# *Anaerobic Biodegradation of Biofuels (Ethanol, Biodiesel, n-Propanol, n-Butanol, and iso-Butanol) in Aquifer Sediment*

**Cherri Adair and John T. Wilson**
*U.S. EPA/ORD/NRMRL*
*R.S. Kerr Center, Ada, OK*

**22nd National Tanks Conference**
**Boston, Massachusetts**
**September 20-22, 2010**

RESEARCH AND DEVELOPMENT

*Building a scientific foundation for sound environmental decisions*

# Why do we care?

Toxicity of the compounds

Biodegradation of the Biofuels can inhibit natural biodegradation of benzene and BTEX compounds

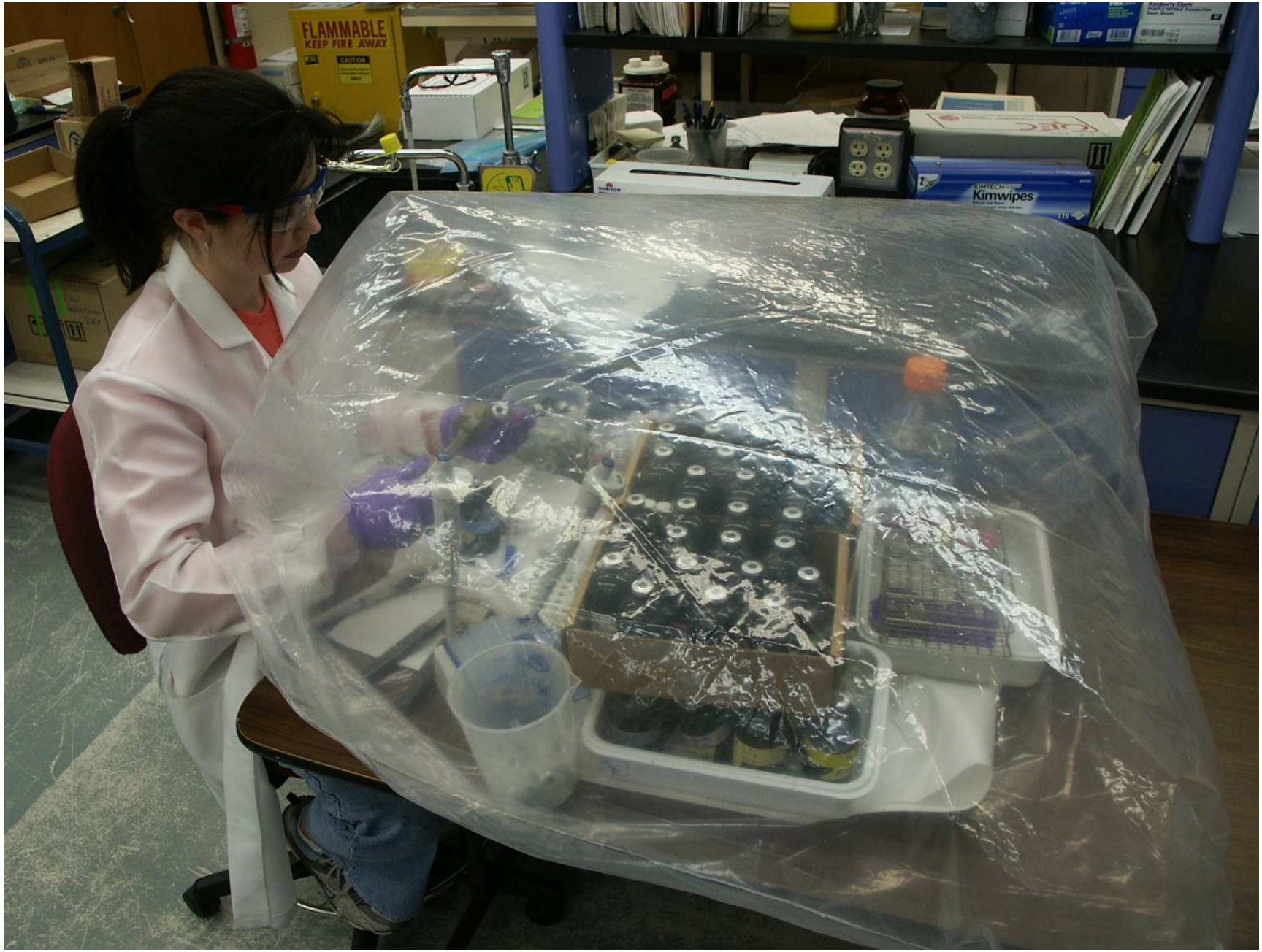
Biodegradation of Biofuels leads to production of methane-

- potential for formation of explosive mixtures
- potential to expedite vapor intrusion of BTEX compounds

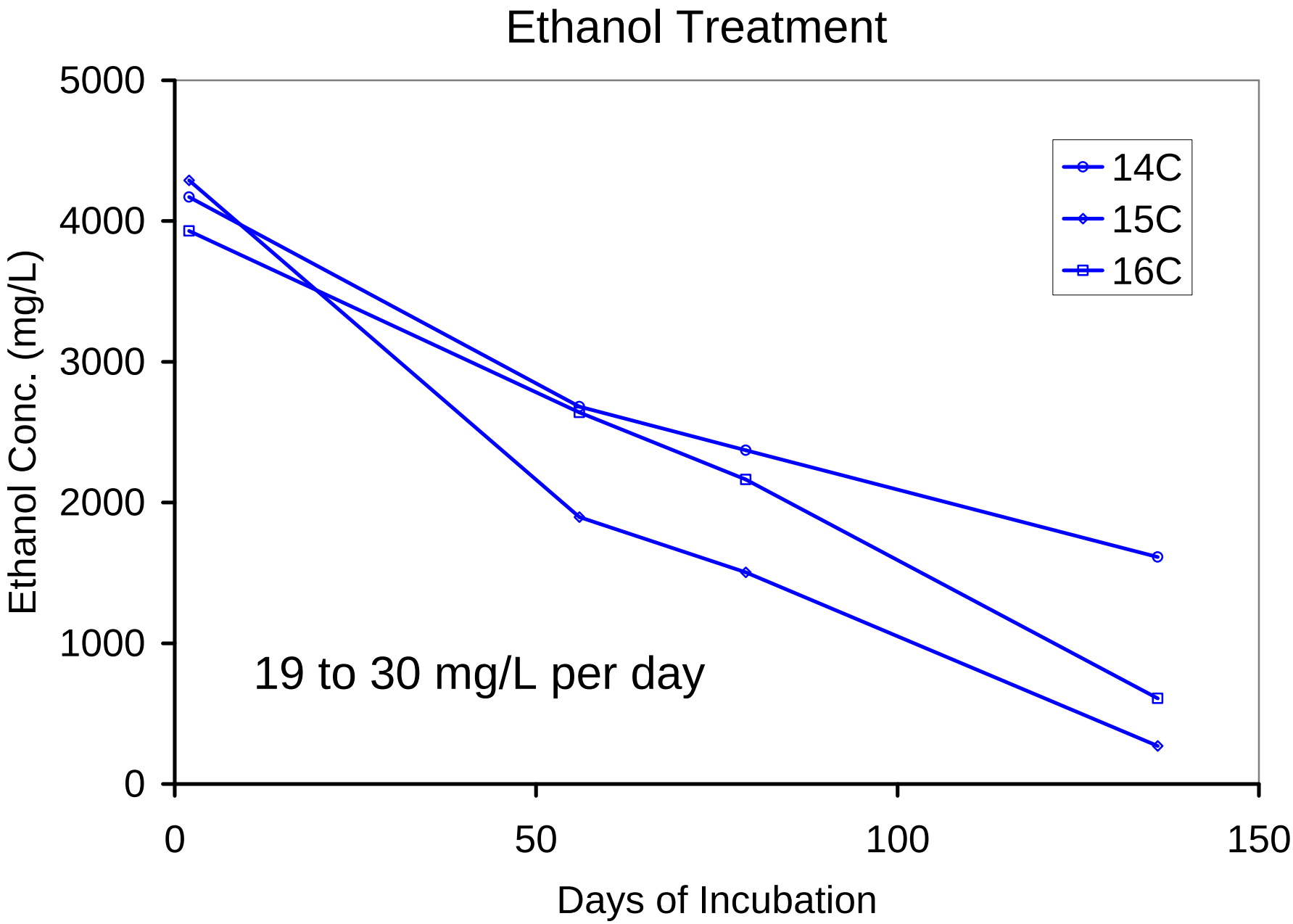
# ***Biofuel Treatments***

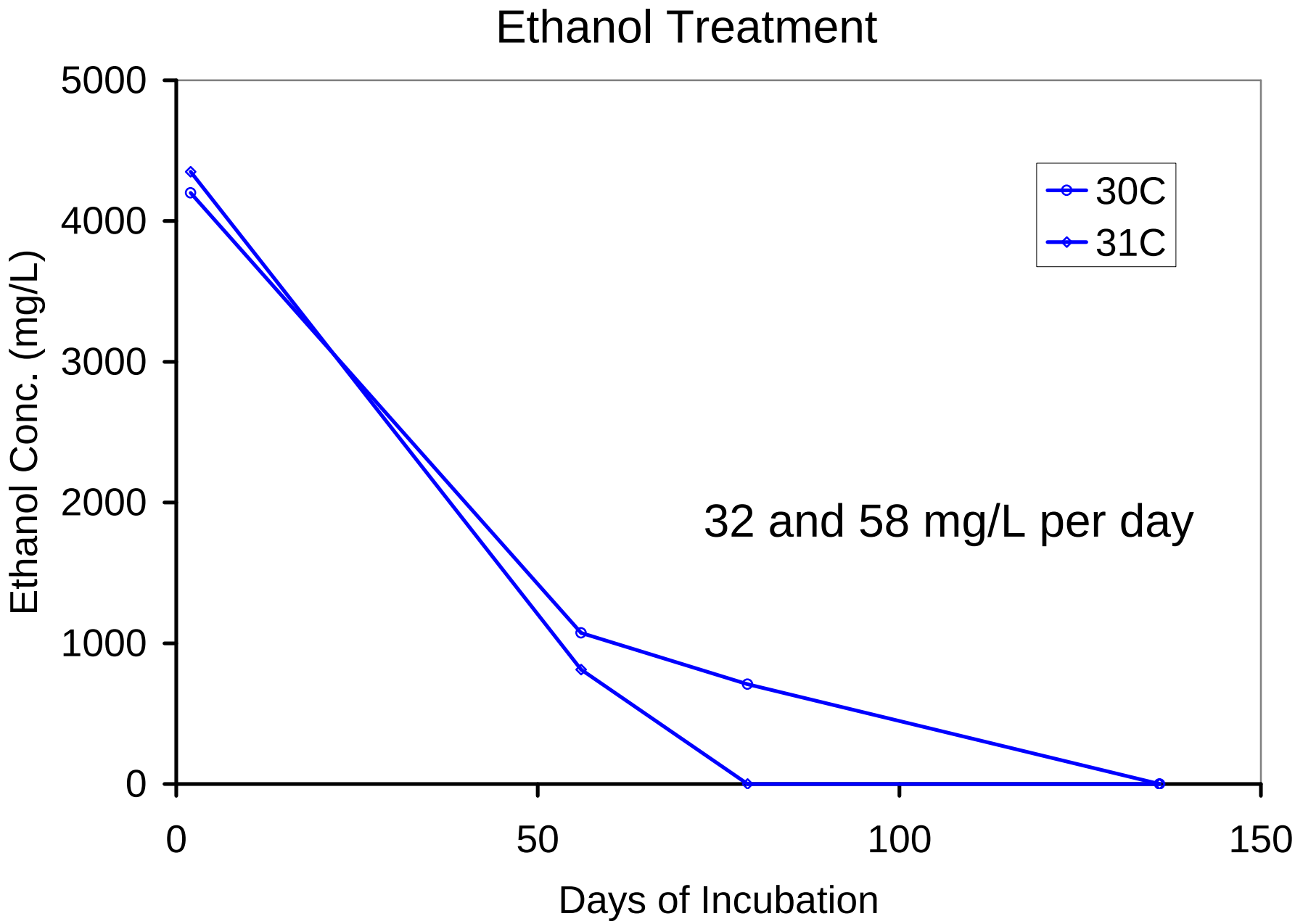
- Ethanol
- *n*-Butanol
- *iso*-Butanol
- *n*-Propanol
- Biodiesel Emulsion


B-03C9-6
UNITED STATES OF AMERICA
LIBERTY
QUARTER DOLLAR


FLAMMABLE
KEEP FIRE AWAY
Kimwipes

# Ethanol


Ethanol Treatment
5000
4000
3000
2000
1000
0
Ethanol Conc. (mg/L)
14C
15C
16C
19 to 30 mg/L per day
0
50
100
150
Days of Incubation


Ethanol Treatment
Ethanol Conc. (mg/L)
5000
4000
3000
2000
1000
0
0
50
100
150
Days of Incubation
30C
31C
32 and 58 mg/L per day

# Ethanol Fermentation to Methane

$$2\ CH_3CH_2OH + 2\ H_2O \rightarrow 2\ CH_3COOH + 4\ H_2$$

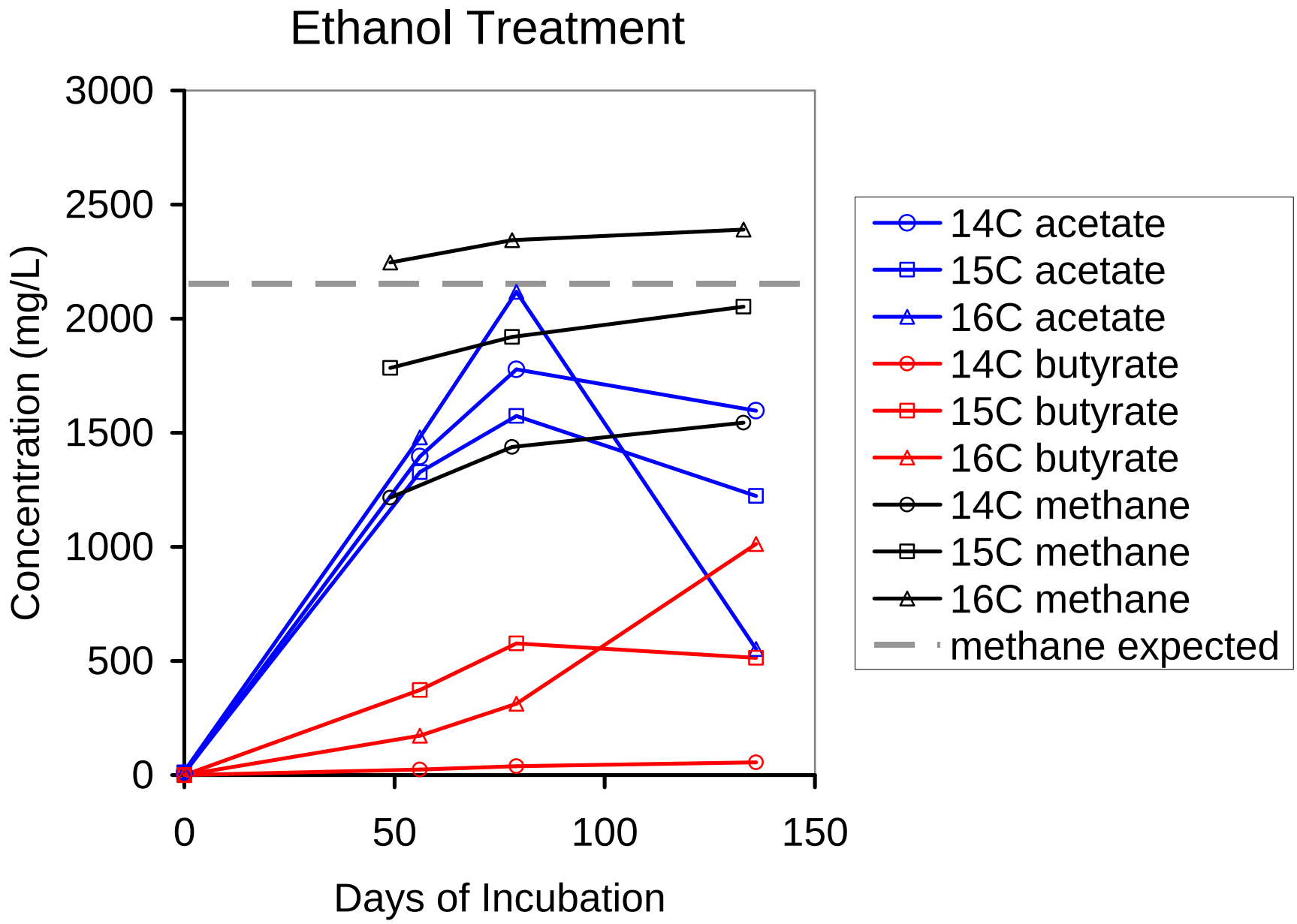
$$2\ CH_3COOH \rightarrow 2\ CO_2 + 2\ CH_4$$

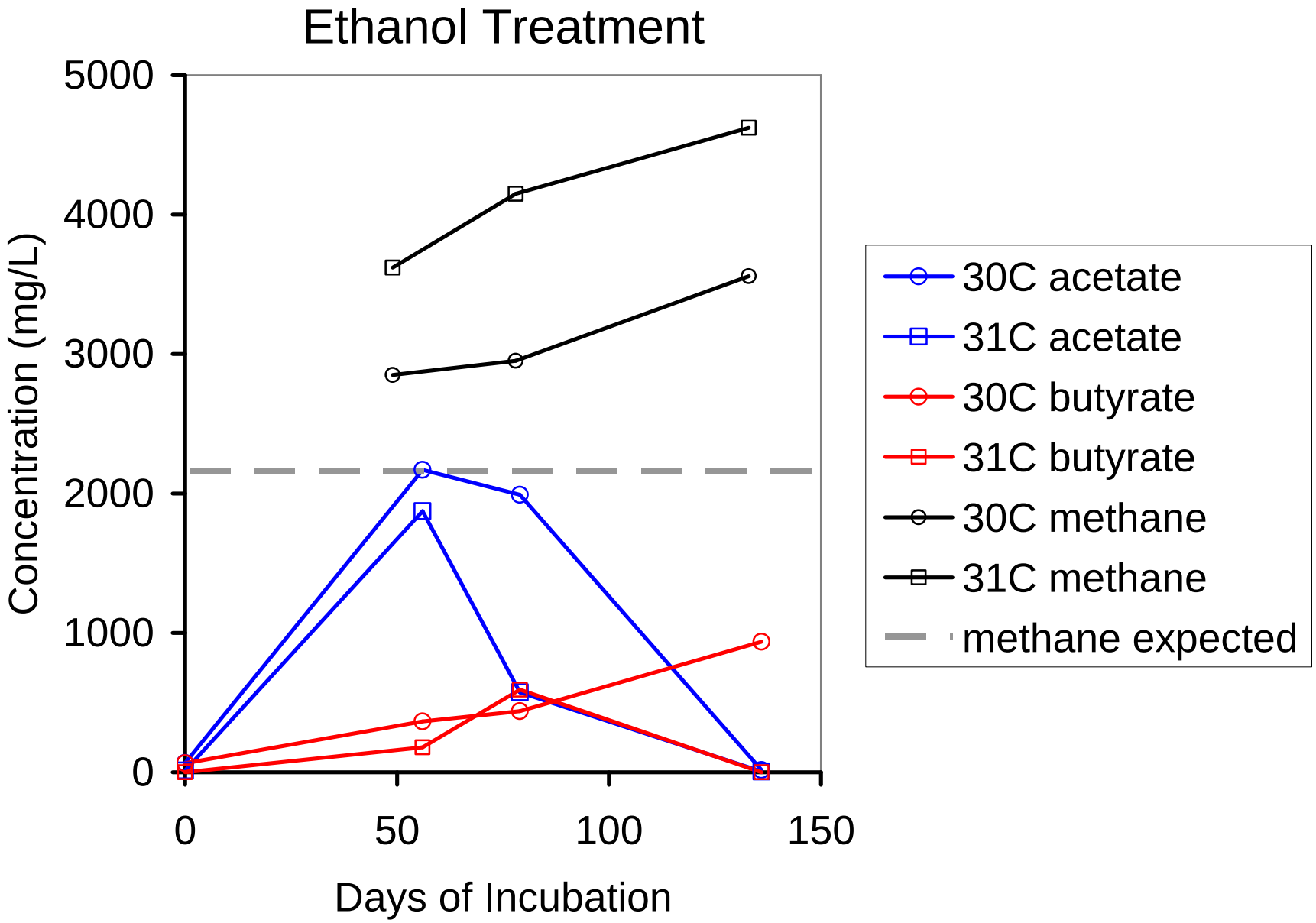
$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$$

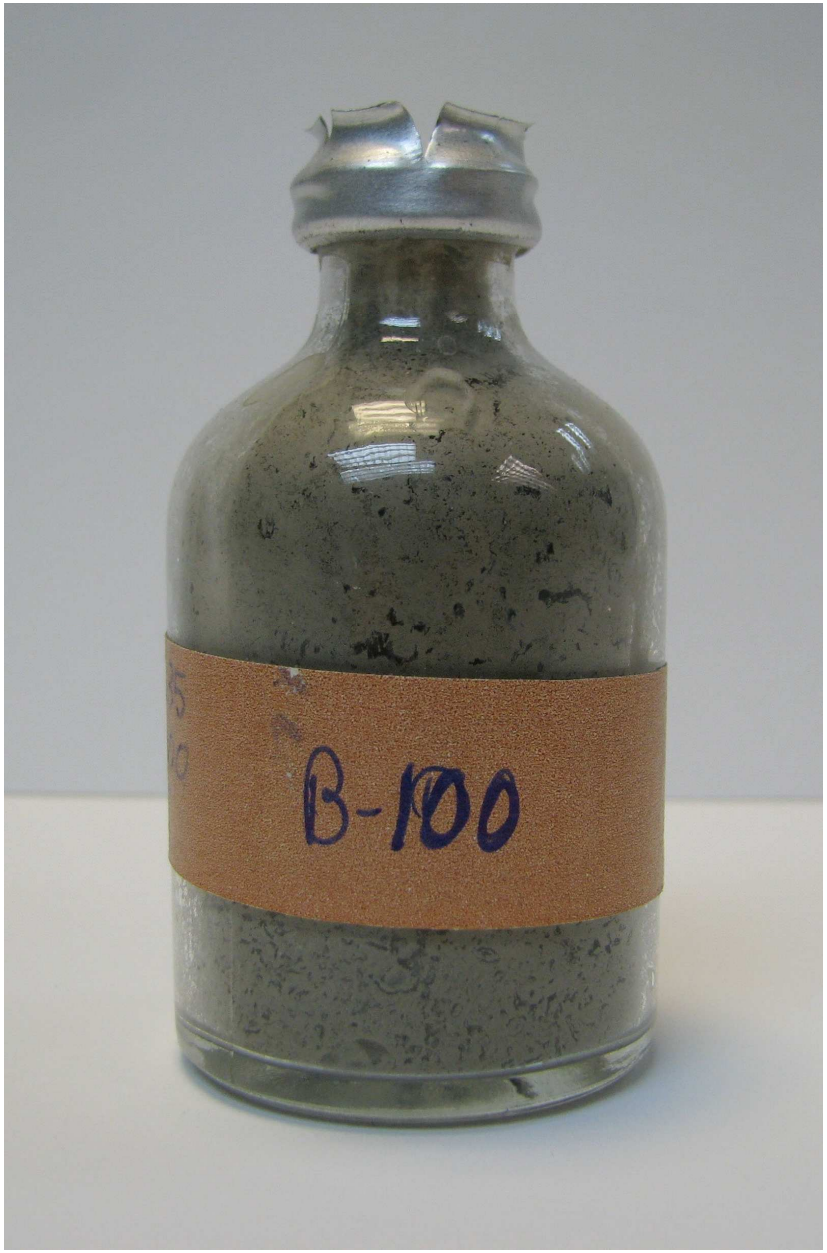
---

$$2\ CH_3CH_2OH \rightarrow 3\ CH_4 + CO_2$$

92 mg/L ethanol produces 48 mg/L methane

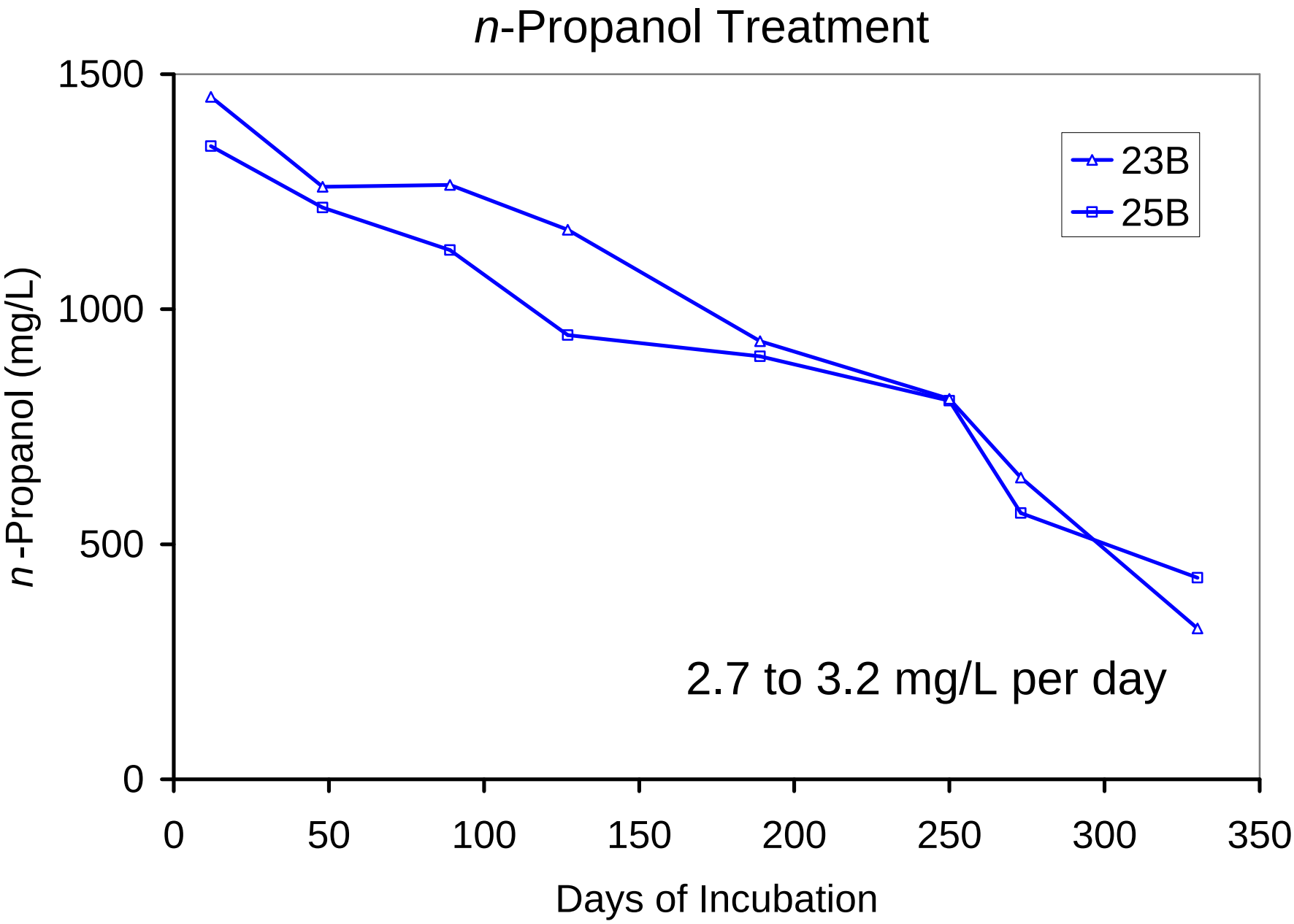

Ethanol Treatment
Concentration (mg/L)
3000
2500
2000
1500
1000
500
0
0
50
100
150
Days of Incubation
14C acetate
15C acetate
16C acetate
14C butyrate
15C butyrate
16C butyrate
14C methane
15C methane
16C methane
methane expected


Ethanol Treatment
Concentration (mg/L)
5000
4000
3000
2000
1000
0
0
50
100
150
Days of Incubation
30C acetate
31C acetate
30C butyrate
31C butyrate
30C methane
31C methane
methane expected


B-100


B-100

# *n*-Propanol


*n*-Propanol Treatment
1500
1000
500
0
*n*-Propanol (mg/L)
23B
25B
2.7 to 3.2 mg/L per day
0
50
100
150
200
250
300
350
Days of Incubation

# Propanol Fermentation to Methane

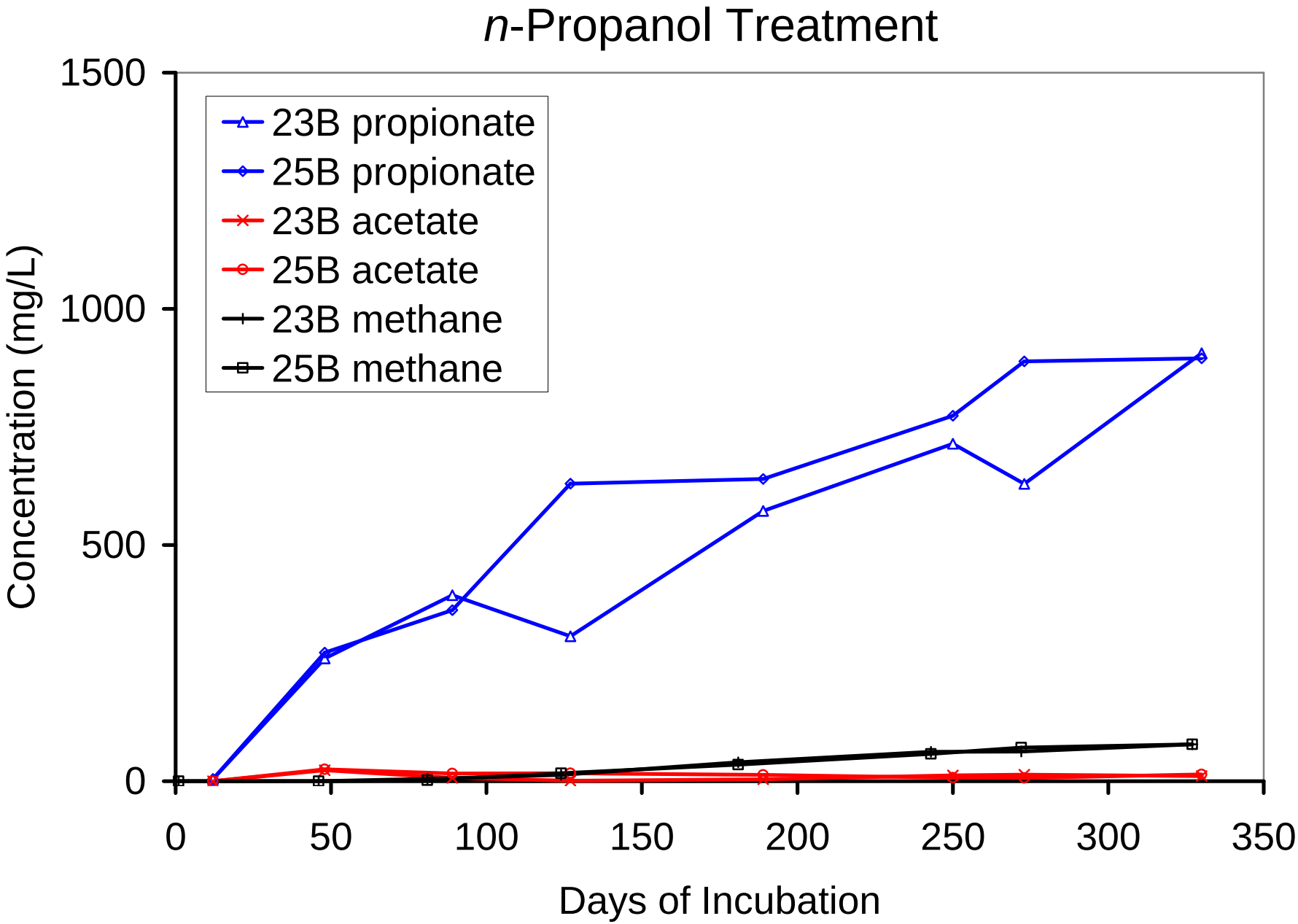
$$CH_3CH_2CH_2OH + H_2O \rightarrow CH_3CH_2COOH + 2\ H_2$$

$$CH_3CH_2COOH + 2\ H_2O \rightarrow CH_3COOH + CO_2 + 3\ H_2$$

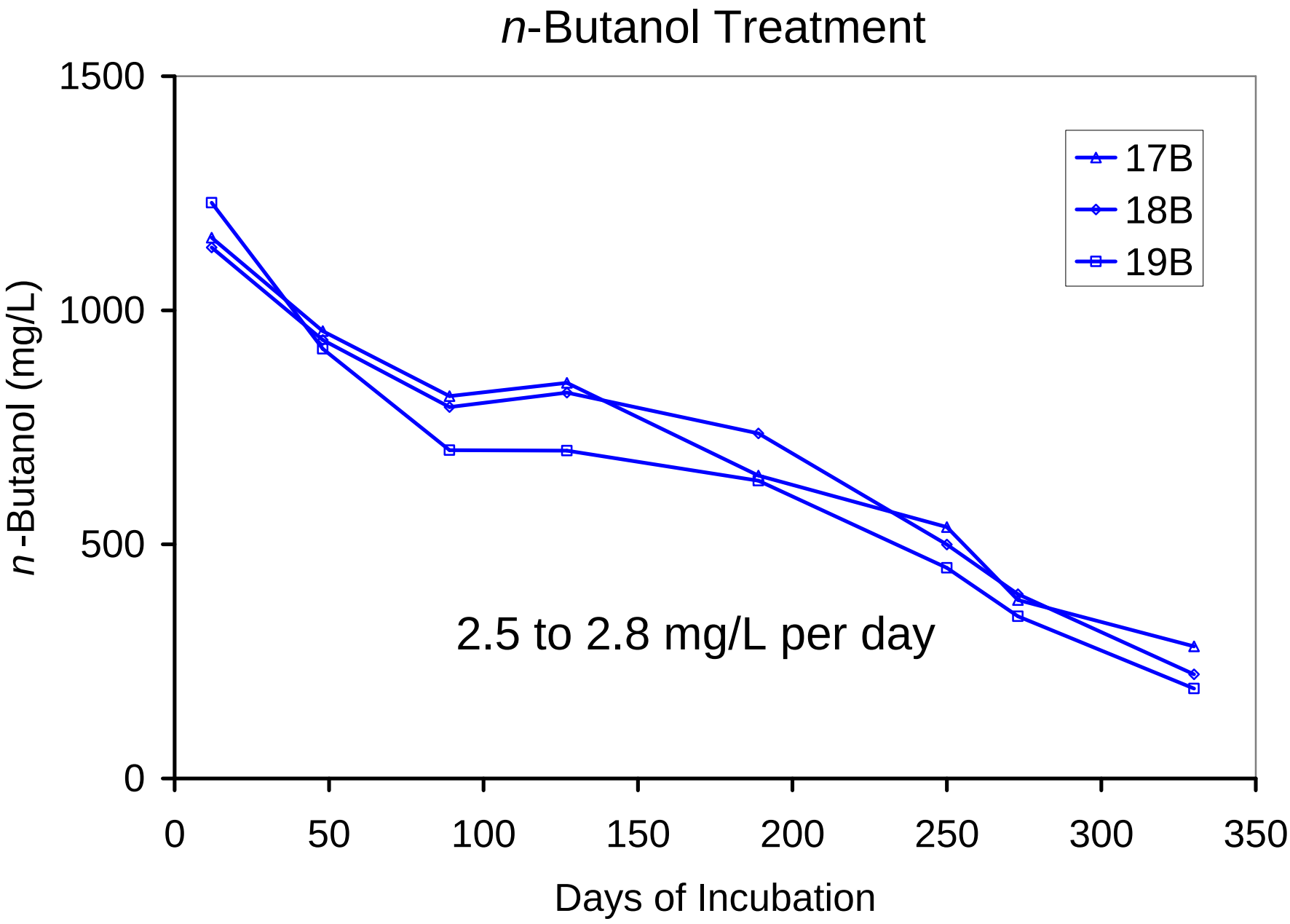
$$CH_3COOH \rightarrow CO_2 + CH_4$$

$$5\ H_2 + 1.25\ CO_2 \rightarrow 1.25\ CH_4 + 2.5\ H_2O$$

60 mg/L propanol produces 36 mg/L methane


*n*-Propanol Treatment
1500
1000
500
0
Concentration (mg/L)
23B propionate
25B propionate
23B acetate
25B acetate
23B methane
25B methane
0
50
100
150
200
250
300
350
Days of Incubation

# *n*-Butanol


*n*-Butanol Treatment
1500
1000
500
0
*n*-Butanol (mg/L)
0
50
100
150
200
250
300
350
Days of Incubation
17B
18B
19B
2.5 to 2.8 mg/L per day

# Butanol Fermentation to Methane

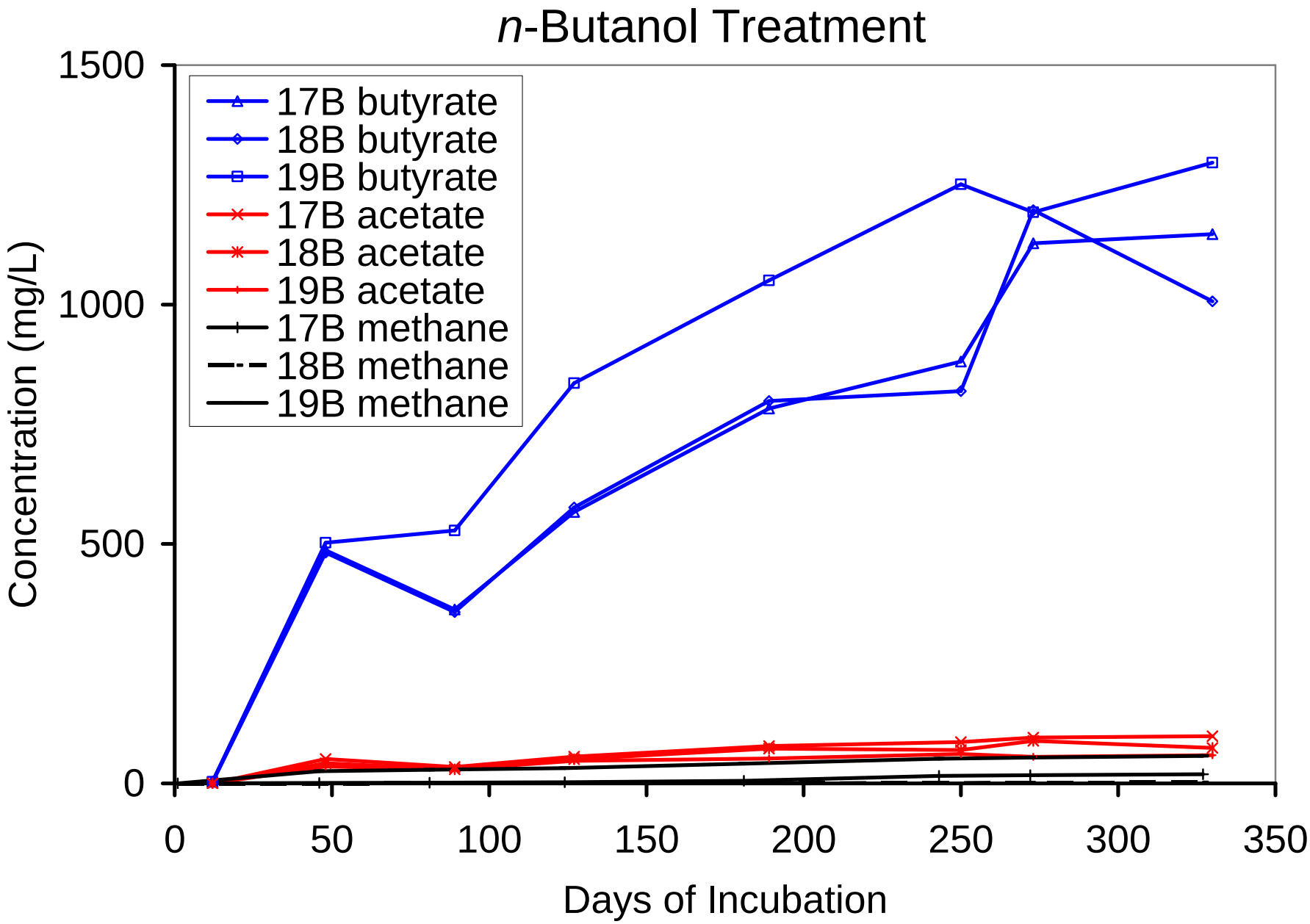
$$CH_3CH_2CH_2CH_2OH + H_2O \rightarrow CH_3CH_2CH_2COOH + 2\ H_2$$

$$CH_3CH_2CH_2COOH + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ H_2$$

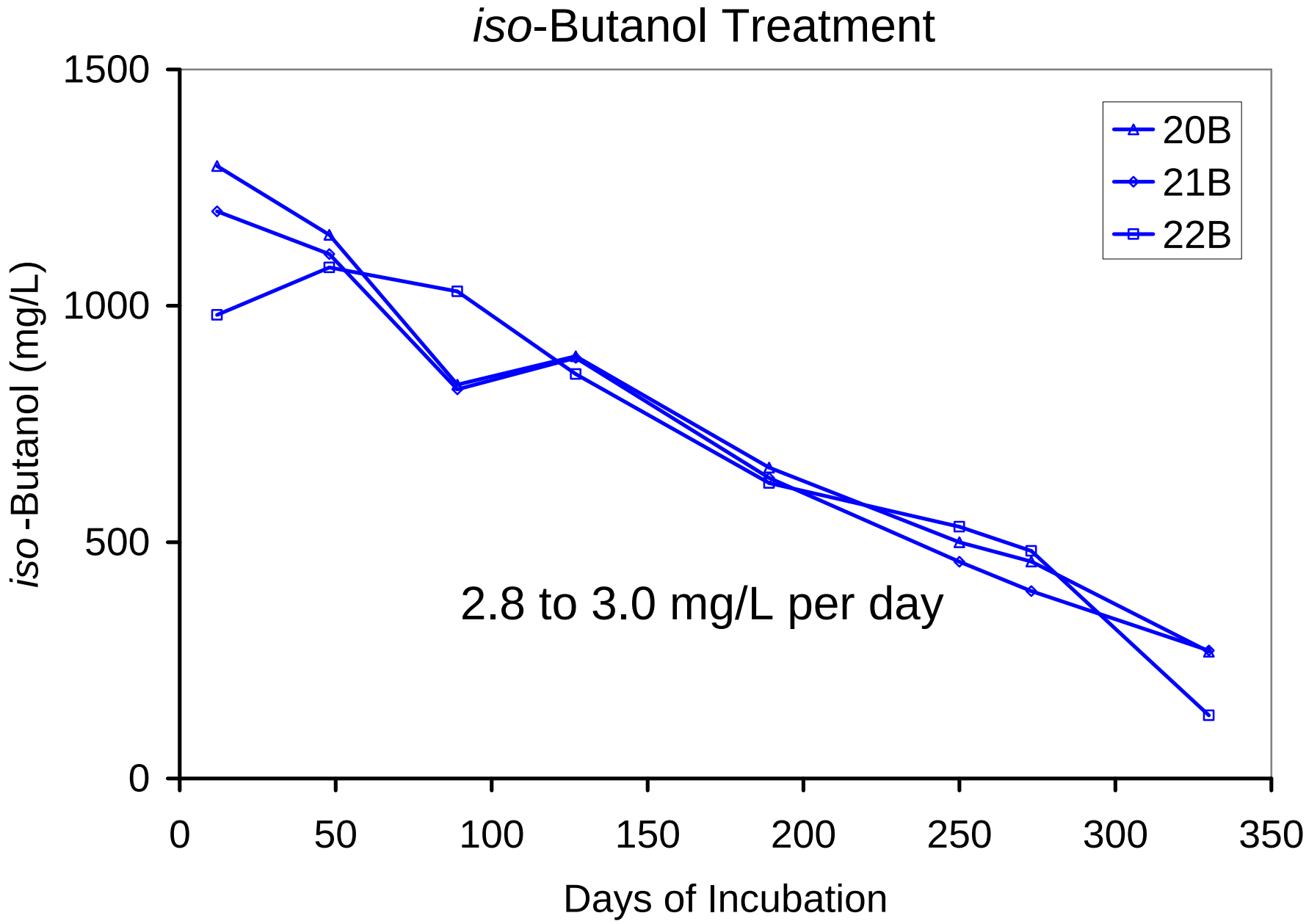
$$2\ CH_3COOH \rightarrow 2\ CO_2 + 2\ CH_4$$

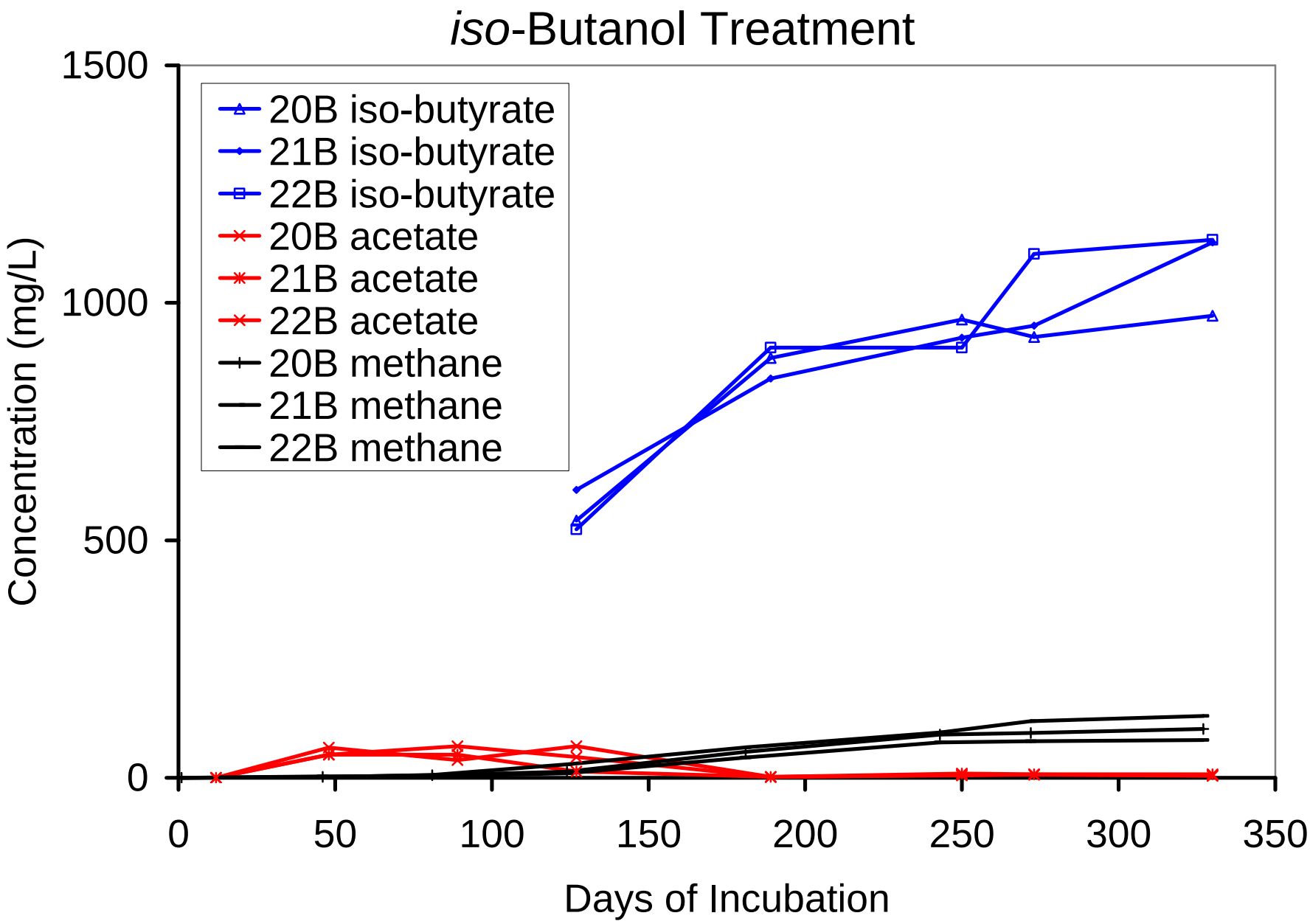
$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$$

74 mg/L butanol produces 48 mg/L methane

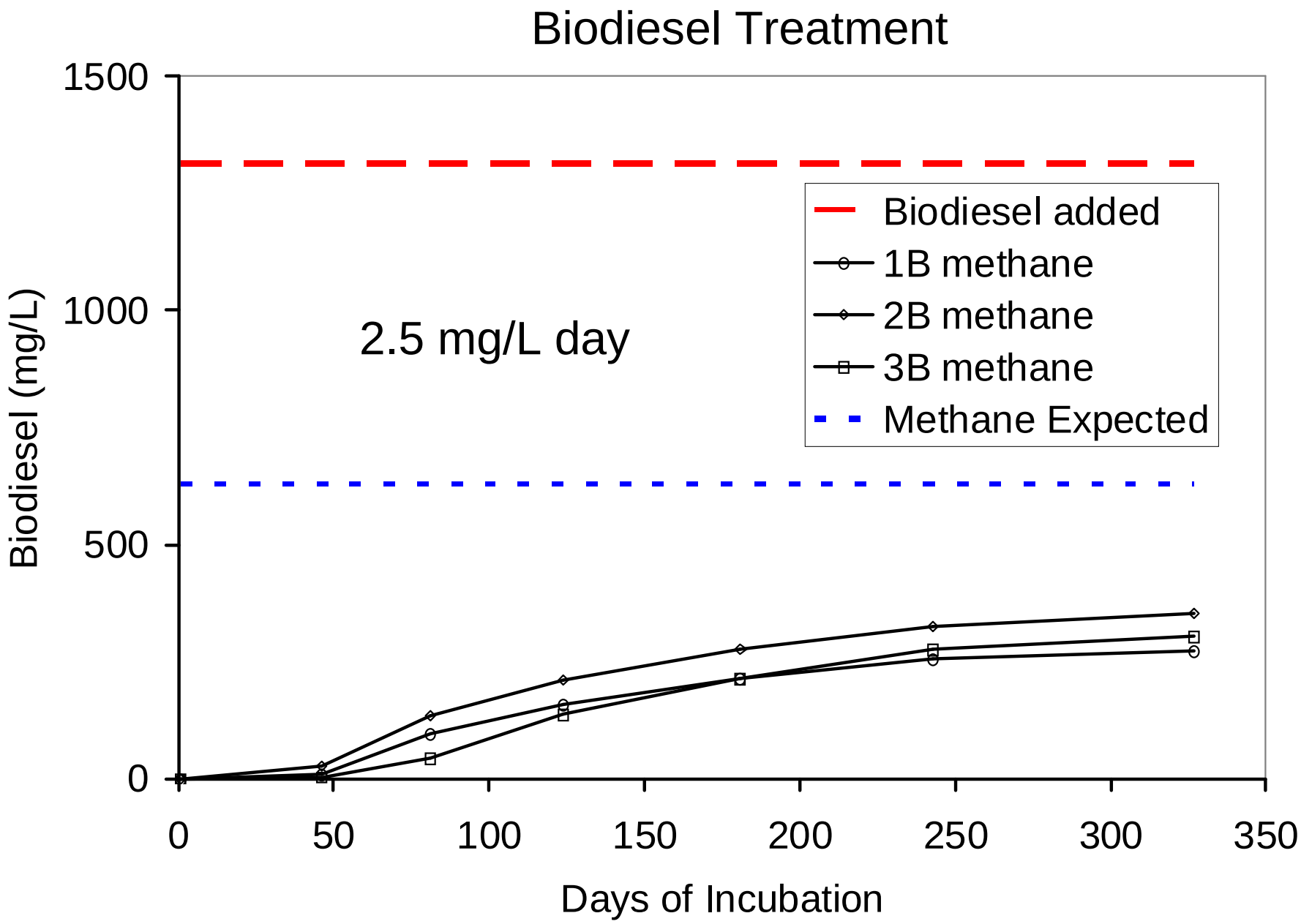

*n*-Butanol Treatment
1500
1000
500
0
Concentration (mg/L)
17B butyrate
18B butyrate
19B butyrate
17B acetate
18B acetate
19B acetate
17B methane
18B methane
19B methane
0
50
100
150
200
250
300
350
Days of Incubation

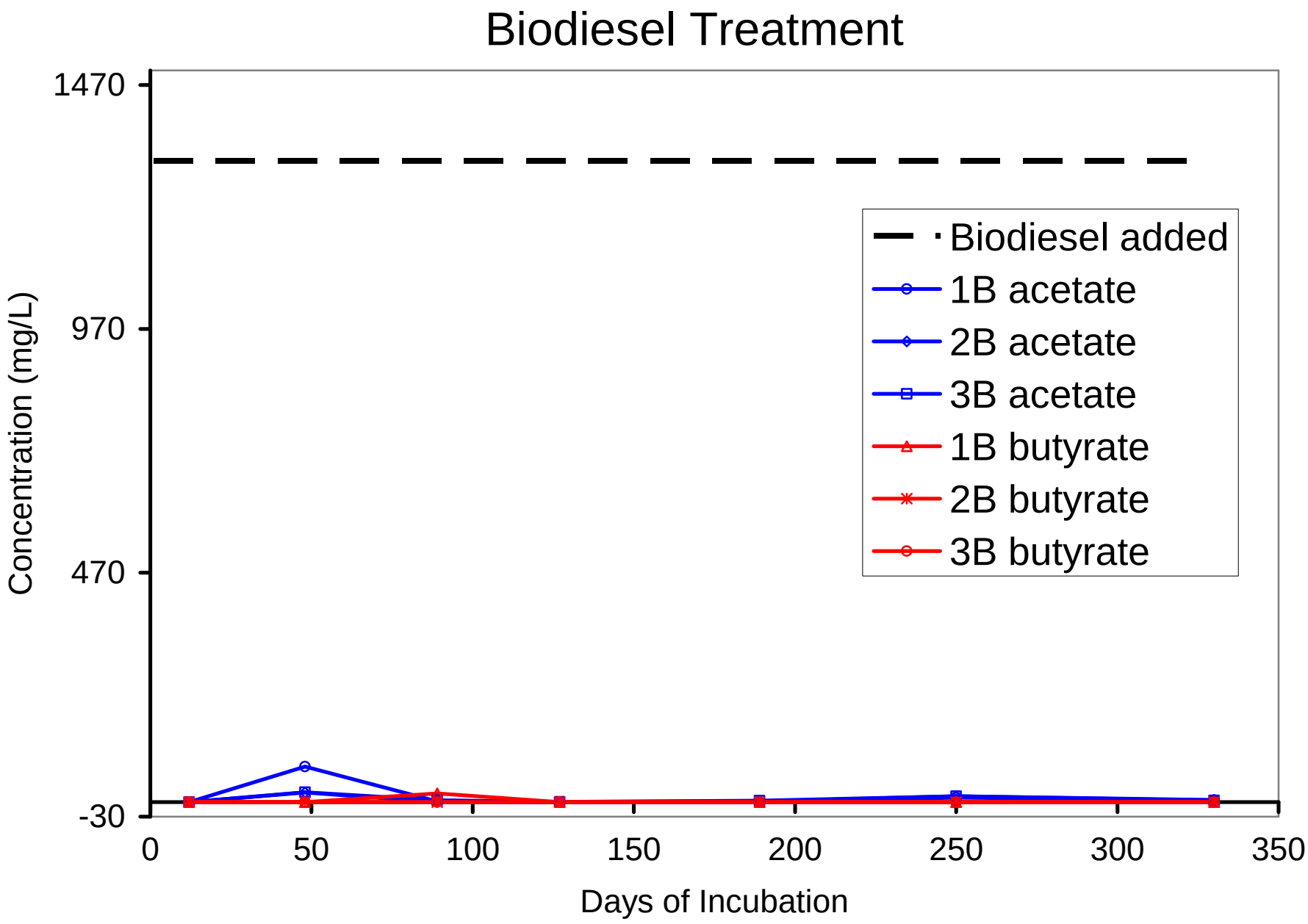
# *iso*-Butanol


*iso*-Butanol Treatment
1500
1000
500
0
*iso*-Butanol (mg/L)
20B
21B
22B
2.8 to 3.0 mg/L per day
0
50
100
150
200
250
300
350
Days of Incubation


*iso*-Butanol Treatment
Concentration (mg/L)
1500
1000
500
0
0
50
100
150
200
250
300
350
Days of Incubation
20B iso-butyrate
21B iso-butyrate
22B iso-butyrate
20B acetate
21B acetate
22B acetate
20B methane
21B methane
22B methane

# Biodiesel


Biodiesel Treatment
1500
1000
500
0
Biodiesel (mg/L)
2.5 mg/L day
Biodiesel added
1B methane
2B methane
3B methane
Methane Expected
0
50
100
150
200
250
300
350
Days of Incubation


Biodiesel Treatment
1470
970
470
-30
Concentration (mg/L)
0
50
100
150
200
250
300
350
Days of Incubation
Biodiesel added
1B acetate
2B acetate
3B acetate
1B butyrate
2B butyrate
3B butyrate

At higher concentrations (near 1000 mg/L) ethanol rapidly fermented to methane at a rate near 20 to 60 mg/L per day.

The rate of fermentation of *n*-propanol, *n*-butanol and *iso*-butanol was slower, near 2 to 3 mg/L per day, and the fermentation stopped at the corresponding fatty acid. Little methane was produced in the first year of incubation.

At concentrations near 1000 mg/L, the rate of fermentation of biodiesel to methane was near 3 mg/L biodiesel per day.

## Why didn't the propionate, butyrate and iso-butyrate degrade?

Did the water go acidic, forming the free acid of propionate, butyrate and *iso*-butyrate? The free acids are toxic. Important at pH <5.

Were the concentrations of $H_2$ too high for anaerobic biodegradation to be thermodynamically feasible? In the microcosms, this is generally true for $H_2$ concentrations > 1 E-4 atmospheres.

| Biofuel | $H_2$ | pH |
|---|---|---|
| | Atmospheres | |
| Ethanol | 2.8 to 27 E-4<br>16 to 130 E-4 | 5.6 to 6.3 |
| *n*-Propanol | 0.7 to 4.1 E-4<br>1.3 to 25 E-4 | 6.2 |
| *n*-Butanol | 43 to 47 E-4<br>18 to 39 E-4 | 5.9 to 6.0 |
| *iso*-Butanol | 0.3 to 8.6 E-4<br>1.7 to 10.2 E-4 | 6.0 to 6.1 |

The fermentation of propanol, or butanol, or *iso*-butanol kept the concentration of $H_2$ too high for propionate, or butyrate or *iso*-butyrate to degrade.

After the alcohols are completely degraded, the fermentation of the organic acids should begin.

We will monitor the microcosms for another year to see if the organic acids start to degrade to form methane.

Why do we care if the rate of biofuel biodegradation is 2 to 3 mg per liter per day, or 20 to 60 mg per liter per day?

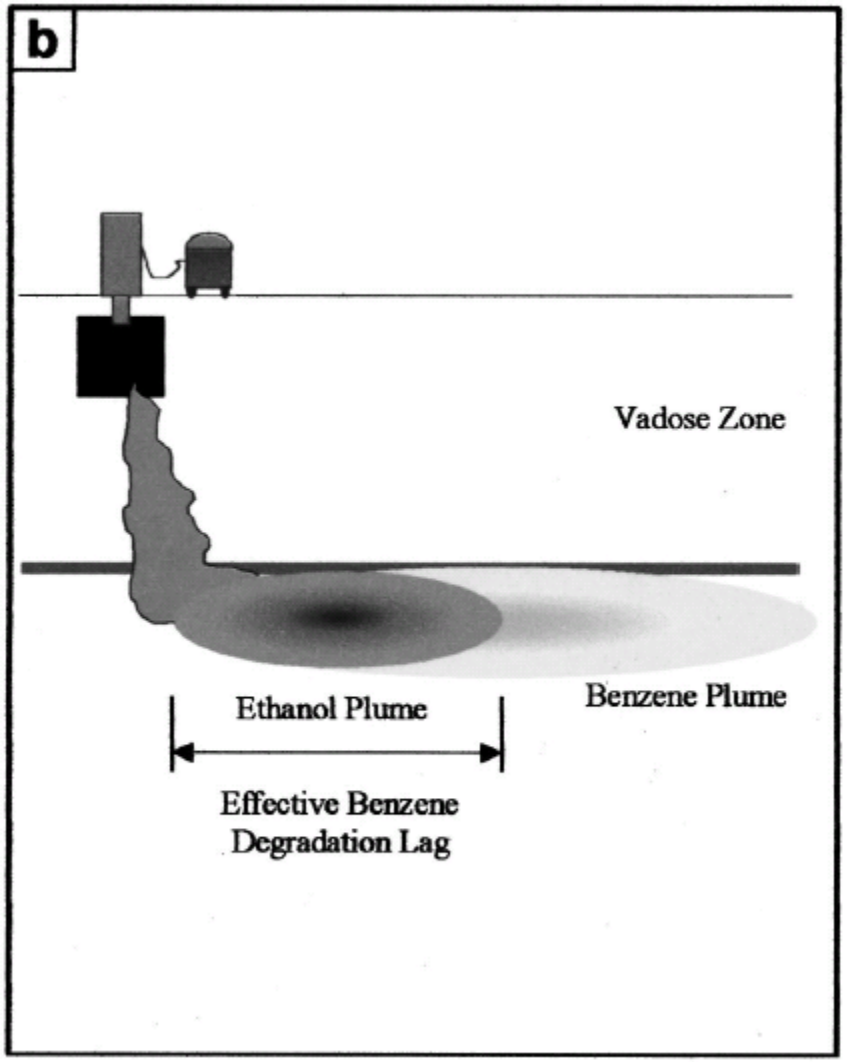
U.S. EPA has developed a simple screening model to describe the interactions of ethanol and benzene in a plume of contaminated ground water.

# *FOOTPRINT*

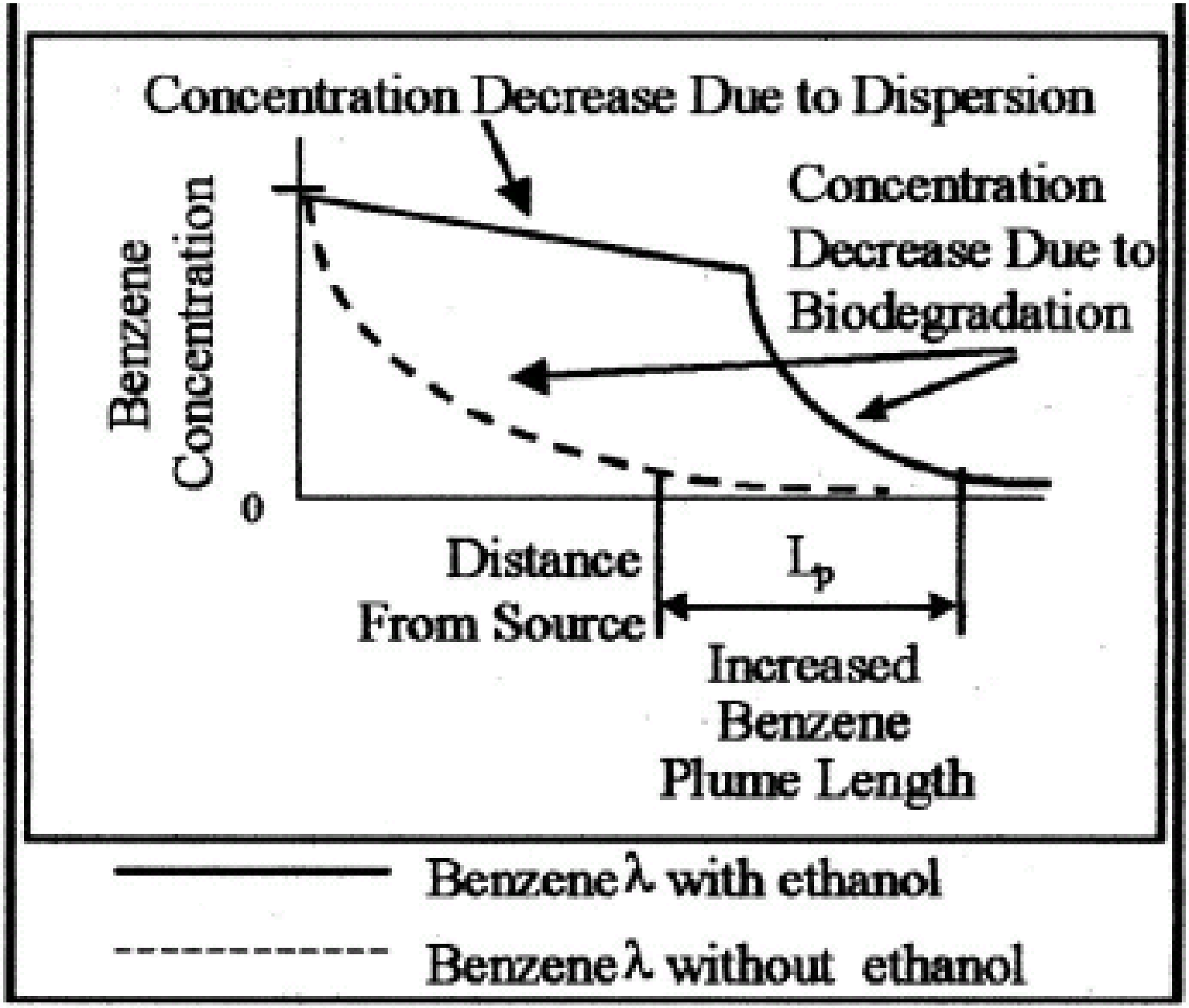
- A screening tool to predict the impact of ethanol on the size of the plume of BTEX compounds resulting from a spill of gasohol
- Based on a conceptual model to estimate the plume area for any BTEX compounds in the presence or absence of ethanol
- Currently, FOOTPRINT is distributed through the CSMoS web site:

  http://www.epa.gov/nrmrl/gwerd/csmos/index.html

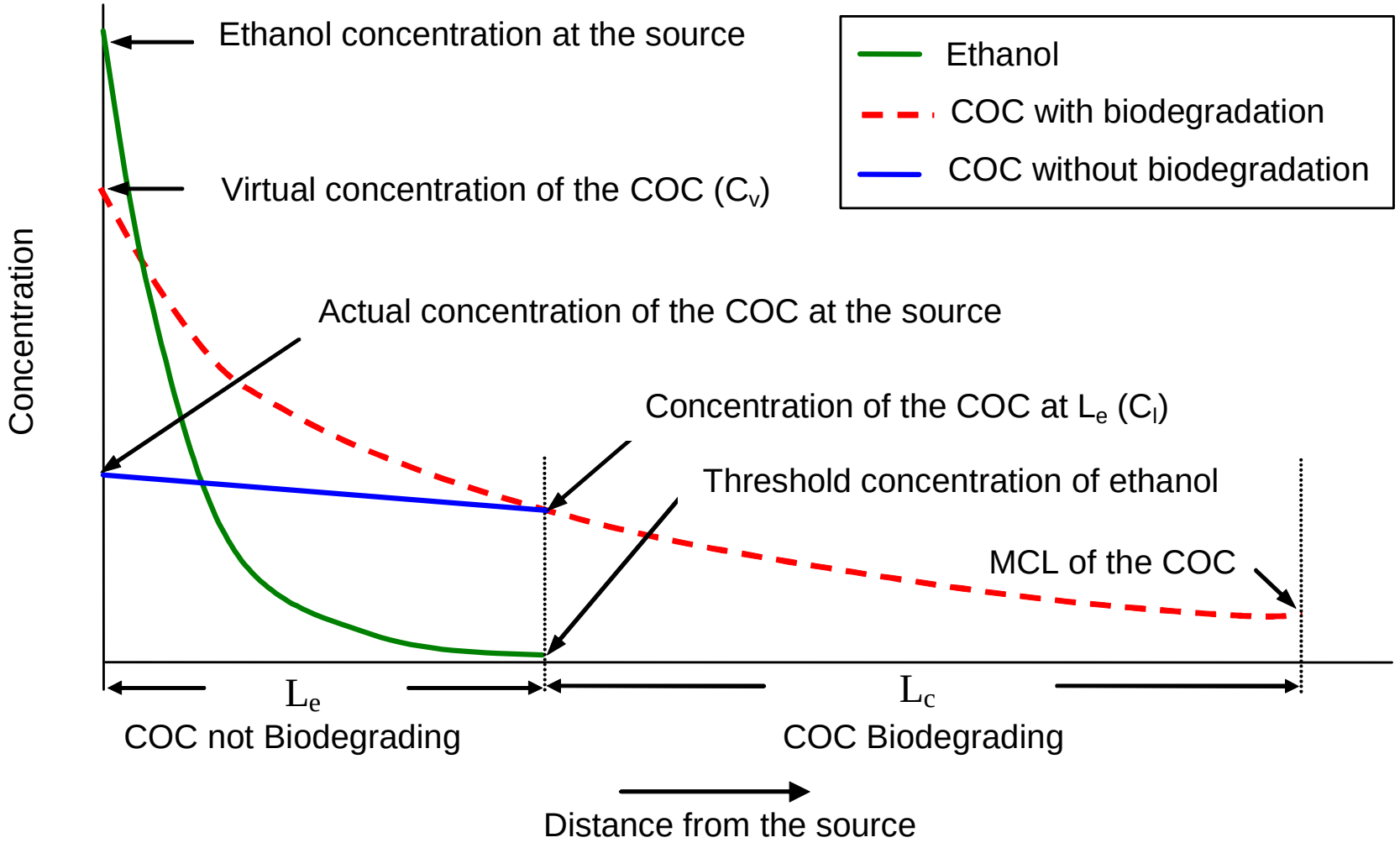
# Conceptual Model of a co-mingled ethanol/benzene plume.




Impact of Ethanol on Benzene Plume, Lengths: Microbial and Modeling Studies. Deeb, R. A., J. O. Sharp, A. Stocking, S. McDonald, K. A. West, M. Laugier, P. J. J. Alvarez, M. C. Kavanaugh, and L. Alvarez-Cohen, 2002, Journal of Environmental Engineering, ASCE, 128(9): 868-875.


Concentration Decrease Due to Dispersion
Concentration Decrease Due to Biodegradation
Benzene Concentration
0
Distance From Source
$L_p$
Increased Benzene Plume Length
Benzene λ with ethanol
Benzene λ without ethanol

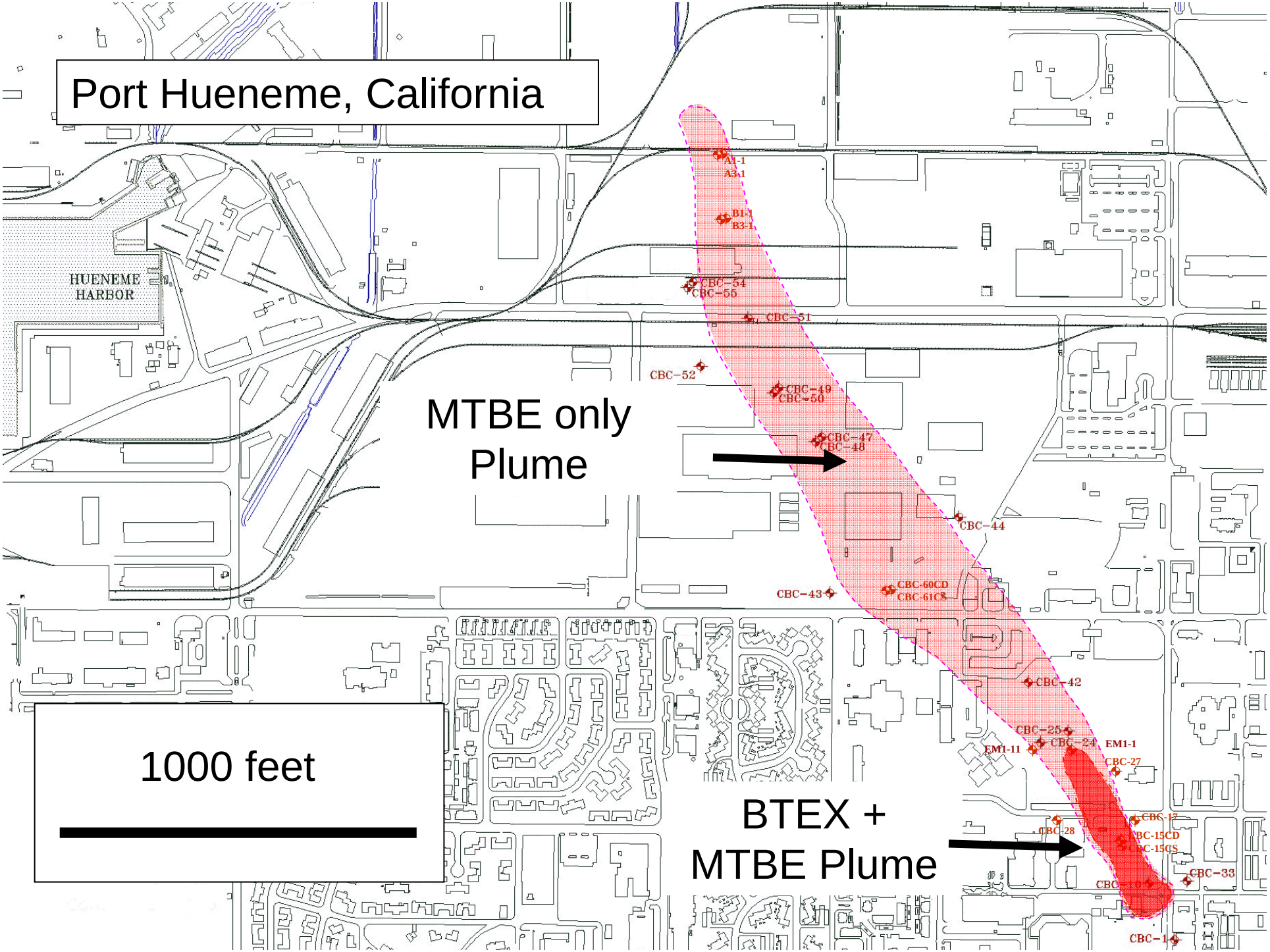
Footprint uses the concept of a virtual concentration to extract an analytical solution based on Domenico (1987).

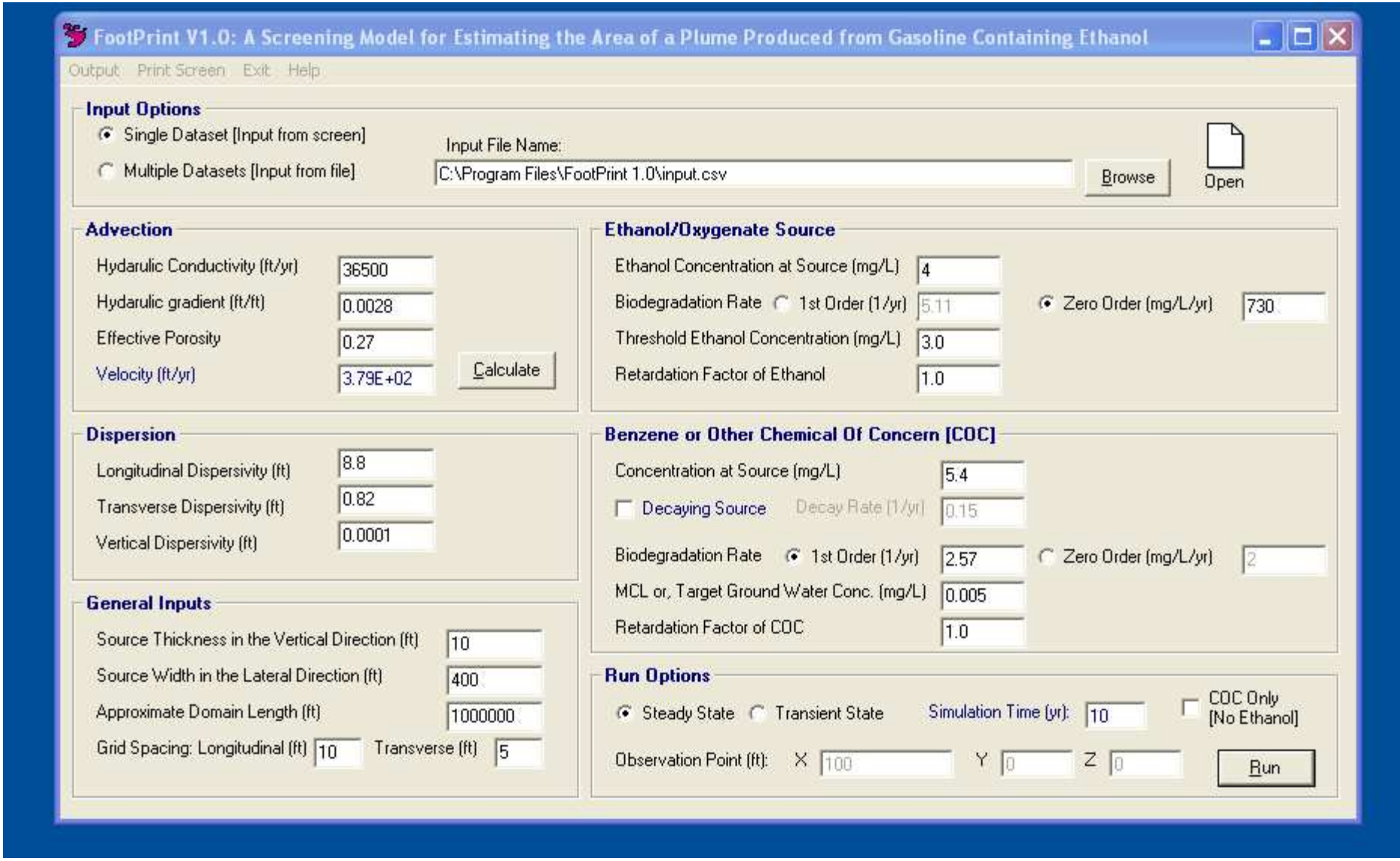

Ethanol concentration at the source
Virtual concentration of the COC ($C_v$)
Actual concentration of the COC at the source
Concentration of the COC at $L_e$ ($C_l$)
Threshold concentration of ethanol
MCL of the COC
Ethanol
COC with biodegradation
COC without biodegradation
Concentration
$L_e$
$L_c$
COC not Biodegrading
COC Biodegrading
Distance from the source

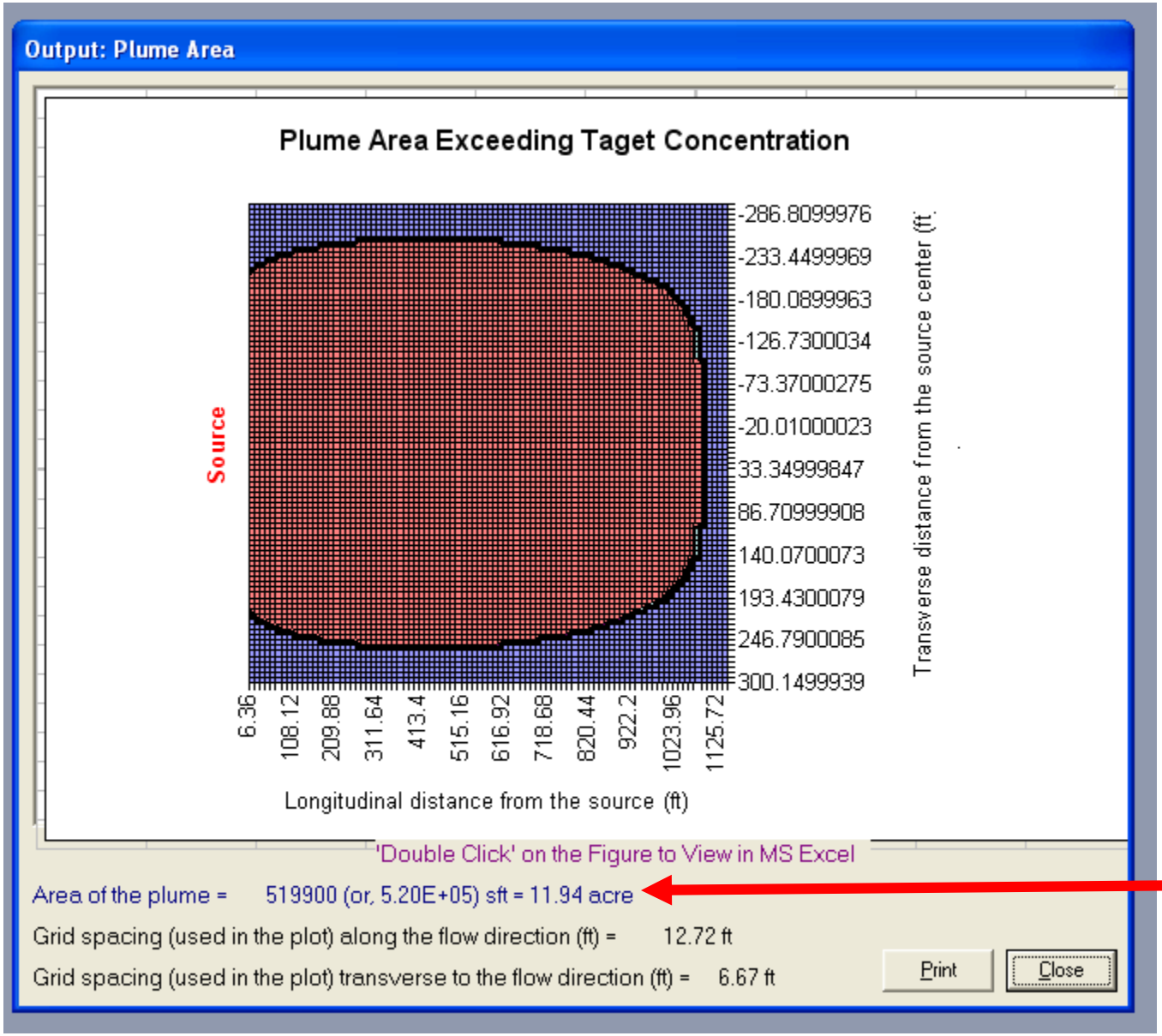
# Field Application

The motor fuel spill at the BX at Naval Base Port Hueneme is an example of a “high risk” site for a biofuel spill.

- Large LNAPL source area (400 feet wide)
- High ground water velocity (300 to 400 feet per year)
- High concentration of Benzene (>5 mg/L)


Port Hueneme, California
HUENEME HARBOR
A1-1
A3-1
B1-1
B3-1
CBC-54
CBC-55
CBC-51
CBC-52
CBC-49
CBC-50
CBC-47
CBC-48
CBC-44
CBC-43
CBC-60CD
CBC-61CS
CBC-42
CBC-25
CBC-24
EM1-11
EM1-1
CBC-27
CBC-28
CBC-17
CBC-15CD
CBC-15CS
CBC-33
CBC-10
CBC-14
MTBE only Plume
BTEX + MTBE Plume
1000 feet


FootPrint V1.0: A Screening Model for Estimating the Area of a Plume Produced from Gasoline Containing Ethanol
Output
Print Screen
Exit
Help
Input Options
Single Dataset [Input from screen]
Multiple Datasets [Input from file]
Input File Name:
C:\Program Files\FootPrint 1.0\input.csv
Browse
Open
Advection
Hydarulic Conductivity (ft/yr) 36500
Hydarulic gradient (ft/ft) 0.0028
Effective Porosity 0.27
Velocity (ft/yr) 3.79E+02
Calculate
Ethanol/Oxygenate Source
Ethanol Concentration at Source (mg/L) 4
Biodegradation Rate 1st Order (1/yr) 5.11
Zero Order (mg/L/yr) 730
Threshold Ethanol Concentration (mg/L) 3.0
Retardation Factor of Ethanol 1.0
Dispersion
Longitudinal Dispersivity (ft) 8.8
Transverse Dispersivity (ft) 0.82
Vertical Dispersivity (ft) 0.0001
Benzene or Other Chemical Of Concern [COC]
Concentration at Source (mg/L) 5.4
Decaying Source
Decay Rate (1/yr) 0.15
Biodegradation Rate 1st Order (1/yr) 2.57
Zero Order (mg/L/yr) 2
MCL or, Target Ground Water Conc. (mg/L) 0.005
Retardation Factor of COC 1.0
General Inputs
Source Thickness in the Vertical Direction (ft) 10
Source Width in the Lateral Direction (ft) 400
Approximate Domain Length (ft) 1000000
Grid Spacing: Longitudinal (ft) 10
Transverse (ft) 5
Run Options
Steady State
Transient State
Simulation Time (yr): 10
COC Only [No Ethanol]
Observation Point (ft): X 100 Y 0 Z 0
Run


Output: Plume Area
Plume Area Exceeding Taget Concentration
Source
-286.8099976
-233.4499969
-180.0899963
-126.7300034
-73.37000275
-20.01000023
33.34999847
86.70999908
140.0700073
193.4300079
246.7900085
300.1499939
Transverse distance from the source center (ft)
6.36
108.12
209.88
311.64
413.4
515.16
616.92
718.68
820.44
922.2
1023.96
1125.72
Longitudinal distance from the source (ft)
'Double Click' on the Figure to View in MS Excel
Area of the plume = 519900 (or, 5.20E+05) sft = 11.94 acre
Grid spacing (used in the plot) along the flow direction (ft) = 12.72 ft
Grid spacing (used in the plot) transverse to the flow direction (ft) = 6.67 ft
Print
Close

| Condition | Plume Footprint (acres) |
|---|---|
| Actual BTEX Plume Area | 10.1 |
| FOOTPRINT Calibration Existing Plume, with no Ethanol | 11.9 |
| FOOTPRINT Prediction E10 spill, Biofuel biodegradation 20 mg/L per day | 14.8 |
| FOOTPRINT Prediction E10 spill, Biofuel biodegradation 2 mg/L per day | 41.9 |