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Rapid and High Sensitive Detection of Bacteria Sensor using a Porous Ion Exchange Film

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1. Introduction

In recent years, food poisoning and endocrine disrupting chemicals problems have been focused. A biological and bacterial sensor with rapid response has been required for monitoring our health conditions and food hygiene. We have succeeded in detecting bacteria high sensitivity and rapidly by sensing device using the porous ion exchange film without concentration operation and culture.

A conventional technique for bacteria counting has to take a long time for more than 24h due to culture of bacteria. We attempted to develop a sensor with a rapid and high sensitivity by using a porous ion exchange film having three dimensional detection aspect as shown in Fig. 1. Conventional biosensors such as ISFET (Ion Sensitive Field Effect Transistor) for protein and DNA have two dimensional detection aspect. We have reported amino acid detection by the impedance change of the ion exchange film. [1)] As this porous ion exchanger has an open-cellular structure with 5~50μm diameter pore, bacteria can easily penetrate into the film. This paper reports electrical characteristics and mechanism of sensing bacteria (*Bacillus subtilis*).

2. Experimental

The porous ion exchange film is synthesized using the styrene and the divinylbenzene (DVB)[2)]. In this film, ion exchange functional groups such as sulfonic acid groups for cation exchanger or quaternary ammonium groups for anion exchanger are located at intervals of about 1nm on porous polymer as shown in Fig. 2. The impedance of the ion exchange film is increased by ions, because proton hopping conduction on the functional groups is prevented due to ion adsorption on the functional groups.

We measured the impedance of the porous film after dipping in solution containing bacteria (*Bacillus subtilis*). The film impedance measurement was carried out at 1kHz. The initial state of the cation (anion) exchange film was made by H^+ (OH^-) substitution using 1mol/ml HCl (NaOH) followed by rinsing with deionized water. The concentration of *Bacillus subtilis* in the solution was examined by absorbance of a UV spectrophotometer. The concentration of bacteria was estimated from interrelation between the film impedance and the absorbance intensity.

3. Results and discussion

The impedance of the anion exchange film drastically increases as compared to that of the cation exchange film after *Bacillus subtilis* solution treatment as shown in Fig. 3. After dipping in the solution, we observed both type exchange films by SEM. We found unexpected results. We could not find *Bacillus subtilis* in the anion exchange film with large impedance increase as shown in Figure 4 (b). However, in the cation exchange film with small impedance increase, *Bacillus subtilis* is observed as shown in Fig. 4 (a).

It is possible that a cell wall of *Bacillus subtilis* has been dissolved, because the inside of the anion exchange film has strong basic. To confirm it, we attempted to dissolve only the cell wall of *Bacillus subtilis* by using a special enzyme (Lysozyme). In the anion film, the impedance of the bacteria solution after mixing with the enzyme increased as well as that of the solution before bacteria solution treatment as shown in Fig. 5.

From these results, it is thought that the cell wall of *Bacillus subtilis* was dissolved in the inside of the anion exchanger because of strong OH^-, and inner ionic contents in *Bacillus subtilis* were liquefied out. The impedance of anion film changed by absorbing ionic contents on the exchange sites as shown in Fig.6.

The anion exchange film of normal type, we have succeeded in detecting *Bacillus subtilis* with a concentration as low as 10^4/ml without culture of *Bacillus subtilis* as shown in Figure 7. By optimizing configuration the film, we can also detect a drastic impedance changing of the film with low concentration as low as 8×10^2/ml as shown in Figure 8. It is probable that DNA of bacteria can be detected with rapid response and high sensitivity by using the novel ion exchange film.

4. Conclusion

We have achieved detection of *Bacillus subtilis* with a concentration as low as 10^2/ml by sensing device using the porous ion exchange film without concentration operation and culture. The detection level is sufficient for practical bacteria counts in beverage and food. There is a high potentiality that the porous ion exchange film is applicable to a high performance device for biological sensing.

5. Reference

1)H.Aoki, K.Miyano, C.Kimura and T.Sugino, IEICE Technical Report ED Vol.149 (2006) 73-78.

2)H.Inoue, K.Yamanaka, A.Yoshida, T.Aoki, M.Teraguchi, T.Kaneko, Polymer, 45, 3 (2004).

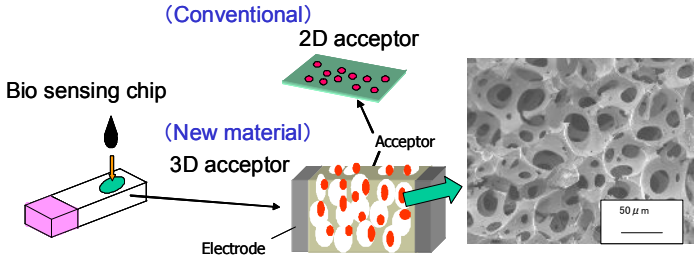


Fig.1 Our sensor image and a photo image of porous ion exchange film

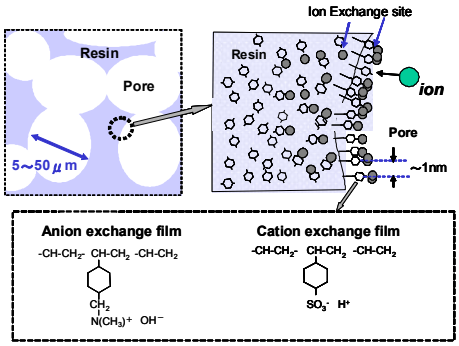


Fig.2 A structure of porous ion exchange film.

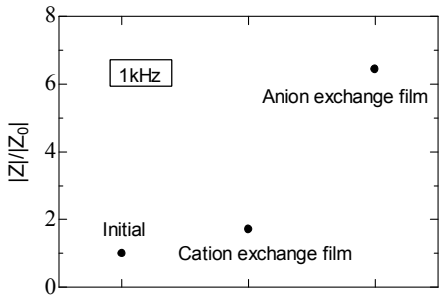
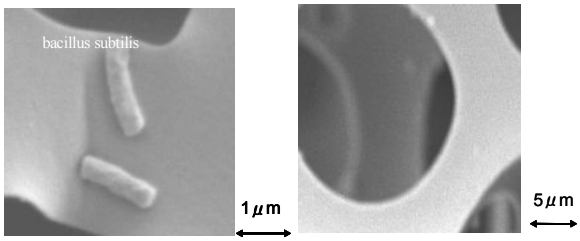


Fig.3 The impedance of the film after *Bacillus subtilis* solution treatment (cation, anion)



(a) Cation exchange film (a) Anion exchange film

Fig.4 The pore surface of (a) cation, (b) anion film after *Bacillus subtilis* solution treatment

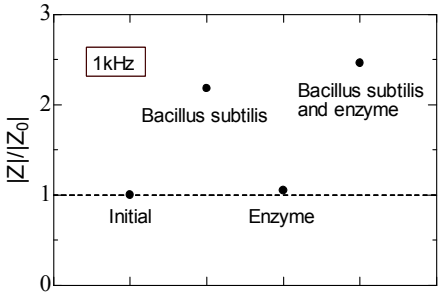


Fig.5 The impedance of the anion exchange film after enzyme treatment

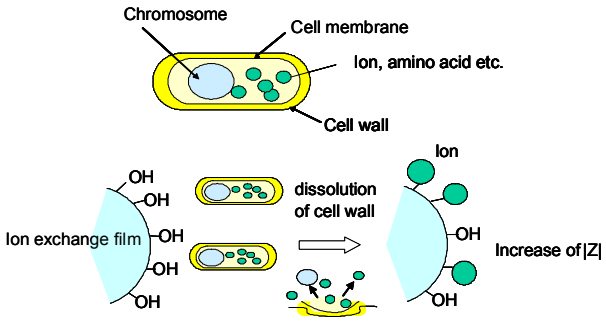


Fig.6 Mechanism of bacteria detection in anion exchange film

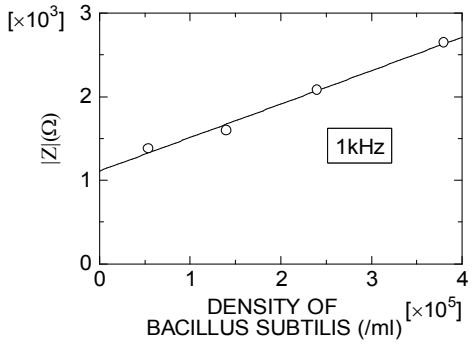


Fig.7 Dependence of the anion film impedance on *Bacillus subtilis* concentration treatment

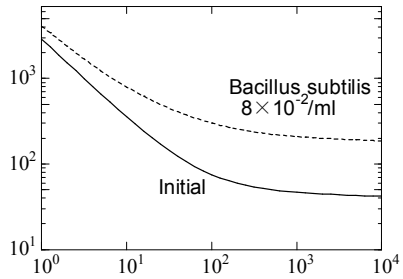


Fig.8 Frequency dependence of the impedance for an optimized film