

Development of Phytoplankton Communities and Common Off-flavors in a Biofloc Technology System Used to Culture Channel Catfish *Ictalurus punctatus*

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Preharvest off-flavors in pond-raised channel catfish

Musty = 2-Methylisoborneol (MIB)

Earthy = Geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol)

Losses due to:

- Additional feed costs
- Delay in stocking a new crop
- Diseases
- Bird depredation
- Water quality deterioration



Microbial sources of geosmin and MIB

Cyanobacteria (blue-green algae)

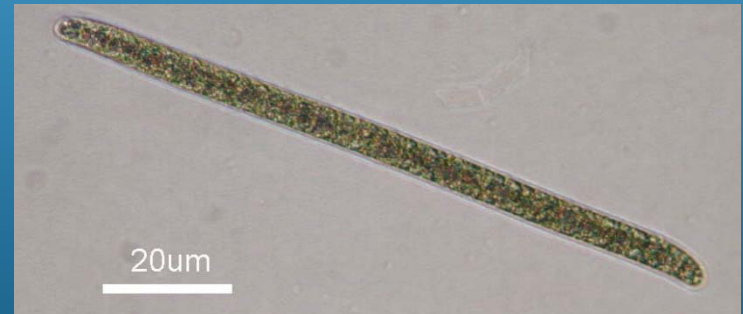
Actinomycetes (*Streptomyces*, *Nocardia*)

Fungi (*Penicillium* spp.)

Myxobacteria (*Myxococcus xanthus*)

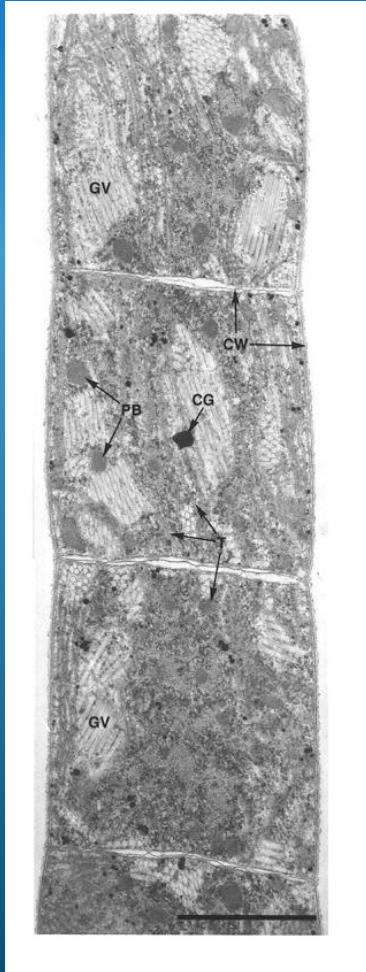
Planktonic cyanobacteria:

- dominate phytoplankton communities (minimal vertical mixing of water column)
- produce toxins
- poor base for aquatic food chains
- poor oxygenators of the water



Planktothrix perornata

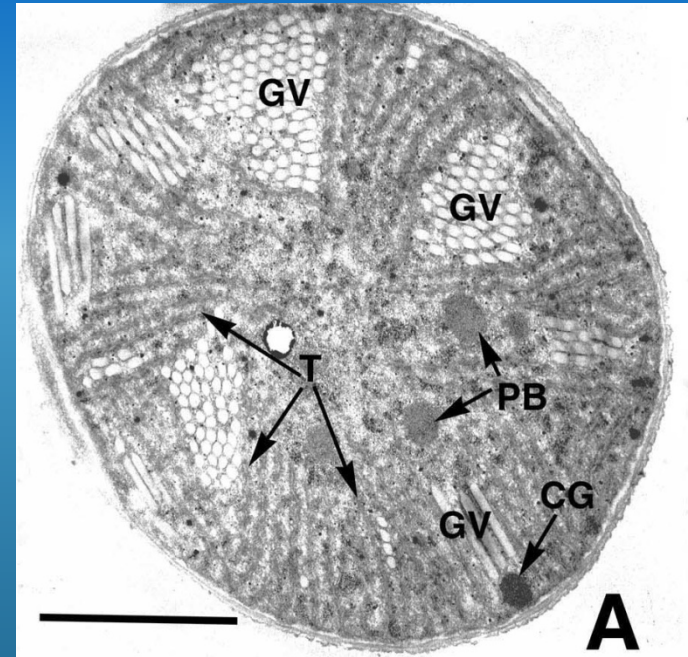
TEM micrograph of *P. perornata*



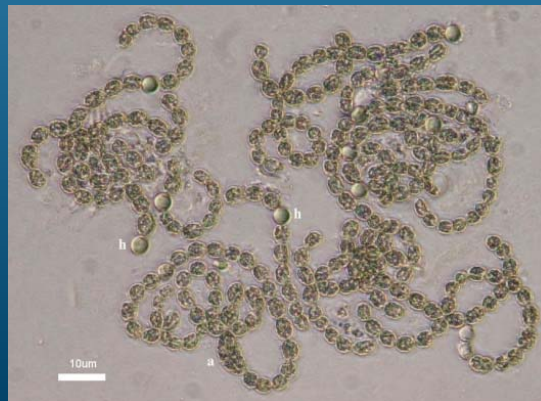
Regulate cell buoyancy via collapse and reformation of gas vacuoles (poorly mixed and stratified ponds)

Increase of water column turbulence:

- direct competition for light with eukaryotic phytoplankton
- disruption of beneficial microbial interactions
- disaggregation of cells and filament damage



Planktothrix perornata



Anabaena circinalis

Biofloc technology system for culture of channel catfish

Nine wood-framed tanks (15.6 m³ water, mean depth 0.81 m)
Lined with high density polyethylene; at USDA-ARS-SNARC
1.865 kW blower/3 tanks provided air through diffuser grid

Each tank:

- 2.5 m³ of pond water
- 0.28 kg 11-37-0 (N-P-K)
- 1.8 kg dried molasses
- 3.4 kg salt to raise chloride concentration >100 mg/L



Biofloc technology system for culture of channel catfish

Fingerling catfish stocking on May 13, 2010:

initial fish biomasses of either 0.4, 0.5, 0.9, 1.4, or 2.5 kg/m³ per tank



Feeding:

- daily to satiation with 32% protein floating feed
- rates of 60.9, 43.1, 69.5, 79.1, and 100.7 g/m³ per week
- range of 323 - 754 lb/acre-day

Catfish harvested on Nov. 10, 2010

Sample collection and analysis

Composite water samples (1 L) were collected biweekly (weekly for chlorophyll *a* determination)

At end of study, 5 catfish collected from each tank

Water samples:

- geosmin and MIB levels using SPME-GC-MS
- phytoplankton identification and enumeration
- chl *a*; chloroform:methanol (2:1) extraction and spectroscopy

Fillet samples:

- geosmin and MIB levels using microwave distillation and SPME-GC-MS



Isolation of actinomycetes from water and biofloc

Serial dilution (1:10) in sterile 0.85% saline water (10^4 , 10^5 , 10^6)

Spread-plate technique; 1% yeast extract-1% dextrose (YD) agar plates

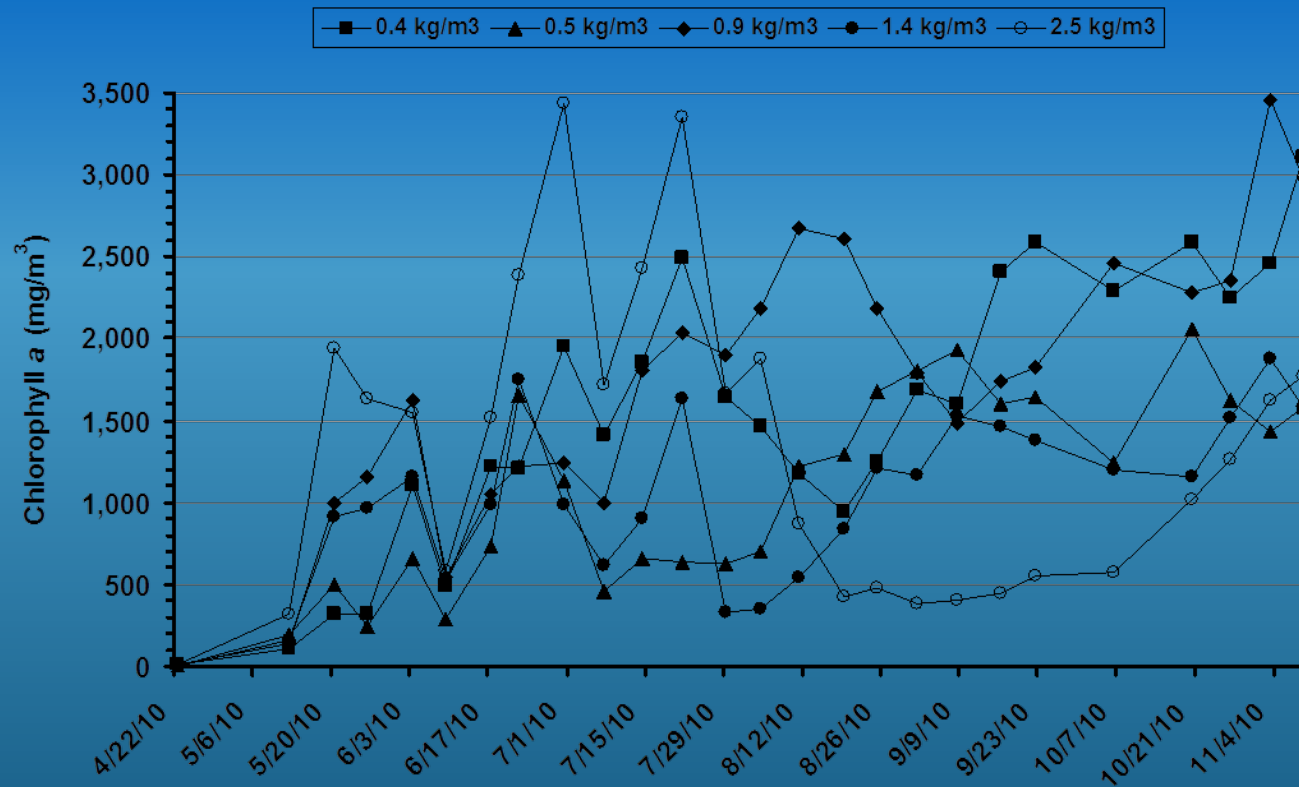
Incubated 7-10 days at 29°C

Colonies with chalky appearance were streaked on YD agar plates for isolation

- olfaction to detect earthy-musty odors
- single colony removed for SPME-GC-MS analysis
- genotypic identification by phylogenetic analysis via 16S rRNA gene sequencing



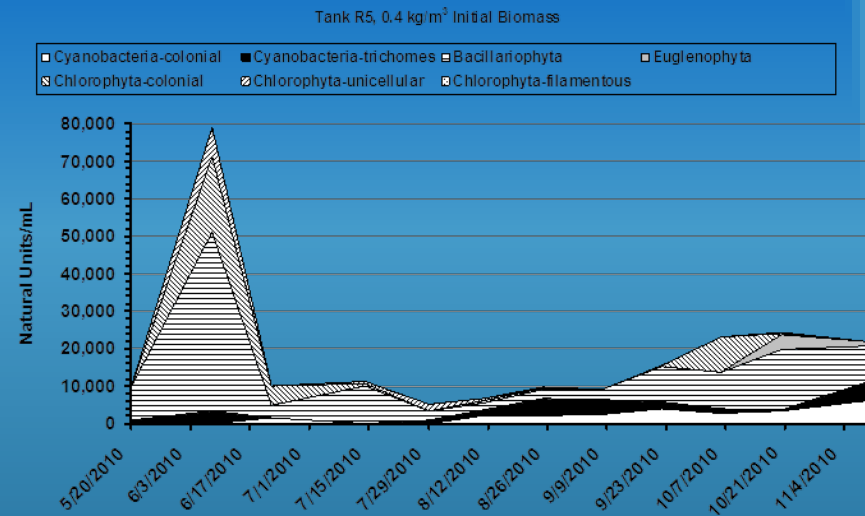
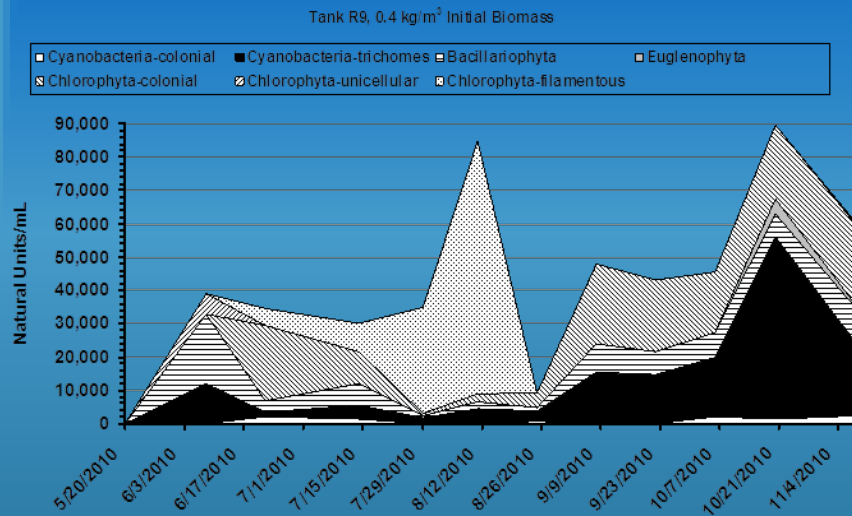
Results: phytoplankton biomass



Mean chlorophyll *a* per tank = 1084 to 2015 mg/m³

In catfish ponds, 342 to 439 mg/m³ (Torrans. 2005. *NAJA*)

Results: phytoplankton community composition

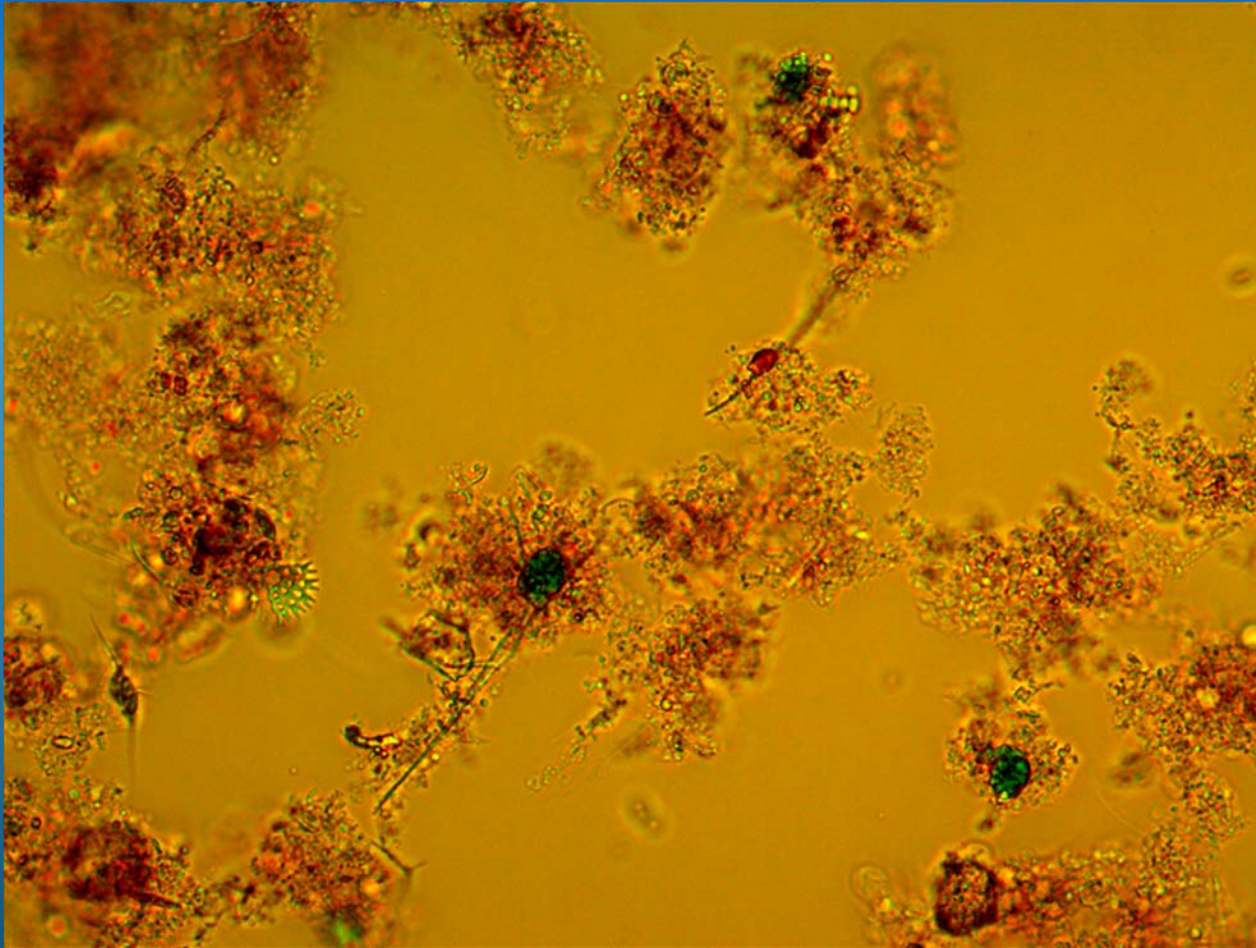


Dominance of groups (cyanobacteria, green algae, diatoms, and euglenoids) was variable among the BFT tanks

Most common:

- five genera of chlorophytes (unicellular and colonial types)
- two genera of cyanophytes (*Coelosphaerium* sp., *Jaaginema subtilissimum*)
- diatoms

Results: filamentous cyanobacteria

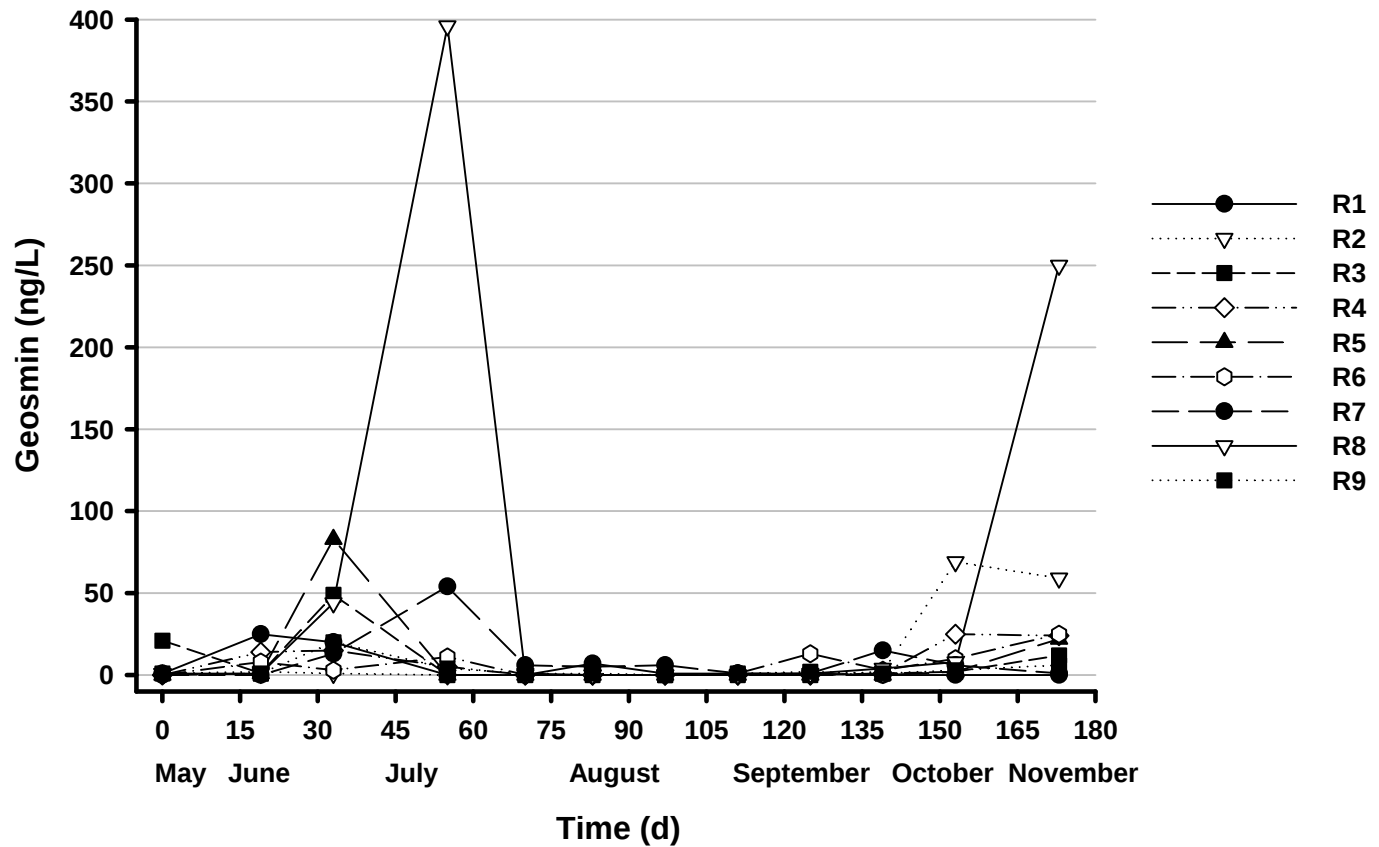


***Jaaginema subtilissimum* (straight trichomes 1-1.5 μm wide)**

Results: filamentous cyanobacteria

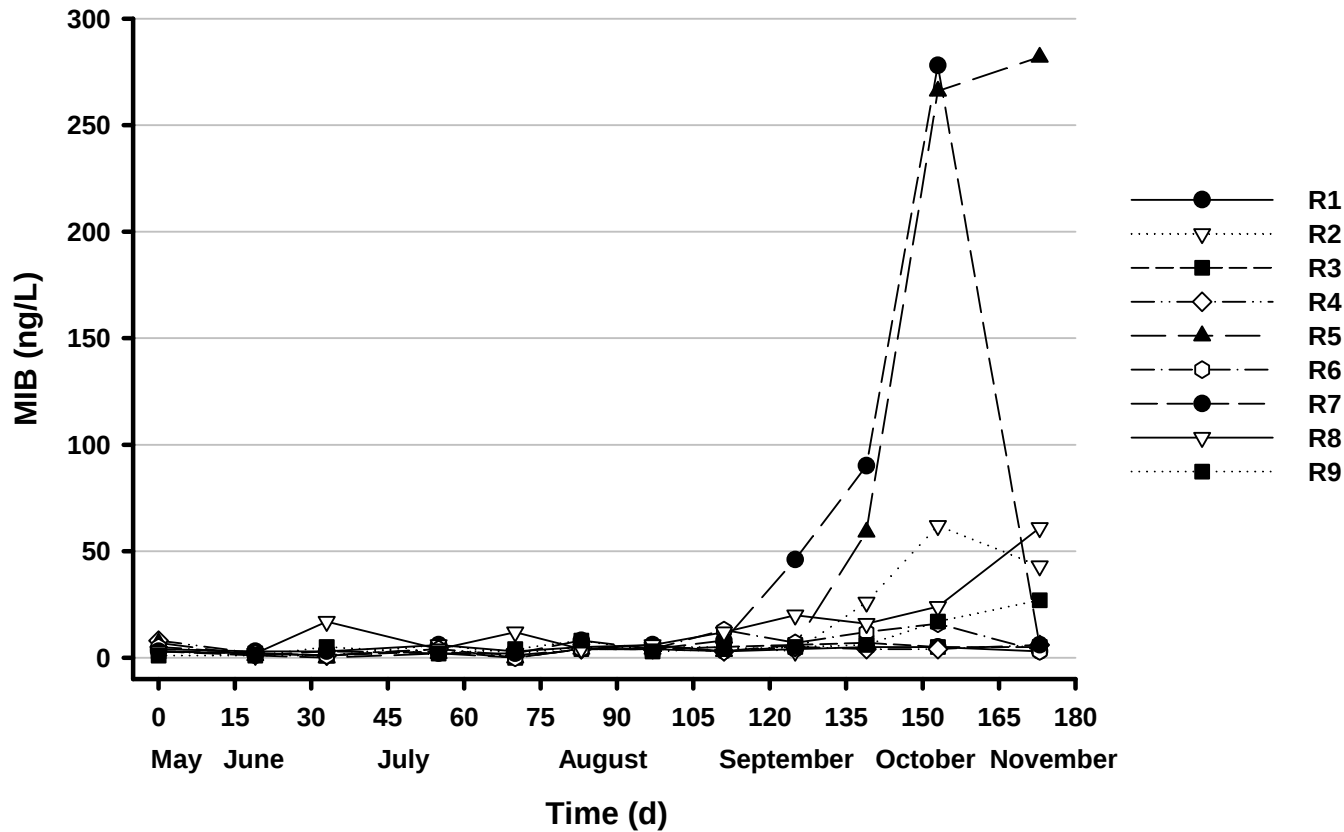


Results: geosmin levels in tank water, 2010



No correlation of geosmin levels with presence of *Coelosphaerium* sp. and *Jaaginema subtilissimum*

Results: MIB levels in tank water, 2010



No correlation of MIB levels with presence of *Coelosphaerium* sp. and *Jaaginema subtilissimum*

Results: geosmin and MIB levels in catfish, 2010

	R1	R2	R3	R4	R5	R6	R7	R8	R9
Geosmin	1.6 (0.3)	3.8 (0.2)	25.4 (2.9)	44.4 (8.4)	482.2 (94.9)	35.8 (4.6)	5.0 (0.7)	60.8 (11.4)	140.8 (17.6)
MIB	55.0 (3.7)	38.6 (5.9)	24.8 (3.1)	33.4 (7.0)	644.0 (91.4)	25.0 (1.4)	20.8 (1.6)	31.6 (4.6)	61.4 (8.1)

R5: Geosmin = 247, 319, 454, 639, and 752 ng/kg;
MIB = 390, 528, 647, 723, and 932 ng/kg

Human sensory detection threshold (ng/kg)

	<u>Average consumer</u>	<u>Flavor tester</u>
MIB	700	100-200
Geosmin	8,400?	250-500

Results: geosmin and MIB levels in catfish, 2011

	R1	R2	R3	R4	R5	R6	R7	R8	R9
Geosmin	33.8	289.6	63.2	98.0	38.4	51.0	24.8	121.4	29.4
	(2.5)	(51.0)	(10.6)	(9.9)	(5.0)	(8.3)	(3.9)	(9.2)	(2.8)
MIB	84.2	20.2	26.4	128.8	21.4	23.4	27.0	19.6	12.0
	(6.5)	(4.4)	(2.5)	(15.7)	(1.4)	(3.5)	(3.2)	(0.5)	(2.0)

R2: Geosmin = 185, 201, 264, 333, and 465 ng/kg;

R4: MIB = 94, 111, 112, 145, and 182 ng/kg

Human sensory detection threshold (ng/kg)

	<u>Average consumer</u>	<u>Flavor tester</u>
MIB	700	100-200
Geosmin	8,400?	250-500

Results: isolation of actinomycetes

2010: only one isolate (*Nocardia* sp.); not a producer of geosmin or MIB

2011: *Nocardia asteroides* isolated; “weak” producer of MIB

<u>Sampling date</u>	<u>Tank</u>	<u><i>N. asteroides</i> propagules (x 10⁵/mL)</u>
6-6-11	R5	0.3
7-11-11	R2	1.0
	R8	1.7
8-8-11	R2	0.1
9-6-11	R2	6.7
10-11-11	R3	1.3
	R5	0.3
10-18-11	R9	6.7

Results: abundance of actinomycete propagules: BFT tanks comparison to earthen ponds

- 1. In water from earthen fish ponds, 3×10^0 cfu/mL to 3.3×10^3 cfu/mL**
(Schrader and Blevins. 1993. *Canadian J. Microbiol.* 39:834)
- 2. In 2011 study with BFT tanks, 0 cfu/mL to 6.7×10^5 cfu/mL**

BFT tanks may promote greater abundance of actinomycete propagules; actinomycetes appear to be minor contributors to geosmin and MIB

Summary

BFT systems favored phytoplankton communities dominated by small colonial types of cyanobacteria, green algae, and diatoms

Geosmin and MIB production/episodes occur within BFT tanks, though less intense and less persistent than in earthen catfish ponds

Geosmin and MIB-related off-flavors in catfish from BFT tanks appear to be less intense and less frequent than in catfish from earthen ponds

Cyanobacteria are most likely the main microbial sources of geosmin and MIB within the BFT tanks

**K.K. Schrader, B.W. Green, and P.W. Perschbacher. 2011.
Development of phytoplankton communities and common
off-flavors in a biofloc technology system used for the
culture of channel catfish (*Ictalurus punctatus*). *Aquacultural
Engineering* 45:188-126.**