Effect of Transmembrane Pressure on Geosmin Release from Cyanobacterial Cell During Microfiltration

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Abstract: The mechanisms of increase in geosmin concentration in MF permeate with filtration time were investigated when inflow to the MF contained cyanobacteria incorporating geosmin inside and outside their cells. Whereas the geosmin concentration in the MF permeate was almost the same as the extracellular geosmin concentration in the MF inflow at the beginning of filtration, it increased and exceeded the extracellular geosmin concentration in MF inflow after 2 h of filtration. A cell viability test using a double-staining method revealed that a portion of the intracellular geosmin, which was incorporated in the cyanobacterial cells and therefore retained on the MF membrane, was released from the cells. This occurred probably because increased transmembrane pressure (TMP) with filtration time forced the cells to compress. However, geosmin release was not explained simply by cell breakage: other possible factors were implied.

Keywords: Cyanobacteria; geosmin; microfiltration; water treatment

INTRODUCTION

An earthy-musty odor is still one of the big problems in the field of drinking water treatment in Japan. Owing to the delay in the development of legal systems to protect the environment in Japan, high concentrations of nutrient salts contained in the effluents from houses and agricultural fields heavily eutrophied lakes and ponds, including drinking water sources. Cyanobacteria accordingly bloomed in the eutrophied lakes and ponds, some of which generate earthy-musty odor compounds: geosmin and 2-methylisoborneol (2-MIB) (Lovell, 1976; Persson, 1980; Burlingame et al., 1986). Because these compounds are hard to remove with conventional drinking water treatment consisting of coagulation, sedimentation, sand filtration, and chlorination processes (Waer, 2006), these compounds contaminated tap water. They can be tasted even at very low concentrations (less than 10 ng/L), leading to many complaints to the water authorities. Twenty million people (approximately one-sixth of the Japanese population) suffered from this odor problem in 1990 (Organization of the Ministry of Health, Labor and Welfare, Japan, 2004), eroding public trust. Even in the present decade, approximately 1.6 million consumers suffer from musty odors in their tap water (Organization of the Ministry of Health, Labor and Welfare, Japan, 2004), accounting for a large portion of complaints to water authorities.

These compounds are generated in cells of cyanobacterium and actinomycetes, and are stored in cells as intracellular compounds. Wu and Jüttner reported that geosmin exists in thylakoid, cell membrane, and particular dissolved proteins, while 2-methylisoborneol exists in particular dissolved proteins (Wu and Jüttner, 1988a). A portion of the intracellular compounds are released from the cells. The reported release ratio of geosmin varies widely: 30% (averaged) from *Anabaena macrospora* (Negoro et al., 1988), 3.8–6.2% from *Oscillatoria tenuis* (Wu and Jüttner, 1988), and 0.2–0.4% from *Fischerella muscicola* (Wu and Jüttner, 1988b).

Some technologies have been applied to the removal of earthy-musty odor compounds. Ozonation is one of the most effective such methods. However, bromate, which the US EPA classifies as a possible carcinogenic substance, was found to be produced during the ozonation process (Asami et al., 1996), which could be a big disadvantage of the process. Ozonation decomposes cells of microorganisms and then forces them to leak their intracellular organic matter, increasing the risk of disinfection byproducts in the following chlorination process (Henderson et al., 2008). Granular activated carbon (GAC) adsorption is also one of the most effective methods, and is usually employed in combination with the ozonation process. However, nematodes are reported to propagate in the GAC bed (Miwai and Morizane, 1988); they might be a possible carrier of pathogenic microorganisms such as bacteria and viruses. Powdered activated carbon (PAC) adsorption has been widely applied for the removal of earthy-musty odor compounds for a long time. However, because the uptake rate of earthy-musty odor compounds is not large, a long contact time is required for their effective removal. Thus a large reaction tank is necessary. In other words, the PAC contact time with the target compounds is actually limited, and a large portion of the PAC is discarded before its adsorption capacity is fully utilized.

To compensate for this disadvantage, submicron-PAC (S-PAC), which is produced by grinding commercially available PAC to finer particles with diameters of less than 1 μm, has been developed and is now being introduced to actual drinking water treatment. Uptake rates of earthy-musty odor compounds for S-PAC are dramatically enhanced by increasing the PAC surface area (Matsui et al., 2007), enabling enhanced utilization of adsorption capacity even in a short contact time. Applied S-PAC is rejected by the subsequent microfiltration (MF) process because the S-PAC is too small to be removed by a conventional rapid sand filter.
However, a phenomenon was observed in a pilot-scale experimental plant employing the S-PAC–MF hybrid system, in which the geosmin concentration in MF permeate gradually increased with filtration time. This increase might be a disadvantage of the hybrid system. Accordingly, the objectives of the present study are (1) to investigate TMP’s effect on intracellular geosmin release, and (2) to investigate whether or not cyanobacterial cells are broken during the MF process.

MATERIALS AND METHODS

Cyanobacteria used
A filamentous cyanobacterium, *Anabaena smithii* (Komárek) Watanabe (NIES824), was provided by the National Institute for Environmental Studies (NIES, Tsukuba, Japan), and then used in the present study. *A. smithii* cells are either barrel-shaped or spherical, with a diameter of approximately 10 µm. The cyanobacterium was cultivated in a 1-L glass vessel in cefixime and tellurite (CT) medium (Watanabe and Ichimura, 1977) with a growth chamber (MLR-351, Sanyo Electric Co. Ltd., Moriguchi, Japan) at 30 ºC.

MF flow experiments
Cyanobacterium *A. smithii* in its stationary phase was used for the MF experiments. Culture medium containing the cyanobacterium was spiked into CT medium in a raw water tank at the proportion of 1:159 (v/v). The cyanobacterium-spiked water was directly fed into a flat sheet ceramic MF module (nominal pore size 0.1 µm, effective filtration area 0.0007 m²; NGK Insulators, Ltd., Nagoya, Japan) by a peristaltic pump in dead-end mode. Geosmin concentrations in the MF inflow and in the MF permeate were measured periodically. After the MF filtration experiment, the ceramic MF membrane on which cyanobacterial cells were accumulated was shaken mildly for more than 5 min in CT medium to withdraw cyanobacterial cells from the membrane. The viability of the withdrawn cells was determined by the cell staining procedure described below.

Analysis of intra- and extracellular geosmin concentration
Each sample was divided and placed into two beakers: one for quantification of total geosmin and the other for quantification of extracellular geosmin. For quantification of total geosmin, cyanobacterial cells were lysed by reacting with 20 mg L⁻¹ sodium hypochlorite for 30 min at room temperature. Then, sodium thiosulfate was added to the solution at a final concentration of 20 mg L⁻¹ to quench the unreacted sodium hypochlorite. Finally, quantification of the geosmin in the mixture after the solution had been filtered by gravity through a glass fiber filter with a 1-µm pore size gave the total geosmin concentration. For quantification of extracellular geosmin, cyanobacterial cells were removed from the sample solution by filtering the solution by gravity through a glass fiber filter with a 1-µm pore size. Quantification of the geosmin in the filtrate gave the extracellular geosmin concentration. The intracellular geosmin concentration was calculated by subtracting the extracellular geosmin concentration from the total geosmin concentration.
Analysis of geosmin by gas chromatography and mass spectrometry

Geosmin concentration was measured with a gas chromatograph – mass spectrometer (GC–MS, QP-2010, Shimadzu Corporation, Kyoto, Japan) equipped with a purge-and-trap system (Aqua PT 5000J Plus, GL Sciences Inc., Tokyo, Japan). GC–MS was performed in selected ion monitoring mode. d₃-Geosmin (Hayashi Pure Chemical Ind., Ltd., Osaka, Japan) was used as an internal standard. Ion fragments of geosmin and d₃-geosmin were detected at m/z 112 and 115, respectively.

Determination of cyanobacterium cell viability

The cells in MF inflow and on the ceramic MF membrane after MF filtration were stained by fluorescence reagents to determine their viability. The cell viability test procedure is based on the integrity of the cell membrane, which is closely related to viability: while cells with an intact cell membrane are stained bright green (Fig. 2) with fluorescein diacetate (FDA; Sigma-Aldrich Co., St. Louis, MO, USA) (Rotman and Papermaster, 1966), cells with a damaged cell membrane are stained orange (Fig. 3) with propidium iodide (PI; Invitrogen, Carlsbad, CA, USA) (Jones and Senft, 1985). The assays were performed according to the manufacturer’s instructions. The stained cells were examined with a fluorescence microscope (BX51 with URFL-T and U-LH100HGAP; Olympus Corporation, Tokyo, Japan). The excitation wavelengths for FDA and PI were 488 nm and 530 nm, and the fluorescence wavelength for FDA and PI were 460 nm and 620 nm, respectively.

RESULTS AND DISCUSSION

The cyanobacterium was completely retained by the MF membrane and then formed a cake layer on the membrane surface, because the cells of the cyanobacteria were larger than the pore size of the MF membrane. The retention caused two phenomena: TMP increased with filtration time (Fig. 4) and the intracellular geosmin accumulated on the MF membrane along with the cyanobacterial cells. Figure 5 shows the changes in extracellular geosmin concentrations in the MF inflow and in the MF permeate. The latter was almost the same as the former and less than the total (intracellular + extracellular) geosmin concentration in the MF inflow at the beginning of filtration, which means that the MF process completely rejected the intracellular geosmin along with the cyanobacterial cells, and that the extracellular geosmin passed through the MF membrane. After 2 h of filtration, the extracellular geosmin concentration in the MF permeate began to increase and exceed that in the MF inflow. This means that the intracellular geosmin was released under increased TMP.

![Fig.2 Cells stained by FDA.](image1)
![Fig.3 Cells stained by PI.](image2)

![Fig. 4 Change in TMP with filtration time.](chart1)
cells had been subjected to the high pressure during the filtration. Further study is needed. Intracellular geosmin may be released physiologically without disruption of cell membranes through stress that the caused the difference between the intracellular geosmin release ratio and the damaged cell ratio. Otherwise, the geosmin, but were not stained with any of the reagents used. This underestimation of damaged cells may have during the filtration and then did not maintain the shape of the cell. The cells would release intracellular extracellular organic matter) as well as the geosmin incorporated in the cell. We imagined that the geosmin softly in the present procedure may include the geosmin that softly attaches to the cell surface (i.e., entrapped in cell breakage on the membrane alone. One possible explanation is as follows: "intracellular geosmin" fractionated in the present procedure may include the geosmin that softly attaches to the cell surface (i.e., entrapped in extracellular organic matter) as well as the geosmin incorporated in the cell. We imagined that the geosmin softly attaching to the cell surface would be detached to the water during filtration even though the cyanobacterial cells were intact, which contributed to the increase in the geosmin concentration in the MF permeate with filtration time. Another possible explanation is that a portion of the cells retained on the MF were completely decomposed on the MF process. Whereas all cyanobacterial cells were intact in the MF inflow, 8% of the cells were damaged on the MF membrane. This indicates that the increased TMP during the filtration probably forced the cells to compress and release the intracellular geosmin. Pietsch et al. (2002) reported a similar phenomenon: intracellular cyanotoxin was released during MF treatment. They concluded that the release was caused by turbulence and pressure gradients in filters (Pietsch et al., 2002). To compare cell damage and the extent of geosmin release, the intracellular geosmin release ratio was calculated as follows: subtracting the extracellular geosmin concentration in the MF inflow from that in the MF permeate and then multiplying the value by the volume of MF permeate gave the amount of released geosmin. Multiplying the intracellular geosmin concentration by the volume of the MF inflow gave the amount of intracellular geosmin inflow. Dividing the amount of intracellular geosmin released by that of the inflow intracellular geosmin gave the intracellular geosmin release ratio. Figure 5 compares the intracellular geosmin release ratio and the damaged cell ratio at different time-averaged TMPs. The damaged cell ratio was calculated by dividing the number of damaged cells by that of all cells on the MF membrane after the filtration. When the applied pressure was small (TMP=5 kPa), no cells on the membrane were damaged and no geosmin release was observed. In contrast, when the applied pressure was increased up to 30 kPa and above, the cyanobacterial cells were damaged on the membrane: the damaged cell ratios were 10% and 8% at 30 and 60 kPa, respectively. This supports our hypothesis that the geosmin release was probably caused by the breakage of the cell membrane. Chow et al. (1997) also reported that the cyanobacterial cells were damaged during MF treatment, and the damaged cell ratio was approximately 20% with TMP of 200 kPa. However, the damaged cell ratios were smaller than the intracellular geosmin release ratios, suggesting that the geosmin release was not simply explained by the cell breakage on the membrane alone. One possible explanation is as follows: "intracellular geosmin" fractionated in the present procedure may include the geosmin that softly attaches to the cell surface (i.e., entrapped in extracellular organic matter) as well as the geosmin incorporated in the cell. We imagined that the geosmin softly attaching to the cell surface would be detached to the water during filtration even though the cyanobacterial cells were intact, which contributed to the increase in the geosmin concentration in the MF permeate with filtration time. Another possible explanation is that a portion of the cells retained on the MF were completely decomposed during the filtration and then did not maintain the shape of the cell. These cells would release intracellular geosmin, but were not stained with any of the reagents used. This underestimation of damaged cells may have caused the difference between the intracellular geosmin release ratio and the damaged cell ratio. Otherwise, the intracellular geosmin may be released physiologically without disruption of cell membranes through stress that the cells had been subjected to the high pressure during the filtration. Further study is needed.

Fig. 5 Change in geosmin concentrations with filtration time. ■: extracellular geosmin in the MF inflow. ×: total geosmin in the MF inflow. ◇: extracellular geosmin in the MF permeate.
CONCLUSIONS
A portion of the intracellular geosmin incorporated in the cyanobacterial cells was released from the cells probably because the increased TMP during the filtration forced the cells to compress. However, comparing the intracellular geosmin release ratio and the damaged cell ratio revealed that the geosmin release was not explained simply by cell breakage; other possible factors were implied.

REFERENCES