

TECHNICAL REPORT

Testing Biodegradability of Aqueous Film Forming Foam Formulations in an Aerobic Environment

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LIST OF ACRONYMS AND ABBREVIATIONS

4:2 FTS	4:2 Fluorotelomer sulfonic acid
8:2 FTS	8:2 Fluorotelomer sulfonic acid
μl	microliter
AFFF	aqueous film forming foam
BDL	below detection limit
CECE	Department of Civil, Environmental, and Construction Engineering
COD	chemical oxygen demand
DGMBE	diethyleneglycol monobutylether
DMDA N-Oxides	dimethyltetradecylamine oxide
ENTX	Department of Environmental Toxicology
IS	internal standard
LC-MS	liquid chromatography-mass spectrometry
LOQ	level of quantification
N-EtFOSAA	N-Ethyl perfluorooctane sulfonamido acetic aci
N-MeFOSAA	N-Methyl perfluorooctane sulfonamido acetic acid
mg/L	milligrams per liter
mL	milliliter
OUR	Oxygen Uptake Rate
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	Perfluorobutanesulfonic acid
PFDA	Perfluorodecanoic acid
PFDoA	Perfluorododecanoic acid
PFDS	Perfluorodecanesulfonic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFNS	Perfluorononanesulfonic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFOSA	Perfluorooctanesulfonamide
PFPeA	Perfluoropentanoic acid
PFPeS	Perfluoropentanesulfonic acid
PFTeDA	Perfluorotetradecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFUdA	Perfluoroundecanoic acid
rpm	revolutions per minute
SDS	sodium lauryl sulfate
sNH ₃	soluble ammonia
sNO ₃	soluble nitrate
sNO ₂	soluble nitrite

TCMP	2-chloro-6-(trichloromethyl)pyridine
tCOD	total chemical oxygen demand
TKN	Total Kjeldahl Nitrogen
tPO ₄	total phosphorus
tTKN	total Total Kjeldahl Nitrogen
TTU	Texas Tech University
VSS	Volatile Suspended Solids

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OBJECTIVE AND SCOPE OF TEST PROGRAM

A test program was designed to assess aerobic biodegradability of seven aqueous film forming (AFFF) formulations in an aerobic environment. Six of the formulations were fluorine free and one was a fluorine-containing reference formulation (Buckeye). Of the fluorine free formulations, four were commercial foams and two were experimental foams supported by SERDP. Following the test plan outlined below, chemical oxygen demand (COD) present in the aerobic testing reactors was monitored after three hours of incubation and every seven days thereafter. Testing was complete in 28 days. Oxygen uptake volume and rate were monitored using an aerobic respirometer. Table 1 shows the abbreviations used for these formulations herein.

Table 1: Abbreviations used for different formulations

Formulation Name	Formulation Type	Abbreviation
National Foam NFD 20-391 Formulation – 1L	SERDP Developmental Formulation	NFD 20-931
Fomtec ENVIRO 2-3% FFF	Commercial PFAS-free Formulation	Fomtec
Buckeye Platinum Plus C6 MILSPEC 3%	Reference C6 Formulation	Buckeye
National Foam AVIO F3 Green KHC 3%	Commercial PFAS-free Formulation	AVIO
NRL 502W Siloxane-based Formulation	SERDP Developmental Formulation	NRL 502
Bio-Ex ECOPOL A 3% FFF	Commercial PFAS-free Formulation	Ecopol
Solberg Re-healing Foam RF3 3%	Commercial PFAS-free Formulation	Solberg

TEST PLAN

Tests were conducted according to a modified Zahn-Wellens (OECD 302B, 1992) test where three different concentrations of each test substance were applied to the biological reactors to check for potential toxicity of the test substances. Duplicate seed and control reactors were operated. Control reactors used acetate as a carbon source.

COD was used as a measure of organic carbon in each reactor. The seed culture was mixed liquor from a local municipal wastewater treatment facility. Tests were conducted at 25°C. Nutrients, trace minerals, and buffer were added to ensure that deficiencies did not affect biodegradation. Nitrification inhibitor (2-chloro-6-(trichloromethyl)pyridine [TCMP]) was added to ensure that oxygen uptake measured was due only to organic degradation. Reactors were incubated for 28

days. Oxygen uptake of all reactors was monitored using an aerobic respirometer. COD was measured after three hours of incubation and every seven subsequent days.

In addition to the biodegradability test reactors monitored for COD, seven sacrificial reactors were set up (one for each AFFF formulation), to allow sampling and analysis of the contents. The additional reactors were operated on respirometers just as with the biodegradation testing reactors with the same biomass, nutrient, and feed concentrations. The mixed liquors in these additional reactors were sampled on days 0, 7, 13, 21, and 28 and sent to Texas Tech University, Lubbock, TX for confirmation of targeted analytes including remaining fluorinated compounds where appropriate.

The test set up for the biodegradation testing reactors and sacrificial reactors is presented in Table 2 and the detailed test plan is provided in Appendix A.

Table 2: Biodegradability Testing Reactors

Set No.	Reactor Name	Bottle No.	Bottle Volume mL	mL Culture	mg VSS/L	mL of Acetate Feedstock	mL Dilute AFFF Formula	COD, mg/L	Final dilutions in % of AFFF main formulation in 60,000 mg/L COD target solution	Final percentages of AFFF main formulation in biodegradation reactors	g VSS/g DOC	TCMP
1	Seed	1, 2	500	100	1000	0	0					Yes
2	Control	3, 4	500	20	200	2	0	240			2.2	Yes
3	AVIO	5	500	20	200	0	1	120	7.66	0.02	4.5	Yes
6		500	20	200	0	1.5	180		0.02	3.0	Yes	
7		500	20	200	0	2	240		0.03	2.2	Yes	
4	ECOPOL	8	500	20	200	0	1	120	15.19	0.03	4.5	Yes
9		500	20	200	0	1.5	180		0.05	3.0	Yes	
10		500	20	200	0	2	240		0.06	2.2	Yes	
5	Fomtec	11	500	20	200	0	1	120	25.75	0.05	4.5	Yes
12		500	20	200	0	1.5	180		0.08	3.0	Yes	
13		500	20	200	0	2	240		0.10	2.2	Yes	
6	Solberg	14	500	20	200	0	1	120	14.07	0.03	4.5	Yes
15		500	20	200	0	1.5	180		0.04	3.0	Yes	
16		500	20	200	0	2	240		0.06	2.2	Yes	
7	SRL 502W	17	500	20	200	0	1	120	10.41	0.02	4.5	Yes
18		500	20	200	0	1.5	180		0.03	3.0	Yes	
19		500	20	200	0	2	240		0.04	2.2	Yes	
8	NFD 20-391	20	500	20	200	0	1	120	38.00	0.08	4.5	Yes
21		500	20	200	0	1.5	180		0.11	3.0	Yes	
22		500	20	200	0	2	240		0.15	2.2	Yes	
9	Buckeye	23	500	20	200	0	1	120	14.99	0.03	4.5	Yes
24		500	20	200	0	1.5	180		0.04	3.0	Yes	
25		500	20	200	0	2	240		0.06	2.2	Yes	
Sacrificial Reactors												
10	AVIO	26	500	20	200	0	1.5	180	7.66	0.02	3.0	Yes
11	ECOPOL	27	500	20	200	0	1.5	180	15.19	0.05	3.0	Yes
12	Fomtec	28	500	20	200	0	1.5	180	25.75	0.08	3.0	Yes
13	Solberg	29	500	20	200	0	1.5	180	14.07	0.04	3.0	Yes
14	NRL 502	30	500	20	200	0	1.5	180	10.41	0.03	3.0	Yes
15	NFD 20-391	31	500	20	200	0	1.5	180	38.00	0.11	3.0	Yes
16	Buckeye	32	500	20	200	0	1.5	180	14.99	0.04	3.0	Yes

COD: Chemical Oxygen Demand, VSS: Volatile Suspended Solids, DOC: Dissolved Organic Carbon, TCMP: 2-chloro-6-(trichloromethyl)pyridine

Initially, neat AFFF products were analyzed for total COD (Table 3), total TKN, soluble ammonia, soluble nitrite, soluble nitrate, and total phosphate. The product with the highest COD was the AVIO formulation with a concentration of 783,200 mg/L. The product with the lowest COD was the NFD 20-391 with a concentration of 158,960. The large differences in COD suggest that some of the formulations would have a greater environmental impact with equivalent release volumes than others if all were mixed and applied at 3% concentrations. Higher COD levels mean a greater amount of oxidizable organic material in the sample, which will reduce dissolved oxygen (DO) levels in the aquatic environment. A reduction in DO can lead to anaerobic conditions, which is deleterious to aquatic life forms. Nitrogen concentrations varied between product formulations as well. AVIO, ECOPOL, and Fomtec had the highest concentrations of organic nitrogen. NFD 20-391 and Buckeye had the highest concentrations of nitrogen in inorganic form nitrate.

Table 3: AFFF formulation general characteristics in mg/L

Formulation	Chemical Formula	COD* (mg/L)	COD** g COD/g Dry Material
NFD 20-931	$C_{1.5}H_{3.1}ON_{0.08}$	158,960	1.42
Fomtec	$C_{1.6}H_{3.7}ON_{0.31}$	233,040	1.33
Buckeye	$C_{1.5}H_{3.2}ON_{0.04}$	400,160	1.51
AVIO	$C_{3.4}H_{7.2}ON_{0.13}$	783,200	2.24
NRL 502	$C_{1.9}H_{3.8}O$	576,320	1.75
ECOPOL	$C_{2.1}H_{4.6}ON_{0.13}$	394,960	1.78
Solberg	$C_{1.3}H_{2.5}ON_{0.021}$	426,400	1.29

*COD: Chemical Oxygen Demand, empirically determined **theoretical calculation of COD

The foams used in testing were also analyzed for targeted analytes prior to the bioreactor testing and every 7-days by liquid chromatography-mass spectrometry (LC-MS). Analytes were first identified through safety data sheets, subsequent analytes were identified by retention times (Table 4). For the non-fluorine foams, 1 milliliter (mL) of sample was centrifuged (3000 revolutions per minute [rpm] for 10 minutes) and then injected directly into the LC-MS (triplicate injections). This method allowed confirmation of the identity of certain chemical components in the non-fluorine foams, allowing targeted analyses of select components in samples from the bioreactors (see below). This analysis was performed at the Texas Tech University (TTU) Department of Environmental Toxicology (ENTX). Neat materials of the identified chemicals (for commercial foams) or neat chemicals within identified chemical classes (for experimental foams) were used as standards to support targeted LC-MS analysis.

The Buckeye (fluorine-containing) foam was analyzed on two different LC-MS instruments. For analysis at ENTX, 950 microliters (μ L) of sample was centrifuged (3000 rpm for 10 minutes), an internal standard (deuterated perfluorohexanoic acid [PFHxA]) was added, and then the sample

was injected into the LC-MS for analysis of select PFAS and non-PFAS analytes. The Buckeye foam was also tested via targeted LC-MS analysis for higher resolution analysis of PFAS components at the TTU Department of Civil, Environmental, and Construction Engineering (CECE). For analysis at CECE, 1,260 μL of reactor water sample and 3 μL of an IS mix were added to HPLC-grade methanol in a 2-mL autosampler vial (triplicate injections). A 22-component PFAS standard was used for the CECE instrument calibration. Components confirmed in this AFFF are listed in Table 5. The major PFAS components in the Buckeye foam were PFHxA, perfluorohexanesulfonic acid [PFHxS], and perfluorobutanoic acid [PFBA].

Table 4: Targeted Analytes of Foams Identified by Safety Data Sheets**Commercial Foams****NFD 20-391**

Hexylene glycol (107-41-5)	4-10%
Sodium laureth sulphate (68891-38-3)	1-4%
1-Propanaminium, N-(3-aminopropyl)-2-hydroxy-N,N-dimethyl-3-sulfo-, N-(C8-18(even numbered) acyl) derivs., hydroxides, inner salts (939-455-3)	4-10%

Fomtec

Diethyleneglycol monobutylether (112-34-5)	5-10%
C12-C14 sulfuric acid esters (9058-18-9)	5-10%
C12-C14 alcohols ethoxylated sulfates (68891-38-3)	1-3%
C12-C14 amines N-oxides (308062-28-4)	<1%

ECOPOL

Diethyleneglycol monobutylether (112-34-5)	10-25%
Alkyl sulfate	0-2.5%
C10-C22 alkyl betaine	0-2.5%
Amphoteric surfactant	0-2.5%

AVIO

Diethyleneglycol monobutylether (112-34-5)	10-30%
Sodium lauryl sulfate (151-21-3)	7-13%
Lauramine oxide (1643-20-5)	7-13%
Dimethyltetradecylamine oxide (3332-27-2)	0-2.5%

Experimental Foams**NRL 502**

3-(polyoxyethylene)propylheptamethyltrisiloxane (67674-67-3)	unknown%
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Solberg

Unknown (NA)	unknown%
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Buckeye

Hexylene glycol (107-41-5)	< 19%
Fluorosurfactants (NA)	< 5%
Hydrocarbon surfactants (NA)	< 5%

Table 5: Components of Buckeye Identified by LC-MS Analysis

Component Name	Concentration (ng/mL)
4:2 Fluorotelomer sulfonic acid (4:2 FTS)	420
6:2 Fluorotelomer sulfonic acid (6:2 FTS)	9126
Perfluorobutanesulfonic acid (PFBS)	26
Perfluorobutanoic Acid (PFBA)	1239
Perfluoroheptanoic acid (PFHpA)	43
Perfluorohexanoic acid (PFHxA)	2870
Perfluorohexanesulfonic acid (PFHxS)	8
Perfluorononanoic acid (PFNA)	< 0.0033
Perfluoropentanesulfonic acid (PFPeS)	58
Perfluorooctanoic acid (PFOA)	< 0.0007
Perfluoropentanoic acid (PFPeA)	342

Samples collected from each sacrificial reactor were analyzed using LC-MS for two to four targeted analytes (Tables 4 and 5) to further assess biodegradation, this was in addition to the testing for COD and nutrients (described above). Samples from the Buckeye reactors were also analyzed for select PFAS.

RESULTS

The results from analysis of the biodegradation testing reactors and sacrificial reactors for each of the foams are presented below.

AVIO

The National Foam formulation showed good biodegradation characteristics with 28-d oxygen uptake equal to approximately 100% of the initial COD added to the reactor (Figure 1). These uptakes exceeded 100% of the initial applied COD by the end of the test. This indicates that actual COD of the formulation was higher than what was measured in the standard COD test (see Addendum #1 for more details). A major portion of organic material was degraded in the first 24 hours of incubation as indicated by the first oxygen uptake rate (OUR) peak in Figure 2. A second portion of organic material required approximately two days of biomass adaptation before it began to be degraded as indicated by the second OUR peak in Figure 2. Measurement of soluble COD concentration over time for all doses approached zero after 28-days of incubation, thereby indicating complete biodegradability (Figure 3). The similarity of the adaptation curves at all concentrations indicate that the formulation was not toxic to the microbial community at any concentration tested. Concentrations of neat formulation used in the biodegradation testing reactors were 96 mg/L, 144 mg/L, and 192 mg/L.

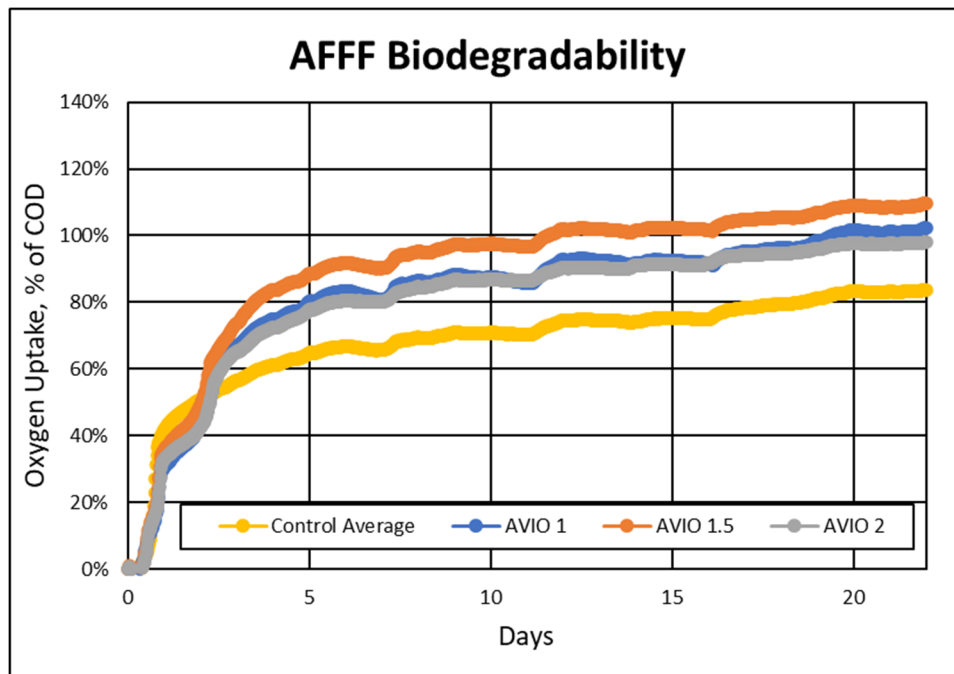


Figure 1: AVIO oxygen uptake curves as %COD

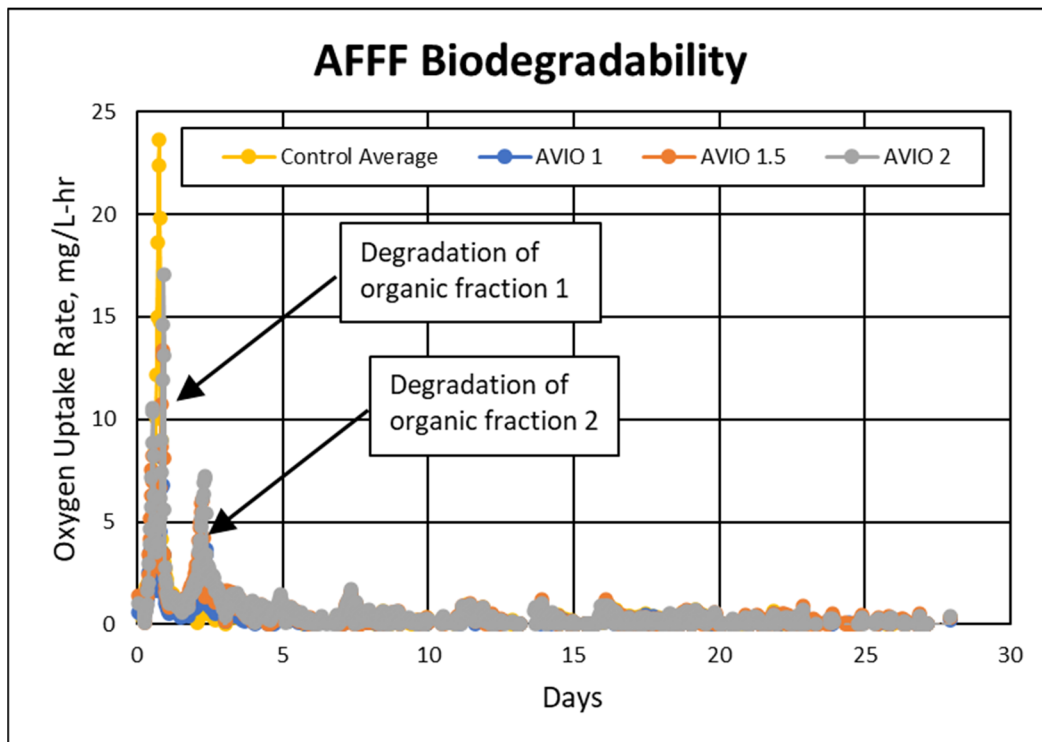


Figure 2: AVIO oxygen uptake rate curves

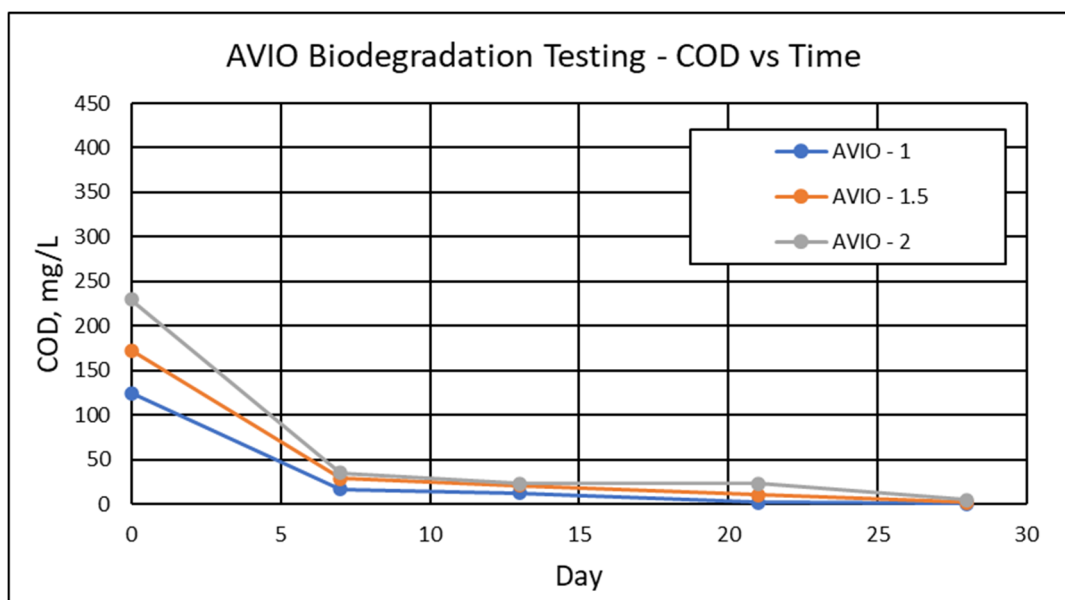


Figure 3: Soluble COD vs time for AVIO formulation

Effluent from the biodegradability testing of the AVIO formulation was analyzed by LC-MS for diethyleneglycol monobutylether (DGMBE), sodium lauryl sulfate (SDS), and dimethyltetradecylamine oxide (DMDA N-Oxides). Results showed that all three of these had dropped to non-detect levels by the seventh day of testing. Results are presented graphically in Figure 4.

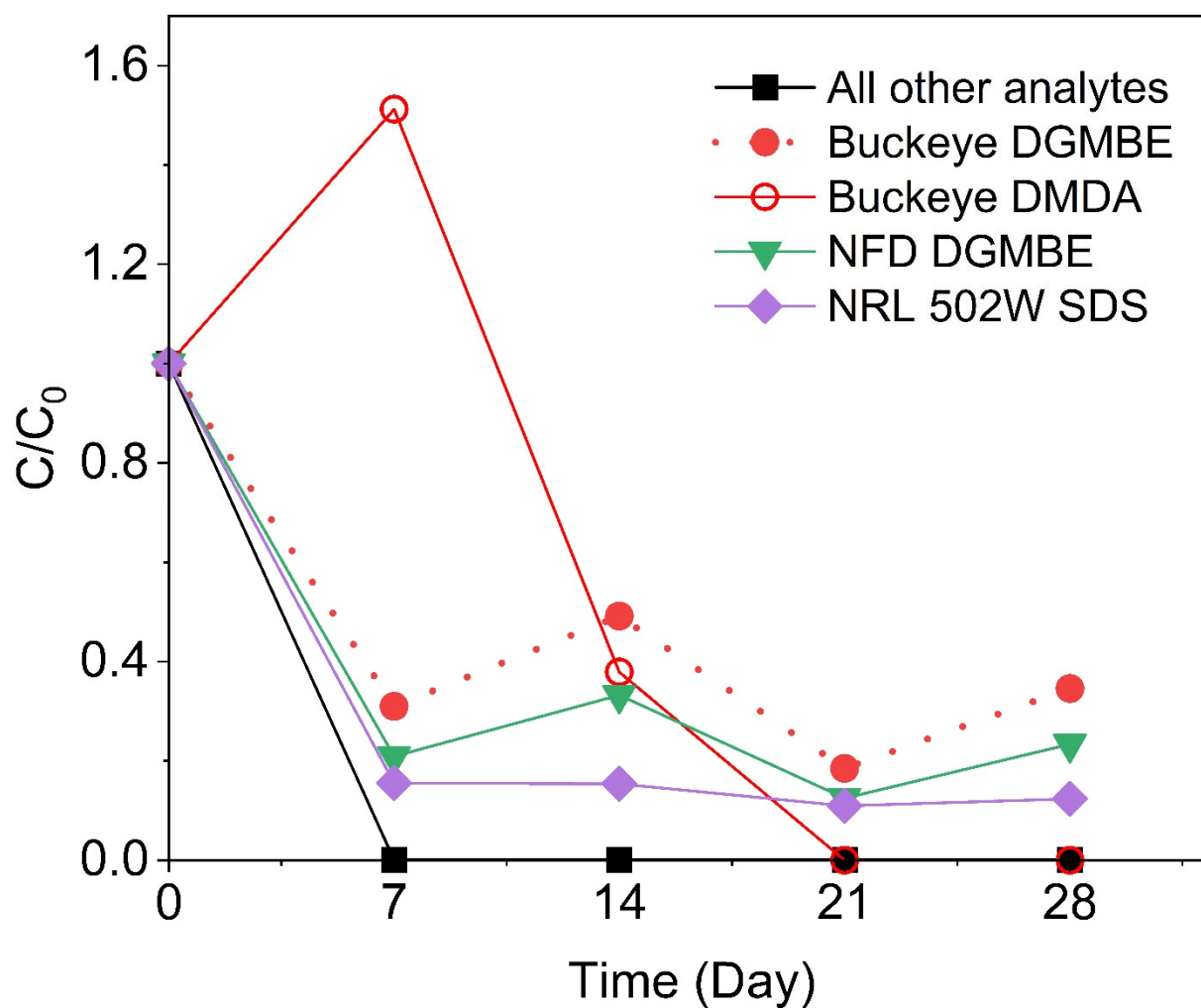


Figure 4: Biodegradation of DGMBE, DMDA, SDS, and HG in PFAS-free and reference AFFF formulations. The black square symbols represent the overall trend observed with most of the compounds with some exceptions which include the decay of DGMBE and DMDA in Buckeye, DGMBE in NFD, and SDS in NRL 502W shown as solid and hollow circles, triangles, and diamonds, respectively.

ECOPOL

The ECOPOL formulation showed good biodegradation characteristics with 28-d oxygen uptake of approximately 100% of the COD (Figure 5). Oxygen uptake rates showed initial biodegradation of a portion of organic material (Figure 6) followed by a second portion that required approximately two days of biomass adaptation before it began to be degraded. Soluble COD concentration for all doses was nearly zero indicating good biodegradability (Figure 7). The similarity of the adaptation curves at all concentrations indicate that the formulation was not toxic to the microbial community at any concentration tested. Concentrations of neat formulation used in these tests were 170 mg/L, 255 mg/L, and 340 mg/L.

Effluent from the biodegradability testing of the ECOPOL formulation was analyzed by LC-MS for DGMBE, SDS, and DMDA N-Oxides. Results showed that all three of these had dropped to non-detect levels by the seventh day of testing. Results are presented graphically in Figure 4.

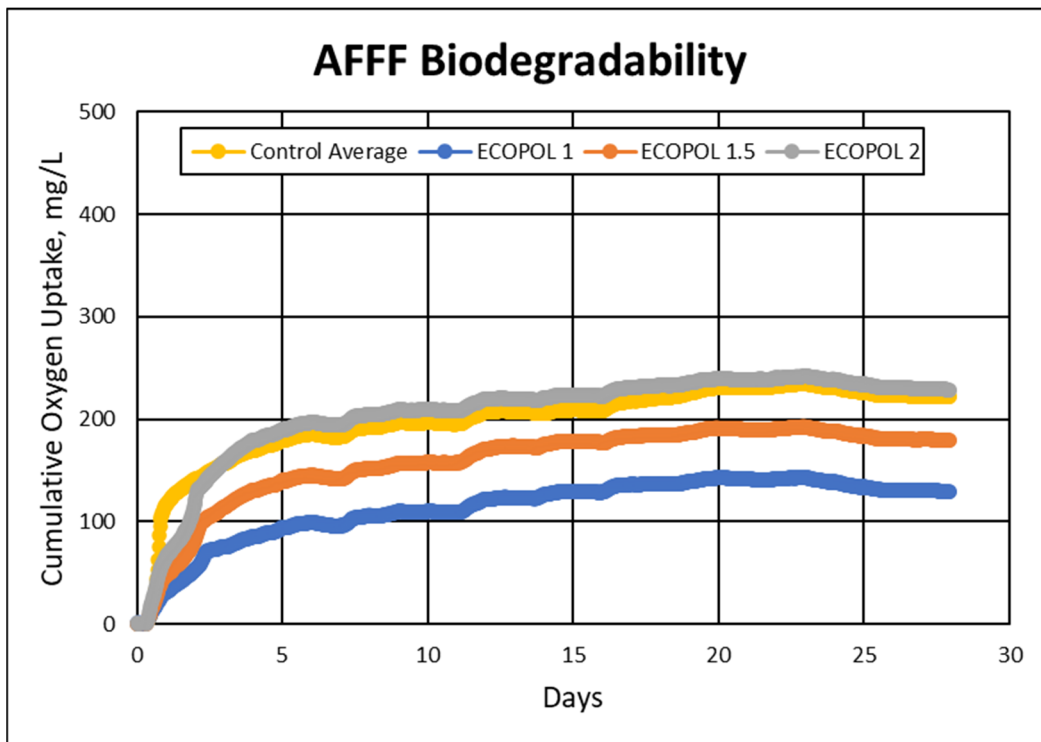


Figure 5: ECOPOL oxygen uptake curves as %COD

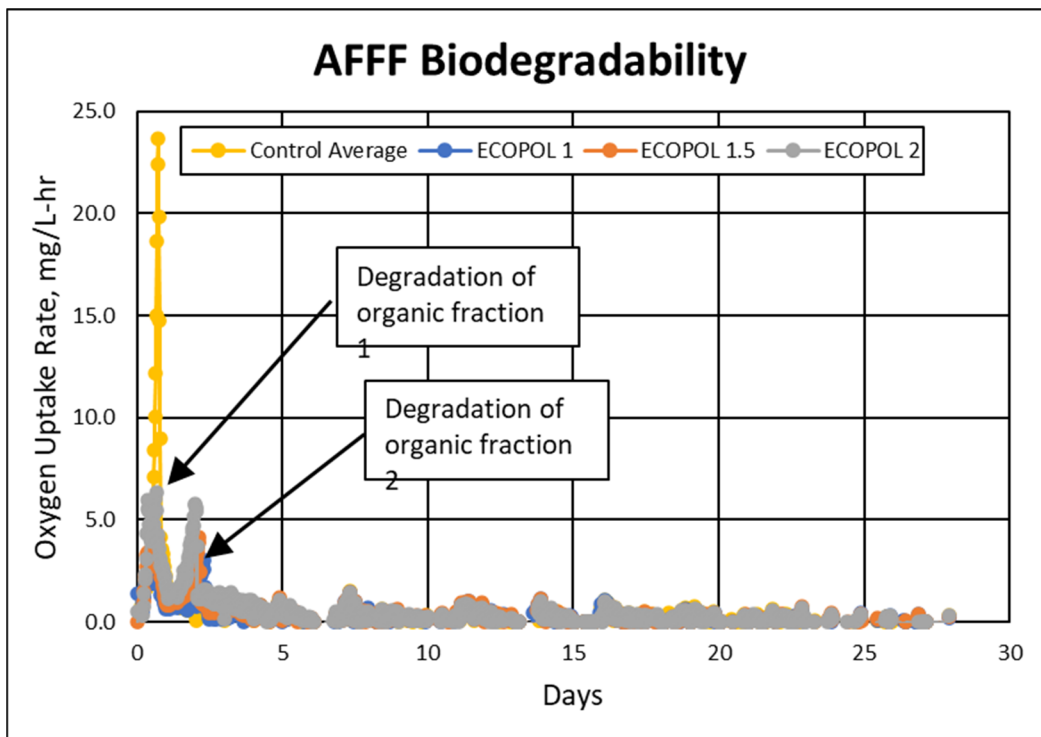


Figure 6: ECOPOL oxygen uptake rate curves

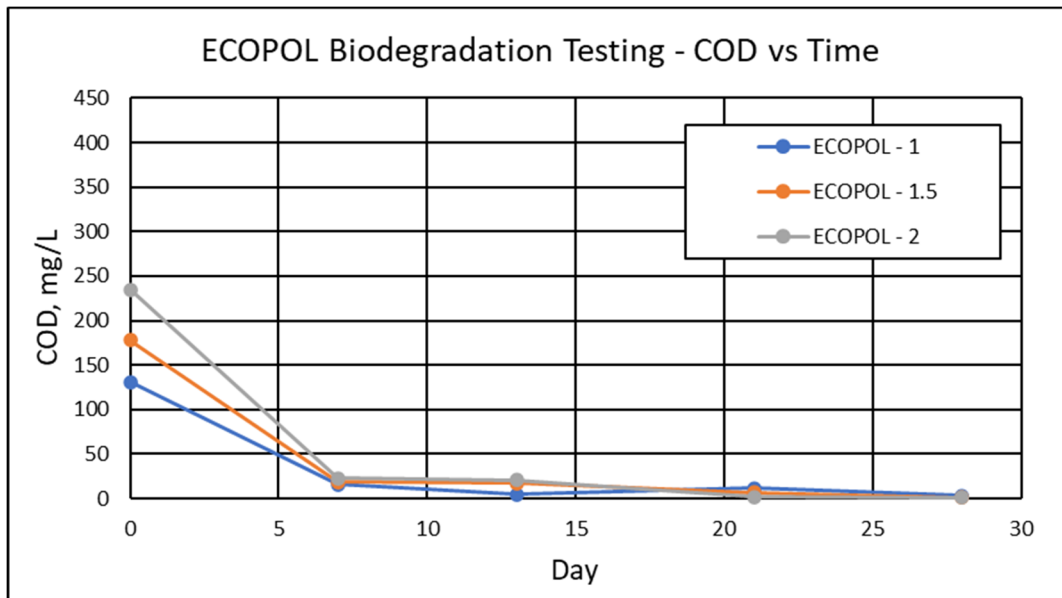


Figure 7: Soluble COD vs. time for ECOPOL formulation

Fomtec

Oxygen uptake as a percent of Fomtec COD was similar for all dose conditions (Figure 8). Oxygen uptakes exceeding 100% of the initial applied COD indicated that the actual COD of the formulation was higher than what was measured in the standard COD test (see Addendum#1 for more information). Oxygen uptake rates showed rapid initial biodegradation of a portion of organic material with a high rate that was similar to the control (Figure 9). A second portion of organic material required approximately two days of adaptation before it began to be degraded. Soluble COD concentrations versus time for all doses approached zero thereby indicating good biodegradability (Figure 10). The similarity of the adaptation curves at all concentrations indicate that the formulation was not toxic to the microbial community at any concentration tested. Concentrations of neat formulation used in these tests were 428 mg/L, 642 mg/L, and 856 mg/L.

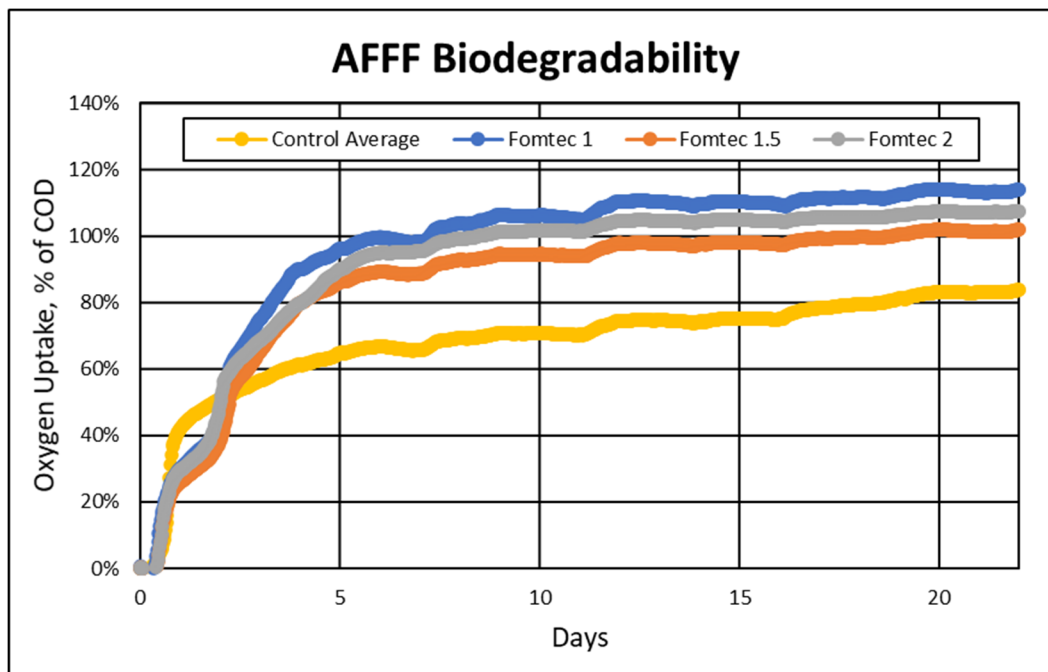


Figure 8: Fomtec oxygen uptake curves as %COD

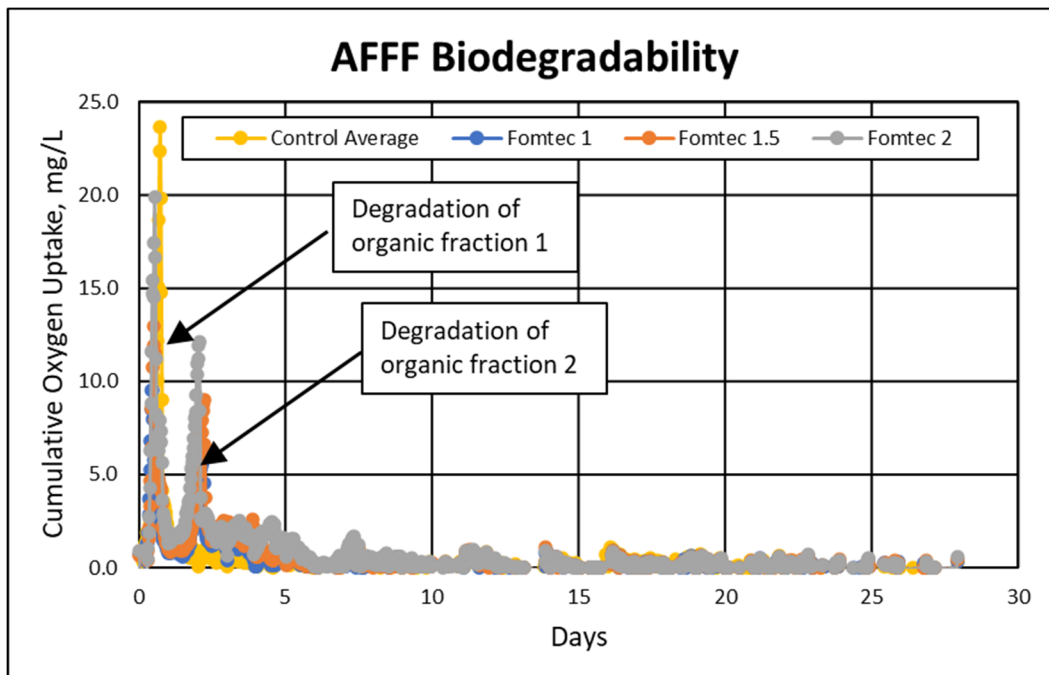


Figure 9: Fomtec oxygen uptake rate curves

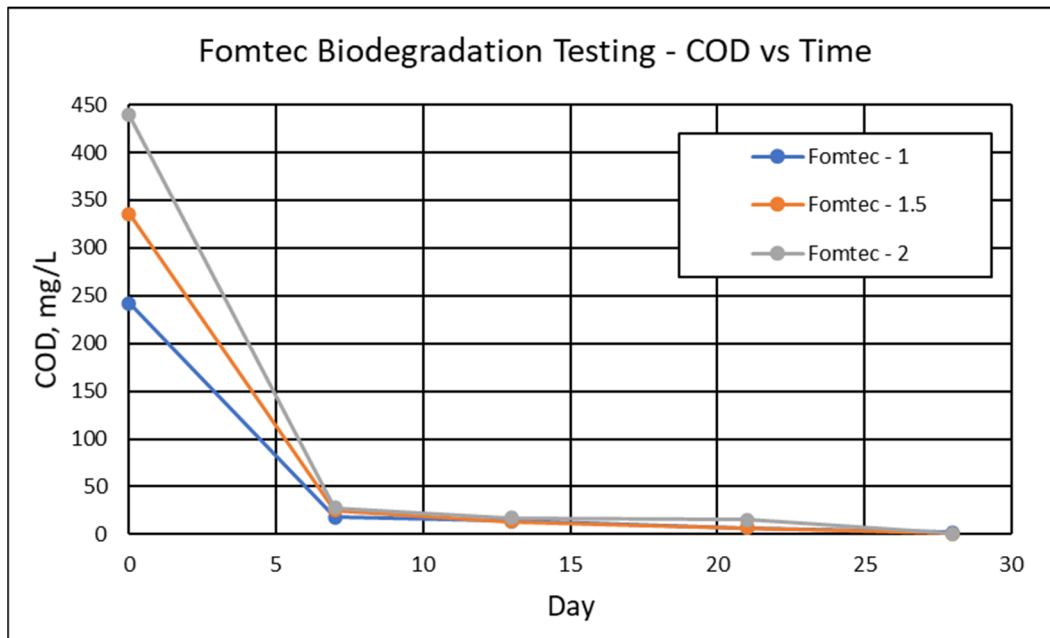


Figure 10: Soluble COD vs time for Fomtec formulation

Effluent from the biodegradability testing of the Fomtec formulation was analyzed by LC-MS for DGMBE, SDS, and DMDA N-Oxides. Results showed that all three of these had dropped to non-detect levels by the seventh day of testing. Results are presented graphically in Figure 4.

Solberg

Oxygen uptake as a percent of the COD was similar to that for the control for the 1.5 mL and 2 mL dose conditions (Figure 11). The higher percentage of oxygen uptake for the 1 mL dose is an outlier. Oxygen uptake rates for the Solberg formulation showed rapid initial biodegradation of a portion of organic material (Figure 12). A second portion of organic material required approximately two days of adaptation before it began to be degraded. Final effluent COD concentration for all doses was nearly zero indicating good biodegradability (Figure 13). The similarity of the adaptation curves at all concentrations indicate that the formulation was not toxic to the microbial community at any concentration tested. Concentrations of neat formulation used in these tests were 196 mg/L, 294 mg/L, and 393 mg/L.

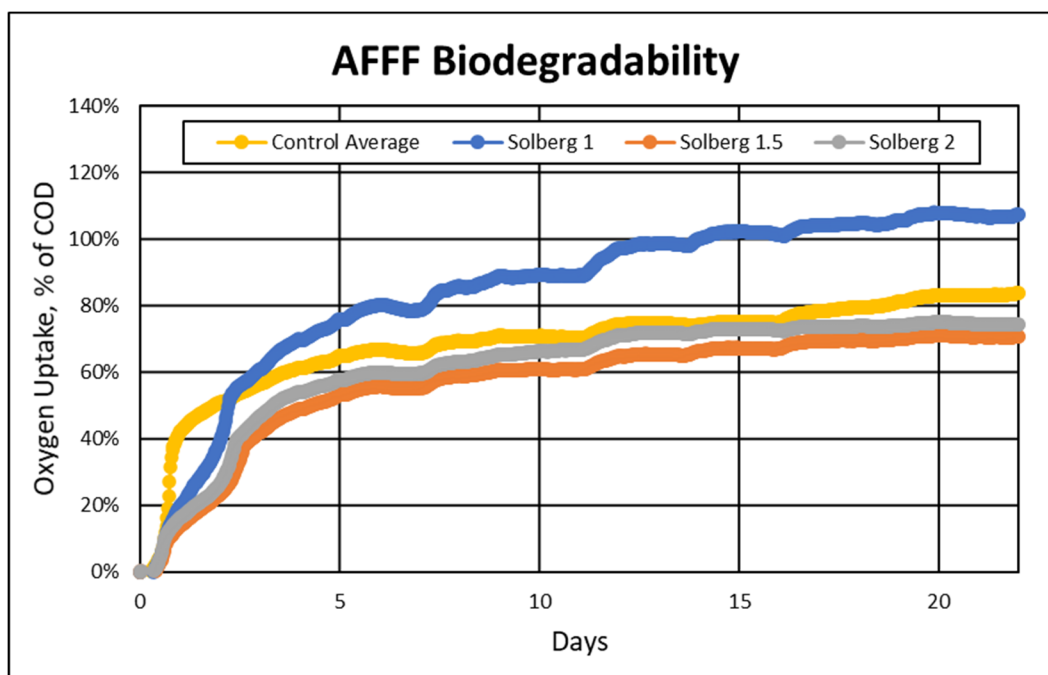


Figure 11: Solberg oxygen uptake curves as % COD

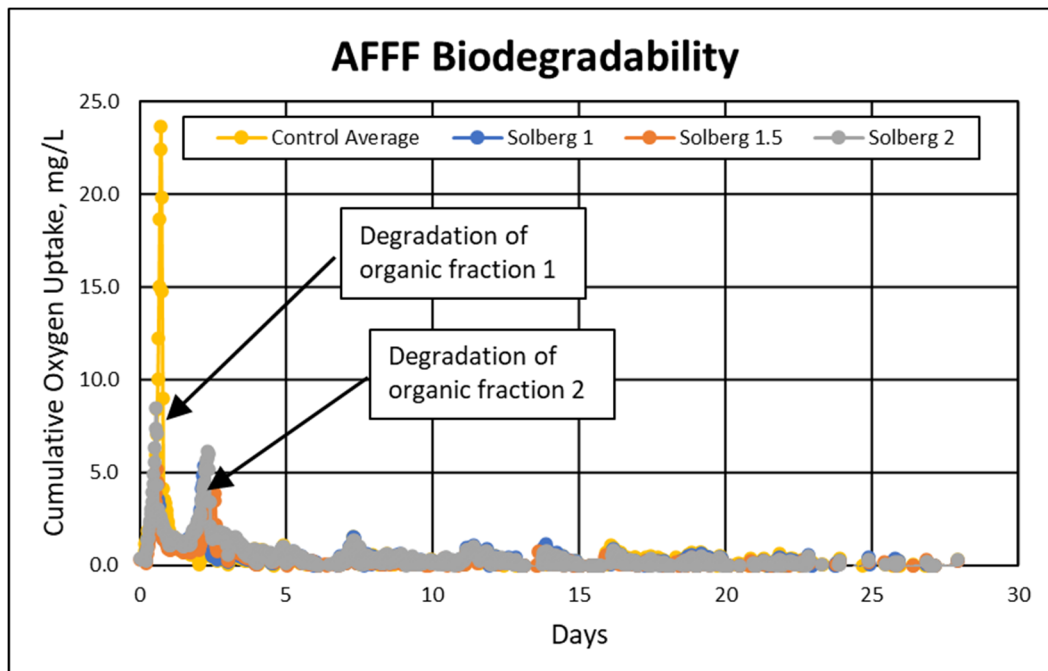


Figure 12: Solberg oxygen uptake rate curves

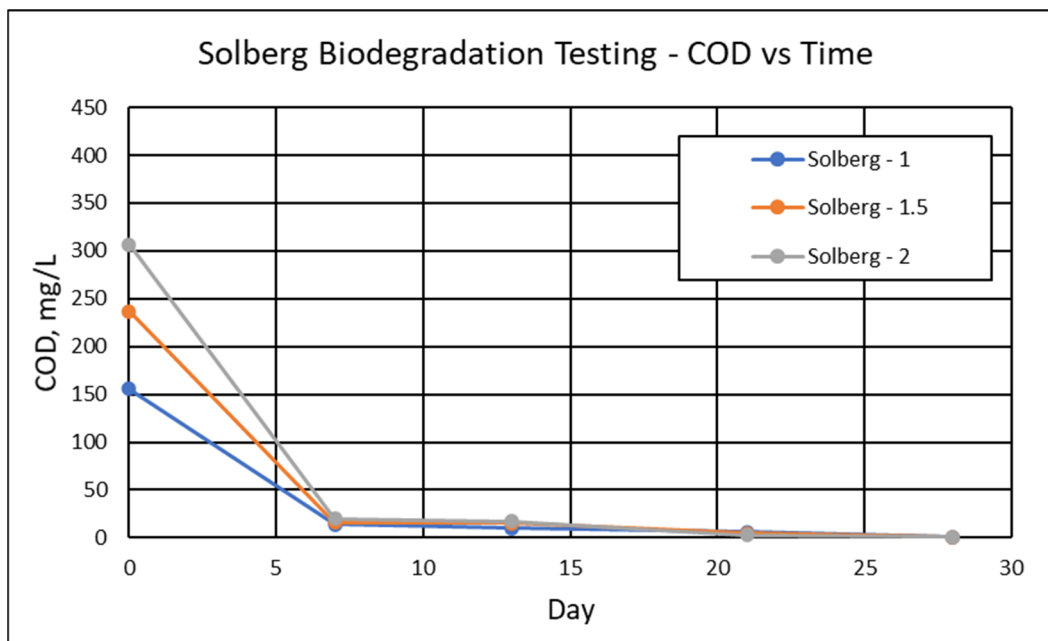


Figure 13: Soluble COD vs time for Solberg formulation

Effluent from the biodegradability testing of the Solberg formulation was analyzed by LC-MS for DGMBE and SDS. Results showed that both of these had dropped to non-detect levels by the seventh day of testing. Results are presented graphically in Figure 4.

NRL 502

Oxygen uptake reactions for the NRL 502W formulation were somewhat variable in the different reactors (Figure 14). This variation can happen at times due to difficulties in dosing viscous material. The 1 mL and 2 mL curves were similar indicating that the 1.5 mL curve is likely an outlier. Uptakes exceeding 100% of the initial applied COD by the end of the test indicated that the actual COD of the formulation was higher than what was measured in the standard COD test (see Addendum #1 for more information). Oxygen uptake rates for the NRL 502W formulation showed slow initial biological degradation through day 3 thereby suggesting that the formulation was undergoing biological hydrolysis (Figures 15). Soluble COD concentrations versus time for the 1.5 mL and 2 mL doses were higher in comparison to other AFFF formulations and were 15 and 17 mg/L, respectively after 28-days of incubation (Figure 16). While these values only represent 8% and 8.5% of the feed COD, they do indicate that part of the organic material in the NRL formulation degraded more slowly than other formulations. Overall, the formulation showed good biodegradability at the end of 28-days. The similarity of the oxygen uptake curves at all concentrations indicate that the formulation was not toxic to the microbial community at any concentration tested. Concentrations of neat formulation used in these tests were 100 mg/L, 155 mg/L, and 200 mg/L.

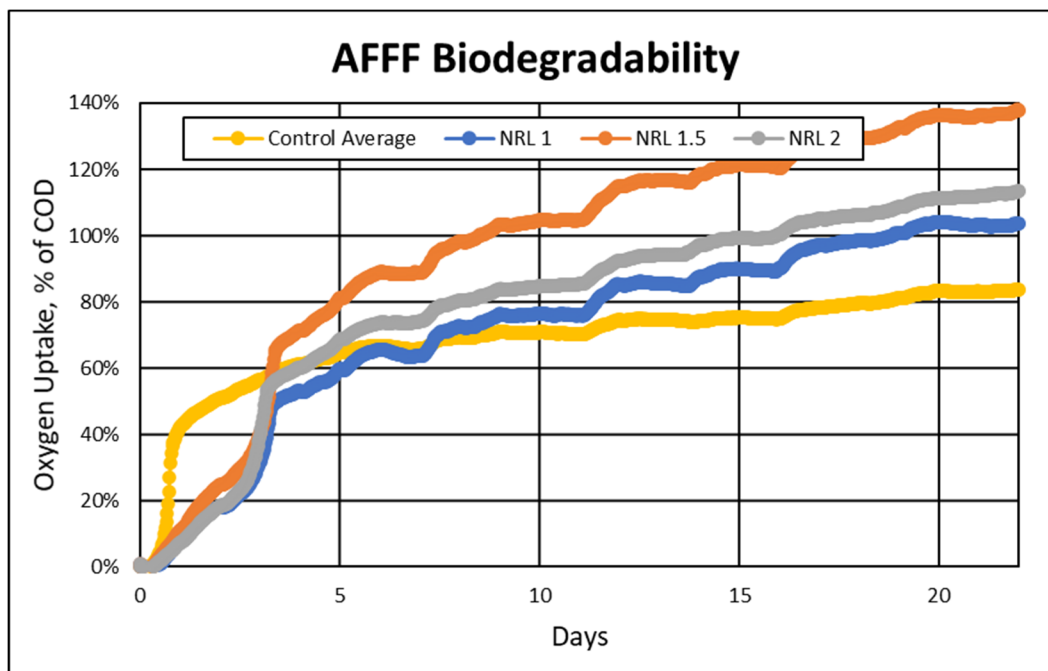


Figure 14: NRL 502W oxygen uptake curves as %COD

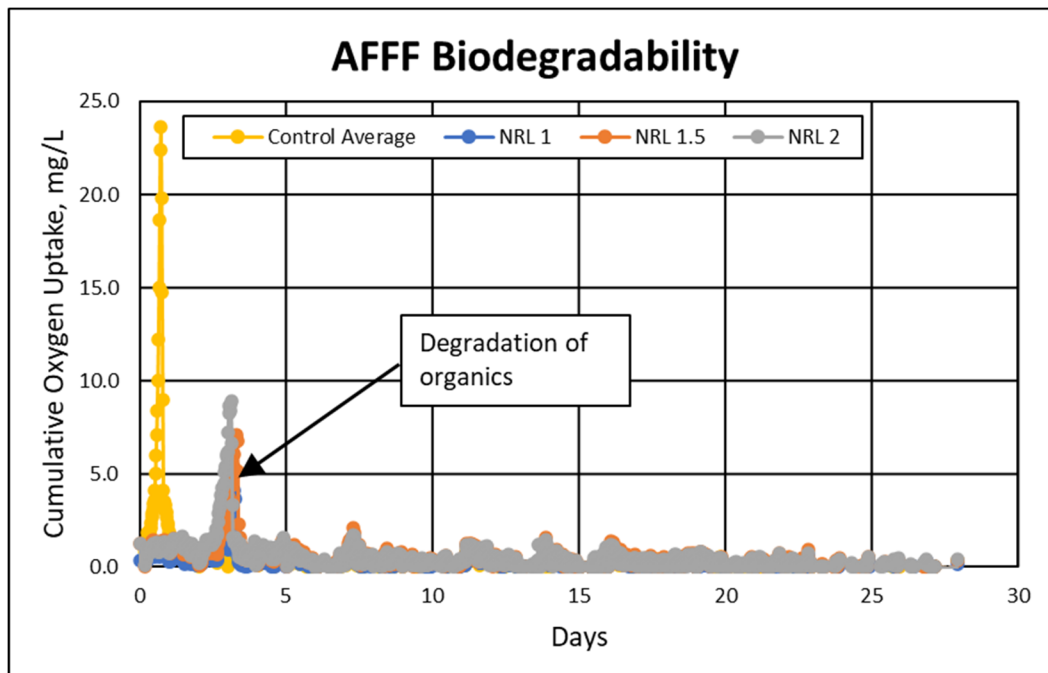


Figure 15: NRL 502 oxygen uptake rate curves

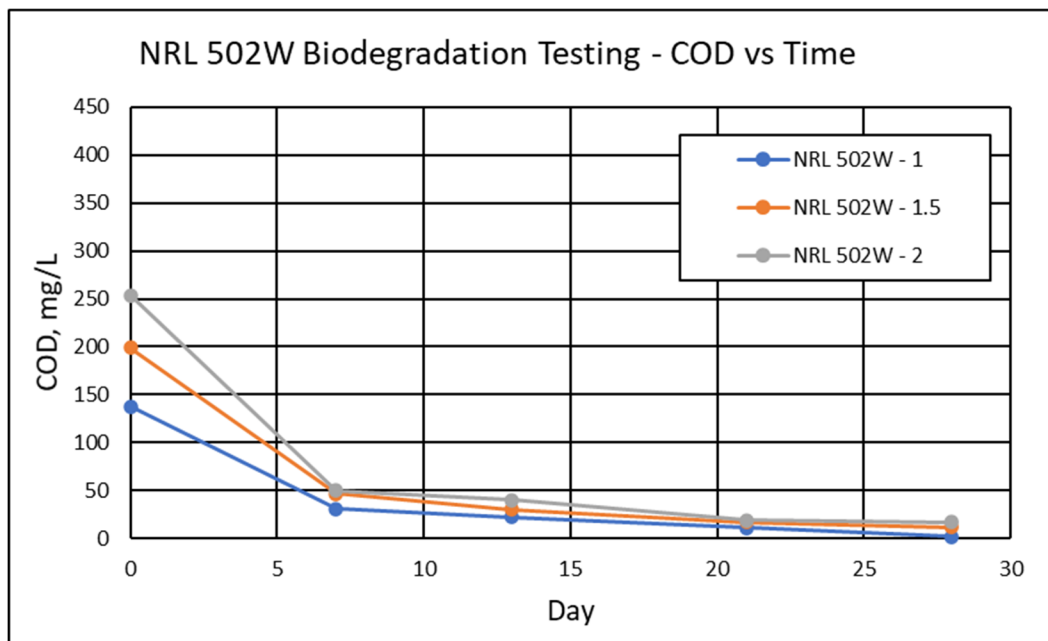


Figure 16: Soluble COD vs. time for NRL 502 formulation

Effluent from the biodegradability testing of the NRL formulation was analyzed by LC-MS for DGMBE, SDS, and DMDA N-Oxides. Results showed that DGMBE and DMDA N-Oxides had dropped to non-detect levels by the seventh day of testing. SDS concentration dropped during the

first seven days of testing but remained at approximately 15% of the initial concentration through the end of the testing. Results are presented graphically in Figure 4.

NFD 20-391

Oxygen uptake as a percent of COD was similar for all dose conditions (Figure 17). Uptakes exceeding 100% of the initial applied COD by the end of the test indicate that actual COD of the formulation was higher than what was measured in the standard COD test (see Addendum #1 for more information). Oxygen uptake rates for the NFD 20-391 formulation showed rapid initial biodegradation of a portion of organic material (Figure 18). A second portion of organic material required approximately four days of adaptation before it began to be degraded. Soluble COD vs. time for all doses approached zero thereby indicating good biodegradability (Figure 19). Similarity of oxygen uptake curves for all concentrations indicates that the formulation was not toxic to the microbial community at any concentration tested. Concentrations of neat formulation used in these tests were 569 mg/L, 854 mg/L, and 1,138 mg/L.

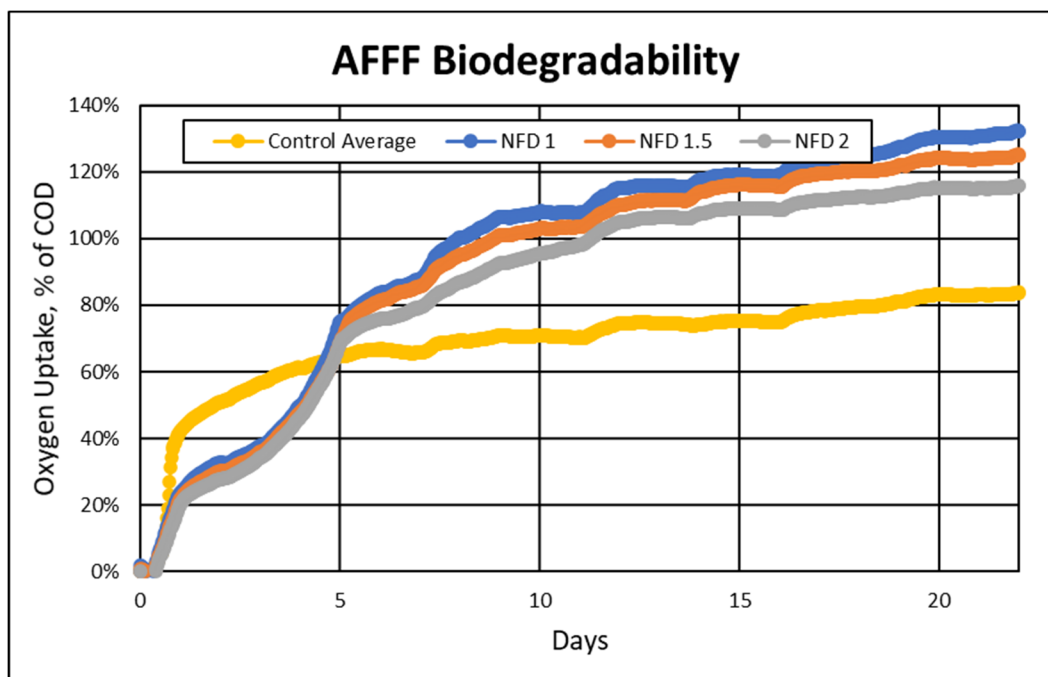


Figure 17: NFD 20-391 oxygen uptake curves as %COD

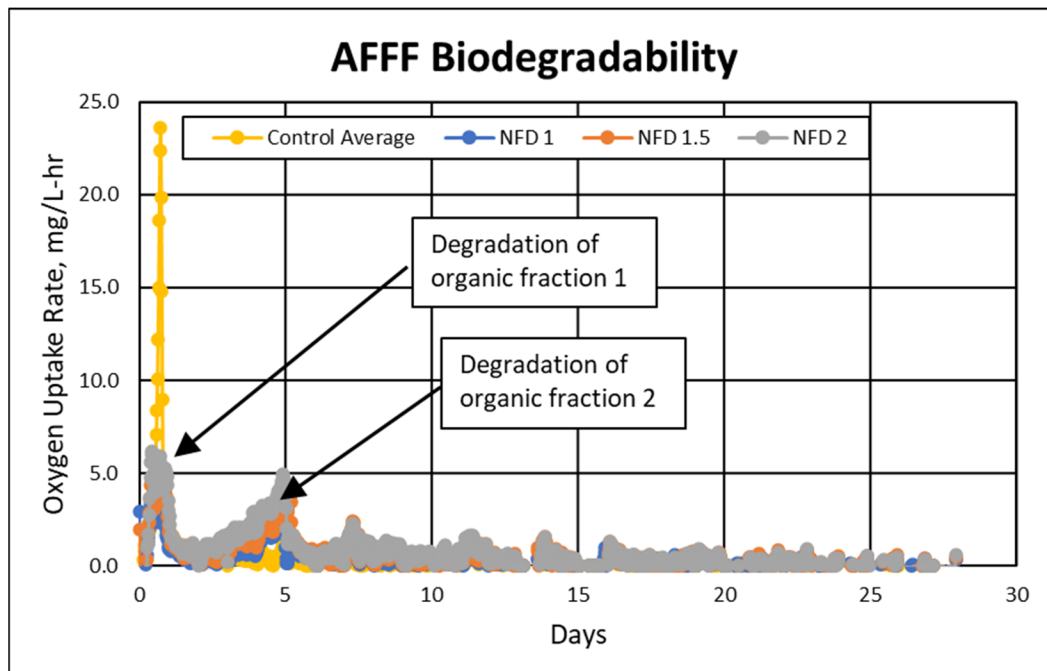


Figure 18: NFD 20-391 oxygen uptake rate curves

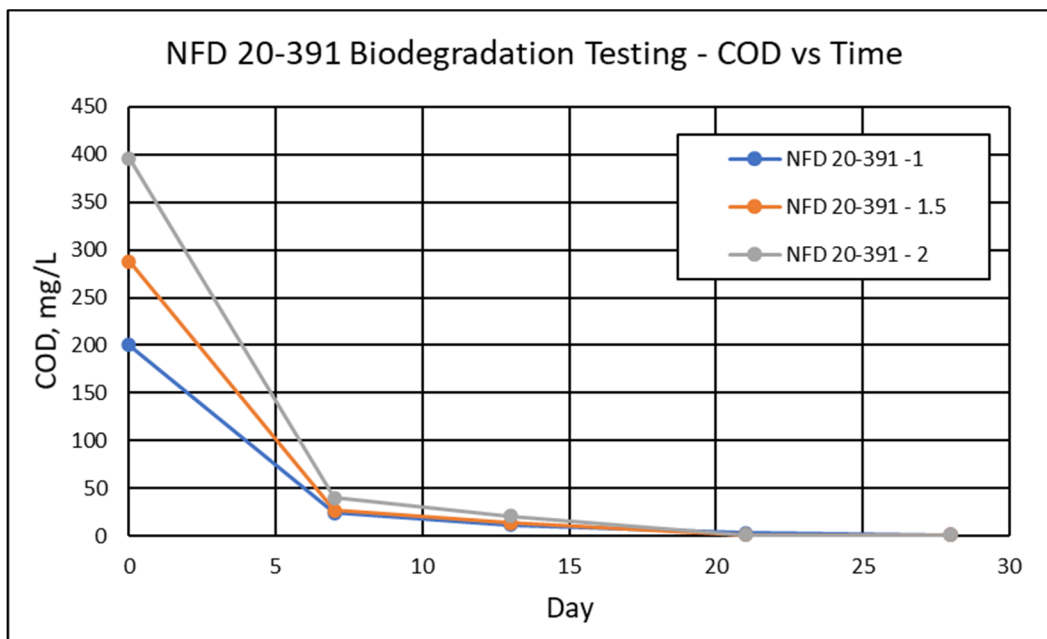


Figure 19: Soluble COD vs. time

Effluent from the biodegradability testing of the NFD 20-391 formulation was analyzed by LC-MS for DGMBE, SDS, and Hexylene Glycol. Results showed that SDS and Hexylene Glycol had dropped to non-detect levels by the seventh day of testing. DGMBE dropped during the first

seven days of biodegradability testing but remained detectable at approximately 20% through the end of the test. Results are presented graphically in Figure 4.

Buckeye

Oxygen uptake as a percent of COD was similar for all dose conditions (Figure 20). Uptakes exceeding 100% of the initial applied COD by the end of the test indicate that actual COD of the formulation was higher than what was measured in the standard COD test (see Addendum #1 for more information). Oxygen uptake rates for the Buckeye formulation showed initial biodegradation of a portion of organic material (Figure 21). A second portion of organic material required five days of adaptation before it began to be degraded. The similarity of the oxygen uptake curves at all concentrations indicate that the formulation was not toxic to the microbial community at any concentration tested. Concentrations of neat formulation used in these tests were 169 mg/L, 254 mg/L, and 338 mg/L.

Soluble COD concentrations versus time for all doses were somewhat higher than for other formulations. Effluent CODs for the 1 mL, 1.5 mL, and 2 mL doses were 7%, 3%, and 3% of the feed CODs respectively (Figure 22). The safety data sheet for the Buckeye formula indicates that the fluorine fraction of the formulation is <5%. The residual COD from these tests may be associated with the fluorine fraction of the formulation. Fluorinated organics have been shown to be recalcitrant to biological degradation both aerobically and anaerobically.

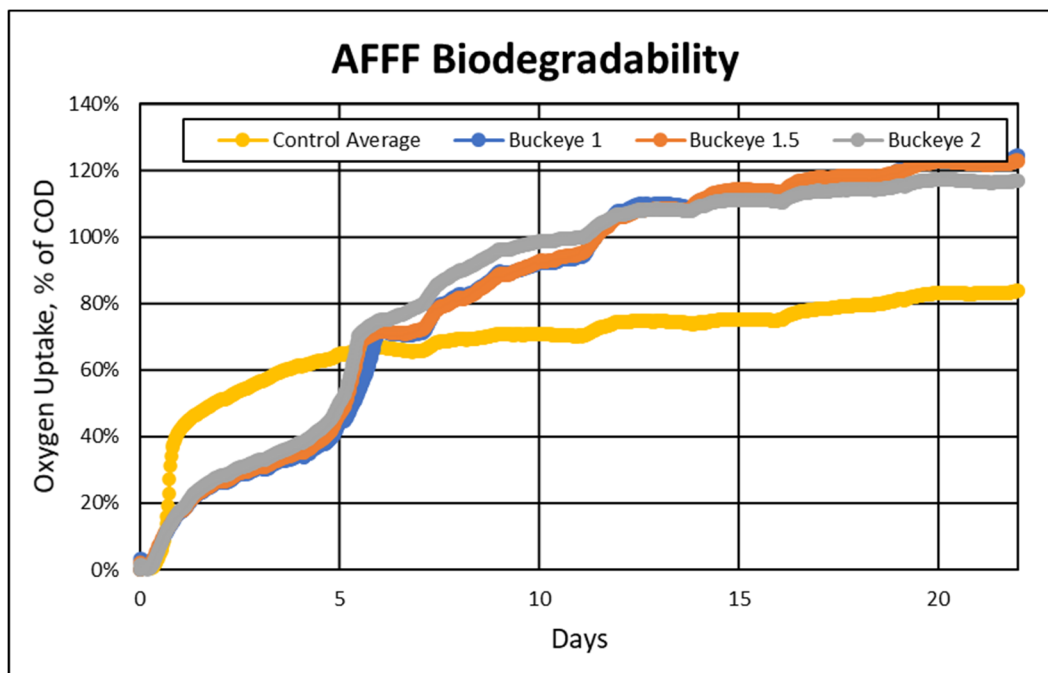


Figure 20: Buckeye oxygen uptake curves as %COD

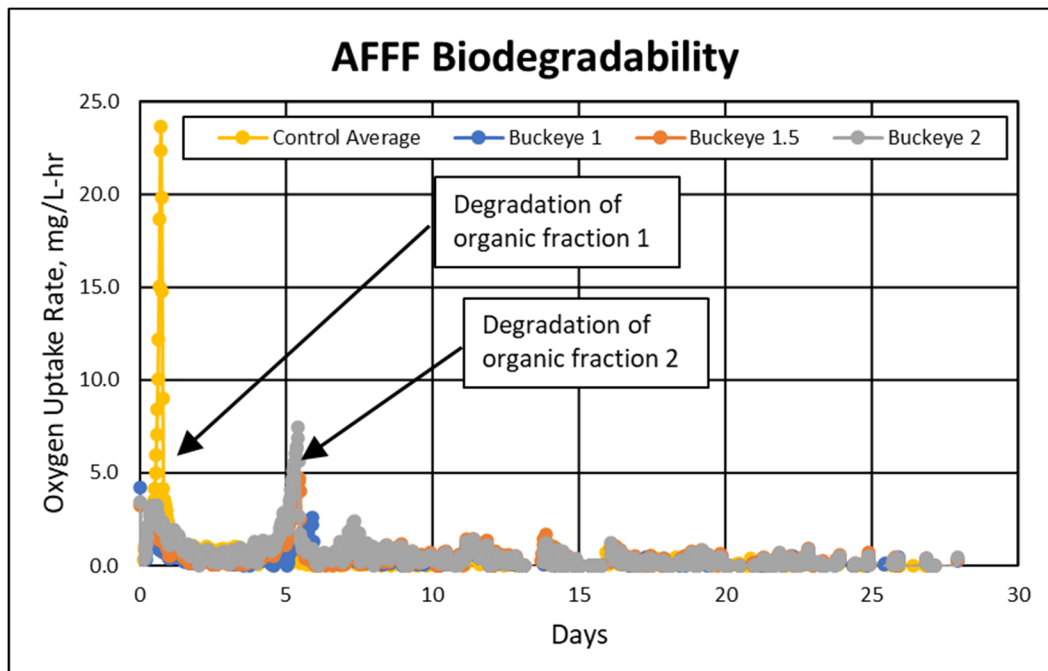


Figure 21: Buckeye oxygen uptake rate curves

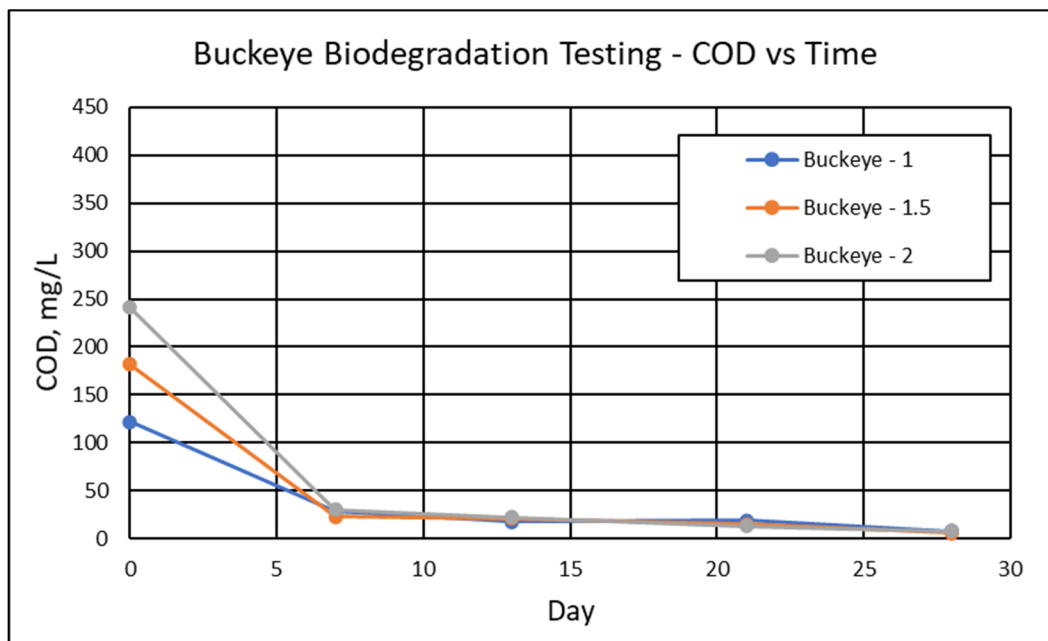


Figure 22: Soluble COD vs. time for Buckeye formulation

Effluent from the biodegradability testing of the Buckeye formulation was analyzed by LC-MS at ENTX for DGMBE, SDS, Hexylene Glycol, DMDA N-Oxides, PFHxA and PFBA. Additionally, for more selective analysis of PFAS analytes in the effluent was conducted at

CECE by LC-MS due the sensitivity being greater. Results showed that DGMBE dropped during the first seven days of biodegradability testing but remained detectable at approximately 30% of the original concentration through the end of the test. SDS and Hexylene Glycol dropped to non-detect levels by the seventh day of testing. DMDA N-Oxides had dropped to non-detect by the twenty-first day of testing (Figure 4). Based on LC-MS analysis conducted at CECE (TTU), shorter chain PFAA was detected at high concentrations in Buckeye with PFHA at ~ 2870 ng/mL and the precursors fluorotelomer sulfonate 6:2 FTS at ~ 9126 ng/mL and 4:2 FTS at 420 ng/mL. Results indicated that both 6:2 FTS and 4:2 FTS were degraded in Buckeye through the 28-day test period while some of the PFAAs including PFHpA, PFHxA, PFPeA, and PFBA were being generated with simultaneously getting degraded (Figure 23). This is consistent with previous biodegradation studies where fluorotelomer precursors have been shown to degrade to PFCAs forming C8-C6 from 8:2 FTS transformation and C6-C4 from a 6:2 FTS (Carrillo-Abad et al., 2018; Shaw et al., 2019; Weber et al., 2017) suggesting a similar pathway for 4:2 FTS to PFBA. From a previous study on the exact Buckeye formulation, there are other precursors present especially 6:2 precursors which have yield to 6:2 FTS generation. In other words, 6:2 FTS is very likely to undergo simultaneous generation and biodegradation. Not shown here, PFHxS concentrations over the course of the 28-day experiment appear to decrease; however, these detection limits are beyond the LOQ and thus not reliable and therefore graphically omitted.

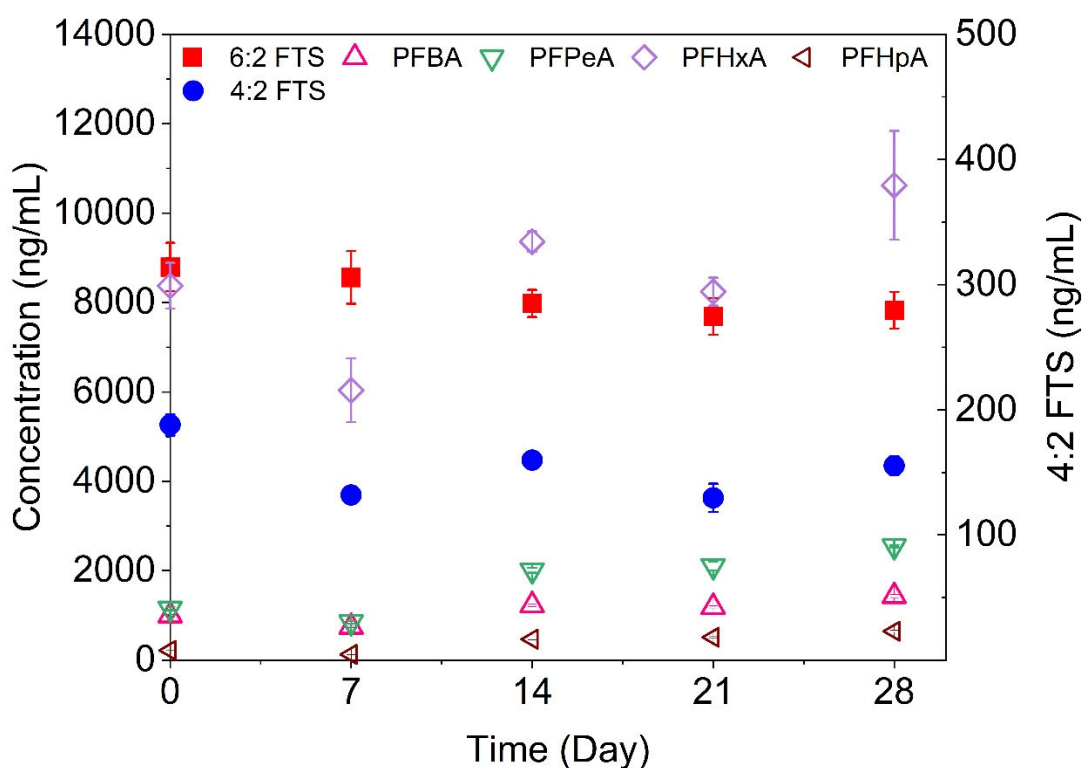


Figure 23: The degradation of tested PFAS in Buckeye AFFF formulation over the reaction period of 28-day. 4:2 FTS concentration is showed in secondary axis.

Biodegradability Testing Final Analyses

All reactors were tested for COD, NH₃, NO₂, NO₃, and pH after 28-days of incubation, with results presented in Table 5. Final pH in the reactors was in the acceptable range for aerobic biodegradation. The presence of ammonia and the absence of nitrite and nitrate show that the nitrification inhibitor was effective over the course of the test and oxygen uptake due to nitrification did not occur.

Table 6: Final analysis for each reactor

Reactor No	Reactor	COD, mg/L	NH ₃ , mg/L	NO ₂ , mg/L	NO ₃ , mg/L	pH
5	National Foam Avio Green KHC 3% - 1	1	16.7	BDL	BDL	7.34
6	National Foam Avio Green KHC 3% - 1.5	2	18.85	BDL	BDL	7.33
7	National Foam Avio Green KHC 3% - 2	5	17.25	BDL	BDL	7.35
8	BIOEX - 1	3	15.95	BDL	BDL	7.34
9	BIOEX - 1.5	1	16.25	BDL	BDL	7.33
10	BIOEX - 2	1	15.05	BDL	BDL	7.38
11	Fomtec - 1	2	26.15	BDL	BDL	7.51
12	Fomtec - 1.5	1	29.05	BDL	BDL	7.6
13	Fomtec - 2	1	32.6	BDL	BDL	7.7
14	Solberg - 1	1	15	BDL	BDL	7.35
15	Solberg - 1.5	1	15.95	BDL	BDL	7.34
16	Solberg - 2	1	14.4	BDL	BDL	7.29
17	SRL 502W - 1	2	15.45	BDL	BDL	7.34
18	SRL 502W - 1.5	12	15	BDL	BDL	7.31
19	SRL 502W - 2	17	14.9	BDL	BDL	7.29
20	NFD 20-391 -1	1	16.6	BDL	BDL	7.27
21	NFD 20-391 - 1.5	1	15.4	BDL	BDL	7.25
22	NFD 20-391 - 2	1	17.2	BDL	BDL	7.23
23	Buckeye - 1	8	17	BDL	BDL	7.33
24	Buckeye - 1.5	6	16.65	BDL	BDL	7.34
25	Buckeye - 2	8	15.8	BDL	BDL	7.33

* BDL = Below Detection Limit

COD: Chemical Oxygen Demand

CONCLUSIONS

- The AFFF formulations had COD ranging from 159,000 mg/L to 783,000 mg/L.
- The COD of the formulations was not precisely determinable by the standard dichromate COD test. COD returned by this test resulted in values that were lower than indicated by biological activity.
- The variation in COD among AFFF formulations suggests that foams would have differing environmental impact if all were mixed at 3% for application as recommended by manufacturers.
- Some formulations had high organic nitrogen content with the Fomtec product having a TKN concentration of 18,800 mg/L.
- The NFD 20-391 and Buckeye formulations had relatively higher inorganic nitrogen (nitrate) concentrations than other formulations. These were 5,360 and 9,920 mg/L, respectively.
- All formulations required biomass adaptation, which was achieved in two days for the AVIO, ECOPOL, Fomtec, and Solberg. Three, four, and five days of adaptation were required for the NRL 502W, NFD 20-391, and Buckeye formulations, respectively.
- The organic fraction for all formulations showed good biodegradability.
- The NRL 502W product had a higher proportion of slowly degradable organics than other formulations.
- The Buckeye product had residual COD values that could be associated with the fluorinated organic portion of the formulation.
- Targeted analysis of fluorine-free products led to detections of Hexylene Glycol (HG), N,N-Dimethyldodecylamine N-oxide (DMDA N-oxide), Diethylene glycol monobutyl ether (DEGMBE), and Sodium dodecyl sulfate (SDS).
- Biodegradation of HG, DEGMBE, DMDA, and SDS followed similar trends of ~ 100% removal within 7-days with some exceptions which include the decay of DEGMBE and DMDA in Buckeye, DEGMBE in NFD, and SDS in NRL 502W.
- The Buckeye product had relatively high concentration of 6:2 FTS which found to undergo biodegradation and yield perfluoroalkyl acids generation.
- To our knowledge, the only ingredient tested for biodegradability with current 28-day biodegradation data in Safety Data Sheets was hexylene glycol, with a value of 81%.

REFERENCES

- Carrillo-Abad, J., Pérez-Herranz, V., Urtiaga, A., 2018. Electrochemical oxidation of 6:2 fluorotelomer sulfonic acid (6:2 FTSA) on BDD: electrode characterization and mechanistic investigation. *J. Appl. Electrochem.* 48, 589–596. <https://doi.org/10.1007/s10800-018-1180-8>
- OECD 302B, 1992. OECD Guidelines for the Testing of Chemicals, Section 3: Environmental fate and behaviour Test No. 302B: Inherent Biodegradability: Zahn-Wellens/ EVPA Test. <https://doi.org/https://doi.org/10.1787/9789264070387-en>
- Shaw, D.M.J., Munoz, G., Bottos, E.M., Duy, S.V., Sauve, S., Liu, J., Van Hamme, J.D., 2019. Degradation and defluorination of 6:2 fluorotelomer sulfonamidoalkyl betaine and 6:2 fluorotelomer sulfonate by *Gordonia* sp. strain NB4-1Y under sulfur-limiting conditions. *Sci. Total Environ.* 647, 690–698. <https://doi.org/10.1016/j.scitotenv.2018.08.012>
- Weber, A.K., Barber, L.B., LeBlanc, D.R., Sunderland, E.M., Vecitis, C.D., 2017. Geochemical and Hydrologic Factors Controlling Subsurface Transport of Poly- and Perfluoroalkyl Substances, Cape Cod, Massachusetts. *Environ. Sci. Technol.* 51, 4269–4279. <https://doi.org/10.1021/acs.est.6b05573>

Appendix A

Test Plan for Testing Ready Biodegradability of Seven AFFF Formulations

There were eight samples. However, only seven were tested:

1. Non-fluorine containing products
 - a. AVIO
 - b. ECOPOL
 - c. Fomtec
 - d. Solberg
 - e. NRL 502
 - f. NFD 20-391
 - g. Angus Fire
2. Fluorine containing product
 - a. Buckeye

All formulations were tested except for Angus Fire. Care and proper PPE (gloves, lab coat, eye protection) must be used when handling these chemicals.

Solutions

1. Acetate control feedstock: 54 ml of glacial acetic acid was added to 600 mL distilled water in a 1L bottle. pH was adjusted to 6.5 using sodium hydroxide. Mixtures was diluted to 1 L using distilled water. COD of feedstock was checked and was approximately 60,000 mg/L.
2. Firefighting foams: A concentration of 60,000 mg/L COD was targeted by making appropriate dilutions of AFFF formulations depending on initial COD values measured for each AFFF formulation (Table 2). Briefly, an initial dilution was made where further dilutions were made to achieve 120, 180, and 240 mg/L COD in bioreactors. These concentrations were randomly selected to meet the criteria for OECD method (OECD 302B, 1992). Each formulation consisted of 50 mL DI water added to a 250 mL bottle. Then 25 mL of neat AFFF product was added and diluted to 100 mL. Next, the COD was measured, and additional tests were shown in analytical table at end of this test plan (Table A1). 6000 mg of COD from dilution was added to each of the AFFFs to a 100 mL bottle (approximately 11 mL – 32 mL depending on the AFFF; Table 2). pH was adjusted to 7.0 using NaOH or HCl. Mixtures was then diluted to 100 mL. Test COD should be 60,000 mg/L. These were the mixtures used during the test setup. Additional tests were performed as shown in analytical table A1. After completion of testing, results were

reviewed, and Table A2 was updated for volumes and concentrations needed for the testing.

3. Test Medium: 40 mL MinI, Min II, 10 mL of nutrient solution and 80 mL phosphate buffer solutions we all added to 16 L of tap water.

Biomass

One gallon of mixed liquor was obtained from the Fayetteville Wastewater Treatment plant. Biomass was aerated after returning to the lab and sample was strained to remove large particles. The sample was then centrifuged to concentrate the solids and centrate was discarded. Tap water was added to the solids to make sample to approximately 20% of original volume and mixed thoroughly to re-suspend solids. The sample was analyzed for total suspended solids (TSS) and volatile suspended solids (VSS) as shown in the Table A1 The concentrated biomass was diluted so that at least one liter of biomass with a concentration of 5,000 mg/L VSS was reached.

Biodegradability Testing

A series of thirty-two reactors were set up as shown in Table A2. Reactors 1 – 25 were used for the biodegradability testing. Reactors 26 – 32 were monitored but used to accumulate sample for shipping to other labs for analysis. 480 mL of test medium was added from Solutions: Step 3 above. VSS and AFFF and other feedstocks were added as shown in Table A2. TCMP, as indicated on the table was added and concentrated seed table (20 mL) was added. Stirring was adjusted to 600 rpm. The bottles incubated at 25 °C for 28 days. On days 7, 14, 21, and 28, 25 ml of mixed liquor from reactors 26 – 31 was removed and the sample was put in a 50 mL centrifuge tube. Tubes were labelled with AFFF and the date sample was taken. Samples were frozen for shipment at the end of the test. On days 7, 14, 21, and 28, 25 mL of sample was removed from reactor 32 and centrifuged. Centrate was poured into a fresh centrifuge tube, labeled with “Buckeye”, dated, and frozen for shipment. Samples were shipped to lab as directed. On days 0 (after 3 hours of incubation), 7, 14, 21 and 28, 10 mL of contents were removed from reactors 1 through 25 and pH, COD, NH₃-N, NO₂-N and NO₃-N measurements were recorded. pH was adjusted to 7 if lower than 6.5 or higher than 8.0 using concentrated NaOH or HCl.

Table A1: Workup for SERDP AFFF

Workup for SERDP AFFF			Dilute AFFF Formulations	Mixed Liquor
Soluble COD	sCOD	mg/L		
Total COD	tCOD	mg/L	x	
Total suspended solids	TSS	mg/L		x
Volatile suspended solids	VSS	mg/L		x
Soluble TKN	sTKN	mg/L as N		
Total TKN	tTKN	mg/L as N	x	
Soluble ammonia-N	sNH ₃ -N	mg/L as N	x	
Soluble nitrate-N	sNO ₃ -N	mg/L as N	x	
Soluble nitrite-N	sNO ₂ -N	mg/L as N	x	
Soluble Phosphorus	sP (or PO ₄)	mg/L as P (or PO ₄)		
Total phosphorus	tP (or PO ₄)	mg/L as P (or PO ₄)	x	
Soluble sulfate	sSO ₄	mg/L		
Soluble color	sColor	mg/L		
Soluble chloride	sCl	mg/L		
Soluble alkalinity	Alk.	mg/L as CaCO ₃		
Total alkalinity	Alk.	mg/L as CaCO ₃		
Total dissolved solids	TDS	mg/L		
Fixed dissolved solids	FDS	mg/L		
pH	pH	su		
ORP	ORP	mV		
FOG	FOG	mg/L		
Soluble lignins	lignins	mg/L		
VFA by GC for anaerobic tests	VFA	mg/L		
Mineral Scan				
Total Quats		mg/L		
Non-Ionic Surfactants		mg/L		
Anionic Surfactants		mg/L		
Long Chain Fatty Acids	LCFA	mg/L		

* Skip soluble parameters if the raw sample is too thick to get a good sample for testing.

Table A2: Biodegradability testing reactors

Set No.	Reactor Name	Bottle No.	Bottle		mg VSS/L	mL of	mL Dilute	COD, mg/L	g VSS/g DOC	TCMP
			Volume mL	mL Culture		Acetate Feedstock	AFFF Formula			
1	Seed	1, 2	500	100	1000	0	0			Yes
2	Control	3, 4	500	20	200	2	0	240	2.2	Yes
3	National Foam Avio Green KHC 3%	5	500	20	200	0	1	120	4.5	Yes
		6	500	20	200	0	1.5	180	3.0	Yes
		7	500	20	200	0	2	240	2.2	Yes
4	BIOEX	8	500	20	200	0	1	120	4.5	Yes
		9	500	20	200	0	1.5	180	3.0	Yes
		10	500	20	200	0	2	240	2.2	Yes
5	Fomtec	11	500	20	200	0	1	120	4.5	Yes
		12	500	20	200	0	1.5	180	3.0	Yes
		13	500	20	200	0	2	240	2.2	Yes
6	Solberg	14	500	20	200	0	1	120	4.5	Yes
		15	500	20	200	0	1.5	180	3.0	Yes
		16	500	20	200	0	2	240	2.2	Yes
7	SRL 502W	17	500	20	200	0	1	120	4.5	Yes
		18	500	20	200	0	1.5	180	3.0	Yes
		19	500	20	200	0	2	240	2.2	Yes
8	NFD 20-391	20	500	20	200	0	1	120	4.5	Yes
		21	500	20	200	0	1.5	180	3.0	Yes
		22	500	20	200	0	2	240	2.2	Yes
9	Buckeye	23	500	20	200	0	1	120	4.5	Yes
		24	500	20	200	0	1.5	180	3.0	Yes
		25	500	20	200	0	2	240	2.2	Yes
Sacrificial Reactors										
10	National Foam Avio	26	500	20	200	0	1.5	180	3.0	Yes
	Green KHC 3%									
11	BIOEX	27	500	20	200	0	1.5	180	3.0	Yes
12	Fomtec	28	500	20	200	0	1.5	180	3.0	Yes
13	Solberg	29	500	20	200	0	1.5	180	3.0	Yes
14	SRL 502W	30	500	20	200	0	1.5	180	3.0	Yes
15	NFD 20-391	31	500	20	200	0	1.5	180	3.0	Yes
16	Buckeye	32	500	20	200	0	1.5	180	3.0	Yes

COD: Chemical Oxygen Demand, VSS: Volatile Suspended Solids, DOC: Dissolved Organic Carbon, TCMP: 2-chloro-6-(trichloromethyl)pyridine

Supplementary Material
Testing Biodegradability of Aqueous Film Forming Foam
Formulations in an Aerobic Environment

February 2022

Testing was performed in December 2020/January 2021 to evaluate the biodegradability of seven aqueous film forming foam (AFFF) formulations. Six of the formulations were experimental non-fluorine-containing and one contained fluorine. The biodegradability testing performed by EnviTreat followed OECD 302B guidance. In this testing, manometric measurement of oxygen uptake by microorganisms was measured as an indicator of how far and how quickly biological degradation of organics were occurring. At the same time, soluble COD measurements were taken periodically as a second measure of how far the biological degradation of organics proceeded and to measure any residual COD that may not be biodegradable.

The COD test used by EnviTreat is the USEPA approved Hach dichromate method. This method follows the Standard Methods 5220 D method. Standard Methods and Hach note that the digestion method typically oxidizes organics to 95% - 100% of their theoretical value. In some cases, organics may be hard to digest. The method specifies that a longer digestion time, four hours instead of two hours, can be used in these cases.

While performing the AFFF testing, the oxygen uptake measured for several of the formulations was higher than the applied COD. This is not possible and indicates that the measured COD was low as would happen if the organics in the sample were not completely digested by the dichromate method (Figure S1). EnviTreat re-tested the COD using the longer, 4-hour digestion method and did see an increase in COD with some of the formulations. The COD increase ranged from 3% to 38% with an average increase of 14%. Applied CODs were adjusted but oxygen uptake still exceeded 100% of applied COD. The fact that the oxygen uptake for the control was typical of readily biodegradable compounds indicates that the respirometer was working properly.

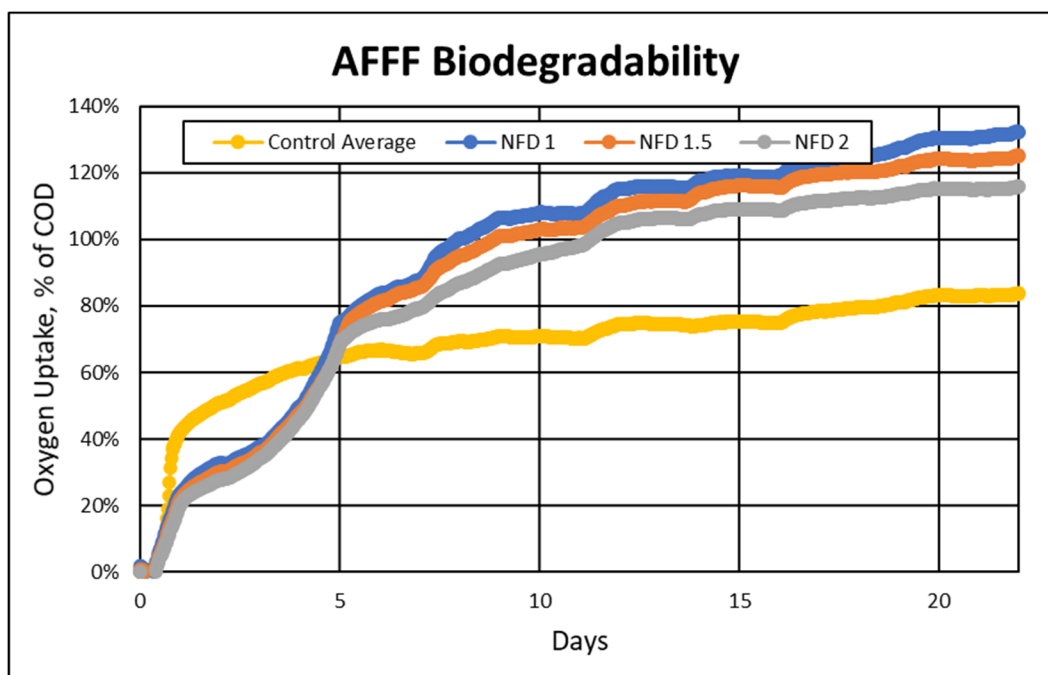


Figure S1: NFD 20-391 oxygen uptake curves as %COD

The dichromate test method does not have a protocol for digesting more than four hours and the purpose of this study was not to develop a COD method that could accurately digest all the organics in the sample. Instead, an elemental analysis was performed on AFFF formulations to determine the proportion of carbon, oxygen, hydrogen, nitrogen, and sulfur in the organic compounds. The method requires drying of the material to be tested prior to measurement. Once the proportions of these elements are known, a chemical formula can be developed, and a theoretical COD may be calculated. The chemical formulas and COD are presented in Table S1.

Table S1: Chemical formula and COD of AFFF formulations calculated from elemental analysis results.

Chemical formula and COD of AFFF formulations calculated from elemental analysis results.

Formulation	Chemical Formula	COD, g COD/g Dry Material
NFD 20-931	$C_{1.5}H_{3.1}ON_{0.08}$	1.42
Fomtec	$C_{1.6}H_{3.7}ON_{0.31}$	1.33
Buckeye	$C_{1.5}H_{3.2}ON_{0.04}$	1.51
AVIO	$C_{3.4}H_{7.2}ON_{0.13}$	2.24
NRL 502	$C_{1.9}H_{3.8}O$	1.75
Ecopol	$C_{2.1}H_{4.6}ON_{0.13}$	1.78
Solberg	$C_{1.3}H_{2.5}ON_{0.021}$	1.29

The total solids and volatile solids of the AFFF formulations were measured and the COD of formulations was calculated based on the volatile solids content. However, these CODs were still low. We suspect that the drying process caused some alcohols and other organics to volatilize leading to a false low solids result in both the elemental analysis as well as the total and volatile solids tests.

Finally, we measured the total organic carbon (TOC) content of each of the AFFF formulations. An approximate COD could be calculated based on the CODs determined and presented in Table S1 and the TOC test results as that test does not rely on drying. The results from this effort also produced COD results that were lower than the results measured in the typical dichromate method. This is likely due to lower carbon content in the chemical formulas due to the evaporation mentioned above.

While the exact COD of the formulations could not be accurately measured, the results of the biodegradability test were clear. Only the range of the COD of the AFFF formulations would be expected to vary slightly with a more accurate COD measurement. Those conclusions were:

- The AFFF formulations had measured COD concentrations ranging from 159,000 mg/L to 783,000 mg/L.
- COD of the formulations were not precisely determinable by the standard dichromate COD test. COD returned by this test resulted in COD values that were lower than indicated by biological activity.
- The variation in COD among AFFF formulations suggests that foams would have differing environmental impact if all were mixed at 3% for application as recommended by manufacturers.
- Some formulations had high organic nitrogen content with the Fomtec product having a TKN concentration of 18,800 mg/L.
- The NFD 20-391 and Buckeye formulations had relatively higher inorganic nitrogen (nitrate) concentrations than other formulations. These were 5,360 mg/L and 9,920 mg/L respectively.

- All formulations required biomass adaptation. Adaptation was achieved in two days for the National Foam, BIOEX, Fomtec, and Solberg formulations. Three, four, and five days of adaptation were required for the NRL 502W, NFD 20-391, and Buckeye formulations respectively.
- The organic fraction for all formulations showed good biodegradability.
- The NRL 502W product had a higher proportion of slowly degradable organics than other formulations.
- The Buckeye product had residual COD values that could be associated with the fluorinated organic portion of the formulation.