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# Identification of conditions underlying production of geosmin and 2-methylisoborneol in a recirculating system

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ABSTRACT

Geosmin and 2-methylisoborneol (MIB) are semi-volatile terpenoid compounds produced as secondary metabolites by benthic and planktonic cyanobacteria, several genera of fungi, and various actinomycetes. These off-flavor compounds pose a heavy economic burden in the aquaculture industry rendering fish unmarketable unless purified by purging with large quantities of clean water. In the present study, the presence of off-flavor compounds was examined in a recirculating aquaculture system (RAS) for tilapia culture. In this zero-discharge system, where water from the fish basins is recirculated through parallel aerobic (drum filter and a trickling filter) and anaerobic treatment loops (sedimentation/digestion basin), concentrations of geosmin and, in particular, MIB were highest in the aerobic treatment loop. Lowest concentrations were detected in the anaerobic treatment loop. This latter finding pointed toward a possible reduction of these compounds in this basin. Two bacterial strains of the streptomycetes family were isolated from the aerobic, organic-rich, drum filter and the nitrifying trickling filter. In vitro tests with these isolates, closely related to *Streptomyces roseoflavus* and *Streptomyces thermocarboxydus*, revealed that MIB production exceeded geosmin production under all conditions tested and was significantly higher under aerobic than under anoxic conditions. Under the latter conditions, with nitrate as an electron donor, the *S. roseoflavus*-like isolate was capable of denitrification. Based on the results obtained in this study, it was concluded that aerobic, organic-rich conditions stimulate the growth of actinomycetes and subsequent production of geosmin and MIB in the system. The observed reduction of these compounds in the anaerobic water treatment component may serve in designing treatment steps aimed at alleviating the problem of geosmin and MIB accumulation in recirculating systems.



## 1. Introduction

Geosmin and 2-methylisoborneol (MIB) are two of the most common compounds that impart an earthy–musty taste and odor to water. In addition to drinking water supplies (Persson, 1983; Silvey and Roach, 1953; Terashima, 1988; Watson, 2004; Yagi, 1983), these compounds are detrimental in many aquaculture facilities (Tucker, 2000). When released into the culture water of such facilities, geosmin and MIB are absorbed through the gills, skin or gastrointestinal tract by lipid-rich fish tissues and often render the fish unmarketable (Howgate, 2004).

Geosmin and MIB are semi-volatile terpenoid compounds produced as secondary metabolites by benthic and planktonic cyanobacteria, several genera of fungi, and various actinomycetes (Wood et al., 2001). While cyanobacteria are often associated with geosmin and MIB production in conventional, outdoor ponds (Tucker, 2000), streptomyces are thought to be the organisms responsible for production of these compounds in indoor recirculating systems. However, this latter assumption has not been verified by experimental evidence.

In both conventional and recirculating aquaculture systems, abatement strategies have mainly been focused on purging off-flavored fish with clean water before marketing (Tucker and van der Ploeg, 1999). In conventional ponds, identification of geosmin and MIB-producing cyanobacteria and an understanding of the conditions promoting their growth, have triggered studies aimed at preventing the proliferation of these organisms (Tucker and van der Ploeg, 1999; Zimba et al., 2001; Schrader et al., 2003). Preventive measures such as these have not been applied in recirculating systems where an understanding of the organisms responsible for geosmin and MIB production and their environmental requirements is lacking.

In the present study, production of geosmin and MIB was examined in a zero-discharge recirculating systems with aerobic and anaerobic treatment components. Production sites of geosmin and MIB within the system were identified and production rates of these compounds were determined in vitro in crude samples from different system components. In addition, two geosmin and MIB-producing

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bacteria of the Streptomyces genus were isolated from the system and examined for the production of these compounds under different environmental conditions.

## 2. Materials and methods

### 2.1. Experimental system

The indoor, zero-discharge recirculating system examined in this study has been previously described by Shnel et al. (2002). Briefly, it comprises 24 culture basins (volume 5 $m^3$, each) of which 12 were operated in a recirculation mode (Fig. 1). Water from the fish basins is led through a mechanical drum filter for removal of organic matter. From here, water is led through two separate treatment stages. One stage, the aerobic treatment stage, consists of a nitrifying trickling filter for ammonia removal followed by an oxygen enrichment step. The second, anoxic treatment stage, consists of a sedimentation/digestion basin in which organic matter derived from the drum filter is digested and where nitrate is denitrified. Geosmin and MIB concentrations in the system were examined during part (January–July, 2003) of a tilapia (*Oreochromis niloticus*×*Oreochromis aureus*) growth cycle lasting from October 2002 until September 2003.

### 2.2. Chemical analyses

Inorganic nutrients were determined monthly during the complete tilapia growth cycle lasting from October, 2002 until September, 2003. Determinations were performed on duplicate samples derived from the effluent of the fish culture basins. Samples for total ammonia nitrogen ($NH_3$-N and $NH_4^+$-N, from hereon referred to as TAN), nitrite, nitrate and phosphorus analyses were filtered through 25 mm glass micro fiber filters immediately after sampling and kept cool at 4 °C until analyzed by colorimetric methods using a Spectrosonic 1001 spectrophotometer (Bausch & Lomb, Rochester, NY). TAN was determined after oxidation to indophenol as described by Scheiner (1976). Nitrite was analyzed using the sulfanilamide reagent according to Strickland and Parsons (1968). Nitrate was determined by ultraviolet spectrophotometry as previously described (APHA 1995). Dissolved reactive phosphorus levels were measured by the ascorbic acid method (Strickland and Parsons, 1968). Organic matter was measured as chemical oxygen demand by oxidation of the organic matter with dichromate in the presence of concentrated sulphuric acid according to APHA (1995).

### 2.3. Analysis of geosmin and MIB

During the period from January until July, 2003, geosmin and MIB were determined monthly on triplicate samples derived from: (a) the outlet of the fish basins, (b) water within the drum filter, (c) water within the digestion basin, (d) trickling filter inlet, and (e) trickling filter outlet. Analysis of these compounds was performed by using the solid phase micro extraction method based on extraction of these compounds onto a StableFlex fiber (Supelco, Bellefonte, PA) from the headspace of 40 mL glass vials, containing 25 mL water sample. After an initial incubation of 10 min in a water bath at 65 °C, fibers were injected through the Teflon faced silicone septa (Supelco, Bellefonte, PA) of the airtight vials. After an extraction period of 20 min, the fibers were introduced for 1.5 min at 250 °C into the splitless operated injector of a HP5890 (Palo Alto, CA) gas chromatograph with a flame ionized detector (GC-FID). The GC was operated with a MDN-5 fused silica capillary column (30 m×0.25 mm) of 0.25 μm film thickness (Supelco, Bellefonte, PA). Helium was used as the carrier gas at constant flow rate of 1 mL $min^{-1}$. Oven temperature was held at 60 °C for 0.5 min from injection, increased to 100 °C at 30 °C $min^{-1}$, followed by an increase to 185 °C at 20 °C $min^{-1}$ and to 250 °C at 40 °C $min^{-1}$ and held at this maximum temperature for 2.3 min. FID temperature was 280 °C. Identification of geosmin and MIB peaks detected by GC-FID was verified by parallel analysis of selected samples with a gas chromatograph coupled to a mass spectrometer detector, (GC–MS model Saturn 2000, Varian Inc. Palo Alto, CA.).

### 2.4. Laboratory incubation of geosmin and MIB-rich organic matter derived from the system

Crude samples of organic matter were collected from the drum filter and trickling filter of the system. Tests were performed on triplicate samples. Ten grams of organic matter were placed in 500 mL Erlenmeyers containing 250 mL distilled water. The Erlenmeyers were

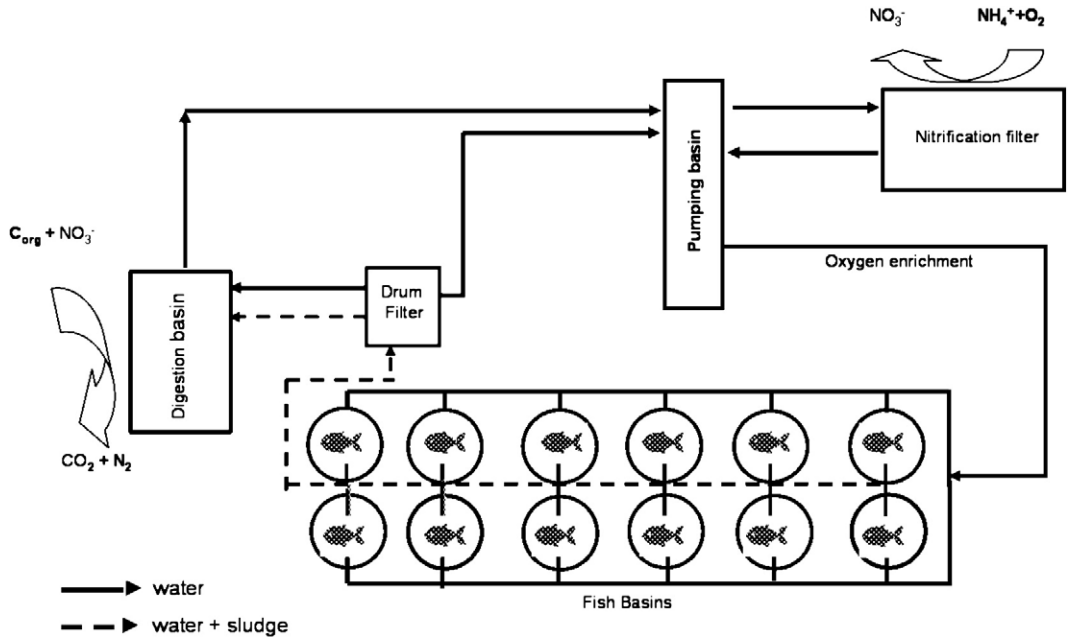


**Fig. 1.** A schematic presentation of the investigated RAS (not to scale). Solid arrows indicate the direction of the water flow and broken arrows the direction of water+sludge flow. The main water purification processes in the various compartments are indicated.