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Biosensing of an indoor volatile organic compound on the basis of fungal growth

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ABSTRACT

A fungal biosensor plate was applied to assessment of the harmfulness of air polluted by formaldehyde. *Alternaria alternata*, *Eurotium herbariorum* and *Aspergillus penicillioides* were used as fungal species. Fungal mycelium length and optical transparency of the biosensor plate were employed as indices of the fungal growth. Formaldehyde in air was detected on the basis of growth inhibition, reflected by suppression of the growth indices. Dynamic range of the measurement was 700–4000 μg m^{-3} or broader. *Eurotium herbariorum* and *Aspergillus penicillioides* were the most suitable fungal species to formaldehyde sensing based on the mycelium length and that based on the transparency, respectively.

1. Introduction

Certain types of chemical substances contained in indoor air are known to cause the sick building syndrome. Thus, those substances need to be detected since they can affect the health of people who are exposed to them for a long period of time (WHO, 1987; Burrell, 1991; Berglund et al., 1992; Hays, 1995; Burr, 1997; Wilke et al., 2004). Accurate qualitative and quantitative analysis of formaldehyde, toluene and other VOCs (volatile organic compounds) of low concentrations is possible by using HPLC (high performance liquid chromatography) and GC/MS (gas chromatography/mass spectrometry). However, these methods have some drawbacks like high cost and burdensome procedure (Gritter et al., 1985). On the other hand, various types of biosensors using microorganisms or living cells have been developed as a technique to assess the general toxicities of harmful substances. The biosensors monitor a cellular activity such as a respiratory or photosynthetic activity, which is inhibited by a toxic substance. Although it is relatively difficult with the biosensors to identify the toxic substance present in a sample and to determine its concentration, those allow measuring toxicity itself. The biosensors are therefore suitable for quick screening of samples in terms of harmfulness. Many of these biosensors are, however, designed to detect toxic substances in liquids (Pandard and Rawson, 1993; Nakanishi et al., 1996; Yamasaki et al., 2004; Shitanda et al., 2005; Maruyama et al., 2006): there are not so many biosensors for the use in air. In this study, to develop a sensor for the gas phase monitoring, we used fungi that live even in air.

We employed formaldehyde as a possible pollutant in indoor air. By passing air containing formaldehyde through a test chamber, we studied its influence on the growth of fungi in the chamber and collected basic data about the fungal biosensors. As a simple method for evaluating the fungal growth, we employed UV (ultraviolet)–visible spectrophotometry. The ultimate objective of this study was to shed light on the relationship between formaldehyde concentration in room air and growth rate of fungi and to develop a simple, effective method of measuring harmful compounds in the gas phase.

2. Materials and methods

2.1. Fungal biosensor plates

Fungal plates were obtained from Institute of Environmental Biology, Japan. The plates have been developed to assess risks of indoor propagation of fungi (Abe, 1993; Abe et al., 1996). In the present work, we used the plates to monitor behavior of fungi that were exposed to formaldehyde. Dry spores of three species of fungi (i.e. *Alternaria alternata*, which is a hygrophilic fungus, *Eurotium herbariorum*, which is less hygrophilic and grows in the humidity range 73–95%, and *Aspergillus penicillioides*, which is xerophilic) were inserted with nutriments between an optically transparent plastic plate and an air-permeable, transparent film (Fig. 1). Spores of each fungal species were dispersed within a circle with 2.8 mm

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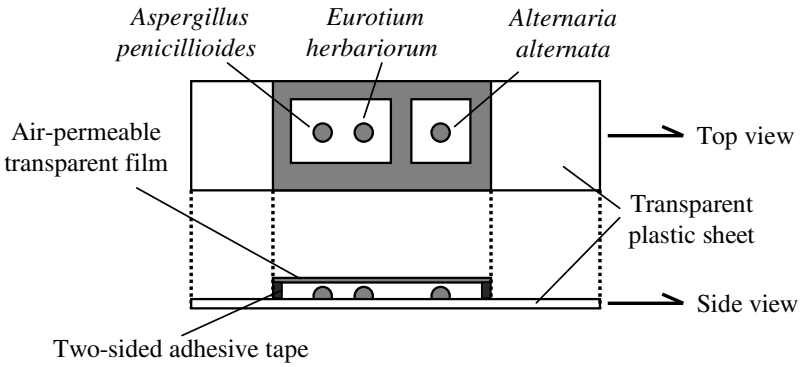

Fig. 1. Outline of the fungal biosensor plate.

diameter (3.5×10^3 spores in each circle). After growth of mycelia, their lengths were measured on the basis of micrographs. The mycelium length was defined in the present work to be the distance from the edge of the circle to the tip of the mycelium as shown in Fig. 2. The "growth index based on mycelium length", L was defined to be average length of the five longest mycelia.

2.2. Test chambers

In this study, a couple of 20-l test chambers (ADPAC: ADvanced Pollution and Air quality Chamber) as shown in Fig. 3 were used to expose the fungal biosensor plate to gaseous samples. The test chamber ADPAC is an apparatus used to measure the amounts of formaldehyde and other VOCs emitted from building materials (Funaki and Tanabe, 2002; Funaki et al., 2003). The basic performances of the test chamber conform to ECA (European Joint Research Report) (ECA, 1991), ISO (International Organization for Standardization) (ISO 16000-9, 2006) and JIS (Japanese Industrial Standards) (JIS A 1901, 2003). The present experimental system consists of two chambers made of stainless steel (SUS 304) and systems for supplying air to the chambers and controlling the humidity of air (Fig. 3). The test chamber consists of a bucket-type container, a chamber cover and a Teflon packing. The chamber was installed in a thermostatic tank for temperature control. The twin chamber system was employed to compare the responses from two biosensor plates, one of which was exposed to polluted air and the other was to clean air.

2.3. Measurements

In this work, we evaluated how formaldehyde influenced the growth of fungi in the biosensor plate. The experiments were carried out three times for each case.

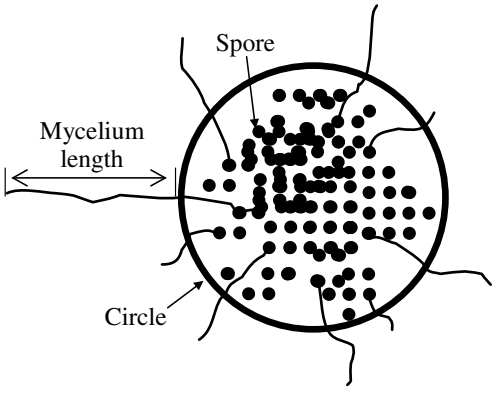

Fig. 2. Definition of the mycelium length.

The chambers were cleaned and heated to 260 °C to remove adsorbed species prior to measurements. We checked that the background concentration of formaldehyde was sufficiently low. Two pieces of the biosensor plate with three species of fungi were placed on a sample stage in each chamber as shown in Fig. 3. Clean air was supplied to one of the test chambers and the formaldehyde of a certain concentration was supplied to the other test chamber. The formaldehyde was supplied as a mixture of 20 ppm formaldehyde in a pure nitrogen and clean air. The air exchange rate was 0.5 h^{-1}, corresponding to the ventilation rate of 0.17 l min^{-1}. At the inlet and outlet of each test chamber, we checked the temperature, humidity and formaldehyde concentration during the measurement. The temperature and relative humidity were maintained at 28 °C and 90% or 80%, respectively. The concentration of the supplied formaldehyde was 0, 100, 700, 2000 or 4000 μg m^{-3} (maintained within ±5% [Ataka et al., 2005]).

Formaldehyde was adsorbed onto a DNPH cartridge (sampling volume = 10 l; Waters, USA) and the concentration of formaldehyde desorbed from the chamber was measured by a HP 1100 HPLC system with an Eclipse XDB-C8 250 × 4.6 mm, 5 μm column and an UV detector of 360 nm wavelength (Agilent Technologies, USA). The mobile phase was a mixture (1:1 by volume) of acetonitrile (HPLC grade, Wako, Japan) and water (purified using a Milli-Q water system, Millipore, USA) and the flow rate was 1 ml min^{-1}. The column teperature was 40 °C and the injection volume was 10 μl. The lower quantitation limit and the lower detection limit were 4.0 and 1.2 μg m^{-3} estimated as 10 and 3 times the standard deviation for the blank of formaldehyde in five DNPH cartridges, respectively. Five-point calibration curves were made for each analysis series. Intercepts of the calibration curves were almost zero and the r^2 values were over 0.999.

Each biosensor plate was taken out of the chamber every 24 h and the fungal growth was observed by an optical microscope (BX51 with a 4 × objective lens and FX380 5 × CCD, Olympus, Japan) or a UV–visible spectrophotometer (Model V-560, JASCO, Japan). The mycelium lengths and the growth index L were evaluated on the basis of the micrographs as described above (see Section 2.1.). Absorbance of the intact biosensor plate was measured by the spectrophotometer at 500 nm. The fungal growth is expected to decrease light transmission of the biosensor plate, giving rise to an absorbance increase.

3. Results and discussion

3.1. Relationship between formaldehyde concentration and mycelium length

Growth of the fungi was observed at different formaldehyde concentrations. Temperature was 28 °C and relative humidity

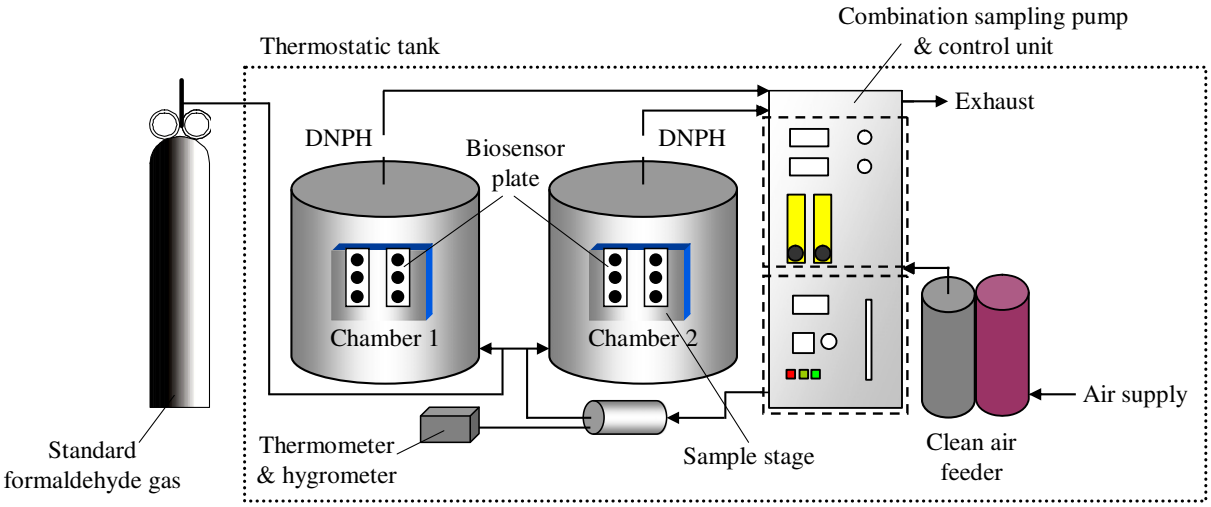

Fig. 3. Experimental system.

was 90% or 80%. Fig. 4 shows typical results for *Aspergillus penicillioides* at 90% relative humidity. A portion of a typical sample selected from six samples (two biosensor plates × three runs; see Section 2.3) is shown in each micrograph. The fungal growth was obviously inhibited by formaldehyde at 700 μg m^{-3} or higher concentrations, although the inhibition was negligible at 100 μg m^{-3} (data not shown).

In order to quantify the inhibition effect, we evaluated average length of the five longest mycelia as the "growth index based on mycelium length", *L*. Changes in the indices of the three types of fungi at different formaldehyde concentrations at 90% relative humidity are shown in Fig. 5. Each data point and each error bar correspond to average and standard deviation ($n = 6$), respectively. Mycelial growth was obvious for the three types of fungi, in the absence of formaldehyde. However, the growth was inhibited in the presence of formaldehyde ($\geqslant$700 μg m^{-3}). The inhibition effect was enhanced as the formaldehyde concentration increased, and the growth was completely inhibited in the presence of 4000 μg m^{-3} formaldehyde, at least for four days. At each formaldehyde concentration, *Eurotium herbariorum* showed the most conspicuous growth, followed by *Aspergillus penicillioides* and *Alternaria alternata* in that order.

The graphs in Fig. 6 were depicted on the basis of the data collected on the third day of the experiment to make the comparison easier. The index *L* and mycelium length-based growth inhibition ratio I_L are plotted against the formaldehyde concentration in Fig. 6a and b, respectively. I_L is defined by Eq. (1).

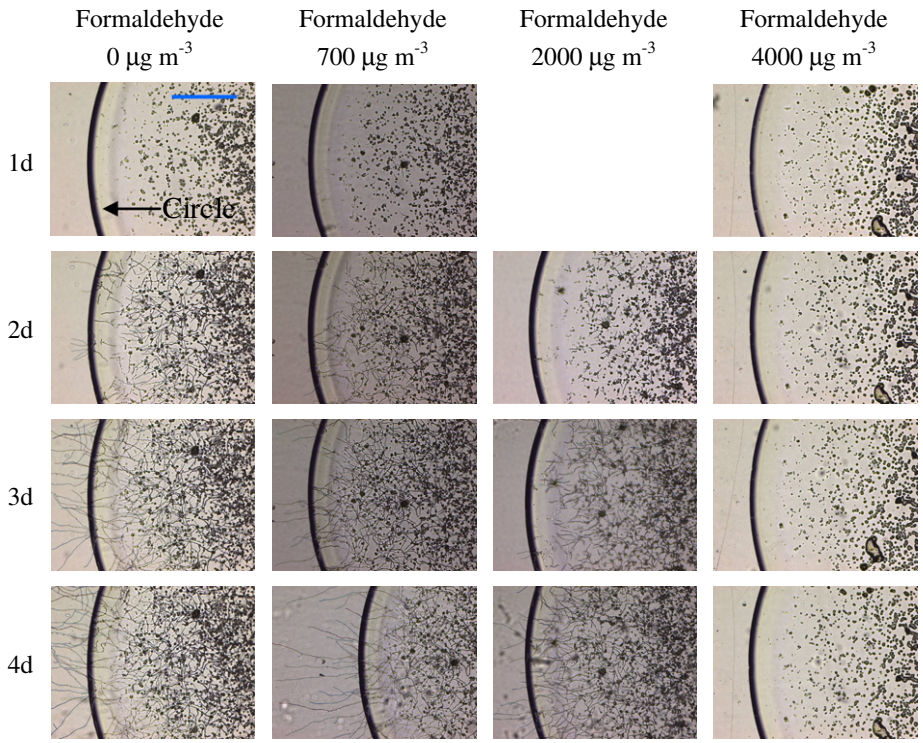

Fig. 4. Growth of *Aspergillus penicillioides* with lapse of time in the presence of 0–4,000 μg m^{-3} of formaldehyde. Temperature was 28 °C and relative humidity was 90%.

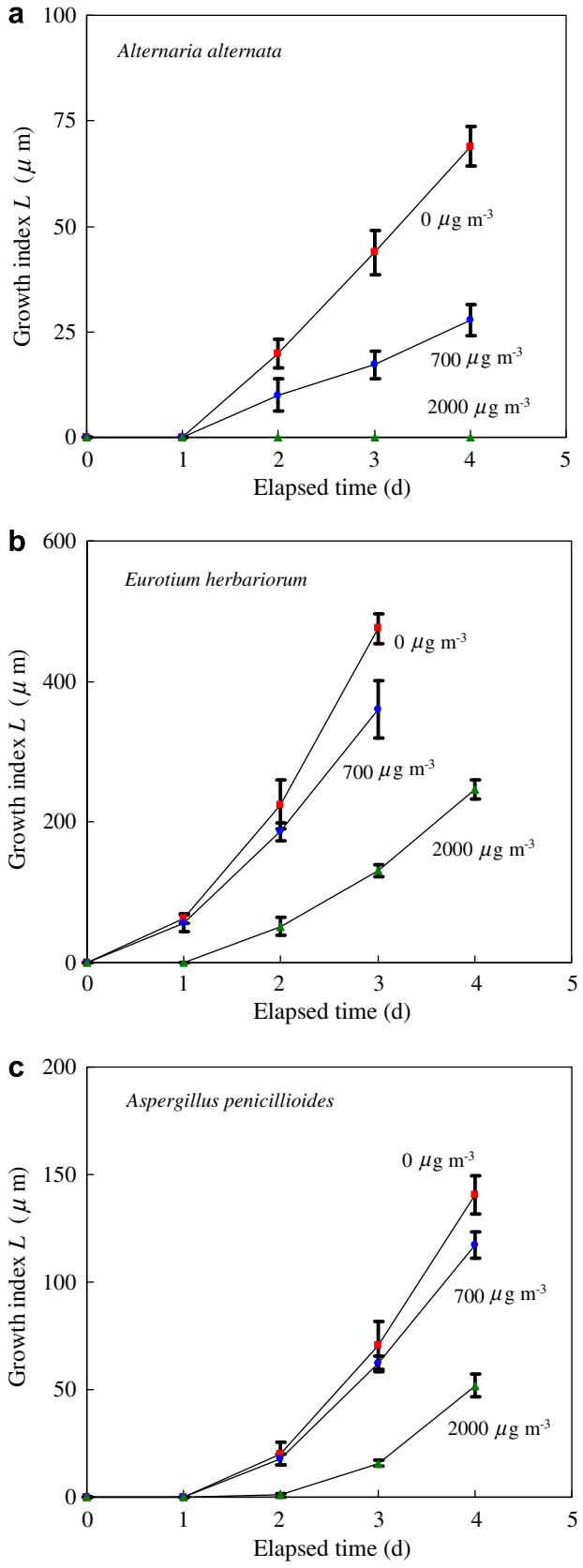

Fig. 5. Mycelial growth of the three fungal species in the presence of 0–2,000 μg m^{-3} of formaldehyde at 28 °C, 90% relative humidity. Growth index L is the average length of the five longest mycelia (see Section 2.1).

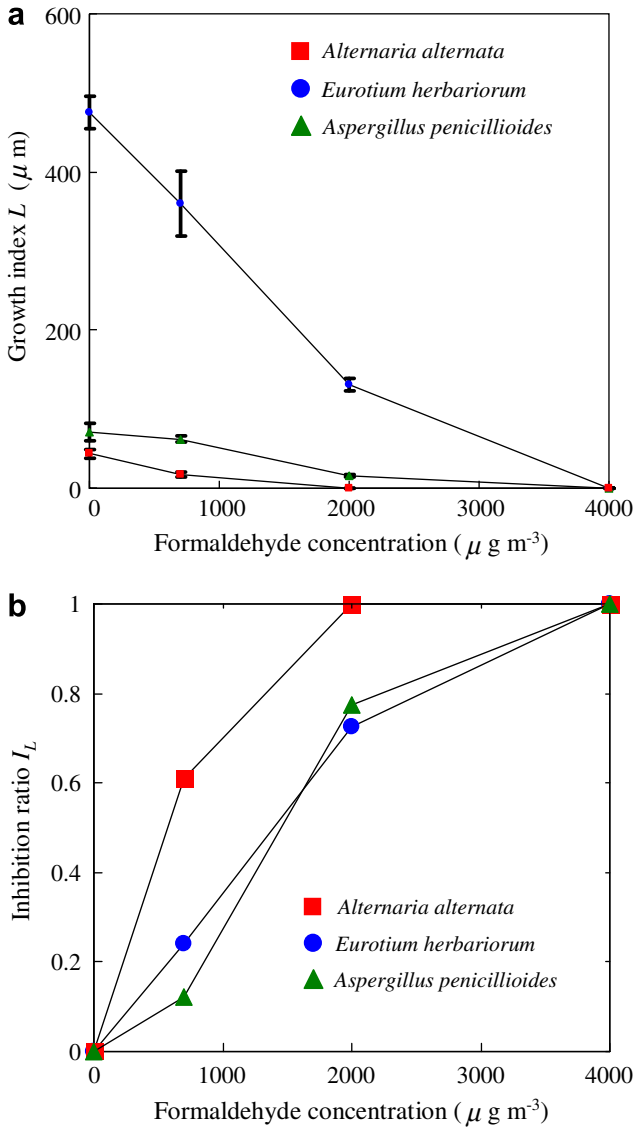

Fig. 6. (a) Growth index L and (b) mycelium length-based growth inhibition ratio I_L (= (L_0–L)/L_0), where L_0 is the growth index in the absence of formaldehyde, as functions of the formaldehyde concentration. The data were calculated from those in Fig. 4 on the third day of the incubation.

$$I_L = \frac{(L_0 - L)}{L_0} \quad (1)$$

where L_0 is the growth index in the absence of formaldehyde.

Fig. 6b indicates that *Alternaria alternata* is the most sensitive fungal species to formaldehyde among the three species examined. However, it grows very slowly, as seen in Fig. 5a. Therefore, *Eurotium herbariorum*, which grows promptly and shows moderate sensitivity, may be the most appropriate species for the detection of formaldehyde on the basis of the microscopic monitoring.

At 80% relative humidity, fungal growth was very slow even in the absence of formaldehyde. On the third day of the experiment, no significant mycelial growth was observed for the three types of fungi. The growth was evident on the fourth day, only for *Eurotium herbariorum* and *Aspergillus penicillioides*. In the presence of 700 μg m^{-3} of formaldehyde, fungal growth was not observed for the three species employed. Thus, it is important to control the relative humidity in the use of the fungal biosensors. For practical

measurements, air may be saturated with moisture so as to keep a high, constant humidity.

3.2. Relationship between formaldehyde concentration and absorbance

The inhibition effect of VOCs such as formaldehyde on fungal growth can thus be evaluated on the basis of the mycelium length. However, the measurement of the precise lengths of several mycelia takes time and effort of an operator. Therefore, as an alternative index, we measured an "absorbance increase of the fungal biosensor plate", A, because we expect that the growth of fungal mycelia should increase absorption and scattering of incident light.

Fig. 7a and b shows dependencies on the formaldehyde concentration of the absorbance increase A at 500 nm and absorbance-based growth inhibition ratio, I_A for *Alternaria alternata* and *Aspergillus penicillioides*. I_A is defined by Eq. (2).

$$I_A = \frac{(A_0 - A)}{A_0} \tag{2}$$

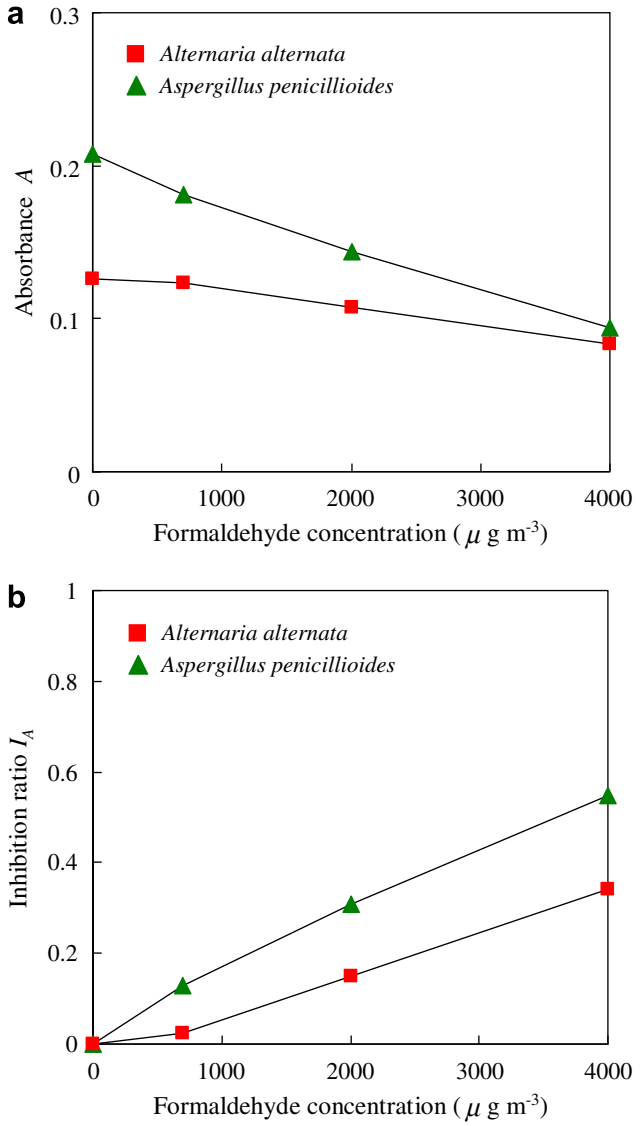

Fig. 7. (a) Absorbance increase A of the biosensor plate at 500 nm and (b) absorbance-based growth inhibition ratio I_A (= $(A_0–A)/A_0$), where A_0 is the absorbance increase in the absence of formaldehyde, as functions of the formaldehyde concentration. The data were obtained on the fourth day of the incubation at 28 °C, 90% relative humidity. The range of the standard deviation was 2–8%.

where A_0 is the absorbance increase in the absence of formaldehyde. The data were collected after four-day incubation at 28 °C, 90% relative humidity.

Absorbance increases during the incubation were obvious (Fig. 7a), indicating that the fungal growth gives rise to the absorbance increase. On the other hand, the absorbance increase was gradually suppressed as the formaldehyde concentration increased, suggesting that the inhibition effect was enhanced. Interestingly, Fig. 7b indicates that *Aspergillus penicillioides*, which was less sensitive than *Alternaria alternata* in the mycelium length-based detection of formaldehyde, was more sensitive than *Alternaria alternata*.

The absorbance increase depends on many different factors besides mycelium lengths (*e.g.* mycelium thickness, spore size, pigment density on the spore and mycelia). Formaldehyde might affect these factors in different ways. For instance, pigment production in *Aspergillus penicillioides* could be much more sensitive to formaldehyde than that of *Alternaria alternata*. A more obvious case might be the absorbance change for *Eurotium herbariorum*. It did not show a monotonic relationship with the formaldehyde concentration. On the basis of the results mentioned above, *Aspergillus penicillioides* is the fungal species that is most suitable to absorbance-based monitoring of formaldehyde.

Sensitivity of the absorbance-based sensing seems to be lower than that of the mycelium length-based sensing. Instead, dynamic range of the former seems to be wider; at least 700–4000 μg m^{-3}.

4. Conclusions

We studied the applicability of the fungal biosensor plates to quantitative assessment of the harmfulness of air polluted by a VOC (i.e. formaldehyde). Fungal mycelium length and absorbance of the fungal biosensor plate were employed as indices of the fungal growth. Formaldehyde in air could be detected on the basis of growth inhibition, reflected by suppressed growth indices. The dynamic range was 700–4000 μg m^{-3} or broader. *Eurotium herbariorum* and *Aspergillus penicillioides* were the most suitable to formaldehyde sensing based on the mycelium length and that based on the absorbance, respectively.

However, for now, it is difficult to detect formaldehyde in air, if its concentration is lower than the guideline value suggested by WHO (World Health Organization) (i.e. 100 μg m^{-3}). If this is the case, formaldehyde should be concentrated on the biosensor plate using an adsorptive material. Introduction of *in situ* monitoring of absorbance instead of the present *ex situ* measurement should also improve the sensitivity. There are some other limitations such as a large equipment. Miniturization of the equipment should not be difficult, although this is beyond the scope of the present work.

The present biosensor plate might also be useful in risk assessment of newly found VOCs or combined toxicity of VOCs. Since different fungal species should have different sensitivities to a specific VOC, on the basis of the difference, in the future, a biosensor array with different fungal species would be used for prediction of the type of toxicity.

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