ABSTRACT

WEAVER, JOSEPH EARL. Effect of Inoculum Source on the Rate and Extent of Anaerobic Biodegradation. (Under the direction of Dr. Morton A. Barlaz and Dr. Francis de los Reyes).

Biodegradable plastics are increasingly available and there is interest their anaerobic biodegradability. As biodegradable plastics used in non-durable products become more prevalent in the marketplace, it is reasonable to expect that a growing portion will be disposed of in landfills. As such, it is desirable to characterize their degradability in an anaerobic ecosystem representative of a landfill.

Tests of anaerobic biodegradability are easier to perform if there is flexibility regarding the source of inoculum. However, the effect of the source of inoculum on test results should be characterized. The goal of this research was to determine if inocula from various sources significantly affected test results.

Four series of reactors were inoculated with either decomposed municipal solid waste (MSW), landfill leachate, anaerobically digested municipal wastewater treatment sludge (*i.e.*, biosolids), or a digestate produced in a lab-scale anaerobic digester fed with the organic fraction of municipal solid waste. Substrates tested were copy paper, newspaper, grass, and one of a number of plastics that were thought to be biodegradable. The reactors were operated to represent optimum conditions for anaerobic biodegradation in a high solids matrix, such as a landfill. Methane production was used to measure biodegradation.

Methane yields from copy paper and grass were insensitive to inoculum source ($p>0.05$). Copy paper inoculated with biosolids, digestate, or MSW produced 222, 214 and 216 mL

CH_4/dry g, respectively. Fresh grass inoculated with leachate or MSW produced a respective 66 and 73 mL CH_4/dry g. Newspaper was sensitive to inoculum source ($p<=0.05$) and when treated with biosolids, organic waste digestate, leachate, or MSW produced 91, 121, 104, and 74 mL CH_4/dry g, respectively. None of the tested plastics were shown to biodegrade.

Degradation rates were also sometimes sensitive to inoculum source. Copy paper inoculated with biosolids, digestate, or MSW decayed at similar rates of 4.7, 4.9 and 5.7 yr^{-1}, respectively. Fresh grass inoculated with leachate or MSW decayed at rates of 26 and 17 yr^{-1}, respectively. Newspaper was more sensitive to inoculum source ($p<=0.05$); it decayed at rates of 9.0, 4.2, 6.7, and 16 yr^{-1} when inoculated with biosolids, digestate, leachate, or MSW, respectively.

Inocula source also influenced the time required for substrates to degrade beyond 3 half-lives. As half-lives, unlike decay rates, include lag time, this metric is useful in assessing the time required to perform a test and obtain sufficient data to determine decay rates. Copy paper inoculated with biosolids, organic waste digestate, and MSW degraded beyond 3 half-live lives after 201, 179, and 159 days, respectively. Copy paper reactors treated with leachate or a second sample of biosolids have been active for over 350 and 275 respective days and have not yet degraded past 3 half-lives. In the latter case, significant lag periods were observed. Fresh grass inoculated with leachate or MSW took 29 and 33 days to degrade beyond 3 half-lives, respectively. Newspaper inoculated with biosolids, organic waste digestate, leachate, or MSW degraded beyond 3 half-live lives after 109, 186, 162, and 56 days, respectively.

The sensitivity of newspaper to inoculum source suggests that the same inoculum for all substrates should be used in a given study if newspaper is chosen as a reference. If flexibility in inoculum source is required, then copy paper inoculated with either MSW or digestate is recommended. In addition to flexibility, the digestate and MSW inocula produced the most reproducible results for methane yield and production rate, respectively.

Effect of Inoculum Source on the Rate and Extent of Anaerobic Biodegradation

by
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DEDICATION

To my wife, Katy, who always told me “You can”, when I thought “I can’t.”

BIOGRAPHY

Joe Weaver comes to environmental engineering via an indirect path. While originally an electrical engineer specializing in computer programming, Joe always had an interest in the sciences, particularly the life sciences. A closely spaced set of conversations about cholera made Joe interested in the disease. As he read, this interest bloomed into a general curiosity about microbiology. Although he enjoyed reading about medical microbiology, his background in engineering led him to applied microbiology in the context of environmental engineering.

After a few years as a hobby microbiologist, during which he started calling the pressure cooker an autoclave and continually threatened to set up a garage-scale anaerobic digester, Joe started taking some local courses in microbiology, environmental science, and environmental engineering. This was part of a long, deliberate process in which he tried to decide if he wanted to change his career and follow his passion. Throughout all of this, he received nothing but support from his wife, friends, family, and teachers. Joe is now a graduate student in the Civil, Construction, and Environmental Engineering Department at North Carolina State University and is experiencing one of the most fulfilling professional periods of his life.

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One of the greatest lessons I've learned as a student is that no work stands alone, nor does any researcher. With fear of leaving anyone out, and in no particular order, my thanks to:

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My family, who told me I wasn't crazy and started my love of learning.

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The Orange Water and Sewer Authority and North Wake Landfill for providing inocula.

Dr. Barlaz devoted a large amount of time guiding me in improving this thesis, a task for which I am very grateful. Any mistakes which remain are my own.

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LIST OF POLYMER ABBREVIATIONS

Traditional

HDPE	High density polyethylene
LDPE	Low density polyethylene
LLDPE	Linear low density polyethylene
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PVC	Polyvinyl chloride

Synthetic and Biologically Derived Polyesters

PCL	Poly(ε-caprolactone)
PHA	Polyhydroxyalkanoate
PHB	Poly(β-hydroxybutyrate)
PHBV	Poly(β-hydroxybutyrate-co-β-hydroxyvalerate)
PLA	Polylactic acid
PLLA	Poly(L-lactide)

Other

CA	Cellulose acetate
PVA	Polyvinyl alcohol

INTRODUCTION

Biodegradable plastics are increasingly present as disposable goods. Examples of merchandise for which there are claims of biodegradability include cutlery, drinking cups, plastic wrap, straws, coffee stirrers, party balloons, and plastic bags. Most of these items will ultimately be disposed of in a landfill. The extent to which these goods degrade in a landfill is of interest, as such, it is desirable to characterize their degradability in a high solids anaerobic ecosystem. Thus it is important to have a standard test to measure anaerobic biodegradability under simulated landfill conditions.

While it is possible to determine the degradation of plastics in an anaerobic environment, most tests use either anaerobically digested organic waste or MSW (ASTM 2012b; Eleazer et al. 1997). Tests would be easier to perform with alternative inocula which may be easier to obtain and not require pre-digestion, such as landfill leachate or stabilized biosolids. However, permitting such flexibility first requires characterizing the effect of inoculum source on test results. The goal of this research was to determine how inocula from four sources (MSW, leachate, digested organic waste, and biosolids) affected methane yields and rates in tests of anaerobic degradation. The test method used represented an environment similar to a landfill and inferred degradation from methane generation.

Conditions which define test environments include temperature, moisture, oxygen, pH, nutrient availability, and the presence of an appropriate microbial community. In practice, environments are classified as either aerobic (*i.e.* compost) (ASTM 2009), or anaerobic (*i.e.* landfill) (ASTM 2012b; ASTM 2012a; Barlaz et al. 1989).

Anaerobic tests may be further categorized based on how degradation is inferred. Methane production is used to infer degradation in respirometric tests and allow easier differentiation between biodegradation and abiotic disintegration. Example respirometric methods include biochemical methane potential tests (Wang et al. 1997) and lab-scale simulation reactors (Barlaz et al. 1989). Non-respirometric tests infer degradation through other parameters, such as mass loss or microscopic examination, and may be easier to perform at the cost of a reduced ability to determine the cause of degradation (Eubeler et al. 2009).

The objective of this research was to examine the effect of inoculum source on respirometric anaerobic biodegradability tests performed using lab-scale landfill simulation reactors. An overview of biodegradable plastics and anaerobic test methods is presented in the next section and is followed by a presentation of the experimental design and methods. The results of the tests are organized by substrate and are followed by a discussion of inocula which includes recommendations for the development of future standards which address the anaerobic biodegradation of plastics in a landfill.

BACKGROUND

The fraction of plastic disposed of in a landfill and factors which make traditional plastics recalcitrant to degradation are discussed. This is followed by an overview of the major classes of biodegradable plastics and the methods by which they degrade. The requirements for an appropriate biodegradation test are then listed and used as a context for describing methods of testing plastic degradation. Finally, methods to produce specific degradable

plastics are explained and the reported abilities of those plastics to anaerobically biodegrade, as determined by the preceding methods, are presented.

Plastic Disposal in the US and Major Classes of Biodegradable Plastic

The total 2010 US disposal of plastics was 2.6×10^7 Mg and was predominantly composed of the resins shown in Figure 1 (EPA 2011). These resins all consist of monomers linked by a long backbone. Many functional units may be attached to the side chain and can be simply hydrogen, as in the case of PE, or an aromatic ring, as in the case of PS. The nature of the backbone may vary and can be purely carbon, as in PE, contain C-N bonds, as in polyamides such as nylon, or contain ester bonds, as in polyesters such as PET. Specific properties of the plastic also depend on cross linking and branching between polymer strands, the polymer's degree of crystallization, and additives such as plasticizers, dyes, and fire retardants. While not a majority portion of disposed resins, biodegradable plastics are increasingly present in the marketplace and can be designed to directly replace traditionally used polymers such as polyethylene and polystyrene.

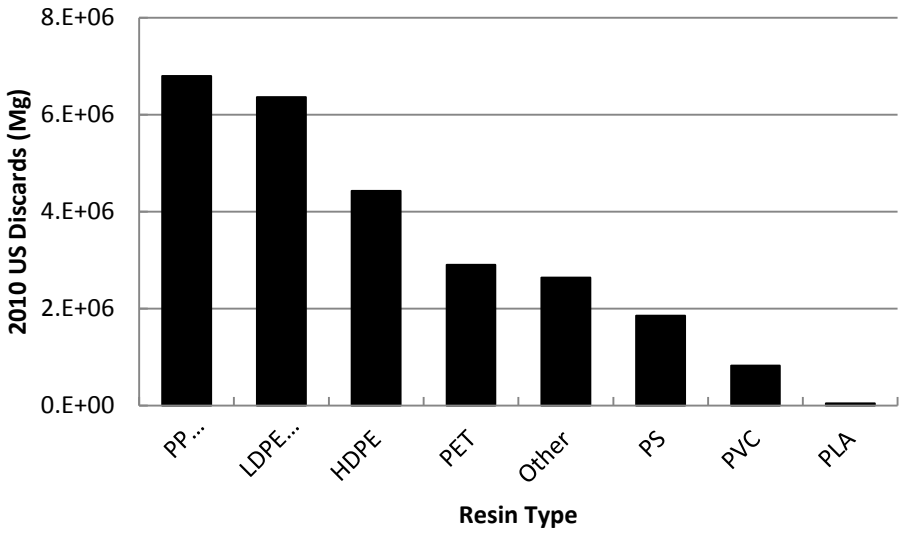


Figure 1 Resins Discarded in US Waste Stream (EPA 2011)

While potentially a rich carbon source for microbes, plastics resist biodegradation, mainly because they are too large to pass through cell membranes and are not readily reduced to lower molecular weight (Abou-Zeid 2000). LDPE, with an average molecular weight of 150000 daltons (Da), must be broken into components of less than 500 daltons for microbial uptake (Chandra & Rustgi 1998). In natural polymers, size reduction is usually accomplished through hydrolysis catalyzed by extracellular enzymes. Plastics lack sites which may be hydrolyzed by microbial enzymes, other than at the end of the polymer backbone, which is usually deep within the hydrophobic matrix of the polymer (Palmisano & Barlaz 1996).

The breakdown of specific plastics may be further hindered by other factors. Steric hindrance occurs when large side chains, such as the phenol groups present in polystyrene, physically block enzymatic attack of the carbon backbone and may be the reason biodegradability decreases inversely with side chain size (Tokiwa et al. 2009). The polymer‘s degrees of cross

linking, branching, and crystallization also hinder degradation. The hydrophobic nature of most plastics makes it difficult for microbes to maintain the prolonged, close attachment required for degradation.

Degradable plastics may be characterized by the general method in which they are intended to degrade. As a consequence of different degradation mechanisms, each class may degrade to a different extent and at a different rate when exposed to different environments.

One method of producing degradable plastics is the insertion of additives into traditional polymers. The additives are intended to enhance biodegradation by catalyzing initial polymer breakdown when exposed to abiotic factors such as heat, oxygen, or ultraviolet light. Without sufficient exposure to the appropriate factor the plastics are not expected to degrade. For example, the Total Degradable Plastics Additive (TDPA) produced by Environmental Products Inc. (EPI) has been shown to induce plastic degradation under only aerobic conditions (Mohee et al. 2008) and should not be expected to induce degradation within an anaerobic environment, such as a landfill.

Instead of an added catalyst, the backbones of another class of biodegradable plastics contain bonds which are directly attacked by the appropriate microbial community. These plastics are not traditional polyolefins but are synthetically produced, as in the case of the Ingeo brand of PLA produced by NatureWorks. Other plastics in this class are based on biologically derived polyesters, such as the Mirel brand of PHA produced by Metabolix. The extent to which these plastics degrade in a landfill can depend upon the higher level structure of the plastic.

For example, amorphous PLA is more biodegradable under anaerobic conditions than crystalline PLA (Kolstad et al. 2012).

Novamont's Mater-Bi represents a third class of plastics produced from chemically modified polysaccharides, often starch. Depending on the particular modification, polysaccharide, and intended use, these plastics may be directly used as a material, physically blended with other polymers, or chemically co-polymerized with other monomers. The specific form, treatment, and blend of the material determine its degradability within a landfill. For example, an increasing degree of acetylation in CA reduces its anaerobic degradability (Puls et al. 2011).

Biodegradation Test Criteria and Methods

When attempting to characterize the breakdown of a polymer, it is important to understand the differences between biodegradation and abiotic degradation and the types and limitations of various test methods.

Early attempts to characterize the breakdown of synthetic polymers were hindered by not clearly differentiating biological and abiotic degradation (Krupp & Jewell 1992). Over the last 20 years, the differences between the two have become better defined. Biodegradation is the biologically induced chemical transformation of substrates to lower molecular weight compounds which maybe be further altered by anabolic incorporation, complete mineralization (*e.g.* to CO_2 or CH_4), or the formation of stable humic matter (Pettigrew & Johnson 1996). Abiotic degradation is any physical disintegration or chemical change in a substrate caused by non-biological environmental factors (Krupp & Jewell 1992).

Non Respirometric Test Methods

Respirometric test methods were used in this research because most non-respirometric methods are limited in that it is difficult to distinguish abiotic degradation from biodegradation. To illustrate these difficulties, this section describes commonly used non respirometric test methods and how, if possible, biodegradation may be distinguished from abiotic degradation.

A common approach in non respirometric methods is to measure changes in material properties after exposing plastics to an environment in which they are predicted to degrade. The assumption is that the change in property is caused by polymer degradation. For example, reduced mass, tensile strength and Young's modulus and increased elongation at break were used to infer the degradation of PCL, PHB, and PLLA in seawater (Tsuji & Suzuyoshi 2002). Changes in other characteristics have been similarly used to measure plastic degradation; examples include decreasing molecular weight of starch-modifed PE (Erlandsson et al. 1997), color changes of PE containing maleic anhydride (Chandra & Rustgi 1997), microscopic detection of pitting and fractures in starch-PE blends (Vieyra et al. 2012), and bulk disintegration of starch-PHBV blends (Imam et al. 1999).

Agar plate tests are a non respirometric test which can demonstrate biodegradation. In these tests, powdered polymer is suspended in agar on an inoculated petri dish and biodegradation is indicated by the formation of clear zones around bacterial colonies (Abou-Zeid 2000). However, solid media tests do not represent specific ecosystems and are usually qualitative. Some work to quantify results by measuring the size and rate of clear zone growth has been

performed by Augusta et al. (1993), but these results are specific to the environment of the plate (*e.g.* enzyme diffusion rates through agar) and are likely not predictive of behavior in a different matrix representing a specific ecosystem.

Unlike agar plates, tests of material properties are not limited to a specific artificial environment; the plastic being tested may be exposed to any suitable environment between measurements. Field tests are an example of exposing the plastic to a highly representative environment; the plastics are buried *in situ* for some time, excavated, and then analyzed. In one field test, a PCL-starch blend was buried in a landfill for 90 days and then tested for degradation (Cho et al. 2011). In that case, different material properties lead to different conclusions; the researchers concluded, based on visual examination and tensile strength, that biodegradation occurred on some parts of the plastic but they also reported that no significant mass loss was observed. In soil burial tests, an additional confounding factor is the need to determine if mass loss, when observed, is due to actual biodegradation or wash-out/offgassing of the soluble and volatile fractions of the tested plastic (Lucas et al. 2008). A final limitation of soil burial tests is that conditions are usually not ideal for maximum degradation rates, so test durations are longer.

In order to determine the cause of degradation, interpret conflicting results, or determine relative degradation of components within a blended polymer, additional tests may be conducted. One set of additional tests are spectroscopic analyses which use characteristics of emitted electromagnetic spectra to determine the chemical properties of a substance. The

presence or absence of a chemical property specific to either a biotic or abiotic degradation process may then be used to infer how the material degraded.

One spectrographic test, Fourier transform infra-red spectroscopy (FTIR), can be used to detect carboxylic acids associated with strong carbonyl peaks. A mechanism of PE degradation proposed by Albertsson et al. (1987) states that carboxylic acids are produced by initial abiotic degradation and will be removed by microbes during biological degradation. An FTIR comparison of samples of modified PE which received abiotic pretreatment treatment and then either biotic or abiotic degradation supports this mechanism (Albertsson et al. 1998). Therefore, if carboxylic acids are detected, it is concluded that biological degradation did not occur. While the FTIR tests which determined the mechanism were performed aerobically, they may also be applicable to anaerobic tests as carboxylic acids are also metabolized by microorganisms in anaerobic environments.

A second test, X-Ray diffraction (XRD), similar in that it is based on chemical differences between abiotic degradation and biodegradation, can be employed by measuring the degree of polymer crystallization. The less crystallized portions of polymers are preferentially degraded by microorganisms. The effect of this preference is that the remaining undegraded polymer will present an increased level of crystallinity when examined via XRD (Chandra & Rustgi 1998). The predicted increase in crystallinity was observed when modified PE was degraded abiotically and biologically (Albertsson et al. 1995).

A third spectrographic test, nuclear magnetic resonance spectroscopy (NMR), can be used to determine if fractions of a blend degrade to different extents. For example, in a blend of

starch, PHB, and PHBV, the ratio between PHB and PHBV remained constant (determined via NMR), while the fraction of starch (shown with FTIR analysis of OH/CH groups) decreased during degradation (Imam et al. 1998). The change in blend composition implies that starch was preferentially degraded in favor of PHB or PHBV and that neither polyester degraded faster than the other; a fact which would be missed by a solely gravimetric analysis of the blend.

Unequal degradation in polymers can also be detected without spectrographic analysis. Chemical separation of the residual contents after a test may be used to determine the relative fractions of undegraded materials. For example, dichloromethane was used as a solvent to separate a residual PHBV-starch blend after degradation (Imam et al. 1999). Different degrees of mass loss between the separated fractions showed the separate degradation behaviors of each component. Another method may be used in the specific case of starch blends. The high proportion of ^{13}C in plant based materials relative to those based on petroleum has been used to determine relative rates of degradation via isotopic analysis. Monitoring the decreased overall proportion of ^{13}C in residuals of starch-synthetic polymer blends undergoing biodegradation can allow statistical determination of preferential degradation of the starch (Sykes et al. 1994).

Respirometric Test Methods

The major assumption of all anaerobic respirometric test methods is that the biodegradable fraction of carbon in a substrate degrades to methane and carbon dioxide and that biodegradation can therefore be determined by measuring methane production. While this

approach more easily distinguishes biodegradation from abiotic degradation, the tests may still be limited in how well they represent the ecosystem of interest and in how well they relate methane production to specific components within the polymer.

A biochemical methane potential (BMP) test is performed using a serum bottle containing a small amount of substrate relative to the amount of liquid inocula and growth media. BMP tests are useful in determining if a material has the potential to degrade anaerobically (Wang et al. 1997). However, a BMP test is also an example of a method which does not fully represent a landfill. The high moisture content and low solids to liquid ratio means that although the bottle is incubated anaerobically under mesophilic temperatures, the environment is unlike that of the high solids matrix of a landfill.

The effect of inoculum source on anaerobic tests has been unclear. An interlaboratory study of BMP-style tests reported no significant difference in methane yield or rate attributable to inoculum source (Raposo et al. 2011). However, a different study indicated test results are influenced by inoculum source (Elbeshbishy et al. 2012). In both studies, the inocula did not differ as greatly as decomposed MSW differs from biosolids or landfill leachate differs from anaerobically digested organic waste. Additionally, both studies focused on BMP-style tests and not those which are more representative of a landfill.

Unlike BMP tests, ASTM test methods specify the use of reactors which reflect the high solids content of landfills. However, they may not accurately represent average landfill conditions in other ways. For example, ASTM D5511 specifies an incubation temperature of 52°C (ASTM 2012a), but Hanson et al. (2010) observed temperature ranges in landfills in

Alaska, British Columbia, Michigan, and New Mexico of 1-33, 13-50, 15-56, and 21-34°C, respectively. ASTM D7475, in contrast, specifies a more probable temperature of 35°C, but also requires an initial accelerated aerobic degradation step which may not accurately reflect aerobic degradation conditions within a landfill (ASTM 2011). Landfill environments are better represented by ASTM D5526 which specifies a temperature of 35°C and does not include an initial aerobic degradation step (ASTM 2012b). ASTM D5526 requires both an inoculum derived from anaerobically digested household organic waste and a co-substrate composed of aerobically stabilized, screened MSW (ASTM 2012b). An alternative, non-ASTM, method which does not require a co-substrate and uses shredded and anaerobically degraded MSW as an inoculum has been used in other research (Federle et al. 2002; Wang et al. 2011; Ress et al. 1998; Eleazer et al. 1997).

In addition to adequately representing the degradation environment, respirometric test methods are most informative if they can relate methane production to the specific compound of interest. Difficulty linking methane production specifically to the substrate is problematic for two reasons. First, most tests are seeded with an inoculum which produces 'background' methane. Background methane must be either minimized or accounted for to avoid overestimating the amount of biodegradation. This can be addressed by running concurrent blank controls in which the inoculum is the only degradable substance and using the methane yield of the controls to adjust experimental results. Second, when testing blended polymers a failure to attribute changes in the system (*i.e.* methane production) to specific fractions of the blend leads to an incomplete understanding of the degradation of the material.

As an alternative to running blank controls, it is possible to link methane production to a particular substrate by using isotope tracers. Radioactive isotopes, such as ^{14}C are specified in ASTM D6776 (ASTM 2002) and have been used to study the degradation of a number polymers in landfill reactors (Ress et al. 1998; Komarek et al. 2003). Tritium can also be used as a tracer and has been used to study PE degradation (Anderson et al. 1997). In addition to radiolabelling, stable isotopes, such as ^{13}C , have been successfully used to trace starch degradation (Sykes et al. 1994). While useful, producing labeled versions of test plastics may not always be technically feasible and can be expensive (Pettigrew & Johnson 1996). Another limitation of isotope tracers is that bias in labeling a certain carbon location of the substrate will result in an analysis biased towards methane production from that particular carbon, rather than the substrate as a whole.

Tiered Testing Approaches

The limitations of test methods can be addressed by performing multiple tests with complementary strengths. Efficiency in testing can be achieved by assuming that if a substance does not degrade under less stringent conditions, then it will also not degrade as tests become more specific. Quick, easy, or inexpensive tests can then be performed first and followed by more complex tests only if the polymer degrades and only if increased test requirements are warranted. A simple two tier test is exemplified by the performance of a BMP test which is only followed by a landfill reactor test when the BMP results indicate degradation. Tiers can be more complex than in the example and a number of proposed tiers have been published (Kyrikou & Briassoulis 2007; Chandra & Rustgi 1998).

Tiered testing is itself limited in that it can fail to identify degradable materials if some characteristic of later, more realistic tiers would allow degradation not observable in earlier tiers. It is also desirable to clearly state if biodegradation is the assumed mechanism in each tier or if the plastics are first abiotically oxidized and biodegradation tests are then only carried out on the oxidized components. An example of the latter case is the test specified by ASTM D7475 and described by Roy et al. 2011 (ASTM 2011). As explained above, ASTM D7475 includes a phase in which the substrate is exposed to oxygen.

Degradation Mechanisms of Specific Plastics

Enhanced plastic degradability is achievable via a number of mechanisms. Understanding the mechanisms of plastic degradation can provide insight regarding why a polymer may exhibit different behavior in anaerobic ecosystems. An overview of the types of biodegradable plastics is given in this section and more thorough reviews have been published (Luckachan & Pillai 2011; Ammala et al. 2010). In general, biodegradable plastics are either traditional polymers which have been modified in a way which promotes hydrolysis or they are inherently hydrolysable materials which can act as substitutes for traditional plastics.

Insertion of Additives

Insertion of a reactive site into the backbone of the polymer is intended to provide a location for chain scission, either by physiochemical or biological means. The intended mechanism of biodegradation is reduction of polymer length to the point where it may be absorbed by the cell membrane of the microorganism and further degraded.

Prodegradants

Physiochemical chain scission may be achieved by abiotic activation of prodegradants inserted into the polymer backbone. Abiotic factors which may activate prodegradants include UV light, oxygen, and heat. While an additive can require a specific abiotic factor, such as oxygen, additional factors, such as increased temperature or moisture, may further accelerate hydrolysis. When these factors are not present, it is likely that the plastic will not significantly degrade.

Many prodegradants contain transition metal carboxylates which can accelerate the production of peroxide species. The peroxides, in turn, participate in reactions which cleave the polymer backbone, resulting in lower molecular weight polymers and soluble, biodegradable molecules (*e.g.* carboxylic acids, alcohols, etc.) (Scott & Wiles 2001). An example of this type of prodegradant is TDPA, which is used to enhance oxidative breakdown of polyolefins and PE (Ammala et al. 2010). Without oxidative pre-treatment a plastic (resin undisclosed) containing 3% TDPA did not produce methane after 35 days of anaerobic, mesophilic testing and did not lose mass after 40 days of aerobic composting (Mohee et al. 2008). In another experiment, thermally pre-treated LDPE containing TDPA tested under dark, aerobic, room temperature conditions produced significantly more CO_2 than a control but not until after approximately 150 days (Chiellini et al. 2003).

Starch copolymer grafting

Biological hydrolysis of the polymer can be made possible by chemically attaching a small biodegradable molecule, such as starch, to the polymer in a process known as graft

copolymerization (Hakkarainen & Albertsson 2004). This process involves chemical bonding and should not be confused with simple physical blending of two polymers. The inserted molecule acts as an initial site for direct hydrolysis by extracellular enzymes. In a 150 day soil burial test, LDPE copolymerized with starch experienced the mass losses listed in Table 1 (Maharana & Singh 2006).

As starch is readily degradable and LDPE is highly recalcitrant, it may be reasonable to assume preferential degradation of the copolymerized starch. Under that assumption, enhanced LDPE degradation should not be assumed to occur unless the loss in mass is greater than the mass of starch in the blend. These assumptions were applied to data from Maharana & Singh (2006) and the results, presented in Table 1, show that change in LDPE mass was below 10%, with the exception of the greatest amount of starch grafting. One explanation of the jump to nearly 50% PE mass loss at 77% grafting is that there is a threshold below which the majority of reduced length LDPE fragments remaining after starch degradation are still too large to be metabolized.

Table 1 Estimated LDPE Mass Loss when Grafted with Starch Copolymer

Sample #	Initial Blend Mass[a] (g)	Percent Starch in Blend[a]	Initial Mass of starch (g)	Initial Mass of LDPE (g)	Final Blend Mass (g)[a]	% mass loss LDPE[b]
I	0.1620	0%	0.0000	0.1620	0.1620	0%
II	0.1371	41%	0.0567	0.0804	0.0722	10%
III	0.2062	44%	0.0914	0.1149	0.1046	09%
IV	0.0878	47%	0.0411	0.0467	0.0437	06%
V	0.0473	77%	0.0363	0.0110	0.0059	46%

a. Data from (Maharana & Singh 2006)
b. Assumes starch fraction degrades preferentially to LDPE. The final mass of PE was calculated as Initial PE mass – (Blend mass loss – Intitial starch mass)

Starch blends

Mixed materials composed of traditional plastic amended with a highly degradable polymer, such as starch, have been proposed as a biodegradable material. The addition of starch is intended to provide attachment sites for microbes and initial areas of biological attack, physically block the length of polymer formation, and, as the starch is degraded, provide a larger surface area for microbial colonization. In a test of a starch/PE blend, gas was produced aerobically and anaerobically but further analysis attributed gas production to starch only and indicated no biodegradation of the PE (Ndon et al. 1992).

Inherently Hydrolysable Polymers

A number of natural or synthetically produced polymers have backbones which require no modification to be degraded by organisms under certain conditions. Optimum degradation conditions are defined both by environment and bioavailability of the hydrolysable portions of the backbone (*e.g.* low bioavailability in crystalline vs. amorphous plastics). Physical

properties of these materials may not be adequate to serve directly as substitutes for traditional plastics. Desired physical properties, such as high melting point and rigidity, may be achieved by chemical alteration or by blending various polymers together.

Polysaccharide based polymers

In contrast to traditional plastics amended with starch, it is also possible to create plastics predominantly composed of biodegradable polysaccharides. It can be the case that while modification enhances physical properties, it also reduces biodegradability.

Modification of cellulose by acetylation produces CA, and the extent of acetylation is termed the degree of substitution (DS). The most common DS of commercially produced CA (*i.e.* exhibiting the most desirable physical properties) is about 2.5 (Puls et al. 2011). Above a DS of 1.65, anaerobic degradation of CA in mesophilic BMP tests was shown to be inhibited while below a DS of 0.99, the anaerobic biodegradability of CA approached that of cellulose (Rivard et al. 1992). In contrast, Gartiser et al. (1998) reported mesophilic BMP degradation of CA at a DS of 2.5 and hypothesized that this may be due to the large surface area of the CA sample tested.

Polyesters and other polymers

Other polymers with hydrolysable backbones may be either synthetically derived or produced biologically. Most of these plastics are polyesters and include PCL, PLA, and the PHA family of bacterial polyesters, including PHB and PHBV. An example of a biodegradable non-polyester is PVA.

PLA is an attractive material because the monomers used in its production (lactic acid and lactide) do not require a fossil feedstock. The crystallinity of PLA can affect degradation and is determined by stereochemistry; PLLA is the L-form of PLA and is considered semicrystalline (Carrubba et al. 2008). As the literature does not always differentiate PLA from PLLA, the following discussion will refer to the polymer as PLA and explicitly state the degree of crystallization. Amorphous (0% crystallinity) and semicrystalline (30-50% crystallinity) samples of PLA were tested by Kolstad et al. (2012) in landfill reactors incubated at 21°C and 35°C. The reactors were operated using slightly different protocols. Neither form of PLA exhibited degradation at the lower temperature after 390 days. After 160 days of incubation at 35°C, the semicrystalline PLA did not degrade while the amorphous PLA achieved approximately 35% degradation. This is interesting as NatureWorks, a major manufacturer of PLA, cites the same research when claiming that PLA does not degrade anaerobically (Natureworks 2013). The degree of crystallinity of the PLA produced by NatureWorks is unclear.

As is apparent from the research presented above, temperature may also affect the anaerobic degradability of PLA. The effect is unclear because the experiments differed beyond incubation temperature; notably the composition of inocula was different. Another experiment which explored effect of temperature is also not definitive for the same reason. Semicrystalline (30-35% crystallinity) PLA was tested by Itävaara et al. (2002) using BMP-style tests conducted at 37°C and in landfill reactors operated at 52°C. PLA took 100 days to reach 60% degradation at 37°C while it degraded to the same extent in 40 days at 52°C. A

third experiment performed by Shi & Palfery (2010) at 35°C and 52°C used the same BMP-style protocol for both temperatures and also shows temperature dependent degradation. However, the PLA was amended with various amounts of plasticizers. Taken together, the three experiments provide evidence that temperature does affect PLA degradation.

PCL is another polyester known to degrade aerobically. When tested in a landfill simulation reactors, PCL began producing significant amounts of methane after approximately 200 days (Federle et al. 2002). In a BMP-style experiment, PCL showed less than 12% degradation in 77 days (Gartiser et al. 1998). In contrast, PCL tested in aerobic composting conditions was 80% degraded within 20 days (Yang et al. 2005).

PHAs are polyesters naturally produced by bacteria, with PHB being a common subset. PHB is produced by organisms to store energy, and it is biodegradable by a number of microbes. Various blends of the PHA family of polymers have been explored as substitutes for traditional plastics. Copolymers of PHB and PHBV have been shown reach 40 to 60% degradation (Gartiser et al. 1998) in BMP style tests. Poly(3-hydroxybutyrate-co-3-hydroxyoctanoate (PHBO), a similar plastic, was shown to reach 45% degradation after 177 days under simulated landfill conditions (Federle et al. 2002).

PVA is not a polyester, it is a vinyl polymer and is notable because, unlike other vinyl polymers, it is considered to be biodegradable under aerobic conditions (Amann & Minge 2012). The biochemical processes which underlay its aerobic degradation have been extensively reviewed (Kawai & Hu 2009). A BMP-style experiment showed less than 10% anaerobic degradation of PVA after 77 days (Gartiser et al. 1998).

EXPERIMENTAL METHODS

Experimental Design

The goal of this research was to determine the effect of inocula source on tests of anaerobic biodegradation in a simulated landfill environment. To accomplish this, 8L landfill reactors were loaded with reference substrates (newsprint, copy paper, grass) and inoculated with a consortium of microorganisms from one of four anaerobic ecosystems known to convert cellulose to methane. The four inocula chosen were anaerobically digested biosolids from a wastewater treatment plant, digestate from anaerobically degraded food and yard waste, leachate from a closed landfill, and anaerobically degraded MSW. Concurrent tests were performed on plastics thought to be biodegradable. The experimental program is summarized in Table 2 and each inoculum is described in the Materials section.

Table 2 Landfill Reactor Contents

Inoculum	Substrates Tested	
Mesophilic Biosolids[a]	Used Copy Paper (UCP I & II)	Lab ID: 11-546, 12-389
	Old Newspaper (ONP)	Lab ID: 11-545
	Grass	Lab ID: 11-544
	Thin Polyethylene Film II	Lab ID: 12-79
	Polystyrene Foam	Lab ID: 12-40
Landfill Leachate	UCP I	Lab ID: 11-546
	ONP	Lab ID: 11-545
	Grass (Old)[b]	Lab ID: 11-544
	Thick Polyethylene Sheet	Lab ID: 12-2
Decomposed Municipal Solid Waste (MSW)	UCP I	Lab ID: 11-546
	ONP	Lab ID: 11-545
	Grass	Lab ID: 11-544
	Thin Polyethylene Film I	Lab ID: 12-1
	Thick Polyethylene Sheet	Lab ID: 12-2
Organic Waste Digestate[c]	UCP II	Lab ID: 12-389
	ONP	Lab ID: 11-545
	Grass (Old)	Lab ID: 11-544
	Thin Polyethylene Film II	Lab ID: 12-79
	Polystyrene Foam	Lab ID: 12-40

a. Provided by Orange Water and Sewer Authority (OWASA), Chapel Hill, NC
b. The grass is noted as old because it was stored at 4°C for 6 months. It appears to have degraded during storage based on the lower methane yields of reactors set up later in the research as discussed in the Results.
c. Provided by Eden Research Laboratory, Albuquerque, NM.

In addition to the landfill reactor tests, BMP tests were performed to determine the methane potential of the reference substrates and the plastics. The contents of each assay are listed in Table 3.

Table 3 Biochemical Methane Potential Test Contents

Substrate	Inoculum (ml)	BMP Media (ml)	Approximate Substrate Mass (g)[a]
Control	15	80	0
Used Copy Paper II	15	80	0.25
Newspaper	15	80	0.5
Grass	15	80	1.0
Thin Polyethylene Film I	15	80	1.0
Thin Polyethylene Film II	15	80	1.0
Thick Polyethylene Sheet	15	80	1.0
Polystyrene Foam	15	80	1.0

a. Target weight per bottle. Substrate in each bottle varied by a maximum of 0.012 g

Based on reactor results in which some plastics appeared to inhibit methane generation, anaerobic toxicity assays (ATAs) were conducted as summarized in Table 4. The ATA test is designed to evaluate whether the presence of a specific material, in this case one of the tested plastics, inhibits methane production from a biodegradable substrate, copy paper in this case. BMP media is not included to reduce dilution of inhibitory compounds.

Table 4 Anaerobic Toxicity Assay Contents

Substrate	Inoculum (ml)	BMP Media (ml)	Approximate Substrate Mass (g)[a]	Approximate Copy Paper Mass (g)[a]
Control	10	0	0	0.125
Thin Polyethylene Film I	10	0	3.75	0.125
Thin Polyethylene Film II	10	0	3.75	0.125
Thick Polyethylene Sheet	10	0	3.82	0.125
Polystyrene Foam	10	0	3.75	0.125

a. Target weight per bottle. Weights in each bottle varied by a maximum of 0.06 g for substrate and 0.0025 g for copy paper.

All reactors, BMP and ATA tests were carried out in triplicate, except for the BMP control which consisted of 5 bottles.

Materials

Reference Substrates

Reference substrates were selected from those which have well defined and consistent anaerobic biodegradation behaviors and are easy to acquire and handle. In addition, substrates were limited to polymers which require an initial hydrolytic depolymerization step to degrade. For example, cellulose would be acceptable, but glucose would not. Used copy paper, old newspaper, and grass fulfill those requirements and were selected as candidate reference materials.

Used copy paper has a ratio of carbohydrates (cellulose + hemicellulose) to lignin between 42 and 84 as calculated from the data summarized by Barlaz (2006) and is generally considered highly degradable. Old newspaper, in contrast, is highly lignified with a carbohydrate to lignin ratio of 2 to 3 (Barlaz 2006) and, due to both the recalcitrance of lignin and reduced bioavailability of carbohydrates, does not mineralize to the same extent as used copy paper (Eleazer et al. 1997). Grass can be highly lignified but the lignin in grass does not retard methane production as greatly as the lignin present in wood and it has been hypothesized by Eleazer et al. (1997) that this is due to the chemical differences between woody and grassy lignin (Akin et al. 1995).

Substrate Collection and Storage

Used copy paper (UCP) was collected from North Carolina State University (NCSU) campus recycling bins. Soiled and glossy pages as well as staples were removed prior to shredding. Collection was performed twice over the course of the experiment because the first UCP sample was inadvertently discarded. The two UCP samples are designated as I and II. Newspaper was collected from free publication kiosks on and near the NCSU campus. Inserts were removed prior to shredding. Both paper samples were shredded using a cross cut paper shredder.

Grass was collected from the NCSU turf grass experimental station by mowing. Grass was stored at 4°C prior to use, with the exception of a sample of grass stored at -85°C and only used in the BMP test. As described in Results, it was suspected that the grass degraded over time during storage at 4°C. As such, subsamples taken after 3 months of storage are

separately designated from earlier subsamples. To evaluate whether grass decomposed during storage at 4°C, a second sample of grass (designated Grass II) was taken from the turf grass lab and has been stored at 4°C for about 7 months. Subsamples were periodically analyzed for cellulose, hemicellulose and lignin content to assess biodegradation during storage

Plastic samples were provided by members of the Plastics Environmental Council (PEC) and cut with scissors to a size comparable to the shredded paper. Two separate samples of thin polyethylene films were provided and are designated as I and II. Thin film I was provided as fully manufactured drapes for surgical microscopes. Polystyrene foam was provided as fully manufactured expanded polystyrene cups. The thick PE sheet and PE film II were provided as flat rolls.

Inocula

Inocula were derived from four sources: biosolids, anaerobically digested organic waste, landfill leachate, and MSW.

Mesophilic biosolids were collected on three occasions from a local municipal wastewater treatment plant. The first two samples were collected within a day of each other and mixed to form the first biosolids inocula. The second sample was acquired to ensure an adequate volume of inocula was loaded into each reactor. The mixing was performed to avoid bias due to any difference between samples. It should be noted that the biosolids underwent thermophilic treatment (50°C to 57°C) stages at the plant before being stored at 45°C with a retention time of 14 days. Mesophilic biosolids were desired to match the incubation

temperature of 37°C used during testing. The risk in using thermophilic biosolids is that the microbial community may not adapt to the mesophilic test conditions or may take exceedingly long to do so. As methane was actively generated in reactors by the time the full treatment history of the biosolids was discovered, it was apparent that they had adequately adapted to the test conditions.

The organic waste digestate was provided by Eden Labs in Albuquerque, NM. A laboratory digester was fed with a mixture of yard and food waste obtained from a composting facility in Albuquerque, NM. The yard waste was partially decomposed prior to feeding to the anaerobic digester. After digestion, a solid sample was obtained from near the digester bottom and a liquid sample was obtained from above the digester's sludge blanket. These two materials were shipped separately and combined for use as an inoculum.

Landfill leachate was pumped from the leachate collection system of a closed landfill in Raleigh, NC. Prior to use, the methanogenic viability of the leachate was verified by placing subsamples of leachate into serum bottles and adding glucose. Methane production after 48 hours indicated the presence of a viable methanogenic community.

Decomposed MSW was obtained from a ~300 L drum maintained under mesophilic conditions at NCSU. The drum contained residential refuse collected from a transfer station in Wake County, NC. Refuse was shredded prior to incubation at 37°C. The refuse was hand sorted before use to remove large non-degradable items including plastic films, cans, glass, and stones. The MSW was considered decomposed and ready for use as an inoculum when methane production was significantly lower than the initial peak. In an attempt to

improve inoculum homogeneity, large non-degradable solids were manually removed from the MSW prior to inoculation.

Landfill Simulation Reactors

Reactors were operated under conditions designed to maximize the rate and extent of decomposition while remaining representative of optimal landfill conditions. Substrates were shredded, methane generation was initiated with an inoculum, leachate was recirculated and neutralized, and nutrient levels were monitored and adjusted as needed. Tests were conducted in a room maintained at 37°C.

Reactor Construction

Reactors were similar in concept to ones previously used to determine the biodegradability of common MSW components (Eleazer et al. 1997). Samples were incubated in 8-L polypropylene mason jars with a sealed, threaded cap and silicone gasket. Jars were modified as shown in Figure 2 by installation of a leachate collection port, liquid inlet, and gas collection port. Gas was collected in flex-foil gas bags fitted with a hose valve. Leachate was collected in a 2-L intravenous bag.

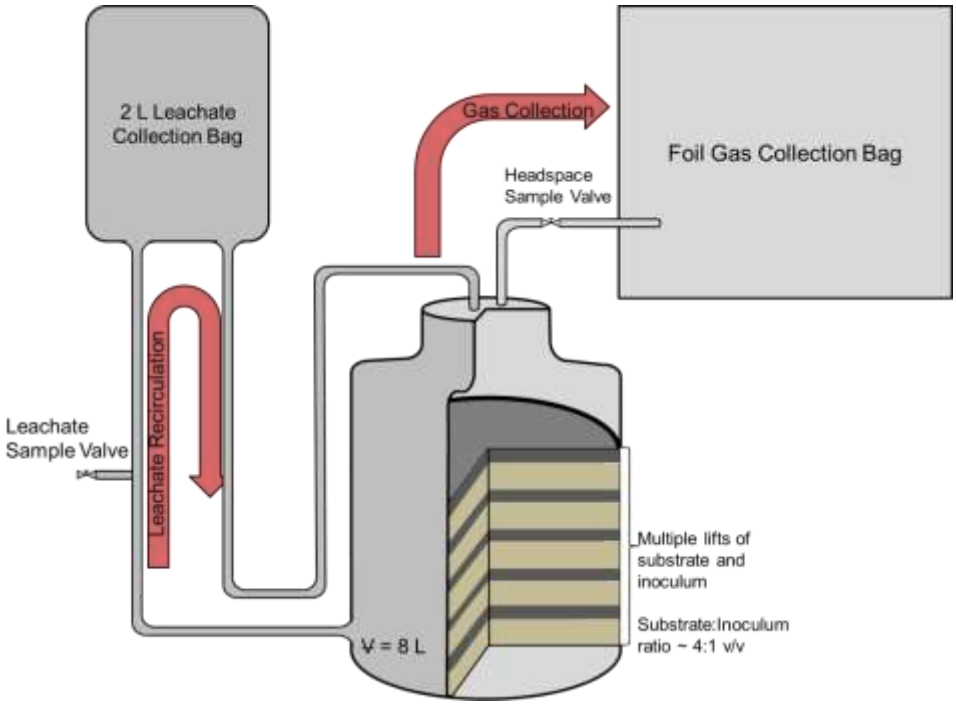


Figure 2 Landfill Simulation Reactor

Assembled reactors were tested for leaks by using a vacuum pump to lower internal pressure to 700 mbar. Reactors experiencing a significant and rapid gain in pressure (more than ~50 mbar in less than 30 minutes) were considered leaky and fixed by replacing fittings or sealing with silicone caulk.

Reactor Loading

Reactors inoculated with solid media were loaded by adding up to 4 alternating layers each of substrate and inocula in a rough volumetric ratio of 4:1 (substrate to inocula) as pictured in Figure 2. Each layer was compacted after addition.

Reactors inoculated with liquids were loaded by adding up to 4 compacted layers of substrate. Equal volumes of inoculum were added to the top of each layer. After substrate was added to all reactors, the remaining inoculum was evenly split between reactors up to the point where additional inoculum would cause the generation of greater than 500 mL of leachate. In cases where inoculum addition was not sufficient to produce 500 mL of leachate, deionized water (DI) was added to reactors until the desired amount of leachate was produced.

A top layer of gravel was added to reactors containing plastics to prevent the substrate from floating and to promote leachate distribution during recirculation. Reactors containing organic waste digestate and reactors containing a grass/biosolids mix did not drain when initially loaded. To facilitate drainage, the reactors were emptied and refilled with a layer of gravel added to the bottom. The addition of gravel was performed on the same day as the initial loading.

Reactors containing plastics were immediately flushed with nitrogen to remove oxygen from the system and wrapped in aluminum foil to prevent photo-oxidation.

Operation and Monitoring

During the initial stages of decomposition, leachate in reactors enters an acid phase as organic acids accumulate. Eventually a balance is reached in the ecosystem of the reactor wherein acid consumption balances acid production. To prevent acid inhibition of methanogens, leachate pH was monitored and adjusted every day until the pH stabilized. Reactors with a pH below 6.7 were adjusted to above that level with 2 M NaOH until the

calculated sodium concentration in the reactor neared 3500 mg/L. Further neutralization, if necessary, was performed with 2 M KOH. After reactors entered an active methanogenic phase, the pH was monitored weekly and then monthly to bimonthly to confirm continued pH stability.

Nutrients in the form of NH_3-N and PO_4-P concentrations were monitored intermittently and adjusted using NH_3Cl and KH_2PO_4 to bring calculated leachate concentrations above targets of 100 mg-N/L and 5 mg-P/L.

Gas was collected in a foil gas sampling bag (SKC Inc, Part Number: NC-2632) and measured from every 3 days to 8 weeks in proportion to gas production. Both the total gas volume and gas composition were measured. Gas volume was determined by evacuating the gas bags into an evacuated cylinder of known volume and monitoring its pressure change. Gas composition was determined using a thermal conductivity detector (TCD) with a SRI gas chromatograph. Gas volumes were corrected to a dry basis at STP and methane production rates and yields are reported as mL CH_4 per dry g of substrate.

When methane production was too low to acquire a sample of sufficient volume from the gas bag, a sample of the reactor headspace was used instead.

Substrate decomposition was considered complete once methane production could no longer be measured. Substrates which did not exhibit significant methane production within 90 days of inoculation were considered complete.

While background methane generation is minimized by using well decomposed inocula at a low inoculum to substrate ratio, some methane attributable to the inoculum is still produced.

Control reactors containing solely the solid inocula were operated to measure background methane. In the case of liquid inocula, controls were run as 100 mL samples in 160 mL serum bottles incubated at 37 °C. The average methane production of the controls was subtracted from the observed methane production of experimental reactors to determine the amount of methane produced from the substrate.

Biochemical Methane Potential

The biochemical methane potential of each substrate was measured in triplicate 160 mL serum bottles filled with dried and ground substrate, liquid media, and an inoculum derived from fresh solid waste. The bottles were incubated at 37°C (Wang et al. 1994). The volume of gas produced was measured approximately monthly using a wetted ground glass syringe. Gas composition was determined with a gas chromatograph. Low (<~5%) methane composition was measured using a flame ionization detector, while other measurements were performed with a TCD. Bottles exhibiting little production were injected with 10 mL of nitrogen to perform GC analysis. After analysis at intermediate timepoints, the headspace was flushed with an 80 %N_2-20% CO_2 mixture and returned to the incubator.

Anaerobic Toxicity Assay

Anaerobic toxicity assays (ATA) were performed to detect inhibition of methane production by the plastics. An ATA, as described by Wang et al. (1997), is performed similar to a BMP but differs in that no media is added to avoid dilution effects, test bottles contain two substrates and an inoculum, and the flushing gas is pure N_2. The two substrates in the test bottles are the test material and a substrate known to degrade anaerobically. Control bottles

contain only the substrate known to degrade. Low methane production relative to controls implies inhibition.

Data Analysis

All reactor methane yields were corrected for background methane generation attributable to the inoculum and converted to a dry basis at STP. The amount of methane in the headspace of each reactor prior to teardown was added to the final cumulative yield measurement and is included in comparisons of ultimate methane yield. The time at which the residual headspace methane was generated is not known and it was applied as a single value at the end of experiment. It was not applied evenly over time because methane production was not constant over the course of the experiment.

Mean values between reactor treatments were compared using statistical inference. Comparisons between two means were performed using unpaired t-tests. The equal variance form of the t-test was used if variances differed by less than a factor of three. Comparisons of equality between greater than 2 means were performed with single factor ANOVA. Comparisons of a specific mean against multiple means were performed by a linear contrast. The null hypothesis in all cases was that means were equivalent. In all interpretations, α has been defined as 5%, therefore p-values less than 0.05 were interpreted as evidence of significant difference.

Substrate decay was modeled as a function of reactive carbon mass by equation 1 given by de la Cruz & Barlaz (2010), where m (kg) is the mass of reactive carbon at time t (yr) and k is the first order decay rate (yr^{-1}).

Reactive carbon is that which will undergo biodegradation. Not all carbon in a substrate will degrade. It may be recalcitrant to hydrolysis, such as unmodified polyethylene, or it may not be bioavailable. For example, cellulose in a newspaper is less anaerobically bioavailable when it is physically surrounded by lignin; only a fraction of the cellulose can be attacked by hydrolytic enzymes, which will result in reduced methane production.

Reactive carbon, by definition, is mineralized to CO_2 and CH_4 and, therefore, the initial mass of reactive carbon m_o can be calculated from the cumulative yield of gas produced, including the correction for residual headspace methane. By the same reasoning, the amount of reactive carbon at any time may be expressed as a function of m_o and the gas yield at that time (m_{CH4} and m_{CO2}) as shown in equation (2).

Equation (1) may be linearized to equation (3) and the first order decay may be determined by linear regression, as illustrated in Figure 3 (de la Cruz & Barlaz 2010). Decay rates for each reactor were determined from methane production data bound by non-zero values closest to 1% and 99% of the measured reactor yield (*i.e.* the approximate middle 98% of the data). This range was selected to constrain the timespan to measurements taken while the substrate was actively degrading. Because the time at which residual headspace methane was produced is unknown, the residual methane correction was not used to calculate the middle 98% of methane production nor was it included as a fitted point. The residual amount of methane was generally small relative to the ultimate yield.

An illustration of a decay rate determination is presented in Figure 3. A majority of R^2 values were above 0.90.

$$\frac{dm}{dt} = -km \quad (1)$$

$$m = m_o - (m_{CH_4} + m_{CO_2}) \quad (2)$$

$$-\mathrm{kt} = \ln(1 - {}^{m_{CH_4}}/_{m_o}) \quad (3)$$

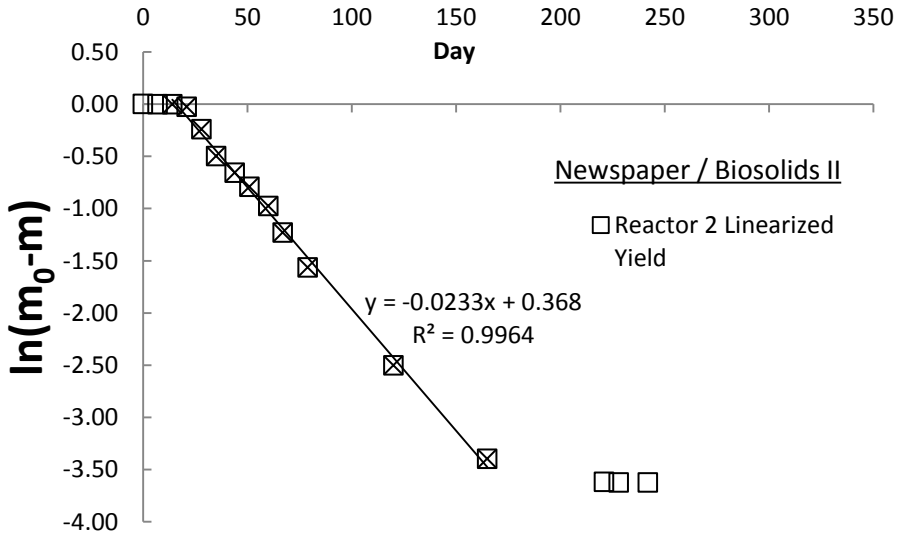


Figure 3 Example Decay Rate Determination

RESULTS

The first part of this chapter presents the performance of the reactors treated with different inocula. Results are organized by substrate in the following order: grass, used copy paper, newspaper, and plastics. Landfill reactor results are followed by BMP and then ATA data.

Landfill Reactors - Performance of Substrates

Methane yields and decay rates for each substrate and inoculum are presented in Table 5, average methane yields over time and methane production rates for each substrate are given in the relevant sections of this chapter.

Table 5 Methane Yields and Decay Rates of Substrates in Landfill Reactors Sorted by Substrate

Substrate	Inoculum	Methane Yield			Decay Rate		
		(ml CH4/g substrate)[a]		CV[b]	k (yr^{-1})[a]		CV[b]
Grass, old	Biosolids II	53	(4.3)	0.08	51	(27)	0.52
Grass, old	Digestate	38[c]	(0.6)	0.02	35[c]	(1.9)	0.05
Grass	Leachate	66	(4.8)	0.07	26	(5.0)	0.19
Grass	MSW	73	(9.3)	0.13	17	(0.18)	0.01
Newspaper	Biosolids II	91	(0.9)	0.01	9.0	(0.56)	0.06
Newspaper	Digestate	121[d]	(3.5)	0.03	4.2[d]	(0.41)	0.10
Newspaper	Leachate	104	(10)	0.10	6.7	(1.7)	0.25
Newspaper	MSW	74	(2.8)	0.04	16	(2.2)	0.14
Used Copy Paper I	Biosolids I	222	(17)	0.08	4.7	(0.73)	0.15
Used Copy Paper II	Biosolids II	100[d]	(46)	0.46	3.2[d,h]	(1.2)	0.38
Used Copy Paper II	Digestate	214[d]	(3.6)	0.02	4.9[d]	(1.7)	0.35
Used Copy Paper I	Leachate	153[c,d]	(1.1)	0.01	3.1[c,d]	(0.29)	0.09
Used Copy Paper I	MSW	216	(24)	0.11	5.7	(0.30)	0.05
Polystyrene Foam	Biosolids II	-0.20[e]	(0.28)	1.41	0	N/A	N/A
Polystyrene Foam	Digestate	0.02[d]	(0.03)	1.19	0[d]	N/A	N/A
Polyethylene Sheet	Leachate	-3.6 [e]	(0.95)	0.27	0	N/A	N/A
Polyethylene Sheet	MSW	-0.24 [e]	(0.06)	0.24	0	N/A	N/A
Polyethylene Film I	MSW	0.96	(0.22)	0.23	0	N/A	N/A
Polyethylene Film II	Biosolids II	-0.09[d, e]	(0.03)	0.35	0[d]	N/A	N/A
Polyethylene Film II	Digestate	1.2	(1.6)	1.41	0	N/A	N/A

a. Average value of three reactors unless otherwise indicated. Standard deviation listed parenthetically.
b. Coefficient of variation - the standard deviation divided by the mean. A lower CV indicates higher reproducibility.
c. The cumulative methane curve for one of the triplicates suggests leakage and this reactor was deleted from the average.
d. Reactors are still running.
e. The negative methane yields indicate that less methane was produced relative to the methane yields from the control containing inoculum only.

Grass

Grass inoculated with either biosolids or digestate yielded less methane relative to yields with either MSW or leachate as the inoculum (Table 5). The trend was true over the course of the experiment, as shown by the average cumulative methane yields in Figure 4. The yields from inoculation with MSW or leachate are not significantly different ($p=0.33$).

The increases in yields at the end of the experiment visible in Figure 4 are a result of accounting for the residual methane in the reactor headspaces at end of the experiment; they do not represent a final burst of methane production. The prominent terminal bump is caused by a combination of large (~5 L) headspaces and methane concentrations (40 to 50%) resulting in a non-trivial residual amount of methane which is further emphasized by the relatively low yields of grass.

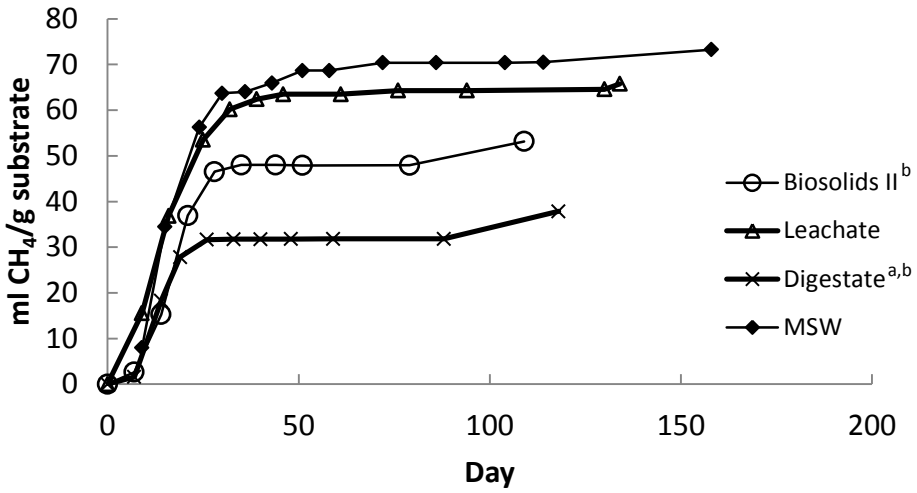


Figure 4 Average Cumulative CH_4 Production of Grass with Various Inocula.

a. The cumulative methane production of one of the triplicates suggests leakage and this reactor was deleted from the average.
b. Older samples stored at 4°C for longer than 3 months.

In addition to methane yield, the rate of decay is an important metric as it has implications for the length of time that a reactor will have to be operated to characterize a substrate. In the case of grass, the decay rate was 17 and 26 yr^{-1} for the MSW and leachate inocula, respectively and was not significantly different ($p=0.13$). The high decay rates correspond to the relatively short period over which the reactors produced methane.

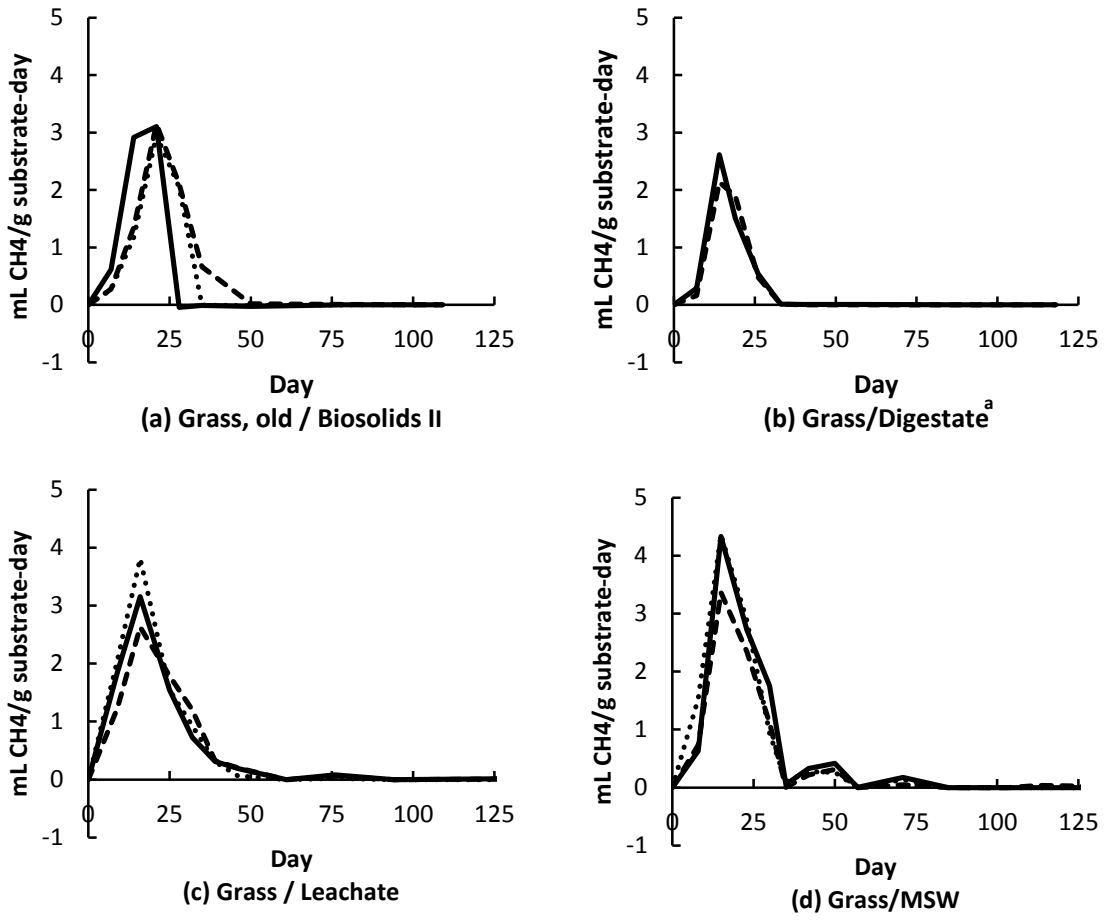


Figure 5 Methane Production Rate of Grass Treated with Different Inocula

a. The cumulative methane production of one of the triplicates suggests leakage and this reactor was excluded from the figure.

One explanation for the lower methane yields associated with biosolids and digestate is that some grass decomposition occurred during storage at 4°C for ~180 days prior to use in reactors. To evaluate whether grass decomposition occurred during storage, a fresh sample of grass was obtained and analyzed as described in Materials. Changes in composition with time are presented in Figure 6 and the data do not support the idea that the grass decomposed

during storage. There is no other apparent reason why the biosolids and digestate inocula would result in a lower yield in the case of grass.

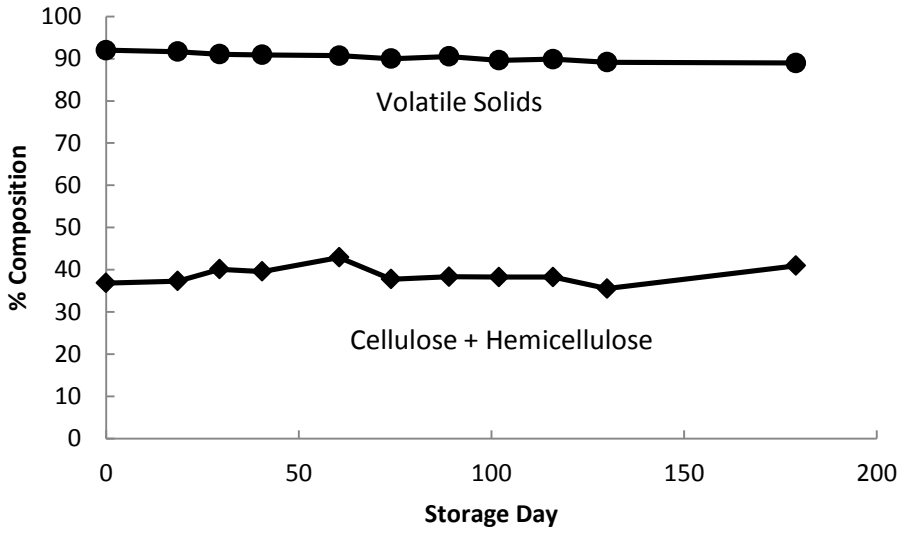


Figure 6 Effect of Storage at 4°C on Grass Composition

Grass presents at least three problems as a potential reference substrate. First, it is probable that methane yields and decay rates vary between grass samples for a number of reasons including differences in grass species, growth stage, and precipitation history prior to collection. Each of those reasons may manifest itself as differences in solids composition such as those shown in Table 6 which compares the second set of fresh grass collected for this research and the grass used in a previous study performed by Eleazer et al. (1997). Interestingly, the grass used in this experiment had a large fraction of organic matter that was not analyzed as cellulose, hemicellulose or lignin in contrast to the samples used by Eleazer et al. (1997). Second, while collecting grass in advance and preserving by drying is possible,

the effect of drying has not been explored to date. Unless grass is collected in advance, it is not available as a substrate in the winter in many regions. Third, the short period of methane production requires frequent (daily, rather than semiweekly to biweekly) gas monitoring to ensure enough data points are collected for meaningful interpretation. As test materials are likely to degrade more slowly than grass, there is little benefit to a reference substrate which degrades in 30 days rather than 90. The only advantage to using grass was that it did not require nutrient supplementation (Table 12, Appendix I).

Table 6 Initial Grass Compositions

Sample	Cellulose %	Hemicellulose %	Lignin %	Volatile Solids %	CHL/VS[a]
Eleazer-1	27	10	28	85	0.77
Eleazer-2	26	15	22	88	0.71
Grass II	22	15	12	92	0.53

a. Ratio of cellulose, hemicellulose, and lignin concentration (CHL) to volatile solids (VS). A ratio of 1.0 would indicate that CHL were the only organics present in the sample.

Used Copy Paper

In the case of copy paper, two different sources were used (I and II) as copy paper I was erroneously discarded. While it is possible that different copy paper samples may cause different results, the performance of the digestate reactors, which contained UCP II, is similar to that of the biosolids I and MSW reactors, which contained UCP I, suggesting that the substrates are similar.

The cumulative methane yields shown in Figure 7 for copy paper I with MSW and biosolids are most comparable because the reactors are complete and these methane yields are similar (p=0.77). As methane production is not complete in the remaining reactors, it is premature to comment on the methane yields.

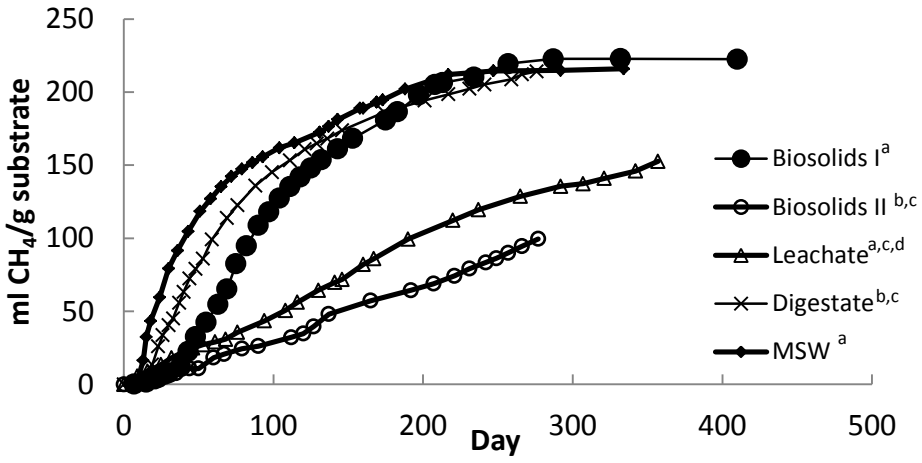


Figure 7 Average Cumulative CH_4 Production of Used Copy Paper I and II with Various Inocula.

a. Used to inoculate UCP I
b. Used to inoculate UCP II
c. Reactors still running.
d. The cumulative methane curve for one of the triplicates suggests leakage and this reactor was deleted from the average.

The average decay rates for the completed copy paper reactors, and the digestate reactor range from 4.7 to 5.7 yr^{-1} and are not significantly different (p=0.65). Although not completed, the digestate reactors were included in this comparison because they have degraded well beyond 3 half-lives and should provide a reasonable estimate of the average

decay rate. This assumption is based on the flattened methane production rates of the reactors (Figure 8c) and an estimated 220 mL CH_4/g based on the biosolids I and MSW yields.

The methane production rates of completed and near completed reactors followed the expected trend of a sharp peak followed by an asymptotic decline, as shown in Figure 8a, c, and e. However, a series of smaller methane production peaks were observed in some reactors, particularly those associated with Biosolids (Figures 8a and 8b). Because the peaks occurred prior to nutrient addition and the release of entrapped gas bubbles would result in only single-point peaks, the cause of the secondary peaks is unexplained. Although the secondary peaks associated with biosolids I account for 16 to 22% of the total yield, the linearized methane production data remains well correlated with the decay rate fits (R^2=0.94).

In the case of leachate, reactors behaved differently from the norm but the methane production rate curves of the two reactors are comparable (Figure 8d); both show a constant rate of moderate (0.5 to 1.0 mL CH_4/g-d) methane production which gradually declines after approximately 200 days. The leachate reactors did not experience an acid phase (Figure 9c) or a clear peak in methane production, which are characteristic of the buildup and consumption of organic acids from hydrolysis as the rate of methanogenesis increases. Although phosphate concentrations were below the target level of 5 mg/L PO_4-P (Table 7), the addition of nutrients to UCP/leachate reactors did not increase methane production rates, which would have been indicated by small peaks in Figure 8d. While hydrolysis appeared to

be limited in UCP/leachate reactors, the data do not conclusively show that hydrolysis was limited by low phosphate concentrations.

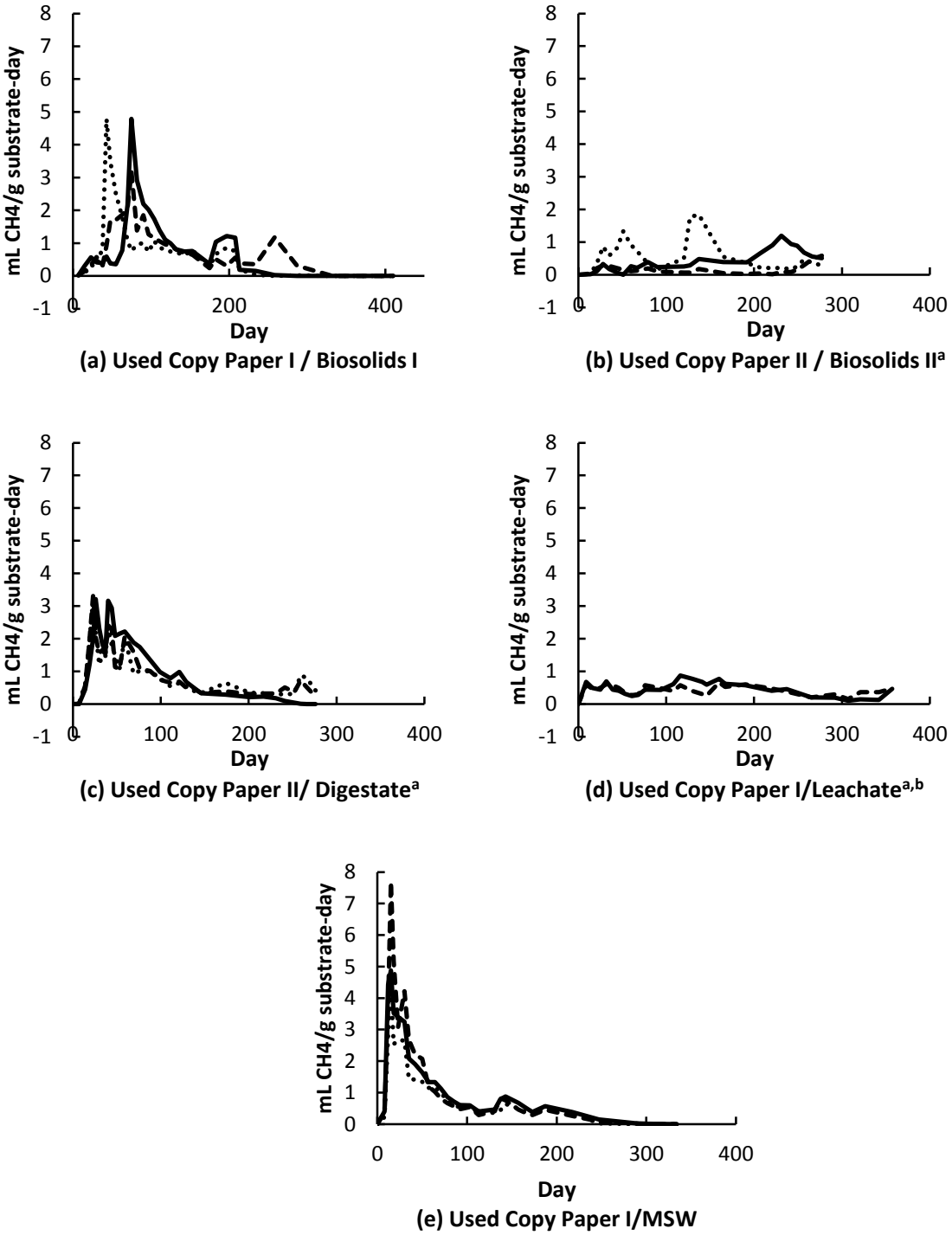


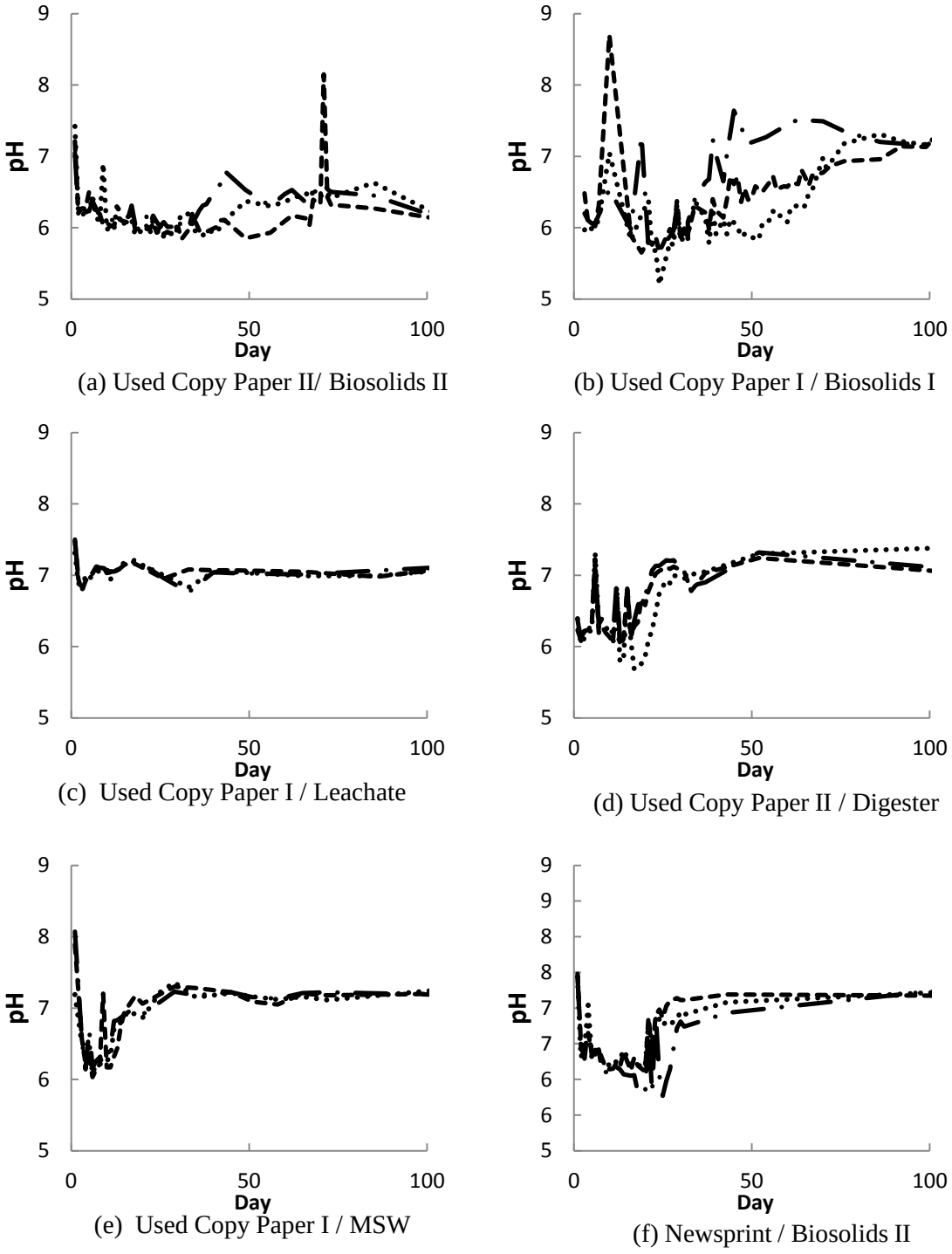
Figure 8 Methane Production Rate of Used Copy Paper Treated with Different Inocula

a. Reactors still running. b. The cumulative methane curve for one of the triplicates suggests leakage and this reactor was not included.

Table 7 Average Nutrient Concentrations of UCP/Leachate Reactors

Experiment Day	63	131	210
NH_3-N (mg/L)	373±10	126±76	16±18
PO_4-P (mg/L)	0.02±0.04	0.3±0.1	0.3±0.1

In the case of biosolids II, UCP reactors did not behave similarly to the norm or to each other. Methane production rates are characterized by either long (100-200 day) lag periods or multiple depressed peaks. The lag periods are associated with prolonged acid phases in which pH stability was not achieved until after an average of 228 days despite daily leachate neutralization until day 43. Neutralization was discontinued after that time as calculated sodium and potassium ion concentrations neared respective toxic concentrations of 3500 and 2500 mg/L of sodium and potassium, respectively (McCarty 1964). The first 100 days of prolonged acid phase are illustrated in Figure 9b and can be contrasted with pH data from other UCP reactors (Figure 9b thru e) and non-UC reactor inoculated with biosolids II (Figure 9f). Because UCP II was not problematic in the digestate reactors and biosolids II was not problematic when used to treat ONP, neither substrate nor inocula appear to be solely responsible for the prolonged acid phase; the cause remains unclear.



(a) Used Copy Paper II/ Biosolids II

(b) Used Copy Paper I / Biosolids I

(c) Used Copy Paper I / Leachate

(d) Used Copy Paper II / Digester

(e) Used Copy Paper I / MSW

(f) Newsprint / Biosolids II

Figure 9 Example Variations in Acid Phase Duration Between Reactors

Newspaper

The average cumulative methane production shown in Figure 10 varied significantly (p=0.0002) between inocula. When inoculated with MSW or digestate, newspaper produced significantly different amounts of methane (p=0.0001). Apparently, the microbial community associated with MSW is less able to degrade newspaper than the communities of other inocula while digestate is more able to fully degrade newspaper than other inocula. Inoculation with leachate or biosolids II produced marginally similar yields (p=0.08) which remain lower than that of the digestate-seeded reactors and higher than that of the MSW-seeded reactors.

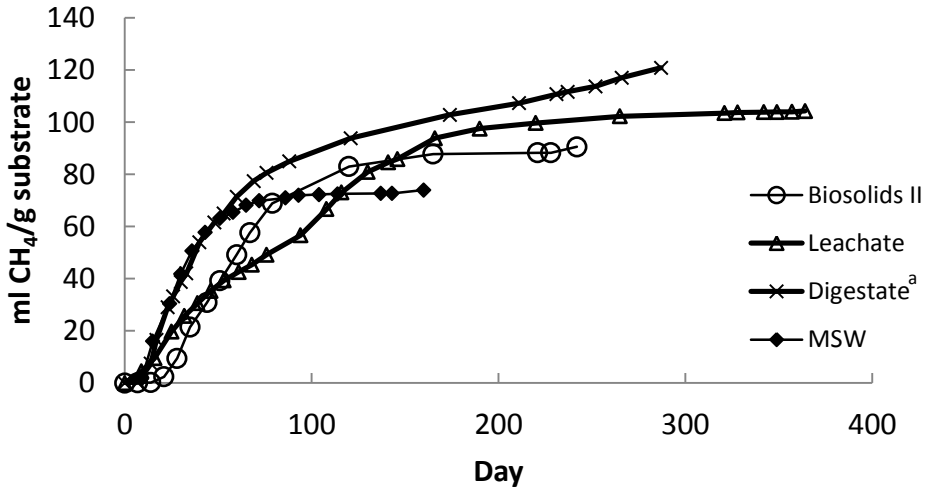


Figure 10 Average Cumulative CH_4 Production of Newspaper with Various Inocula.

a. Reactors still running.

Decay rates for newspaper range from 4.1 to 16 and are significantly different (p=0.0002). MSW, which completed earliest, showed the highest decay rate, while digestate reactors are still actively producing methane.

Since both decay rates and yields of newspaper vary with inocula, it is not surprising that the methane production rate curves also vary (Figure 11). Although inoculation with biosolids II, digestate, or MSW generally produces a peak followed by an asymptotic decline, the treatments differ in the specific size and shape of the peak and decline. As illustrated in Figure 11a and b, biosolids II and digestate had similar peak methane production periods and differed mainly in their period of decelerated methane production. Biosolids II gradually declined to zero methane production, while digestate rapidly fell to a low, but non-zero constant methane production rate. MSW differed from both biosolids II and digestate in that the peak production period lasted approximately half as long and accounted for most of the methane produced, as the asymptotic decline was very sharp (Figure 11d).

Unlike the other inocula, leachate did not result in a true peak period. Instead, methane production rates averaged approximately 0.5 mL CH_4/g-d for six months and then gradually approached zero over an additional six months (Figure 11c). This is similar to the behavior observed when leachate was used to inoculate used copy paper (Figure 8d). The concentrations of phosphate in the leachate reactors were low (Table 8), suggesting nutrient limitation. Nutrients were added only on days 103 and 144, and peaks in methane production occurred on day 108 and 166 (Figure 11c). The peaks could be attributed to the added nutrients. While, degradation in ONP/leachate was likely hydrolysis limited, and the data

suggest that low phosphate concentrations may have been the cause, there is not sufficient data to conclude that this was the case.

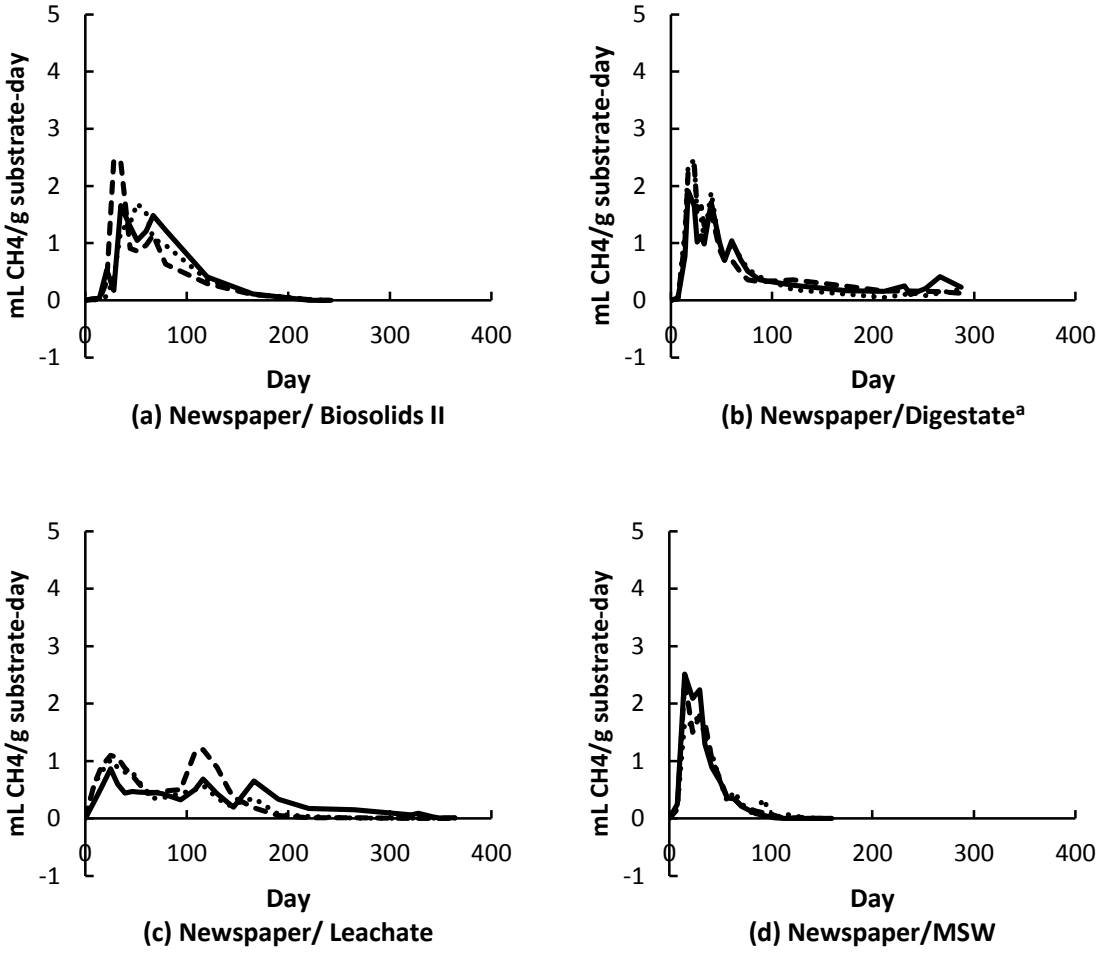


Figure 11 Methane Production Rate of Newspaper Treated with Different Inocula

a. Reactors still running

Table 8 Average Nutrient Concentrations of ONP/Leachate Reactors

Experiment Day	63	131	210
NH_3-N (mg/L)	322±72	205±84	201±39
PO_4-P (mg/L)	0±0	0.3±0	1.7±0.7

Newspaper appears sensitive to inoculum source; with the exception of leachate, the sensitivity mainly reflects differences in behavior after peak methane production. Figure 12 shows the same cumulative yields as Figure 10, but with individual data markers removed and the 'shoulders' corresponding to peak methane production highlighted. The post-peak methane production period for each curve corresponds to the portions to the right of the shoulder. The yields of newspaper immediately after peak production are ~83, 85,93, and 71 mL CH_4/g for biosolids II, digestate, leachate, and MSW, respectively. While digestate and MSW are still at the respective high and low end of the range, the post shoulder yields, unlike ultimate yields, are similar ($p=0.12$).

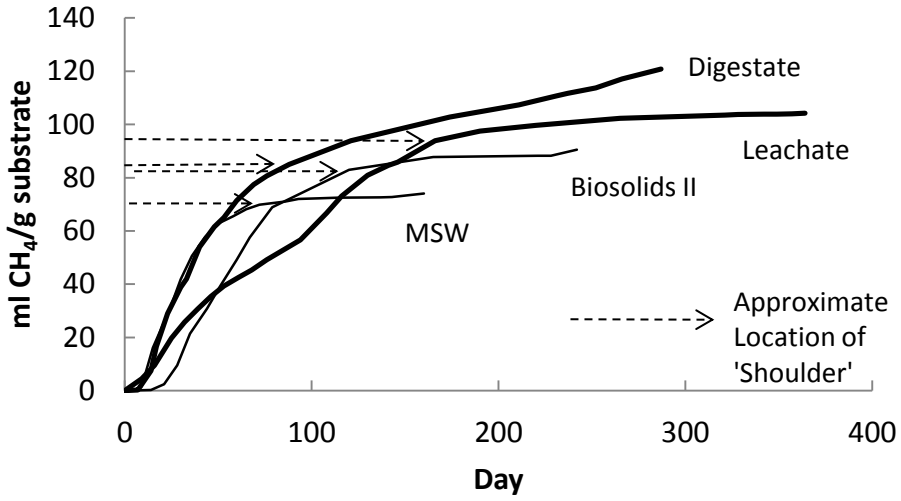


Figure 12 Shoulder Points and Post-Peak Methane Yields of Newspaper with Various Inocula.

The flat line of MSW after the shoulder can be interpreted as all bioavailable substrate being consumed. The linear, but not flat, post shoulder behavior of other curves indicates that hydrolysis of newspaper continued. Since the yields at the shoulder were similar and since MSW produced little methane after the shoulder, it is likely that the post-peak hydrolysis was associated with portions of the newspaper that the MSW was unable to degrade. The differing amounts of methane production after the shoulder may therefore represent the relative ability of inocula to degrade the less bioavailable portions of newspaper.

Continued degradation of less readily available portions of newspaper can also explain the varying asymptotic behaviors shown in the methane production rate curves; the ‘long-tail’ degradation associated with digestate in Figure 11b corresponds to the long, linear post-shoulder yield seen in Figure 12.

Plastics

The methane yields of four plastics were also tested in the reactor system and, as summarized in Table 5, there was no significant methane production from any of the materials with any inocula. In several cases, negative yields were reported. Negative yields may be caused by either inhibition or an artifact of correcting yields for background methane production attributable to the inoculum. Negative yields associated with biosolids II were the only plastic yields significantly different from controls ($p<0.05$).

To further explore whether the plastics were inhibitory, these materials were evaluated in anaerobic toxicity assays that are designed to evaluate whether a substrate inhibits methane generation. The results of the ATA work are presented in a separate section.

Since plastics yielded little to no methane after at least 90 days, the viability of the microbial ecosystem was tested by dosing one member of each reactor with a glucose solution. Control reactors were also spiked in the case of solid inocula. Methane generation attributable to the glucose varied and is listed in Table 9. All spiked reactors exhibited a pH drop and most showed an increased methane concentration in comparison with the non-spiked reactors (Appendix II). Production of methane from glucose indicates that the reactors were inactive due to the lack of a biodegradable substrate, and not due to the lack of a viable microbial ecosystem.

Table 9 Glucose Spike Methane Recovery

Substrate	Inoculum	Glucose Dose (g)	Theoretical Methane ($ml\ CH_4$)	Methane Recovery[b] ($ml\ CH_4$)	%
Control	MSW	5.4	2003	694	35%
Thick Polyethylene	MSW	5.4	2003	63	3%
Thin Polyethylene I	MSW	5.4	2003	227	11%
Control	Eden	7.1	2668	149	6%
Thin Polyethylene II	Eden	7.1	2668	157	6%
Polystyrene Foam[a]	Eden	7.1	2668	67	2%
Thick Polyethylene	Leachate	5.4	2003	128	6%
Thin Polyethylene II[a]	Biosolids II	7.1	2668	3	0%
Polystyrene Foam	Biosolids II	7.1	2668	84	3%

a. Reactors not yet taken down. As headspace methane is not accounted for, the actual recovery rate may be higher.
b. Headspace not flushed before spike. Methane present assumed to be derived from glucose. Recovery is based only on gas production and does not address dissolved or intermediate species.

While very little methane was recovered in case of thin PE II inoculated with biosolids II, the pH drop and rebound observable in Figure 25, Appendix II suggests that glucose was metabolized to organic acids and these organic acids were removed from the system. In addition, the fact that all other reactors inoculated with biosolids produced methane implies that the inoculum was capable of methane production at the beginning of the experiment. The low recovery of methane from the spiked reactor may be underestimated as the methane in the headspace has not yet been accounted for. However, it should be noted that the negative plastic yields from bioreactors were statistically ($p<0.05$) different from controls.

Glucose spike testing was originally intended to be qualitative. The reduced amount of methane observed compared to the theoretical yield from added glucose may indicate partial inhibition, but the test was not originally designed for that analysis. In particular, the headspaces of the reactors were not flushed prior to the spike and gas measurements were not taken as frequently as would be appropriate. Background methane attributable to the inoculum was based on methane production from the appropriate controls. Although the pH did rapidly drop during the tests, the fact that it also returned to neutral indicates that the microbial community was viable.

Biochemical Methane Potential

The BMP of each substrate after 254 days of incubation at 37°C and 121 days of incubation at 52°C are presented in Table 10. The average yields from landfill reactors are also presented for comparison and are the same as those listed in Table 5.

Table 10 Biochemical Methane Potentials at Day 254

Substrate	Gas Yield (ml CH_4 / g substrate)[a]							
	BMP-37°C		BMP-52°C[e]		Biosolids[b]	Digestate	Leachate	MSW
Used Copy Paper[c]	166[II]	(1)			222[I], 100[II]	214[II]	153[I]	216[I]
Newspaper	103	(3)			91	121	104	74
Grass	65	(3)			53[d]	38[d]	66	73
PS Foam	2.1	(0.6)	13	(0.1)	-0.2	0.02		
Thin PE Film I	1.3	(0.4)	8	(0.2)				0.96
Thin PE Film II	0	N/A			-0.09	1.2		
Thick PE Sheet	0	N/A					-3.6	-0.24

a. Average value of 3 tests. Standard deviation presented in parenthesis.
b. Biosolids II, except in the case of Used Copy Paper I
c. Used copy paper sample is denoted with the superscript I and II
d. Old grass sample
e. Data provided by Yinglong Guan (unpublished)

The used copy paper BMP results are unusual; the yield is below the average measured in landfill reactors and it is generally expected that a BMP test will result in higher methane yields than a landfill reactor. The cause of this result is unknown.

With the exception of leachate ($p=0.96$), yields from newspaper reactors were significantly different from the BMP ($p<=0.05$). As the BMP inoculum is cultured using ground MSW as a substrate, it is interesting that the yield more closely reflects that of leachate than MSW. Because the BMP culture has a low solids content, this may be due to a lack of hydrolytic bacteria strongly attached to a solid matrix. An alternative explanation is that the substrate in finely ground in a BMP but is coarsely shredded in a landfill reactor; the ground substrate may be more bioavailable and therefore produce more methane.

Unlike copy paper and newspaper, yields from landfill reactors containing fresh grass were not significantly different from the BMP ($p=0.36$).

Although the first sample of PE film produced some methane in a BMP test, 1.3 mL CH_4/g is well below the theoretical amount of 1200 mL CH_4/g predicted by the Buswell equation (Buswell & Mueller 1952). The same argument holds for the small amount of methane produced by the PS foam. The other plastics did not produce methane significantly ($p>0.05$) different than the controls, which is similar to results from landfill reactors in that the plastics did not degrade.

Increased incubation temperature did result in increased methane production from the PS foam and a PE film. Unlike the mesophilic test, which used a culture fed with ground MSW, the thermophilic tests were inoculated with thermophilic biosolids from a wastewater treatment plant. While it is possible that the difference in inocula is the cause of the different methane yields, the results from landfill reactor tests do not support that interpretation. It is more likely that the difference in temperature is responsible for increased methane production. As explained in the background section, increased degradation at higher temperatures could be caused by either thermally activated oxidation or by decreased plastic crystallinity. While the methane produced remains insignificant in comparison to the theoretical yield, the difference between the two results illustrates the effect of temperature on the results of tests of plastic degradation. The potential inhibitory effects seen with biosolids II were not observed in the thermophilic BMP test. While sampled at different points in treatment, inocula from each test were obtained from the same treatment plant.

Anaerobic Toxicity

The results of incubating plastics for 83 days in an anaerobic toxicity assay test are presented in Table 11. Some bottles have results reported for 208 days of incubation because they were allowed to continue incubating throughout this research and were tested for additional methane production prior to measuring pH. Polyethylene film I appears to inhibit methane production at low dilution. Interestingly, this was the only polyethylene substrate to produce even a small amount of methane in the BMP test at 52°C (Table 10). One explanation for the difference is that the high moisture level of BMP led to dilution of inhibitory compounds. However, that hypothesis does not explain the similar positive yield when the plastic was tested in landfill reactors where the liquid to solid ratio is such that dilution would not be significant (Table 5). It should also be noted that although it does not explain the lack of inhibition, both positive yields are small (~0.1% of theoretical).

Reproducibility between tests was poor in the case of PE II and PS. While the average methane yield from either set of bottles was approximately that of copy paper, individual bottles either produced more or less methane than the two controls.

The pH of each bottle except for PS-A was measured at the end of this research to gain insight regarding the test results. All but one (PE-Film II A) were either below pH 6.1 or above pH 8.2, well outside the optimum range for methanogenesis. Interestingly, pH extremes and variability do not always correspond with either inhibited bottles, as shown with PS, or with differences between replicates, as shown with PE I. As the pH was not measured over the course of the experiment, it is unknown if the final pH accurately

characterizes the environment of the bottles during the main phase of methane production. Regardless of the cause or effect, the variable and extreme pH values are undesirable and suggest that the ATA test, as performed, is flawed.

The low reproducibility between replicates, presence of a failed control, non-neutral pH of many bottles, and discrepancy between this test and the landfill reactor and BMP tests casts doubt on the ATA results. Additional ATA experiments, perhaps modified to better maintain neutral pH, should be performed to better evaluate potential inhibition.

Perhaps the most interesting result is the high methane yield in PS-A which is 50% greater than the theoretical yield (~300 mL/g) of the used copy paper and may indicate degradation of the PS. The result is being tested by treating a fresh sample of the PS in a BMP-style test using an inoculum derived from contents of the PS-A bottle.

Table 11 Anaerobic Toxicity Test of Modified Plastics

Test Bottle	Periodic Methane Production (ml) Test Period (days)				Total Methane Yield (ml/g UCP)	T-Test[a]	Average Methane Yield (ml/g UCP)[d]	CV[b]	pH[e]
	0 to 15	16 to 50	51 to 83	84 to 208[f]					
Control A	4.7	16	1.2	3.95	204				8.48
Control B	6.2	25	0.47	0.34	250	N/A	227 (23)	0.10	8.64
Control C[c]	3.1	0.61	0	nm	30				5.57
PE Film I A	3.1	0.86	0.02	nm	31				8.31
PE Film I B	4.0	1.2	0.06	nm	42	0.04	34 (5)	0.15	5.60
PE Film I C	3.6	0.1	0	nm	30				8.63
PE Film II A	7.3	7.1	29	nm	344				7.89
PE Film II B	5.7	2.1	0.29	nm	64	0.42	248 (130)	0.53	8.22
PE Film II C	6.2	34	1.7	nm	337				8.22
PE Sheet A	3.5	19	3.4	nm	204				5.75
PE Sheet B	4.1	16	12	nm	253	0.35	239 (26)	0.11	5.68
PE Sheet C	2.7	24	6.0	nm	261				5.34
PS Foam A	0.3	11	46	3.7	480				nm[g]
PS Foam B	0	1	0.3	0.1	10	0.37	166 (221)	1.33	6.03
PS Foam C	0	1	0.3	0	9				5.90

a. Unpaired one tailed test of null hypothesis that yield is greater than or equal to control.
b. Coefficient of variation - normalized measure of spread obtained by dividing standard deviation by mean. Lower value indicates less spread.
c. Probable failed bottle, not used in calculations
d. Standard deviation in parenthesis.
e. pH of bottle contents at end of experiment. In order to extract sufficient liquid, non-control bottles were rinsed with a small (<20 mL) amount of DI prior to pH measurement.
f. Prior to measuring pH, bottles were tested for final methane production if overpressure was > 5 mL, nm indicates bottle was not measured.
g. Not measured –to generate an inoculum for another test, bottle contents were treated with BMP media, which eliminated the ability to measure pH.

DISCUSSION AND RECOMMENDATIONS

The goal of this research was to determine the effect of inoculum source on tests of anaerobic biodegradation. The behavior of different inocula is discussed first and this is followed by some practical observations regarding inocula and substrates. The final section of this chapter recommends substrate and inocula pairs based on desired test characteristics.

Comparison of the Behavior of Alternate Inocula on the Same Substrate

The objective of this section is to evaluate the extent to which there are trends in the behavior of the different inocula. For example, if the decay rate was consistently higher for one of the tested inocula, this could suggest that it is the most desirable for testing.

In Figure 13, decay rates were normalized by dividing by the maximum rate on a per substrate basis. Paper substrates (*i.e.* UCP and ONP) inoculated with MSW are associated with decay rates near the maximum. However, it should be noted that the high decay rate of ONP when inoculated with MSW is probably caused by the lower methane yield (and implied lower extent of degradation) of that combination. While inoculum source affects decay rate, there is no clear trend independent of substrate.

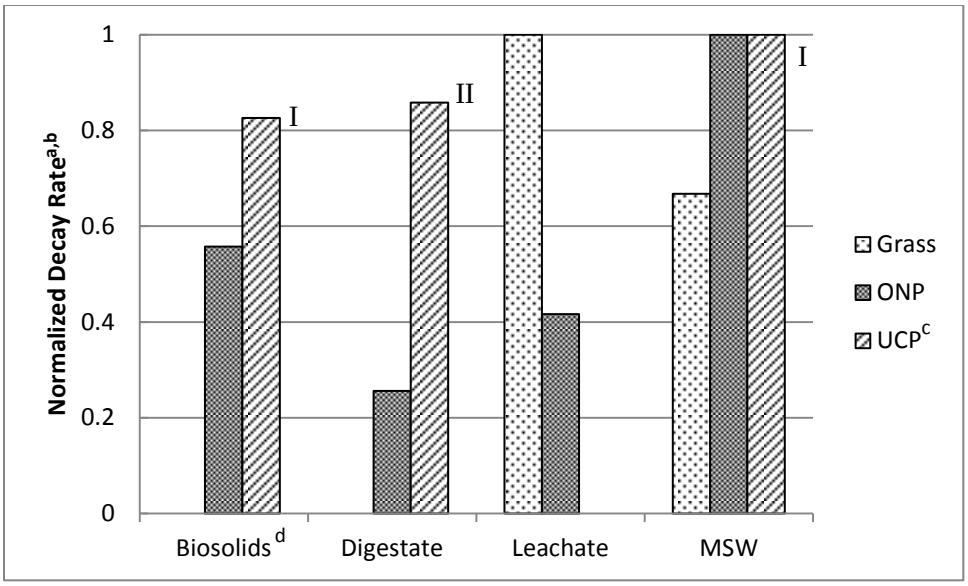


Figure 13 Normalized Decay Rates for Inocula/Substrate Pairs

a. To allow comparisons between substrates, the rates have been normalized to unity by dividing by the maximum rate on a per substrate basis.

b. Results have not been presented for old grass, reactor sets which have not yet degraded beyond 3 half-lives, or substrates which did not degrade.

c. The sample of UCP (I or II) is designated next to the column for the appropriate reactor set.

d. Biosolids I and II in the case of UCP and ONP, respectively.

Methane yields for each inoculum and substrate pair have been normalized by dividing by the maximum yield on a per substrate basis and are shown in Figure 14. As in the case of decay rates, no inoculum exhibited a consistent effect independent of substrate.

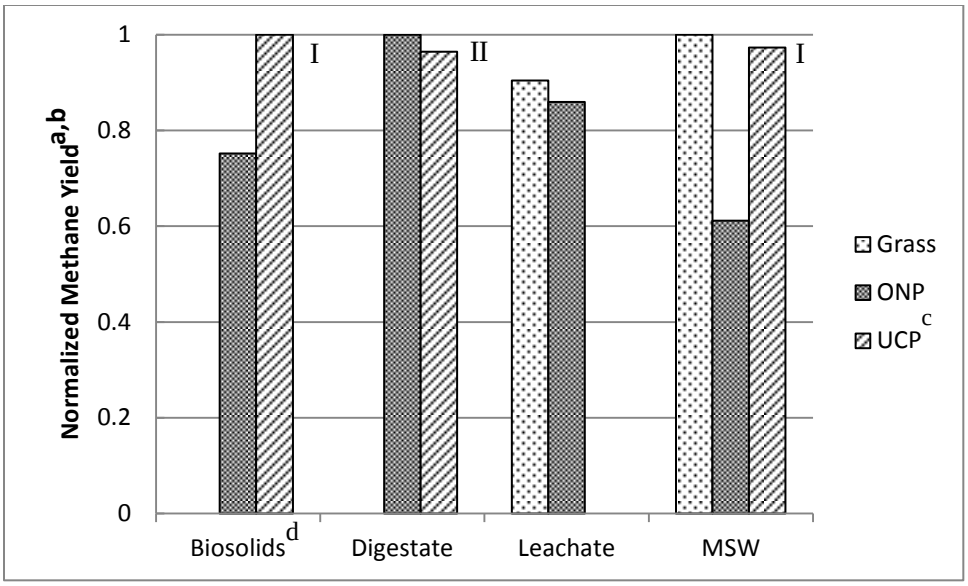


Figure 14 Normalized Methane Yields for Inocula/Substrate Pairs

a. Yields have been normalized to unity by dividing by the maximum yield .

b. Results have not been presented for old grass, reactor sets which have not yet degraded beyond 3 half-lives, or substrates which did not degrade.

c. The sample of UCP (I or II) is designated next to the column for the appropriate reactor set.

d. Biosolids I and II in the case of UCP and ONP, respectively.

While inocula did not exhibit trends in gas yield or rate, a trend in reproducibility is apparent; digestate yields were consistently reproducible (Figure 15). While the reproducibility of other individual combinations (*e.g* biosolids/ONP) may have been better, no other inoculum was as consistent between substrates. This trend does not hold for the reproducibility of plastics listed in Table 5. The poor reproducibility of plastics is, however, due to the very low yields of those reactors and is not attributable to inoculum source.

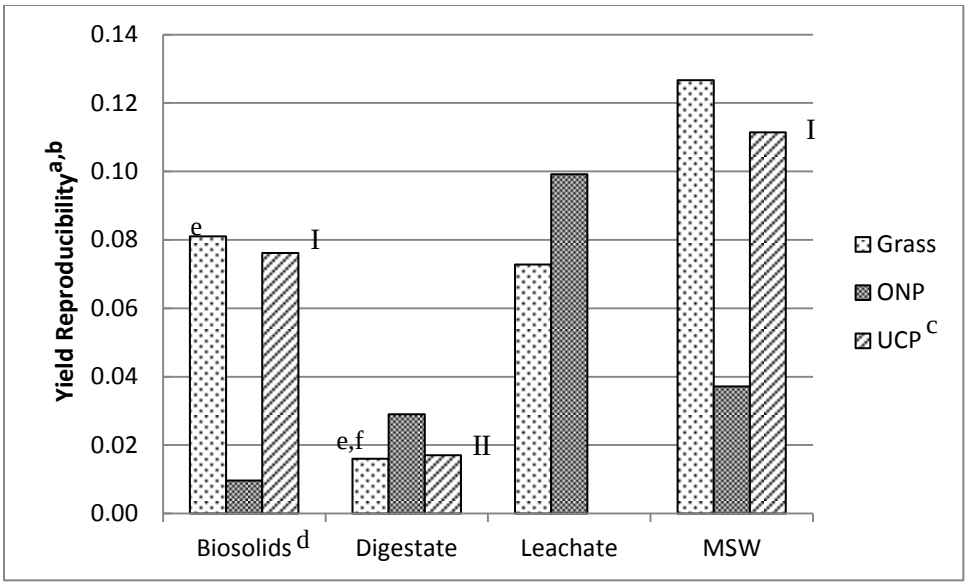


Figure 15 Reproducibility of Methane Yields for Inocula/Substrate Pairs

a. Reproducibility as measured by coefficient of variation (the standard deviation divided by the mean). A lower CV indicates higher reproducibility.
b. Results have not been presented for reactor sets which have not yet degraded beyond 3 half-lives or substrates which did not degrade. As the values have not been normalized, old grass data have been included.
c. The sample of UCP (I or II) is designated next to the column for the appropriate reactor set.
d. Biosolids I in the case of UCP and biosolids II for ONP and grass.
e. Old grass sample.
f. Cumulative methane production of one of the triplicates suggests leakage and this reactor was deleted from the average.

Although not as apparent as the trend in yield reproducibility, there was also a trend in decay rate reproducibility. Even though it had the second worst ONP reproducibility, MSW was the only inoculum in which the reproducibility of every decay rate was below 0.15 (Figure 16). With the exception of grass, decay rates associated with biosolids were also below 0.15. The reproducibility of the grass decay rate was high (>0.5) for biosolids and variable between inocula. The short degradation time (and, therefore small amount of data) of grass may be one reason for such variability.

Methane production rates are related to decay rates, and it is reasonable to assume that similar methane production rate curves, such as those for UCP/digestate in Figure 8c, correlate to reproducible decay rates. However, the reproducibility of UCP/digestate decay rates is the worst (>0.2) of the three listed. The low reproducibility is because although all three reactors experienced similar peak behavior, one reactor, represented by the solid line in in Figure 8 c, completed at least 50 days earlier than the others and has a correspondingly higher decay rate (7.8 yr^{-1}). The other two reactors, which are still active, experienced a longer and 'thicker' asymptotic decline and have decay rates of 5 and 4.4 yr^{-1}.

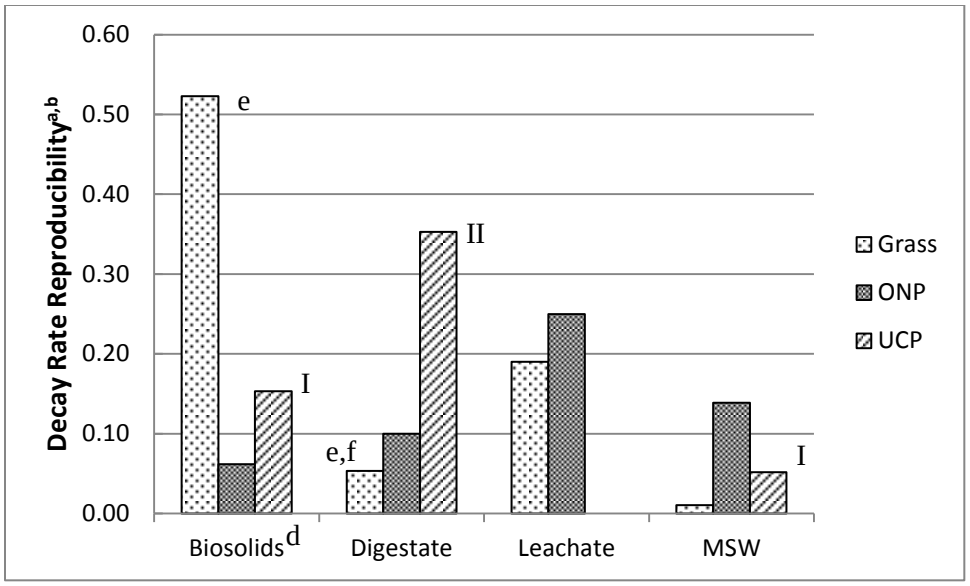


Figure 16 Reproducibility of Decay Rates for Inocula/Substrate Pairs

a. Reproducibility as measured by coefficient of variation (the standard deviation divided by the mean). A lower CV indicates higher reproducibility.
b. Results have not been presented for reactor sets which have not yet degraded beyond 3 half-lives or substrates which did not degrade As the values have not been normalized, old grass data has been included.
c. The sample of UCP (I or II) is designated next to the column for the appropriate reactor set.
d. Biosolids I in the case of UCP and biosolids II for ONP and grass.
e. Old grass sample.
f. The cumulative methane production of one of the triplicates suggests leakage and this reactor was deleted from the average.

Decay rate calculations do not include lag times so they are not ideal estimators of how long an experiment must run. An alternative metric is the time required to reach three half-lives where this time includes any lag time associated with the initiation of methane production. It is assumed that after three half-lives of degradation enough data has been collected to provide reasonable estimates of decay rates. As illustrated in Figure 17, the duration of an experiment is usually influenced by both substrate and inoculum. In the case of paper substrates, digestate experiment time appears independent of substrate. The time to complete

MSW experiments was consistently short, with every reactor set completing in less than 6 months and with a low variance between triplicate reactors.

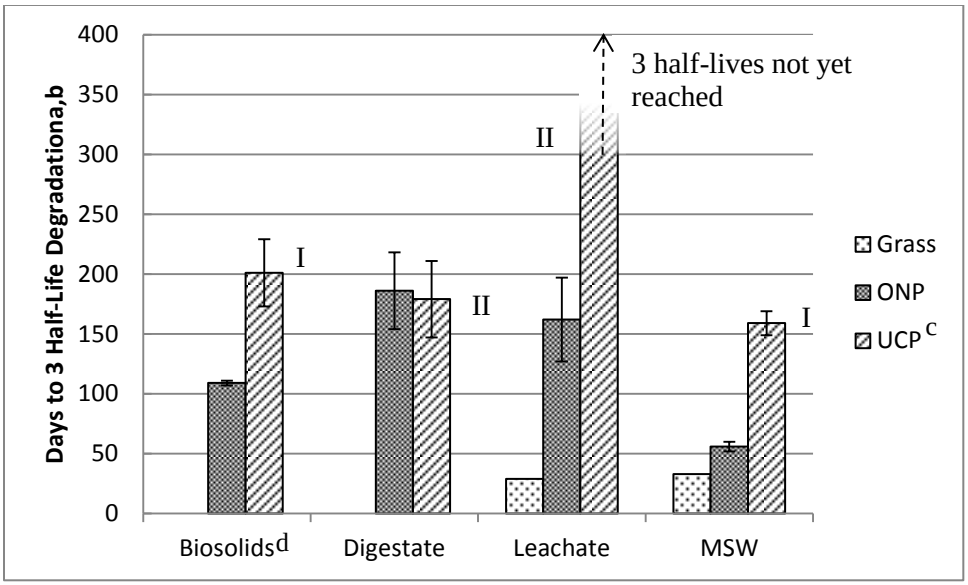


Figure 17 Time to Reach 3 Half-lives of Degradation for Inocula/Substrate Pairs

a. Number of days to reach to 87.5% (*i.e.* three half-lives) of total methane yield. Bars are 1 standard deviation.
b. Results have not been presented for old grass, reactor sets which have not yet degraded beyond 3 half-lives or substrates which did not degrade. In the case of incomplete UCP reactors, the maximum methane yield was estimated to be 215 mL CH_4/dry mg.
c. The sample of UCP (I or II) is designated next to the column for the appropriate reactor set.
d. Biosolids I in the case of UCP and biosolids II for ONP.

Practical Observations

Both of the liquid inocula (leachate, biosolids) are widely available and required no laboratory pre-digestion or maintenance, but both require more labor during the experiment. While the nutrient limitation suspected in the case of landfill leachate is not conclusively proven by the data, it would be prudent to frequently monitor and adjust nutrient levels. This

may have to be performed more often than practical. Biosolids do not require as frequent nutrient adjustment, but are associated with longer acid phases during which the reactor leachate pH must be adjusted daily.

MSW and both liquid inocula are only available for use if there is a nearby cooperative landfill or wastewater treatment plant. In contrast, the organic waste digestate does not require a third party, but, like MSW, requires maintaining an active culture of inocula. Unlike MSW, the digestate was homogenous and did not require manual removal of large non-degradable objects prior to inoculation.

The specific combination of used copy paper and biosolids resulted in poor reactor performance. Reactors containing this combination experienced long periods of acid production before significant methane production and the second set of these reactors were unstable to the point where the data they produced were mainly usable as an indicator that copy paper and biosolids should not be used.

Used copy paper is widely available but was the only substrate to result in unstable reactors. It produces a large amount of gas, which allows for easier measurement and less noise caused by experimental error. However, unless inoculated with digestate, used copy paper required at least as twice as long an experiment duration as other substrates.

Old newspaper produces a moderate amount of gas over a moderate time span when compared to the other substrates. However, newspaper was the substrate most affected by inoculum source.

Recommendations

The major tradeoffs in selection of anaerobic test substrates and inocula are between reproducibility, experiment time, ease of inocula use, and flexibility in inocula choice.

If reproducibility is to be maximized while still allowing a choice of inoculum, either MSW or digestate are suggested as inocula and copy paper should be selected as a substrate. The potential instability of copy paper was not observed with MSW and digestate, and methane yields and production rates from copy paper were not sensitive to inoculum choice. While biosolids exhibited good reproducibility when used with newspaper, the sensitivity of newspaper results to inoculum makes this an undesirable choice. In terms of methane yield, digestate is more reproducible than MSW while the converse is true in the case of decay rate determination.

While grass provided the shortest experiment time, it cannot be considered a suitable substrate for the reasons explained in Results. Aside from grass, newspaper inoculated with MSW provides the shortest duration experiments. The drawback to combining MSW and newspaper is that the results are significantly different from those of newspaper combined with other inocula. Additionally, the duration is shorter than the three months recommended before concluding that plastic will not degrade. The combinations which satisfy the most criteria are copy paper inoculated with either digestate or MSW. As such, copy paper inoculated with MSW or digestate, while taking longer, is the suggested combination.

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APPENDICES

APPENDIX I – NUTRIENTS

Nitrogen, in the form of ammonia, and phosphorous, in the form of phosphate were monitored the measured concentrations are presented in by substrate Table 12 through Table 18. Reactors with concentrations below 100 mg/L NH_3-N and 5 mg/L PO_4-P were adjusted as described in Experimental Methods.

Table 12 Nutrient Concentrations of Leachate from Grass Reactors

Experiment Day		7	18	21	28	37	63	122
Inoculum	Nutrient (mg/L)[a]							
Biosolids II[d]	NH_3-N	1280[b]		1075±35[c]	1160±137			
	PO_4-P	135[b]		130±18[c]	73±10			
Digestate[d]	NH_3-N		483±10			503±14		
	PO_4-P		35±3			28±3		
Leachate	NH_3-N						1120±166	
	PO_4-P						26±1.5	
MSW	NH_3-N							891±101
	PO_4-P							24±2.5

a. Average of 3 values, standard deviation follows ±.
b. Only one reactor measured.
c. Two reactors measured, excludes reactors measured on day 7.
d. Grass sample was stored at 4°C for longer than 3 months.

Table 13 Nutrient Concentrations of Leachate from Used Copy Paper Reactors

Experiment Day		0-25	26-50	51-101	101-150	151-200	201-300
Inoculum	Nutrient (mg/L)[a]						
Biosolids I	NH_3-N					27±35	197±114
	PO_4-P					10±6	21±2
Biosolids II	NH_3-N	253±79	11±14		51±47		
	PO_4-P	0.3±0.3	1.5±1.0		3±0		
Digestate	NH_3-N	0.4±0.2	5.3±6.7	483±10	4.3±3.2		
	PO_4-P	0.4±0.6	36.7 ±30.4	35±3	6.0±0		
Leachate	NH_3-N			373±10	126±76		16±18
	PO_4-P			0.02±0.04	0.3±0.1		0.3±0.1
MSW	NH_3-N				2.3±1.5	4.7±2.9	66±65
	PO_4-P				0.8±0.2	1.4±0.3	8.3±2.0

a. Average of 3 values, standard deviation follows ±.

Table 14 Nutrient Concentrations of Leachate from Newspaper Reactors

Experiment Day		0-25	26-50	51-101	101-150	151-200	201-300
Inoculum	Nutrient (mg/L)[a]						
Biosolids II	NH_3-N	239±42	50±56		27±42		
	PO_4-P	3.3±1.5	3±2		9.3±10		
Digestate	NH_3-N	1.6±2.1	19±25		11±14		
	PO_4-P	0.8±0.2	13±1		2.4±1.1		
Leachate	NH_3-N			322±72	205±84		201±39
	PO_4-P			0±0	0.3±0		1.7±0.7
MSW	NH_3-N				124±77		
	PO_4-P				1.1±0.6		

a. Average of 3 values, standard deviation follows ±.

Table 15 Nutrient Concentrations of Leachate from PS Foam Reactors

Experiment Day		0-25	26-50
Inoculum	Nutrient (mg/L)[a]		
Biosolids II	NH_3-N	624±63	481±214
	PO_4-P	64±7	56±17
Digestate	NH_3-N	61±12	91±37
	PO_4-P	1.2±0.7	4.7±1.5

a. Average of 3 values, standard deviation follows ±.

Table 16 Nutrient Concentrations of Leachate from Thick PE Sheet Reactors

Experiment Day		51-101	101-150
Inoculum	Nutrient (mg/L)[a]		
Leachate	NH_3-N	754±108	
	PO_4-P	4.0±0.8	
MSW	NH_3-N		0.9±1.0
	PO_4-P		0.6±0.2

a. Average of 3 values, standard deviation follows ±.

Table 17 Nutrient Concentrations of Leachate from Thin PE Film Reactors

Experiment Day		0-19	20-50	51-150	101-150
Inoculum	Nutrient (mg/L)[a]				
Biosolids II (Film II)	NH_3-N	728±63			
	PO_4-P	42±20			
Digestate (Film II)	NH_3-N	20±3.8	99±7		
	PO_4-P	0.2±0.1	5.3±0.6		
MSW (Film I)	NH_3-N				272±72
	PO_4-P				4.6±0.3

a. Average of 3 values, standard deviation follows ±.

Table 18 Nutrient Concentrations of Leachate from Control Reactors

Experiment Day		0-19	20-50	51-150	101-150
Inoculum	Nutrient (mg/L)[a]				
Digestate	NH_3-N	93±9	65±21		
	PO_4-P	0.9±0.2	7.7±1.5		
MSW	NH_3-N				685±58
	PO_4-P				8.2±1.9

a. Average of 3 values, standard deviation follows ±.

APPENDIX II – GLUCOSE SPIKES

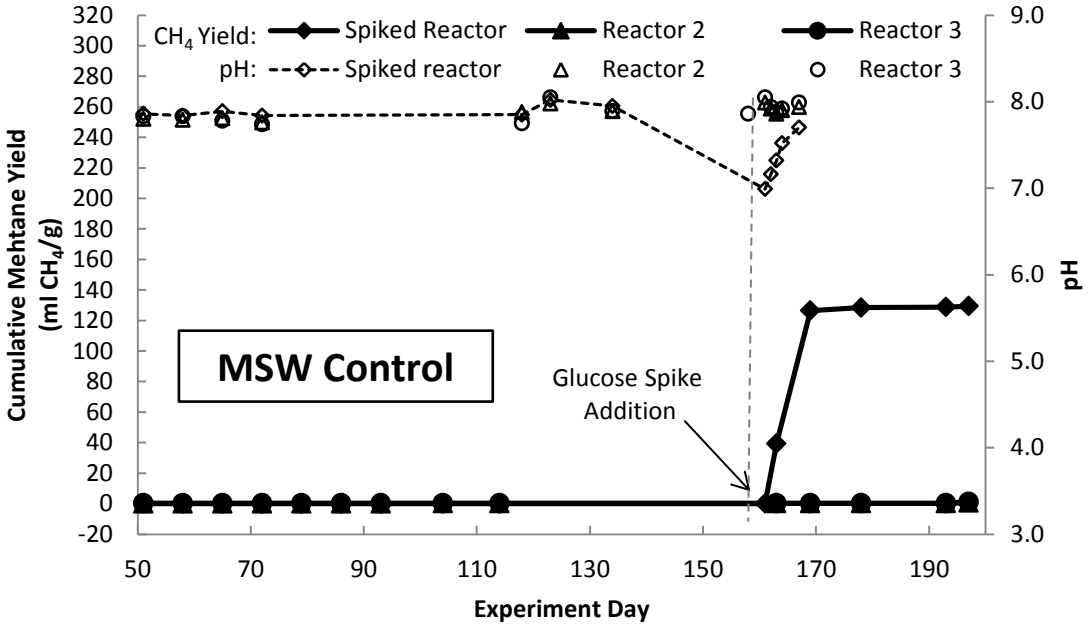

Figure 18 Glucose Spike Effects on MSW/Control Reactors

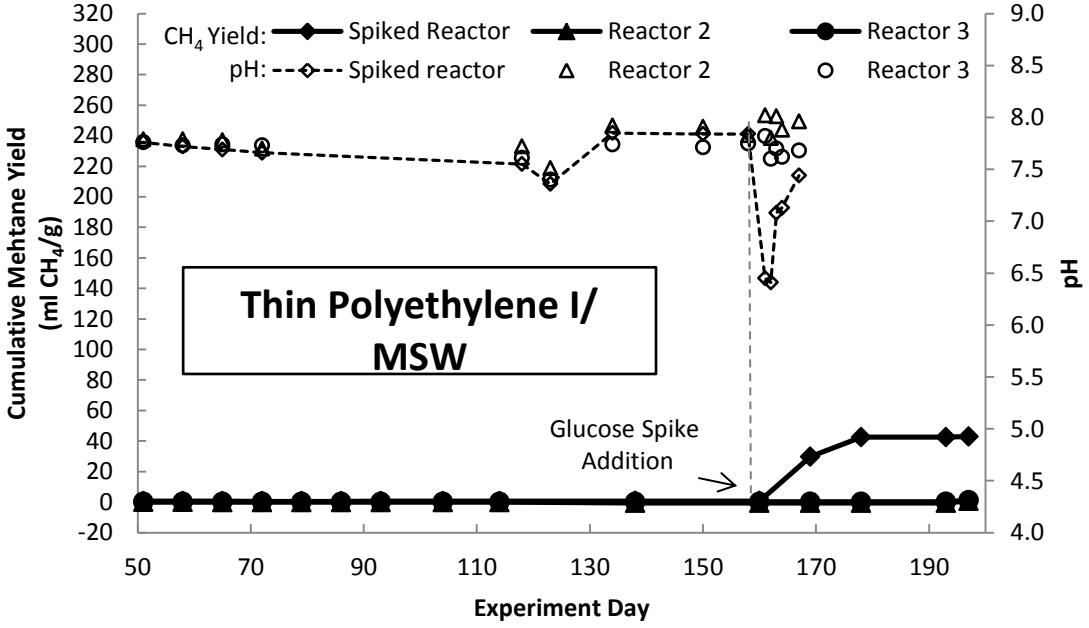

Figure 19 Glucose Spike Effects on Thin Polyethylene I/MSW Reactors

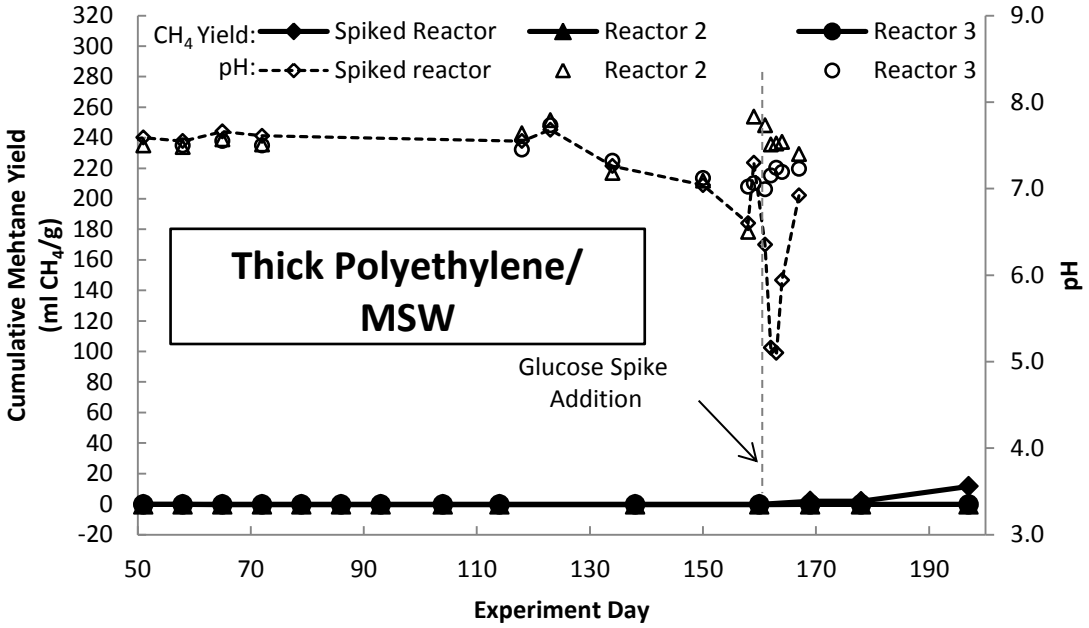

Figure 20 Glucose Spike Effects on Thick Polyethylene/MSW Reactors

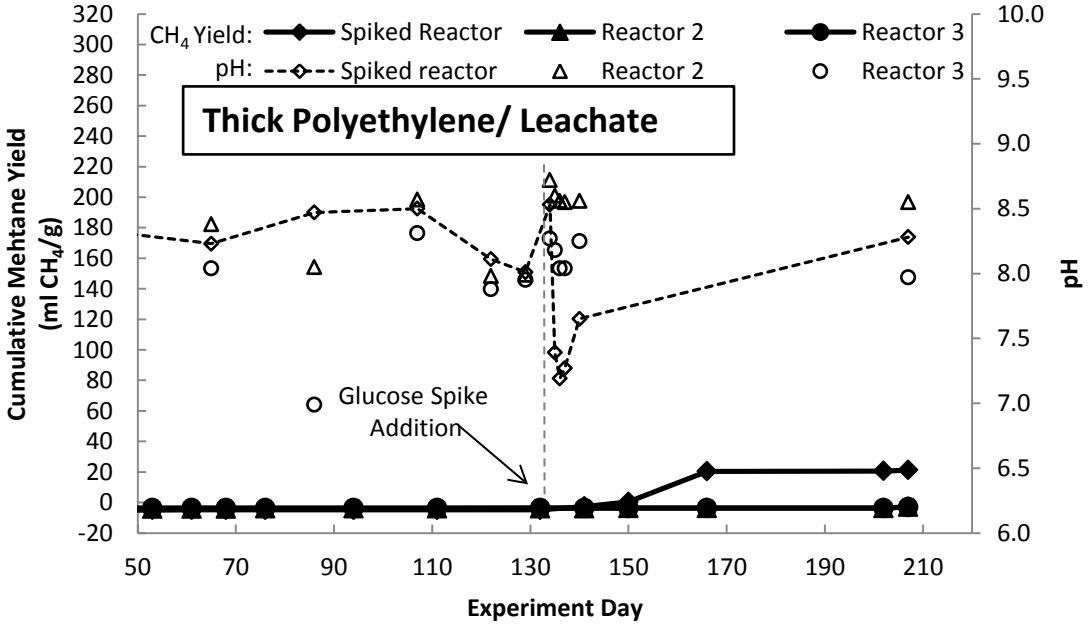

Figure 21 Glucose Spike Effects on Thick Polyethylene/Leachate Reactors

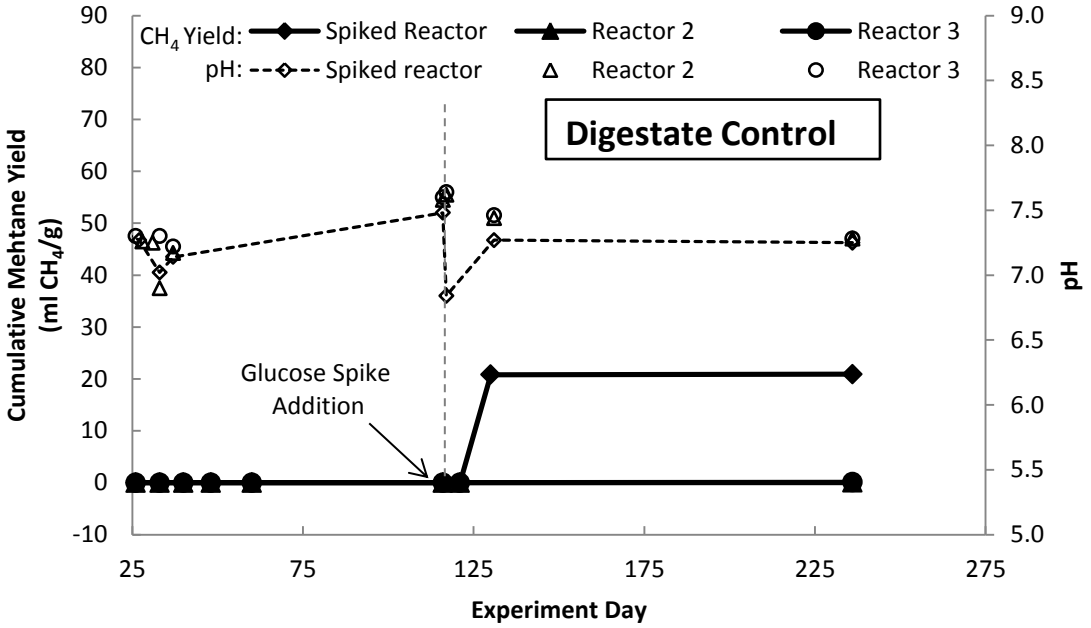

Figure 22 Glucose Spike Effects on Digestate Control Reactors

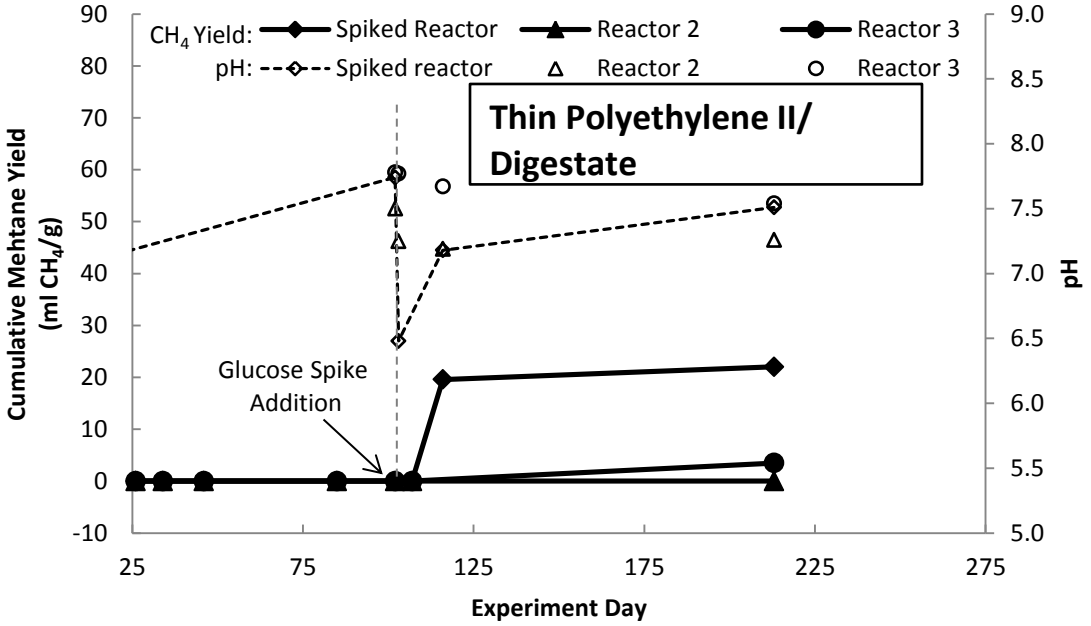

Figure 23 Glucose Spike Effects on Thin Polyethylene II/Digestate Reactors

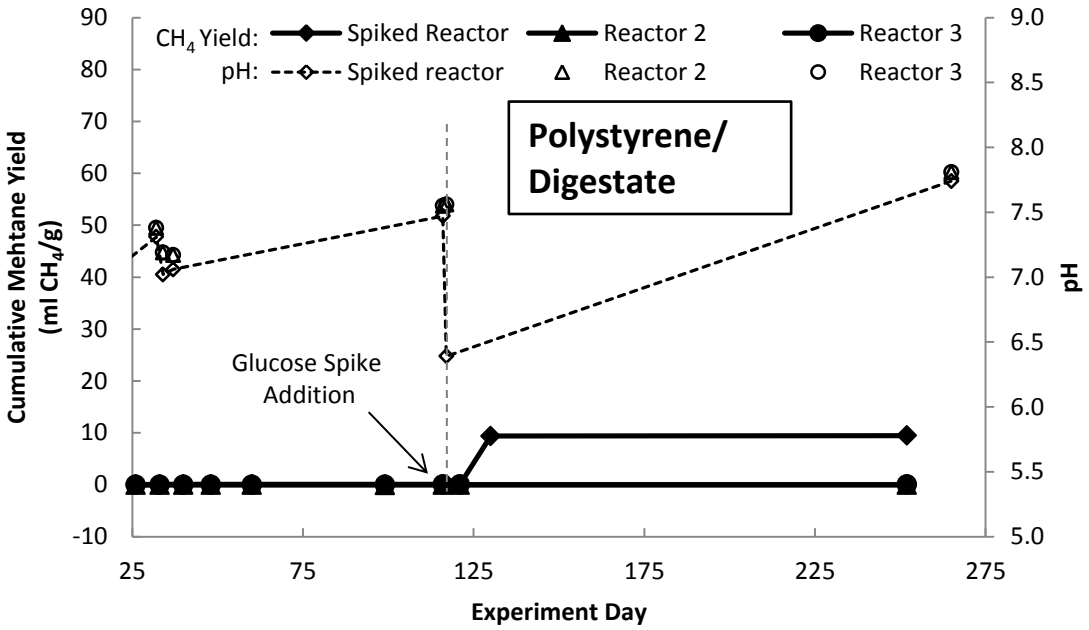

Figure 24 Glucose Spike Effects on Polystyrene/Digestate Reactors

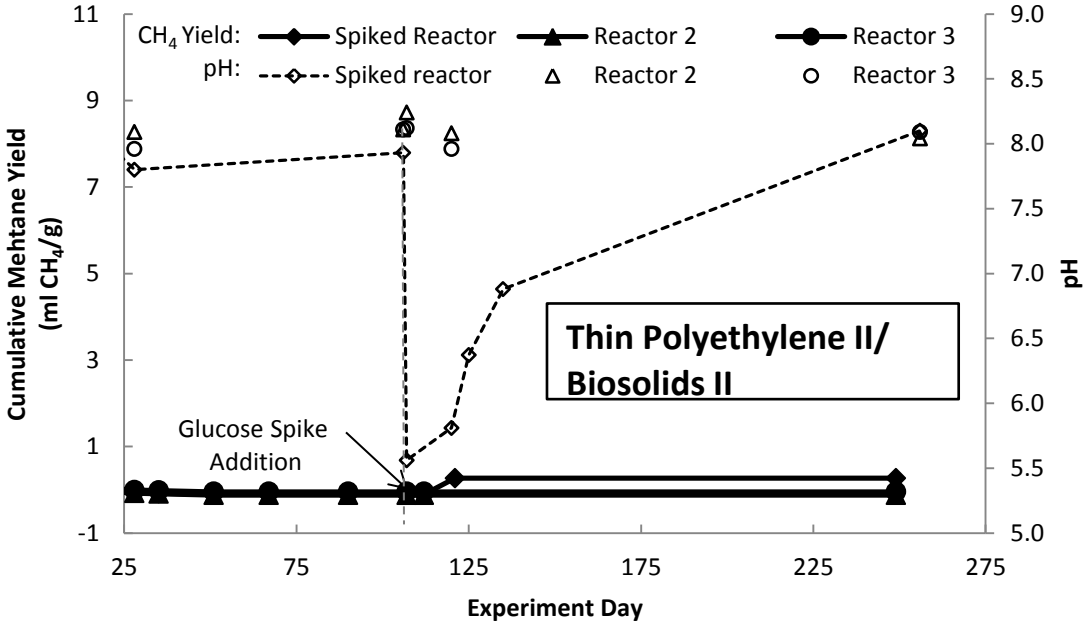

Figure 25 Glucose Spike Effects on Thin Polyethylene II/Biosolids II Reactors

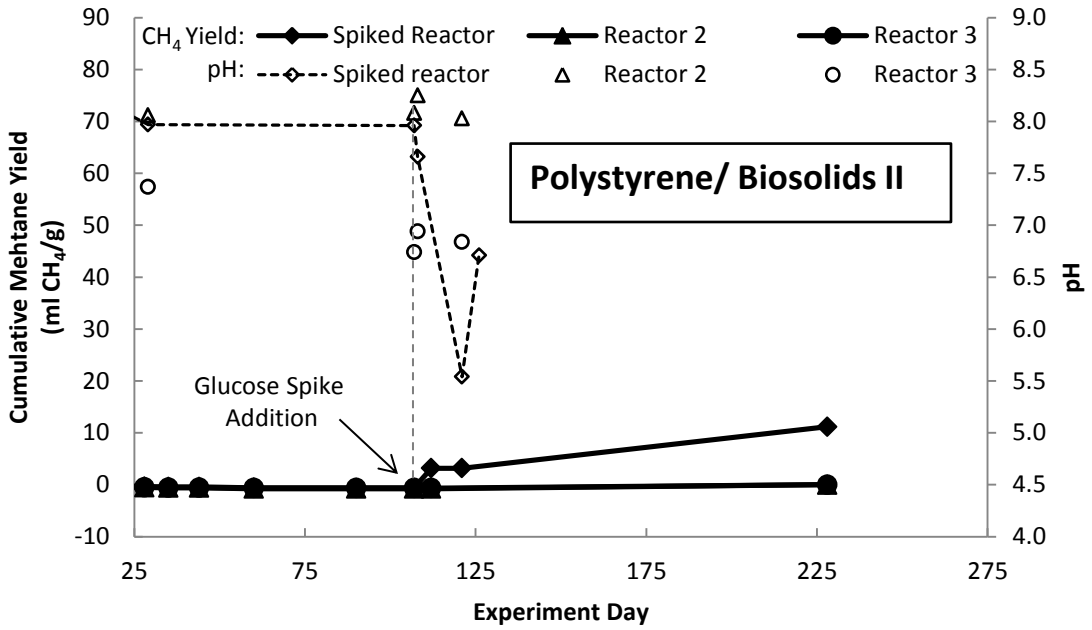

Figure 26 Glucose Spike Effects on Polystyrene/Biosolids II Reactors