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Effect of growth phase of cyanobacterium on release of intracellular geosmin from cells during microfiltration process

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Abstract. During low-pressure membrane treatments of cyanobacterial cells, including microfiltration (MF) and ultrafiltration (UF), there have reportedly been releases of intracellular compounds including cyanotoxins and compounds with an earthy-musty odor into the water, probably owing to cyanobacterial cell breakage retained on the membrane. However, to our knowledge, no information was reported regarding the effect of growth phase of cyanobacterial cells on the release of the intracellular compounds. In the present study, we used a geosmin-producing cyanobacterium, Anabaena smithii, to investigate the effect of the growth phase of the cyanobacterium on the release of intracellular geosmin during laboratory-scale MF experiments with the cells in either the logarithmic growth or stationary phase. Separate detection of damaged and intact cells revealed that the extent of cell breakage on the MF membrane was almost the same for logarithmic growth and stationary phase cells. However, whereas the geosmin concentration in the MF permeate increased after 3 h of filtration with cells in the logarithmic growth phase, it did not increase during filtration with cells in the stationary phase: the trend in the geosmin concentration in the MF permeate with time was much different between the logarithmic growth and stationary phases. Adsorption of geosmin to algogenic organic matter (AOM) retained on the MF membrane and/or pore blocking with the AOM were greater when the cells were in the stationary phase versus the logarithmic growth phase, the result being a decrease in the apparent release of intracellular geosmin from the stationary phase cells. In actual drinking water treatment plants employing membrane processes, more attention should be paid to the cyanobacterial cells in logarithmic growth phase than in stationary phase from a viewpoint of preventing the leakage of intracellular earthy-musty odor compounds to finished water.

Keywords: algogenic organic matters; Anabaena; earthy-musty odor; logarithmic growth phase; stationary phase

1. Introduction

Cyanobacteria are ubiquitous in the aquatic environment, including rivers, lakes, and ponds. Some cyanobacteria produce compounds such as 2-methylisoborneol and geosmin (Peterson *et al*. 1995, Saadoun *et al*. 2001, Huang *et al*. 2007, Li *et al*. 2012) that have an earthy-musty odor that

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leads to complaints from consumers about the bad taste of tap water. Other cyanobacteria produce a variety of cyanotoxins (Lam *et al*. 1995, Chow *et al*. 1999, Gijsbertsen-Abrahamse *et al*. 2006, Campinas and Rosa 2010) that can have a severe impact on human health. These compounds are synthesized and then stored within cyanobacterial cells, but the cells exude a portion of the compounds.

Removing cyanobacterial cells without damaging their cell membranes is very important during treatment of drinking water because intracellular compounds may be released into the water through breaches in the cell membrane if the membrane is damaged. Actually, the chlorination process causes breakage of the cell membrane and concomitant release of intracellular compounds (Ando *et al*. 1995, Peterson *et al*. 1995, Daly *et al*. 2007, Zamyadi *et al*. 2012). It is difficult to remove dissolved organic compounds by conventional drinking water treatment processes, which consist of coagulation, sedimentation, and rapid sand filtration (Waer 2006). It is therefore reasonable and advisable to remove cyanobacterial cells prior to chlorination, especially when the concentration of cyanobacteria becomes excessive in semi-enclosed bodies of water that are sources of drinking water.

Compared with other particles, cyanobacteria are relatively difficult to remove during conventional drinking water treatment, because algogenic organic matter (AOM) inhibits coagulation (Widrig *et al*. 1996, Sano *et al*. 2011). Many researchers have reported that conventional drinking water treatment does not cause cell breakage (Peterson *et al*. 1995, Chow *et al*. 1999, Sun *et al*. 2012), whereas Pietsch *et al*. (2002) have reported that cyanobacterial cells are damaged during the treatment process and that intracellular compounds are then released into the water.

Low-pressure membrane treatment processes including microfiltration (MF) and ultrafiltration (UF) are expected to effectively remove cyanobacterial cells (Chow *et al*. 1997, Dixon *et al*. 2011, Sorlini *et al*. 2013) because the diameters of the pores in the membranes are much smaller than the diameters of cyanobacterial cells. Cyanobacterial cells have been reportedly damaged during the process of membrane filtration (Chow *et al*. 1997, Gijsbertsen-Abrahamse *et al*. 2006, Campinas and Rosa 2010, Matsushita *et al*. 2010, Dixon *et al*. 2011), and the filtrate is then contaminated with compounds exuded by the cells. Shear forces associated with the use of centrifugal pumps to feed water to the filter (Gijsbertsen-Abrahamse *et al*. 2006) and pressure gradients formed in the filter (Matsushita *et al*. 2010) have been suspected of causing damage to cell membranes. Although the growth phase of cyanobacteria is generally known to have an impact on the amount and composition of both intracellular and extracellular AOM (Pivokonsky *et al*. 2006, Henderson *et al*. 2008), there have been no investigations into the effect of growth phase on the release during membrane filtration of intracellular organic compounds such as cyanotoxins and compounds that produce earthy-musty odors. In the present study, we investigated the release during microfiltration of geosmin by the cyanobacterium *Anabaena smithii* grown in both logarithmic growth and stationary phases. We discuss the factors that contributed to the release of geosmin by *A. smithii*.

2. Materials and methods

2.1 The cyanobacterium

A geosmin-producing cyanobacterium, *A. smithii* (NIES 824), was obtained from the National

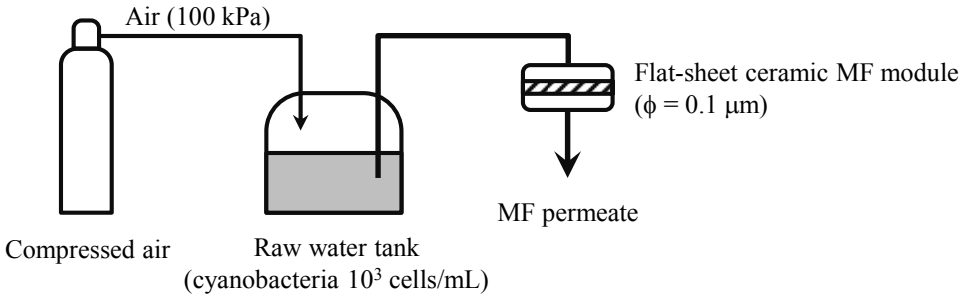


Fig. 1 Experimental setup for laboratory-scale MF experiments

Institute for Environmental Studies (NIES, Tsukuba, Japan) and then cultivated in 1-L glass vessels in cefixime and tellurite (CT) medium (TAPS, 400 mg/L; $Ca(NO_3)_2 \cdot 4H_2O$, 150 mg/L; KNO_3, 100 mg/L; β-Na_2 glycerophosphate$\cdot 5H_2O$, 50 mg/L; $MgSO_4 \cdot 7H_2O$, 40 mg/L; vitamin B_{12}, 0.1 μg/L; biotin, 0.1 μg/L; thiamine HCl, 10 μg/L; PIV metal solution, 3 mL/L (PIV metal solution was prepared by dissolving following chemicals to 100 mL of Milli-Q water: $FeCl_3 \cdot 6H_2O$, 19.6 mg; $MnCl_2 \cdot 4H_2O$, 3.6 mg; $ZnSO_4 \cdot 7H_2O$, 2.2 mg; $CoCl_2 \cdot 6H_2O$, 0.4 mg; $Na_2MoO_4 \cdot 2H_2O$, 0.25 mg; $Na_2EDTA \cdot 2H_2O$, 100 mg)) (Watanabe and Ichimura 1977). A small portion (10 mL) of the culture medium in which the cyanobacterial cells had been well cultivated was then transferred to a fresh CT medium (1 L), and then the cells were successively cultivated in the same manner. The successive cultivation was repeated several times, and a portion of culture medium was withdrawn from each cultivation at different growth phases for laboratory-scale MF experiments described below. The growth phase of the cyanobacterium for the MF experiments was determined from the curve of chlorophyll a concentration versus time (ISO 1992) (data not shown: 10-25 d and 30-60 d of cultivation were defined as logarithmic growth and stationary phases).

2.2 Laboratory-scale MF experiments

Fig. 1 shows the experimental setup for laboratory-scale MF experiments. Culture medium containing cyanobacterial cells was diluted with CT medium in a raw water tank to a density of 10^3 cells/mL. Extracellular and intracellular geosmin concentrations in the raw water tank for the logarithmic growth phase cells were 35 and 116 ng/L on average, whereas those for the stationary phase cells were 58 and 103 ng/L on average, respectively. The cyanobacterium-spiked CT medium was fed in a dead-end mode directly into a flat-sheet ceramic MF module (nominal pore size 0.1 μm; effective filtration area 7 cm^2; NGK Insulators, Ltd., Nagoya, Japan) with a constant pressure of 100 kPa provided by compressed air. No pump was used to feed the water. The MF experiments lasted for 8 h without backwashing. Geosmin concentrations in the MF inflow and in the MF permeate were measured periodically during the MF experiments.

After the 8-h filtration, the cyanobacterium-spiked CT medium in the raw water tank was replaced with fresh CT medium containing 100 ng/L of d_3-geosmin, and this medium was fed into the MF module in the same manner to investigate the adsorption of geosmin on the retentate that had accumulated on the surface of the MF membrane during the MF experiments. The MF filtrate was collected in a beaker until 50 mL of the d_3-geosmin-containing CT medium had been filtered. The d_3-geosmin concentration in the MF filtrate was then measured. The extent of adsorption of geosmin was evaluated by comparing the d_3-geosmin concentrations in the MF inflow and

permeate.

2.3 Fractionation of intracellular and extracellular geosmin

Each sample withdrawn from the MF inflow 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, sodium hypochlorite was added to the sample solution at 20 mg-Cl_2/L to release the intracellular geosmin into the water by breaking down the cell membranes of the cyanobacteria. After the reaction had been allowed to proceed for 30 min at room temperature, an excess between the total and extracellular geosmin concentrations. amount of sodium thiosulfate was added to the mixture to quench the unreacted sodium hypochlorite. Actually, geosmin is not oxidized with chlorination (Lalezary *et al*. 1986, Srinivasan and Sorial 2011), and we confirmed that 20 mg-Cl_2/L of chlorine dose and 30 min of contact time were enough for the elution of the intracellular geosmin to the water (data not shown). The total geosmin concentration (intracellular + extracellular geosmin) was equated to the concentration of geosmin in the mixture after the mixture had been passed by gravity through a glass fiber filter with a pore size of 1 μm (GA-100, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). For quantification of extracellular geosmin, cyanobacterial cells were removed from the sample solution by passing the solution by gravity through a GA-100 glass fiber filter. The extracellular geosmin concentration was equated to the geosmin concentration in the filtrate. The intracellular geosmin concentration was equated to the difference

2.4 Quantification of geosmin

Geosmin and d_3-geosmin were quantified with a gas chromatography-mass spectrometry (GC-MS) analytical system (Agilent 7890A gas chromatograph; Agilent 5975C mass spectrometry detector; Agilent Technologies, Palo Alto, CA, USA) equipped with a purge-and-trap sample concentrator (Eclipse 4660, OI Analytical, College Station, TX, USA). A capillary column (HP-5MS: length 30 m; i.d. 250 μm; thickness 0.25 μm; Agilent Technologies) was used for sample separation. The temperatures of the ion source and transfer line were 200 and 180°C, respectively. GC-MS was performed in the selected ion-monitoring (SIM) mode. D_3-geosmin and 2-methylisoborneol were used as internal standards for geosmin and d_3-geosmin quantifications, respectively. The detected fragment ions of geosmin, d_3-geosmin, and 2-methylisoborneol occurred at m/z 112, 115, and 95, respectively.

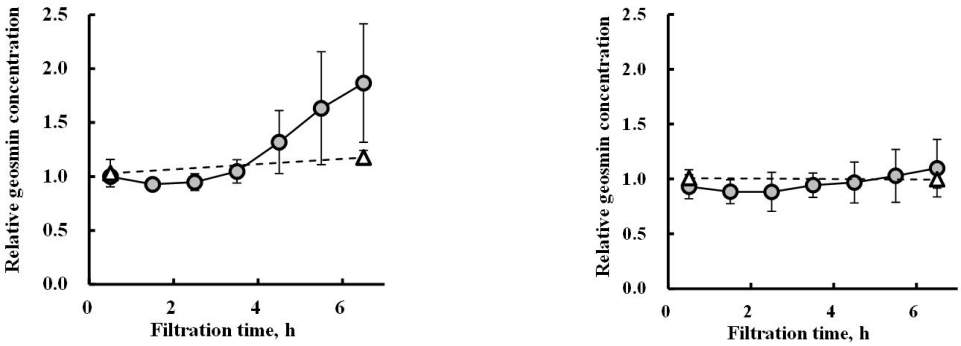
2.5 Damaged/intact cell detection

To visualize damaged cells, cyanobacterial cells were stained with SYTOX Green Nucleic Acid Stain (Invitrogen, Ltd., Paisley, UK) as follows (Sato *et al*. 2004). SYTOX Green (5 mM in DMSO) was diluted 1:100 with TE buffer (0.5 M EDTA, 20 μL; 1 M tris-HCl, 100 μL; distilled water, 10 mL). A suspension of cyanobacteria was added to the mixture so that the final concentration of SYTOX Green was 0.5 μM, and the mixture was then incubated at 37 °C for 5 min in the dark. The damaged cyanobacterial cells were detected with a fluorescence microscope (BX51, Olympus Corp., Tokyo, Japan) with an excitation wavelength of 470-495 nm (light green fluorescence). Intact cyanobacterial cells were detected by their autofluorescence when exposed to light with an excitation wavelength of 530-550 nm (red fluorescence) (Sato *et al*. 2004). At least 400 cells in random fields were counted for viability calculations in each experiment.

3. Results and discussion

3.1 Effect of growth phase on geosmin release during MF

Fig. 2(a) shows the changes in the geosmin concentrations in MF permeate and inflow observed during the MF treatments with cyanobacterial cells in their logarithmic growth phase. The vertical axis represents the ratio of the geosmin concentrations (extracellular geosmin concentrations in MF permeate and inflow) to the initial extracellular geosmin concentration in MF inflow at 0.5 h of filtration. Geosmin is synthesized in cyanobacterial cells in the same metabolic pathway used to synthesize the hydrophobic phytol tail of chlorophyll (Naes and Post 1988), and some of the synthesized geosmin is exuded from the cells. The MF inflow contained both extracellular geosmin and intracellular geosmin. Because the pore size of the MF membrane (0.1 μm) was much smaller than the cyanobacterial cell used in this study (6-12 μm in width, 4-10 μm in length), any cyanobacterial cells must have been completely retained on the MF membrane. No cells were observed during microscopic examination of the MF permeate. The intracellular geosmin was accordingly retained on the MF membrane along with the cyanobacterial cells. In contrast, the extracellular geosmin was not retained on the MF membrane itself because the geosmin molecule is small enough to pass through the membrane's pores. Accordingly, the extracellular geosmin concentration in the MF permeate (gray circles) was almost the same as that in the MF inflow during the early stage of the filtration (< 3 h). The concentration of extracellular geosmin in the MF inflow (white triangles) did not change during the filtration experiment significantly (p = 0.30). In contrast, the extracellular geosmin concentration in the MF permeate began to increase after 3 h and finally exceeded that in the MF inflow after 4 h. This increase was most likely caused by the fact that some of intracellular geosmin retained on the MF membrane along with the cyanobacterial cells was released into the liquid phase, probably as a result of cell breakage. Campinas and Rosa (2010) have reported that intracellular microcystins were released



(a) Logarithmic growth phase (b) Stationary phase

Fig. 2 Changes in the extracellular geosmin concentrations in MF permeate and inflow during the filtration of: (a) logarithmic growth phase (LP); and (b) stationary phase (SP) cells. The vertical axis represents the ratio of the geosmin concentrations (extracellular geosmin concentrations in MF permeate and inflow) to the initial extracellular geosmin concentration in MF inflow at 0.5 h of filtration. Gray circles and white triangles represent extracellular geosmin concentrations, respectively, in the MF permeate and inflow. Error bars for LP and SP indicate the standard deviations of 5 and 8 runs, respectively

from cyanobacterial cells during the ultrafiltration of water that contained *Microcystis aeruginosa* and that the release was greater at the end of the filtration. The fact that a similar trend in the release of geosmin was observed for *A. smithii* (Matsushita *et al*. 2010) is in agreement with our results.

In contrast, during the MF treatments with cyanobacterial cells in their stationary phase (Fig. 2(b)), the extracellular geosmin concentration in the MF permeate seemed to slightly increase with time, but the extent of increase was much smaller than that observed with cyanobacterial cells in their logarithmic growth phase. Moreover, no significant difference (p = 0.16) was observed between the extracellular geosmin concentration in the MF permeate and the MF inflow. The trend in the extracellular geosmin concentration in MF permeate with time was much different between the logarithmic growth and stationary phases, which may be attributable to the difference in the extent of cell breakage.

3.2 Effect of growth phase on cell breakage during MF

Firstly, to evaluate the extent of the force received by the cells on the MF membrane during the filtration, we counted the number of cells constituting one trichome after the cells retained on the MF membrane had been recovered at the end of the filtration experiment by gently shaking the MF membrane in fresh CT medium, and then compared it with that in the MF inflow. Fig. 3 shows the comparison in the number of cells per trichome in the MF inflow and retentate. For the logarithmic growth phase cells, one trichome in the MF inflow consisted of 28.7 ± 9.9 cells. In contrast, the number of cells per trichome recovered from the MF membrane was 14.2 ± 6.4. The number of cells per trichome in the MF retentate was significantly smaller than that in the MF inflow (p = 0.014): the length of trichome roughly halved on the membrane. This result clearly showed that during the filtration period the cyanobacterial cells were subjected to some forces, possibly the shear force caused by the pressure gradients formed on the MF membrane, to dissociate the adhesion between the cells constituting the trichome. For the stationary phase cells, one trichome in the MF inflow consisted of 14.0 ± 9.7 cells, which was smaller than that for the logarithmic growth phase cells. The similar observation that the length of trichome shortened with increasing cultivation time has been reported by other researchers (Saker *et al*. 1999, Hawkins *et al*. 2001).

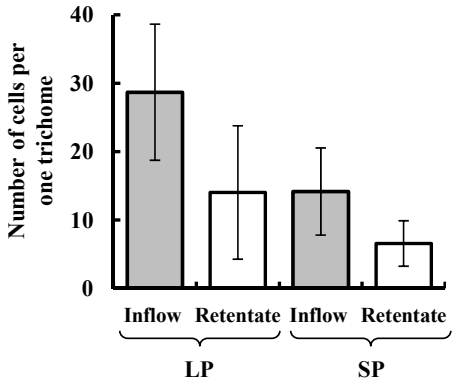


Fig. 3 Comparison in number of cells per one trichome between inflow and retentate. LP and SP mean logarithmic growth and stationary phases, respectively. Error bars for LP and SP indicate the standard deviations of 5 and 8 samples, respectively

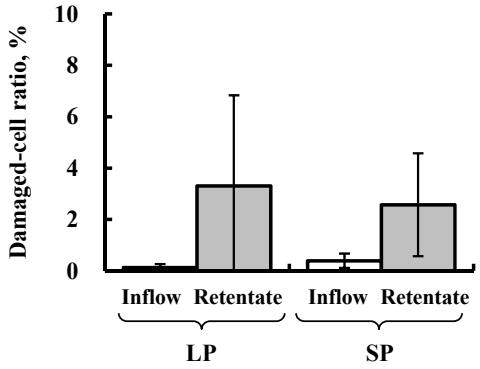


Fig. 4 Damaged-cell ratios in logarithmic growth phase (LP) and stationary phase (SP) cells. Error bars for LP and SP indicate the standard deviations of 5 and 8 samples, respectively

The number of cells per trichome in the MF retentate was 6.6 ± 3.3, which was significantly smaller than that in the MF inflow (p = 0.014) in the same manner for the logarithmic growth phase cells. The length of trichome was roughly halved during the filtration also for the stationary phase cells: the extent of the reduction in the cell length was almost the same between the logarithmic growth and stationary phases. The observation could show the extents of the force received by the cells on the MF membrane during the filtration were similar for both the logarithmic growth and stationary phases. However, the number of cells constituting one trichome may decrease when the cells were recovered from the MF membrane. Accordingly, direct observation of cell integrity is required.

Secondly, to clearly show the extent of possible cell breakage on the MF membrane, we separately counted damaged and intact cyanobacterial cells in the MF inflow and retentate. Fig. 4 shows the damaged-cell ratio for the cyanobacterial cells in the MF inflow and retentate during the logarithmic growth and stationary phases. Whereas the damaged-cell ratios for the cyanobacterial cells in the MF inflow (white columns) were less than 0.4% during both growth phases, the damaged-cell ratio for the cyanobacterial cells recovered from the MF membrane (gray columns) were approximately 3%. This result clearly indicates that the cyanobacterial cells were damaged on the MF membrane during the filtration process. Chow *et al*. (1997) have also reported the breakage of cyanobacterial cells during MF and UF treatments; their damaged-cell ratios were in the range of 3-17%. These values are greater than those in the present study, possibly because their applied pressure (200 kPa) during the MF experiments was twice as large as ours. Alternatively, the difference in the damaged-cell ratios may be due to the fact that they studied a different species of cyanobacterium, *Microcystis aeruginosa*.

In the present study, the damaged-cell ratio for the cyanobacterial cells recovered from the MF membrane were 3.3 ± 3.5% and 2.6 ± 2.0% in the logarithmic growth and stationary phases, respectively. The damaged-cell ratios were not significantly different (p = 0.64) in both growth phases, although we had expected that the ratio was larger in the logarithmic growth phase cells than in the stationary phase cells: the growth phase of the cyanobacteria did not affect the extent of cell breakage. Nevertheless, the extent of geosmin release during the MF treatment was greater for the logarithmic growth phase cells than for the stationary phase cells (Fig. 2); the observations seemed discrepant.

3.3 Effect of growth phase on geosmin adsorption to AOM

Several studies have reported that AOM is retained on MF/UF membranes and enhances membrane fouling (Campinas and Rosa 2010, Qu *et al*. 2012, Zhang *et al*. 2013). In the present study, we experimentally investigated the possible adsorption of geosmin to the AOM retained on the membrane surface by supplying d_3-geosmin to the membrane after the filtration experiments (Fig. 5). When we used a virgin membrane in the adsorption experiments, a slight decrease (12%) in d_3-geosmin concentration was observed, probably owing to the adsorption of the d_3-geosmin to the membrane, the membrane module and the tubes. When we used a membrane through which water containing logarithmic-growth-phase cyanobacterial cells had been filtered, the d_3-geosmin concentration decreased by 29%. This result clearly indicates that additional d_3-geosmin adsorbed to the AOM and accumulated on the membrane. Many polysaccharides contained in AOM have been reported to be adsorptive and to have the capacity to adsorb chemicals and particles. Similar adsorption to AOM has been reported for extracellular microcystin (Campinas and Rosa 2010). Alternatively, the reduction in the pore size of the membrane with AOM deposition onto the membrane pores may contribute the geosmin rejection (Dixon *et al*. 2011). When we tested a membrane filter through which water containing stationary-phase cyanobacterial cells had been filtered, the d_3-geosmin concentration decreased by 43%. A greater percentage of the d_3-geosmin therefore adsorbed to the membrane through which stationary phase cells were filtered than to the membrane through which logarithmic growth phase cells were filtered, or the membrane pores may be blocked with the AOM at greater extent when the stationary phase cells were filtered. The amount of AOM per cyanobacterial cell has been reported to vary widely between species, but generally the amount tends to increase with the age of the cells (Pivokonsky *et al*. 2006, Huang *et al*. 2007, Henderson *et al*. 2008). Moreover, Zhang *et al*. (2013) reported that the AOM obtained from a later phase of algal growth had higher affinity for the ceramic membrane compared with the AOM from an earlier growth phase, and that consequently led to more severe irreversible membrane fouling. There was probably a greater amount of AOM to which the d_3-geosmin could adsorb and/or AOM that could block the membrane pores in the stationary phase than in the logarithmic growth phase cyanobacteria, the result being enhanced adsorption and/or retention of the d_3-geosmin in the experiment with stationary phase cells, respectively. Because of this enhanced adsorption/retention, the extent of geosmin release was smaller from the stationary phase than the logarithmic growth phase culture, even though the extent of cell damage was almost the

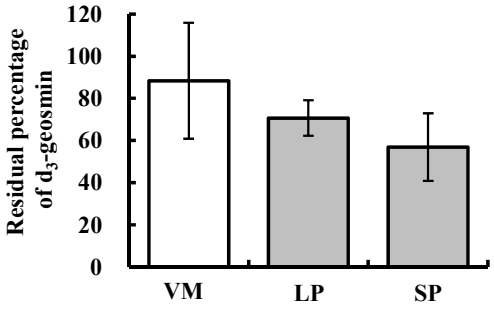


Fig. 5 Difference in adsorption of d_3-geosmin to the retentate that accumulated on the MF membrane. Error bars for virgin membrane (VM), logarithmic growth phase (LP) cells, and stationary phase (SP) cells indicate standard deviation of 2, 8, and 8 samples, respectively

same in the two phases. In actual drinking water treatment plants employing membrane processes, more attention should be paid to the cyanobacterial cells in logarithmic growth phase than in stationary phase from a viewpoint of preventing the leakage of intracellular earthy-musty odor compounds to finished water.

4. Conclusions

(1) The extent of cyanobacterial cell breakage on MF membranes was almost the same for logarithmic growth and stationary phase cells. The growth phase of the cyanobacteria did not affect cell breakage.
(2) Even though the extent of cell breakage was almost the same, the release of intracellular geosmin into the MF permeate was greater from the logarithmic growth phase than the stationary phase cells.
(3) Adsorption of geosmin to AOM retained on the MF membrane and/or the extent of pore blocking with AOM were greater for stationary phase versus logarithmic growth phase cells, the result being a decrease in the apparent release of intracellular geosmin from stationary phase cells.

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References

Ando, A., Miwa, M., Kajino, M. and Tatsumi, S. (1995), "Removal of musty-odorous compounds in water and retained in algal cells through water purification process", *Water Sci. Technol.*, **25**(2), 299-306.

Campinas, M. and Rosa, M.J. (2010), "Evaluation of cyanobacterial cells removal and lysis by ultrafiltration", *Separ. Purif. Technol.*, **70**(3), 345-353.

Chow, C.W.K., Panglisch, S., House, J., Drikas, M., Burch, M.D. and Gimbel, R. (1997), "A study of membrane filtration for the removal of cyanobacterial cells", *J. Water Suppl.: Res. Technol. – AQUA*, **46**(6), 324-334.

Chow, C.W.K., Drikas, M., House, J., Burch, M.D. and Velzeboer, R.M.A. (1999), "The impact of conventional water treatment processes on cells of the cyanobacterium *Microcystis aeruginosa*", *Water Res.*, **33**(15), 3253-3262.

Daly, R.I., Ho, L. and Brookes, J.D. (2007), "Effect of chlorination on *Microcystis aeruginosa* cell integrity and subsequent microcystin release and degradation", *Environ. Sci. Technol.*, **41**(12), 4447-4453.

Dixon, M.B., Richard, Y., Ho, L., Chow, C.W.K., O'Neill, B.K. and Newcombe, G. (2011), "A coagulation–powdered activated carbon–ultrafiltration – multiple barrier approach for removing toxins from two Australian cyanobacterial blooms", *J. Hazard. Mater.*, **186**(2-3), 1553-1559.

Gijsbertsen-Abrahamse, A.J., Schmidt, W., Chorus, I. and Heijman, S.G.J. (2006), "Removal of cyanotoxins by ultrafiltration and nanofiltration", *J. Membr. Sci.*, **276**(1-2), 252-259.

Hawkins, P.R., Putt, E., Falconer, I. and Humpage, A. (2001), "Phenotypical variation in a toxic strain of the phytoplankter, *cylindrospermopsis raciborskii* (nostocales, cyanophyceae) during batch culture", *Environ. Toxicol.*, **16**(6), 460-467.

Henderson, R., Parsons, S.A. and Jefferson, B. (2008), "The impact of algal properties and pre-oxidation on

solid–liquid separation of algae", *Water Res*., **42**(8-9), 1827-1845.
Huang, W.-J., Lai, C.-H. and Cheng, Y.-L. (2007), "Evaluation of extracellular products and mutagenicity in cyanobacteria cultures separated from a eutrophic reservoir", *Sci. Total Environ*., **377**(2-3), 214-223.
ISO (1992), ISO 10260, water quality – measurement of biochemical parameters – spectrometric determination of the chlorophyll-a concentration; ISO, Geneva, Switzerland.
Lalezary, S., Pirbazari, M. and McGuire, M.J. (1986), "Oxidation of five earthy-musty taste and odor compounds", *J. Am. Water Works Assoc*., **78**(3), 62-69.
Lam, A.K.Y., Prepas, E.E., Spink, D. and Hrudey, S.E. (1995), "Chemical control of hepatotoxic phytoplankton blooms: Implications for human health", *Water Res*., **29**(8), 1845-1854.
Li, L., Gao, N., Deng, Y., Yao, J. and Zhang, K. (2012), "Characterization of intracellular & extracellular algae organic matters (AOM) of *Microcystis aeruginosa* and formation of AOM-associated disinfection byproducts and odor & taste compounds", *Water Res*., **46**(4), 1233-1240.
Matsushita, T., Kobayashi, Y., Nakamura, K., Matsui, Y. and Ohno, K. (2010), "Effect of transmembrane pressure on geosmin release from cyanobacterial cell during microfiltration", *Water Practice Technol*., **5**(3), DOI: 10.2166/wpt.2010.049
Naes, H. and Post, A. (1988), "Transient states of geosmin, pigments, carbohydrates and proteins in continuous cultures of *Oscillatoria brevis* induced by changes in nitrogen supply", *Arch. Microbiol*., **150**(4), 333-337.
Peterson, H.G., Hrudey, S.E., Cantin, I.A., Perley, T.R. and Kenefick, S.L. (1995), "Physiological toxicity, cell membrane damage and the release of dissolved organic carbon and geosmin by *Aphanizomenon flos-aquae* after exposure to water treatment chemicals", *Water Res*., **29**(6), 1515-1523.
Pietsch, J., Bornmann, K. and Schmidt, W. (2002), "Relevance of intra- and extracellular cyanotoxins for drinking water treatment", *Acta Hydroch. et Hydrobiol*., **30**(1), 7-15.
Pivokonsky, M., Kloucek, O. and Pivokonska, L. (2006), "Evaluation of the production, composition and aluminum and iron complexation of algogenic organic matter", *Water Res*., **40**(16), 3045-3052.
Qu, F., Liang, H., He, J., Ma, J., Wang, Z., Yu, H. and Li, G. (2012), "Characterization of dissolved extracellular organic matter (DEOM) and bound extracellular organic matter (BEOM) of *Microcystis aeruginosa* and their impacts on UF membrane fouling", *Water Res*., **46**(9), 2881-2890.
Saadoun, I.M.K., Schrader, K.K. and Blevins, W.T. (2001), "Environmental and nutritional factors affecting geosmin synthesis by *Anabaena* SP.", *Water Research*, **35**(5), 1209-1218.
Saker, M.L., Neilan, B.A. and Griffiths, D.J. (1999), "Two morphological forms of *Cylindrospermopsis raciborskii* (cyanobacteria) isolated from Solomon dam, Palm island, Queensland", *J. Phycol*., **35**(3), 599-606.
Sano, D., Ishifuji, S., Sato, Y., Imae, Y., Takaara, T., Masago, Y. and Omura, T. (2011), "Identification and characterization of coagulation inhibitor proteins derived from cyanobacterium *Microcystis aeruginosa*", *Chemosphere*, **82**(8), 1096-1102.
Sato, M., Murata, Y., Mizusawa, M., Iwahashi, H. and Oka, S. (2004), "A simple and rapid dual-fluorescence viability assay for microalgae", *Microbiol. Culture Collect*., **20**(2), 53-59.
Sorlini, S., Gialdini, F. and Collivignarelli, C. (2013), "Removal of cyanobacterial cells and microcystin-LR from drinking water using a hollow fiber microfiltration pilot plant", *Desalination*, **309**, 106-112.
Srinivasan, R. and Sorial, G.A. (2011), "Treatment of taste and odor causing compounds 2-methyl isoborneol and geosmin in drinking water: A critical review", *J. Environ. Sci*., **23**(1), 1-13.
Sun, F., Pei, H.-Y., Hu, W.-R. and Ma, C.-X. (2012), "The lysis of *Microcystis aeruginosa* in $AlCl_3$ coagulation and sedimentation processes", *Chem. Eng. J*., **193-194**, 196-202.
Waer, M.A. (2006), "Removal of musty-odorous compounds in water and retained in algal cells through water purification process", *Opflow*, **32**(6), 3-6.
Watanabe, M.M. and Ichimura, T. (1977), "Fresh- and salt-water forms of *Spirulina platensis* in axenic cultures", *The Bulletin of Japanese Society of Phycology*, **25**(suppl.), pp. 371-377.
Widrig, D.L., Gray, K.A. and McAuliffe, K.S. (1996), "Removal of algal-derived organic material by preozonation and coagulation: Monitoring changes in organic quality by pyrolysis-GC-MS", *Water Res*., **30**(11), 2621-2632.

Zamyadi, A., Ho, L., Newcombe, G., Bustamante, H. and Prévost, M. (2012) "Fate of toxic cyanobacterial cells and disinfection by-products formation after chlorination", *Water Res*., **46**(5), 1524-1535.
Zhang, X., Fan, L. and Roddick, F.A. (2013), "Influence of the characteristics of soluble algal organic matter released from *Microcystis aeruginosa* on the fouling of a ceramic microfiltration membrane", *J. Membr. Sci*., **425-426**, 23-29.

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