STEAM HYDROLYSIS AND ANAEROBIC DIGESTION OF BIODEGRADABLE (POLYLACTIC ACID) PACKAGING WASTE

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
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To God who gave to me a wonderful family and friends always ready to give support, moral and advice

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Biodegradable plastic is now being used for packaging purposes to avoid the environmental burden of oil based polymers. However, after usage the biodegradable polymer waste when disposed takes longer to degrade than typical organic wastes.

The same mechanical characteristics that make this plastic appealing to use make it undesirable in composting operations. Relatively high temperature industrial composting conditions are required to treat this waste. Most municipalities lack such facilities. Therefore, the majority of biopolymer wastes are sent to landfills, which is contrary to the purpose of using biodegradable polymers.

Research has suggested pretreatment as a solution for accelerating degradation of biopolymers. Among proposed pretreatment, hydrolysis has been suggested as the most promising.

In this research kinetics of hydrolysis of polylactic acid at temperatures above and below the melting point of the polymer, and mass loading of PLA during hydrolysis was studied to explore the extent of degradation of the material on reaction.

In all cases studied, loss in mass and degradation of polymer was observed. It was most noticeable when 2.5 grams and 7.5 grams of sample was exposed for 120 minutes at 160 ºC. At the end of the treatment no solids were present, and the molecular weight average (MW) reduced to 900 and 1217 respectively.

Previous work done in our laboratory discovered that it was possible to digest PLA in a thermophilic anaerobic digester and this would serve to eliminate waste while producing methane. In this work benefits of anaerobic digestion of PLA using an adapted microbial flora were studied. Results of this work suggest that PLA can be directly digested anaerobically without need for hydrolysis pretreatment. The digestion of hydrolyzed PLA was very fast, and depending on the time of exposure the lag time was between 0.64 and 3.5 days, when compared to a lag time of 25 days when using raw PLA. Anaerobic digestion yielded 94 -98% of theoretical methane yield, indicating almost complete biogasification of the material.

CHAPTER 1
INTRODUCTION

Success of plastics is evidenced by its use in many types of packaging throughout the world. However, plastic waste is often viewed as an environmental burden. Several authors have defined polyolefin plastics as energy sinks since the material is made from fuel stocks and requires even more energy to convert fuel into plastics. The three most important polyolefins used from production of petrochemicals are polyethylene, polypropylene and polybutadiene (Hatch et al., 1981).

Traditional sources for olefins production in Europe and in the United States are naphtha, gas oil and liquid gas petroleum. These raw materials must undergo energy intensive transformations to obtain ethylene, propylene, or butadiene. Also, other materials are added to olefins to form more complicated structures, such as nitrogen to propylene to obtain acrylonitrile, oxygen to obtain epoxies, etc. (Hatch et al., 1981).

A polymer is a macromolecule with large numbers of repeating units. Homopolymers are made from one building block. Copolymers are made from more than one building block produced by the addition polymerization reactions (Wojciechowski et al. 1986).

Polymers are classified depending on type of monomer (polyolefins, polyesters, polyamides, etc.); type of formation reaction (condensation or addition polymerization); type of thermal behavior (thermosetting and thermoplastics); and type of utilization, (thermosetting, thermoplastics, fibers, engineering plastics, etc.). Polymer data are usually given under the name of the polymer such as polyethylene, polystyrene, or by the type of monomer as acrylic or polyester.

Design of material with specific useful live requires choice of specific monomers to obtain copolymer with the desirable hydrophilic characteristics (Le Digabel and Averous, 2006; Yew et al., 2006)

In an effort to relieve the real or perceived environmental burden of petroleum based polymers, work is being done to develop biologically derived plastics. Polylactic acid (PLA), which is produced from renewable plant resources, has recently experienced increased utilization as an alternative to petro-derived polymers in order to reduce their impact on the environment (Tsuji, 2008).

PLA belongs to the family of aliphatic polyesters commonly made from ά-hydroxy acids, which includes polyglycolic acid or polymandelic acid, and are considered biodegradable and compostable.

PLA is a polyester polymer produced by the condensation of lactic acid that is derived from microbial fermentation of renewable agriculture resources, such as glucose from corn, sucrose from cane sugar, lactose from cheese whey, and cellulose from waste papers (Ho et al., 1999). PLA is a thermoplastic, with high strength and high modulus, which makes it useful for the industrial the industrial packaging or the biocompatible/bioabsorbable medical device market.

It is one of the few polymers in which the stereochemical structure can easily be modified by polymerizing a controlled mixture of the L- or D- isomers to yield high molecular weight amorphous or crystalline polymers that can come in contact with food and are generally recognized as safe (GRAS)(Garlotta, 2001).

L- Polylactic acid (PLLA) has also been attracting much interest as an alternative to commercial polymers such as polyethylene, polypropylene, polyethylene

terephthalate, and polystyrene. PLLA is also a superior material for feedstock recycling into L-lactic acid by hydrolysis and into L, L-Lactide by pyrolysis (Mohd-Adnan et al., 2008).

Hydrolysis is a process by which polymers undergo chemical degradation by being split by addition of water; the polymer must contain hydrolysable covalent bonds such as ester, ether, anhydride, amide, carbamide (urea), ester amide (urethane) etc. Rate and extent of hydrolysis (degradation) depends on parameter such as water activity, temperature, pH and time (Le Digabel and Averous, 2006)

PLA degradation occurs in the presence of water provoking a hydrolysis of the ester bonds (Lucas et al, 2008). The rate of degradation depends on size and shape of the article, the isomer ratio, and temperature hydrolysis (Garlotta, 2001).

Today many cups and containers manufactured with PLA are going straight to landfills because most municipalities do not possess expertise or equipment to handle PLA. Nevertheless, this “biodegradable” plastic does not degrade quickly in landfills (Tokiwa et al., 2004). This research objective is study of the degradation of PLA due to hydrolysis and possible recovery of valuable by-products.

CHAPTER 2
HYDROLYSIS AND BIODEGRADATION OF POLYLACTIC ACID (PLA)

Currently, PLA is used for medical applications including wound closure, prosthetic implants, and drug release (Tokiwa and Calabia, 2006); as controlled-release devices for herbicides and pesticides; and as a plant growth enhancer (de Jong et al., 2001). Developing applications for PLA include degradable plastics (cast films, blown films, and rigid containers), fibers and non-wovens, and paperboard coatings. PLA-degradable plastics have a projected U.S. market of 2.5 to 3.4 million tons/year (sales volume, $3.1 to 4.4 billion/year) and are expected to compete with hydrocarbon-based thermoplastics, such as polystyrene, polypropylene, polyethylene, and polyethylene-terephthalate, on a cost and performance basis (Ho et al., 1999).

PLA has high mechanical strength, thermal plasticity, fabricability, biodegradability, and biocompatibility. It has been proposed as a renewable, degradable plastic for uses in service ware, grocery, waste and composting bags, mulch films and controlled release matrices for fertilizers, pesticides and herbicides.

Generally, PLA polymers are made into useful items using thermal processes, such as injection molding and extrusion. Therefore, its rheological properties, especially its shear viscosity are important to processes such as film blowing, paper coating, injection molding, sheet forming and fiber spinning.

In order for PLA to be processed on large-scale production lines in applications such as injection molding, blow molding, thermoforming, and extrusion, the polymer must possess adequate thermal stability to prevent degradation and maintain molecular weight and properties.

Pure PLA undergoes thermal degradation at temperatures above 200 ºC (392 ºF) by hydrolysis, lactide reformation, oxidative main chain scission, and inter- or intramolecular transesterification reactions (Jamshidi et al., 1988). The most widely used method for improving PLA processability is based on melting point depression by random incorporation of small amounts of lactide enantiomers of opposite configuration into the polymer (i.e. adding a small amount of D-lactide to the L-lactide to obtain PDLLA). Unfortunately, the melting point depression is accompanied by a significant decrease in crystallinity and crystallization rates (Garlotta, 2001). PLA can be easily degraded by enzymatic or alkali hydrolysis in compost, but its rate of degradation in soil is not high (Ohkita, 2006). Also, PLA plastics are sensitive to moisture and heat, which limits applications for the plastic (Ho et al., 1999).

Polymeric materials that are exposed to outdoor conditions (i.e. weather, ageing and burying) undergo degradation from mechanical actions/interactions and light, thermal and chemical reactions (Helbing et al., 2006; Ipekoglu et al., 2007).

Thermal degradation of thermoplastic polymers occurs at the melting temperature when the polymer is transformed from solid to liquid (159 – 178 ºC for L- PLA depending on molecular weight).

Biodegradable polymers such as L-PLA are semicrystalline polymers, they posses amorphous and crystalline regions. Structural changes take place at their glass transition temperature (T_g) (i.e. 50 º C for L-PLA), the mobility and volume of polymeric chains are modified. Above T_g (rubbery state), disorganization of chains facilitate chemical and biological reactions. Below T_g (glassy state), formation of spherulites may take place, generating inter-spherulitic cracks and brittleness.

Hydrolysis is another way by which polymers undergo chemical degradation. PLA hydrolysis occurs in the presence of water provoking a hydrolysis of ester bonds. (Lucas et al., 2008)

Hydrolytic degradation of the polymer matrix is affected by the amount of crystallinity in the samples. It has been shown that highly crystalline PLA will take months, sometimes years, to hydrolyze fully to lactic acid, whereas an amorphous sample degrades in weeks. This is due the impermeability of crystalline regions.

Pure poly(D-lactide) or poly(L-lactide) has an equilibrium crystalline melting point of 207 ºC, but typical melting points are in the 170 ºC – 180 ºC range. This is due to small and imperfect crystallites, slight racemization, and impurities (Kharas et al., 1994; Kricheldorf et al., 1996).

In PLA hydrolytic degradation, degradation varies depending on time, temperature, molecular weight, impurities, and catalyst concentration. Catalyst and oligomers decrease degradation temperature and increase degradation rate of PLA. In addition catalysts cause viscosity and rheological changes (Garlotta, 2001). PLA Hydrolysis is catalyzed by both acid and base. (Gopferich et al., 1996)

Hydrolytic degradation of massive amorphous poly (DL-lactic acid) devices was shown to proceed heterogeneously, proceeding faster inside than at the surface (Vert et al., 1994).

In the interior there is a larger contribution of auto-catalysis. Initially, hydrolysis of ester bonds proceeds homogenously trough the matrix. During degradation, two factors are of importance. First, degradation causes an increase in the number of carboxylic acid chain ends, which are known to auto-catalyze ester hydrolysis. Second, only

oligomers, which are soluble in the surrounding aqueous medium, can escape from the matrix. As aging proceeds, soluble oligomers, which are close to the surface, can leach out before they fully degrade, whereas those located in the core of the matrix remain entrapped. This yields a low pH in the core which, in turn, results in accelerated degradation rates (Vert et al., 1994).

Degradation of semi-crystalline PLLA involves even more complex processes. It was reported by Fischer et al. 1973 that the hydrolytic degradation occurs in two stages. In the first stage, water diffuses into the amorphous regions resulting in random hydrolytic scission of ester bonds. The degree of crystallinity can even increase as degradation proceeds. The second stage starts when most of the amorphous regions have been degraded. Hydrolytic attack then progresses from edges towards the center of crystalline domains. A retardation in degradation has been observed during degradation of intrinsically amorphous poly (DL-LA) by the formation of a crystalline phase of an oligomeric stereocomplex as an intermediate. This intermediate setereocomplex is highly resistant to hydrolysis (S.J. de Jong et al., 2001).

Biodegradation of polymeric materials includes several steps and the process can stop at each stage (Pelmont., 1995).

- The combined action of microbial communities, other decomposer organisms or/and abiotic factors fragment the biodegradable materials into tiny fractions. This step is called biodeterioration (Eggins and Oxley, 2001; Walsh, 2001).
- Microorganisms secrete catalytic agents (i.e. enzymes and free radicals) able to cleave polymeric molecules progressively reducing molecular weight. This process generates oligomers, dimmers and monomers. This step is called depolymerisation.
- Some molecules are recognized by receptors of microbial cells and can move across membranes. Other molecules stay in the extracellular surroundings and can be the object of different modifications.

- In the cytoplasm, transported molecules integrate into microbial metabolism to produce energy, new biomass, storage vesicles and numerous primary and secondary metabolites. This step is called assimilation.
- Concomitantly, some simple and complex metabolites may be excreted and reach the extracellular surroundings (e.g. organic acids, aldehydes, terpens, antibiotics, etc.) Simple molecules such as CO_2, N_2, CH_4, H_2O and different salts from intracellular metabolites that are completely oxidized are released in the environment. This stage is called mineralization.

The term “biodegradation” indicates the predominance of biological activity. However, in nature, biotic and abiotic factors act synergically to decompose organic matter. Several studies about biodegradation of some polymers show that the abiotic degradation precedes microbial assimilation. Consequently, the abiotic degradation must not be neglected (Lucas et al., 2008).

Several studies have shown that certain proteases including proteinase K, pronase and bromelain have been found to increase the rate of degradation of PLA Williams (1981) was the first to report biodegradation of PLA by Proteinase K, a fungal serine protease of *Tritirachium album.*

PLA can be degraded as well by microbes; however, PLA degraders appear to be scarce in the environment. A burial test indicated that PLA was not readily degraded when samples were buried under soil for 20 months (Tokiwa et al., 2004).

Pranamuda et al., (1997) found that PLA degrading organisms are sparsely distributed in soil environments and found only one, an actinomycete *Amycolatopsis sp.* that degraded PLA in culture at 30 °C. Hakkarainen et al., (2002) found that PLA films were degraded to a powder after 5 weeks in a mixed culture of compost microorganisms at 30°C whereas the film in abiotic medium looked intact. They also found PLA molecular weights, especially number average molecular weight (Mn) were reduced to a greater extent in the biotic medium, probably due to cleavage near the

chain ends. Other authors claim that initial degradation is due to abiotic hydrolysis only followed by biotic assimilation of breakdown products. PLA is completely mineralized to CO_2, water and a small amount of biomass after 4-6 weeks in compost (Shogren et al., 2002) (in composting aerobic conditions prevails.)

Under anaerobic conditions, organic matter usually degrades in four stages: a) hydrolysis, b) acidogenesis, c) acetogenesis, and d) methanogenesis. During hydrolysis molecules split and become smaller and soluble resulting in conversion of carbohydrates, fats and proteins into sugars, fatty acid and amino acids. This chemical reaction requires water, and is aided by temperature and enzymes. Later, during acidogenesis, simpler compounds undergo fermentation carried out by acidogenic bacteria, and produce volatile fatty acids, hydrogen and carbon dioxide. Later, during acetogenesis, acetic acid, hydrogen, and carbon dioxide are produced. During methanogenesis, the final products of the anaerobic digestion are obtained. These are methane and carbon dioxide.

Little information was available for the anaerobic degradation of PLA, and most of the information available pertains to composting. PLA can be degraded in a composting environment where it is hydrolyzed into smaller molecules (oligomers, dimmers and monomers) after 45-60 days at 50-60 ºC (Urayama et al., 2002).

Anaerobic digestion could be a valid alternative for PLA degradation. The anaerobic digestion will lead to a complete mineralization of PLA which is much better than the non-degradation happening in land fills and the energy consumption required performing high temperature composting. . Therefore, studies were done to determine feasibility of anaerobic digestion of PLA in order to produce methane.

CHAPTER 3
POLYLACTIC ACID HYDROLYSIS

Introduction

Characteristics that make PLA a desirable material (i.e. excellent mechanical properties, stability and durability) make it resistant to degradation. As a result, PLA has a low biodegradability rate compared to composted organic waste, this prevents commercial compost operators from receiving it.

Several pretreatments have been proposed as a solution with hydrolysis being most effective (Vargas et. al. 2007). Hydrolysis may offer an opportunity for monomer recovery.

The chapter describes effects of temperature and moisture on PLA. Two temperatures and three ratios of PLA-water were studied to better understand hydrolytic degradation of PLA.

Background

Polylactic acid (PLA) is a bio-degradable thermoplastic polymer that is beginning to be produced on large scale from fermentation of corn to lactic acid and subsequent polymerization (Shoegren et. al 2001).

Biodegradable polyesters, such as PLA are under investigation for biomedical applications including orthopedic fixture materials, degradable sutures, absorbable fibers and pharmaceutical applications such as controlled-release devices (de Jong et. al. 2001). PLA has been used as a matrix for the controlled-release of drugs and as scaffolds on which living tissue can regenerate (Khang et al., 2003). Also, PLA has been proposed as a renewable degradable plastic for use in service-ware, grocery,

waste and composting bags, mulch films, and controlled release matrices for fertilizers, pesticides and herbicides (Qi Fang et. al. 1999).

The percentage of poly(L-lactide) (PLLA) and poly D-lactide (PDLA) in polymer blends affect crystal structure, melting point, and glass transition of PLA (Okihara et al.,1991; Garlotta 2002). A 50/50 PLLA/PDLA in polymer blend can have a different crystal structure from that of pure PLLA or PDLA (Sasaki, 2003). The 50/50 blend can form a stereo complex, which is a complex between PLLA and PDLA. The stereo complex structure of the 50/50 PLLA/PDLA blend has a glass transition temperature of 65-72 ºC (Tsuji, 2002;2005) and a melting point of 220-230 ºC, which are higher than those of pure PLLA and pure PDLA (Tsuji, 2002; Sarasua, 2005).

Several methods have been proposed for PLA degradation. However, Vargas et al. (2009) compared several degradation methods including gamma irradiation, electron beam irradiation and steam treatment (hydrolysis). Results of this study showed hydrolysis as the most effective pretreatment to degrade polylactic acid.

Hydrolytic degradation proceeds either at the surface (homogeneous) or within the bulk material (heterogeneous) and is controlled by a wide variety of compositional and property variables such as matrix morphology, chain orientation, chemical composition and stereochemical structure, sequence distribution, molecular weight and distribution, presence of residual monomers, oligomers and other low molecular weight products, size and shape of the specimen, oxygen, microorganisms, enzymes, pH and temperature (Hakkarainen, 2002).

Degradation occurs in stages, the first being diffusion of water into the material, hydrolysis of ester bonds and lowering of molecular weight followed by intracellular

uptake of lactic acid oligomers, and catabolism. Rates of hydrolysis increase with water content and temperature and are catalyzed by free carboxyl groups of the hydrolyzed PLA ends. Hydrolysis is actually faster in the interior of a thick sample since carboxylic acid concentrations are higher than at the exterior due to leaching of the acidic PLA oligomers into the surroundings aqueous medium.

In abiotic aqueous environments degradation proceeds through hydrolysis of the ester bond, this reduces molecular weight polymer to intermediate degradation products (insoluble and soluble oligomers) and, finally, lactic acid is formed as the ultimate degradation product of abiotic hydrolysis (Shogren et. al. 2003).

Material and Methods

Feedstock

PLA waste was created using commercial thermoformed cups (Fabri-Kal , Inc ., Kalamazoo, MI) obtained from TREEO Center at the University of Florida (Figure 3-1).

Feedstock Preparation

2 packages of 50 cups each were ground in a hammer mill. After grinding pieces were cut manually until homogeneous pieces of approximately 1” by 0.25” were obtained.

Hydrolysis Protocol

- Step 1: three concentrations of PLA were tried, so 2.5 grams, 7.5 grams and 30 grams in 30 grams of water.
- Step 2: to perform hydrolysis a Mathis BFA-24 Beaker Dryer with PLC Univision (Werner Mathis USA Inc., Concord,NC) was used.
- Step 3: Waste samples were deposited in the Mathis BFA-24 200 mL vials (working pressure 4 bar) with 30 grams of D.I. water.
- Step 4: Vials were purged for 2 minutes using nitrogen gas, and then tightly closed.

- Step 5: For each concentration duplicates were made.
- Step 6: Vials were loaded into the machine.
- Step 7: Mathis dyer was programmed to stay at 121 °C, and the waste was treated for 120, 240, 360, 480 and 720 minutes.
- Step 8: Vials were allowed to cool down.
- Step 9: Once vials were at room temperature, 15 mL of liquid sample were taken from each vial and filtered using Whatman syringe 0.45 µm filters. The rest of the vial including solids was placed in an oven at 95 °C for 48 hours to drive off water.
- Step 10: 2 mL from the filtered liquid was taken from the vials containing the liquid sample, and placed into a clear borosilicate glass screw-neck 12x32 mm numbered vials (sample used for lactic acid determination).
- Step 11: Solids were taken from the oven and 10 mg sample were weighed and placed into 10 mL screw-neck pyrex vials; 10 mL of tetrahydrofuran (THF) HPLC grade was added to each vial. The vial was agitated and warmed for complete dissolution of the sample (sample used for molecular weight determination).
- Step 12: remaining solids were weighed and then stored.
- Steps 1 to 6 were repeated for different concentrations (2.5, 7.5 and 30 grams)
- Step 13: the Mathis dyer was then programmed to remain at 160 °C and the waste was treated for 30, 60, 90, and 120 minutes.
- Steps 8 to 11 were repeated for each concentration.

Analysis

A Hitachi UV reverse phase HPLC was used to determine lactic acid. The mobile phase used was a solution of sulfuric acid. Chromatograms of samples treated at 121 °C for more than 360 minutes needed to be diluted. For this analysis at time 0, lactic acid concentration was zero.

A Waters GPCV2000 gel permeation chromatograph was used to determine molecular weight of the different samples. tetrahydrofuran (THF) was used as the mobile phase in this case. Initially a sample of raw PLA was analyzed.

Results

In all cases reduction of molecular weight and growth in lactic acid concentration was observed (Figure 3-2, 3-3, 3-4). Figure 3-2 summarizes degradation of polylactic acid at 121 ºC for 2.5 grams and 7.5 grams of PLA loading, respectively. The plot is an average of molecular weights for two runs. The molecular weight at the start of each PLA loading was determined to be 1.21×10^5 grams. For the sample with 2.5 grams, molecular weight decrease rapidly to 2.8×10^4 grams in 120 minutes and continued to decrease further to 1.09×10^4 grams at 360 minutes. At the end of the run (720 minutes) molecular weight was 5.57×10^3 grams. Similar observations were noted for PLA loading of 7.5 grams. After 720 minutes the molecular weight was 4.7×10^3 for 7.5 grams of PLA loading (Table 3-1).

Figure 3-2 and 3-3 summarizes degradation of polylactic acid at 160ºC for samples made with 2.5 grams and 7.5 grams PLA, respectively. The molecular weight at the start of each PLA loading was determined to be 1.21×10^5 grams. For samples with 2.5 grams PLA the molecular weight decreased rapidly to 7.85×10^3 grams in 30 minutes and continued to decrease to 5.92×10^3grams at 45 minutes. At the end of the run (120 minutes) molecular weight dropped to 9.27×10^2 grams. A similar observation was noted for PLA samples of 7.5 grams. After 120 minutes, molecular weight was 7.24×10^2grams (Table 3-2).

Figure 3-5A summarizes the increase in lactic acid concentration at 121 ºC for 2.5 grams and 7.5 grams of PLA, respectively. The plot is an average concentration of lactic acid for two runs. Initial lactic acid concentration was determined to be 0. Samples with 2.5 grams PLA lactic acid concentration increased to 1.30×10^{-3} M in 120 minutes and continued to increase further to 1.02×10^{-2} M at 240 minutes. At the end of the run (720

minutes) the lactic acid increases to 3.2 x 10^{-1} M. A similar observation was noted for 7.5 grams PLA samples. After 720 minutes the lactic acid concentrations was 1.11 M. Figure 3-5B shows lactic acid trends at 160 ºC for 2.5 grams and 7.5 grams of PLA loading respectively. The plot is an average of lactic acid production for two runs. Lactic acid concentration at the start of each PLA loading was determined to be 0 M. For samples with 2.5 grams PLA lactic acid concentration increase to 1.1 x 10^{-2} M in 30 minutes and continued to increase to 7.0 x 10^{-2} M and 1.5 x 10^{-1} M. At the end of the run (120 minutes) lactic acid concentration was 3.51 x 10^{-1} M. A similar observation was noted for PLA loadings of 7.5 grams. After 120 minutes the lactic acid concentrations was 4.43 x 10^{-1}.

A first order model was proposed to describe the kinetics of degradation. Equation (3-1) is a general expression for first order kinetics.

$$r = -\frac{d[A]}{dt} = k[A] \tag{3-1}$$

where A: concentration of the reactant (mol)
k: rate constant (min^{-1})
n: order of the reaction
t: time (min)

This expression was linearized to determine constant k (slope). Figure 3-7shows a normalized plot of rate of change of molecular weight with respect to time.

$$\ln [A] = -kt + \ln [A]_0 \tag{3-2}$$

where Ao: initial concentration of the reactant (mol)
k: rate constant (min^{-1})
n: order of the reaction
t: time (min)

Table 3-3. shows constants, k and n for 121 and 160ºC. Fig 3-6 shows the calculation of energy of activation (Ea) and table 3-3, exhibits the summary of the obtained values.

To understand kinetics of lactic acid production it was proposed to determine the order of the reaction. The equation below is a general expression to determine first order reaction.

$$dP/dt = k[A] \quad (3\text{-}3)$$

where A: concentration of the reactant (mol)
P: concentration of the product (mmol/L)
k: rate constant (min^{-1})
t: time (min)

This expression was linearized to determined constant k (intercept). Figure 3-7 shows a semi-log plot of rate of change of molecular weight with respect to time.

$$Ln\ [P] = [A]\ e^{-kt} \quad (3\text{-}4)$$

where A: concentration of the reactant (mol)
P: initial concentration of lactic acid (mmol/L)
k: rate constant (min^{-1})
t: time (min)

Table 3-4 shows constants, k and n for 121 and 160ºC.

Discussion

Molecular Weight Degradation

The reduction in molecular weight happens with simultaneous loss of PLA mass. Since the matter can not be destroyed, the loss in PLA mass suggests the formation of another product as effect of degradation.

Literature suggests that moisture breaks the ester bonds of PLA producing a reduction in chain size. Degradation of polylactic acid shows an exponential trend (Fig 3-2 and 3-3). Therefore, first order kinetics were applied. For this experiment molecular

weight average, Mw, was used. Many authors have been experimenting with kinetics models for PLA hydrolytic degradation; however, temperature, concentration, heat exposures vary, which results in different kinetics rate constants (k), and energy of activation (Ea).

Activation energy (Ea) was estimated using values of k at 121 and 160 ºC. Ea calculated was 7.4×10^4 J/mol (Table 3-3) and the k values were 7.7×10^{-3} and 5.9×10^{-2} min^{-1}, respectively. Tsuji et al. (2004) reported an Ea value of 7.52×10^4 J/mol when working with a range of temperatures of 37-97ºC. Mohd-Adnan et al. 2008 reported different values for autocatalytic random hydrolysis being considered as the main reaction mechanism. This study suggests that employment of unsuitable reactions mechanisms to analyze kinetics can produce large deviations in Ea value. k values at 100 ºC were 8.4×10^{-5} and 7.2×10^{-4} s^{-1} at 130°C resulting in an Ea of 8.72×10^4 J/mol

Lactic Acid Formation

Production of lactic acid happened as a result of PLA degradation. Lactic acid production did not follow the same trend of PLA degradation. Most of the degradation of PLA happened in the first 120 minutes when the heat exposure was 121 ºC and 30 minutes when the heat exposition was 160 ºC. However, the concentration of lactic acid at 120 minutes and 30 minutes when using a heat exposure of 121 ºC and 160 ºC was low. Production of lactic acid shows an exponential trend (Figure 3-4). First order kinetics fits well to data after initial delay. Initial composition of the waste is unknown, but for effect of calculations it was assumed PLA after hydrolysis yielded only lactic acid. Knowing the exact amount of PLA and water added and using PLA density of 1.25 g/mL initial Molar solution were calculated, and then compared with the results of HPLC analysis. At 121 °C the maximum recovery (7.5 grams and 720 minutes) was about

47% and at 160 °C the maximum recovery (7.5 grams and 120 minutes) was about 20%. An increase in acetic acid concentration was observed when working at 160 °C. Hydrolysis of optically active PLLA in the melt may cause racemization and decomposition of lactic acids due to high temperature Tsuji et al. (2003). PLA is not 100% lactic acid Garlotta et al. (2001) in his literature review of polylactic acid shows several compositions for starch-PLA plasticizers; overall the standard lactic acid concentration in PLA ranges from 60-75%.

Effect of PLA Concentration on Hydrolysis

Results suggest that the amount of PLA treated affected the hydrolysis. In this particular experiment, a better fit to a first order kinetics was observed for samples with 7.5 grams of PLA; this phenomenon repeats with both heat exposures (121 ºC and 160 ºC). Zhang et al., 1994 conducted studies on polylactic acid degradation and suggested that polymer degradation rate is determined by polymer reactivity with water and accessibility of ester groups to water and catalyst (carboxylic end groups).

Effect of Temperature on Hydrolysis

It can be seen from table 3-1 and 3-2 the molecular weight degradation occurs at a faster rate when temperature is elevated to 160 ºC from 120 ºC. More importantly, with respect to the concentration of PLA loading, time required to achieve complete degradation of PLA is reduced by almost seven times at 120 minutes. This suggests that at 121 ºC there is likely a formation of intermediate products that reduce the rate of hydrolysis. Tsuji et al., 2008 and Mohd-Adnan et al, 2008 suggested factors influence hydrolysis kinetics such as crystallinity and optical purity.

Conclusions

PLA hydrolysis is a complex reaction. Temperature, concentration, molecular weight, crystallinity, size, thickness may affect the reaction. A first order model was applied for this reaction. Values for activation energy compared well to literature values.

Figure 3-1. Cup used for obtaining feedstock

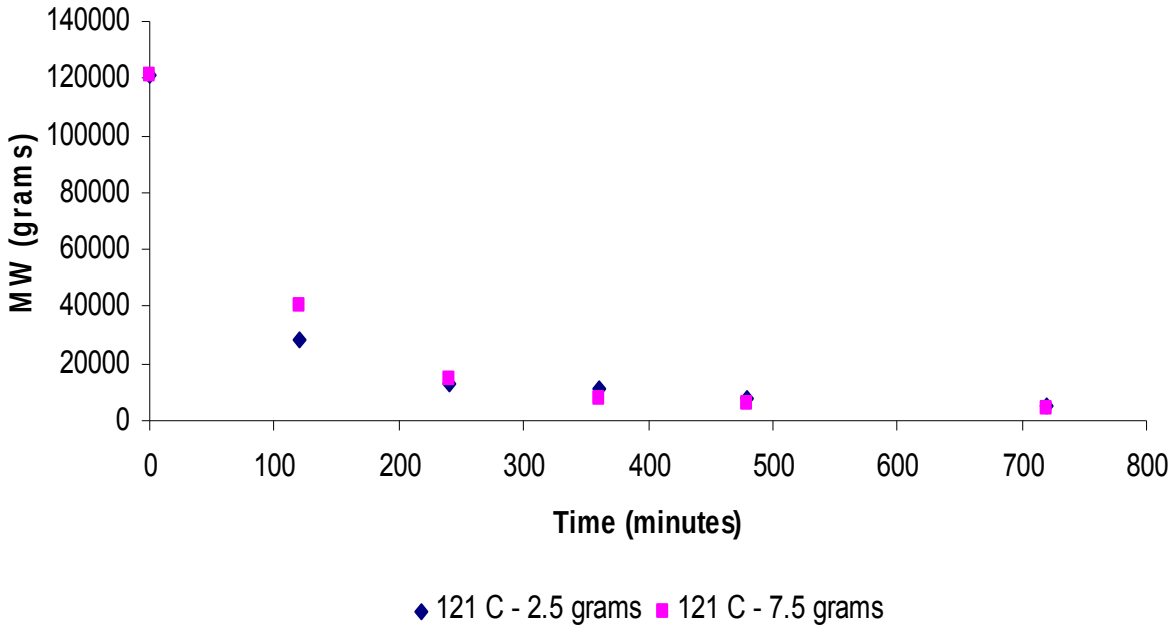


Figure 3-2. MW degradation 121ºC

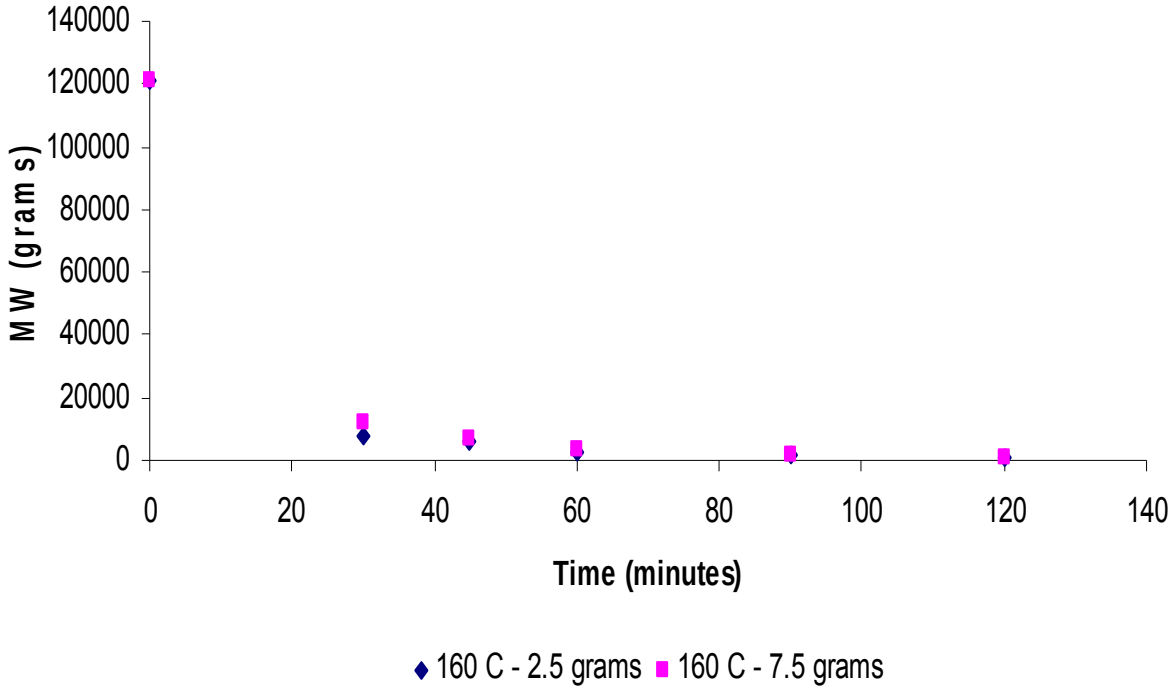
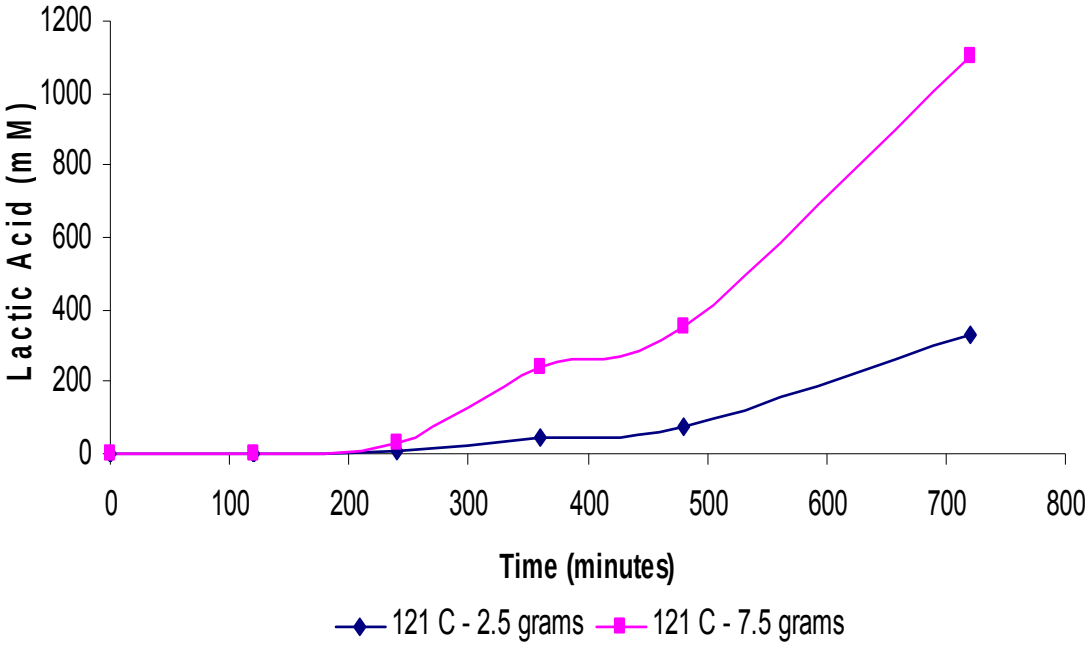


Figure 3-3. MW degradation 160ºC



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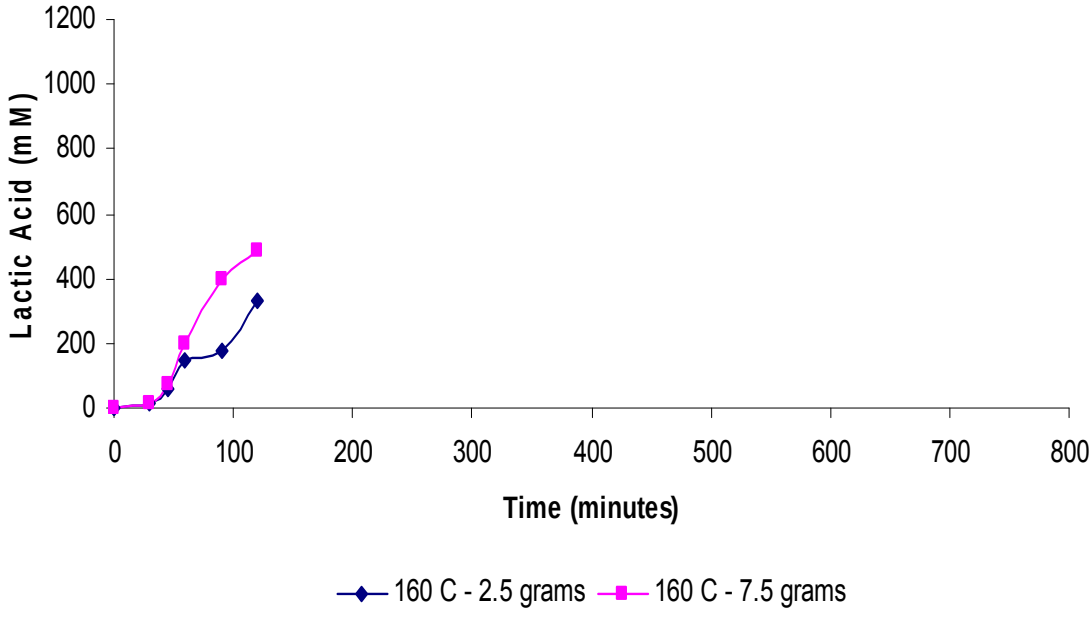
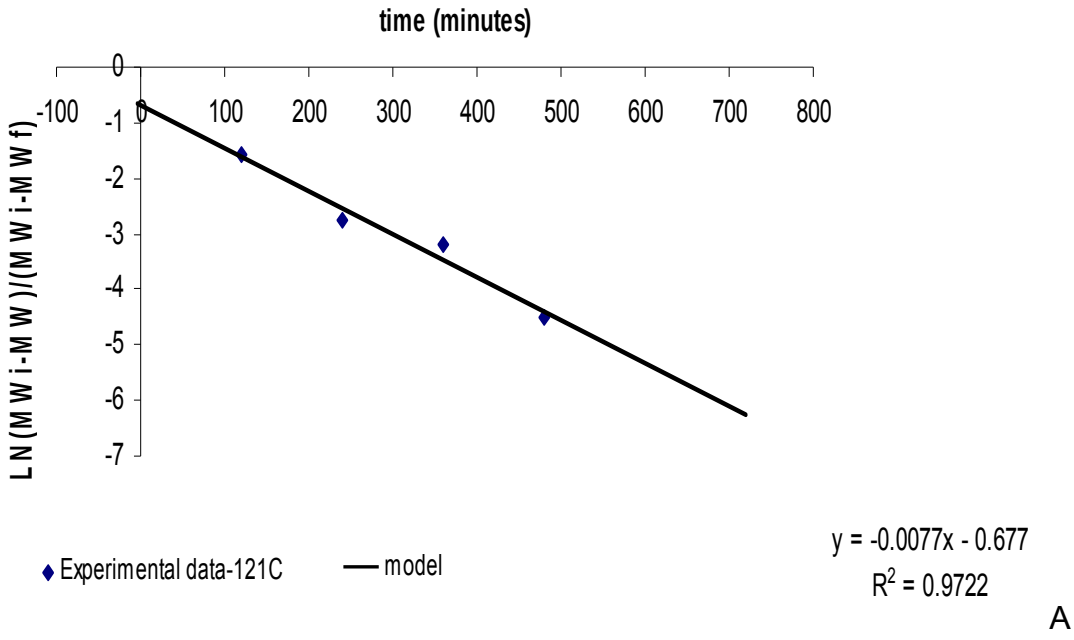


B

Figure 3-4. Variation in lactic acid concentration as a function of time. A) Lactic acid concentration while working at 121 ^{o}C. B) Lactic acid concentration while working at 160 ^{o}C



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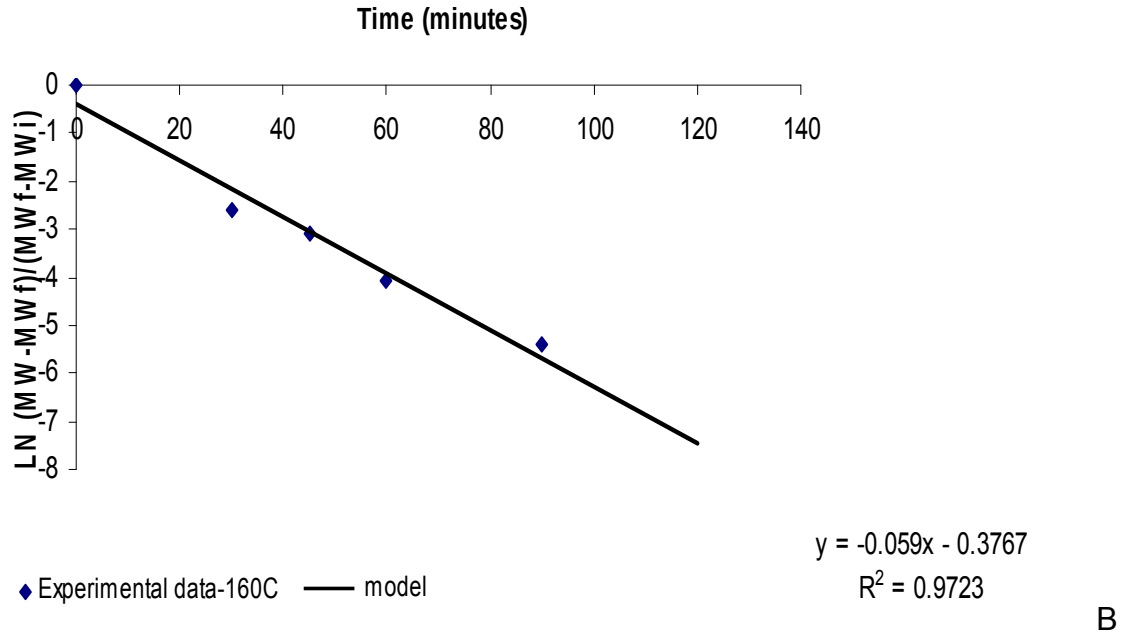


B

Figure 3-5. Linearized plot of rate of change with respect to time A) Model and Experimental data at 121 ºC B) Model and Experimental data at 160ºC

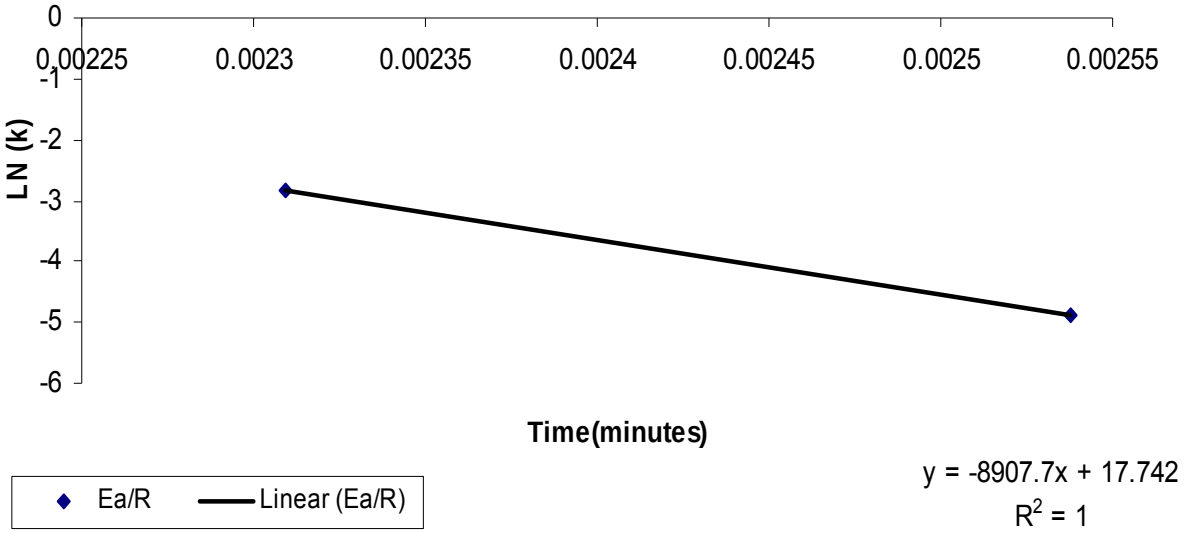
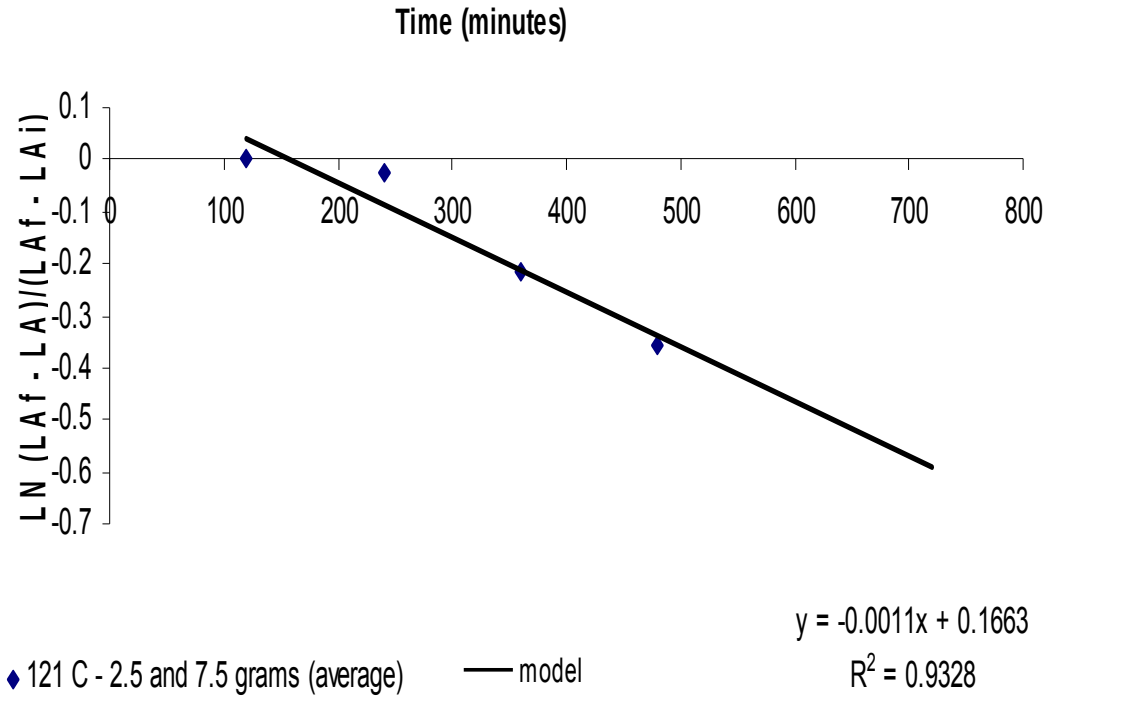


Figure 3-6. Determination of Ea for MW degradation



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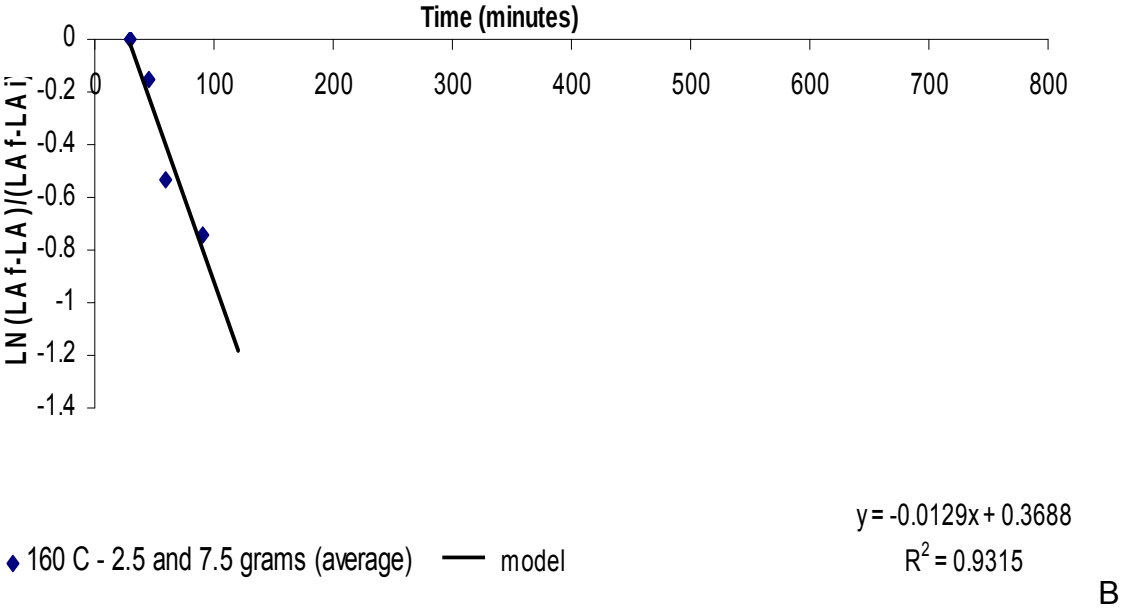


B

Figure 3-7. Lactic acid concentration plotted as first order equation A) 2.5 and 7.5 grams 121 ºC B) 2.5 and 7.5 grams 160ºC

Table 3-1. Molecular weight degradation of PLA at 121ºC

PLA (grams)	2.5	7.5
Time (min)	Mw (g/mol)	Mw (g/mol)
0	1.21×10^5	1.21×10^5
120	2.80×10^4	4.03×10^4
240	1.29×10^4	1.49×10^4
360	1.09×10^4	7.87×10^3
480	8.10×10^3	6.36×10^3
720	5.57×10^3	4.70×10^3

Table 3-2. Molecular weight degradation of PLA 160ºC

PLA (grams)	2.5	7.5
Time (min)	Mw (g/mol)	Mw (g/mol)
0	1.21×10^5	1.21×10^5
30	7.85×10^4	1.18×10^4
45	5.92×10^3	6.85×10^3
60	2.39×10^3	3.50×10^3
90	1.36×10^3	1.51×10^3
120	9.27×10^2	7.24×10^2

Table 3-3. Molecular weight PLA degradation constant k and Ea

k (min^{-1})	T (K)	Ea (kJ)
$7.7*10^{-3}$	394	74.1
$5.9*10^{-2}$	433	

Table 3-4. Lactic acid first order rate constants

Mass (grams)	Temp(°C)	k
2.5 - 7.5 (average)	121	$1.10*10^{-3}$
2.5 - 7.5 (average)	160	$1.29*10^{-2}$

Table 3-5. Primary transition temperatures of selected PLA copolymers[a]

Copolymer ratio	Glass transition temp (ºC)	Melting temperature (º C)
100/0 (L/D,L)-PLA	63	178
95/5 (L/D,L)-PLA	59	164
90/10 (L/D,L)-PLA	56	150
85/15 (L/D,L)-PLA	56	140
80/20 (L/D,L)-PLA	56	(125)[b]

[a] Data from Garlotta, 2002
[b]Melting point achieved by strain crystallization

Table 3-6. Effect of processing conditions on mechanical properties of PLA copolymers[a]

Copolymer ratio (L/D,L)-PLA	Process condition	Tensile strength MPa	Young's modulus GPa	Elongation (%)	MW
100/0	Injection molded, crystallized	64.8	4.0		800000
90/10	Injection molded, amorphous	53.4	1.03	4.6	
90/10	Injection molded, crystallized	58.6	1.29	5.1	
90/10	Extruded, biaxially oriented, strain crystallized	80.9	3.41	41.2	145000
90/10	Extruded, biaxially oriented, strain crystallized, heat set	70.1	2.76	20.7	145000
95/5	Extruded, biaxially oriented, strain crystallized	68.6	1.88	56.7	120000
95/5	Extruded, biaxially oriented, strain crystallized, heat set	60.7	1.63	63.8	120000
80/20	Injection molded, amorphous	51.7	2.1	5.7	268000
80/20	Extruded, biaxially oriented, strain crystallized	84.1	2.9a4	18.2	268000
80/20	Extruded, biaxially oriented, strain crystallized, heat set	80.1	2.54	32.3	268000

[a] Data from Garlotta, 2002

CHAPTER 4
ANAEROBIC DIGESTION OF HYDROLYZED AND NON-HYDROLYZED POLYLACTIC ACID

Introduction

Plastics play an important role as a result of their many applications such as packaging. However, because of their persistence in the environment, and the increased cost of solid waste disposal due to the reductions in available landfill space as well as the potential hazards from waste incineration, polymers have become a waste management problem. Thus, biodegradable polymers – as a potential partial solution of these problems – were developed during the last decade.

Despite being a compostable polymer, currently PLA waste is being sent to landfills. Landfills do not offer an environment for efficient PLA degradation. Landfills are made to serve as a perennial containment for waste. Landfills are not just one compact mass of dirt; they are layers of dirt and wastes. Landfills begin with a lining at the bottom (concrete or plastic), mineral sealing layer, a protective layer, drain, drainage layer, and garbage being placed on the top of it, then a new layer of dirt and impermeable material is leveled for cover; thus minimizing moisture ingress. Liquid collection systems are installed below and above the liners so that any leachate which leaks through or is retained on them can be recovered. Deposition of wet wastes is reduced to a practical minimum. In the first instance, landfill gas should be avoided as far as practicable as they represent a potential risk to people.

Invariable material in the landfill begins to degrade over time generating landfill gas. Although landfill gas recovery is seen as one of the end-of-the-pipe solution to the problem of escaping landfill gas. The trend in a number of countries is to discourage or prohibit landfilling of organic wastes so that any future methane generation in the sites

would be minimal or negligible. The cost of landfilling are high; a study done in the United Kingdom on urban and rural municipal waste disposal indicated that costs range between $ 11.25 and $ 33.75 per ton (UNEP, 1995) . In conventional landfills due to the prolonged persistence of adverse conditions for microbial growth, it takes decades for the waste to degrade and yield methane. Moreover, the gas production is not sustained and is subjected to temporal and spatial variations across the landfill. Anaerobic digestion technologies have been developed for accelerating the biological degradation of the wastes either in bioreactor landfills or in-vessel systems.

In anaerobic digestion process, organic compound like carbohydrates, fats and proteins are mineralized to biogas through the syntrophic action of several groups of microorganism. The process occurs in nature in anaerobic environments in the absence of molecular oxygen, like wetlands, rice fields, intestines of animals, aquatic sediments, and manures, and is responsible for carbon cycling in these environments. The engineered process is called anaerobic digestion (Lai et al., 2008). Anaerobic Digestion of PLA will help not only to save space and money, but it will produce some energy in exchange.

Previous studies conducted by (Vargas et al., 2009) have proven that biodegradability of PLA can be increased by hydrolysis of PLA.

The Biochemical Methane Potential (BMP) assay is a procedure developed to determine the methane yield of an organic material during its anaerobic decomposition by a mixed microbial flora in a defined medium. This assay provides a simple means to monitor relative biodegradability of substrates (Owens and Chynoweth, 1993).

The aim of this study was to determine, the BMP of polylactic acid that was hydrolyzed using different duration of heat exposure and comparing the obtained results with available literature on anaerobic composting/digestion of PLA.

Background

The fermentation process in which organic material is degraded and biogas (composed of mainly methane and carbon dioxide) is produced, is referred to as anaerobic digestion. Anaerobic digestion processes occur in many places where the organic material is available and redox potential is low (zero oxygen). The amount of excess sludge produced is very small and well stabilized, hence even the so called granular anaerobic sludge produced in the bioreactor has economic value (Van Lier et al, 2008).

The complete mineralization to methane and CO_2 of the polymer under anaerobic conditions involves successions of syntrophic associations (Schmotz et al., 2006). Hence, most anaerobic biodegradation studies of polyesters have focused on mixed and unspecified populations such as diverse anaerobic sludges and sediments (Budwill et al., 1992; Reischwitz et al., 1998).

From the literature, it was realized that anaerobic biodegradation of PLA depends on temperature, microbial cultures, pH, agitation, surrounding biomass, and whether the medium is liquid or solid (Abou-Zeid et al, 2001).

Several reports showed that the crystalline part of the PLA was more resistant to degradation than the amorphous part, and that the rate of degradation decreases with an increase in crystallinity (Tsuji et al., 2001). The degradation behavior of polymers also depends on their number average molecular mass (Mn) or weight average

molecular mass (Mw). Polymer molecules, even of the same type, come in different sizes; so we have to take an average of some kind (weight average molecular weight):

$$\bar{M}_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}$$

N_i: number of molecules
M_i: Molecular weight

The number average molecular weight is the ordinary arithmetic mean or average of the molecular weights of the individual macromolecules.

$$\bar{M}_n = \frac{\sum_i N_i M_i}{\sum_i N_i}$$

N_i: number of molecules
M_i: Molecular weight

High molecular weight polymers are degraded at a slower rate than those with low molecular weights (Tokiwa and Suzuki 1978). The melting temperature (T_m) of polyesters has a great effect on enzymatic degradability.

The biodegradability of PLA depends on the environment to which it is exposed. In human or animals bodies, it is believed that PLA is initially degraded by hydrolysis and the soluble oligomers formed are metabolized by cells. Upon disposal in the environment, it is hydrolyzed into low molecular weight oligomers and then mineralized into CO_2 and water by microorganisms present in the environment (Lunt 1998). Soil burial tests show that degradation of PLA in soil is slow and that it takes a long time for degradation to start. For instance, no degradation was observed on PLA sheets after 6 weeks in soil (Okhita and Lee 2006). On the other hand, PLA can be degraded in a composting environment where it is hydrolyzed into smaller molecules (oligomers,

dimmers and monomers) after 45-60 days at 50-60 ºC. These smaller molecules are then degraded into CO_2 and water by microorganisms in the compost (Tokiwa et al., 2006).

PLA degraders have a limited distribution and rather scarce in the soil environment compared with those that degrade poly hydroxyl butyrate (PHB), poly caprolactone (PCL) and poly butylene succinate (PBS). The population of these polyester-degrading microbes decreased in the order of PHB = PCL > PBS > PLA. A burial test comparison of PLA and other polyesters, e.g. PHB, PCL and PBS, indicated that PLA was not readily degraded when the PLA samples were buried under soil for 20 months (Tokiwa and Jarerat, 2004).

Microbial degradation of PLA was first published by Pranamuda et al. (1997) using an *actinomycete Amycolatopsis* strain isolated form soil. Since then, quite a number of *Amycolatopsis* strains have been isolated as PLA degraders. Ikura and Kudo (1999) analyzed 50 samples collected from soil, pond, rivers but only two strains were capable of degrading more than 50% of L-PLA film in the liquid medium. The sequence of the strain is closely related to *Amicolatopsis mediterranei*. Another L-PLA degrading microorganism, *Amycolaptosis* sp strain K104-1 was isolated from 300 soil samples.

In addition to *Amycolaptosis*, several *actinomycetes* belonging to *Lentzea, Kibdelosporangium*, *Streptoalloteichus* and *Saccharothrix* are also capable of degrading PLA.

Out of 14 fungal strains tested, only two strains of *F. moniliforme* and one strain of *Penicillium roqueforti* could assimilate lactic acid and racemic oligomer products of PLA, but no degradation was observed on PLA (Tokiwa and Calabia, 2006).

The microbial vulnerability of polymers is attributed to the biosynthesis of lipases, esterases, ureases and protases. Enzymes involved in deterioration require the presence of cofactors such as, presence of cations present in the material matrix and coenzymes synthesized by microorganisms, for the breakdown of specific bonds. The biodeterioration of thermoplastic polymers could proceed by two different mechanisms (i.e., bulk and surface erosion). PLA proceeds by bulk erosion, in the case of bulk erosion, fragments are lost from the entire polymer mass and the molecular weight changes due to the bond cleavage. This lysis is provoked by chemicals such as, water, acid, bases, transition metals and radicals, or by radiation but not by enzymes. They are too large to penetrate throughout the matrix framework (Lucas et al., 2008).

Several studies have shown that certain proteases, including proteinase K, pronase and bromelain have been found to increase the rate of degradation of PLA while estereases do not (Hakkarainen et al., 2002; MacDonald et al. 1996).

Torres et al., 1999, found growth of fungal mycelia on racemic PLA plates after 8 weeks in soil. Urayama et al., 2002, found only a 20% decrease in molecular weight of PLA (100% L) plates after 20 months in soil while a 75% decrease was noted for PLA (70% L). Ho and Pometto 1997, found about 20% of a PLA film was mineralized to CO_2 after 182 days in a laboratory respirometer charged with soil at 28ºC. It was found that PLA films had weight loses varying form 0 to 100% after burial in soil for 2 years depending on PLA type and location (Shogren et al, 2002).

Vargas et al., (2009), found that PLA samples appeared to be much more vulnerable to themophilic anaerobic biological degradation when PLA was pretreated (gamma radiation, electron beam, etc). However, at 37 ºC, untreated (no pretreatment)

PLA showed negligible weight loss under anaerobic conditions after 180 days. Temperature of incubation was a key factor for anaerobic biodegradation of PLA. In this work methanogenesis was initiated after 21 days of incubation at 58ºC, and this was found to be the quickest breakdown of PLA based on literature review.

This study focuses on direct hydrolysis of PLA at 160 °C and mass loading of 2.5 grams of PLA and anaerobic degradation of hydrolyzate after pretreatment.

Material and Methods

Feedstock

Waste was created using commercial thermoformed cups (Fabri-Kal, Inc., Kalamazoo, MI) obtained from TREEO Center at the University of Florida (Figure 3-1).

Anaerobic Digestion Protocol

The mixed microbial flora preparation and the PLA pretreatment was carried out according to the following protocols.

Mixed microbial flora (inocolum) preparation

Vargas et al., 2009, reported good results when thermally treated PLA and untreated PLA was subjected to anaerobic digestion using thermophilic conditions. In this experiment we first adapted the mixture of microbial flora (inoculum) to acidic feed (i.e. lactic acid) at thermophilic conditions.

To adapt the microbial flora, a 5 L reactor (digestor) was used (Figure 4-1). Using a batch operation the digestor was fed with silage sorghum (fermented). It is well known that fermented sorghum develop organic acids such as lactic acid. After the digestion ceased lactic acid 80 % (L) was added to the digestor. Lactic acid was added daily and the concentration was slowly increased.

The pH and gas produced by the digestor was measured daily. Aliquots of 2.5 mL of lactic acid addition per day was determined as maximum for the system. The inoculum was under adaptation for 210 days.

Feedstock preparation

The created waste was subjected to Hydrolysis. Three set of samples were prepared using duplicates (Table 4-1).

After the hydrolysis Molecular Weight and Lactic Acid content was measured in the samples. The samples with higher ratio of lactic acid recuperation were selected. Best results were achieved when working with concentration of 2.5 grams of PLA with 30 grams of water with thermal exposure of 160ºC (Table 4-2).

BMP preparation

The three hydrolyzed samples and untreated PLA were placed in glass serum bottles (cap 280 mL). 100 mL of the adapted thermophilic inoculum and nutrients were added (Table 4-3). The bottles were sealed with butyl rubber stopper and crimp with aluminum caps; set at 55ºC in a Lab-Line L-C Incubator (Lab-Line Instruments,Inc., Melrose Park,IL).

Analysis

Every 4 days, gas production was measured using a syringe and composition was determined using a Gas Partitioner Chromatograph model 1200 (Fisher Scientific Philadelphia, PA) adapted with a thermal conductivity detector. Biochemical methane potential (BMP) of PLA was expressed as a yield of methane per gram of PLA sample loaded into BMP bottles, and was determined in accordance to ASTM E1 196 (ASTM,1996). Also, Chemical Oxygen Demand (COD) was measured weekly as a way to know if the reaction was taking place or had stopped.

Results

Hydrolyzed PLA showed a faster digestion than “raw” PLA. Figure 4-2A compares the milliliters of methane produced per gram of hydrolyzed PLA. It can be seen that the slope of the samples differs depending on the time they were exposed to heat (Figure 4-3); also, they differ in the amount of methane produced. From all the hydrolyzed samples under anaerobic digestion; the sample with 60 minutes of heat exposure showed better results. PLA samples with 120 minutes of heat exposure at 160 °C showed a 16.34% of methane concentration after 4 days, reaching maximum methane concentration of 54.83% after 23 days. The total amount of methane produced by this sample was 44.92 mL CH_4 /g PLA. PLA samples with 60 minutes of heat exposure at 160 °C showed a 15.20% of methane after 4 days, reaching a maximum methane concentration of 58.47% after 38 days. The total amount of methane produced by this sample was 160.65 mL of CH_4 / g PLA. PLA samples with 30 minutes of heat exposure at 160 °C showed an 11.40% of methane concentration after 4 days, reaching a maximum methane concentration of 49.03% after 27 days.

Raw PLA digestion is shown in Figure 4-2B. Raw PLA samples without any heat treatment showed a 7.90% of methane concentration after 8 days, reaching a maximum methane concentration of 50.80% after 42 days.

Discussion

Previous experiments conducted by Vargas et al., 2009, reported for untreated (raw) PLA hydrolysis and acidification to occur during the first 21 days of anaerobic digestion, and a production of 187 cc CH_4/g PLA after 56 days at 58 °C. In this experiment we started to have methane production as soon as 4 days after the

anaerobic digestion began and a production of 232 mL CH_4 / g PLA after 54 days at 50°C.

Theoretical methane yield was calculated to be 339 mL CH4/g PLA at standard conditions of temperature and pressure. This value was calculated using stoichiometry of the assumed reactions that governed the anaerobic process.

$$C_3H_6O_3 + 3\ O_2 \rightarrow 3\ O_2 + 3\ H_2O$$

$$\frac{3 \times 32}{90} \rightarrow \text{COD} = 1.067$$

$$1.067\ \frac{\text{g COD}}{\text{g PLA}} \times 0.35\ \frac{\text{L } CH_4}{\text{g COD}} = 373 \times (298/328) = 339\ \text{mL } CH_4 / \text{g PLA (STP)}$$

For all the hydrolyzed PLA methane production was calculated assuming 100% of lactic acid concentration in PLA. Therefore, in two grams aliquot of a 2.5 grams of PLA and 30 grams of water mixture the expected amount of methane would be:

$$2.5\ \frac{\text{g PLA}}{32.5\ \text{g mixture}} \times 2\ \text{g mixture} = 0.154\ \text{g PLA}$$

$$0.154\ \text{g PLA} \times 1.067\ \frac{\text{g COD}}{\text{g PLA}} = 0.164\ \text{g COD}$$

$$0.164\ \text{g COD} \times 0.35\ \frac{\text{L } CH_4}{\text{g COD}} = 57.45\ \text{mL } CH_4$$

Figure 4.2A shows experimental data collected when digesting hydrolyzed PLA for 30, 60 and 120 minutes.

The performance of the BMPs was evaluated by fitting the cumulative methane production data to the modified Gompertz equation (Koppar and Pullammanappallil, 2008). The Gompertz equation describes cumulative methane production from batch digesters assuming that methane production is a function of bacterial growth (Table 4.5)

Samples with heat exposure of 120 and 60 minutes were completely done when these results were written. However, the collected data in the other two BMP's, 30 minutes and raw, are good enough to predict the final methane production; the final methane production were calculated to be 143.1 and 323.62 mL respectively.

The methane production differs considerably between the samples treated 30 and 60 minutes to the one treated 120 minutes. Also, the methane production differs considerably between the raw PLA and the samples treated 30 and 60 minutes. The difference in methane production between the samples treated for 30 and 60 minutes are rather low.

This difference in methane production may be due to the feedstock used in each of the BMPs. When digesting raw PLA a methane production of 339 mL of methane per gram of PLA was predicted; however, the actual yield is 323.62 mL. The error between Gompertz model value and the theoretical value is 4.5%. A theoretical value of methane for samples with heat exposure of 30 and 60 minutes is hard to determine since in both cases the feedstock was part solid and part liquid. The PLA sample with heat exposure of 120 minutes was liquid; upon normalizing the results the lactic acid production is 291.68 mL of methane per gram of PLA. Assuming that the sample was just monomer (lactic acid) the expected gas production was 339 mL. The error between the

experimental value and the theoretical value was 6.0%. In all the cases the methane production is less than the stoichiometic theoretical value. The theoretical value used was calculated assuming 100% of lactic acid concentration in the PLA used. Nevertheless, this is not the case. The amount of lactic acid in samples varies from 60 to 80% depending on fillers and plasticizers (Garlotta, 2001). The used fillers and plasticizers may have a different behavior than lactic acid in anaerobic digestion.

These results suggested that adapted bacteria and thermophilic anaerobic digestion should be used when anaerobically digesting PLA. From the results hydrolysis pretreatment seems to expedite the reaction, and more importantly, it can be understood that previous hydrolysis is not needed when using an adapted microbial flora.

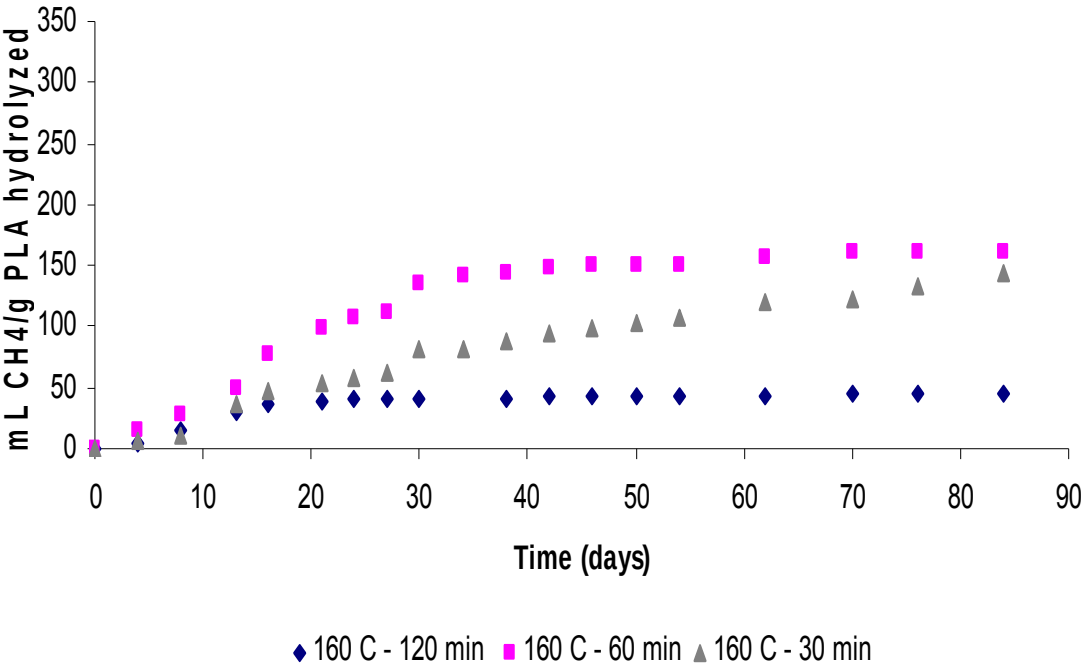
From literature we know that some strains of Actinobacteria (i.e. Amycolatopsis) and Firmicutes (i.e. *Bacillus stearothermophilus, Geobacillus thermocatenulatus* and *Paenibacillus amylolyticus* strain *TB-13*) have been effective when digesting PLA anaerobically. In a study of the UF thermophilic inocolum used for this experiment Actinobacteria and Firmicutes were present in the mixed flora; however, the identification analysis does not indicate the presence of the family of bacteria described in the literature.

This suggested that the concentration of the particular bacteria was negligible when the adaptation of the bacteria started, but separate adaptation can increase the concentration of appropriate bacteria. On the other hand, it may be possible that more than one kind of *Actinobacteria* and *Firmicutes* are able to produce Proteases, Lipases and Estereases that are needed to break down PLA.

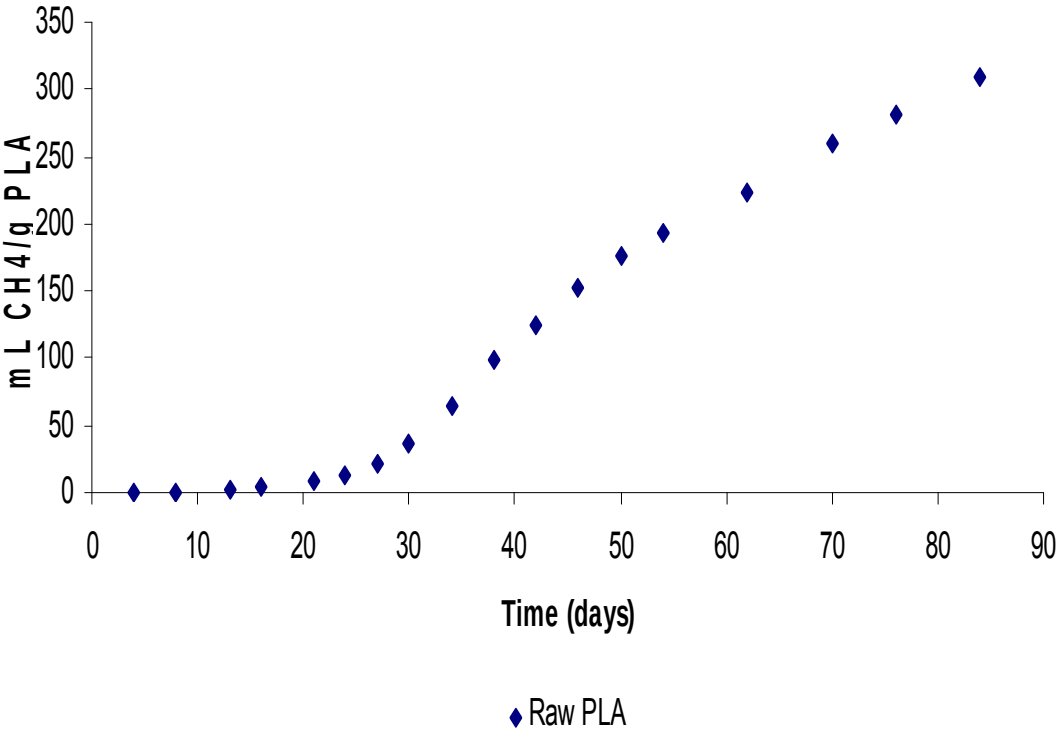
Future Work

To evacuate doubts whether if another *Actinobacteria* and *Firmicutes* are able to produce Proteases, Lipases and Estreases an identification study in the adapted incolum is suggested.

Figure 4-1. Mixed microbial flora adaptation



A



B

Figure 4-2. PLA anaerobic digestion. A) Hydrolyzed PLA. B) Raw PLA.

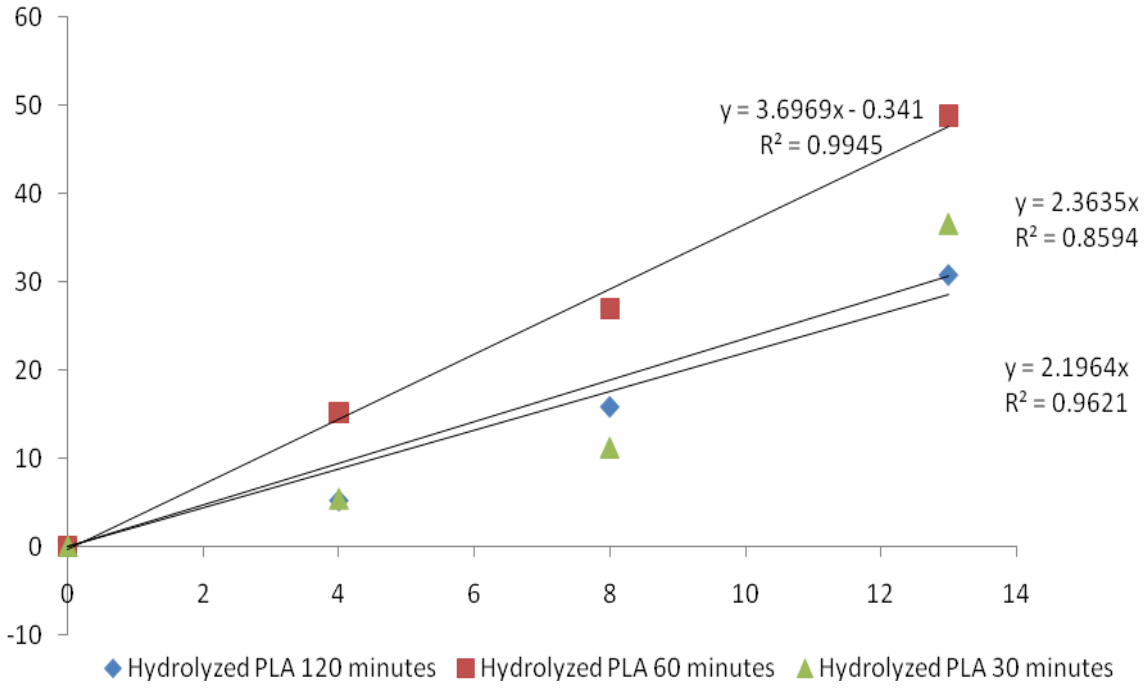


Figure 4-3 Initial methane production rate from hydrolyzed PLA.

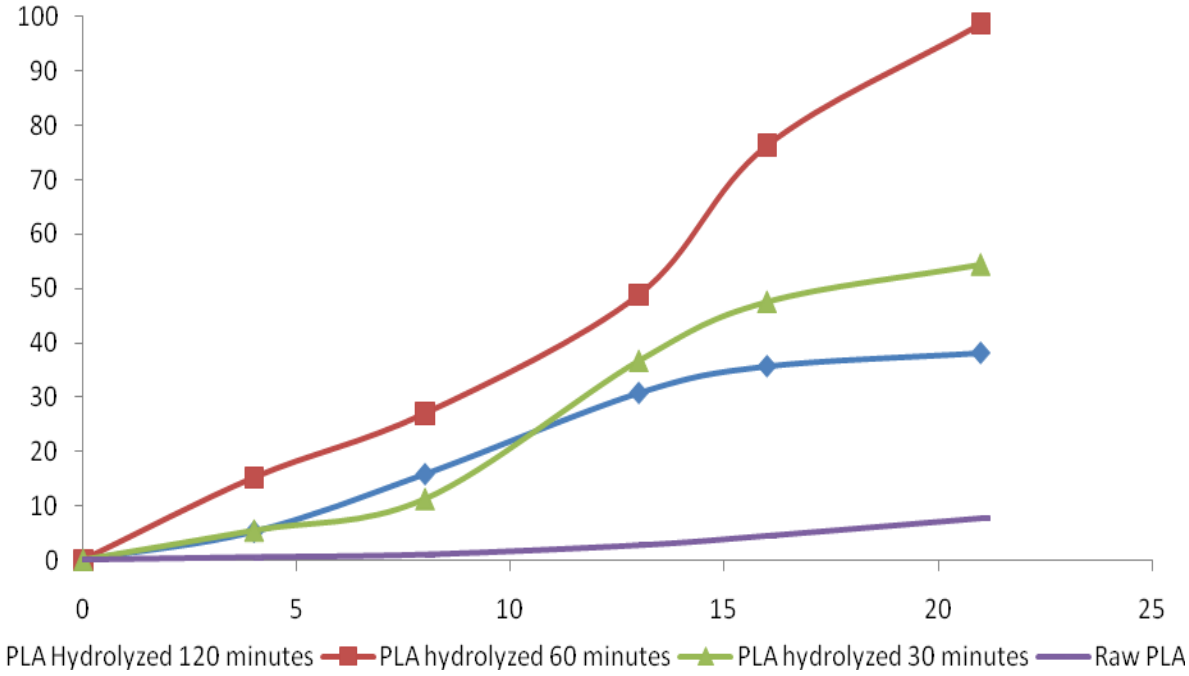


Figure 4-4 Methane production from hydrolyzed PLA and raw PLA in the first 21 days of anaerobic digestion.

Table 4-1. Hydrolyzed selected samples

PLA (grams)	H2O (grams)	Temperature (ºC)	Retention time (min)	Number
2.494	31.566	160.00	30.00	M16
2.584	30.549	160.00	60.00	M18
2.534	30.512	160.00	120.00	M20
2.61	31.336	160.00	30.00	M11
2.539	30.665	160.00	60.00	M13
2.552	30.665	160.00	120.00	M15

Table 4-2. Lactic acid concentration and percent recovery of hydrolyzed selected samples

Number	Lactic Acid (mM)	Recovery (%)
M16	0.03	1.28
M18	0.32	12.26
M20	0.71	27.87
M11	0.03	1.14
M13	0.35	13.59
M15	0.78	30.68

Table 4-3. Content of BMP bottles

Number	$PLA_{hydrolyzed}$ (grams)	Inocolum (mL)
RAW	2.0	100
M16	2.4	100
M18	2.1	100
M20	2.4	100
RAW	2.0	100
M11	2.1	100
M13	2.1	100
M15	2.1	100

Table 4-4. PLA molecular weight after hydrolysis

Number	MW(grams)
RAW	1.21×10^5
M16	2.87×10^3
M18	7.93×10^2
M20	6.60×10^2
RAW	1.21×10^5
M11	3.60×10^3
M13	1.53×10^3
M15	8.22×10^2

Table 4-5 Summary of performance of hydrolyzed and non hydrolyzed PLA

Feedstock condition	Temperature anaerobic digestion °C	CH_4 $yield_{experimental}$ mL CH4/g PLA	Gompertz parameters (model)[a]			Duration to produce 95% CH4 yield potential
			P^b	$R_m{}^b$	λ^b	
			mL CH_4 Kg VS^{-1}	mL CH_4 Kg $VS^{-1}day^{-1}$	days	
Raw PLA	55		323.62	6.99	25.07	86.92
30 minutes-pretreatment	55		143.1	2.4	0.64	83.48
60 minutes-pretreatment	55	160.65	158.28	5.67	3.47	85.25
120 minutes-pretreatment	55	44.92	42.23	3.14	2.79	40.11

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