SR-IR microscopic observation of Biofilms

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Biofilms have complex structure composed of microbes and matrix on surface in humid environments. We observed 2D mapping of molecular vibrational spectra with an IR microscope to analyze the structure.

The sample of biofilm, which was grown on a glass substrate set in an artificial pond in Ritsumeikan University, was used. A drop of the sample liquid was put on an Al-coated slide glass and dried in air. IR spectra and 2D mapping were observed with synchrotron radiation IR (SR-IR) microspectroscope beamline (BL-15) in the SR center of Ritsumeikan University. IR spectra were collected over mid-infrared region of 4000 to 800 cm^{-1} at a resolution of 4cm^{-1} in reflection mode. Each spectrum was accumulated by 128 times. An observed area was mapped by 20 μm in x and y directions using a 20 μm x 20 μm square aperture and a 32x cassegrain objective.

Fig. 1(a) shows a video image of the sample and the rectanglar inset is the observed area. Figure 1(b) is the mean spectrum of the observed area. Two sharp peaks at 3345 and 1060 cm^{-1} are assigned to ν(OH) and ν(CO), respectively. The features can be mainly observed in the spectra of crystalline cellulose such as cell wall of the algae [1]. The two molecular vibrations were distributed about center of the observed area as shown in Fig. 2 (a) and (b), corresponding to the rod-like object about the center in the Fig. 1(a). The rod therefore could be an alga. On the other hand, the aggregate of the grains was on both sides of the alga as shown in Fig. 1(a). The mean spectrum of the aggregate did not have the sharp peaks as seen in that of alga but had a feature of the band of ν(OH) in 3200 to 3700 cm^{-1} broader than that of the alga (Fig. 3). Figure 4 shows an IR image of the band of 1145-1120 cm^{-1}. The band is assigned to ν(CO) of glycocalyx which is one of extracellular polymeric substances (EPS) produced by bacteria in biofilms [2]. The band is distributed not only to both side of the alga but also on the alga and biofilms were found to be surrounded by the alga. In summary, we could observe biofilms around an alga, but need more detailed analysis on interface between algae and biofilms for further study of growth mechanisms of biofilms on alga.

Acknowledgements

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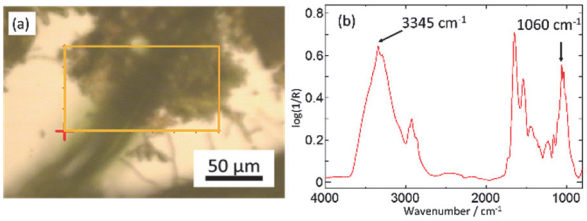

Fig. 1 (a) a video image of the sample and an observed area is surrounded by the rectanglar. (b) is a mean IR spectrum in the observed area in (a).

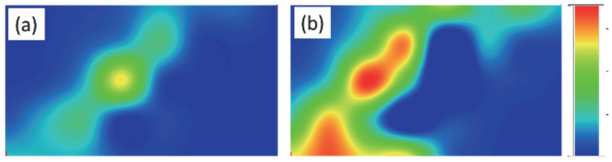

Fig. 2 IR mapping images of ν(OH) at 3345 cm^{-1} (a) and ν(CO) at 1060 cm^{-1} (b).

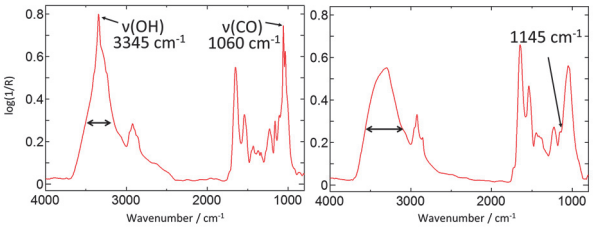

Fig. 3 (a) the mean spectrum of the alga. (b) the mean spectrum of the aggregate in Fig. 1 (a).

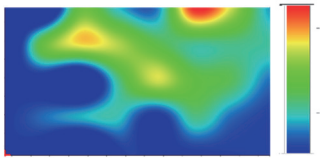

Fig. 4 An IR mapping image of ν(CO) band of 1145-1120 cm^{-1}.

References

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[2] H-Y. N. Holman et al., Anal. Chem., 81 (2009) 8564-8570.