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:

\[5 \text{ mg MtBE/L} \times 0.14 \text{ mg VSS/mg MtBE} = 0.7 \text{ mg VSS/L}\]

- Need to retain as much biomass as possible to achieve treatment goals
Initial Bench Top Porous Pot Reactor Studies
Schematic of Porous Pot Bioreactor

- Air Supply
- Solids Removal
- Nutrient Feed
- Buffer Feed
- MTBE Feed
- Recirculating Water Bath
- Aqueous Effluent
- Sampling Port
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

Influent MTBE
Effluent MTBE
Effluent TBA

1 µg/L MTBE

Influent MTBE
Effluent MTBE
Effluent TBA
MTBE Biodegradation as a Function of Biomass Concentration

Biomass Concentration, mg/L

Concentration, mM

pH 8.4

1 µg/L MTBE

10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
0 200 400 600 800 1000 1200

Biomass Concentration, mg/L
MTBE/DIPE-Fed Reactor
MTBE/DIPE-Fed Reactor

20-d sludge age < > no sludge wasting

Concentration, mM

0 100 200 300 400 500
time, days

Inf MTBE
Inf DIPE
Eff MTBE
Eff TBA
Eff DIPE

1 µg/L MTBE

10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6}

Inf MTBE
Inf DIPE
Eff MTBE
Eff TBA
Eff DIPE

20-d sludge age < > no sludge wasting

1 µg/L MTBE
## Summary of System Performance: All Active Reactors

<table>
<thead>
<tr>
<th></th>
<th>Influent MTBE, mg/d</th>
<th>MTBE in Effluent, mg/d</th>
<th>MTBE in Exhaust Air, mg/d</th>
<th>Percent Removed</th>
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</thead>
<tbody>
<tr>
<td>MTBE Alone</td>
<td>355.50</td>
<td>0.013</td>
<td>0.002</td>
<td>99.99</td>
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<tr>
<td>MTBE and DEE</td>
<td>177.75</td>
<td>0.033</td>
<td>0.003</td>
<td>99.98</td>
</tr>
<tr>
<td>MTBE and DIPE</td>
<td>177.75</td>
<td>0.018</td>
<td>0.002</td>
<td>99.99</td>
</tr>
<tr>
<td>MTBE and Ethanol</td>
<td>177.75</td>
<td>0.010</td>
<td>0.002</td>
<td>99.99</td>
</tr>
<tr>
<td>MTBE and BTEX</td>
<td>202.35</td>
<td>0.060</td>
<td>0.002</td>
<td>99.97</td>
</tr>
</tbody>
</table>
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

MTBE or TBA Concentration, mM

Time, hours

- MTBE
- TBA
- MTBE, killed

MTBE-Spiked Serum Bottle

0.5 mM
TBA Spiked
0.65 mM

Concentration, mM

TBA

TBA, Killed

time, hours

0 1 02 03 04 05 06 0

0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70

0.65 mM
TBA-Spiked Serum Bottle: 0.65 mM

The diagram shows the mass of carbon over time for different carbon forms:
- Total Carbon
- Inorganic Carbon
- Aqueous C (MTBE & TBA)
- Dissolved Org. C

The graph plots the mass of carbon in mg against time in hours, ranging from 0 to 60 hours.
MTBE & TBA Spiked Serum Bottle
0.5 mM MTBE, 0.60 mM TBA

Concentration, mM

0 0.2 0.4 0.6 0.8 1.0

Time, hours

MTBE
TBA
MTBE, Killed
TBA, Killed

0 10 20 30 40 50 60 70 80 90 100
MTBE/TBA Spiked Serum Bottle

- Total Carbon
- Inorganic Carbon
- Aqueous C (MTBE & TBA)
- Dissolved Org. C

Time, hours:
0.0  2.0  4.0  6.0  8.0  10.0  12.0  14.0  16.0  18.0  20.0

Mass of carbon, mg:
0.0  2.0  4.0  6.0  8.0  10.0  12.0  14.0
Summary of Serum Bottle Experiments

- Complete carbon balance obtained: all MTBE converted to $\text{CO}_2$ and $\text{H}_2\text{O}$ with no buildup of intermediates
- As MTBE degrades, TBA first increases, then declines to undetectable levels
- Rate-liming step is mineralization of TBA
Performance of Membrane Bioreactor
MBR vs. Activated Sludge

**AS SYSTEM**

- **INFLUENT**
- **AERATION**
- **CLARIFIER**
- **WASTE**
- **EFFLUENT**

**MBR SYSTEM**

- **INFLUENT**
- **AERATION**
- **WASTE**
- **MEMBRANE**
- **EFFLUENT**
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 m²
  - 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

Day
0 50 100 150 200 250 300 350
Concentration, mg/L
10^-5 10^-4 10^-3 10^-2 10^-1 10^0 10^1
Effluent MTBE
Effluent TBA
Influent (sp)
Influent MTBE

Concentration, mg/L
10^-5 10^-4 10^-3 10^-2 10^-1 10^0 10^1
Day
0 50 100 150 200 250 300 350
Effect of Biomass Retention

Day 0 50 100 150 200 250 300 350

Effluent Concentration, mg/L

10^0 10^-1 10^-2 10^-3 10^-4 10^-5

VSS Concentration, mg/L

0 1000 2000 3000 4000 5000

Effluent MTBE
Effluent TBA
VSS
Dissolved Organic Carbon

DOC Concentration, mg/L

Day

Influent to GAC
Dechlorinated Tap Water before MtBE addition
Effluent from MBR
MBR Performance When Influent MtBE Reduced to 1 mg/L
MBR Performance, 1 mg/L MtBE in Feed

Concentration, mg/L

Inf (sp)
Inf MtBE
Eff MTBE
Eff TBA

Time, days
DOC in Effluent Fed 1 mg/L MtBE

- Influent DOC Corrected for MtBE Carbon
- Effluent DOC

Concentration, mg/L vs. time, days
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

<table>
<thead>
<tr>
<th>Day</th>
<th>DGGE Band</th>
<th>Microorganism</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>3</td>
<td><em>Nitrospina</em></td>
</tr>
<tr>
<td>29</td>
<td>5</td>
<td><em>Sphingomonas</em></td>
</tr>
<tr>
<td>42</td>
<td>6</td>
<td><em>Sphingomonas</em></td>
</tr>
<tr>
<td>73</td>
<td>8</td>
<td><em>Sphingomonas</em></td>
</tr>
<tr>
<td>111</td>
<td>9</td>
<td><em>Sphingomonas</em></td>
</tr>
<tr>
<td></td>
<td>Porous Pot Culture</td>
<td><em>Flavobacteria-Cytophaga</em></td>
</tr>
</tbody>
</table>
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 \( \alpha \)-Proteobacteria

- Predominant species in MBR
- \textit{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 \textit{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

Concentration, µg/L

MtBE and tBA Degradation
BTEX Degradation

Concentration, µg/L

Time, days

0 10 20 30 40 50 60 70 80 90

- Benzene
- Toluene
- E-Benzene
- Xylene

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

[Graph showing the biodegradation of MtBE over time]

Influent MTBE
Effluent MTBE
Effluent TBA

Concentration, mg/L

Time, days
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

<table>
<thead>
<tr>
<th>Flow</th>
<th>Stripping</th>
<th>MBR</th>
<th>BCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mgd</td>
<td>2.11</td>
<td>1.76</td>
<td>1.72</td>
</tr>
<tr>
<td>0.3 mgd</td>
<td>0.88</td>
<td>0.93</td>
<td>0.91</td>
</tr>
<tr>
<td>1.0 mgd</td>
<td>0.41</td>
<td>0.54</td>
<td>0.76</td>
</tr>
</tbody>
</table>

*Cost of *ex-situ* treatments, $/1000 gal*

*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

John R. Morrison
Maher Zein
Gregory J. Wilson
Amy Pruden
Marie A. Sedran
Makram T. Suidan

Department of Civil & Environmental Engineering
University of Cincinnati
THANK YOU