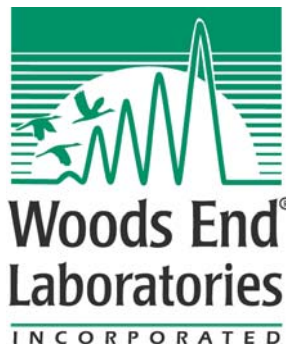


**Report:**  
**Determination of Anaerobic Biodegradation of  
BioFlex PVC Film by ASTM D-5526 Procedure**

for

UltraFlex Systems, Inc.  
1578 Sussex Turnpike, Bldg 4  
Randolph NJ 07869

Prepared by:



August 28, 2007

## Executive Summary

Bioflex film #7450 provided by the client was tested for biodegradation according to ASTM procedure D-5526-94 — *Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under Accelerated Landfill Conditions*. The test was conducted at 35°C for 104 days, at three moisture levels in an inoculum substrate prepared from municipal solid waste. *Apparent net decomposition* of the test material was calculated from the cumulative biogas-carbon evolved from the test vessels after subtracting the biogas-carbon evolved from the blank inoculum substrate at the same dry-matter level.

Apparent net decomposition of the Bioflex material at the end of the test period at the 45% dry-matter (DM) test conditions was 14.5%. Bioflex decomposition at 35% DM rose to 18% at day-35, then declined steadily to negative values, indicating inhibition of the inoculum substrate decomposition at that point. Biogas evolution from the Bioflex vessels at 60% DM was slower than the corresponding blanks for the first 60 days, with cumulative net decomposition of negative 9% indicating slight inhibition of the inoculum substrate.

The cellulose powder reference material showed 16% net decomposition in the 60% DM conditions, and net negative decomposition (apparent inhibition) at 35% and 45% DM.

Biogas evolution from the blanks indicated that total decomposition of the organic carbon in the inoculum substrate was 21%, 28%, and 18% in the 35%, 45%, and 60% dry-matter conditions, respectively.

## Materials and Methods

The biodegradation test was conducted in 125cc septum bottles, incubated in a 35°C water bath. The inoculum substrate consisted of municipal solid waste (MSW) that had undergone a two-month period of composting and curing in windrows with mechanical ventilation. The compost was further conditioned by screening through a 4-mm sieve, then curing in the laboratory at room temperature for seven days in an open plastic bag. Each test vessel contained 10 grams fresh weight of this inoculum, plus 2.2ml of an active anaerobic biogas seed culture that had been maintained by monthly feeding with municipal foodwaste.

The Bioflex material #7450 was supplied by UltraFlex as sheets of 0.67mm approximate thickness. Each test vessel contained 2.00 grams of Bioflex cut into approximately 140 3mmx5mm pieces. Positive reference controls were set up with 1.00 grams of 20-micron cellulose powder (Sigma-Aldrich, p/n 310697, batch #08622CD). Blank controls were set up with only the inoculum substrate. All treatments were set up in triplicate. The contents for each vessel were mixed then adjusted to the designated dry-matter level by addition of de-ionized water, then thoroughly mixed again. After introduction into the test bottles, each bottle was sealed with a septa-cap, and the incubation was begun. The test materials parameters are shown in Table 1.

**Table 1.** Properties of BioFlex test material, cellulose powder positive control, and inoculum substrate in ASTM 5526 procedure.

<b>Property</b>	<b>Inoculum Substrate</b>	<b>BioFlex</b>	<b>Cellulose Powder</b>
Dry Matter, %	73.0	100	100
Volatile Solids, % of DM	76.7		
Total Carbon, % of DM	40.8	40	40
TN, % of DM	1.21		
C:N	34		
pH	6.35		
Quantity in Vessel, g. fresh	10.0	2.00	1.00
Total Carbon, grams in Vessel	2.98	0.8	0.4
Maximum Possible Biogas, ml.	6084	1633	817

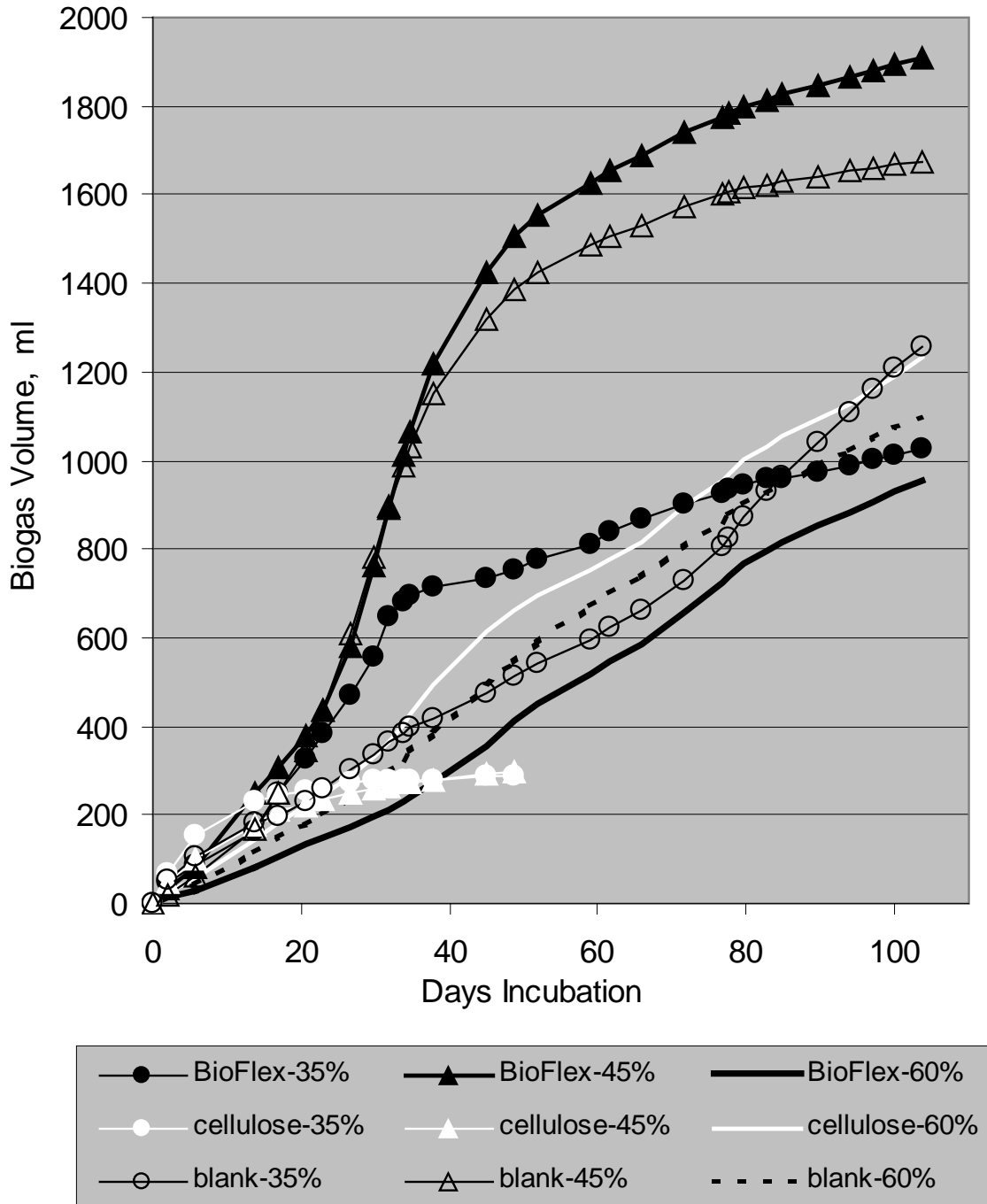
Biogas evolution was measured two times per week by insertion through the septum with a 25-gauge needle attached to a digital pressure transducer (Sper Scientific Ltd.). At approximately weekly intervals the composition of the biogas was determined by non-dispersive infra-red sensor (until day 27) or gas chromatography (after day 27). The pressure readings were converted to biogas volume using equations derived from calibration of the pressure sensor with the appropriate bottle headspace volumes: ml gas = (bars pressure)\*(calibration slope). Biogas volumes in the procedure blanks were subtracted from the Bioflex and reference control gas volumes to determine the net decomposition or inhibition due to the presence of the test materials.

## Results

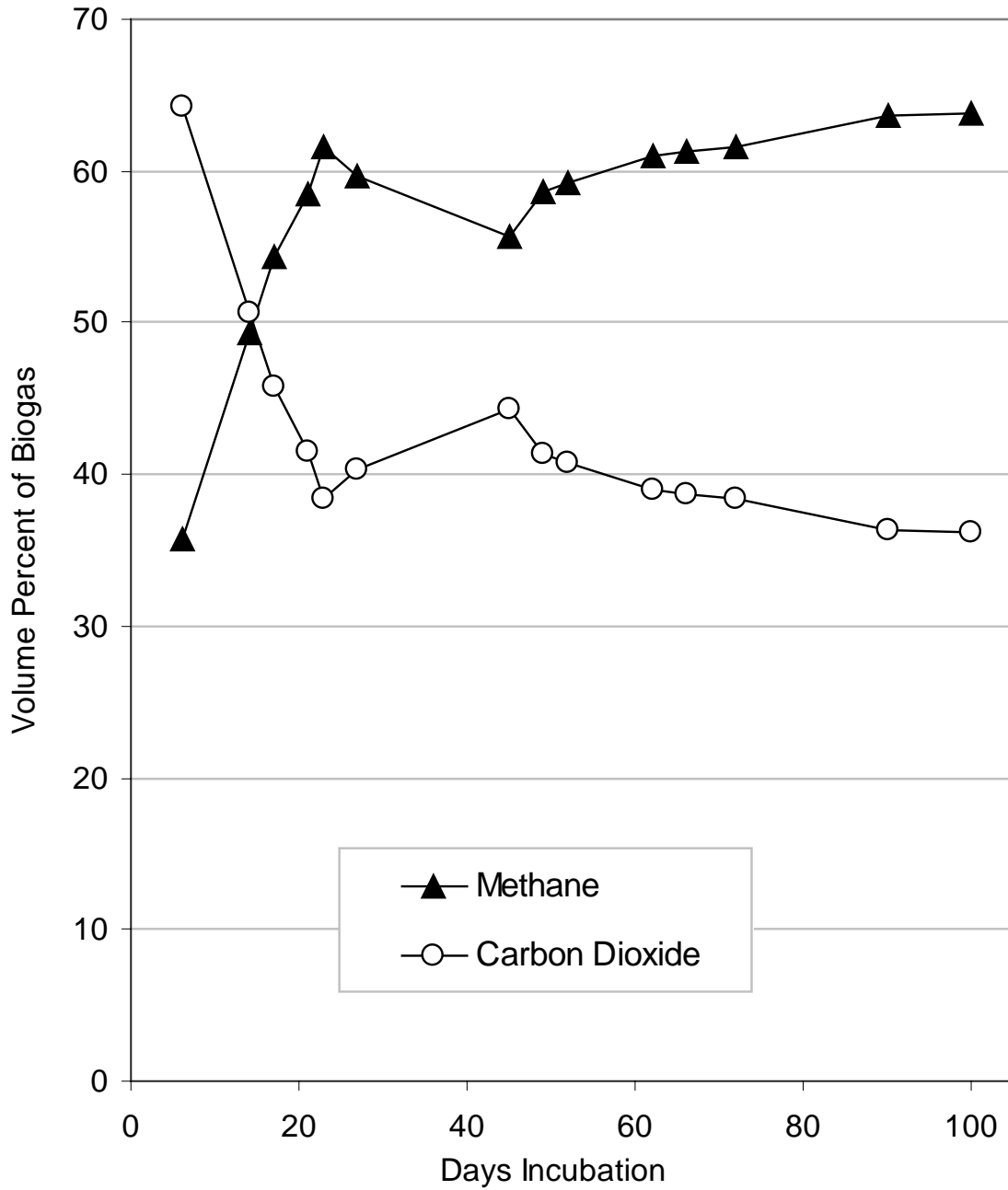
The pressure readings indicating biogas evolution by each of the nine test vessels are listed in Table 2. After 62 days of incubation two of the blank and Bioflex vessels at 35% dry matter and all three of the cellulose reference vessels at both 35% and 45% dry matter were discontinued because biogas production had ceased. The course of total biogas evolution for all nine treatments is shown in Figure 1. Biogas composition followed the normal course of increasing proportion of methane (Table 3 and Figure 2).

Net decomposition for the Bioflex and positive cellulose control at all three dry-matter levels is shown in Figure 3. Net decomposition is the total C-loss from the test vessels (test material plus substrate), minus the carbon loss of the blank substrate at the corresponding moisture level. Positive values or slopes indicate net decomposition of the BioFlex or cellulose powder. Negative values or declining slopes indicate inhibition of the substrate decomposition in the test vessels with respect to the substrate in the corresponding blanks. At the end of the test period apparent net decomposition of the Bioflex material at the 45% dry matter test conditions was 14.5% (standard deviation = 2.81, 95% confidence interval = 0.1). Bioflex decomposition at 35% dry matter rose to 18% at day-35, and then declined steadily to negative values, indicating inhibition of the inoculum substrate decomposition. Biogas evolution from the Bioflex vessels at 60% dry matter was slower than the corresponding blanks for the first 60 days, with cumulative net decomposition of negative 9% indicating slight inhibition of the inoculum substrate. The cellulose powder reference material showed 16% net decomposition in the 60% TS conditions and net negative decomposition (apparent inhibition) in the 35% and 45% TS conditions.

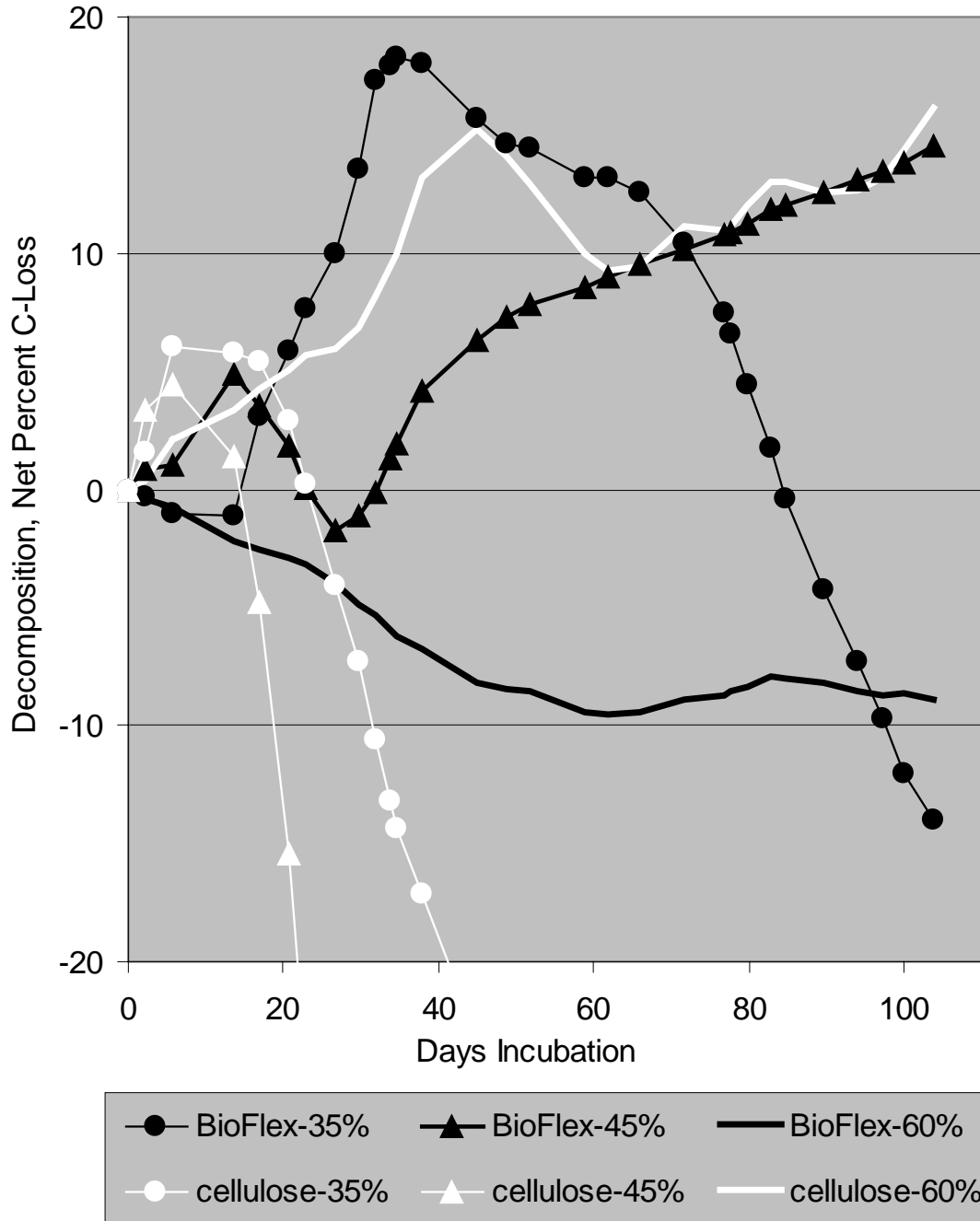
Biogas evolution from the blanks indicated that total decomposition of the organic carbon in the inoculum substrate was 21%, 28%, and 18% in the 35%, 45%, and 60% dry-matter conditions, respectively (Figure 4).



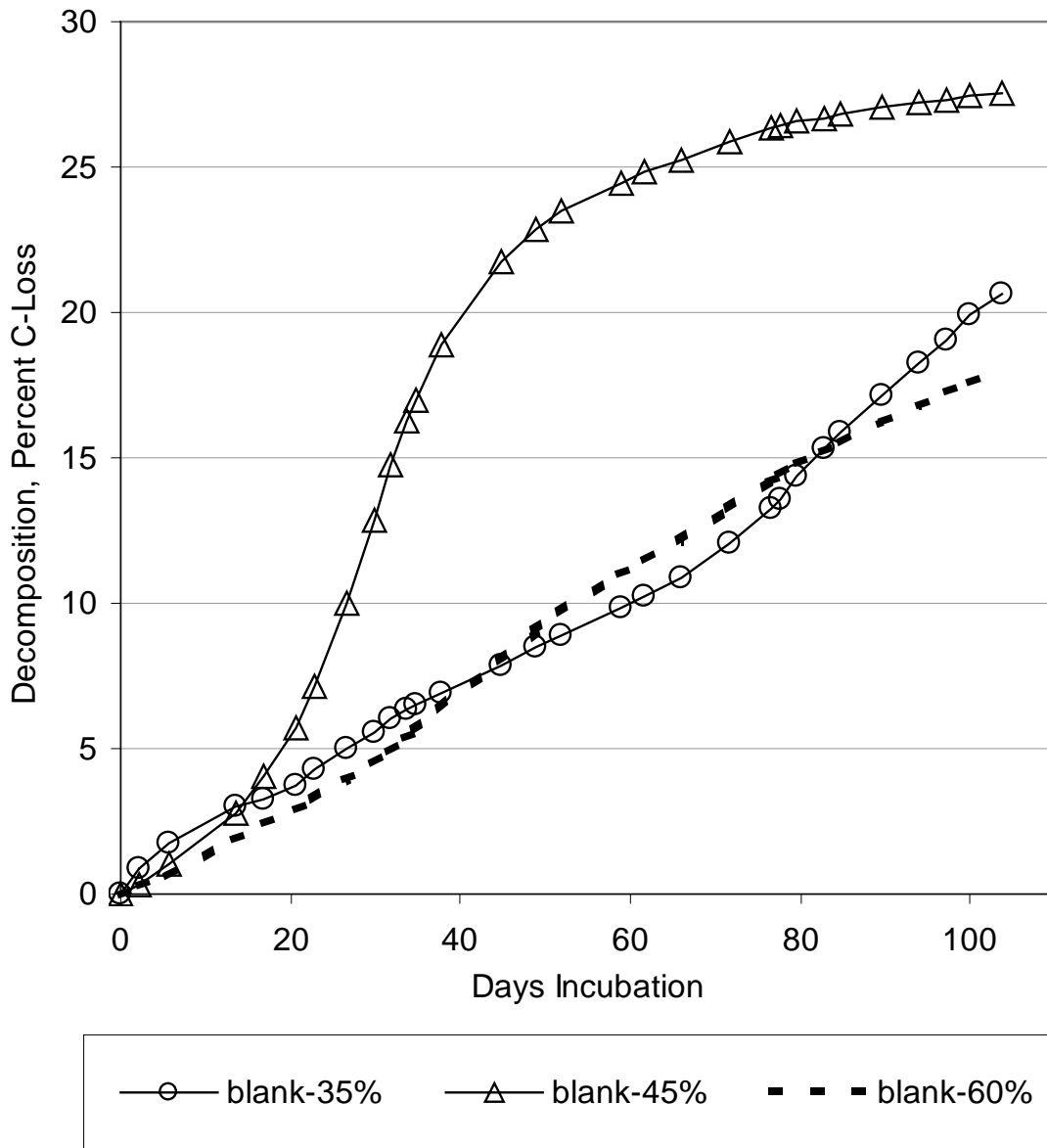
**Figure 1.** Cumulative biogas collected from BioFlex test material, cellulose powder positive control, and inoculum substrate blank in ASTM 5526 procedure. Each point is the mean of three reps. The cellulose positive controls at 35% and 45% dry matter were discontinued after 49 days because biogas evolution had ceased.



**Figure 2.** Composition of biogas evolved from BioFlex test material, cellulose powder positive control, and inoculum substrate blank in ASTM 5526 procedure. Each point is the mean of all test vessels.



**Figure 3** Percent carbon loss from BioFlex test material and cellulose powder positive control, in ASTM 5526 procedure, calculated from total biogas carbon evolution as a percent of the initial total carbon. The legend indicates the test material and the percent dry-matter. Decomposition is the total C-loss from the test vessels (test material plus substrate), minus the carbon loss of the blank substrate at the corresponding moisture level. Positive values or slopes indicate net decomposition of the BioFlex or cellulose powder. Negative values or declining slopes indicate inhibition of the substrate decomposition in the test vessels with respect to the substrate in the corresponding blanks. Each point is the mean of three reps.



**Figure 4.** Cumulative carbon loss from inoculum substrate in ASTM 5526 procedure, calculated from total biogas carbon evolution as the percent of initial carbon content. The legend indicates the percent dry-matter. Each point is the mean of three reps.

**Table 2. Biogas pressure readings**

days	blank 35 A	blank 35 B	blank 35 C	blank 45 A	blank 45 B	blank 45 C	blank 60 A	blank 60 B	blank 60 C	BFlex 35 A	BFlex 35 B	BFlex 35 C	BFlex 45 A	BFlex 45 B	BFlex 45 C	BFlex 60 A	BFlex 60 B	BFlex 60 C	cell . 35 A	cell . 35 B	cell . 35 C	cell . 45 A	cell . 45 B	cell . 45 C	cell . 60 A	cell . 60 B	cell . 60 C
0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.1	0.442	0.450	0.462	0.122	0.300	0.124	0.166	0.176	0.172	0.440	0.424	0.430	0.300	0.310	0.318	0.162	0.094	0.106	0.588	0.578	0.594	0.420	0.424	0.416	0.196	0.198	0.200
5.7	0.438	0.436	0.458	0.366	0.426	0.280	0.166	0.162	0.166	0.384	0.440	0.350	0.386	0.392	0.368	0.120	0.116	0.108	0.778	0.812	0.794	0.452	0.452	0.418	0.316	0.312	0.232
13.7	0.418	1.000	0.700	0.502	1.340	0.824	0.628	0.630	0.572	0.710	0.662	0.704	1.424	1.460	1.458	0.466	0.352	0.468	0.812	0.610	0.652	0.666	0.718	0.684	0.906	0.840	0.346
16.9	0.118	0.480	0.130	0.526	0.738	0.764	0.260	0.342	0.208	0.074	0.110	0.748	0.550	0.522	0.420	0.232	0.168	0.270	0.188	0.012	0.120	0.254	0.280	0.232	0.358	0.348	0.300
20.6	0.166	0.556	0.272	0.696	0.928	0.904	0.220	0.350	0.208	0.154	0.278	0.680	0.632	0.696	0.536	0.238	0.152	0.266	0.132	0.072	0.072	0.106	0.118	0.084	0.336	0.340	0.270
22.9	0.104	0.150	0.268	0.846	0.688	0.692	0.146	0.216	0.150	0.164	0.196	0.540	0.470	0.514	0.504	0.130	0.104	0.160	0.082	0.064	0.070	0.096	0.050	0.212	0.244	0.240	0.164
26.7	0.082	0.112	0.400	1.566	1.470	1.430	0.300	0.400	0.288	0.250	0.170	0.752	1.120	1.228	1.454	0.230	0.162	0.278	0.100	0.062	0.118	0.120	0.160	0.096	0.342	0.390	0.310
29.8	0.028	0.056	0.278	1.650	1.218	1.476	0.322	0.314	0.228	0.134	0.058	0.810	1.378	1.486	1.818	0.178	0.112	0.224	0.028	0.034	0.070	0.104	0.116	0.074	0.382	0.360	0.318
31.8	0.014	0.036	0.250	0.968	1.050	0.938	0.214	0.226	0.158	0.100	0.024	0.794	1.088	1.090	1.226	0.142	0.100	0.166	0.000	0.000	0.034	0.050	0.062	0.038	0.354	0.244	0.272
33.7	0.000	0.036	0.188	0.746	0.842	0.790	0.216	0.258	0.236	0.040	0.036	0.294	1.038	0.988	1.016	0.160	0.122	0.202	0.002	0.002	0.016	0.048	0.050	0.036	0.430	0.306	0.242
34.7	0.000	0.026	0.090	0.338	0.402	0.384	0.110	0.138	0.140	0.028	0.028	0.140	0.474	0.468	0.438	0.100	0.060	0.122	0.000	0.000	0.000	0.024	0.026	0.026	0.252	0.184	0.062
37.8	0.000	0.034	0.198	0.934	1.004	1.018	0.296	0.402	0.306	0.022	0.012	0.164	1.330	1.358	1.258	0.280	0.202	0.326	0.000	0.000	0.000	0.034	0.040	0.026	0.716	0.524	0.428
44.8	0.066	0.088	0.510	1.492	1.320	1.530	0.786	0.974	0.792	0.098	0.094	0.168	1.882	1.742	1.708	0.794	0.534	0.680	0.086	0.056	0.070	0.103	0.142	0.136	1.114	0.960	0.918
48.8	0.000	0.038	0.344	0.558	0.620	0.560	0.544	0.496	0.462	0.022	0.030	0.200	0.760	0.728	0.704	0.580	0.384	0.442	0.016	0.008	0.012	0.040	0.040	0.046	0.472	0.344	0.458
51.8	0.008	0.004	0.234	0.304	0.354	0.326	0.374	0.318	0.322	0.020	0.013	0.208	0.452	0.394	0.376	0.362	0.258	0.374	0.014	0.000	0.000	0.000	0.024	0.032	0.296	0.198	0.292
58.9	0.000	0.000	0.492	0.492	0.552	0.508	0.704	0.684	0.600	0.018	0.026	0.322	0.680	0.628	0.586	0.584	0.518	0.550	0.000	0.000	0.004	0.022	0.032	0.032	0.530	0.352	0.534
61.7	0.004	0.000	0.224	0.166	0.184	0.156	0.252	0.262	0.216	0.018	0.006	0.222	0.246	0.240	0.198	0.206	0.252	0.256	0.002	0.000	0.000	0.000	0.000	0.000	0.234	0.128	0.232
65.9			0.332	0.184	0.264	0.194	0.416	0.294	0.278			0.254	0.272	0.388	0.242	0.346	0.368	0.300							0.418	0.222	0.388
71.7			0.630	0.296	0.404	0.346	0.678	0.488	0.446			0.320	0.414	0.558	0.330	0.680	0.552	0.622							0.904	0.328	0.742
76.7			0.658	0.190	0.218	0.262	0.590	0.442	0.458			0.238	0.342	0.386	0.230	0.588	0.440	0.586							0.756	0.202	0.514
77.7			0.182	0.046	0.046	0.054	0.110	0.084	0.086			0.046	0.076	0.078	0.044	0.120	0.088	0.116							0.152	0.034	0.126
79.7			0.410	0.084	0.070	0.098	0.254	0.182	0.196			0.102	0.146	0.146	0.098	0.264	0.220	0.244							0.388	0.102	0.326
82.7			0.498	0.098	0.000	0.076	0.106	0.284	0.218			0.112	0.158	0.164	0.110	0.262	0.254	0.280							0.404	0.108	0.308
84.7			0.304	0.068	0.052	0.072	0.178	0.154	0.158			0.000	0.108	0.100	0.066	0.152	0.164	0.152							0.214	0.090	0.182
89.7			0.696	0.120	0.092	0.116	0.368	0.344	0.320			0.146	0.208	0.200	0.154	0.318	0.332	0.312							0.446	0.180	0.340
93.9			0.588	0.096	0.076	0.104	0.318	0.306	0.246			0.150	0.184	0.188	0.132	0.270	0.250	0.232							0.390	0.198	0.296
97.1			0.446	0.064	0.048	0.070	0.258	0.208	0.194			0.098	0.134	0.134	0.098	0.188	0.218	0.202							0.356	0.178	0.250
99.9			0.444	0.062	0.046	0.066	0.198	0.184	0.160			0.110	0.138	0.114	0.078	0.164	0.198	0.192							0.316	0.218	0.236
103.8			0.396	0.044	0.050	0.076	0.212	0.282	0.200			0.114	0.200	0.150	0.104	0.200	0.208	0.218							0.446	0.342	0.276
total			11.1	13.6	14.8	14.2	9.4	9.6	8.2	2.7	2.6	9.2	16.6	16.9	16.1	8.5	7.0	8.5	2.8	2.3	2.6	2.5	2.7	2.6	12.7	8.5	9.6
mean		11.1			14.2			9.1			9.2			16.5			8.0			2.6			2.6			10.2	
stdev				0.59				0.76						0.40			0.87			0.26			0.10			2.18	

Table 3. Methane and carbon dioxide content of weekly biogas samples collected from the ASTM 5526 test of Bioflex. Gases were measured in pooled samples by non-dispersive infra-red sensors until day 27. For the remainder of the trial analyses were by gas chromatography on samples from each test vessel. Each point in the table is the mean of three reps. The values shown have been normalized to 100% total for the two gases for each sample.

Days	6	14	17	21	23	27	45	49	52	62	66	72	90	100	
<b>Methane</b>															
blank-35%	39	56	59	59	60	65	54	57	56	59	60	60	68	66	
blank-45%	39	56	59	59	60	65	60	60	60	62	61	62	63	64	
blank-60%	39		59	60	60	67	59	60	60	60	61	61	61	61	
BioFlex-35%	34		60	63		62	52	57	61	64	65	64	67	68	
BioFlex-45%	34	63		63	64	62	60	60	60	62	62	63	64	65	
BioFlex-60%	34				64		60	61	60	61	62	62	61	62	
cellulose-35%	34	30	48	54		52	50								
cellulose-45%	34	36	48	54		52	50								
cellulose-60%	34	56	48	54	61	52	56	57	57	59	59	59	60	60	
means	36	49	54	58	62	60	56	59	59	61	61	62	64	64	
<b>Carbon Dioxide</b>															
blank-35%	61	44	41	41	40	35	46	43	44	41	40	40	32	34	
blank-45%	61	44	41	41	40	35	40	40	40	38	39	38	37	36	
blank-60%	61		41	40	40	33	41	40	40	40	39	39	39	39	
BioFlex-35%	66		40	37		38	48	43	39	36	35	36	33	32	
BioFlex-45%	66	37		37	36	38	40	40	40	38	38	37	36	35	
BioFlex-60%	66				36		40	39	40	39	38	38	39	38	
cellulose-35%	66	70	52	46		48	50								
cellulose-45%	66	64	52	46		48	50								
cellulose-60%	66	44	52	46	39	48	44	43	43	41	41	41	40	40	
means	64	51	46	42	38	40	44	41	41	39	39	38	36	36	