MtBE Biodegradation in High Biomass Reactors

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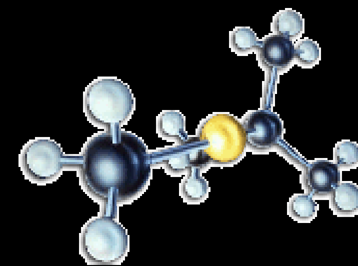
Topics To Be Discussed

- Brief background
- Initial bench top studies with continuous flow porous pot reactors
  - MtBE as sole carbon source
  - MtBE in combination with other carbon sources
  - Kinetics of MtBE biodegradation
- Bench top MBR performance
- Microbiology (culture ID)
- Pilot-scale gravity-flow reactor
- Porous pot study of Millville, NJ Superfund Site
- Economic Analysis

Background: MtBE Properties

- **Low odor and taste threshold**
  - **53 μg/L odor threshold**
  - **20-40 μg/L taste threshold**



- **Highly soluble in water (55 g/L) with limited sorptivity to aquifer solids**

- **Low yields of MtBE-degrading cultures**
  - **0.20-0.28 g cells/g MTBE (Salanitro *et al.*, 1994)**
  - **0.09-0.12 g cells/g MTBE (Fortin & Deshusses, 1999)**
  - **0.083 g VSS/g MTBE (G. Wilson *et al.*, 2001)**

Consequences of Low Yield

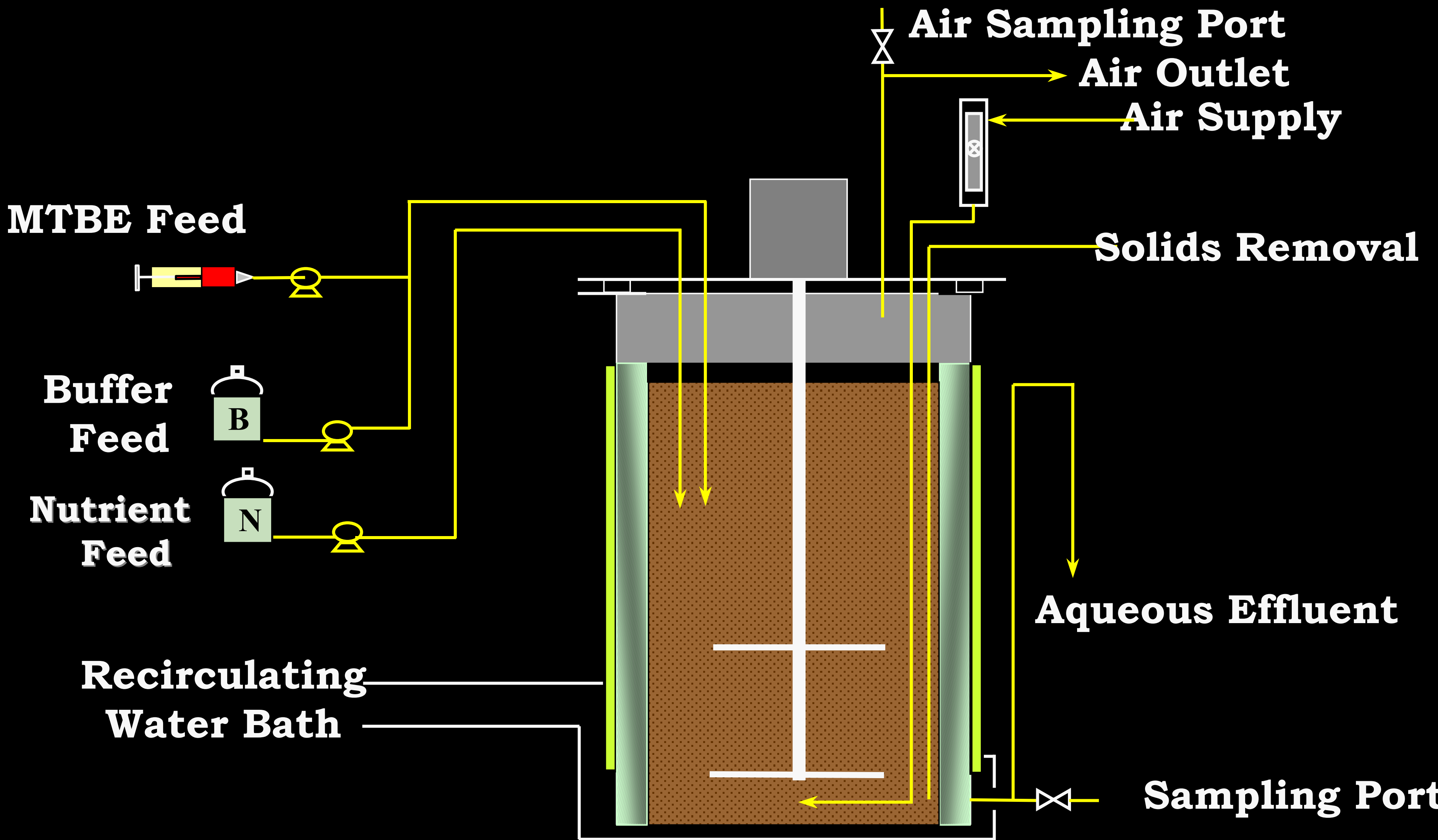
- Expected biomass levels in a typical, conventional reactor if influent MtBE is 5 mg/L:

  5 mg MtBE/L x 0.14 mg VSS/mg MtBE = 0.7 mg VSS/L

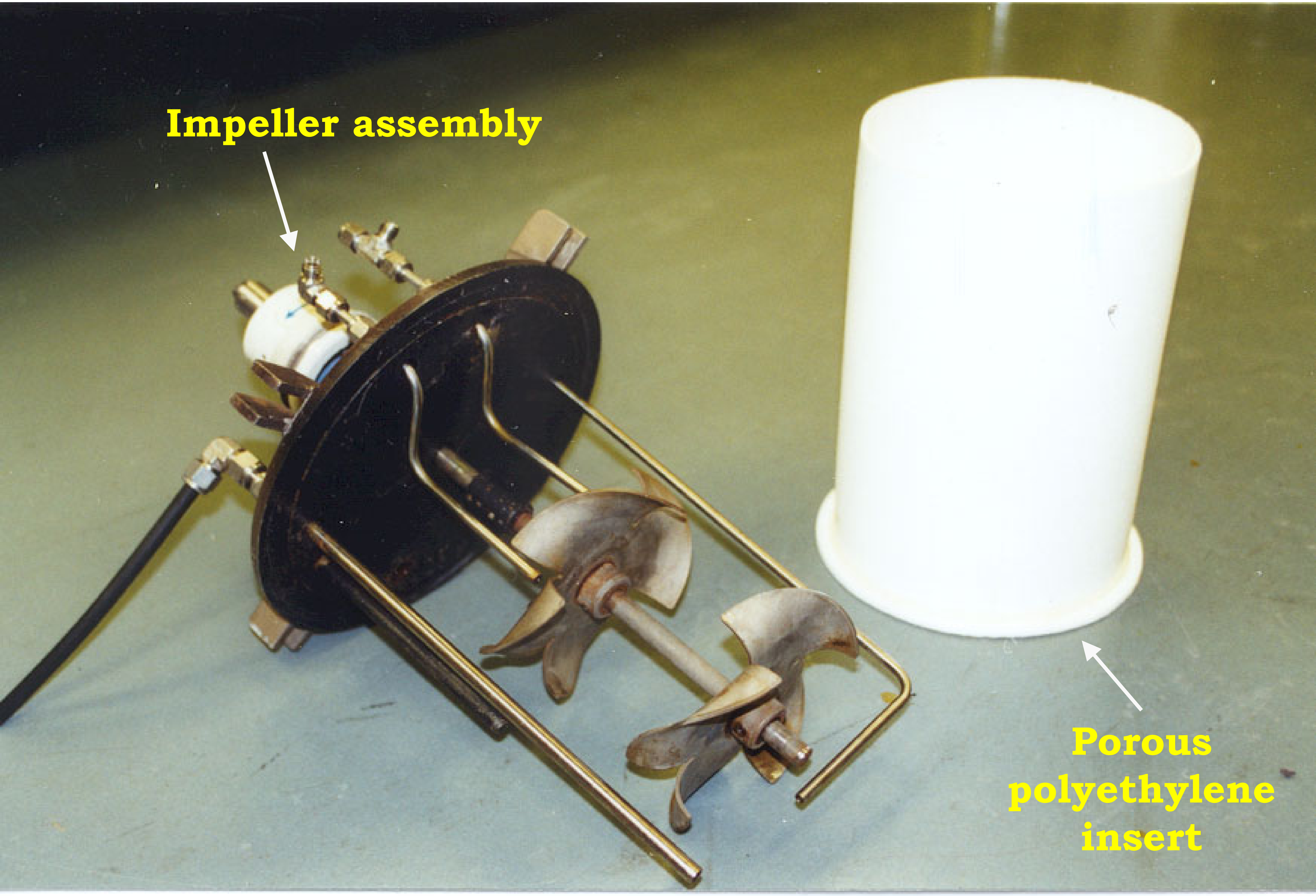
- Need to retain as much biomass as possible to achieve treatment goals

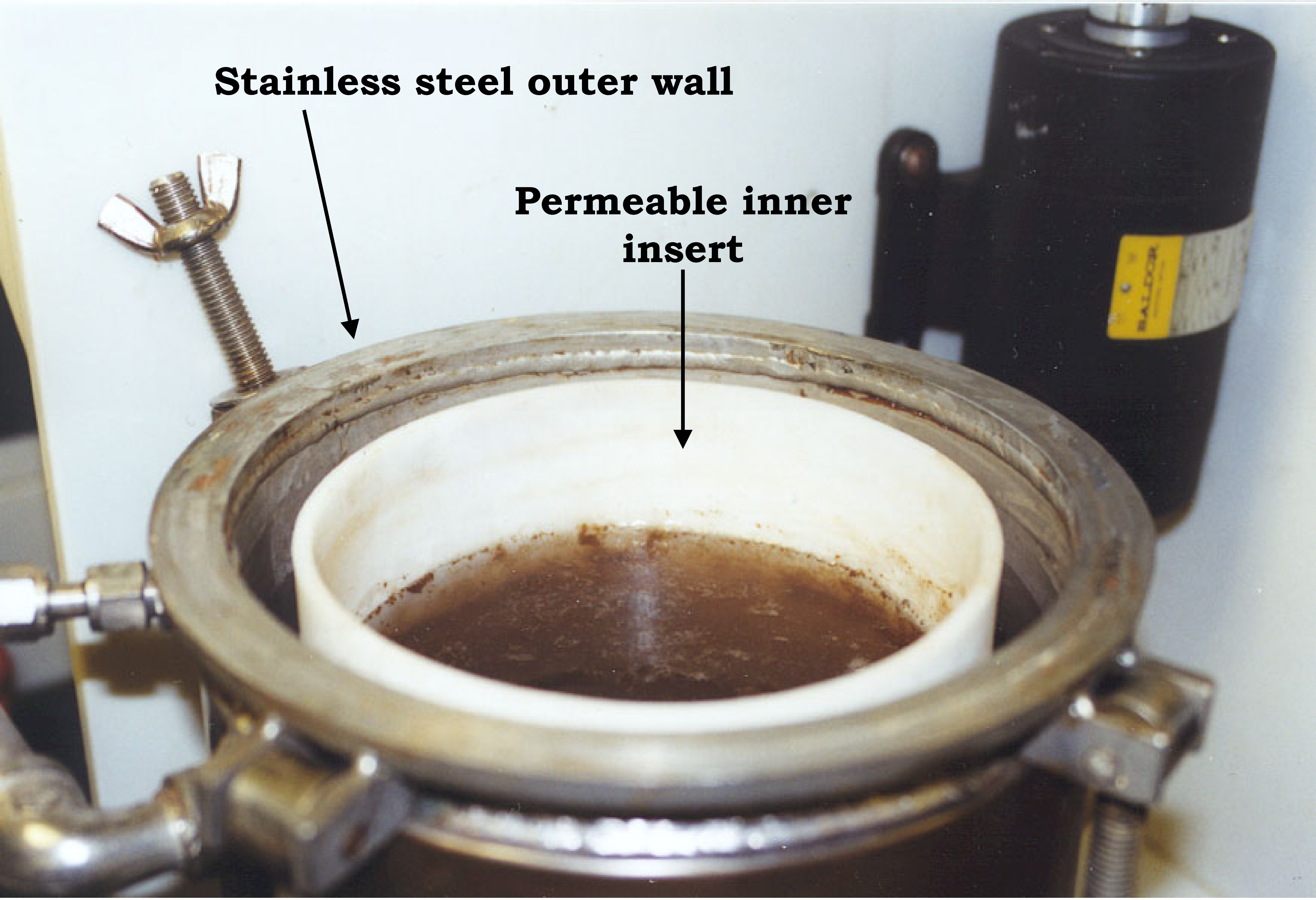
Initial Bench Top Porous Pot Reactor Studies

Schematic of Porous Pot Bioreactor




Porous Pot Chemostats


Impeller assembly
Porous polyethylene insert


Stainless steel outer wall
Permeable inner insert

Operating Conditions of Porous Pot

- 6-L aeration volume in 12-L capacity vessel
  - Porous pot insert made of polyethylene
- Feed flow rate: 2.4 L/day
  - HRT = 2.5 days
- Initial solids wastage rate: 5%/day
  - SRT = 20 days
- Wastage rate substantially reduced after ~115 days (solids wasted only due to sampling)

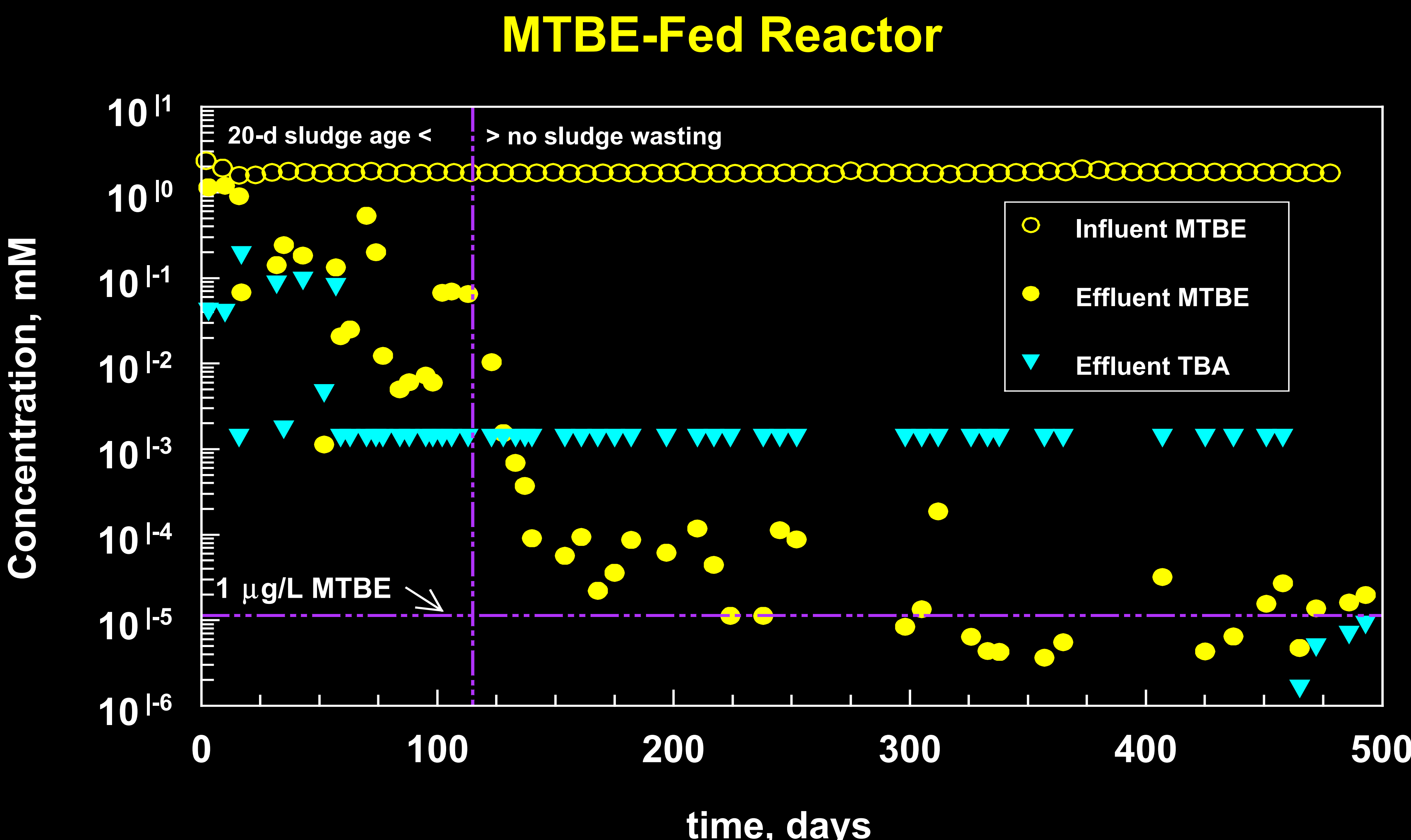
Operating Conditions

- Influent carbon source: MtBE alone or in combination with DEE, DIPE, EtOH, BTEX
- Substrate concentrations (COD = ~420 mg/L):
  - 150 mg/L when MtBE alone
  - 75 mg/L when MtBE combined with other carbon sources
- Temperature = 20°C
- HRT = 2.5 days
- pH = 7.5 to 8.1
- Dissolved Oxygen > 3 mg/L

System Startup: Seed Culture

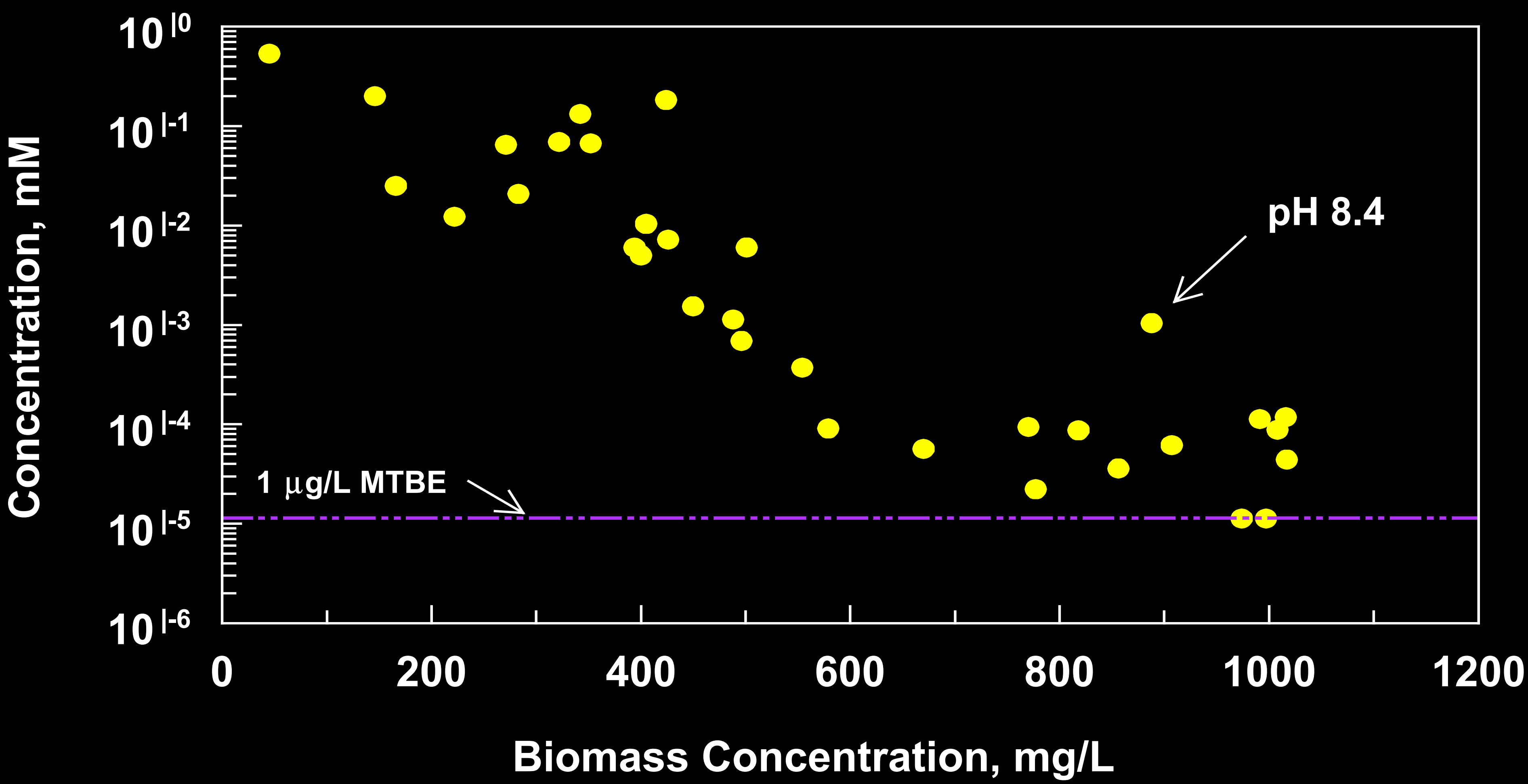
- 2 L mixed liquor from Mill Creek Sewage Treatment Plant, Cincinnati, OH
- 600 mL of mixed liquor from Shell Dev't. Corp. Refinery, Houston, TX
- 140 mL of aquifer material wash water, Port Hueneme, CA

Performance Results from Initial Porous Pot Studies

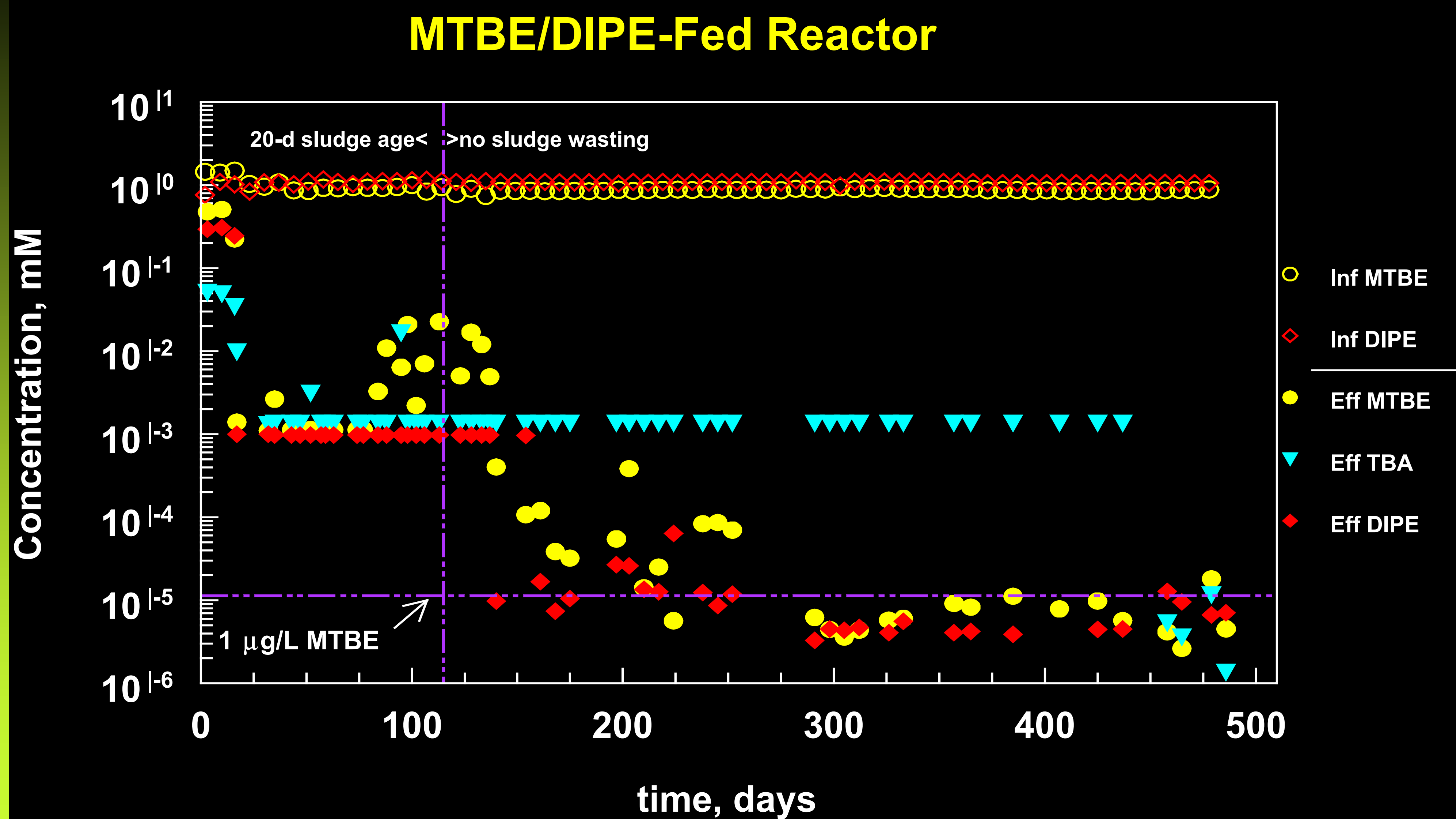


MTBE-Fed Reactor
Concentration, mM
time, days
20-d sludge age <
> no sludge wasting
1 μg/L MTBE
Influent MTBE
Effluent MTBE
Effluent TBA
10 l1
10 l0
10 l-1
10 l-2
10 l-3
10 l-4
10 l-5
10 l-6
0
100
200
300
400
500

MTBE Biodegradation as a Function of Biomass Concentration



MTBE/DIPE-Fed Reactor



MTBE/DIPE-Fed Reactor
20-d sludge age<
>no sludge wasting
Concentration, mM
10 |1
10 |0
10 |-1
10 |-2
10 |-3
10 |-4
10 |-5
10 |-6
1 µg/L MTBE
0
100
200
300
400
500
time, days
Inf MTBE
Inf DIPE
Eff MTBE
Eff TBA
Eff DIPE

Summary of System Performance: All Active Reactors

	Influent MTBE, mg/d	MTBE in Effluent, mg/d	MTBE in Exhaust Air, mg/d	Percent Removed
MTBE Alone	355.50	0.013	0.002	99.99
MTBE and DEE	177.75	0.033	0.003	99.98
MTBE and DIPE	177.75	0.018	0.002	99.99
MTBE and Ethanol	177.75	0.010	0.002	99.99
MTBE and BTEX	202.35	0.060	0.002	99.97

Summary of Continuous Flow Porous Pot Experiments

- At high biomass concentrations, MTBE biodegraded ~99.99% in *presence or absence* of other carbon sources

- COD and carbon analysis confirmed **mineralization** of MTBE and its intermediates, with the effluent carbon virtually all in inorganic form

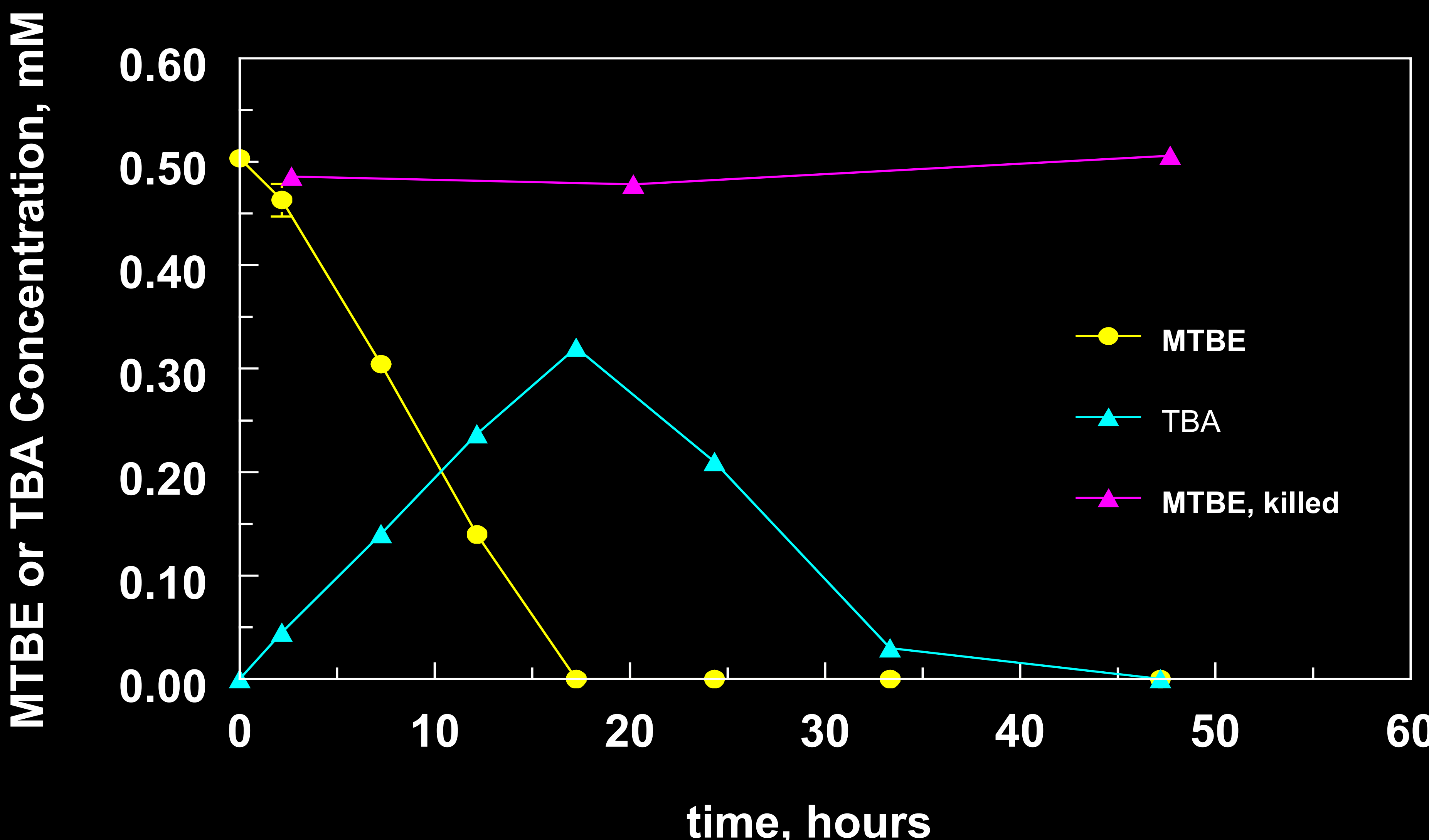
- No significant loss of MTBE from the control **abiotic** reactor, indicating good system integrity

Serum Bottle Batch Experiments

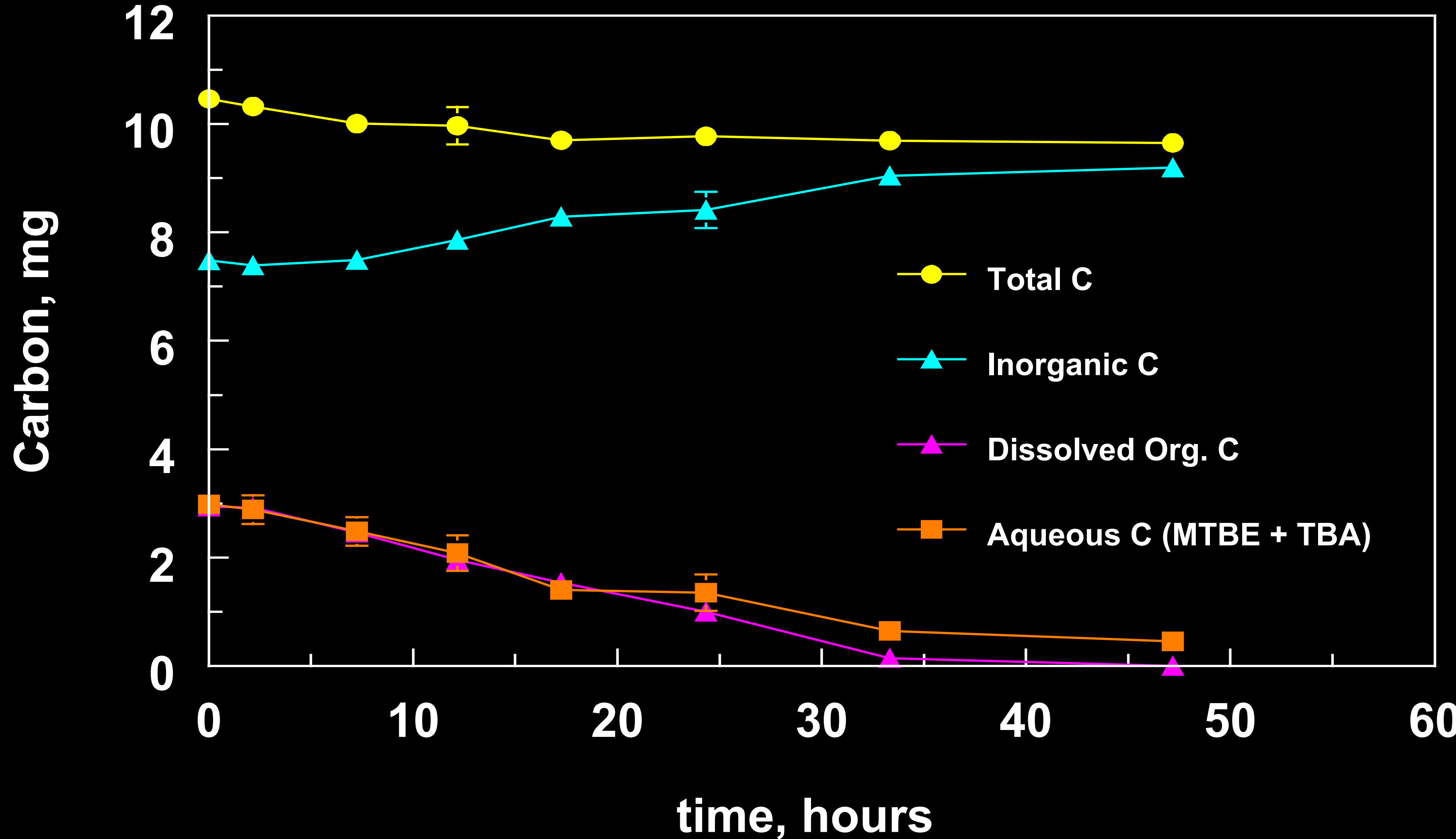
Experimental Conditions

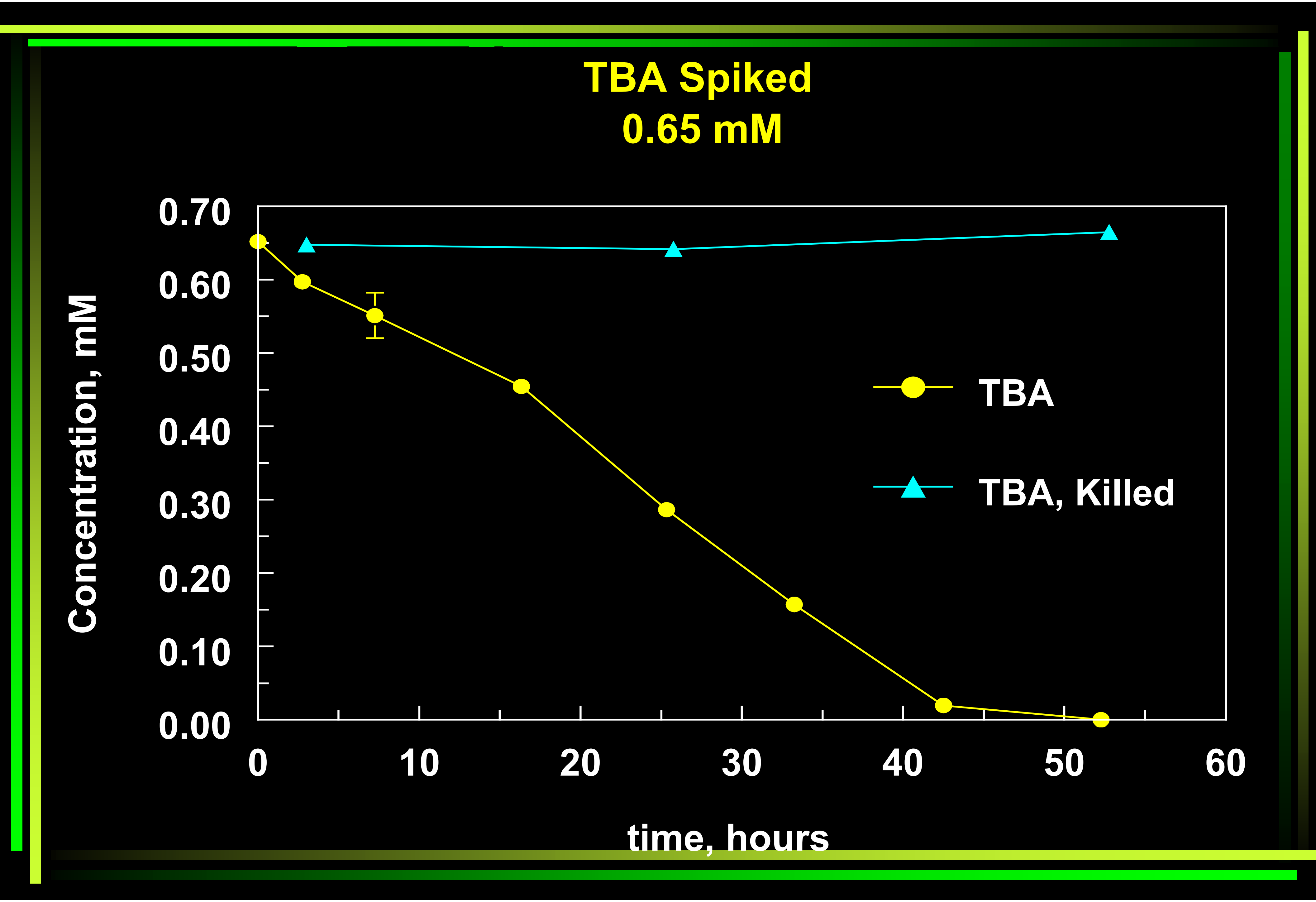
- 3 substrates fed to separate serum bottles in triplicate
  - MTBE alone
  - TBA alone
  - MTBE + TBA
- 3 Feed Concentrations
  - 5, 15, and 45 mg/L (0.057, 0.17, 0.51 mM)
- Analyze for substrates and intermediates during biodegradation

MTBE-Spiked Serum Bottle
0.5 mM

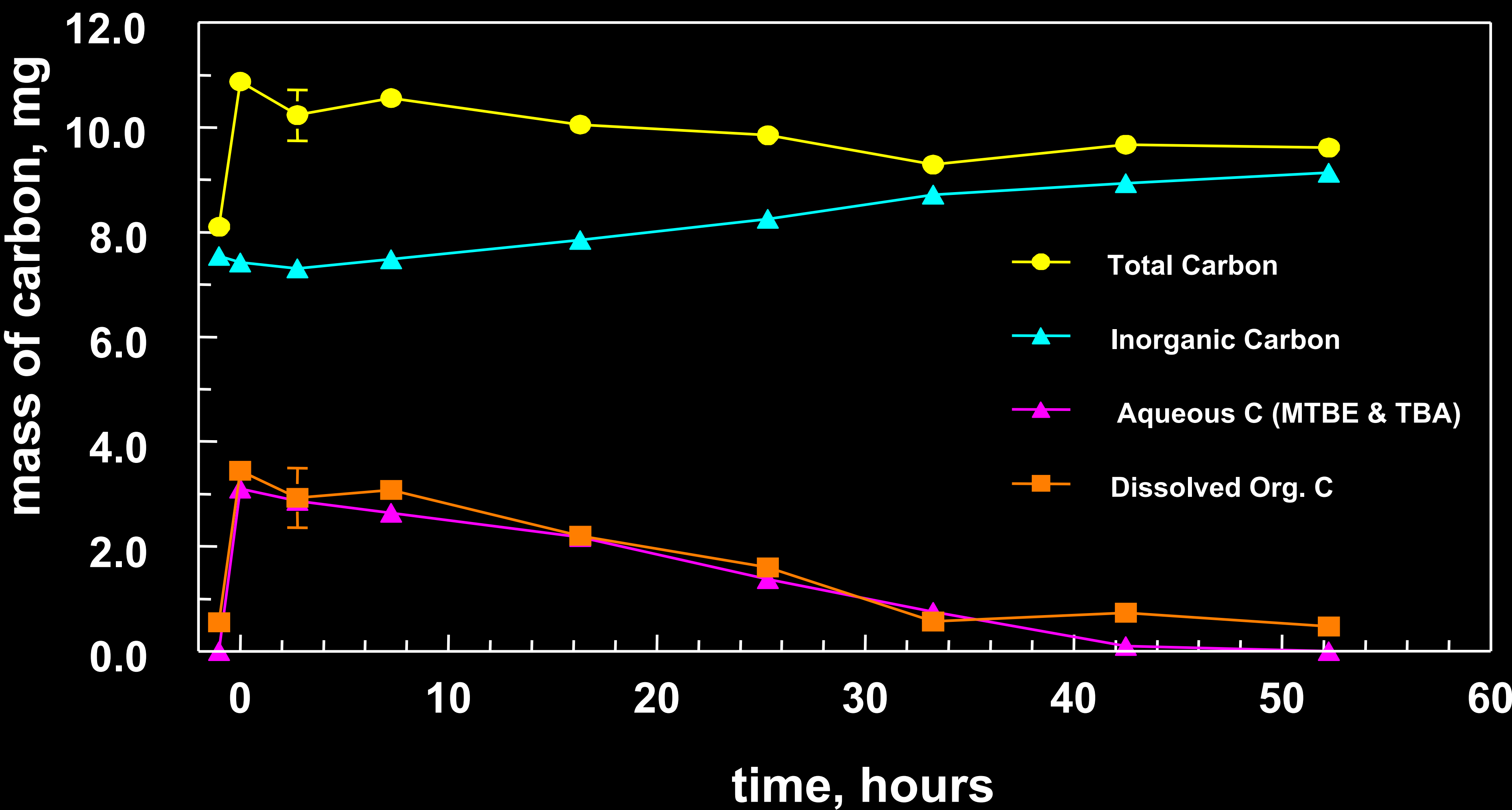


Carbon Balance
0.5 mM

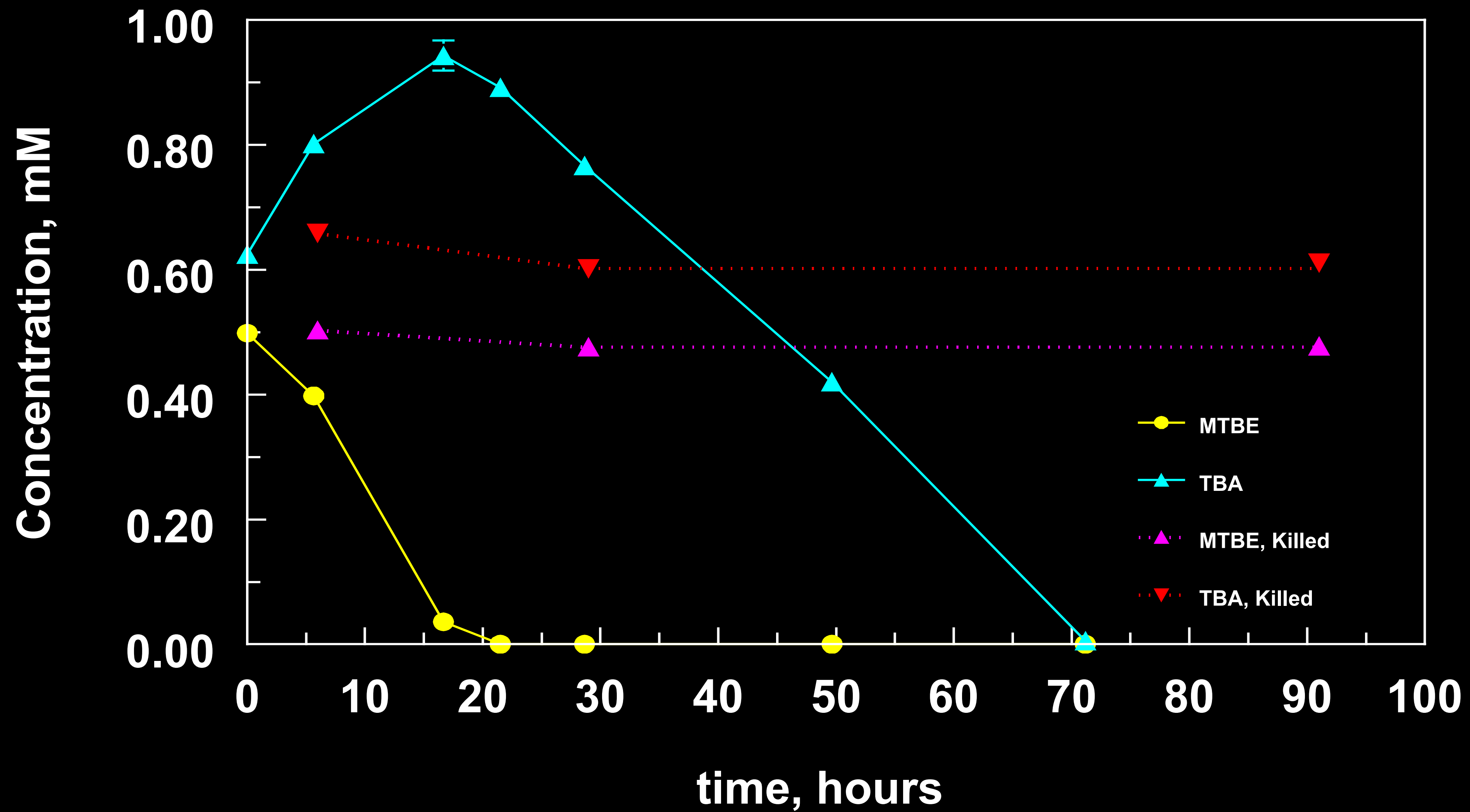



TBA Spiked
0.65 mM
Concentration, mM
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
10
20
30
40
50
60
time, hours
TBA
TBA, Killed

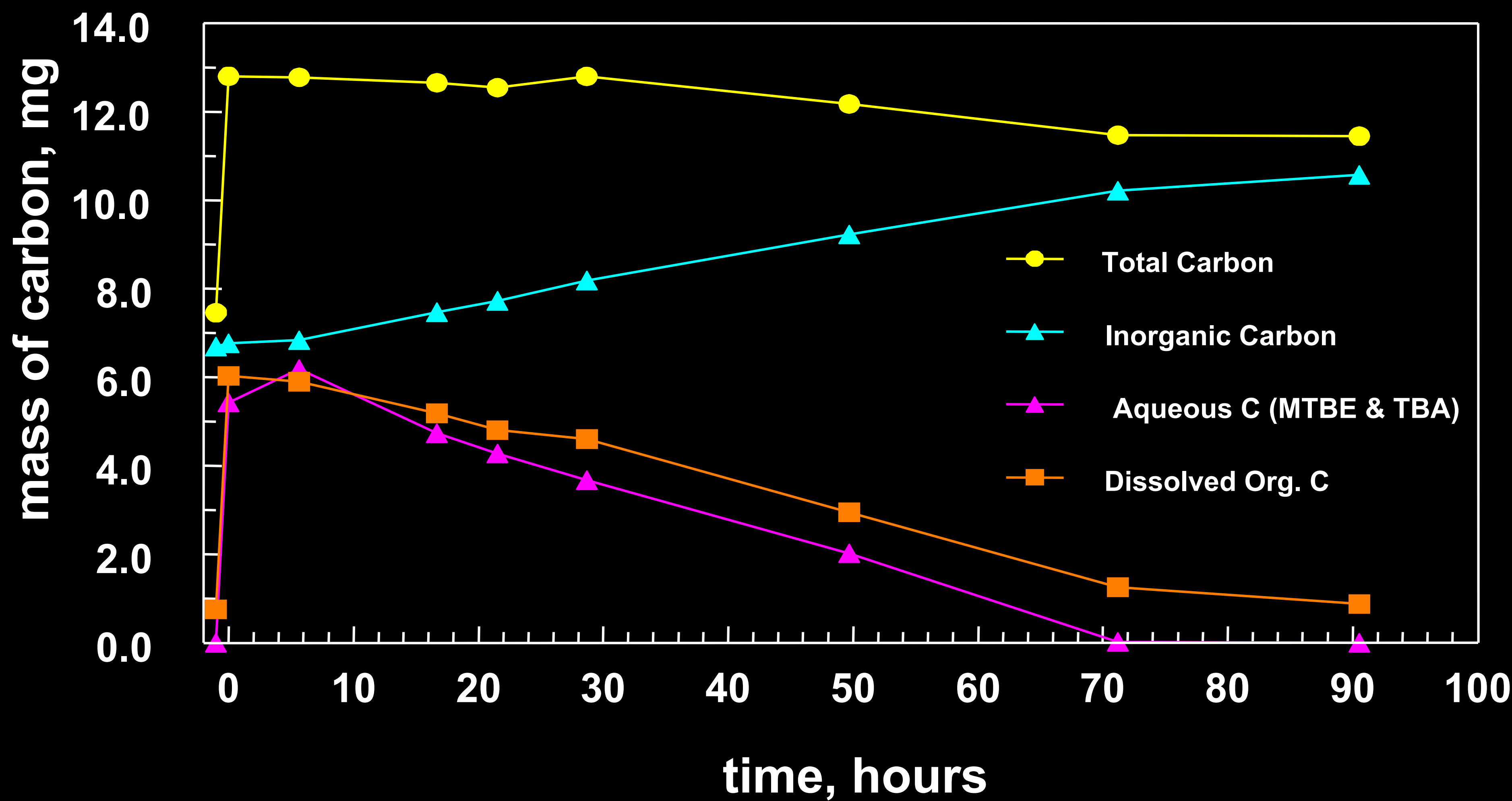
TBA-Spiked Serum Bottle: 0.65 mM



MTBE & TBA Spiked Serum Bottle
0.5 mM MTBE, 0.60 mM TBA



MTBE/TBA Spiked Serum Bottle

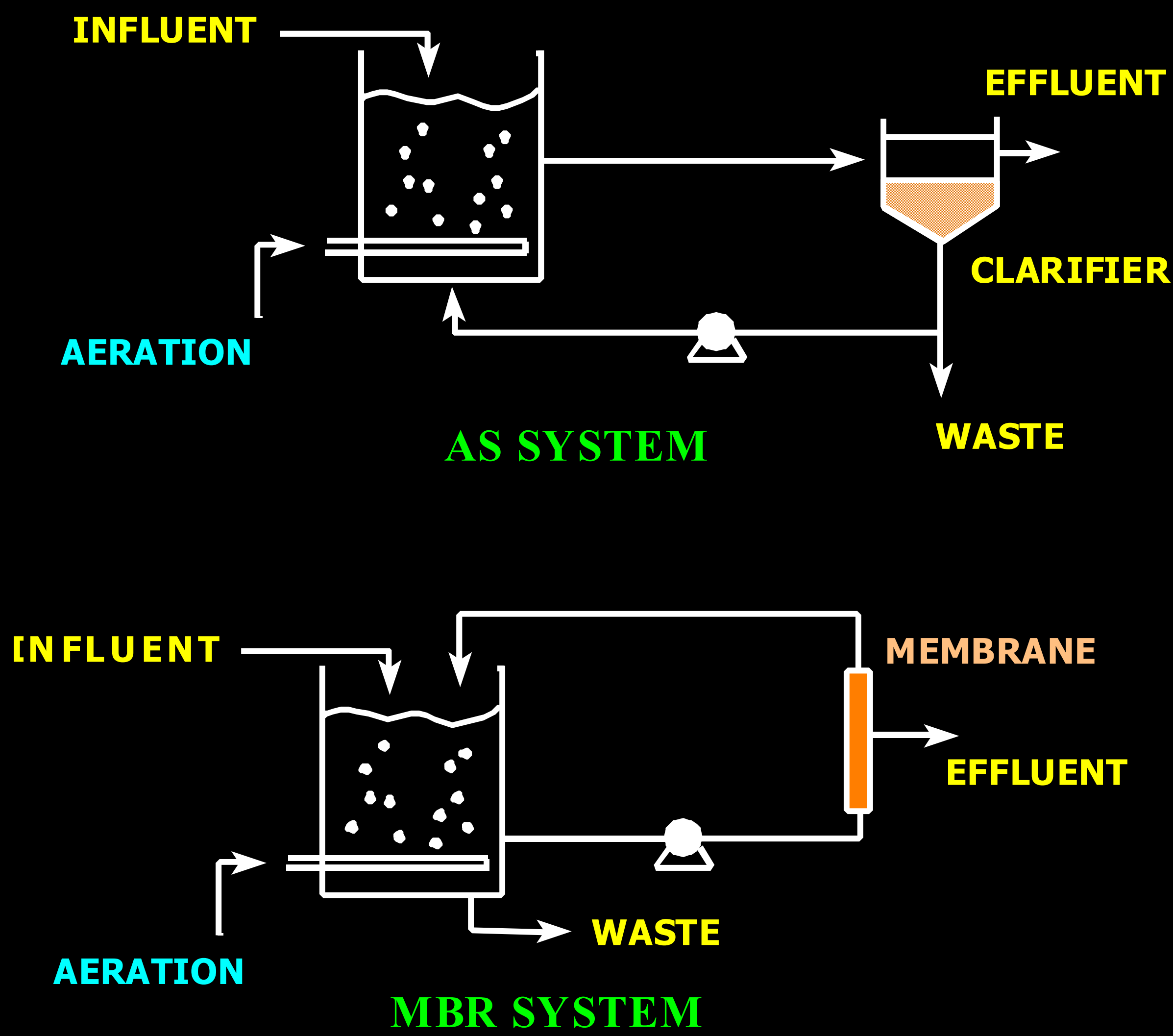


Summary of Serum Bottle Experiments

- Complete carbon balance obtained: all MTBE converted to CO_2 and H_2O with no buildup of intermediates
- As MTBE degrades, TBA first increases, then declines to undetectable levels
- Rate-limiting step is mineralization of TBA

Performance of Membrane Bioreactor

MBR vs. Activated Sludge



Characteristics of Membrane Bioreactors (MBR)

- **Advantages:**
  - Close control of solids
  - Extremely high effluent quality
  - Compact design
- **Disadvantages**
  - Fouling
  - Cost (both capital and operating)
  - High shear stress

Treatment Approach Used

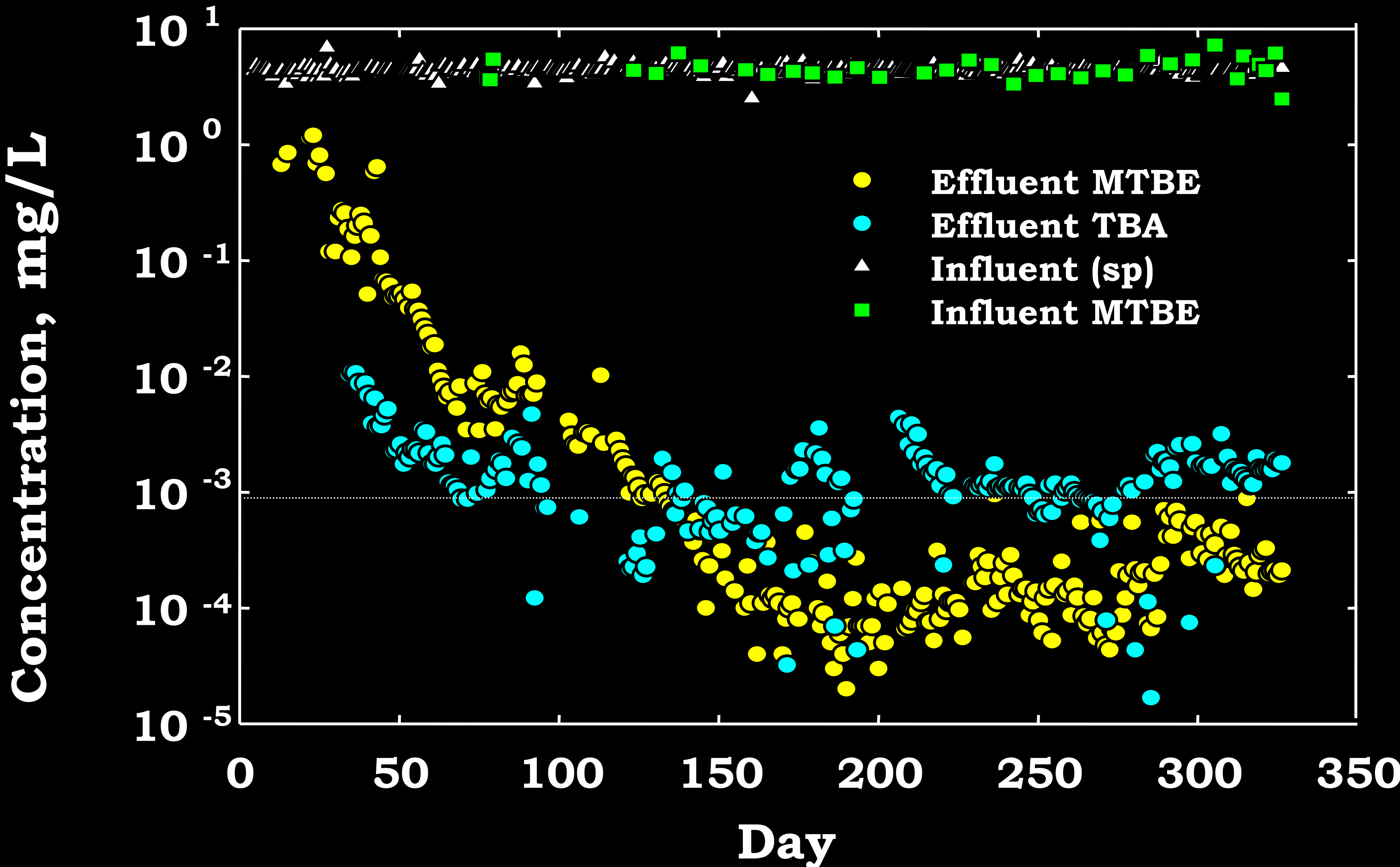
- Ultrafiltration membrane
  - Tech-Sep Kerasep™ (Rhone Poulenc, France)
  - External cross-flow, tubular ceramic membrane
  - Pore size = 0.02 μm (300 kDaltons)
  - Total surface area of 0.085 m^2
  - Volume of reactor = 6 L
  - Operated at 6 L/h or 1 h HRT
- Mixed culture from previous bench top porous pot chemostats fed MtBE
- Feed water = dechlorinated Cincinnati tap water

Summary of Operating Conditions

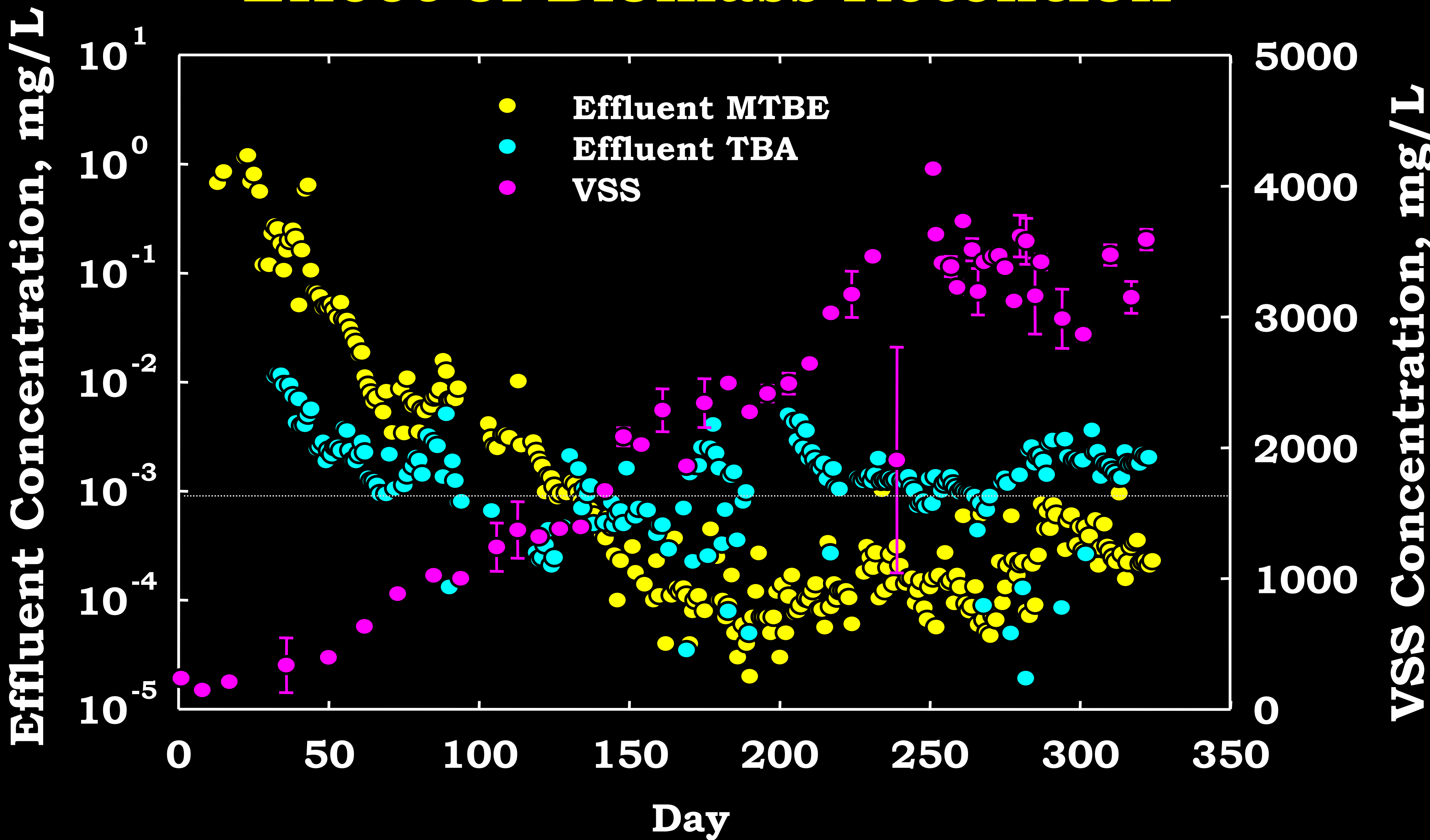
• Feed MtBE concentration	5 mg/L
• Hydraulic Retention Time	1 hour
• Temperature	18-20°C
• pH	7.2-7.8
• Dissolved oxygen	> 3 mg/L
• Solids retention time	>100 days

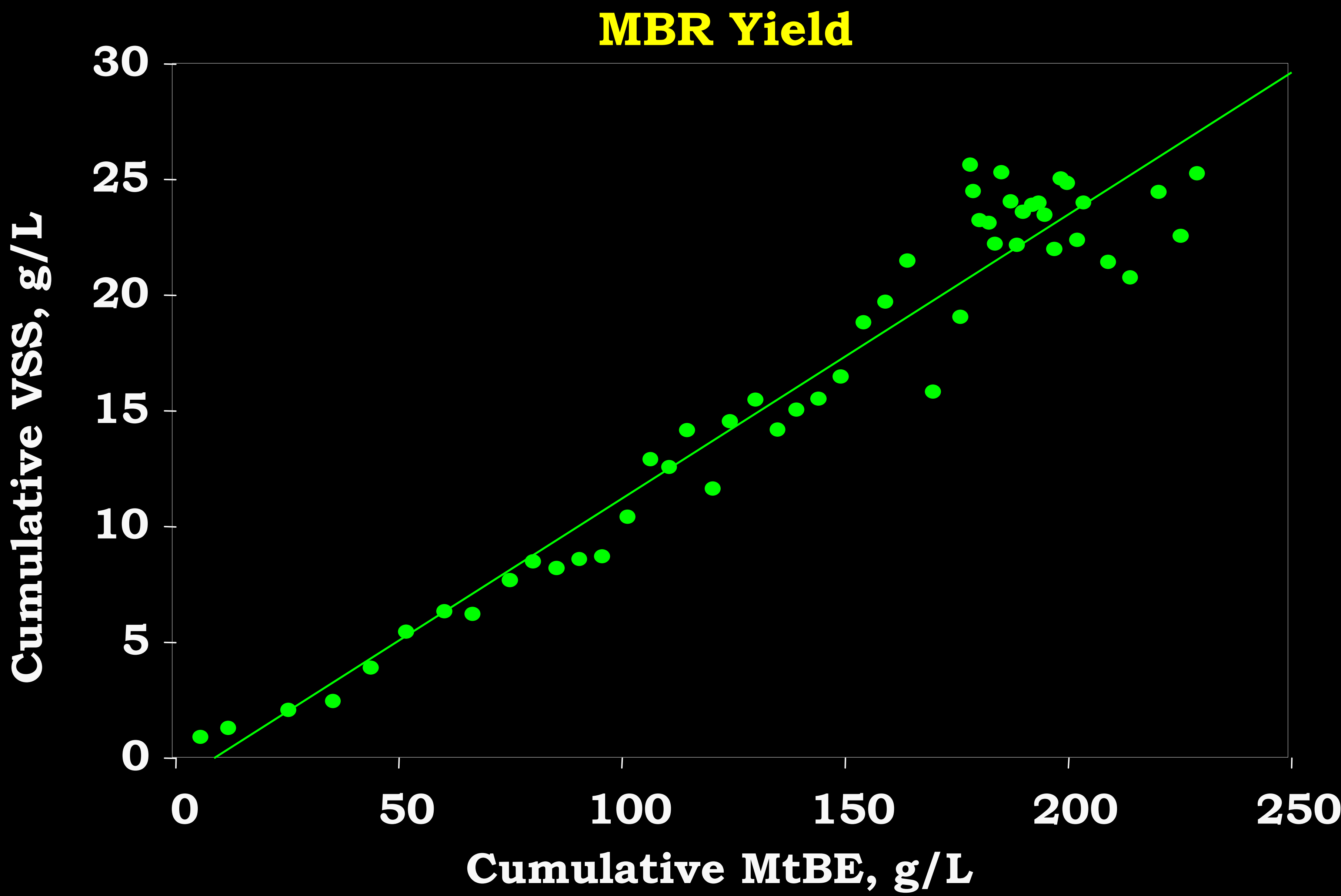
MBR Performance Results

MBR Performance, 5 mg/L MtBE in Feed



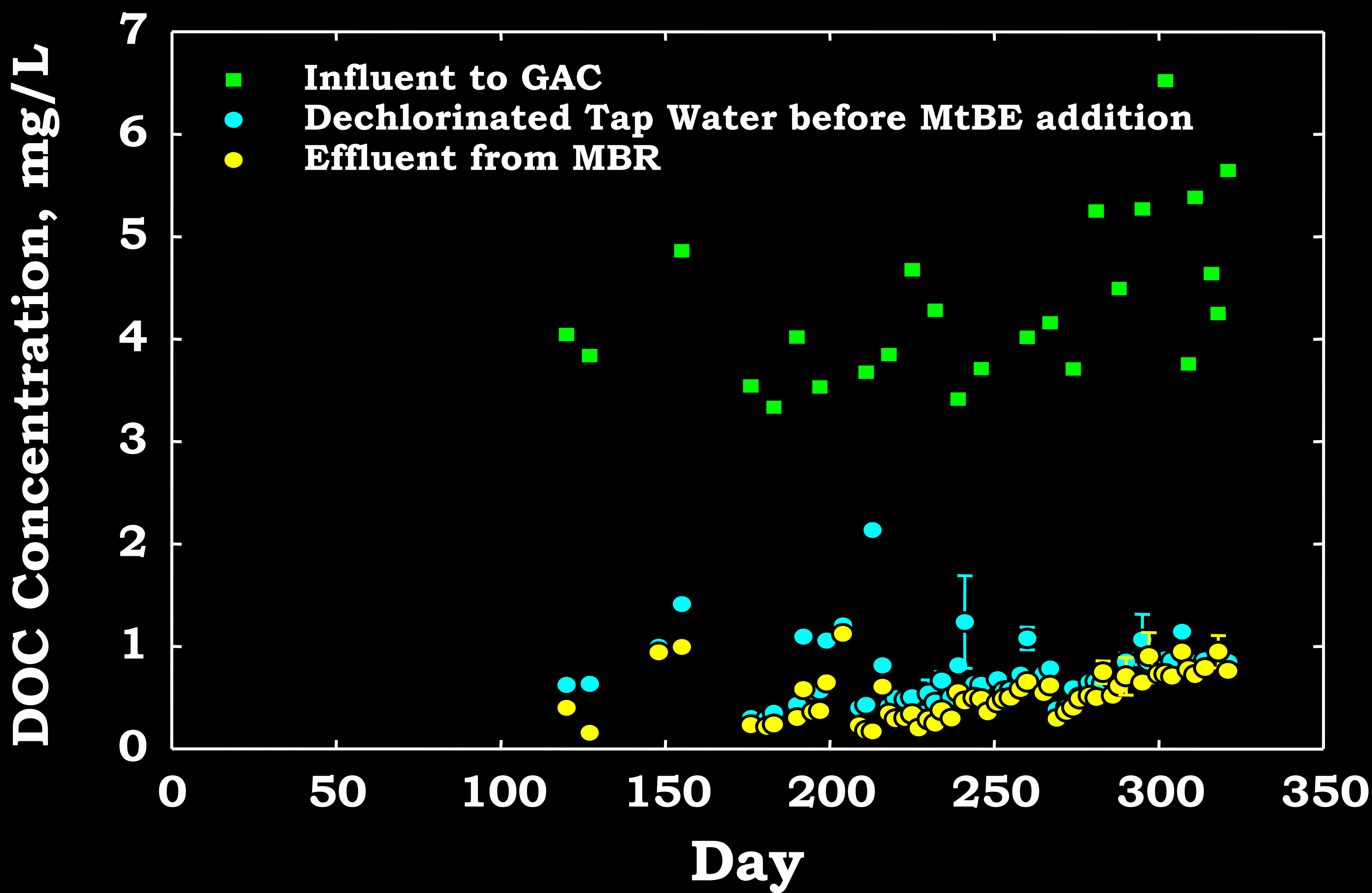
Effect of Biomass Retention





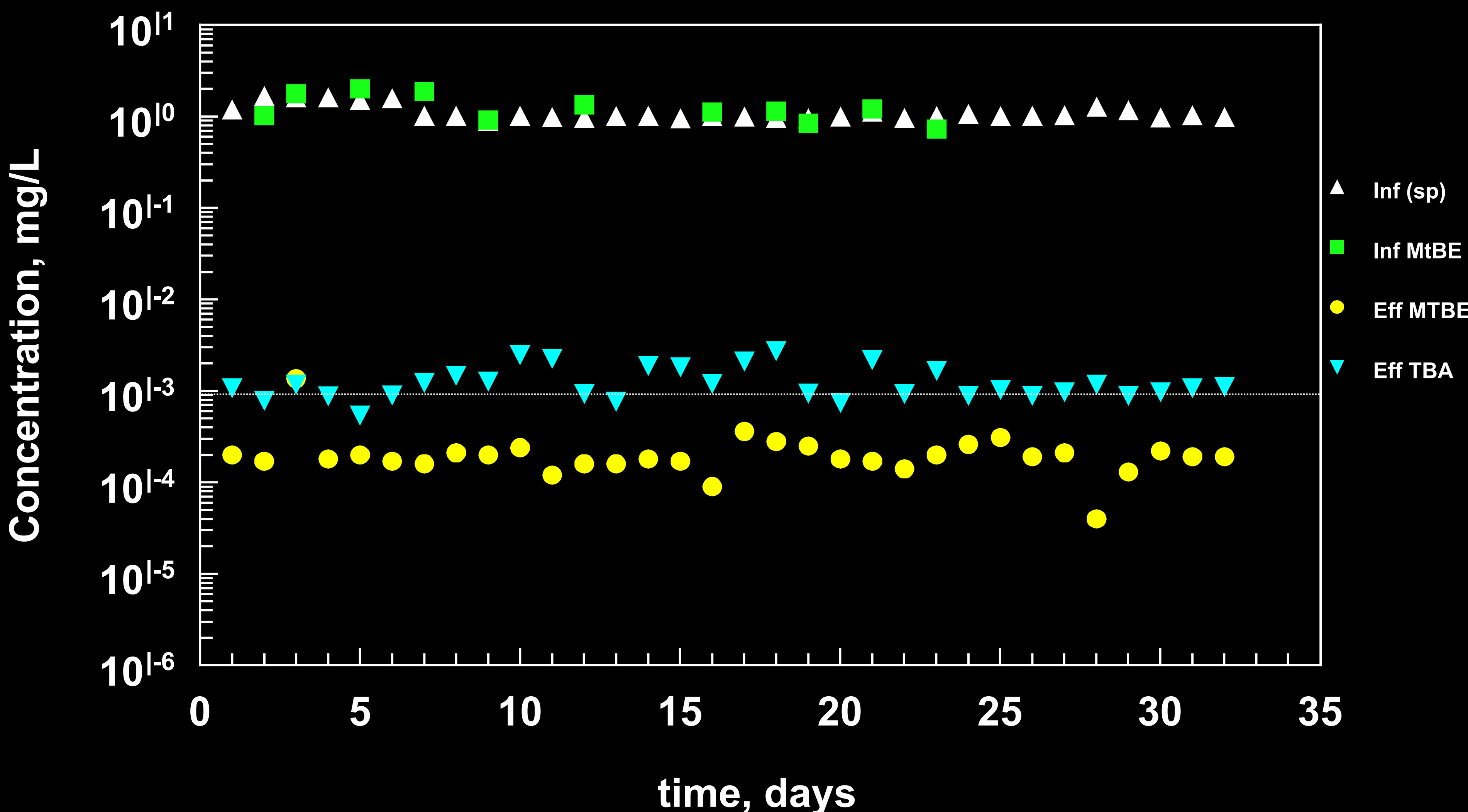
MBR Yield
30
25
20
15
10
5
0
0
50
100
150
200
250
Cumulative VSS, g/L
Cumulative MtBE, g/L

Dissolved Organic Carbon

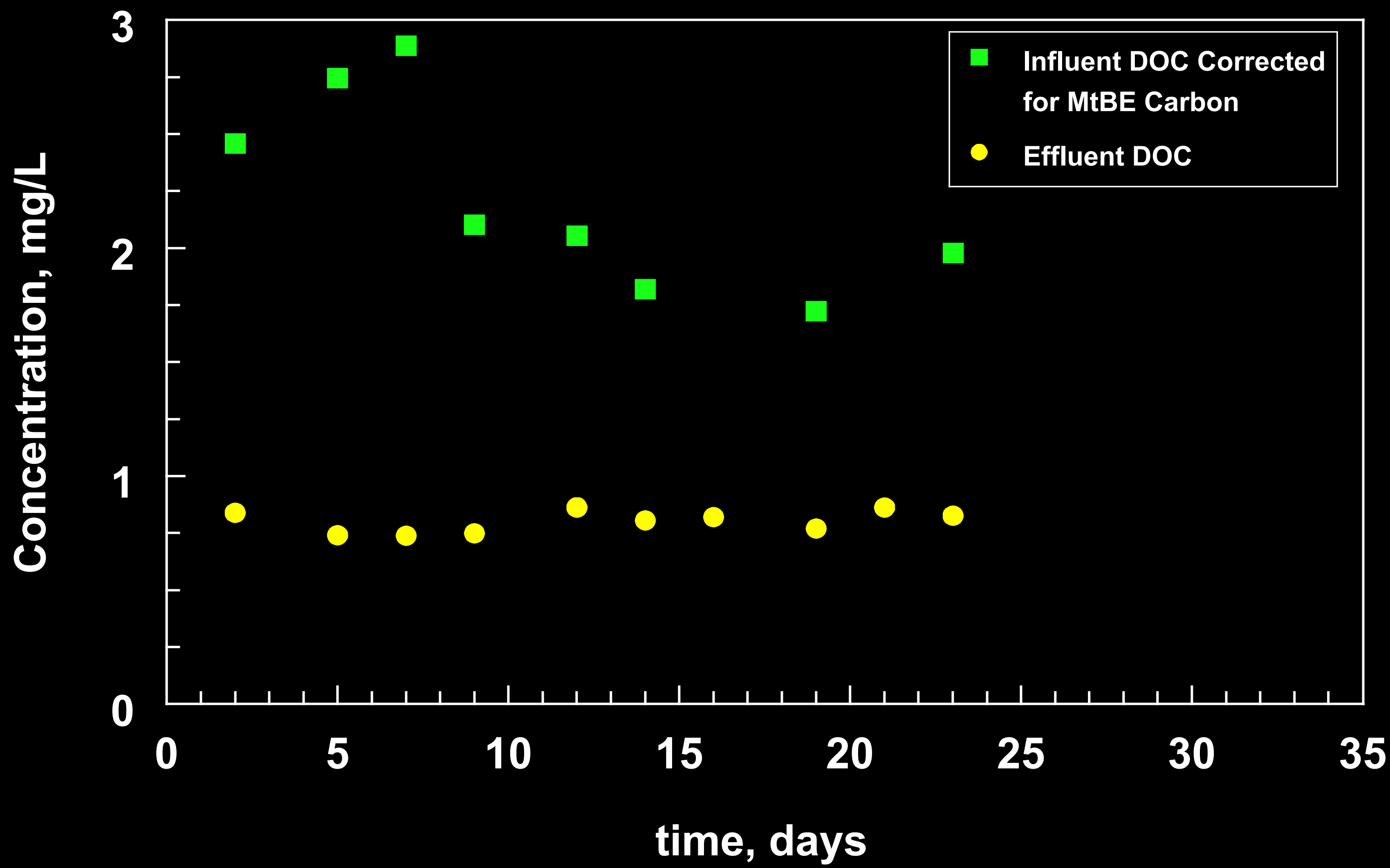


MBR Performance When Influent MtBE Reduced to 1 mg/L

MBR Performance, 1 mg/L MtBE in Feed



DOC in Effluent Fed 1 mg/L MtBE



Identification of Active Microbial Species in MBR

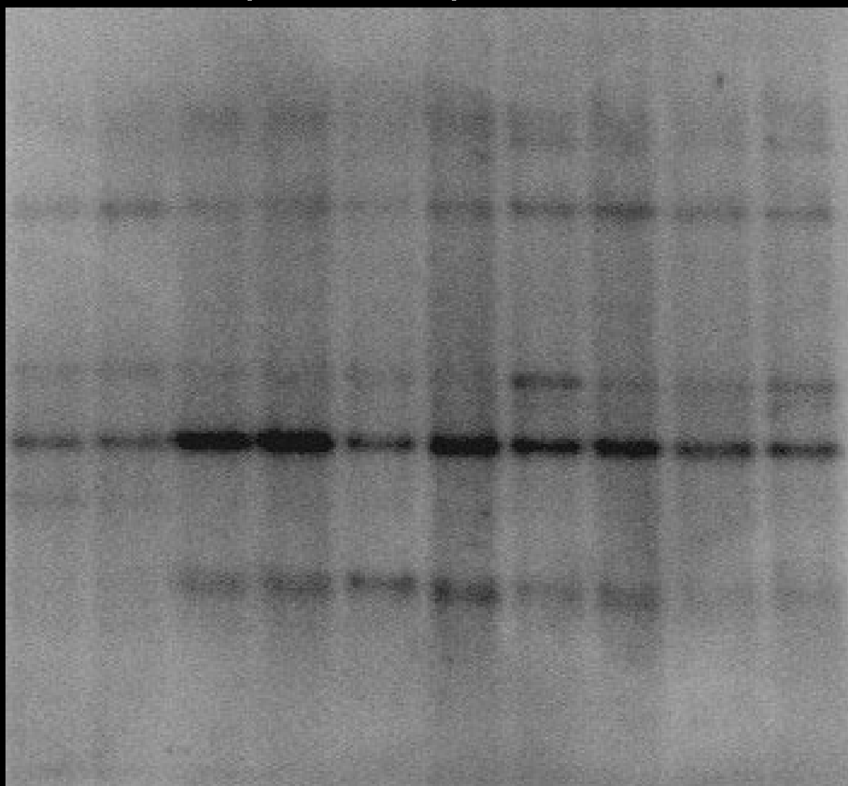
Denaturing Gradient Gel Electrophoresis (DGGE)

- Molecular tool targeting 16S rDNA
- Resolves DNA from mixed culture based on melting and electrophoretic mobility behavior
- Banding pattern gives a snapshot of community structure
- One band ~ one microorganism

Bacterial Identification by DGGE Pattern in MBR

Day

16 29 42 73 111



DGGE Band	Microorganism
3	*Nitrospina*
5	*Sphingomonas*
6	*Sphingomonas*
8	*Sphingomonas*
9	*Sphingomonas*
Porous Pot Culture	*Flavobacteria-Cytophaga*

Characteristics of *Cytophaga-Flexibacteria* Found in the Porous Pot Systems

- Dominant in activated sludge systems
  - Good floc-formers, settle readily
- Implicated in biodegradation of PAHs, phenols, and other substituted aromatics
- Abundant in organically rich, not oligotrophic, environments
  - Presence in groundwater not documented
    - Could partially explain why field studies have been so inconsistent in observing biodegradation of MTBE in aquifers

Characteristics of *α-Proteobacteria*

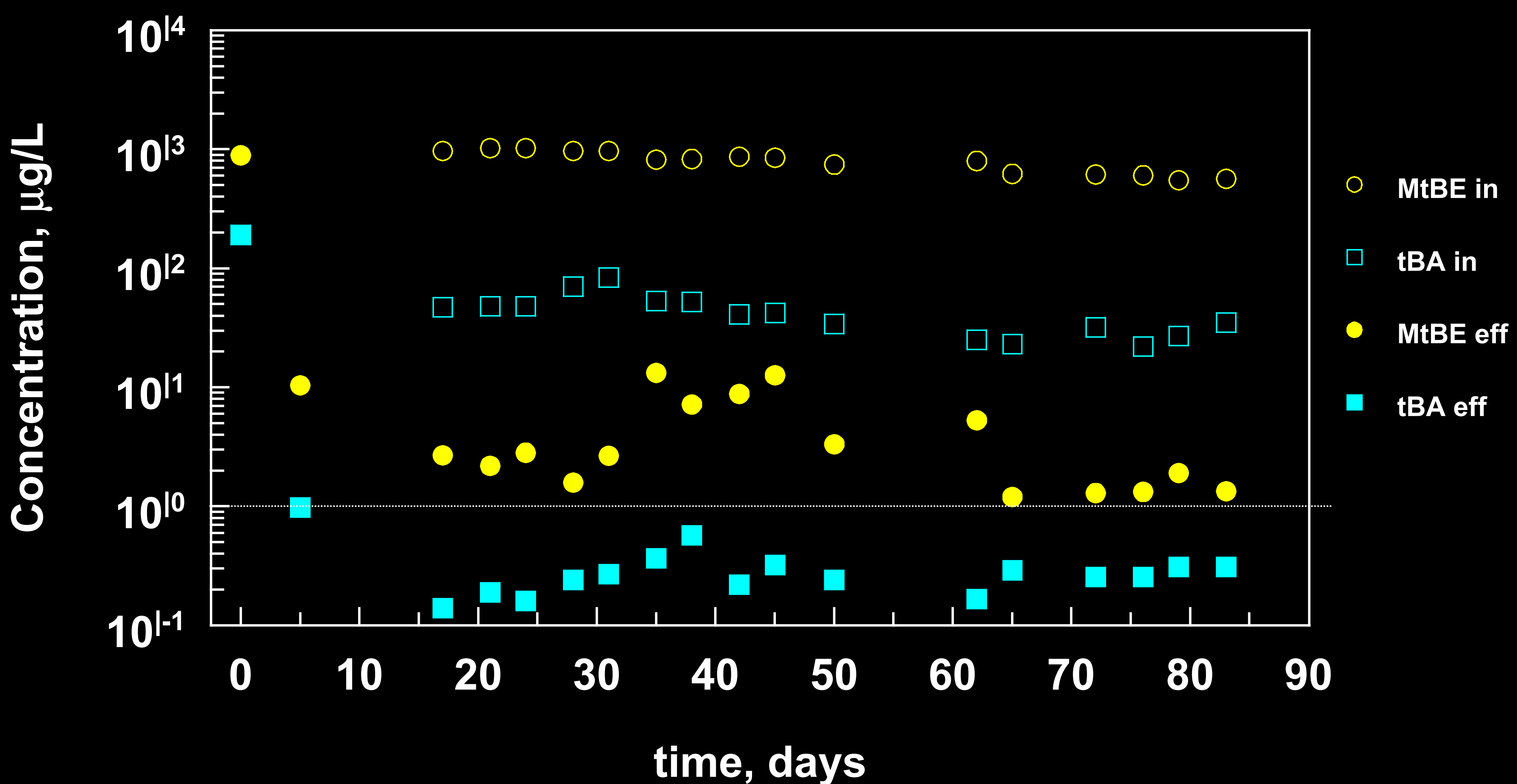
- Predominant species in MBR
- *Sphingomonas* spp.
  - Known degraders of complex substrates like PCP
  - Often detected in subsurface samples
  - Can readily attach to surfaces
  - Highly hydrophobic, good oil degraders
  - Not subject to shear stress as are *Cytophaga*
- Since only present in porous pot reactors with dual substrates, most likely responsible for biodegradation of the alternate substrate

Bench Top Study of Millville, NJ Superfund Site Groundwater

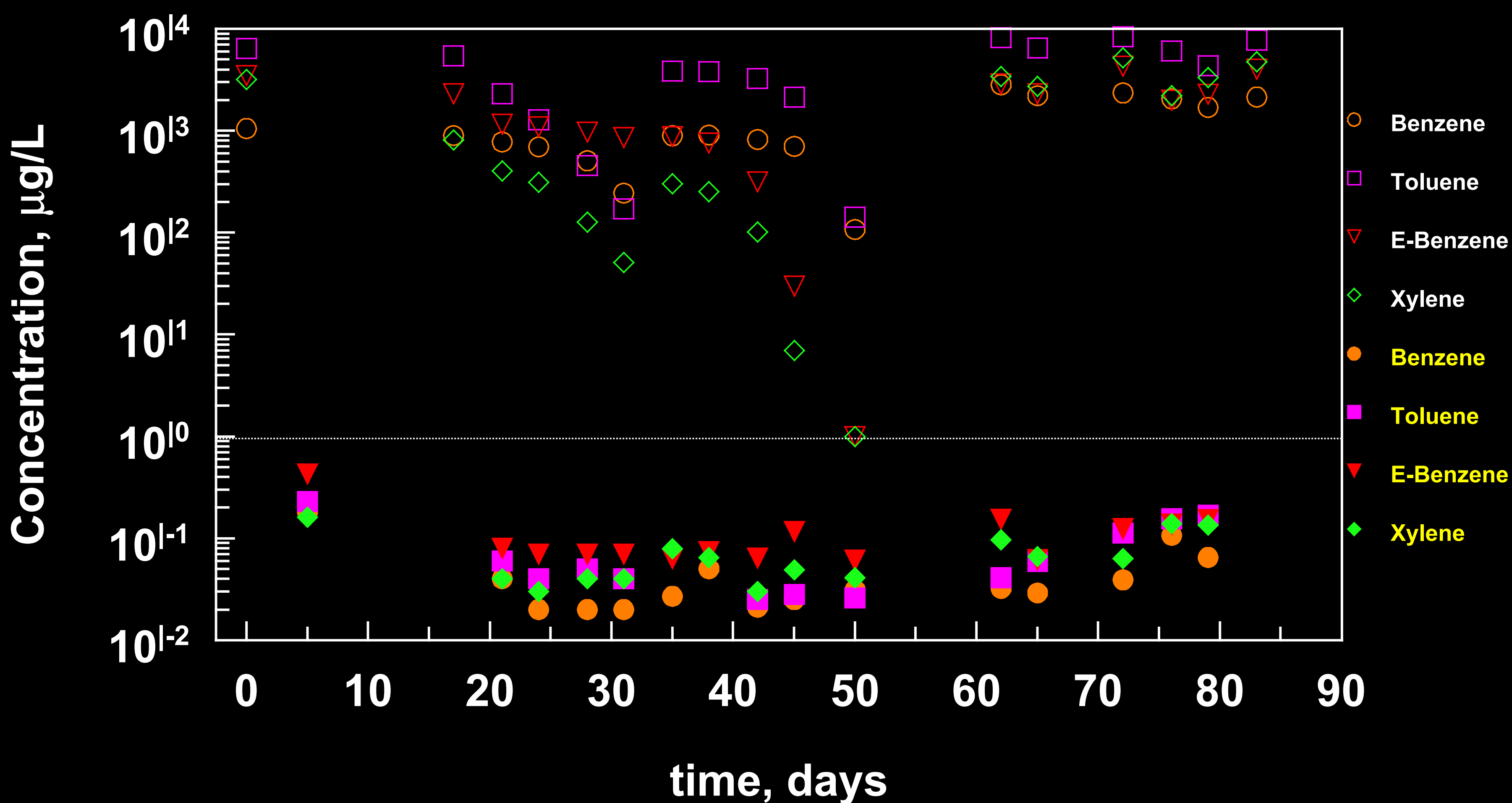
Millville, NJ Study

- MGP site with co-mingled plume of PAHs and fuel hydrocarbons from gasoline spill
- Groundwater shipped every month as feed to porous pot chemostats
- Reactors inoculated with acclimated MtBE cultures and oil degraders
- Reactors operated at 2 different flow rates: 4.8 and 8.5 L/d

MtBE and tBA Degradation



BTEX Degradation

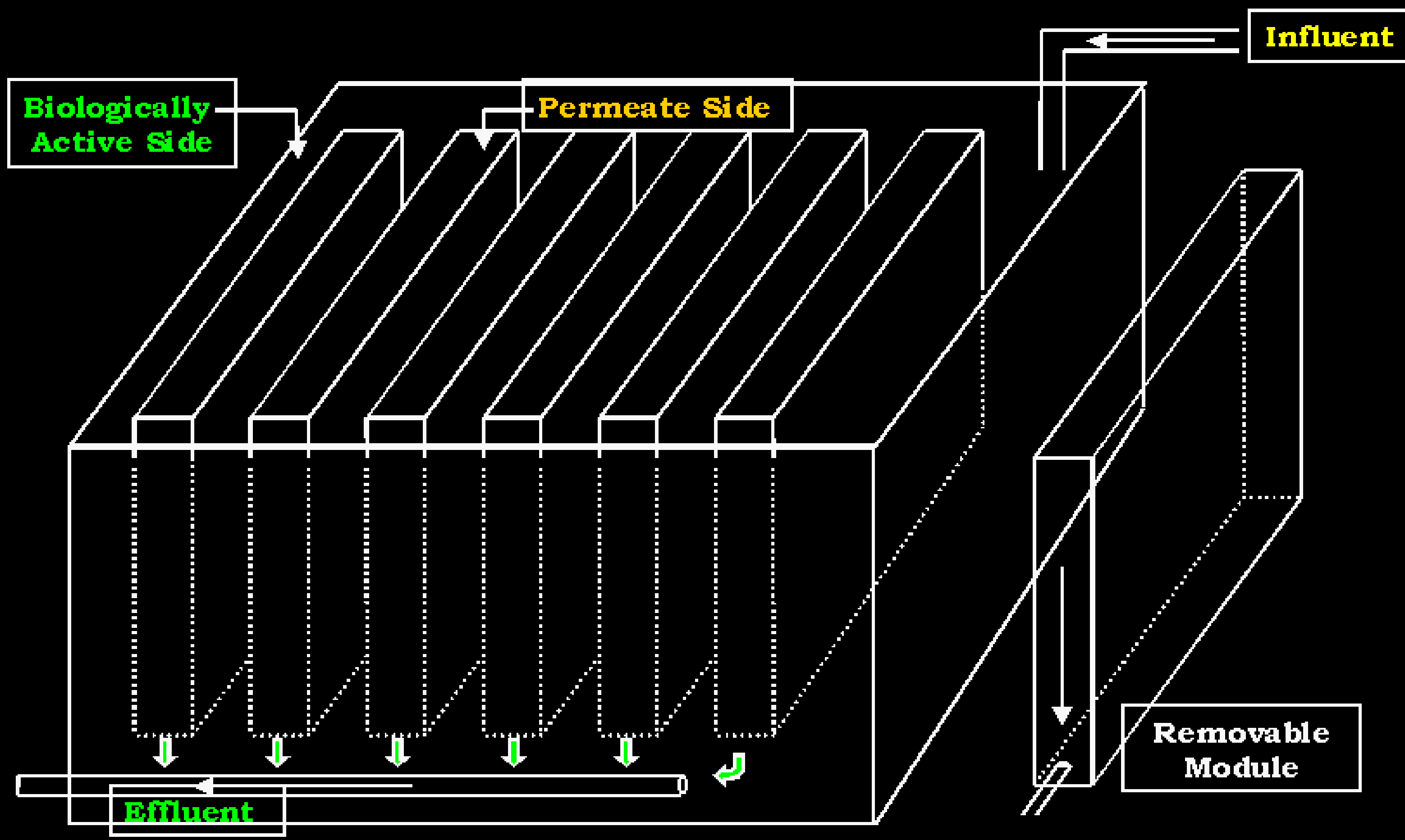


Pilot-Scale Biomass Concentrator Reactor (BCR) Performance

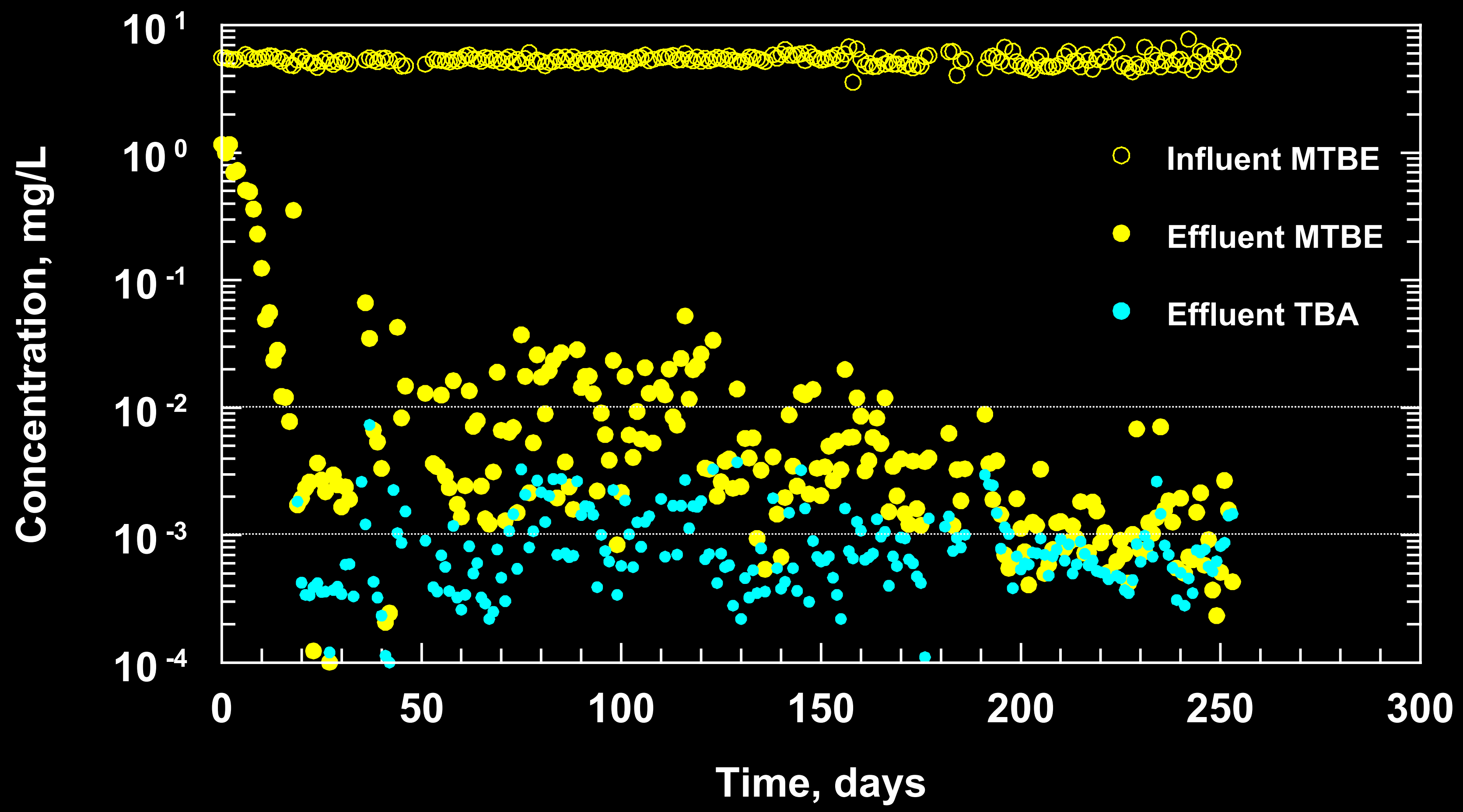
Description of BCR

- 0.5 m^3 capacity system built last year (1.0 m^3 total volume)
- Applied for joint UC/EPA patent
  - Design conceptually based on porous pot reactor
    - Much higher surface area, allowing for greater flow rates under gravity
    - Flow rate of 2,500 L/d (4.8 h HRT)
    - Started up in May, 2001
    - Producing effluent MtBE and tBA concentrations of ~1 μg/L since September, 2001 (5 mg/L in feed)
    - Will increase flow to 5,000 L/d (2.4 h HRT) and reduce MtBE in feed by half
    - Planned for use in a field study at Millville, NJ next year

Schematic Diagram of Biomass Concentrator Reactor



Biodegradation of MtBE in BCR



Economic Evaluation of *Ex-Situ* Reactors

- Comparison of MBR and BCR with Air Stripping
  - Assumptions:
    - 2 mg/L MtBE influent
    - 5 μg/L MtBE effluent
    - 3 GROUNDWATER flow rates (0.1, 0.3, and 1.0 mgd)
    - Air stripping equipped with GAC off-gas treatment

Economic Evaluation of *Ex-Situ* Reactors

Cost of *ex-situ* treatments, $/1000 gal*			
Flow	**Stripping**	**MBR**	**BCR**
0.1 mgd	2.11	1.76	1.72
0.3 mgd	0.88	0.93	0.91
1.0 mgd	0.41	0.54	0.76

*Estimates by Richard Scharp

Economic Comparison

- BCR had not been optimized when the cost analysis was done (costs based on prototype configuration only)
  - Preliminary studies indicate that the BCR can be operated at substantially lower HRTs, which will result in greatly reduced costs
- Thus, biotreatment appears to be cost competitive with air stripping

Conclusions: Ex-Situ Biotreatment

- **Porous Pot Chemostat and Batch Studies**
  - **Maintaining high biomass, aerobic conditions, and pH between 7.2 and 7.7 key for consistent performance**
  - **MtBE and tBA degradation is neither positively nor negatively affected by presence of BTEX**
    - **All compounds were degraded to << 1 μg/L whether MtBE, tBA, or BTEX were sole carbon sources or in combination**

Conclusions: MBR Study

- Effluent quality excellent and consistently below California advisory limits (5 μg/L)
  - Average 0.33 μg/L MtBE
  - Median 0.18 μg/L MtBE
- DOC effluent quality comparable to dechlorinated Cincinnati tap water
- Low cellular yield (0.12 g cells/g MTBE)
- Good performance at 5 and 1 mg/L in feed
- Several advantages over conventional treatment

Collaborators at the University of Cincinnati

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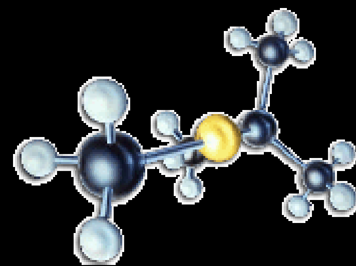

THANK YOU