

Degradation potential of musty odour in a drinking water source by a biofilm method

Norio Sugiura, Hiroko Isoda and Takaaki Maekawa

ABSTRACT

The purpose of this study was to evaluate the degradation potential of 2-methylisoborneol (MIB) and its producer by a biofilm process. The cyanobacteria *Phormidium tenue* was isolated from the biofilm in a biological treatment facility for water supply. MIB was efficiently removed by the facility and the removal ratio was about 70% during the investigation period of 2 years. There was a favourable correlation between the removal rate of *P. tenue* and the number of micro-organisms, especially ciliates on the biofilm. But a high rise of pH value due to sudden proliferation of cyanobacteria induced a reduction in removal of *P. tenue* and MIB. It was found that the ciliate protozoa and pH value were very important factors for the removal of MIB and *P. tenue*.

Key words | biofilm, cyanobacteria, degradation, 2-methylisoborneol (MIB), musty odour, micro-organism

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INTRODUCTION

The occurrence of musty odour in water supplies is mainly due to the presence of two compounds, 2-methylisoborneol (MIB) and geosmin, which are produced by actinomycetes and filamentous cyanobacteria (Tabachek *et al.* 1975; Gerber 1983). In 1993, complaints regarding drinking water containing nuisance odours reached over 20 million in Japan. However, these offensive musty odorous compounds are difficult to remove by conventional water treatment methods. In 1993, the guidelines for MIB and geosmin in drinking water were recommended to be decreased to below 20 ng l⁻¹ for the powdered activated carbon (PAC) treatment method, and to below 10 ng l⁻¹ for the granular activated carbon (GAC) treatment method, respectively by The Welfare Ministry of Japan 1993). To improve polluted water, various treatment processes have been employed in water purification plants. Oxidation treatments based on addition of chlorine or potassium permanganate, or the use of ozonation have been commonly applied, but these treatments caused serious problems such as increases in organic chlorine compounds like trihalomethanes (THM). Peroxide compounds are suspected carcinogens.

In adsorption treatment, PAC and GAC are widely used because MIB and geosmin are efficiently removed over a short time (Lalezary 1986).

More activated carbon (AC) can also favourably adsorb other naturally occurring dissolved organic carbon compounds (DOC), such as humic and fulvic acids, metabolites from algae, and detritus substances from algae. Therefore, in the presence of these competitive substances, the removal capacity for the musty odour compounds can be significantly reduced, resulting in a shortage of AC capacity due to rapid saturation (Sugiura *et al.* 1997a). Consequently, the need to increase the injection amount, and frequent exchanges of AC leads to operational difficulties and elevates costs. Biological treatment has been introduced in Western Europe and Japan, since it was shown that biological activated carbon, sand filtration, stone, and expanded plastic media with aeration were clearly effective for the removal of dissolved organic substances including nuisance tastes and odorous compounds (Rittmann 1990).

Although the use of biofiltration processes is not so prevalent in Japanese drinking water treatment plants,

strengthening of standards for THM, organohalides and other water treatment by-products, and also stricter standards for tastes and odorous compounds will accelerate the employment of biological treatment. Since the 1970s, the eutrophicated Lake Kasumigaura, which is used as a drinking water source, has annually experienced the occurrence of musty odour episodes caused by proliferation of the filamentous cyanobacteria *Phormidium tenue* during spring and fall, and the growth of actinomycetes of the genus *Streptomyces* in the lake sediment from summer to fall (Yagi 1985; Sugiura *et al.* 1987).

In Kasumigaura water purification plant, biological treatment combined with a conventional treatment process was conducted in 1985. As a result, the musty odour has been effectively removed by the biological treatment. However, the role and function of biofilms associated with odour reduction and the roles of micro-organisms in such biofilms remain unclear.

The purpose of this study was to evaluate the removal efficiency and degradation potential by a biofilm of *P. tenue* and its metabolite, MIB.

MATERIALS AND METHODS

Biological treatment facility

A schematic diagram of the biological treatment facility and the contact material for the biofilm is shown in Figure 1. Daily maximum water supply quantity to the facility is $160,000 \text{ m}^3 \text{ d}^{-1}$ with a hydraulic retention time of 2 h. The facility was packed with a honeycomb tube as a non-adsorbing contact material, which was made of polyvinyl chloride with a thickness of 0.1 mm and a 13-mm cell size. The raw water was taken from Kihara area of Lake Kasumigara and continuously fed to the plant. The experiment was conducted from April 1992 to September 1993. Sampling was carried out two or three times every month.

Test for degradation potential of the biofilm

The extent of MIB degradation by the biofilm was expressed as degradation potential. Samples of the biofilm were scraped from the biological treatment facility on

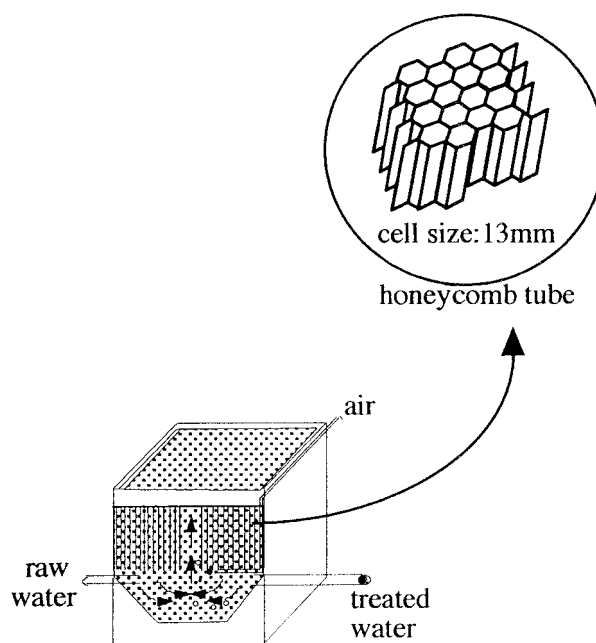


Figure 1 | Schematic diagram of biological treatment facility.

10 May 1992. Each sample of the biofilm, ranging from 100 mg l^{-1} to 900 mg l^{-1} , was inoculated into two series of 150 ml vials containing 100 ml of MIB solution ($500 \mu\text{g l}^{-1}$), adjusting with sterile deionized water through membrane filter (pore size: $0.2 \mu\text{m}$, Millipore Co.). In the test for the time course of MIB degradation by the biofilm, the initial inoculum amount of the biofilm was settled to 500 mg l^{-1} as wet weight, while in the test on effect of biofilm amount and bacterial number on the removal of MIB, the initial inoculum amount was adjusted to 100 mg l^{-1} , 200 mg l^{-1} , 500 mg l^{-1} and 900 mg l^{-1} , respectively. The vials were sealed with an aluminium stopper and automatically gyrated at 100 rpm in a 25°C water bath for 7 d. In the test for seasonal MIB removal by biofilm, the experimental method was performed by the same method described above, but the concentration of biofilm was adjusted to 500 mg l^{-1} .

Analysis and enumeration of micro-organisms

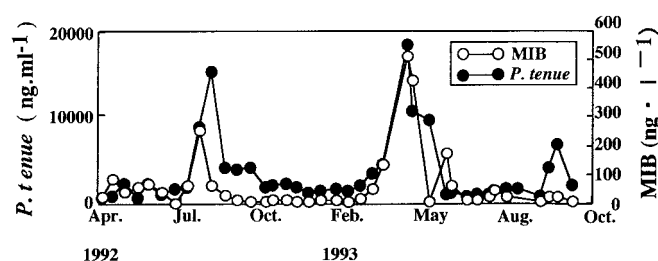
MIB concentration was quantitatively analysed with a GC/MS (Hewlett Packard 5890) equipped with a purge

Table 1 | Analytical conditions for MIB by GC/MS

Item	Condition	
Column	NB-130 m	
Detector	GC/MSD	
Carrier gas	He 1 ml/min	
MS interface temp.	280°C	
Purge and trap	Teckmer LSC-3000	
Sample vol.	20 ml	
Trap material	Tenax 1	
Standby temp.	40°C	
Purge time	12 min	
Dry purge	1 min	
Desorb.	6 min	
Cool down	-50°C	
Inject	4 min, 200°C	
Bake	10 min, 210°C	
Oven programme		
Initial temp.: 50°C, Time: 2 min		
Rate (°C/min)	Final temp. (°C)	Final time (min)
10	190	0
20	280	3

and trap apparatus (Tekmer LSC-3000). Analytical conditions are shown in Table 1. MIB concentration was determined by comparison with a calibration curve for characteristics mass fragment peaks (m/z : 95, 108, 135) of the sample source. The MIB detection limit was 1 ng l^{-1} .

Identification and enumeration of *P. tenue* and micro-organisms, respectively, were performed by a photomicroscope. The isolated monoxenic strains of *P. tenue*

**Figure 2** | Seasonal fluctuation of *Phormidium tenue* and MIB concentration in Lake Kasumigaura.

were cultivated in M-11 medium with the same method described in a previous report (Sugiura 1986), followed by a check for the odorous compound with GC/MS under the same analytical conditions described above. Heterotrophic bacteria were cultivated in a duplicate petri dish in nutrient agar at 25°C for 7 d and were enumerated by the colony forming unit (CFU) method.

RESULTS AND DISCUSSION

The Kasumigaura Water Purification Plant has suffered from continuous musty odour occurrence, resulting in many complaints from consumers. To take measures at the water source, algal fluctuations in the lake have been periodically investigated. From 1992 to 1993, *P. tenue* was always present, though with cell densities from 20 to $18,000 \text{ ng} \cdot \text{ml}^{-1}$ (Figure 2). The maximum cell density in 1992, $15,000 \text{ ng} \cdot \text{ml}^{-1}$, was observed in August, while in 1993 the maximum, $18,000 \text{ ng} \cdot \text{ml}^{-1}$, was observed in March. As growth and change of relative prevalence of algal species in shallow lakes commonly depend on water temperature, we investigate the correlation between the growth of *P. tenue* and water temperature. However, we found no correlation.

The concentration of the odour-causing compound MIB ranged from 5 to 530 ng l^{-1} in raw water from 1992 to 1993 and the highest peak was detected in March 1993, coinciding with the highest density of *P. tenue*. In previous

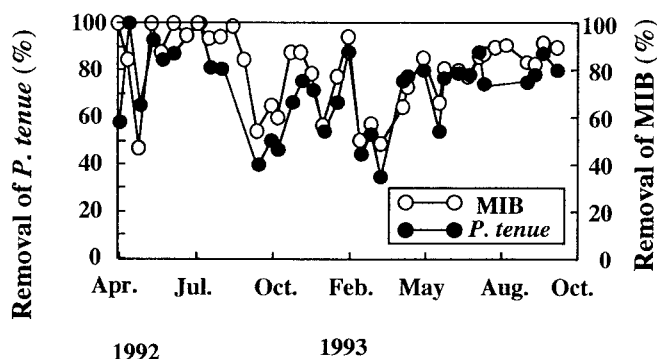


Figure 3 | Removal of *Phormidium tenue* and MIB concentration in a biological treatment facility.

in vitro studies the authors confirmed that the isolated strain produced only MIB in culture. Therefore, we concluded that *P. tenue* exclusively contributed to the *in situ* production of MIB. Given the musty odour occurrence and *P. tenue*'s role in producing MIB, the dominant odoriferous compound, the importance of reducing *P. tenue* from the water supply is understandable.

The waterworks used the biological treatment facility as a means of pretreatment so that the musty odour nuisance, as well as algae from the water source, could not be discharged through the following conventional treatment processes. Removal of *P. tenue* ranged from 47 to 100% (avg. 75.6%) and that of MIB from 41 to 100% (avg. 68.5%) (Figure 3). After April 1993, the removal efficiency of both parameters was higher than that of the previous same season.

This biofilm process with the honeycomb tube reduced *P. tenue* and the musty odour of MIB, although the removal ratio became low in October 1992 and March 1993. The lower removal might be caused by the detachment of functional micro-organisms on the biofilm contributing to the predation and degradation of *P. tenue* and its metabolite, MIB, because the thickness of biofilm was very thin at this period.

In order to understand the removal process of *P. tenue* and MIB, it is necessary to examine which factors are correlated with the removal in the biological treatment apparatus. The *P. tenue* removal velocity was calculated from the following formula:

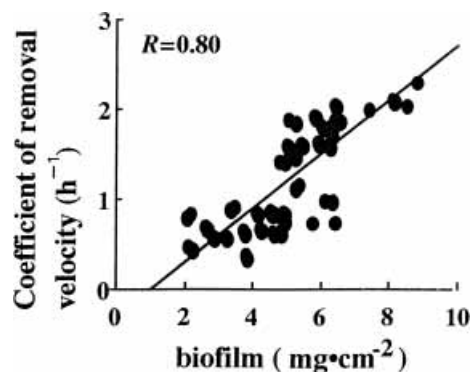


Figure 4 | Correlation between removal velocity of *Phormidium tenue* and biofilm amount.

$$N = N\theta \cdot e^{-kt}$$

$N\theta$: *P. tenue* in raw water ($\text{ng} \cdot \text{ml}^{-1}$),

N : *P. tenue* in treated water ($\text{ng} \cdot \text{ml}^{-1}$),

t : retention time,

k : removal velocity coefficient.

The *P. tenue* removal velocity increased with increase of biofilm density (Figure 4).

The correlation coefficient R was 0.80, which showed a high positive correlation. The removal velocity reached a maximum of 2.0 h^{-1} when the biofilm density was 8.1 mg cm^{-2} . Thus, the biofilm density was closely related to the degradation of *P. tenue*.

Various micro-organisms in the biofilm may contribute to predation and degradation of *P. tenue* and its metabolite MIB. Therefore, it is necessary to obtain information about which factors in the biofilm are correlated with the removal of *P. tenue*. A good correlation was observed between the removal velocity of *P. tenue* and the number of micro-organisms in the biofilm (Figure 5). The maximum removal velocity reached 2.0 h^{-1} , when micro-organism density reached 840 cm^{-2} .

The high correlation suggests that micro-organisms in the biofilm greatly contributed to predation on *P. tenue*.

Moreover, a high positive correlation was observed between the removal velocity of *P. tenue* and the number of ciliates (Figure 6). Among these micro-organisms, ciliated protozoa were most frequently observed under favourable conditions for the removal, ciliate species,

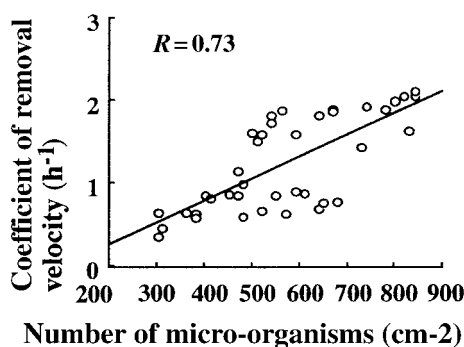


Figure 5 | Correlation between removal velocity of *Phormidium tenue* and micro-organisms.

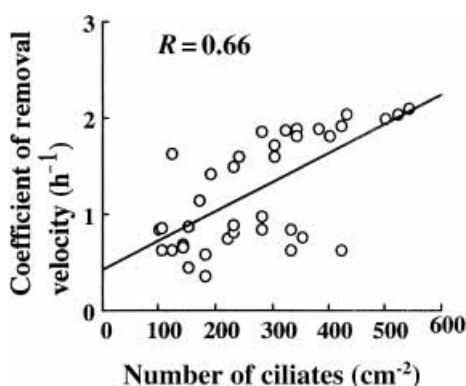


Figure 6 | Correlation between removal velocity of *Phormidium tenue* and ciliates.

such as *Tetrahymena*, *Holostichia*, *Stylonichia* and *Pleuronema*, were frequently observed in the biofilm (Ouchiyama 1995).

As a result, our sequential observation led us to conclude that the ciliates played an important role in the degradation. To establish efficient removal conditions for the musty odour occurrence, it is important to get information about the degradation potential of MIB by the biofilm. We investigated the seasonal effect of the biofilm's MIB degradation potential in detail (Figure 7).

MIB was effectively degraded over a time course of 7 d and the reduction rate was the highest at 7 d. After 7 d, MIB was not reduced below $110 \mu\text{g l}^{-1}$. It was not clear why a high concentration of MIB was maintained; some nutrients needed for the degradation by micro-organisms, especially bacteria, might not be in adequate supply.

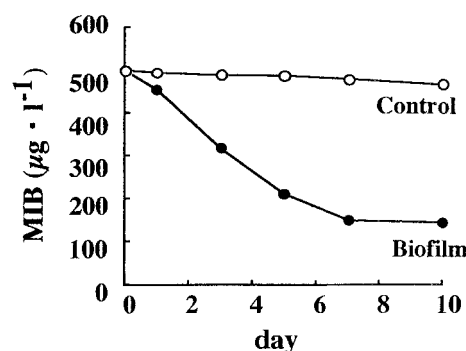


Figure 7 | Time course of MIB degradation by biofilm. Initial inoculum amount of biofilm: 500 mg l^{-1} .

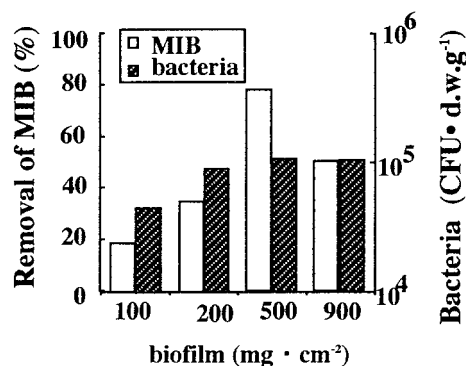


Figure 8 | Effect of biofilm amount and bacteria numbers on removal of MIB.

We fixed the inoculation time for degradation potential experiments at 7 d.

The greatest MIB removal for a 7 d incubation was observed in the 500 mg l^{-1} inoculum (Figure 8).

MIB degradation potential in the bioreactor with the maximum amount of biofilm (900 mg l^{-1}) was explicitly lower than that with 500 mg l^{-1} . The lower removal ratio might be due to the reduction of degradation activation of MIB by bacteria owing to the higher number of coexisting micro-organisms in the inoculum of 900 mg l^{-1} . The biofilm sampled in April and December degraded more than 90% of MIB, but those sampled in August and October degraded only 60–70% (Figure 9). The lower removal ratio by the biofilm sampled in summer and autumn in 1992 might be caused by high pH owing to the proliferation of cyanobacteria, *Microcystis* species. Subsequently, the relationship between the pH of the

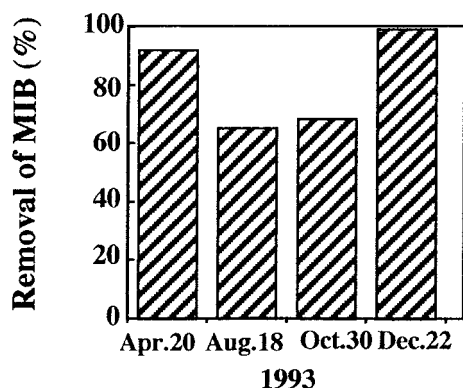


Figure 9 | Seasonal effect of biofilm on removal of MIB.

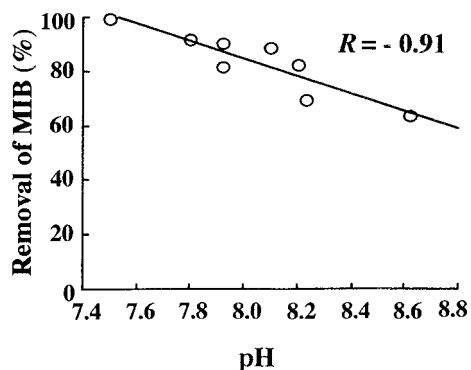


Figure 10 | Effect of pH on removal of MIB.

water in the biological treatment and the removal of MIB was surveyed (Figure 10).

MIB degradation decreased with the increase in pH value. Moreover, microscopic observation demonstrated that the rise of pH induced a decrease in the ciliate species in the biofilm. We concluded that ciliated protozoa and pH were very important factors for the efficient removal of *P. tenue* and its metabolite, MIB, in the biofilm process. Therefore, these findings would contribute to the determination of suitable operational conditions for biofilm treatment and the prevention of musty odour occurrence.

Inamori *et al.* (1990) showed that a ciliated *Trichymostoma cucullulus* actively ingested the strain, resulting in a reduction of MIB by over 20% in a continuous culture experiment for a retention time of 48 h. Sugiura *et al.* (1997b) showed that the isolated micro-flagellate, *Monas guttula*, from the same biological plant

effectively grazed on viable *P. tenue* (98% removal ratio) and degraded MIB (42% removal in 48 h in batch culture experiment). In the report of Izaguirre *et al.* (1988), MIB was degraded efficiently in a culture consisting of various bacterial communities but reconstituting the degradation activity by mixing pure cultures of the bacteria failed. Thus, *in vitro* studies have provided some information about the removal and degradation characteristics of *P. tenue* and MIB by micro-organisms and bacteria. Therefore, it was considered that in the biofilm process *P. tenue* and its intracellular MIB were mainly predated and degraded by protozoa such as ciliates, subsequently the MIB released from *P. tenue* could be assimilated by the aggregated bacteria on the biofilm in the biological treatment facility. However, practical assessments of the removal efficiency and the role of the microbial community are urgently required because utilization of water sources for various purposes is ahead of theoretical elucidation.

Although we indicated the importance of two factors, such as ciliate density on the biofilm and pH value in the biofilm process, development of more effective biological treatment will be facilitated by *in vitro* and *in situ* studies to explore the effects of physical, chemical, other biological factors and carriers for inhabiting micro-organisms, as well as degradation characteristics of micro-organisms in biofilms.

CONCLUSIONS

1. The concentration of the musty odorous compound, MIB, was closely correlated with the growth of *P. tenue* in Lake Kasumigaura and these were efficiently removed by biological treatment combined with conventional water supply treatment.
2. In the removal of *P. tenue*, there was a highly positive correlation between the removal velocity of *P. tenue* and micro-organisms, especially ciliate species on the biofilm in the biological treatment facility.
3. The biofilm during spring and winter in 1992 rapidly degraded MIB.

4. MIB removal ratio was reduced by high pH values due to proliferation of cyanobacteria, such as *Microcystis* and *Oscillatoria*.
5. It was found that abundant ciliate species on the biofilm and pH near neutral were very favourable factors for efficient removal of MIB and *P. tenue*.

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First received 17 September 2001; accepted in revised form 1 February 2002