Biodegradation and Toxicity of Materials in the Marine Environment Supplied by Mirel Plastics Through Respirometry Experimentation According to ASTM D6691 and Polytox Toxicity Testing

Grade P1003

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Introduction

This report details the laboratory findings for the determination of the marine biodegradability of polymer samples using respirometry. These samples were supplied by Mirel Plastics. The samples were tested in accordance with ASTM D6691 "Standard Test Method for Determining Aerobic Biodegradation of Plastics Materials in the Marine Environment by a Defined Microbial Consortium or Natural Seawater" to analyze the amount of biodegradation (percent mineralization) as a function of time. According to ASTM Specification D7081-05, "Standard Specification for Non-Floating Biodegradable Plastics in the Marine Environment", the plastics must demonstrate that 30% or more of the organic carbon is converted to carbon dioxide (mineralization) using ASTM D6691 within 180 days of testing at 30˚C. This is just one of numerous requirements that must be met in order for the material to be classified as "marine biodegradable" according to the ASTM specification.

Experimental Set-up

Sample Preparation

To prepare the polymer samples for testing, each polymer was cryogenically milled to a fine powder using a SPEX Certi-Prep 6750 cryogenic mill. Three 20.00mg test samples were then created from the milled polymer in order to test the polymer in triplicate. In order to determine the percentage of sample that has mineralized (biodegraded) during the test, the initial amount of carbon in the sample must be determined. The polymer samples were sent to Galbraith Laboratories in Knoxville, Tennessee for carbon content determination. This test was run in duplicate and the average value was taken as the carbon content of the sample. The average percent mineralization of the polymer samples during the test would be calculated using this

data. Table 1 lists the measured carbon content for each sample based on analysis at Galbraith Laboratories.

Table 1: Carbon Content Analysis Results of Samples

Sample	Carbon Content (%)
P1003	**55.16**

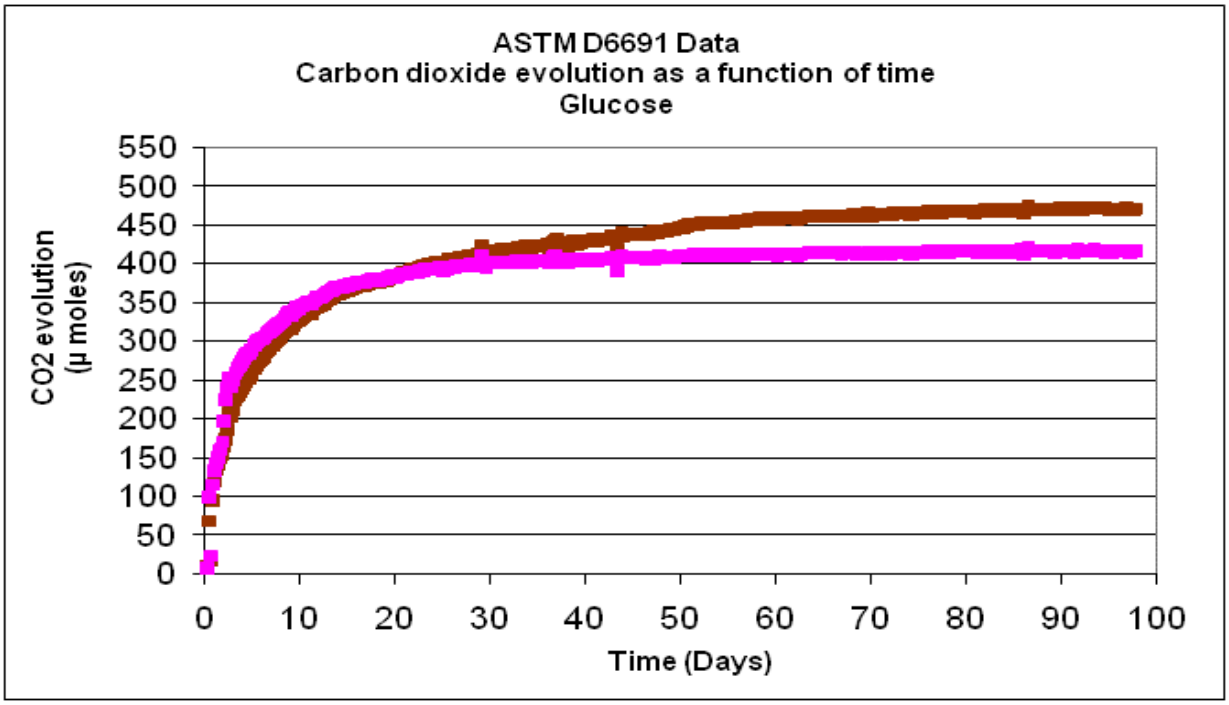
ASTM 6691 Laboratory Testing

Biodegradation in the marine environment was examined through respirometry experimentation (ASTM D6691) where natural sea water was used as the experimental medium. This water was collected from Hampton Beach, NH in July of 2010. Columbus Instruments respirometers were used to measure carbon dioxide evolution (mineralization) as a function of time. Glucose, a known biodegradable material, was used in three sample chambers in order to confirm that the respirometer was operating properly. Chambers containing only the natural seawater were used as a negative baseline control. Please see ASTM D6691 for a more detailed description of the experimental procedure.

Results

Presented in Figure 1, is the carbon dioxide evolution curve of the glucose material that demonstrates excellent biodegradability in the marine environment. This data confirmed that the respirometer was operating properly and exemplifies the shape of an excellent mineralization curve. The measured results for biodegradation testing of the P1003 sample, according to ASTM D6691 is presented in Figure 2. This sample was run in triplicate and the data is shown. The data in the figure has been baseline corrected according to the results obtained by running negative

control samples. Any carbon dioxide evolution measured from the negative control sample is considered to be an experimental baseline and is subtracted from the final test results.



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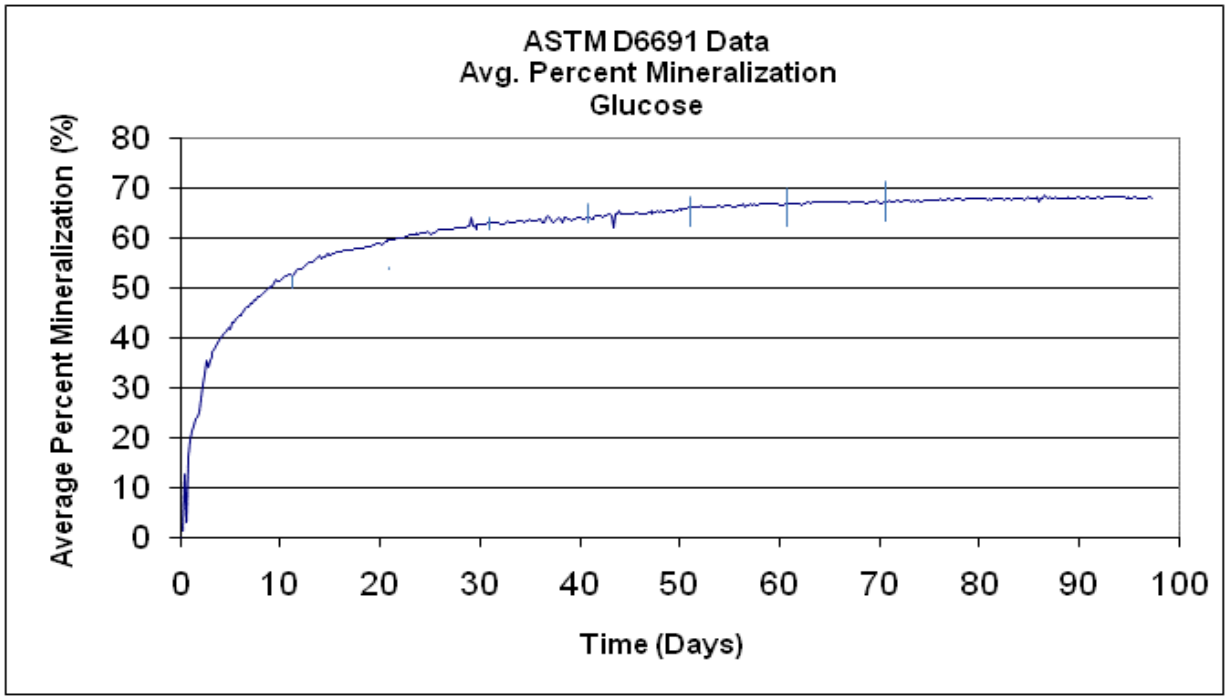

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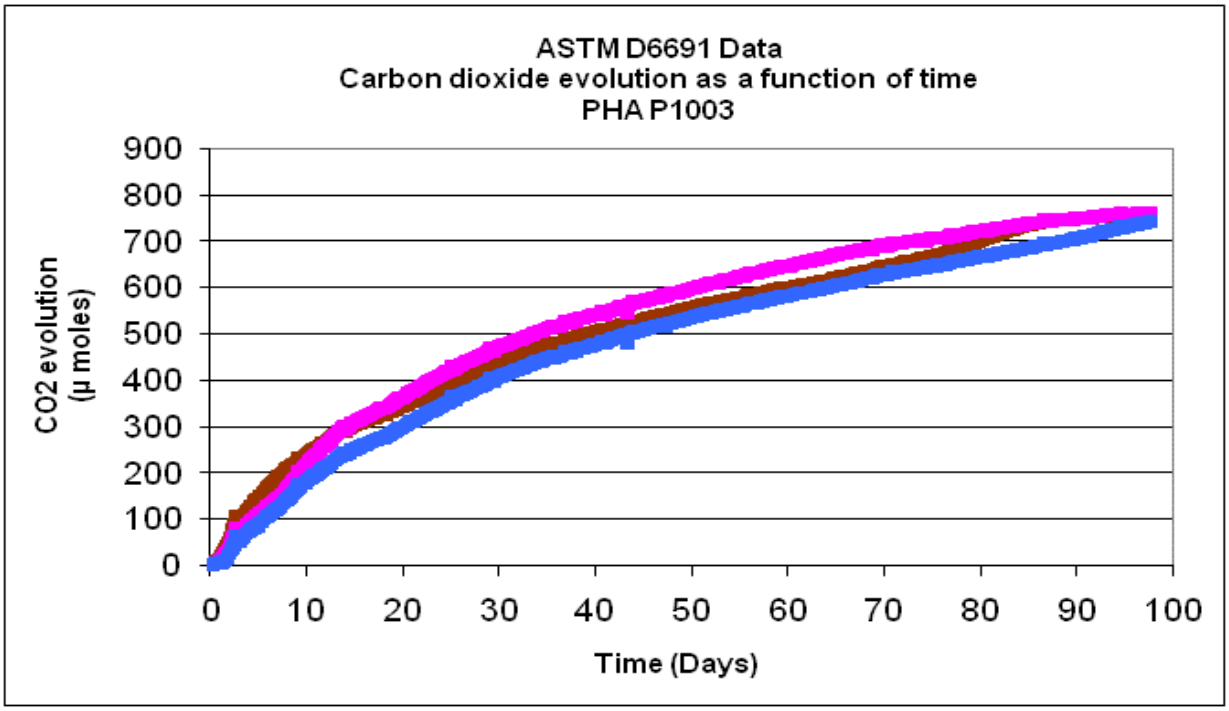
Figure 1: (a) Carbon Dioxide Evolution of glucose as a function of time (b) Average percent mineralization of glucose as a function of time



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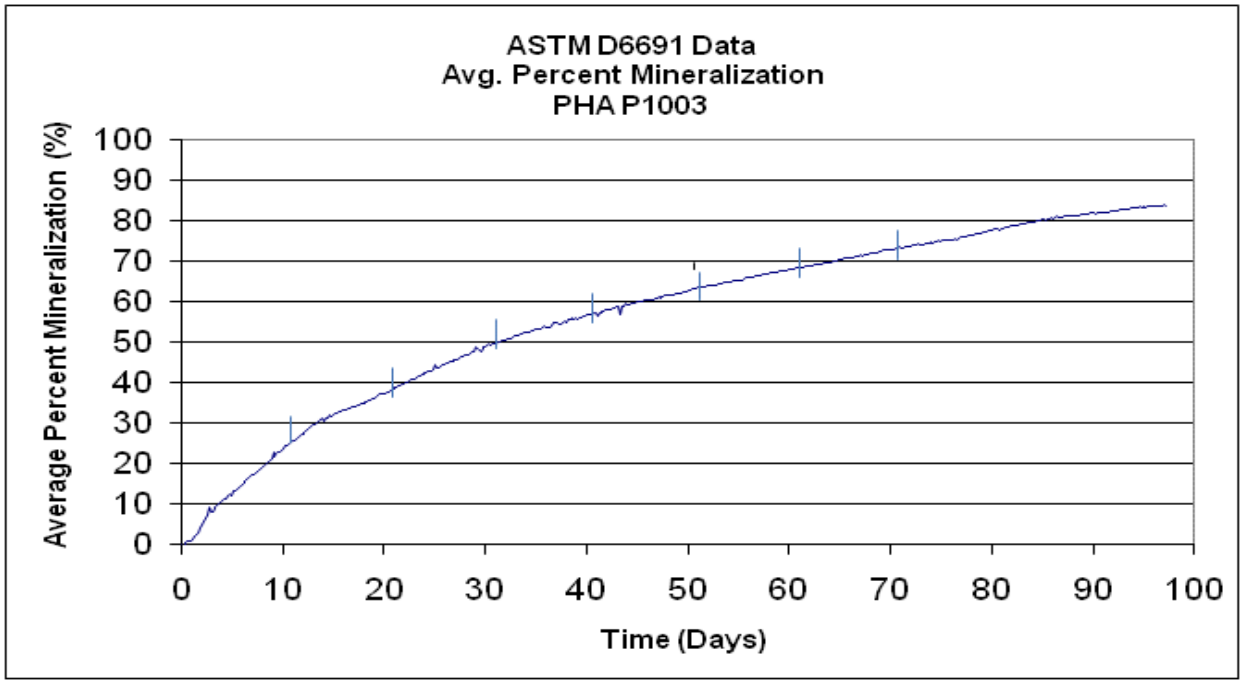

(b)

Figure 2: (a) Carbon Dioxide Evolution of Mirel PHA P1003 as a function of time (b) Average percent mineralization of Mirel PHA P1003 as a function of time

Toxicity Testing

Besides the need to test these samples for biodegradability in the marine environment, it is just as important to determine the toxicity of the samples. The EPA has approved the Polytox® test as an accepted way of measuring the toxicity of samples to the marine environment. This test uses specialized microbial cultures to measure the respiration rate under defined conditions in the presence of the sample. This respiration rate is the amount of oxygen consumed by the aerobic cultures. If the respiration rate of the test solution is lower than the measured baseline rate, then the test solution is considered inhibitory to the microorganisms and toxic. All test samples were subjected to natural seawater at a concentration of 1g/L which was amended with 5g/L nitrogen and 1g/L phosphorous as nutrients. Sample solutions were consistently stirred throughout the duration of the test and were measured at time intervals of 3 days and 1 month to determine the immediate and long-term effects of the sample on its environment.

The baseline activity of the Polytox cultures in natural sea water was determined prior to the toxicity testing. To do this, 500mL of pH adjusted (7.0) natural sea water was aerated using standard house-supplied laboratory air for 30 minutes. One vial of the Polytox culture was poured into a BOD bottle. 50mL of the air saturated seawater was then poured into the vial and the contents were slowly hand mixed for 30 seconds by swirling the contents of the bottle. Additional pre-aerated seawater was then added to the bottle to a level that was just above the bottom of the ground glass joint. The dissolved oxygen probe was inserted into the BOD bottle and stirring was initiated using the stirring mechanism on the probe. The dissolved oxygen reading was recorded every two minutes and the following equation was used to determine the dissolved oxygen uptake rate of the polytox culture in seawater ($DOUR_S$):

$DOUR_S = (DO19_S – DO21_S)/2$ Minutes = mg/L/min ($DO19_S$ = Dissolved Oxygen at 19 minutes)

The same procedure was then used to calculate the background rate of respiration of the sample itself in the absence of the Polytox populations. The natural seawater itself was tested without the addition of the Polytox population. Through this method, the following equation was then used to determine the baseline dissolved oxygen uptake rate ($DOUR_B$):

$DOUR_B = (DO19_B – DO21_B)/2$ Minutes = mg/L/min ($DO19_B$ = Dissolved Oxygen at 19 minutes)

Finally, the toxicity tests of the samples were run after the samples had been allowed to sit in the seawater test solution for time intervals of 3 and 30 days. To begin, 500mL of the natural sea water, which had been in contact with the sample, was transferred to a separate bottle where it was aerated for 30 minutes. The toxicity test was then carried out on the samples according to the procedure that was used to measure the baseline activity of the samples. This test yielded the dissolved oxygen uptake rate of the test samples according to the following equation:

$DOUR_T = (DO19_T – DO21_T)/2$ Minutes = mg/L/min ($DO19_T$ = Dissolved Oxygen at 19 minutes)

The following equation was used to calculate the corrected dissolved oxygen uptake rate for the sample to account for any background activity ($DOUR_B$):

$DOUR_C = DOUR_T - DOUR_B$

The percent inhibition of the test samples to the Polytox populations was calculated using the following equation:

$\%\ Inhibition = 1 - DOUR_C / DOUR_S \times 100$

A negative percentage result indicates a stimulation of the test culture while a positive result indicates inhibition of the oxygen uptake rate and therefore is considered to be toxic to the Polytox populations.

Results

Table 2 summarizes the results of this testing for the Mirel P1003 resin that was provided to the U.S. Army Natick Soldier Research Development and Engineering Center (NSRDEC).

Table 2: Summary of Polytox Testing Results

Sample	Oxygen Inhibition % (3 days)*	Oxygen Inhibition % (30 days)*
Mirel P1003 Resin	-2.17	-69.06

*** Values greater than (+) 5% Indicate Possible Toxicity**

The results presented in Table 2 indicate that the Mirel P1003 resin stimulated the test culture and therefore is not considered toxic by the Polytox toxicity testing method.