Report for 2002FL2B: Biological Transformation of 2-Methylisoborneol (MIB) for Improved Water Quality

- Other Publications:
  - Lauderdale, C., D. Mazyck, P. Chadik, and A. Lindner. 2003. Microbial Transformation Potential of 2-Methylisoborneol: Potential Drinking Water Applications. 5th Annual Environmental Research Poster Symposium, Department of Environmental Engineering Sciences, April 17, 2003.
  - Lauderdale, C., D. Mazyck, P. Chadik, and A. Lindner. 2002. Microbial Transformation Potential of 2-Methylisoborneol: Potential Drinking Water Applications. 5th Annual Environmental Research Poster Symposium, Department of Environmental Engineering Sciences, April 14, 2002.

Report Follows:

Title: Biological Transformation of 2-Methylisoborneol (MIB): Investigation of Remediation for Improved Water Quality

Investigators: Angela Lindner, Univ. of Florida, Gainesville, Florida Water Resources Research Center,
David Mazyck, Univ. of Florida, Gainesville, Florida Water Resources Research Center

Congressional District: 5

Focus Categories: WQL, TRT, SW

Descriptors: Activated carbon, Adsorption and Exchange, Algae, Anaerobic Treatment, Bacteria

Problem and Research Objectives: One of the most problematic taste and odor molecules in drinking water is 2-methylisoborneol (MIB), a product of cyanobacteria, microorganisms that exist in natural waters. MIB has been detected in drinking waters in Australia, England, the U.S. and Japan (Ashitani et al., 1988; Juttner, 1995; Suffet et al., 1996; Zimmerman et al., 1995). While not harmful to human health, this chemical confers a musty taste and odor to the water at a very low odor threshold concentration (OTC) of less than 10 ng/l, and, because consumers value the aesthetic qualities of drinking water, current research has focused on the development of cost- and performance-effective methods for MIB removal (Pirbazari et al., 1992). Conventional treatment methods, including chlorination and flocculation-sedimentation, have not been shown to be capable of consistently removing MIB below its OTC; therefore, alternative methods are being sought. One such approach is biological removal of MIB. Previous studies have shown that depletion of MIB by pure cultures is possible; however, no single study has shown that complete removal (<OTC) is possible by either pure or mixed populations (Izaguirre et al., 1988; Kim et al., 1997; Namkung and Rittmann, 1987; Tanaka et al., 1996). The overall objective of this study was to examine the effectiveness of microbial cultures isolated from a variety of natural sources to remove MIB. The results reported herein involve first-year experiments focusing on the isolation and characterization of MIB-degrading populations and the development of a protocol for MIB analysis.

Objectives: The overall hypothesis guiding this work is that MIB-degrading populations can be isolated from a variety of sources, including from raw surface water and activated sludge. To this end, the broad objectives of this work are as follows:

1. Isolate microbial cultures from various water sources (Lake Manatee raw water and return activated sludge obtained from the University of Florida waste water treatment facility) capable of utilizing MIB as their sole carbon and energy source.

2. Conduct phenotypic characterization of all isolated cultures and compare these characteristics to those of known degraders.

3. Develop and test a protocol using Solid Phase Micro Extraction (SPME) and GC/MS analysis to determine MIB depletion in microbial microcosms.

Methodology: *Isolation, Characterization, and Growth Measurement of MIB-degrading Microorganisms.* Mixed cultures of MIB degraders were isolated from water obtained from the Manatee County Water Treatment Facility and the University of Florida Wastewater Treatment Facility. Samples from each water source were used to inoculate nutrient broth containing 8 mg/l MIB. This solution was then recycled through a filter packed with anthracite until a biological film was formed. The filter effluent was then used to inoculate a minimal medium (See Appendix I for recipe) that contained 8 mg/l of MIB as the sole substrate. Positive growth was verified by observing an increase in turbidity of the effluent using UV/V is spectrophotometry (λ = 600 nm). Known degraders of MIB, *Pseudomonas putida* (ATCC # 12633) and *Bacillus subtilis* (ATCC # 6051), were obtained from the American Type Culture Collection and cultured in a liquid mineral medium that contained MIB as the sole substrate. Solid culturing was performed by streaking from the liquid cultures onto mineral medium agar plates that were subsequently incubated in a 4-liter dessicator containing an open beaker of 5 ml of 100 mg/l MIB solution. The contents of the dessicator were incubated at 30° C for 96 hours before colony development was observed. Each isolated mixed culture was tested for the presence of the catalase enzyme and peptidoglycan in the cell wall, while the cellular morphologies were determined using light microscopic techniques.

Protocol Development of MIB Analysis. The protocol for the analysis of MIB depletion was developed using the Solid Phase Micro Extraction (SPME) procedures described in Standard Method 6040D. For each microcosm, 9 g of NaCl were added to a clean 40 ml glass vial with a screw top septum cap. 30 ml of the liquid culture were then added to the vial, and immediately capped. Sufficient internal standard (2-isopropyl-3-methyoxy pyrazine and 2-isobutyl-3-methyoxy pyrazine) was then injected into the septum to make the concentration of each standard 20 ng/l. The solution was then mixed to dissolve as much NaCl as possible (~15 seconds). Each vial was then placed into a water bath heated to 65 degrees. The SPME needle was then inserted through the septum into the sample headspace while ensuring that the fiber is fully retracted. The fiber was left extended over each sample for 30 to 35 minutes to ensure equilibrium had been reached. The fiber was then removed and blotted dry to avoid getting water into the injector during sample desorption. The SPME needle was subsequently inserted into the injector of the GC and was left in the injector for 10 minutes. The needle was cooled before taking the next sample.

Principal Findings and Significance: *Isolation, Characterization, and Growth Measurement of MIB-degrading Microorganisms.* The initial phase of this project entailed the isolation of mixed cultures capable of growth on MIB as a sole substrate. The previously published optimal initial conditions for the isolation of MIB degraders proved to be ineffective for the isolation of cultures from Manatee County Water Treatment Facility and the University of Florida Wastewater Treatment Facility ((Izaguirre et al., 1988; Tanaka et al., 1996). After several months of unsuccessful isolation attempts using previously described methods, alternative isolation techniques were employed. Successful isolation occurred by concentrating the source water

microbial populations in a biofilm on anthracite columns. Samples taken from the effluent of these columns were capable of displaying positive signs of growth on MIB in liquid culture as shown by an increase in turbidity. Growth rates for each culture were determined using side-arm flasks and UV/VIS spectrophotometry at 600 nm. Figure I illustrates the growth of each mixed culture isolated from the biological columns.

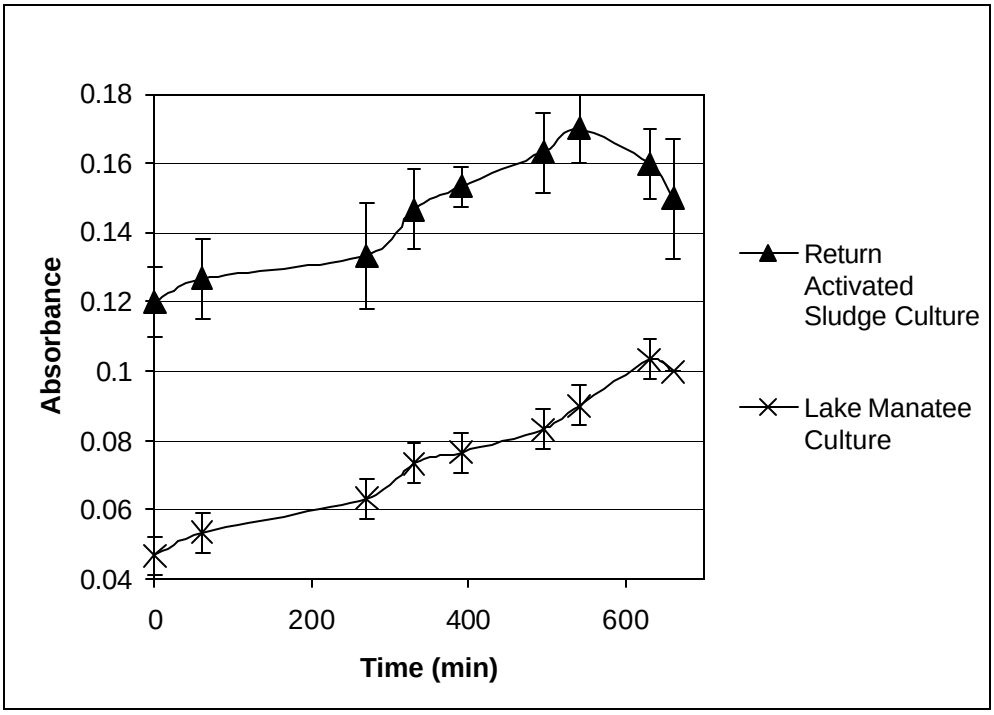

Figure I: Mixed Culture Growth Curves (Each data point represents an average of three measurements, and the error bars represent the 95% standard deviation of these points.)

The growth rates calculated for the Lake Manatee mixed culture and return activated sludge mixed culture were 0.0012 min^{-1} and 0.0006 min^{-1}, respectively. This illustrates that both isolated mixed cultures are capable of growth on MIB, albeit slowly. The growth of the two known degraders was also assessed for comparative purposes. Table I summarizes the growth rate and doubling time for each culture investigated.

Table I: Growth Characteristics of Isolated Mixed Cultures and Known Degraders

Culture Name	Doubling Time, min	Growth Rate, min^{-1}
Bacillus subtilis	630	0.0012
Pseudomonas putida	693	0.001
Mixed (Return Activated Sludge)	1155	0.0006
Mixed (Lake Manatee)	578	0.0013

As shown in Table I, the mixed culture obtained from Lake Manatee has growth characteristics similar to those of the known degraders. The difference in the growth characteristics between the two mixed cultures is perhaps due to previous acclimation of the Lake Manatee cultures to MIB, which is prevalent in their natural environment.

A comparison of the colony and cell characteristics of each culture studied may provide further insight into their relative potentials to degrade MIB. Table II provides the limited colony and cell characteristics of isolates grown on minimal medium agar in the presence of MIB.

Table II: Colony Characteristics of Isolated Mixed Cultures and Known Degraders

Culture Name	Characteristic			
	Colony Shape	**Colony Color**	**Colony Elevation**	**Cell Morphology**
Return Activated Sludge				
Colony 1	amorphous	mucoid	flat	rod-shaped
Colony 2	round w/ defined edges	yellow	raised	coccus
Lake Manatee Raw Water	round w/ defined edges	white	flat	rod-shaped
Bacillus subtilis	round w/ defined edges	white	flat	rod-shaped
Pseudomonas putida	round w/ defined edges	mucoid	flat	rod-shaped

Two dominant MIB-using colonies were isolated from the return activated sludge. The first colony isolated possessed an amorphous shape, a mucoid-like color, and was composed of bacillus-shaped cells. The second colony type isolated from the return activated sludge possessed round and defined edges, a yellow color, and cells that are coccus in shape. The Lake Manatee raw water yielded only one colony type that was round in shape, white in color, and possessing cells that are bacillus in shape. Consistent full growth for all colonies was observed in approximately 3 days. Comparison of these isolated microorganisms with the well-studied *B. subtilis* and *P. putida* shows similar cell and colony characteristics.

The remaining phenotypic characterization of the isolated cultures included a gram stain and catalase test. Table III summarizes these results for each culture.

Table III: Gram Stain and Catalase Test Results

Culture Name	Characteristic	
	Gram Stain	**Catalase**
Return Activated Sludge		
Colony 1	+	+
Colony 2	-	+
Lake Manatee Raw Water	+	+

The results from the gram stain test show that both mixed cultures have populations that are Gram positive; however, there is one colony in the activated sludge mixed culture that has an outer cell wall that is Gram negative. Both isolated cultures also showed the presence of populations capable of expressing the catalase enzyme as a means of protection when exposed to hydrogen peroxide.

Protocol Development of MIB Analysis. The initial protocol development for a method to measure the MIB depletion of a microbial culture was conducted by Mr. Roy Sirengo in his Master's Project in the department of Environmental Engineering at the University of Florida, entitled "Quantification and Removal of Taste- and Odor-Causing Compounds in Drinking Water: A Study of MIB and Geosmin." In this study, growth measurements were taken of a P. putida liquid culture, and headspace samples were collected for SPME analysis. The preliminary results provided in this study show a correlation between MIB depletion with population growth. Figure II shows the data collected from this experiment.

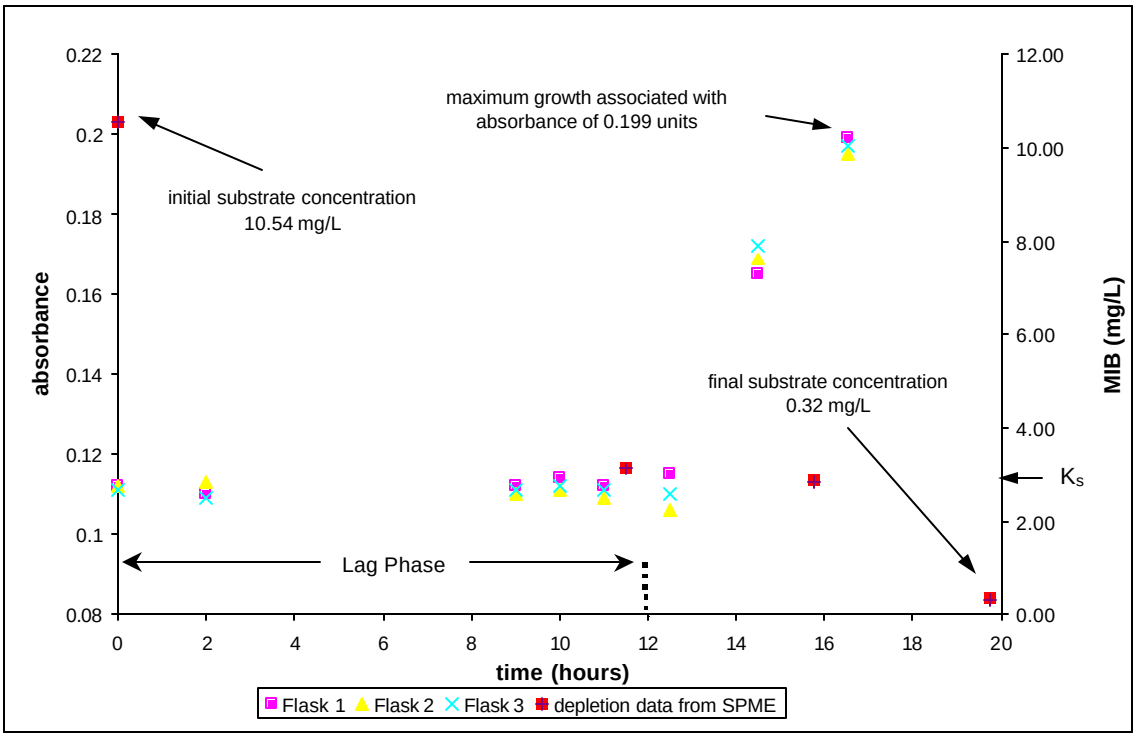

Figure II: Cell Growth and MIB Depletion Using UV/VIS Spectrophotometry and Solid Phase Micro Extraction Analysis

The initial MIB concentration of 10.54 mg/l was reduced to 0.32 mg/l after 19 ¾ hours. These results corroborate those collected in the growth studies showing *P. putida is* capable of using MIB as a sole carbon source; however, as described below, future work will focus on continued development of the SPME-based analytical method for more precision.

Future Work: Current and future work on this project include all of the following tasks:

1. Complete the microbial characterization by performing phylogenetic studies on each isolated culture using PCR-based methods.

2. Determine the MIB-transformation potential of both known MIB-degraders and the isolated mixed cultures using solid phase microextraction (SPME) coupled with GC/MS.

3. Identify any intermediate compounds formed as a result of pure- and mixed-culture transformation activity using GC/MS methods and assess their odor characteristics.

4. Summarize all results by assessing the overall biodegradation potential of isolated cultures.

The anticipated completion of this project is August 2003 and the primary deliverables of this work will be a thesis report and a publication targeted for the journal of *Water Research*.

References:

Ashitani, K. Y. Hishida, and K. Fujiwara. (1988) Behavior of musty odorous compounds during the process of water treatment. *Water Science and Technology* 20(8/9): 261-267.

Izaguirre, R.,L. Wolfe, and E.G. Means. (1988) Bacterial Degradation of 2-Methylisoborneol. *Water Science and Technology* 20(8/9): 205-210.

Juttner, F. (1995) Physiology and biochemistry of odorous compounds from freshwater cyanobacteria and algae. *Water Science and Technology* 31(11): 69-78.

Kim, Youngsug, Yeongho Lee, Chai S. Gee and Euiso Choi. (1997) Treatment of Taste and Odor Causing Substances in Drinking Water. *Water Science & Technology* 35(8): 29-36

Namkung Eun and Bruce E. Rittmann. (1987) Removal of taste and odor causing compounds by biofilms grown on humic substances. *Journal of the American Water Works Association* 79(7): 107-112.

Pirbazari, M., H.S. Borow, S. Craig, V. Ravindran, and M.J McGuire. (1992) Physical chemical characterization of five earthy musty smelling c compounds. *Water Science & Technology* 25(2): 81-88.

Suffet, I.H, Ana Corado, David Chou, Michael J. Maguire, and Steve Butterworth. (1996) AWWA taste and odor survey. *Journal of the American Water Works Association* 88(4): 166-180.

Tanaka. A, T. Oritani, F. Uehara, A Saito, H. Kishita, Y. Niizeki, H. Yokota and F Fuchigami. (1996) Biodegradation of a musty odour component, 2-methylisoborneol. *Water Research* 30(3): 759-761.

Zimmerman, W. J., C. M. Soliman and B. H. Rosen. (1995) Growth and 2-methylisoborneol production by the cyanobacterium *Phorium* LM 689. *WaterScience and Technology* 31(11): 181-186.

Appendix I: Components of Mineral Medium

Species	Concentration (mg/l)
NH_4Cl	50
K_2HPO_4	100
$MgSO_4$	50
$CaCl_2$	20
$FeCl_3$	1
MIB	8

Student Assistants: Chance Lauderdale, Master's of Engineering, Environmental Engineering Sciences. Anticipated Date of Graduation: Fall 2003

Roy Sirengo, Master's of Engineering, Environmental Engineering Sciences. Graduation Date: September, 2002