

Chapter 2

Conditioning Films

Hideyuki Kanematsu and Dana M. Barry

Abstract Planktonic bacteria tend to move toward material surfaces in oligotrophic environments, since carbon compounds as nutrients exist there. The bacterial movement is called chemotaxis and is driven by the existence of nutrients on material surfaces. The nutrients on material surfaces are called “conditioning films”. At the beginning stage of research activities about the phenomenon, it was partly hypothetical due to a lack of high-accuracy direct observation methods. However, the recent development of instrumental analyses enables researchers to observe the existence and behaviors of “conditioning film” in situ. In this chapter, the historical development of measurement and theoretical aspects for conditioning film are surveyed. Then, some advanced analytical techniques and examples of their applications will be introduced.

As described in the former section, the driving force of bacterial movement from their environment to material surfaces is nutrients. Fortunately, nutrients exist on material surfaces. The nutrients for bacteria should be carbon compounds. It is considered the film-like matter of the nano-order scale. It must not be a homogenous film, but non-contiguous and inhomogeneous matter, even though it is called a “conditioning film.” From the viewpoint of material surfaces, the conditioning film could be considered as carbon compounds adsorbing specifically on material surfaces. It appears that Baier [1] and Loep and Neihof [2] first proposed the existence of conditioning films. However, around that time period, they did not make it clear whether the existence of organic compounds was a prerequisite for bacterial attachment. At any rate, organic substances being adsorbed on material surfaces when the materials were immersed in aquatic phases were their findings, particularly in a marine environment. Baier [3] pointed out glycoproteins as an important component of conditioning films; Loeb and Neihof [2]

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mentioned humic substances; Taylor [4] added proteins, lipids, nucleic acid, polysaccharides, and aromatic amino acids; and Zaidi et al. [5] proposed the possibility that it might be composed of many other unspecified macromolecules on material surfaces. Exopolysaccharide (transparent exopolysaccharide) was also suggested as one of the components of conditioning films [6].

Based on all these investigations, the formation of a conditioning film is considered as a multistep process. Based on their observations, Compere et al. [7] and Poleunis et al. [8, 9] suggest that proteins would form first, followed by carbohydrates, if stainless steel specimens are immersed in a marine environment. Carbohydrates are the main components of conditioning films; glucose is the most well-known substance. Carbohydrates universally exist in every metabolic pathway. Usually, they are metabolized and stored as adenosine triphosphate (ATP) in cells, and are called storage carbohydrates. According to some researchers [10, 11], storage carbohydrates constitute almost half of all dissolved organic carbon, for example, in a marine environment. Many kinds of organisms metabolize those carbohydrates and become a source of organic matter [12–15]. The explanation described above belongs to a marine environment. However, a similar process would exist for all other oligotrophic environments [16].

Adsorbed carbohydrates on material surfaces generally contain uronic acid, pyruvate, sulfate, and proteins [17]. They would affect the chemical environments and change some characteristics such as surface charge, wettability, surface free energy, surface roughness, etc. These changes affect the following step, i.e., the attachment of bacteria, etc., tremendously [4, 7, 18]. The schematic illustration for the formation of a conditioning film is shown in Fig. 2.1.

All of these investigations were basically related to marine biofilms. Usually, it has been very hard for researchers to confirm the existence of a biofilm. The reason

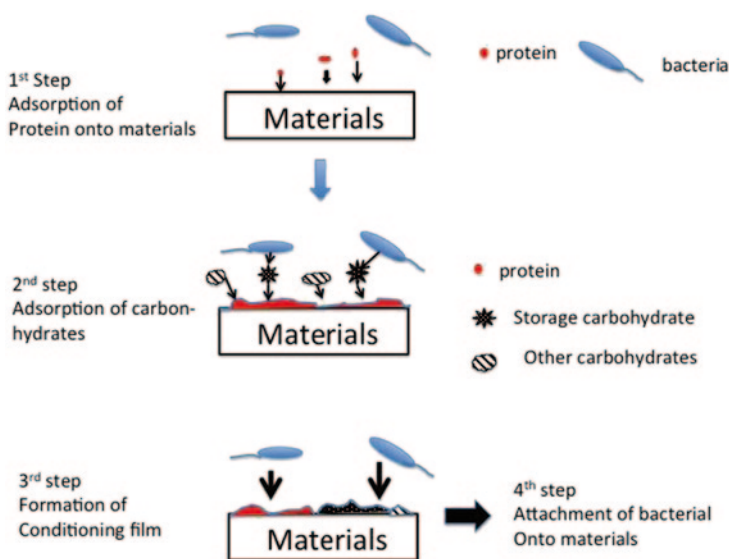


Fig. 2.1 Concept of conditioning film formation on materials in a marine environment



Fig. 2.2 General processing of gas chromatography

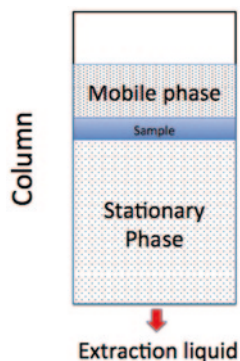
could be partly attributed to the lack of in situ and direct observation apparatuses in the past. The film would often exist as a nano-order film. In addition, the experimental method would be very complicated. This might be the second reason for the retardation of investigations in this field. From the viewpoint of inherent characteristics of conditioning films, it would form on material surfaces inevitably. The contrast specimen that does not have any conditioning film at all could not be made. Therefore, it is very hard to ascertain the influence and involvement of conditioning films for biofilm formation.

However, some researchers have even tried to tackle with the aforementioned topics. With regard to the concrete evidence of conditioning films, the elemental analyses by themselves seem to be insufficient. The analyses of organic compounds cannot be avoided. From this viewpoint, three representative analytical methods—Chromatography, FTIR and TOF-SIMS—can be mentioned.

One of them is chromatography. It is one of the methods to separate and refine substances chemically. It utilizes the differences in adsorption, electric charge, mass, hydrophobicity, etc., among substances. When a substance (mobile phase) moves on a surface or in a substance (stationary phase), the former is broken and separated into its constituents. Chromatography can be divided into two types from the viewpoint of stationary phases—solid chromatography, using solid substances as a stationary phase, and liquid chromatography, using liquid as the stationary phase. On the other hand, chromatography can be divided into three types from the viewpoint of mobile phases. When the mobile phase is gas, it is called gas chromatography. When liquid is used as the mobile phase, it is called liquid chromatography. When supercritical fluid is used as the mobile phase, it is called supercritical chromatography.

Gas chromatography has been utilized in the analyses of carbohydrates. Figure 2.2 shows the schematic principle. Carrier gas such as nitrogen, argon, hydrogen, helium, etc., is introduced into the control part of the carrier gas. Then it enters the column through the introduction part. In the column, the substances are separated and identified by the detector. Generally, gas chromatography needs the target sample in the gas form. Therefore, the substance is restricted to generally being available as a gas. This characteristic makes gas chromatography less universal than liquid chromatography. However, it has high merits for the highly accurate analyses particularly of carbohydrates as well as aliphatic acids and alcohols, since they can be separated due to the difference in boiling points. As already described, the dominant components of conditioning films are often carbohydrates. Therefore, this method has been often utilized to analyze conditioning films [19–21]. Garg et al. [22] utilized this method for the samples in a marine environment (Dona Paula Bay in Indian Ocean), combining their results with a statistical method. Then, this

Fig. 2.3 General process of liquid chromatography



research group, mainly in India, indicated that the conditioning film in the marine environment was composed of rhamnose, arabinose, xylose, ribose, and galactose.

On the other hand, proteins might dominantly occupy the conditioning film inside the human body [23]. To analyze them, liquid chromatography might be more convenient. Figure 2.3 shows the schematic principle. The substances in the sample are forced to penetrate into the stationary phase by the force of a mobile phase. They move through clearances in the stationary phase. However, the constituents of the sample are separated due to the differences in interaction with the stationary phase. Usually, high-performance liquid chromatography (HPLC) is used.

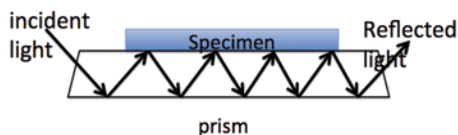
Originally, the mobile phases were moved by a natural drop. However, that might take a long time. Therefore, mobile phases are nowadays moved by pumps forcefully to shorten the time for processing. It is called HPLC. Usually, liquid chromatography belongs to this advanced category. The technique is now used to identify the conditioning films formed on ureteral stents and so on [24, 25].

The third chromatography is supercritical chromatography, where supercritical fluid is used instead of usual liquids. This method can accelerate the measurement time more than HPLC. However, there are only few concrete examples of its application for biofilm research at this point.

Even though chromatography has been used for the measurement of conditioning films very often so far, these analytical techniques were carried out to identify conditioning films, being apart from their substrates. Hence, the accuracy of the results might be deteriorated to some extent. On the other hand, using some analytical methods, it is possible to observe the conditioning film on substrate materials directly.

Fourier transform infrared spectroscopy (FT-IR) is one of the infrared spectroscopies (IRs). IR is generally the analytical technique where a specimen is irradiated with infrared light, and the penetrated/reflected light is measured to analyze the material's structure and to quantify the abundance. Generally, the infrared light is absorbed by materials due to their molecular vibrations and rotations, while ultra-violet light is absorbed due to the electron transitions. The IR is classified into two types, nondispersive and FT-IR. For the former, a grating disperses the penetrated

Fig. 2.4 The schematic illustration for the principle of ATR-FTIR



light and each frequency is detected. For the latter, the penetrated light is detected without dispersion by an interferometer and calculated by Fourier transformation on computers. Being compared with the dispersion type, FT-IR has the following advantages: (1) high-speed measurements—since the measurements at all frequencies can be carried out simultaneously, the measurements do not need a long time. (2) High signal/noise (S/N) ratio—since this technique does not need any slits, the amount of energy reaching the detector can be large. As a result, a high S/N ratio can be achieved. (3) High resolution—as for the wave number, high resolution can be achieved just by increasing the moving distance of the moving mirror. (4) It is easy to enlarge the area of wave numbers available for measurements. The enlargement can be achieved by changing the light source, detector, etc. It is usually very hard for the dispersion-type measurement to achieve.

However, the sample must still be separated from the substrate when one uses the usual FT-IR. From this viewpoint, this technique is still similar to the various other chromatographies. To achieve the direct measurement on a substrate, the attenuated total reflectance–Fourier transform IR (ATR-FTIR) is highly recommended [26]. Figure 2.4 shows the schematic principle for ATR-FTIR. Usually, the incident infrared light penetrates into powder samples. However, ATR-FTIR utilizes reflected lights. As shown in Fig. 2.4, infrared light entering the prism with high refractive index penetrates in the vicinity of a sample specimen. Then it is reflected. The total reflected light is measured and analyzed with Fourier transformation. This method enables wet material surfaces to be measured. Also, the thin surface layer can be measured. The information about the interface between the organic substance on the surface and other materials can be obtained. Fortunately, many peaks corresponding to proteins, e-DNA, polysaccharides, etc., have been elucidated so far. Therefore, the analyses following the measurements are nowadays pretty easy. Recently, the ATR-FTIR has been combined with other methods, and lots of valuable information about conditioning films have been collected and investigated [27–29].

TOF-SIMS is also a promising analytical method in the future. It stands for time-of-flight secondary ion mass spectrometry. TOF-SIMS belongs to the category of surface analysis that can study organic substances in the vicinity of material surfaces. When the primary ion is irradiated on the material surface, parts of molecules existing on the material surface are ionized and emitted as a secondary ion. The secondary ion is analyzed by mass spectrometry. For example, Bautista et al. [30] investigated the protein adsorption in conditioning films, using the apparatus and X-ray Photoelectron Spectroscopy (XPS).

The experimental confirmation for conditioning films has been difficult so far. However, the progress of high-accuracy analyses has enabled us to analyze the in

situ information about the vicinity of material surfaces. This type of information will continue to be updated and improve our knowledge and understanding of conditioning films as well as biofilms.

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