the solid residuals on samples extracted from batch and 6-gallon reactors, and also leachate of 6-gallon reactors to make sure TAV5 still exist.

For re-isolation of TAV5 after culturing under different conditions, and at the end of reactor operation, a pipette tip was gently touched to the bacterial colony and any visible material was wiped away and discarded. The pipette tip, containing a small number of bacterial cells, was used to directly inoculate the polymerase chain reaction (PCR mix). Each 50 μl reaction mix contained 0.2 μM of each primer, 1x colorless GoTaq reaction buffer, 2 mM $MgCl_2$, 0.2 mM of each deoxynucleoside phosphate and 1.25 U of GoTaq Flexi polymerase (Promega). PCR amplification was performed using a Veriti thermocycler (Applied Biosystems) and consisted of one cycle of 95 °C for 3 min to lyse the cells, followed by thirty cycles of 95 °C for 45 s, 55 °C for 30 s, 72 °C for 45 s with a final elongation at 72 °C for 5 min. Five μl of the PCR product was electrophoresed on a 0.8% agarose gel using 1x Tris Acetate EDTA buffer (VWR), and stained with ethidium bromide 1 μl of 10 mg/ml per 50 mL of agarose gel matrix.

For DNA extraction from solid samples, DI water was added to the samples in a 50 mL tube; then samples were stored at −20 °C until DNA extraction. Thawed samples were homogenized and taken for DNA extraction. Five zinc-plated steel BB pellets were added to thawed sample and shaken for 1 min to homogenize. 250 μl of homogenized sample supernatant was harvested and DNA extraction performed, with quantitation done via spectrophotometry. Qiagen Vacuum Manifold was used to process DNA samples. Samples with low DNA yield were re-extracted by scaling up the input to a 2 mL sample, with all other reagents adjusted proportionally. Final homogenate was captured on a single silica column and eluted with 50 μl of solution from QIAamp Power Fecal DNA Kit (QIAGEN, Germantown, MD).

For the 40% TAV5 wood waste reactor, which grew fungi, fungal hyphae were harvested from cultures using an inoculating loop and stored in 500 μl of 100% ETOH. Samples were identified via Sanger sequencing of the ITS region of ribosomal DNA gene segment, as defined by primer ITS1f (5′ CTTGGTCATTTAGAGGAAGTAA) (Earth Microbiome Project, 2019) and ITS2 (5′GCTGCGTTCTTCATCGATGC) (White et al., 1990).

100 μl aliquots of the fungal samples were spun down, ethanol was discarded, and the sample was air dried. Hyphae were suspended in 180 μl of buffer ATL with 20 μl of Proteinase K solution using the QiAmp DNA mini extraction kit (Qiagen), as per manufacturer's instructions. DNA was eluted in 100 μl of buffer AE (10 mM Tris pH 8) and a 5 μl aliquot was amplified via PCR in a 25 μl reaction mix containing 0.4 μM of each primer, 1x colorless GoTaq reaction buffer, 3 mM $MgCl_2$, 0.8 mM of each deoxynucleoside phosphate and 2.5 U of GoTaq Flexi polymerase (Promega). PCR amplification was performed using a Veriti thermocycler (Applied Biosystems) and consisted of one cycle of 94 °C for 2 min, followed by thirty cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s, with a final elongation at 72 °C for 5 min. Five μl of the PCR product was electrophoresed on a 0.8% agarose gel using 1x Tris Acetate EDTA buffer (VWR), and stained with ethidium bromide 1 μl of 10 mg/ml per 50 mL of agarose gel matrix.

2.5.4. Sequence analysis

PCR amplicons were sequenced using the dideoxy chain-termination method (Sanger et al., 1977) and Big Dye v3.1 chemistry (Thermo Scientific). The reaction products were visualized using the model 3130xL capillary electrophoresis system (Applied Biosystems). The PCR amplicons were sequenced at least one time for each strand using the appropriate PCR primer. Sequences were aligned, and high-quality consensus sequence data was selected and compared to the NCBI BLAST database for bacterial or fungal isolate identification. Matches were considered significant if the identify score was greater than 95%.

3. Results and discussion

3.1. Batch-scale reactor results: Optimal addition of TAV5

The question of optimal amount of TAV5 arises because traditional landfill microorganisms would also be present with TAV5, including methanogens. Although increasing TAV5 might facilitate faster lignin decomposition, it could prove counterproductive in terms of methane generation. At larger percentages of TAV5, we hypothesize that TAV5 may outcompete methanogens for food, meaning that the TAV5 would consume the carbon, releasing it as CO_2 without generating methane. Hence, it was hypothesized that an optimal addition of TAV5 will exist, in order to maximize methane generation.

Reactors seeded with TAV5 and AD microorganisms achieved higher cumulative methane compared to reactors seeded with AD microorganisms alone (0% TAV5), for each of the three kinds of separated waste (paper, yard, and wood) (Fig. 1). For paper and TAV5 grown under microaerophilic conditions (2% oxygen, Fig. 1(a)), 10% TAV5 shows the highest cumulative methane, followed by 20% and 30% on down to 90%, in a regularly decreasing trend. 10% TAV5 appears to represent the optimum balance between the rate of lignin breakdown and methane generation; with a higher percentage of TAV5, more lignin was broken down, but fewer microorganisms proportionally were available to generate methane. Since the total percentage of lignin in mixed paper waste is relatively low (around 15.9% (Komilis, 2003)), it may have been possible to achieve good lignin break down with a lower percent of TAV5. For paper and TAV5 grown under aerobic conditions (21% oxygen, Fig. 1(b)), the batch reactor seeded with 40% TAV5 had the highest cumulative methane generation. If TAV5 cultured at 20% oxygen is not as effective at breaking down lignin, perhaps a greater ratio was needed compared to the TAV5 grown at 2% oxygen.

For yard waste, 40% TAV5 gave the highest cumulative methane, for the TAV5 grown under both microaerophilic and aerobic conditions (Fig. 1(c) and (d)). It is likely that 40% TAV5 represented the optimum balance between microorganisms that structurally modify lignin and those that produce methane for yard waste. According to Komilis and Ham (2003), the lignin percentage in yard waste is around 43.8%, compared to 15.9% for paper. The optimal ratio of TAV5 grown under microaerophilic conditions for yard waste (40%) may have been greater than that for paper waste (10%) for TAV5, due to the higher lignin content in yard waste. For yard waste, however, there was not a consistent trend in the methane generated by TAV5 percents other than 40%, like there was for paper and TAV5 grown under microaerophilic conditions. This might have been due to different types of yard waste being inside different bottles, such as different type of leaves. Waste size also may have differed, resulting in different surface areas for microorganisms to access.

For wood waste for TAV5 grown under microaerophilic conditions (Fig. 1(e)), 70% TAV5 gave the maximum cumulative methane

production. The trend was consistent, with 60% TAV5 giving the second highest methane generation, 50% giving third, and 40% giving fourth. According to Wang et al. (2016), hard woods have a lignin content of 27.6–30.2%, and soft woods have a lignin content of 32.4–42.4%. These ranges are slightly lower than the lignin content for yard waste (43.8%), yet a greater proportion of TAV5 microorganisms was required for optimal methane production for microaerophilically-grown TAV5. The higher proportion of TAV5 being required may have been due to the kind of lignin in wood being particularly difficult to structurally modify, compared to the type of lignin in yard waste. However, for the batch scale reactors seeded with TAV5 grown under aerobic conditions (Fig. 1(f)), 30% TAV5 has the highest cumulative methane generation. The lower percent compared to the microaerophilically-grown TAV5 might be due to a different type of wood with lignin that was easier to structurally modify, or the size of the wood in compared to other wood size in other batch reactors. Particle size reduction may increase the bioavailability of carbon compound for wood (Krause et al., 2018). For wood waste reactors with 20% aerobically-grown TAV5, fungi growth was observed after day 45. ITS-PCR analysis and microscopic identification were done on these fungi samples, and methanotroph microorganisms were identified. The methanotrophs likely consumed methane, which can explain the low amount of methane generation for this reactor.

The microaerophilically-grown TAV5 generated greater methane for paper waste and wood waste, but the aerobically-grown TAV5 generated greater methane for yard waste (Table 1). Overall, paper generated the highest amount of methane per kg, with wood waste second, and yard waste third. Paper has the highest methane generation, likely because it has the largest amount of cellulose and lowest amount of lignin and has larger surface area per unit volume of waste for the microbes to access, compared to yard waste and particularly wood waste. Similarly, Jeon et al. (2007) found that paper waste generated more methane than wood waste. Eleazer et al. (1997) found methane production from office paper and corrugated containers to exceed that for grass, leaves and branches; methane production from grass, however, was found to be greater than that from coated paper and old newsprint.

Wood waste produced the second highest methane generation in our batch tests. Eleazer et al. (1997) found biochemical methane potential (BMP) of branches (similar to wood) to be higher than that of leaves, although lower than that of grass. A high percent of leaves in the yard waste could explain the lower yield of the yard waste compared to wood found in our study. In addition, methane generation for hardwoods is higher than that of softwoods due to the different lignin structure, and methoxy groups in softwood and yard waste. If the wood chips were oak or elm, two common kinds of hardwoods in North Central Texas, this could also explain the higher methane yield compared to yard waste.

Yard waste had the lowest methane generation in the batch tests, likely because of the higher lignin percentage in yard waste compared to wood and paper. The specific of plant for leaves and grass, as well as the relative amounts of leaves vs. grass, can make a difference in methane generation from yard waste as well. The lower methane yields overall compared to other studies may have been due the fact that other studies often report BMP values, which represent optimal conditions in terms of nutrients, waste particle size, and moisture. In addition, BMP studies typically report methane volume per mass of volatile solids. Our values are per mass of dry solids. Since dry solids include both volatile and non-volatile solids the cumulative methane values would be lower per dry solids.

For microaerophilically-grown TAV5, 10% TAV5 produced more methane for mixed waste (2 paper: 1 yard: 1 wood) and (1 paper: 1 yard: 2 wood) compared to 0% TAV5 and 40% TAV5 (Table 1). This makes sense, given that 10% microaerophilically-grown TAV5 produced the most methane for batch tests containing only paper. Even for the reactors containing (1 paper: 1 yard: 2 wood), total methane production from paper would be expected to be greater than that from wood: CH_4 production from paper is an order of magnitude greater for paper than wood, and two orders of magnitude greater than yard, on a per weight basis (Fig. 1). For mixed (1 paper: 1 yard: 1 wood) and mixed (1 paper: 2 yard: 1 wood), 40% microaerophilically-grown TAV5 produced the most CH_4, which is surprising, given that CH_4 production from paper with 10% TAV5 was the greatest for the separate waste reactors.

For the aerobically-grown TAV5, 10% produced the most methane for mixed waste, which contrasts with 40% and 30% being the optimum ratios for aerobically-grown TAV5 for separated waste. However, for the mixed waste reactors with 40% aerobically-grown TAV5, methanotroph fungi growth was observed. For all 4 combinations of mixed waste in Table 1, the microaerophilically-grown TAV5 produced greater methane than the aerobically-grown TAV5; however, the methane generation in the reactors with aerobically-grown TAV5 was reduced by the fungi growth.

In summary, addition of TAV5 increased methane production from paper, yard, wood, and mixed waste. The batch reactor tests demonstrated that TAV5 can be grown under aerobic conditions

Table 1
Comparison of TAV5 percent generating the greatest cumulative methane, for various waste substrates and TAV5 grown at 2% O_2/40 °C and TAV5 grown at 20% O_2/room temperature.

Waste	TAV5 % @2% O_2	Cumulative CH_4 generation, L/kg dry solids	% greater CH_4 generation by TAV5 grown at 2% O_2 compared to TAV5 grown at 20% O_2
Paper	10%*	12.0	43%
Yard	40%	0.0772	–23%
Wood	70%	0.677	7%
Mixed 2:1:1	10%	1.92	26%
Mixed 1:1:1	40%	1.85	255%
Mixed 1:2:1	40%	1.79	92%
Mixed 1:1:2	10%	1.85	10%
Waste	TAV5 % @20% O_2	Cumulative CH_4 generation, L/kg dry solids	
Paper	40%	8.42	
Yard	40%	0.101	
Wood	30%	0.631	
Mixed 2:1:1	10%	1.53	
Mixed 1:1:1	10%	0.520	
Mixed 1:2:1	10%	0.933	
Mixed 1:1:2	10%	1.68	

*10% TAV5 indicates that the ratio of weights of TAV5 to anaerobic digester microorganisms was 0.1.

at room temperature (which would not require a heated anaerobic chamber), and then successfully generate methane from lignin-containing waste under conditions typical of a landfill (conditions of 1–2% oxygen at 40 °C). Although microaerophilically-grown TAV5 generated greater CH_4 for paper waste and wood waste, aerobically-grown TAV5 generated greater CH_4 for yard waste.

3.2. 6-Gallon reactor results

For the 6-gallon reactors, methane varied between 40 and 60% and carbon dioxide varied between 25 and 50% (Rahimi, 2019). Reactors seeded with TAV5 and AD microorganisms produced 34%, 277%, and 49% more methane from yard, wood, and mixed waste (yard, wood, paper), respectively, compared to reactors with AD microorganisms alone (Fig. 2). Mixed waste, due to paper content, generated more methane than the wood and yard waste reactors. Similar to the batch tests, methane production from the 6-gallon wood reactors was higher than that for yard waste, with the exception of the wood waste reactor seeded with 40% TAV5, which experienced fungal growth, as discussed below.

Rashid et al. (2017) conducted a study to determine whether bacterial isolates from MSW soil would enhance gas production. The isolates increased gas production from soil with 1% pine powder and compost supplemented with 10% softwood chips by a factor of 5–10 and a factor of 3, respectively. Soil with pine powder differs considerably from solid waste. The factor of 3 for wood chips, which are more representative of solid waste, is comparable to the 297% (almost a factor of 3) increase that we observed for wood waste. However, landfill soil microorganisms are already present in landfills, so adding them to actual landfilled solid waste would not be expected to enhance gas production.

We compared our cumulative methane generation values with those from the literature (Table 2). The literature values are from a review paper on methane generation potential from municipal solid waste with 258 references (Krause et al., 2016). The ranges of values reported in the literature are quite broad, because CH_4 produced depends on the specific kind of waste (e.g. St. Augustine grass vs. Bermuda grass), as well as the conditions of the test (e.g. moisture content, waste particle size, temperature). Cumulative methane generation values for our yard waste 6-gallon reactors (13.2 and 17.7 L/kg without and with TAV5, respectively) fall within the range of literature values for mixed yard waste (5–143 L/kg). Similarly, cumulative methane generation values for the wood waste 6-gallon reactors (6.4 and 25.4 L/kg without and with TAV5, respectively) fall within the range of literature values for wood (0.5–193 L/kg). Finally, cumulative methane generation values for the mixed waste 6-gallon reactors (37.9 and 56.6 L/kg without and with TAV5, respectively) fall within the range of literature values for paper, yard waste, and wood (0.5–342 L/kg). Cumulative methane generation values measured in this study were not at the high ends of the ranges, likely because many of the literature values are from BMP studies, which use optimal conditions, as discussed above.

Table 3 shows the results of CHN percent of samples from 6-gallon reactors. The samples with 0% TAV5 and 10% TAV5 were from the mixed waste reactor (1:1:1); samples with 40% TAV5 were from separated waste reactors. For paper waste, addition of 10% TAV5 reduced the organic carbon present in the sample by 50% compared to AD microorganisms only (0% TAV5). For yard

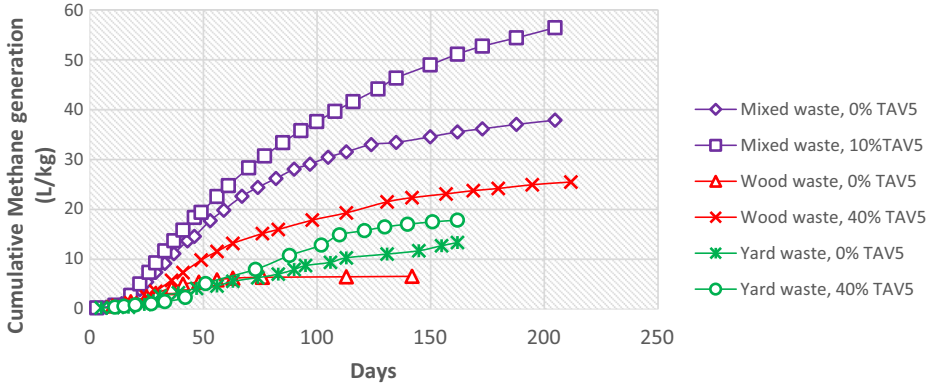


Fig. 2. Cumulative methane generation for 6-gallon reactors containing mixed, yard, and wood waste, with and without TAV5 (values reported in L/kg dry solids).

Table 2
Comparison of methane generation from wastes in 6-gallon reactors, with and without TAV5, with literature values.

Waste general category	Waste sub-category	Cumulative methane generation (L/kg)		
		From literature*	This study**	
			Without TAV5	With TAV5
Paper	Newspaper	40–110		
	Cardboard	152–272		
	Office paper	217–342		
	Magazines	43–165		
Yard Waste	Branches	63		
	Grass	128–334		
	Leaves	31		
	Mixed yard waste	5–143	13.2	17.7
Wood		0.5–193	6.4	25.4
1:1:1 mixture of paper, yard, and wood			37.9	56.6

* Krause et al., 2016.
** Values for this study reported in L/kg dry solids.

Table 3
Carbon-hydrogen-nitrogen composition for wastes with and without TAV5 addition.

Waste	Microorganisms	%C	%C differences	%H	%N
Paper	0% TAV5, AD only	14.1		1.38	0.07
	10% TAV5	7.0	−50%	0.31	0.55
Yard	None	46.1		4.96	1.44
	0% TAV5, AD only	21.3	−54%	2.08	0.48
	10% TAV5	19.2	−58%	1.96	0.38
	40% TAV5	19.1	−59%	1.82	0.36
Wood	None	42.9		5.09	0.66
	0% TAV5, AD only	36.2	−16%	5.46	0.12
	10% TAV5	19.2	−55%	4.63	0.33
	40% TAV5	26.7	−38%	6.12	0.18

Table 4
Acid soluble lignin for wastes with and without TAV5 addition.

Waste	Microorganisms	Acid Soluble Lignin (mg/g)	Acid soluble differences%
Paper	None	1.49	
	0% TAV5, AD only	1.16	−22%
	10% TAV5	0.58	−61%
Yard	None	3.13	
	0% TAV5, AD only	2.74	−12%
	10% TAV5	1.82	−42%
	40% TAV5	1.77	−43%
Wood	None	1.64	
	0% TAV5, AD only	1.60	−2%
	10% TAV5	1.50	−9%
	40% TAV5	1.12	−32%

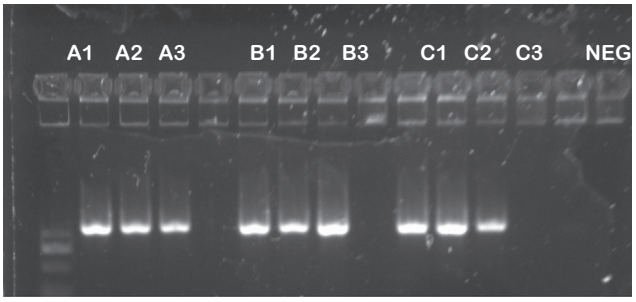


Fig. 3. 16 s rRNA gene PCR results for original TAV5 (A1–A3), TAV5 cultured at 2% O_2 and 40 °C (B1–B3), TAV5 cultured at room temperature and 20% O_2 (C1–C3) and control (NEG).

waste, AD microorganisms alone decreased organic carbon by 54% compared to the initial sample (no microorganisms); addition of 10% and 40% TAV5 reduced it by only 4% and 5% more, respectively. For wood waste, AD microorganisms alone (0% TAV5) lowered organic carbon by 16% compared to the initial sample (no microorganisms); addition of 10% and 40% TAV5 decreased it by 39% and 22% more, respectively. Hence, TAV5 was most effective in facilitating carbon break-down for paper and wood waste.

By comparison, De la Cruz et al. (2014) found that typical anaerobic microorganisms decreased carbon content of soft wood by 16% under mesophilic conditions, which is the same as the percent reduction measured in this study. Wang and Barlaz (2016) found carbon conversions of 17.1–28.5% for hard woods, and 0–9.5% for soft woods.

Table 4 shows the changes in acid soluble lignin (ASL) from undegraded waste for wastes seeded with AD microorganisms alone and various mixes of AD and TAV5 microorganisms in 6-gallon reactors. Percent differences in ASL were calculated based on initial samples (no microorganisms). For paper waste, AD microorganisms alone reduced ASL by 22% compared to the initial sample (no microorganisms); addition of 10% TAV5 decreased ASL by an added 39%. For yard waste, AD microorganisms alone lowered ASL by 12% compared to the initial sample (no microorganisms); addition of 10% and 40% TAV5 reduced ASL by an additional 30% and 31%, respectively. Thus, for yard waste, 40% TAV5 did not decrease lignin substantially more than 10% TAV5, which is surprising in view of the fact that 40% TAV5 generated the greatest methane in the batch tests. For wood waste, AD microorganisms alone reduced ASL by 2% compared to the initial sample (no microorganisms); addition of 10% and 40% TAV5 lowered it by an additional 7% and 30%, respectively. Hence, TAV5 was most effective in lowering ASL for paper waste (additional 39%), followed by yard waste (additional 30–31%) and then wood waste (additional 7–30%). Differences in lignin type for different waste types account for differences in percent change; wastes with 2 methoxy groups tend to be easier to structurally modify (Caulfield et al., 1990). The percent reductions in lignin are specific to the yard and paper mixes tested and would vary for yard waste of different kinds of leaves and grasses, and different mixes of paper.

3.3. Microorganism analysis results – TAV5

Prior to this study, TAV5 had been cultured only at room temperature and 2% O_2. After culturing TAV5 at 2% O_2/40 °C and 20% O_2/room temperature in this study, microorganism identification was performed by 16 s rRNA PCR amplification, in order to confirm that the microorganisms cultured at the new conditions were the same strain as the original organism. Results in Fig. 3 show the microorganisms cultured at the new conditions do indeed both belong to the original *Opitutaceae* bacterium TAV5 strain.

At the end of 6-gallon reactor operation (1–2% oxygen levels), microorganisms were regrown on Agar and identification via 16 s rRNA PCR amplification were performed. The results confirmed that TAV5 (which were cultured at 20% O_2) were still alive after more than 40–100 days in conditions of 1–2% oxygen. This demonstrated that TAV5 microorganisms cultured at 20% O_2 could be introduced into low-level oxygen conditions and survive and increase methane generation. This is important because it will lessen the cost for growing large volumes of TAV5 for seeding landfills, because it could be grown in open lab space rather than in anaerobic chambers.

3.4. Microorganism analysis results – Other

Samples were collected from 7 of the batch reactors (due to cost constraints, analysis could not be run for all reactors) at the end of operation. For each of the 6 batch reactor sets shown in Fig. 1(a)–(f), the reactor which generated the most methane was chosen for microorganism identification, along with the mixed waste batch reactor that generated the most methane (7th sample). For the 6-gallon reactors, samples were collected at the end of operation from the wood waste and mixed waste reactors that generated the most methane. For each sample, the percent of microorganism

Table 5
Relative frequency of microorganism phyla in selected samples from batch and 6-gallon reactors at end of operation.

Sample				Cumulative Methane, L/kg		Microorganism Relative Frequency, %				
Waste	TAV5 wt %, initial	% O_2	Reactor Size		TAV5	Acidobacteria	Archaea (methanogenic)	Bacteroidetes (hydrolytic)	Firmicutes (hydrolytic, acidogenic, acetogenic)	Proteobacteria (hydrolytic, acidogenic)
Sludge inoculum					0	0	0	25	22	49
Yard	40%	20%	Batch	0.101	0.05	0.12	1.3	2.1	68	15
Yard	40%	2%	Batch	0.077	0.06	0.00	3.7	12.9	48	14
Paper	10%	2%	Batch	12.0	3.30	0.71	1.7	4.5	18	40
Paper	40%	20%	Batch	8.4	0.05	0.01	1.0	8.5	51	8
Wood	30%	20%	Batch	0.63	0.11	0.32	0.2	4.6	5	55
Wood	70%	2%	Batch	0.68	0.03	0.02	1.4	0.3	94	3
Mixed 2:1:1	10%	2%	Batch	1.9	0.07	0.02	4.6	19.1	31	18
Wood	40%	1%	6-gal	25.4	3.22	0.55	1.4	4.1	5	45
Mixed 1:1:1 – wood	10%	1%	6-gal	56.6	0.94	1.21	1.0	10.9	5	60
Mixed 1:1:1 – yard	10%	1%	6-gal	56.6	2.49	1.12	0.6	12.3	2	60
Mixed 1:1:1 – paper	10%	1%	6-gal	56.6	0.07	0.00	2.9	13.9	44	2

in each phylum was identified via 16 s rRNA PCR amplification. Table 5 summarizes results (phyla with the greatest percents of those involved in anaerobic degradation), along with cumulative methane generation. Analysis of sludge from a Village Creek Wastewater Treatment Plant anaerobic digester (source of seed) is also presented for comparison (Sabnis, 2014). Percents for each sample do not add to one hundred due to presence of other minor phyla not shown.

As shown in Table 5, phyla of microorganisms that have been associated with the various phases of anaerobic degradation (hydrolysis, acidogenesis, acetogenesis, methanogenesis) were identified (Joubert and Britz, 1987; Metcalf & Eddy, 2004; Deublein and Steinhauser, 2008). Compared to the sludge inoculum, at the end of reactor operation, *Archaea* increased for all the reactors, and *Bacteroidetes* decreased. *Firmicutes* and *Proteobacteria,* increased in some cases and decreased in others.

Of the batch reactors, the paper reactor with 10% TAV5 grown at 2% O_2 has the highest methane generation (12 L/kg), as well as the highest TAV5% at end of reactor operation (3.3%). However, other trends relating methane generated to microbial composition are not observable: reactors with the highest methane generation do not consistently have high percents of microorganisms from any particular phyla.

4. Conclusions and recommendations

Addition of TAV5 increased methane production from paper, yard, and wood waste, as well as reduced the lignin percent. At optimal ratios of TAV5, 49%, 34%, and 297% more methane was generated from mixed waste (paper, yard and wood), yard waste, and wood waste, respectively, compared to reactors seeded with anaerobic digester microorganisms alone. TAV5 was most effective in facilitating acid-soluble lignin break-down for paper waste (additional 39%), followed by yard waste (additional 30–31%) and then wood waste (additional 7–30%).

This research also demonstrated that TAV5 can be grown under aerobic conditions at room temperature (which would not require a heated anaerobic chamber), and then successfully decompose lignin under conditions typical of a landfill (1–2% oxygen and 20 and 40 °C, both within the range of temperatures found in landfills). Although microaerophilically-grown TAV5 generated greater methane for paper waste and wood waste, aerobically-grown TAV5 generated greater methane for yard waste.

For TAV5 cultured under microaerophilic conditions, the optimum ratios of TAV5 to AD microorganisms were 10%, 40%, and 70% for paper, yard, and wood waste, respectively, and 10% or 40% for mixed wastes. For TAV5 cultured under aerobic conditions, the optimum ratios of TAV5 to AD microorganisms were 40%, 40%, and 30% for paper, yard, and wood, respectively, and 10% for all mixed wastes. The ratios do not vary predictably with % lignin content of the waste.

Future research should include testing the ability of TAV5 to increase methane production from yard, wood, and paper waste using a small-scale system under field conditions. It is recommended that TAV5 for this testing be grown under aerobic conditions at room temperature, as was demonstrated in this study, which would be more cost-effective than growing it in a heated anaerobic chamber. Field testing could be conducted in small landfill test cells constructed adjacent to an existing landfill, to take advantage of on-site waste and equipment. Pairs of cells would be filled with the same waste (yard, paper, wood, and mixed), one seeded with TAV5 and the other not.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank Dr. Syed Qasim Endowed Professorship for providing support for laboratory supplies and analyses for this project; Dr. Victoria Chen, Professor of Industrial, Manufacturing, and Systems Engineering at UTA, for her guidance; Dr. Kimberly Bowles, Director of the Life Sciences Core Facility at UTA, for her assistance; and Paul Shover, former Civil Engineering Department Lab Technician, for his invaluable help.

References

Barlaz, M., Ducoste, J., Benson, C.; Castaldi, M.; Luettich, S. 2016. Understanding and predicting temperatures in municipal solid waste landfills. In: Proceedings of the Global Waste Management Symposium, Indian Wells, California, January 31–February 3, 2016.

Brune, A., 2014. Symbiotic digestion of lignocellulose in termite guts. Nature 12, 168–180.

Caulfield, D.F., Passaretti, J.D., Sobczynski, S.F. (Eds.), 1990. Materials interactions relevant to the pulp, paper, and wood industries. In: Proceedings, Materials Research Society Symposium 197, 11–20.

Chun, S.K., 2014. The influence of air inflow on CH_4 composition ratio in landfill gas. J. Mater. Cycl. Waste Manage. 16, 172–177.

De la Cruz, F.B., 2014. Chemical changes during anaerobic decomposition of hardwood, softwood, and old newsprint under mesophilic and thermophilic conditions. J. Agric. Food Chem. 62, 6362–6374.
Deublein, D., Steinhauser, A., 2008. Biogas from Waste and Renewable Resources. Wiley-VCH, Weinheim.
Earth Microbiome Project, 2019. ITS Illumina Aplicon Protocol. http://www.earthmicrobiome.org/protocols-andstandards/its/ (Accessed 6/19).
Eklund, B., Anderson, E.P., Walker, B.L., Burrows, D., 1998. Characterization of landfill gas composition at the fresh kills municipal solid-waste landfill. Environ. Sci. Technol. 32, 2233–2237.
Eleazer, W.E., Odle III, W.S., Wang, Y.S., Barlaz, M.A., 1997. Biodegradability of municipal solid waste components in laboratory-scale landfills. Environ. Sci. Technol. 31 (3), 911–917.
Gray, L.D., Ketring, K.L., Tang, Y.W., 2005. Clinical use of 16S rRNA gene sequencing to identify *Mycoplasma felis* and *M. gateae* associated with feline ulcerative keratitis. J. Clin. Microbiol. 43 (7), 3431–3434.
Gupta, P., Singh, R.S., Sachan, A., Vidyarthi, A.S., Gupta, A., 2012. Study on biogas production by anaerobic digestion of garden-waste. Fuel 95, 495–498.
Hartz, K.E., Klink, R.E., Ham, R.K., 1982. Temperature effects: methane generation from landfill samples. J. Environ. Eng. Div. 108 (4), 629–638.
Infinite Enzymes. 2019. https://infiniteenzymes.com/.
Isroi, Millati, R., Syamsiah, S., Niklasson, C., Cahyanto, M.N., Ludquist, K., Taherzadeh, M.J., 2011. Biological pretreatment of lignocelluloses with white-rot fungi and its applications: a review. Bioresources 6, 5224–5259.
Jang, H.M., Park, S.K., Ha, J.H., Park, J.M., 2013. Microbial community structure in a thermophilic aerobic digester used as a sludge pretreatment process for the mesophilic anaerobic digestion and the enhancement of methane production. Bioresour. Technol. 145, 80–89.
Jayasinghe, P.A., Hettiaratchi, J.P.A., Mehrotra, A.K., Kumar, S., 2011. Effect of enzyme additions on methane production and lignin degradation of landfilled sample of municipal solid waste. Bioresour. Technol. 102, 4633–4637.
Jeon, E., Bae, S., Lee, D., Seo, D., Chun, S., Lee, N., Kim, J., 2007. Methane generation potential and biodegradability of MSW components. Sardinia 2007 Eleventh International Waste Management and Landfill Symposium.
Joubert, W.A., Britz, T.J., 1987. Characterization of aerobic, facultative anaerobic, and anaerobic bacteria in an acidogenic phase reactor and their metabolite formation. Microbial. Ecol. 13 (2), 159–168.
Karanjekar, R.V., Bhatt, A., Altouqi, S., Jangikhatoonabad, N., Durai, V., Sattler, M.L., Hossain, M.D.S., Chen, V.C.P., 2015. Estimating methane emissions from landfills based on rainfall, ambient temperature, and waste composition: the CLEEN model. Waste Manage. 46, 389–398. https://doi.org/10.1016/j.wasman.2015.07.030.
Komilis, D.P., Ham, R.K., 2003. The effect of lignin and sugars to the aerobic decomposition of solid wastes. Waste Manage. 23 (5), 419–423.
Kotak, M., Isanapong, J., Goodwin, L., Bruce, D., Chen, A., Han, C.S., Huntemann, M., Ivanova, N., Land, M.L., Nolan, M., Pati, A., Woyke, T., Rodrigues, J.L.M., 2015. Complete genome sequence of the opitutaceae bacterium strain TAV5, a potential facultative methylotroph of the wood-feeding termite reticulitermes flavipes. Genome Announcements 3, 1.
Krause, M.J., Chickering, G.W., Townsend, T.G., Reinhart, D.R., 2016. Critical review of the methane generation potential of municipal solid waste. Crit. Rev. Environ. Sci. Technol. 46 (13), 1117–1182.
Krause, M.J., Chickering, G.W., Townsend, T.G., Pullammanappallil, P., 2018. Effects of temperature and particle size on the biochemical methane potential of municipal solid waste components. Waste Manage. 71, 25–30.
Lee, T.K., Van Doan, T., Yoo, K., Choi, S., Kim, C., Park, J., 2010. Discovery of commonly existing anode biofilm microbes in two different wastewater treatment MFCs using FLX Titanium pyrosequencing. Appl. Microbiol. Biotechnol. 87, 2335–2343.
Lim, J.W., Wang, J.Y., 2013. Enhanced hydrolysis and methane yield by applying microaeration pretreatment to the anaerobic co-digestion of brown water and food waste. Waste Manage. 33 (4), 813–819.
Metcalf & Eddy, Inc., 2004 Wastewater Engineering: Treatment and Reuse. Fourth Edition, revised by Tchobanoglous, G., Burton, F.L. Stensel, H.D., McGraw Hill.
Mills, T.Y., Sandoval, N.R., Gill, R.T., 2009. Cellulosic hydrolysate toxicity and tolerance mechanisms in Escherichia coli. Biotechnol. Biofuels 2, 26.
Rahimi, H., 2015 Effect of Termite Gut Microbe TAV5 on Methane Production from Municipal Solid Waste Degradation. Master's Thesis, University of Texas at Arlington.
Rahimi, H., 2019. The Effect of Termite Gut Microorganism TAV5 Ratios on Lignin Degradation and Methane Generation from Paper, Yard, and Wood Waste. Ph.D. Dissertation. University of Texas at Arlington.
Rashid, G.M.M., Duran- Pena, M.J., Rahmanpour, R, Sapsford, D, Bug, T.D.H. 2017. Delignification and enhanced gas release from soil containing lignocellulose by treatment with bacterial lignin degraders. Applied Microbiology ISSN. 123, 159–171.
Ren, N.Q., Wang, A.J., Cao, G.L., Xu, J.F., Gao, L.F., 2009. Bioconversion of lignocellulosic biomass to hydrogen: potential and challenges. Biotechnol. Adv. 27 (6), 1051–1060.
Ress, B.B., Calvert, P.P., Pettigrew, C.A., Barlaz, M.A., 1998. Testing anaerobic biodegradability of polymers in a laboratory-scale simulated landfill. Environ. Sci. Technol. 32 (6), 821–827.
Rijal, Nava, 2014. Impact of Rainfall Intervals and Waste Compaction on Methane Generation Rate. Master's Project, University of Texas at Arlington.
Ryba, S.A., Burgess, R.M., 2002. Effects of sample preparation on the measurement of organic carbon, hydrogen, nitrogen, sulfur, and oxygen concentrations in marine sediments. Chemosphere 48 (1), 139–147.
Sabnis, M.S., 2014. Investigation of how microbes involved in anaerobic digestion of vinasse change as functions of temperature, vinasse composition, and time. Ph. D. Dissertation, University of Texas at Arlington.
Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. 74 (12), 5463–5467.
Seidl, M., 2009. Decay Fungi Overview, https://www.emlab.com/resources/education/environmental-reporter/decay-fungi-overview/ (Accessed 5/19).
Sindhu, R., Binod, P., Pandey, A., 2016. Biological pretreatment of lignocellulosic biomass – an overview. Bioresour. Technol. 199, 76–82.
Stevenson, B.S., Eichorst, S.A., Wertz, J.T., Schmidt, T.M., Breznak, J.A., 2004. New strategies for cultivation and detection of previously uncultured microbes. Appl. Environ. Microbiol. 70 (5), 4748–4755. https://doi.org/10.1128/AEM.70.8.4748-4755.2004.
United States Environmental Protection Agency (EPA), 2014. Advancing Sustainable Materials Management: 2014 Fact Sheet. https://www.epa.gov/sites/production/files/2016-11/documents/2014_smmfactsheet_508.pdf (Accessed 6/19).
University of Florida, 2019. Eastern Subterranean Termite. http://entnemdept.ufl.edu/creatures/urban/termites/native_subterraneans.htm (Accessed 12/19).
Wang, X., Barlaz, M.A., 2016. Decomposition and carbon storage of hardwood and softwood branches in laboratory-scale landfills. Sci. Total Environ. 557–558, 355–362.
Watanabe, H., Tokuda, G., 2010. Cellulolytic systems in insects. Ann. Rev. Entomol. 55, 609–632.
White, T.J., Bruns, T., Lee, S., Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR protocols: a guide to methods and applications 30 (pp. 315–322). New York: Academic Press.
Wong, L.J., H'ng, P.S., Wong, S.Y., Lee, S.H., Lum, W.C., Chai, E.W., Wong, W.Z., Chin, K. L. 2014. Termite digestomes as a potential source of symbiotic microbiota for lignocelluloses degradation: a review. Pakistan J. Biol. Sci. 17(8): 956–963.
Xu, W., Fu, S., Yang, Z., Lu, J., Rongbo, G., 2018. Improved methane production from corn straw by microaerobic pretreatment with a pure bacteria system. Bioresour. Technol. 259, 18–23.
Yuan, X., Wen, B., Ma, X., Zhu, W., Wang, X., Chen, S., Cui, Z., 2014. Enhancing the anaerobic digestion of lignocellulose of municipal solid waste using a microbial pretreatment method. Bioresour. Technol. 154, 1–9.
Zabed, H.M., Akter, S., Yun, J., Zhang, G., Awad, F.N., Qi, X., Sahu, J.N., 2019. Recent advances in biological pretreatment of microalgae and lignocellulosic biomass for biofuel production. Renew. Sustain. Energy Rev. 105, 105–128.
Zuroff, T.R., Curtis, W.R., 2012. Developing symbiotic consortia for lignocellulosic biofuel production. Appl. Microbiol. Biotechnol. 93, 1423–1435.