Physiological Studies of MIB- and Geosmin-Producing Cyanobacteria
Isolated from the Phoenix Drinking Water Supply System

Alan Fortuna¹, Qiang Hu¹, Milton Sommerfeld¹ and Paul Westerhoff²
¹Department of Plant Biology and ²Department of Civil and Environmental Engineering, Arizona State University, Tempe, Arizona

Introduction
Laboratory experiments were designed to investigate factors associated with the production of 2-methylisoborneol (MIB) and geosmin by two cyanobacteria, Pseudanabaena sp. and Phormidium sp., which were isolated from the Phoenix water supply system. These two volatile metabolites have been identified as the primary causes of musty and earthy tastes and odors (off-flavors) occurring in the Phoenix drinking water supply system during the summer and fall seasons. The effects of environmental conditions (i.e., temperature, light intensity, and lack of light) on cyanobacterial proliferation, cell autolysis, and the subsequent release of MIB and geosmin were investigated. Understanding the basic physiological responses of these MIB- and geosmin-producers to environmental factors is critical for development of effective field control measures to reduce or eliminate the off-flavors in the Phoenix drinking water supply system and also to be able to predict when off-flavor episodes are likely to occur.

Materials and Methods
Organisms: The cyanobacteria Pseudanabaena sp. was isolated at several sites along the Arizona Canal (e.g. Scottsdale Road, Central Avenue and Deer Valley). Phormidium sp. was isolated from the Verde River below Bartlett Lake and the Arizona Canal at Central Avenue. The two organisms were maintained in culture flasks containing BG-11 growth medium at 20 °C and 40 µmol m⁻² s⁻¹ of light.
Methods: All experiments were carried out in a batch mode. Temperature experiments were conducted in 100-mL flasks containing 40 mL algal culture. Flasks were placed onto a custom-designed thermogradient table varying in temperature from 10 to 35 °C (Fig. 1A). Light intensity experiments were conducted in 20-mL glass test tubes containing 10 mL of culture. Culture tubes were placed in a growth chamber providing light gradients ranging from 0 to 100 µmol m⁻² s⁻¹ of light (Fig. 1B). Algal growth was monitored by measuring chlorophyll a and protein concentrations at designated time intervals. MIB and geosmin concentrations in algal cells and in culture medium were measured by gas chromatography and mass spectrometry (GC/MS) analysis.

Results
1. Identification of MIB- and Geosmin Producers
Isolated and purified Pseudanabaena sp. and Phormidium sp. were confirmed by GC/MS analysis to synthesize and release MIB and geosmin, respectively (Fig. 2).

2. Effect of Temperature
Both cyanobacteria exhibited temperature-dependent growth: the higher the temperature, the higher the growth rate at the range of temperatures tested (Fig. 3). Likewise, the higher temperatures also stimulated the biosynthesis and release of MIB and geosmin. Accordingly, both the highest cell-bound and the highest released MIB and geosmin into culture medium were detected in cultures at the higher temperatures tested (Fig. 4).

3. Effect of Light
While chlorophyll-a concentration was highest at lower light levels, total protein content was greatest at the highest light levels for both species (Fig. 5). This discrepancy is likely due to the reduced need for chlorophyll-a at higher light levels. Odorous compound production was maximized at mid-range light levels (between 20 and 50 µmol m⁻² s⁻¹ of light). Both organisms retained a greater concentration of cell-bound MIB and geosmin than they released into the environment (Fig. 6). Biological responses of cyanobacterial producers of MIB and geosmin (off-flavor compounds) is critical to developing effective measures to predict when off-flavor episodes may occur, and to reduce or eliminate these off-flavors in the Phoenix water supply system. Greater knowledge of the physiological responses of cyanobacterial producers of MIB and geosmin (off-flavor compounds) is critical to developing effective measures to predict when off-flavor episodes may occur, and to reduce or eliminate these off-flavors in the Phoenix water supply system.

Acknowledgements
We greatly acknowledge the support and assistance of the City of Phoenix, the Salt River Project and the Central Arizona Project.

Materials and Methods
Organisms: The cyanobacteria Pseudanabaena sp. was isolated at several sites along the Arizona Canal (e.g. Scottsdale Road, Central Avenue and Deer Valley). Phormidium sp. was isolated from the Verde River below Bartlett Lake and the Arizona Canal at Central Avenue. The two organisms were maintained in culture flasks containing BG-11 growth medium at 20 °C and 40 µmol m⁻² s⁻¹ of light.
Methods: All experiments were carried out in a batch mode. Temperature experiments were conducted in 100-mL flasks containing 40 mL algal culture. Flasks were placed onto a custom-designed thermogradient table varying in temperature from 10 to 35 °C (Fig. 1A). Light intensity experiments were conducted in 20-mL glass test tubes containing 10 mL of culture. Culture tubes were placed in a growth chamber providing light gradients ranging from 0 to 100 µmol m⁻² s⁻¹ of light (Fig. 1B). Algal growth was monitored by measuring chlorophyll a and protein concentrations at designated time intervals. MIB and geosmin concentrations in algal cells and in culture medium were measured by gas chromatography and mass spectrometry (GC/MS) analysis.

Results
1. Identification of MIB- and Geosmin Producers
Isolated and purified Pseudanabaena sp. and Phormidium sp. were confirmed by GC/MS analysis to synthesize and release MIB and geosmin, respectively (Fig. 2).

2. Effect of Temperature
Both cyanobacteria exhibited temperature-dependent growth: the higher the temperature, the higher the growth rate at the range of temperatures tested (Fig. 3). Likewise, the higher temperatures also stimulated the biosynthesis and release of MIB and geosmin. Accordingly, both the highest cell-bound and the highest released MIB and geosmin into culture medium were detected in cultures at the higher temperatures tested (Fig. 4).

3. Effect of Light
While chlorophyll-a concentration was highest at lower light levels, total protein content was greatest at the highest light levels for both species (Fig. 5). This discrepancy is likely due to the reduced need for chlorophyll-a at higher light levels. Odorous compound production was maximized at mid-range light levels (between 20 and 50 µmol m⁻² s⁻¹ of light). Both organisms retained a greater concentration of cell-bound MIB and geosmin than they released into the environment (Fig. 6).

4. Effect of Darkness (Decomposition)
Cultures were grown under optimal growth conditions (26 °C and 30 µmol m⁻² s⁻¹ of light) for two weeks. Thereafter, culture tubes for both species were placed in a dark chamber. Measurements were taken daily for biomass (chlorophyll-a content) and cell-bound and released MIB/Geosmin concentrations. These experiments showed increased cellular release of odorous compounds 4 days after dark incubation and continued for two weeks. Odorous compound levels in the medium doubled over this time span for both species, while total cellular content dropped to almost zero (Fig. 7). This suggests that cell death and subsequent lysis contributes to rapid increases in MIB/Geosmin levels in the environment.

Summary
- Two cyanobacteria, Pseudanabaena sp. and Phormidium sp., isolated from the Arizona Canal and Verde River are confirmed producers of 2-methylisoborneol (MIB) and geosmin, respectively.
- Over the temperature range encountered in nature, both Pseudanabaena and Phormidium accumulated more biomass (protein) at the highest light levels, whereas MIB and geosmin production were the highest at mid-range light levels.
- Intracellular MIB and geosmin concentrations generally increased with an increase in biomass. Concomitant with intracellular accumulation, a corresponding increase in release of MIB and geosmin occurred.
- Exponential growth phase cultures of Pseudanabaena and Phormidium placed in the dark exhibited a rapid decline in intracellular MIB and geosmin and a rapid increase in released MIB/geosmin. Cell lysis and decomposition of MIB/geosmin producers may produce large spikes of these compounds in water supplies.
- Greater knowledge of the physiological responses of cyanobacterial producers of MIB and geosmin (off-flavor compounds) is critical to developing effective measures to predict when off-flavor episodes may occur, and to reduce or eliminate these off-flavors in the Phoenix water supply system.

Acknowledgements
We greatly acknowledge the support and assistance of the City of Phoenix, the Salt River Project and the Central Arizona Project.