Hideyuki Kanematsu · Dana M. Barry
Editors

Biofilm and Materials Science

Springer

Biofilm and Materials Science

Hideyuki Kanematsu • Dana M. Barry
Editors

Biofilm and Materials Science

Springer

Editors
Hideyuki Kanematsu
Deputy President
Professor, Department of Materials Science & Engineering, National Institute of Technology, Suzuka College
Suzuka, Mie
Japan

Dana M. Barry
Research Professor, Department of Chemical & Biomolecular Engineering
Senior Technical Writer & Editor, Center for Advanced Materials Processing (CAMP)
Clarkson University
Potsdam, New York
USA

ISBN 978-3-319-14564-8 978-3-319-14565-5 (eBook)
DOI 10.1007/978-3-319-14565-5

Library of Congress Control Number: 2015930840

Springer Cham Heidelberg New York Dordrecht London
© Springer International Publishing Switzerland 2015
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made.

Printed on acid-free paper

Springer International Publishing is a brand of Springer

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Since the early stage of the creation of life, bacteria have always been main actors on this globe, even though other organisms have evolved by adjusting to various environments throughout history. Sometimes bacteria have caused epidemics and the loss of life to many people. However on a daily basis, many of them have played important roles in producing foods and decomposing many materials (including those that are toxic) back to nature, etc. From that viewpoint, bacteria have been close companions to human beings, either good or bad. Actually, we did not know about their essence so much until recent times.

It was in the late 1970s that the notion of biofilm was first proposed. We found out gradually that bacteria in biofilm are the usual form, and that the planktonic bacteria just floating in air and aqueous systems, or growing in a certain culture are the rare ones. Also we have recently learned how different both types of bacteria are from each other.

Early on, research about bacteria was carried out in the medical field. Then the work expanded to the area of environmental science. Through research and development, a different notion called "quorum sensing," the phenomenon of communication among bacteria was introduced into the mechanism of biofilm formation. Originally bacteriologists investigated quorum sensing quite separately, not relating it to biofilm. In the 1980s, another new revolutionary notion and technology came about. The former was molecular systems based on small subunit ribosomal RNA (SSU rRNA) genes. The latter was the quantitative community analysis for environmental microbes established by Dr. Norman Pace and his group. Not only biofilm, but also nature itself is composed of viable, but nonculturable (VBNC) bacteria, so it is very hard to solve the biofilm problems with a pure culture technique since Koch. The notion and technology in biofilm research were also introduced. The knowledge and information about biofilm have skyrocketed since then.

However, when we try to solve the daily industrial problems, the materials science and engineering fields seem to have the most important knowledge and information to tackle them, especially since biofilm generally forms at the interface between the microbe (environment) and materials. As described in many chapters of this book again and again, the biofilm formation starts by the attachment of bacteria to a surface. To solve the industrial problems, one should approach them from

both the environmental side and the materials side. However, there have been few investigations from the viewpoint of materials science and engineering. Our original aim was to complete the missing link between bacteriology and materials science. Since this is not the usual approach, our book was designed to be relatively fundamental so it is appropriate for beginners such as undergraduate students and nonbiological practical engineers. However, we believe this book also measures up to the expectations of graduate students, professional researchers, and high-level practical engineers. The authors worked hard as a team to prepare a book that meets the needs of a diverse audience.

Part I contains the fundamental parts. The life cycle of biofilm is composed of multisteps. Therefore, the process is divided into some steps and each is described in detail, respectively. In this part, readers will learn the basic knowledge about biofilm and prepare to move to the next part. Part II introduces many practical problems encountered in various industries on a daily basis. In this section the reader will get a chance to find the unexpected relationship between biofilm and industrial problems. Hopefully this part will be filled with many surprises. And Part III is composed of knowledge and information about solving problems of biofilm. In some chapters, you will learn how to remove and control biofilm. In other chapters, you will learn how to measure and evaluate biofilm properly and precisely. Even though some of these techniques are still under investigation, you will find out many evaluation methods available for materials scientists and engineers.

For many chapters of this book, the editors, Kanematsu and Barry, took the responsibility of writing them. However, many brilliant professionals were invited to join us and write their own chapters. You will get a chance to confirm their names and their parts in the Table of Contents. The editors removed the unnecessary and redundant expressions and descriptions in order to prepare a complete and integrated scholarly book. Each chapter is informative and written as a stand-alone, so the reader can begin anywhere in the book depending upon his/her interests and needs.

As we already stated, biofilm is still not fully understood and its meaning and importance for materials science and engineering are still underestimated. Therefore, we consider the readers of this book to be like sailors traveling to an unknown sea with *Biofilm and Materials Science* as a guide. We the editors and authors hope from the bottom of our hearts that this book will be a good guidebook and compass for your work and research (which may at times resemble traveling in an unknown sea). Bon voyage, all!

November 21, 2014

Hideyuki Kanematsu and Dana M. Barry,
(book editors)
On behalf of the authors

Acknowledgments

We the editors would like to thank all of the book chapter authors, the National Institute of Technology, Suzuka College and its President Yasutsugu Nitta, Clarkson University, CAMP (the Center for Advanced Materials Processing), Clarkson University's Department of Chemical & Biomolecular Engineering, and Ansted University, for their greatly appreciated support. We also thank the Iron and Steel Institute of Japan (ISIJ) and its Division of Process Evaluation and Material Characterization. Many ideas and suggestions were obtained in the forum and lots of information came from our activities there. We would like to thank the Iketani Science and Technology Foundation for their financial support too. Their funding to us made it possible for us to discuss closely and vividly on a face-to-face basis with each other.

Some information contained in this book was obtained from work carried out for national funding projects in Japan. We appreciate the Japan Society for the Promotion of Science (JSPS) for Grant-in-Aid for Scientific Research (KAKENHI, particularly No.21360372 and No. 24656460). Also we appreciate the New Energy and Industrial Technology Development Organization (NEDO) for their two big national projects. The first one is entitled the Development of Anti-fouling Mirror by Bio-related Technology for Heliostat and the second is entitled Research & Development for Physical Treatment of Scales in Geo-thermal Power Generation Utilizing Hot Springs Heat.

We express our sincere appreciation to Ms. Ania Levinson and Springer Verlag. Their kind offer (invitation) made it possible for us to launch on this international book project together. Their patience and trust encouraged us throughout the duration of this exciting project.

In addition, we would like to thank our families, Dr. Kanematsu's parents (Shoji and Michiko Kanematsu), his wife (Reiko), and children (Hitomi and Hiroyuki); Dr. Barry's parents (Daniel and Celia Malloy), her husband (James), and children (James, Brian, Daniel, and Eric), for their continued interest and support.

Hideyuki Kanematsu (PhD), Book Editor
Dana M. Barry (PhD), Book Editor

Contents

Part I Biofilm, Microbiology, and Genomics

1 Introduction 3
Hideyuki Kanematsu and Dana M. Barry

2 Conditioning Films 9
Hideyuki Kanematsu and Dana M. Barry

3 Movement of Bacteria Towards Material Surfaces 17
Hideyuki Kanematsu and Dana M. Barry

4 Adhesion of Bacteria 23
Katsutoshi Hori

5 Microstructures of Biofilm 35
James Chapman

6 Detachment of Bacteria 45
James Chapman

7 Genomics Approach 53
Hajime Ikegai

8 General Biological Biofilm Observation and Evaluation 61
Hideyuki Kanematsu and Dana M. Barry

Part II Biofilm and Industrial Problems

9 Corrosion and Biofilm 69
Reza Javaherdashti

10 Cooling Water 79
Dana M. Barry and Hideyuki Kanematsu

11 Ships and Marine Structures 85
Daisuke Kuroda

12 Biofilm Formation on Medical Devices and Infection: Preventive Approaches 93
Sitaraman Krishnan

13 Hygiene Problems and Food Industry 109
Satoshi Fukuzaki

14 Environmental Problems: Soil and Underground Water Treatment and Bioremediation 117
Hideyuki Kanematsu and Dana M. Barry

15 Energy Problems—Fuel Cell 125
Nobumitsu Hirai

16 Bioreactors in Industries and Biofilm 135
Dana M. Barry and Hideyuki Kanematsu

17 Fish Reef, Seaweed Bed, and Other Construction Applications 141
Hideyuki Kanematsu and Dana M. Barry

18 Contamination and Clean Surface of Materials 147
Hideyuki Kanematsu and Dana M. Barry

Part III Solving Industrial Biofilm Problems

19 Chemical Cleaning 155
Satoshi Fukuzaki

20 Physical Removal of Biofilm 163
Dana M. Barry and Hideyuki Kanematsu

21 Antibacterial Effect of Materials and Biofilm 169
Hideyuki Kanematsu and Dana M. Barry

22 Immersion Tests 175
Daisuke Kuroda

23 Artificial Biofilm Formation on the Laboratory Scale 181
Dana M. Barry, Hideyuki Kanematsu and Paul B. McGrath

24 New Evaluation Techniques for Biofilm in Materials Science 187
Hideyuki Kanematsu and Dana M. Barry

25 Future Scope 193
Hideyuki Kanematsu and Dana M. Barry

Part I
Biofilm, Microbiology, and Genomics

Chapter 1
Introduction

Hideyuki Kanematsu and Dana M. Barry

Abstract What is biofilm and how does it form? How are materials involved in the formation process? In this chapter, the authors provide answers or clues to these basic questions. Biofilm is a result of many complicated steps. It includes the formation of a conditioning film on a material's surface, the movement of bacteria, an attachment process, the growth on material surfaces, and the breakdown finally. All of these processes seem to be repeated a number of times. In this chapter the whole process is presented and briefly described, to provide the reader with a quick view of the process and general knowledge about biofilm.

In the late 1970s, we became familiar with the concept of biofilm. The finding could be attributed to a great medical scientist in Canada named Dr. John William Costerton (1934–2012). When he first proposed the new concept most of the responses which he had were cynical smiles or complete disregard. The notion was filled with revolutionary and contradictory contents against conventional ones like the great finding Koch and Pasteur had in bacteriology. It seems that Dr. Costerton had tough days to develop his new discipline. His door for a brighter future was almost closed. However, the notion and concept have been accepted gradually and nowadays they are indispensable for us to deal with bacteria-related phenomena. The concept "biofilm" is essential for bacteriology and the related phenomena. Now, what is biofilm?

The definition of biofilm is versatile. The expression differs from researcher to researcher and it can be described in different ways, depending on the case. The simplest, however, somewhat vague definition is that "biofilm is a film derived from an organism." Actually, it is a film-like matter on material surfaces. In the strict meaning, it is not any homogenous film, but a kind of inhomogeneous and

H. Kanematsu (✉)
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

D. M. Barry
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, USA
e-mail: dmbarry@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_1

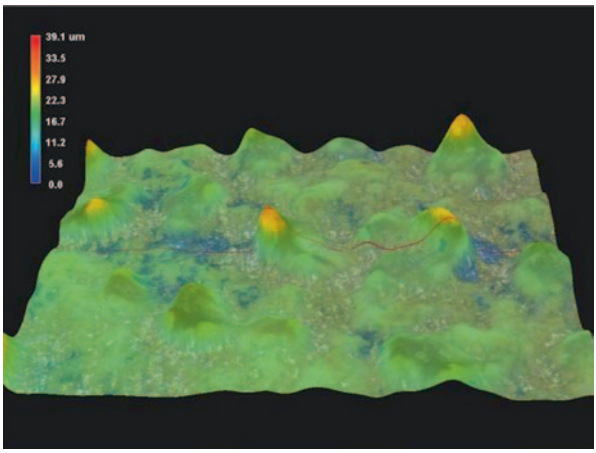


Fig. 1.1 Biofilm on steel, observed by an optical microscope [1]

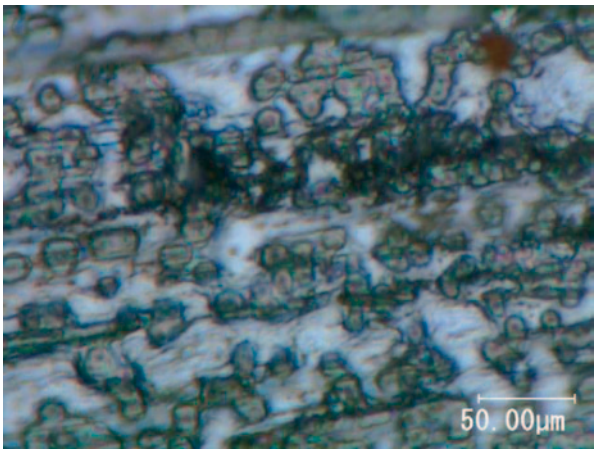


Fig. 1.2 Biofilm on a steel surface observed from the perpendicular direction by an optical microscopy [2]

discontinuous film-like matter. People often say that "seeing is believing." This rule can be applied to this case. A simple example is provided. Figure 1.1 shows biofilm formed on steel.

As shown in the figure, biofilm is a tower-like inhomogeneous film. One of the main biofilm components is water. Actually, researchers have been telling that the water would account for 80 % and more of biofilm. From this viewpoint, it could be grasped as "water film." Figure 1.2 shows biofilm formed on another piece of steel, which was observed from the perpendicular direction by an optical microscope.

The tiny bubble-like matters are biofilm. If an observer is not used to biofilm, he or she might not recognize it and miss the good opportunity to observe it correctly. So you might see it as water film on materials. Other than water, biofilm contains polysaccharides and proteins. Also bacteria themselves are in it, of course.

Now we know what biofilm looks like. It is a tower-like, inhomogeneous film-like matter on material surfaces. It is composed mainly of dominant water,

polysaccharide/protein and bacteria themselves. However, is it really a new finding for us human beings? To tell the truth, Leeuwenhoek (1632–1723), the Father of Microbiology, observed biofilm appearing on his own teeth. However, he did not realize it clearly as anything from bacteria, even though he enjoyed the observation in a microbial world and paved the way to the new discipline. The basic notion about the relationship with bacteria was lacking. We had to wait until Costerton proposed it in the late twentieth century. From this viewpoint, the finding about the biofilm by Costerton should be considered a proposition of a new concept. So how does biofilm form by bacterial activity?

Bacteria are usually found floating in oligotrophic environments. Such floating bacteria are called planktonic bacteria. Until Costerton proposed the concept of biofilm, people considered planktonic bacteria to be the "normal state." However, this was wrong. The floating planktonic bacteria are always seeking nutrients to survive. Their nutrients are usually carbon compounds. In most cases they exist on the surface of various materials and form what is called a conditioning film. Bacteria move toward the surface due to the concentration gradient of nutrients. Not only the nutrients, but other factors such as electricity, magnetism, and light, can cause bacteria to move in a certain direction. We define such bacterial movement determined by a certain directional exogenous stimulus as taxis. There are many kinds of taxes. Barotaxis is caused by pressure, galvanotaxis by electricity, magnetotaxis by magnetism, geotaxis by gravitation, hydrotaxis by water, phototaxis by light, rheotaxis by flow, thermotaxis by temperature, and so on. Some taxes may appear for a certain bacterial species. Movement (taxis) that is caused by nutrients is generally called chemotaxis. It is relatively common. Bacteria move toward materials' surfaces by chemotaxis, driven by the existence of conditioning film there.

Also bacteria move to materials' surfaces anyway to obtain the nutrients there. However, it is not so easy for them to attach to the material surface because they must overcome some obstacles such as flow, surface conditions, coulomb forces, hydrophobicity/hydrophilicity, etc. Eventually, the bacteria attach to the materials. However, they sometimes detach from the surface.

For a while, the bacteria continue the attachment and detachment process and finally, the number of bacteria begins to increase, as a result of the dominant attachment process. When the bacteria population reaches a certain value, they simultaneously excrete polysaccharides. The phenomenon is called quorum sensing. Originally, each microorganism (of bacteria) gives a certain signal protein out from its body along with other proteins. The amount of the signal protein called AI (auto inducer) tends to increase with the amount of bacteria on a material's surface. Therefore, the amount of AI reaches a certain value when the bacterial number also reaches a certain threshold value. When AI concentration exceeds a critical threshold value, it stimulates a certain part of the gene, which leads to the simultaneous excretion of polysaccharides. It is really just like a conversation among bacteria. They use chemical transmittance for the conversation. In this way, the polysaccharide is generated and bacteria begin to exist in the matrix of slimy polysaccharide on materials' surfaces. The biofilm forms at this point.

In the biofilm, bacteria can exist easier than those in the planktonic state. Fortunately for them (and perhaps unfortunately for us human beings in many cases), they can catch more nutrients, resist chemicals such as biocides, antibiotics, etc., and protect themselves from flow and bigger organisms which might move them away from their stable states in various ways. From this point of view, biofilm could be considered a survival strategy for bacteria. As described already, we human beings thought in the past that the planktonic bacteria were the usual state for bacteria. However we are realizing that bacteria within biofilms are the usual case for them and planktonic state bacteria should be considered an exception. Actually, some researchers pointed out that several tens of percentages for the phenotype could be changed from the planktonic state to the biofilm one. Usually, bacteria's size is around 1 μm in the longitudinal direction. However, they tend to shrink to 60–80 % of the original length in biofilm. In this way, bacteria seem to change their existence, adjusting themselves to an energy-saving one, so that they get a chance to survive. This is very important for humans to know in order to deal with bacteria, especially since the usual state of bacteria is within biofilms and their characteristics are changed drastically in various ways. Koch and Pasteur devised a culture and incubation technique for bacteria based on knowledge about the planktonic state of bacteria. However, it may have a number of limitations. One might consider the concept of biofilm as a kind of Copernican Revolution.

The biofilm formed on materials' surfaces incorporate many matters from the environment, such as mineral matters, organic matters, etc., as well as the interaction with substrates. In this way, the biofilm grows with the passage of time. The growth of biofilm depends on many factors such as temperature, other environmental conditions, the kind of substrates, etc. The flow is particularly very important. Actually, it is one of the most important factors to determine the biofilm structure and its characteristics. This correlation has been investigated by many researchers so far. When biofilm grows, the interaction between the substrate and biofilm becomes more remarkable. From the viewpoint of materials science, this stage is particularly important.

However, biofilm does not grow eternally. At a certain point, the collapse occurs inevitably. It seems that many factors might be involved in the breakdown of biofilm. These factors are still being investigated at this point. When the biofilm is broken, the biofilm bacteria are released into the environment again and their "dormant" state is changed to that of planktonic bacteria again. They move to seek nutrients and find material surfaces to attach to and grow on. The process is repeated. Therefore, the biofilm can be considered a kind of reservoir for planktonic bacteria. As shown in Fig. 1.3, the process of biofilm formation through breakdown is repeated and during the process there are many kinds of correlations between biofilm and substrate materials.

As shown in the figure and described above, the biofilm formation process is composed of multisteps and is very complicated. It means that one needs to know the process in detail and correctly in order to understand the correlation between biofilm and substrate materials.

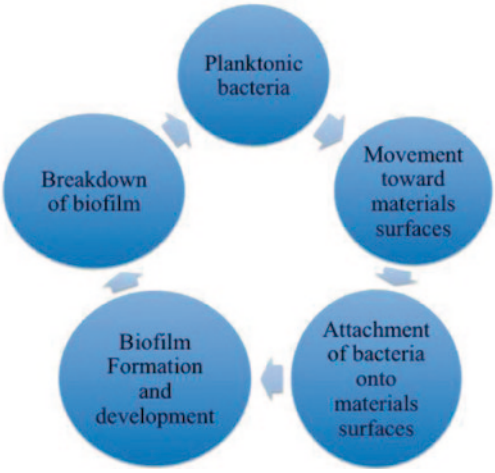


Fig. 1.3 Multisteps for biofilm formation and development

At first biofilm research and investigations were carried out from the viewpoint of medical science, since bacteriology would be the best and the most appropriate discipline for biofilm. In addition to that, Costerton himself who proposed the notion of biofilm was firstly a scientist in medical science. Then the topic got attention in the area of environmental science. However, the investigation and study of biofilms in materials science and engineering have not been carried out enough. The causes could be attributed to many reasons. One of the main reasons is that the concept of biofilm is unfamiliar to materials scientists and engineers. Also, they may feel that the topic is too biological and not relevant to their fields. When we come to think about this, they must know that the opposite is true.

Many phenomena in materials science and engineering relate to biofilm formation and growth. For example, corrosion has a close relationship to biofilm. Microbiologically influenced corrosion has been a discipline during this half century and has a close relation with biofilm formation. In addition to the special case of corrosion of metallic materials, general corrosion in the atmosphere may have a certain connection to biofilm formed by bacterial activity. Since the biofilm can retain a certain amount of water on materials' surfaces, it may get a chance to bring about corrosion in a dried atmosphere more easily. If such an indirect correlation between biofilm and metallic corrosion can be counted for the causes, then the influence of biofilm on metallic materials would be tremendous and huge.

The problem of scale growth on the walls of cooling pipes is also one of the serious problems in the materials science and engineering field. Since the inner walls of cooling pipes provide some appropriate growing conditions, biofilm could form and develop there very easily. As microfouling builds up on the material's surface, it forms slime and scale, which impact and reduce a pipe's ability for heat exchange. This leads to operational performance deterioration in the contaminated system [3]. Finally, it would lead to an increase in CO_2 due to the ineffective heat exchange capability.

The following chapters present and discuss detailed information about concepts, topics, etc., relating to biofilms from various viewpoints. The direction, intention, and aims of this book are very important, especially to the present and future materials scientists and engineers who will tackle the problems of biofilm.

References

1. Ikegai H, Kanematsu H (2009) Worked up from some sources for presentations
2. Kanematsu H et al (2012) Worked up from some sources for presentations
3. Kanematsu H (2014) Biofilm/Biofouling Problems & CO2 Reduction, in ICAT News Letter 2014, ICAT (Institute of Carbon Accountants and Traders)

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]

H. Kanematsu (✉)
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

D. M. Barry
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, US
e-mail: dmbarry@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_2

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 expolysaccharide) 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

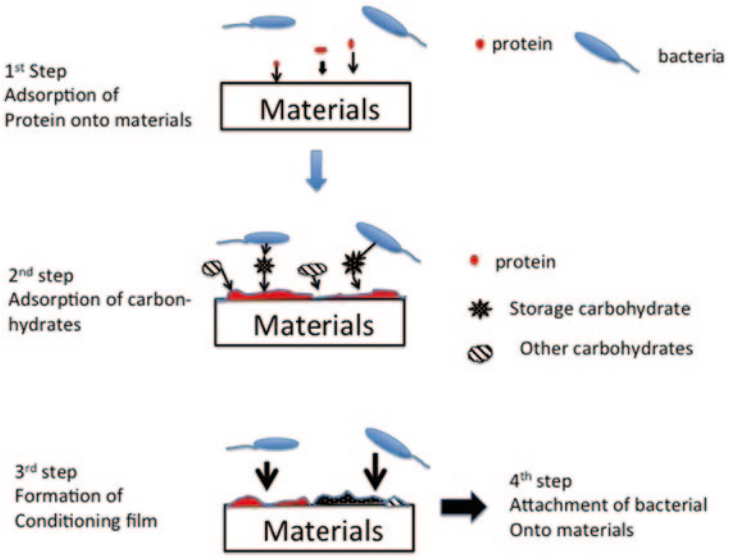


Fig. 2.1 Concept of conditioning film formation on materials in a marine environment

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

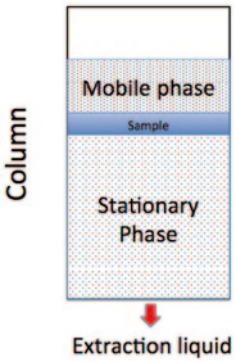


Fig. 2.3 General process of liquid chromatography

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 ultraviolet 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

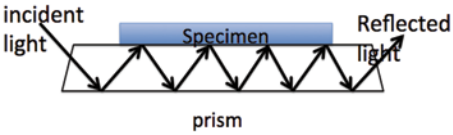


Fig. 2.4 The schematic illustration for the principle of ATR-FTIR

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.

References

1. Baier RE (1972) Influence of the initial surface condition of materials on bio adhesion. In: Third Inter Cong Marine corrosion and Fouling, Gaithersburg, The USA, p. 633–639
2. Loeb GI, Neihof RA (1975) Marine conditioning films. Adv Chem Ser 145:319–335
3. Baier RE (1980) Substrate influences on adhesion of microorganisms and their resultant new surface properties. In: Bitton G, Marshall KC (eds) Adsorption of microorganisms to surfaces. Willey, New York, pp 59–104
4. Taylor GT, Zheng D, Lee M, Troy PJ, Gyananath G, Sharma SK (1997) Influence of surface properties on accumulation of conditioning films and marine bacteria on substrata exposed to oligotrophic waters. Biogouling 11:31–57
5. Zaidi BR, Bard RF, Tosterson TR (1984) Microbial specificity of metallic surfaces exposed to ambient seawater. Appl Environ Microbiol 48:519–524
6. Berman T, Passow U (2007) Transparent Exopolymer Particles (TEP); an overlooked factor in the process of biofilm formation in aquatic environments, Nature proceedings
7. Compere C, Bellon-Fontaine MN, Bertrand P, Costa D, Marcus P, Poleunis C, Pradier CM, Rondot B, Walls MG (2001) Kinetics of conditioning layer formation on stainless steel immersed in seawater. Biofouling 17:129–145
8. Poleunis C, Compere C, Bertrand P (2002). Time-of-flight secondary ion mass spectrometry: characterization of stainless steel surfaces immersed in natural seawater. J Microbiol Methods 48:195–205
9. Poleunis C, Rubio C, Compere C, Bertrand P (2003) ToF-SIMS chemical mapping study of protein adsorption onto stainless steel surfaces immersed in saline aqueous solutions. Appl Surf Sci 203–204:693–697
10. Pakulski JD, Benner R (1994) Abundance and distribution of carbohydrates in the ocean. Limnol Oceanogr 39:930–940
11. Amon RMW, Benner R (2003) Combined neutral sugars as indicator of the diagenetic state of dissolved organic matter in the Arctic Ocean. Deep sea Res 50:151–169
12. Cowie GI, Hedges JI, Prahl FG, De Lange GJ (1984) Carbohydrate sources in a coastal marine environment. Geochim Cosmochim Acta 48:2075–2087
13. Skoog A, Benner R (1997) Aldoses in various size fractions of marine organic matter: implications for carbon recycling. Limnol Oceanogr 42:1803–1813
14. D'Souza F, Bhosle NB (2001) Variation in the composition of carbohydrates in the Dona Paula bay (west of India) during May/June 1998. Oceanol Acta 24:221–237
15. Khodse VB, Fernandes L, Gopalkrishna VV, Bhosle NB, Fernandes V, Matondkar SGP, Bhushan R (2007) Distribution and seasonal variation of concentrations of particulate carbohydrates and uronic acids in the northern Indian Ocean. Mar Chem 103(3–4):327–346
16. Tareq SM, Ohta K (2011) Distribution of combined monosaccharides in sediments from the Lake Rawa Danau, West Java, Indonesia: sources and diagenetic fate of carbohydrates in a tropical wetland. Geochem J 45:1–13
17. Decho AW (1990) Microbial exopolymer secretions in open ocean environments: their role(s) in food webs and marine progresses. Oceanogr Mar Biol Ann Rev 28:73–153
18. Little B, Zsolnay ZA (1985) Chemical fingerprinting of adsorbed organic materials on metal surfaces. J Colloid Interface Sci 104:79–86
19. Bhosle NB, Sawant SS, Garg A, Wagh AB (1995) Isolation and partial chemical analysis of expolysaccharides from the marine fouling diatom *Navicula subinflata*. Bot Mar 38:103–110

20. D'Souza F, Bhosle NB (2003) Analysis of microfouling products formed on metallic surfaces exposed in a marine environment. Biofouling 19(2):95–107
21. D'Souza F, Garg A, Bhosle NB (2005) Seasonal variation in the chemical composition and carbohydrate signature compounds of biofilm. Aquat Microb Ecol 41:199–207
22. Garg A, Jain A, Bhosle NB (2009) Chemical characterization of the marine conditioning film. Biodeter Biodegr 63(1):7–11
23. Canales BK, Higgins LA, Markowski T, Anderson L, Li QA, Monga M (2009) Presence of five conditioning film proteins are highly associated with early stent encrustation. J Endourol 23(9):1437–1442
24. Wollin TA, Tieszer C, Riddell JV, Denstedt JD, Reid G (1998) Bacterial biofilm formation, encrustation, and antibiotic adsorption to ureteral stents indwelling in humans. J Endourol 12(2):101–111
25. Lange D, Elwood CN, Chew BH (2011) Biomaterials in urology—beyond drug eluting and degradable—a rational approach to ureteral stent design. In: Pignatello R (Ed) Biomaterials—Physics and Chemistry, p. 459–474
26. Humbert F, Quiles F (2011) In-situ study of early stages of biofilm formation under different environmental stresses by ATR-FTIR spectroscopy. In: Mendez-Villas A (Ed) Science against microbial pathogens; communicating current research and technological advances. Formatex Research Center, Badajoz
27. Delille A, Quiles F, Humbert F (2007) In situ monitoring of the nascent *Pseudomonas fluorescens* biofilm response to variations in the dissolved organic carbon level in low-nutrient water by attenuated total reflectance-Fourier transform infrared spectroscopy. Appl Environ Microbiol 73(18):5782–5788
28. Lorite GS, Rodrigues CM, de Souza AA, Kranz C, Mizaikoff B, Cotta MA (2011) The role of conditioning film formation and surface chemical changes on *Xylella fastidiosa* adhesion and biofilm evolution. J Colloid Interface Sci 359:289–295
29. Neubauer D, Scharpf J, Pasquarelli A, Mizaikoff B, Kranz C (2013) Combined in situ atomic force microscopy and infrared attenuated total reflection spectroelectrochemistry. Analyst 138(22):6695–7040
30. Torres BBE, Carvalho ML, Seyeux A, Zanna S, Cristiani P, Tribollet B, Marcus P, Frateur I (2014) Effect of protein adsorption on the corrosion behavior of 70Cu–30Ni alloy in artificial seawater. Bioelectrochemistry 97:34–42

Chapter 3
Movement of Bacteria Towards Material Surfaces

Hideyuki Kanematsu and Dana M. Barry

Abstract Bacteria in planktonic states tend to move towards material surfaces. The main reason or driving force for the natural motion should be attributed to chemotaxis, where bacteria move in the direction of high-density nutrition. Carbon compounds adsorb on material surfaces and exist there generally as a very thin film. They attract planktonic bacteria in oligotrophic environments. However, various other factors would affect the bacterial movement in the vicinity of material surfaces. Flow is one of them. It could help bacteria and nutrients approach the material's surface and also produce shear stress to make the biofilm stable, stronger, and thinner. The geographical configuration of a material's surface is also a very important factor. In addition to them, chemical structures and properties (hydrophobicity–hydrophilicity, surface tension, and energy), the components of interfaces, etc. would affect the movement of bacteria in the vicinity of surfaces. In this chapter, those factors are explained and discussed.

Biological Viewpoint

Bacteria generally move by the stimulation from outside. Such a characteristic is called the 'taxis'. Taxis are defined as inherent bacterial action driven by external directional stimuli. There are many kinds of external stimuli and bacterial taxis. To name a few, pressure, electricity, and magnetism could make bacteria move in a certain direction very often. The corresponding taxis are called barotaxis, galvanotaxis, and magnetotaxis, respectively. Among them, bacteria have a certain taxis that external chemicals would drive bacteria. This kind of taxis is called chemotaxis. Generally, the bacterial movement from their environment to a material's surface

H. Kanematsu (✉)
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

D. M. Barry
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, USA
e-mail: dmbarry@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_3

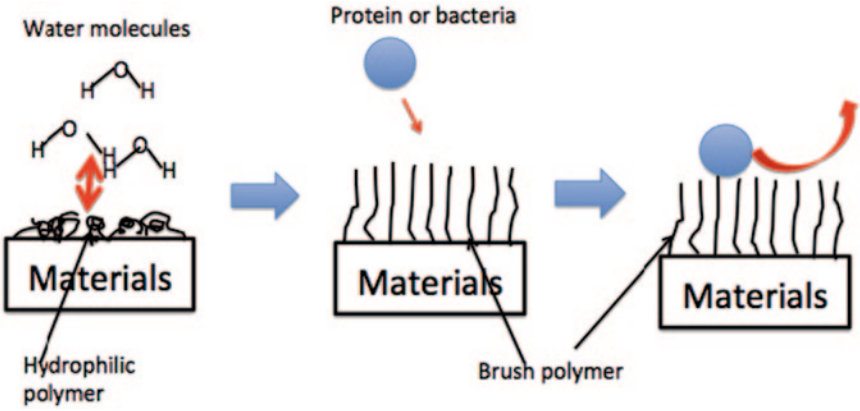


Fig. 3.1 Brush polymer layer and attached bacteria or protein

has a close relation with chemotaxis. As already described, the conditioning film composed of carbon compounds is expected to exist on material surfaces generally. This 'film' would play a role as source of chemotaxis. The detailed information and knowledge were already described in the former chapter (Chap. 2).

Chemical or Metallurgical Viewpoint

In the early studies, some researchers realized that the chemical situation other than a biological one would also affect the biofilm formation to some extent. Costerton et al. [1] agreed that the biofilm formation would be affected by the substrates. In the relatively early stage of biofilm research, Absolom et al. [2] insisted that the balance among hydrophobicity, hydrophilicity, and surface tension of the bacteria could determine the extent of biofilm formation. From the chemical viewpoint, hydrophobicity seems to be a favourable characteristic for bacterial adsorption to the surface. If the material's surface would be hydrophobic, then hydrophobic bacteria would favourably attach to them from the viewpoint of energy. Since the surface area resulted from the coalescence between two hydrophobic substances, it would become smaller than that before, generally speaking. For the energy to distort the water structure would be smaller with the surface area. In the same way, hydrophilic surfaces might attract bacteria with hydrophilic characteristics. However, according to the results of polymer coatings [3], some hydrophilic polymers attached to hydrophilic surfaces would react with water molecules and could get the brush-like geographical structures, shown in Fig. 3.1. Then it might repel proteins or bacteria by bouncing them back. As described in Chap. 2, material surfaces could get the polymers before the bacterial attachment. Therefore, the polymers existing on a material's surface in advance might work as a repellent against bacteria and protein.

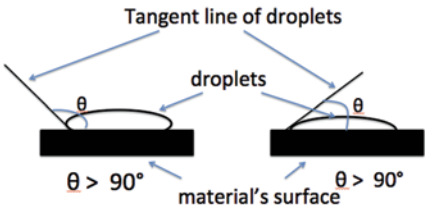


Fig. 3.2 Contact angles and wettability

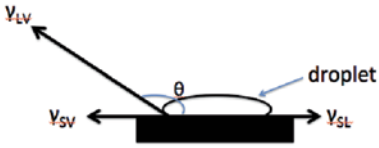


Fig. 3.3 Young's equation

From the viewpoint, hydrophobic surfaces might be more favourable for bacterial attachment than hydrophilic ones.

Usually, hydrophobicity–hydrophilicity characteristics and the transition between them could be evaluated by contact angles. Figure 3.2 shows the general sketch of a droplet, when it is placed on a material's surface. Hydrophobic droplets tend to hang together without extending on the surface. The extent depends on hydrophobicity. If it would be higher, the angle formed by the tangent line of the curve and the solid surface on the side of the droplet would also be larger.

The angle is called contact angle and is a sort of indication for hydrophobicity–hydrophilicity. The equation 3.1 shows the relation among three interfacial tensions, γ_{SV}, γ_{SL} and γ_{LV}

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} cos(\theta) \tag{3.1}$$

Each force corresponds to that for solid–gas (γ_{SV}), solid-liquid (γ_{SL}) and liquid gas (γ_{LV}) interface. They are shown in Fig. 3.3 schematically. Unfortunately, it is very hard to testify the effect of hydrophobicity–hydrophilicity on bacterial attachment. There are mainly three reasons for the difficulty. First, the difficulty to evaluate the characteristics of material surfaces and the attached components onto material surfaces could be mentioned. Generally, the parameter, contact angle, is a macroscopic value. However, the components contributing to the biofilm formation such as bacteria, protein, and other molecules are microscopic. Therefore, the measurement relating to contact angles could not cover the phenomena precisely. Second, the measurement of hydrophobicity for bacteria is difficult to be measured. Usually, it is not achieved by measuring contact angles. Usually, it is estimated by the rate of adsorption into hydrophobic liquid in water. The difficulty of this experimental technique makes the standardized evaluation more complicated.

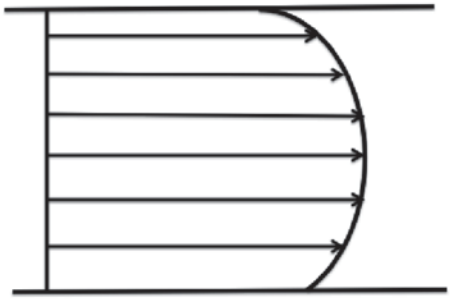


Fig 3.4 Schematic illustration for laminar flow

On the other hand, metals existing on material surfaces seem to give more concrete and clearer effects about biofilm formation. Kanematsu et al. [4] showed that the capability of biofilm formation depends on the substrate metals. They defined the biofilm formation capability of metals as the extent of staining by crystal violet, when they were immersed into culture media of *Pseudomonas aeruginosa*. They observed that the capability differed from metal to metal. Iron especially seemed to have the highest capability to form biofilm on it. The researchers attributed this to the strong affinity between iron ion and bacteria in most cases. Even though the mechanism for the special affinity has not been clear yet, this is a very interesting truth from the viewpoint of practical application.

Again, one would have the difficulty to testify the effect of substrate elements or structures on biofilm formation, since geographical topography would generally affect the result tremendously. Generally, high-surface roughness would give bacteria favourable opportunities to attach on the surface very easily [5, 6]. One should be very careful to prepare the specimens, so that the surface roughness would be almost the same among specimens, when investigating the effect of elements, chemicals, and substances, on biofilm formation.

Physical Viewpoint

In the former section, we see how the material's surface would affect the biofilm formation seriously. However, the biofilm formation is not so simple, so that we could estimate it only from the viewpoint of a material's surface. Some other important factors would be involved. The flow is one of them and the precise analyses could be achieved by the knowledge of fluid dynamics. The motion for the transport is generally composed of mass transport, thermal effects, and gravitational effect.

As for mass transport, laminar and turbulent flows are the main factors and affect the bacterial motion in different ways. Laminar flow always has the flow lines parallel to the pipe wall and they never cross each other. The distribution of flow rate in the case is shown in Fig. 3.4. In the figure, the arrow corresponds to the flow rate and their length shows how large the rate is for each point. The flow rate increases

Fig 3.5 Schematic illustration for turbulent flow

with the perpendicular distance to the pipe wall. It reaches a maximum value at the centreline of the pipe. The friction between the flow body and pipe wall causes the phenomena. On the other hand, turbulent flow is basically intensive and various vortexes are generated in it.

The turbulent flow is very strong and has many vortexes in it, as shown in Fig. 3.5

To estimate which type of flow would occur, Reynolds number (Re) is usually used. Re is defined as follows.

$$\mathrm{Re} = (\mathrm{pvd}) / \mu \tag{3.2}$$

Where d is the density of the fluid, v is the velocity of the fluid, and p is a constant length in the system such as the diameter of the fluid pipe, and μ is the viscosity coefficient of the fluid. Usually, the laminar flow is realized when Re is less than 2000. On the other hand, the turbulent flow is realized when Re is greater than 4000. When Re is between 2000 and 4000, the mixed flow would occur.

The area close to a material's surface does not have such a rapid flow. Actually, the flow rate at the pipe's inner surface is close to zero. That is because the viscous force is dominant over the inertia force, etc., the main force in the bulk flow. The area is called the boundary layer. Researchers classify the vicinity of material surfaces as sub layers within the boundary layer.

In the laminar flow, boundary layers are generally well developed. It means there are relatively wide areas in the vicinity of a surface where the flow rate is very low and mass transportation is difficult to occur. In such a situation as it relates to biofilm formation, two matters could not be provided to the surface enough. One is the nutrient and the other is bacteria. As a result, the biofilm formed in the laminar flow is generally less compact, but thicker, since the flow rate in the boundary layer is relatively low.

On the other hand, there are many vortexes in turbulent flow. In such a case, the approach of bacteria and the supply of nutrients would be relatively easy. The boundary layer does not develop so much. The biofilm formed in turbulent flow is thin, but dense generally speaking. The difference of biofilm characteristics seems to depend on the flow rate in the vicinity of a surface [7, 8].

As described above, many factors affect bacterial attachment even before the attachment process begins. This stage is very important for controlling biofilm formation and needs further investigation.

References

1. Costerton JW, Marrie TJ, Cheng K–J (1985) Phenomena of bacterial adhesion. In: Savage DC, Fletcher M (eds) Bacterial adhesion, pp 3–43. Plenum, New York
2. Absolom DR, Lamberti FV, Policova Z, Zingg Wl, van Oss CJ, Neumann AW (1983) Surface thermodynamics of bacterial adhesion. Appl Environ Microbiol 46:90–97
3. Knetsch Menno LW, Koole Leo H (2011) New strategies in the development of antimicrobial coatings: the example of increasing usage of silver and silver nanoparticles. Polymers 3:340–366
4. Kanematsu H, Ikigai H, Yoshitake M (2009) Evaluation of various metallic coatings on steel to mitigate biofilm formation. Int J Mol Sci 10(2):559–571.
5. Gharechahi M, Moosavi H, Forghani M (2012) Effect of surface roughness and materials composition on biofilm formation. J Biomat Nanobiotechnol 3:541–546.
6. Preedy E, Perni S, Nipiĉ D, Bohinc K, Prokopovich P (2014) Surface roughness mediated adhesion forces between borosilicate glass and gram-positive bacteria. Langmuir 30(31):9466–9476
7. Huang J, Hao OJ, Al-Ghusain IA et al (1992) Biological fixed film systems. Water Environ Res 64:359–366
8. Santos R, Callow ME, Bott TR (1991) The structure of Psudomonas fluorescens biofilms in contact with flowing systems. Biofouling 4:319–336

Chapter 4
Adhesion of Bacteria

Katsutoshi Hori

Abstract Bacterial adhesion is the initial step in colonization and biofilm formation. Conventional physicochemical approaches based on Lifshitz–van der Waals, electrostatic, and acid–base interactions provide important models of bacterial adhesion but have a limited capacity to provide a complete understanding of the complex adhesion process of real bacterial cells. In conventional approaches, bacterial cells, whose surfaces are structurally and chemically heterogeneous, are often described from the viewpoint of their overall cellular properties. Cell appendages such as polysaccharide chains and proteinous nanofibers have an important function bridging between cells and the substratum in conventional adhesion models, but sometimes cause deviation from the models of cell adhesion. In reality, cell appendages are responsible for specific and nonspecific cell adhesion to biotic and abiotic surfaces.

Theory of Bacterial Adhesion

1. Conventional model based on the DLVO theory

Bacteria are about 0.5–2 μm in size, that is, nearly in the range of colloidal particles. Therefore, bacterial adhesion has been described by the DLVO theory [1], which originally described the interaction of a colloidal particle with a surface. According to this theory, the total interaction between a surface and a particle is the summation of their van der Waals and Coulomb interactions [2]. Since the van der Waals attractive force is dominant in the vicinity of a surface, particles cannot separate from the surface by Brownian motion, and therefore adhere irreversibly. In contrast,

K. Hori (✉)
Nagoya University, Nagoya, Japan
e-mail: khori@nubio.nagoya-u.ac.jp

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_4

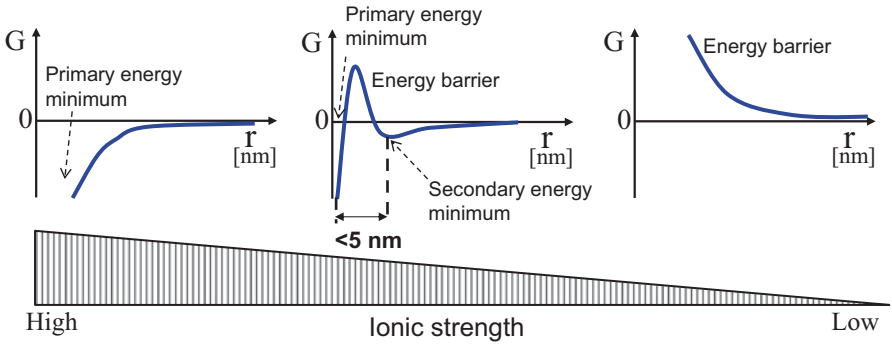
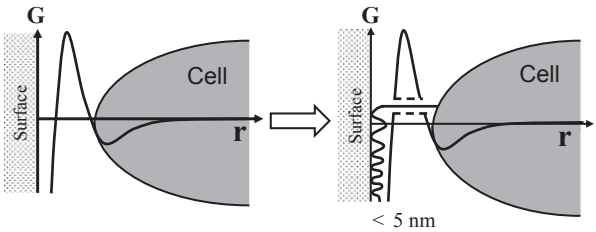


Fig. 4.1 Total interaction energy between a bacterial cell and a surface depending on ionic strength

the Coulomb interaction becomes dominant at a distance away from the surface because the van der Waals force decreases sharply with distance.

In the presence of a charged particle in an aqueous solution, counter ions against the surface charge are attracted by the particle, forming an electric double layer. As bacteria and natural surfaces in aqueous solution are usually negatively charged [3], repulsive electrostatic energy is caused by overlap of the electrical double layers of bacterial cells and the substratum [1, 3–6]. This repulsive energy increases as the ionic strength of an aqueous solution decreases because shielding of the surface charges by the ions in the electrical double layers lessens. At low ionic strengths, when a bacterial cell approaches a surface, there is an energy barrier which bacterial cells cannot surmount by swimming or Brownian motion (Fig. 4.1) [1, 7–9]. In these conditions, there is a shallow secondary energy minimum outside of the energy barrier. The distance from the surface to the secondary energy minimum is usually within several nanometers, depending on the ionic strength. In the first step of cell adhesion, a bacterial cell comes to this position by its motility or Brownian motion, and adheres to the surface reversibly (Fig. 4.2). In the following step, the bacterial cell uses nanofibers, such as pili and flagella, or produces exopolymeric substances (EPS), which can pierce the energy barrier due to their small radii, for bridging between the cell and surface. When the energy barrier becomes higher and farther from the substratum at lower ionic strengths, however, it becomes difficult for the nanofibers and EPS to reach the substratum, and bacterial cells become un-

Fig. 4.2 Conventional two-step adhesion model on the basis of the DLVO theory



able to adhere. On the contrary, at high ionic strengths, the energy barrier disappears and bacterial cells easily and rapidly attain irreversible adhesion. Many researchers have found a link between decreasing bacterial adhesion and decreasing ionic strength, which is consistent with the DLVO theory [3, 10–17].

2. Thermodynamic approach and extended DLVO theory

The thermodynamic approach is based on the surface free energies of the interacting surfaces [18, 19], which are calculated using the following equation:

$$\Delta G_{\mathrm{adh}} = \gamma_{\mathrm{sm}} - \gamma_{\mathrm{sl}} - \gamma_{\mathrm{ml}} \tag{4.1}$$

where γ_{sm}, γ_{sl}, and γ_{ml} are the solid–microorganism, solid–liquid, and microorganism–liquid interfacial free energies, respectively. Adhesion is favored if the free energy is negative as a result of adhesion. However, it should be noted that thermodynamics principally assumes that the process is reversible, which is often not the case [20]. Based on the DLVO theory, bacterial adhesion has been described as a two-phase process including an initial, instantaneous, and reversible physical phase (phase one) and a time-dependent and irreversible molecular and cellular phase (phase two) [21]. However, the thermodynamic approach cannot be applied to the adhesion in phase one at the secondary energy minimum, where a new cell–substratum interface is not formed [20, 22]. Thus, the thermodynamic approach, in which the formation of a new cell–substratum interface at the expense of the substratum–medium and cell–medium interfaces is calculated, contrasts with the DLVO theory, in which the interaction energy is distance dependent. However, the thermodynamic approach helps to explain a common observation: bacteria with a hydrophobic cell surface prefer hydrophobic material surfaces; those with a hydrophilic cell surface prefer hydrophilic surfaces [21].

Obviously, hydrophobicity is an important factor that greatly affects bacterial adhesion and the self-agglutination of bacterial cells [21–27]. Hydrophobic interaction between two apolar moieties immersed in water is the sole consequence of the hydrogen-bonding energy of cohesion of the water molecules surrounding these moieties [28]. Hydrogen-bonding can be viewed as a form of more general electron-donor/electron-accepter interactions, namely, Lewis acid–base interactions [29]. According to van Oss, the surface tension (γ) consists of the Lifshitz–van der Waals component γ^{LW} and the Lewis acid–base component γ^{AB} [30]. γ^{LW} comprises the London dispersion force, the Keesom dipole–dipole force, and the Debye dipole-induced dipole force. van Oss developed the extended DLVO theory, in which the hydrophobic/hydrophilic interactions and osmotic interaction also are included [31, 32]. Since the osmotic interaction is negligibly small in bacterial adhesion, the total adhesion energy can be expressed as:

$$\Delta G^{\mathrm{adh}} = \Delta G^{\mathrm{vdW}} + \Delta G^{\mathrm{dl}} + \Delta G^{\mathrm{AB}} \tag{4.2}$$

where ΔG^{vdW} is the Lifshitz–van der Waals interaction, ΔG^{dl} is the electric double layer interaction, and ΔG^{AB} relates to acid–base interactions. The latter component

introduces a component that, in principle, describes attractive hydrophobic interactions and repulsive hydration effects. The influence of the acid–base interactions is enormous compared with electrostatic and Lifshitz–van der Waals interactions. In some cases, the extended DLVO theory seems to qualitatively predict experimental adhesion results better than the classical DLVO theory; the extended DLVO theory predicts very strong interaction due to acid–base interactions leading to an extremely deep minimum without an energy barrier, whereas adhesion is not expected to occur according to the classical DLVO theory [4, 20, 33]. However, the distance dependence of acid–base interactions is also relatively short-ranged. Acid–base interactions and the electric double layer interaction decay exponentially from the value at close contact [32], and calculations have shown that a distance between the interacting surfaces of less than 5 nm is required before acid–base interactions can become operative [4].

3. Estimation of surface potential of bacterial cells

To estimate the height of the energy barrier, ζ-potentials of bacterial cells have been calculated from the electrophoretic mobility (EPM) by using the Smoluchowski equation. However, it has been found that in many bacterial strains, the EPM approaches a nonzero value with increasing ionic strength [34, 35]. This suggests that the ζ-potential is inappropriate for accurately measuring the surface potential of bacterial cells. Ohshima et al. [36–40] have pointed out that the Smoluchowski equation can be applied to only rigid particles having no polymers, and have developed a model that describes the EPM of particles having a soft polymer surface layer in which diffuse double layer ions and water molecules can freely penetrate. Morisaki et al. [34, 35] used the Ohshima model to estimate accurate surface potentials of actual bacterial cells. In the Ohshima model, EPM (μ) is described by the following formula and the EPM change approaching a nonzero value along with ionic strength can be explained:

$$\mu = \left(\frac{\varepsilon_r \varepsilon_0}{\eta}\right)\left(\frac{\Psi_0 / \kappa_m + \Psi_{DON} / \lambda}{1/\kappa_m + 1/\lambda}\right) + \frac{zeN}{\eta \lambda^2} \tag{4.3}$$

where ε_r is the relative permittivity of the medium in which the cells suspended, ε_0 is the permittivity of a vacuum, η is the viscosity of the medium, Ψ is the surface potential of the particles, Ψ_{DON} is the Donnan potential of the polymer layer, κ_m is the Debye–Hückel parameter of the polymer layer, z is the valence of the charged groups in the polymers, e is the electron charge, N is the density of the charged groups, and λ is the softness parameter, which has dimensions of reciprocal length. It is reasonable that bacterial cells are recognized as soft particles that are covered with or encapsulated by polymers, for example, polysaccharides and proteins. By applying the Ohshima model to bacterial cells, it has been revealed that the actual surface potential of bacterial cells is much smaller than the ζ-potential calculated from the Smoluchowski equation and that the energy barrier disappears or is sufficiently low for bacterial cells to surmount it even at low ionic strengths, where, by

applying the ζ-potential to the DLVO theory, a high-energy barrier had been thought to prevent direct interaction of a cell and a surface [34]. However, the high-energy barrier preventing the cells from irreversibly adhering to a surface does seem to be present under very-low ionic strength conditions even for a "soft" particle [34].

Cell Surface Structure Mediating Bacterial Cell Adhesion

Unlike simple colloidal particles, a bacterial surface is structurally and chemically heterogeneous. For example, gram-negative bacteria have an outer membrane (OM) consisting of a lipid bilayer containing lipopolysaccharide (LPS) at the outer layer of the OM with significant variations in the coverage density and local distribution [41–43]. Various kinds of OM proteins are heterogeneously embedded in the OM and many of them protrude out of cells, forming into cell appendages. Pili and flagella are typical cell appendages, have lengths from hundreds of nanometers to several micrometers, forming long fibrous structures with diameters of several nanometers to tens of nanometers. These bacterionanofibers not only pierce the energy barrier described by the DLVO theory and/or tether a cell body to surfaces but also cause deviation of cell adhesion behavior from that predicted by the DLVO theory. In addition, although different structures on the same cell individually contribute to the net cell character, they are often described in terms of their combined contribution to the overall cellular properties. A hydrophobic part localized at a bacterionanofiber against the hydrophilic main cell surface specifically orients the cell at the interface [23].

4. Polysaccharides

LPS is composed of lipid A, core polysaccharides, and an outermost region of O antigen units [44, 45] and is important for bacterial initial surface adhesion [46]. The common antigen (A-band) polymer is composed of 10–20 repeating α-D-rhamnose units [47], while the serotype-specific antigen (B-band) is composed of di- to pentasaccharide repeating units [48]. The B-band has much longer polysaccharides than the A-band as well as a higher concentration of amino sugars, but a low concentration of sulfate and rhamnose [49]. The B-band has been shown to form hydrogen bonds of approximately 2.5 kT J (where k is Boltzmann constant and T is absolute temperature) each with mineral surfaces. It is suggested that 1000 or fewer such bonds are sufficient to anchor a cell firmly to a surface [50]. Simoni et al. [51] calculated the interaction energy between cells of *Pseudomonas* sp. and surfaces in a sand column and concluded that there was an energy barrier of several 100 kT J per cell at about 20 nm and that LPS hindered close contact of cells with surfaces. Consequently, they hypothesized that LPS would bind the cells to the surface from a distance of about 20 nm, and assumed that LPS could form hydrogen bonds with the substratum.

EPS is composed of primary polysaccharides and other macromolecules such as proteins, DNA, lipids, and humic substances [52]. The EPS matrix that keeps the

microorganisms together in biofilms is responsible for adhesion to a given surface [53, 54]. Interactions between EPS and surfaces are caused by noncovalent bonds, such as electrostatic attraction and hydrogen bonds [55]. The binding force of these interactions is weak compared with a covalent C–C bond. However, these weak interactions are multiplied by the large number of binding sites available in the macromolecules, and the total binding force exceeds that of covalent C–C bonds [56].

5. Bacterionanofibers

The most well-known proteinous adhesin of gram-negative bacteria is a hair-like nanofiber called a pilus or fimbria [57]. (The latter (*pl*: fimbriae) is a synonym for the former (*pl*: pili).) Pili are complexes consisting of several kinds of protein subunits, and a single protein subunit called a major pilin stacks by hundreds, being arranged into a helix to form a long rod-shaped body. The tip of a pilus contains several different proteins capped by the adhesin. There are several kinds of pili, which are varied in function, molecular structure, localization on a cell surface, and mechanisms of secretion and assembly.

Type IV pili have been studied most in terms of the relationship with biofilm formation. This type of pili has been reported to be involved in both nonspecific adhesion to abiotic surfaces for colonization and biofilm formation, and specific binding to target molecules in hosts [58]. Type IV pili extend from the poles of a bacterial cell and mediate the movement of bacteria over surfaces without the use of flagella. These movements are known as social gliding in *Myxococcus xanthus* and twitching in organisms such as *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae* [59]. These surface motilities are thought to contribute to enlargement and development of microcolonies and biofilms.

Type 1 pili and P pili are also well-known adhesive fimbriae, which are present on gram-negative bacteria such as *Escherichia coli* and *Salmonella* species. Unlike polar type IV pili, these types of pili are peritrichate and not responsible for twitching motility. They are important virulence factors of pathogens for adhesion to host cells. They are secreted and assembled by the chaperone-usher pathway, in which a periplasmic chaperone and an OM assembly platform, the "usher," are required (Fig. 4.3a) [57, 60–62]. In *E. coli* strains, the structural pilus subunits of Type 1 pili and P pili are encoded by *fim* and *pap* gene clusters, respectively, together with the chaperone and the usher in the respective operons. Major pilins of type 1 pili and P pili are FimA and PapA, respectively. An adhesin subunit, FimH in type 1 pilus or PapG in P pilus, is located at the distal end of each pilus fibril. These pili are assembled in a top–down fashion, with the adhesin subunit incorporated first. These adhesin subunits bind specifically to target molecules such as sugars or glycolipids of hosts; for example, FimH is the mannose-specific adhesin [63].

Recently, autotransporter adhesins (ATADs), which are nonfimbrial adhesins, have attracted considerable attention as a new class of virulence factors [64]. Unlike long, polymeric, hair-like pili, they are short monomeric or oligomeric nanofibers seen in gram-negative bacteria. The designation "autotransporter" comes from the translocation mechanism known as the type V secretion system, which is one of the most widely distributed secretion systems among gram-negative bacteria [61]. To

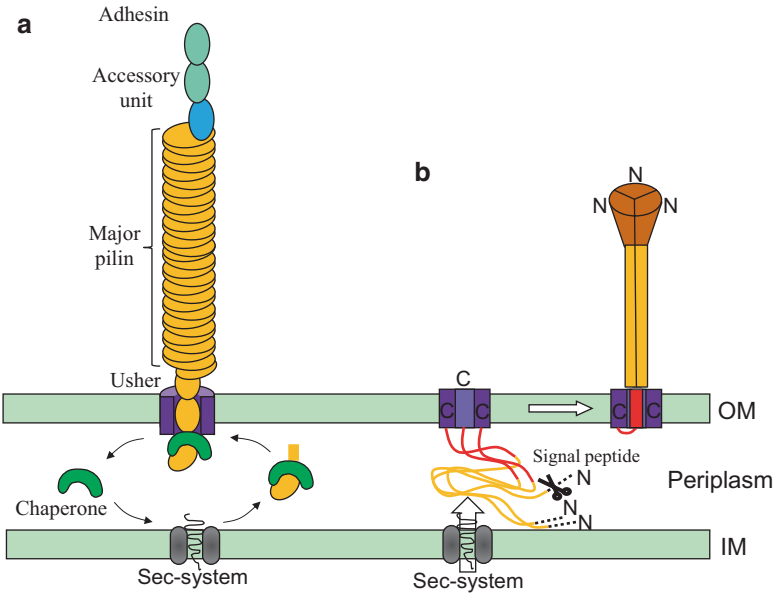


Fig. 4.3 Overviews of the translocation and assembly of nanofiber proteins. **a** Type 1 pili **b** TAAs

date, two variations have been reported in ATADs; one is monomeric autotransporters, and the other is a subfamily known as trimeric autotransporter adhesins (TAAs) [65]. In both types, after their secretion into the periplasm across the IM by the Sec-dependent system and procession of an N-terminal signal peptide, the C-terminal translocator domain is inserted into the OM and forms a β-barrel structure.

Virtually all monomeric types undergo a cleavage event that separates the passenger domain from the translocator domain [64]. This cleavage is often autoproteolytic, and the passenger domain remains either noncovalently associated with the cell surface or can be released into the extracellular milieu. By contrast, in all TAAs that have been examined to date, the passenger domain remains covalently linked to the translocator domain, which therefore becomes the membrane anchor domain [65]. The translocator domains in both monomeric types and TAAs consist of 12 transmembrane β-strands, which are arranged in an antiparallel fashion in the OM and form a β-barrel pore with a hydrophilic inside and a hydrophobic outside.

Molecular organizations of TAAs have a common feature with variations; they form homotrimeric structures with a head-stalk-membrane anchor architecture in the orientation of the N-terminal toward the C-terminal, forming a "lollipop"-shaped structure [66, 67]. Therefore, the length of the nanofiber depends on that of the polypeptides, which vary in size from about 300 a.a. to over 3000 a.a. Whereas the head and stalk domains are diverse and found in different combinations, the membrane anchor is the only domain that is homologous for all TAAs and thus defines this family. The membrane anchor contains four transmembrane β-strands that construct the fused trimeric 12-stranded β-barrel and a short-coiled coil segment,

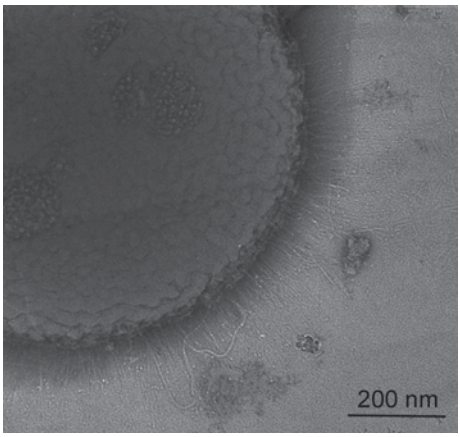


Fig. 4.4 AtaA nanofiber on *Acinetobacter* sp. Tol 5 cells

which is considered to occlude the β-barrel pore after export and folding of the passenger domain (Fig. 4.3b). The high sequence diversity and distinct mosaic-like structures of TAAs have lead to difficulties in the annotation of their sequences. Recently, however, Szczensny and Lupas [68] have developed a workflow referred to as daTAA for the accurate domain annotation of TAAs. This useful web-based annotation platform contributes to the better understanding of the molecular structures that control cell-surface interactions.

Most of the characterized TAAs are responsible for the adhesion of gram-negative pathogens to the biotic surfaces of host tissues. As examples of representative TAAs, YadA from *Yersinia enterocolitica*, BadA from *Bartonella henselae*, and Vomps from *Bartonella quintana* bind to epithelial cells and various extracellular matrix proteins including collagen, fibronectin, laminin, and so on [69–72]. Unlike these reported TAAs, AtaA from *Acinetobacter* sp. Tol 5, which is a nonpathogenic bacterium showing high adhesiveness and autoagglutinating nature [73–76] (Fig. 4.4), mediates adhesion to various abiotic surfaces ranging from hydrophobic plastics to hydrophilic glass and stainless steel [77]. Tol 5 cells deviate from the DLVO theory due to AtaA nanofibers. In addition, the wild-type cells expressing these nanofibers attain irreversible adhesion immediately under conditions in which adhesion of ΔataA mutant cells is still reversible [75]. Tol 5 cells are considered to be able to attain irreversible adhesion rapidly without approaching the vicinity of the substratum through the long-distance interaction mediated by the nanofibers (Fig. 4.5).

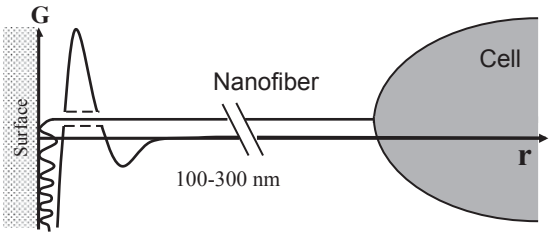


Fig. 4.5 One-step adhesion model mediated by long nanofibers as seen in *Acinetobacter* sp. Tol 5

Caulobacter crescentus

The gram-negative bacterium *Caulobacter crescentus* is one of the first colonizers of submerged surfaces in aquatic environments [78]. *C. crescentus* cells attached to a surface are capable of resisting washing with strong jets of water, suggesting that the attachment of single cells is extremely strong [79]. This bacterium has a dimorphic life cycle consisting of a stage as a swimming swarmer cell followed by a stage as a nonmotile sessile cell that has a polar adhesive nanofiber approximately 100 nm in diameter and several micrometers in length [80, 81]. This adhesive nanofiber is called a "stalk" and tipped by holdfast, a polysaccharide adhesin that mediates the strong adhesion of the stalked cells to surfaces. The stalk is an extension of the cell envelope containing IM, PG, and OM, whereas the holdfast is composed of extracellular polysaccharides and additional components such as proteins [82]. Tsang et al. [83] reported that adhesion strength between the holdfast and the substrate is at least 68 N/mm^2, which is the strongest ever measured for a biological adhesive. Polymers of N-acetylglucosamine, which are an important component in the holdfast, were revealed to play a critical role in the adhesive force [82].

References

1. Marshall KC, Stout R, Mitchell R (1971) Mechanism of the initial events in the sorption of marine bacteria to surfaces. J Gen Microbiol 68:337–348
2. Hiemenz PC, Rajagopalan R (1997) Principles of colloid and surface chemistry. Marcel Dekker, New York
3. van Loosdrecht MCM, Lyklema J, Norde W, Zehnder AJB (1989) Bacterial adhesion: a physicochemical approach. Microb Ecol 17:1–15
4. Bos R, van der Mei HC, Busscher HJ (1999) Physico-chemistry of initial microbial adhesive interactions-its mechanisms and methods for study. FEMS Microbiol Rev 23:179–230
5. Ong YL, Razatos A, Georgiou G, Sharma MM (1999) Adhesion forces between *E. coli* bacteria and biomaterial surfaces. Langmuir 15:2719–2725
6. Van Loosdrecht MCM, Lyklema J, Norde W, Schraa G, Zehnder AJ (1987) Electrophoretic mobility and hydrophobicity as a measured to predict the initial steps of bacterial adhesion. Appl Environ Microbiol 53:1898–1901
7. Quirynen M, Bollen CM (1995) The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature. J Clin Periodontol 22:1–14
8. Jones GW, Isaacson RE (1982) Proteinaceous bacterial adhesins and their receptors. Crit Rev Microbiol 10:229–260
9. Van Loosdrecht MCM, Zehnder AJB (1990) Energetics of bacterial adhesion. Experientia 46:817–822
10. Abu-Lail NI, Camesano TA (2003) Role of ionic strength on the relationship of biopolymer conformation, DLVO contributions, and steric interactions to bioadhesion of *Pseudomonas putida* KT2442. Biomacromolecules 4:1000–1012
11. Bolster CH, Mills AL, Hornberger GM, Herman JS (2001) Effect of surface coatings, grain size, and ionic strength on the maximum attainable coverage of bacteria on sand surfaces, J. Contam. Hydrol 50:287–305

12. Bunt CR, Jones DS, Tucker IG (1995) The effects of pH, ionic strength and polyvalent ions on the cell surface hydrophobicity of *Escherichia coli* evaluated by the BATH and HIC methods. Int J Pharm 113:257–261
13. Jewett DG, Hilbert TA, Logan BE, Arnold RG, Bales RC (1995) Bacterial transport in laboratory columns and filters: influence of ionic strength and pH on collision efficiency. Water Res 29:1673–1680
14. Li Q, Logan BE (1999) Enhancing bacterial transport for bioaugmentation of aquifers using low ionic strength solutions and surfactants. Water Res 33:1090–1100
15. Otto K, Elwing H, Hermansson M (1999) Effect of ionic strength on initial interactions of *Escherichia coli* with surfaces, studied on-line by a novel quartz crystal microbalance technique. J Bacteriol 181:5210–5218
16. Vigeant MA, Ford RM (1997) Interactions between motile *Escherichia coli* and glass in media with various ionic strengths, as observed with a three-dimensional-tracking microscope. Appl Environ Microbiol 63:3474–3479
17. Zita A, Hermansson M (1994) Effects of ionic strength on bacterial adhesion and stability of flocs in a wastewater activated sludge system. Appl Environ Microbiol 60:3041–3048
18. Absolom DR, Lamberti FV, Policova Z, Zingg W, Van Oss CJ, Neumann AW (1983) Surface thermodynamics of bacterial adhesion. Appl Environ Microbiol 46:90–97
19. Busscher HJ, Weerkamp AH, Van der Mei HC, van Pelt AW De Jong HP, Arends J (1984) Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. Appl Environ Microbiol 48:980–983
20. Hermansson M (1999) The DLVO theory in microbial adhesion. Colloids Surf B 14:105–119
21. An YH, Friedman RJ (1998) Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. J Biomed Mater Res 43:338–348
22. Katsikogianni M, Missirlis YF (2004) Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur Cell Mater 8:37–57
23. Marshall KC, Cruickshank RH (1973) Cell surface hydrophobicity and the orientation of certain bacteria at interfaces, Arch. Mikrobiol 91:29–40
24. Rijnaarts HHM, Norde W, Bouwer EJ, Lyklema J, Zehnder AJB (1995) Reversibility and mechanism of bacterial adhesion. Colloids Surf B 4:5–22
25. Zita A, Hermansson M (1997) Effects of bacterial cell surface structures and hydrophobicity on attachment to activated sludge flocs. Appl Environ Microbiol 63:1168–1170
26. Zita A, Hermansson M (1997) Determination of bacterial cell surface hydrophobicity of single cells in cultures and in wastewater in situ. FEMS Microbiol Lett 152:299–306
27. Olofsson AC, Zita A, Hermansson M (1998) Floc stability and adhesion of green-fluorescent-protein-marked bacteria to flocs in activated sludge. Microbiology 144:519–528
28. Van Oss CJ (1995) Hydrophobicity of biosurfaces origin, quantitative determination and interaction energies. Colloids Surf B 5:91–110
29. Van Oss CJ (1991) The forces involved in bioadhesion to flat surfaces and particles their determination and relative roles. Biofouling 4:25–35
30. Van Oss CJ, Chaudhury MK, Good RJ (1987) Monopolar surfaces. Adv Colloid Interface Sci 28:35–64
31. Van Oss CJ (1989) Energetics of cell-cell and cell-biopolymer interactions. Cell Biochem Biophys 14:1–16
32. Van Oss CJ (1993) Acid-base interfacial interactions in aqueous media. Colloids Surf A 78:1–49
33. Meinders JM, Van der Mei HC, Busscher HJ (1995) Deposition efficiency and reversibility of bacterial adhesion under flow. J Colloid Interface Sci 176:329–341
34. Morisaki H, Nagai S, Ohshima H, Ikemoto E, Kogure K (1999) The effect of motility and cell-surface polymers on bacterial attachment. Microbiology 145:2797–2802
35. Takashima S, Morisaki H (1997) Surface characteristics of the microbial cell of *Pseudomonas syringae* and its relevance to cell attachment. Colloids Surf B 9:205–212
36. Ohshima H (1995) Electrophoretic mobility of soft particles. Colloids Surf A 103:249–255
37. Ohshima H, Kondo T (1991) On the electrophoretic mobility of biological cells. Biophys Chem 39:191–198

38. Ohshima H (1994) Electrophoretic mobility of soft particles. J Colloid Interface Sci 163:474–483
39. Ohshima H, Makino K, Kato T, Fujimoto K, Kondo T, Kawaguchi H (1993) Electrophoretic mobility of latex particles covered with temperature-sensitive hydrogel layers. J Colloid Interface Sci 159:512–514
40. Ohshima H (1995) Electrophoresis of soft particles. Adv Colloid Interface Sci 62:189–235
41. Atabek A, Camesano TA (2007) Atomic force microscopy study of the effect of lipopolysaccharides and extracellular polymers on adhesion of *Pseudomonas aeruginosa*. J Bacteriol 189:8503–8509
42. Walker SL, Redman JA, Elimelech M (2004) Role of Cell Surface Lipopolysaccharides in *Escherichia coli* K12 adhesion and transport. Langmuir 20:7736–7746
43. Kotra LP, Golemi D, Amro NA, Liu GY, Mobashery S (1999) Dynamics of the lipopolysaccharide assembly on the surface of *Escherichia coli*. J Am Chem Soc 121:8707–8711
44. Caroff M, Karibian D (2003) Structure of bacterial lipopolysaccharides. Carbohydr Res 338:2431–2447
45. Rocchetta HL, Burrows LL, Lam JS (1999) Genetics of O-antigen biosynthesis in *Pseudomonas aeruginosa*. Microbiol Mol Biol Rev 63:523–553
46. Davey ME, O'Toole G A (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64:847–867
47. Arsenault TL, Hughes DW, Maclean DB, Szarek WA, Kropinski AMB, Lam JS (1991) Structural studies on the polysaccharide portion of a-band lipopolysaccharide from a mutant (Ak1401) of *Pseudomonas aeruginosa* strain PAO1. Can J Chem 69:1273–1280
48. Burrows LL, Charter DF, Lam JS (1996) Molecular characterization of the *Pseudomonas aeruginosa* serotype O5 (PAO1) B-band lipopolysaccharide gene cluster. Mol Microbiol 22:481–495
49. Rivera M, McGroarty EJ (1989) Analysis of a common-antigen lipopolysaccharide from *Pseudomonas aeruginosa*. J Bacteriol 171:2244–2248
50. Jucker BA, Harms H, Hug SJ, Zehnder AJB (1997) Adsorption of bacterial surface polysaccharides on mineral oxides is mediated by hydrogen bonds. Colloids Surf B 9:331–343
51. Simoni SF, Harms H, Bosma TNP, Zehnder AJB (1998) Population heterogeneity affects transport of bacteria through sand columns at low flow rates. Environ Sci Technol 32:2100–2105
52. Flemming HC, Wingender J, Griebe T, Mayer C (2000) Physico-chemical Properties of Biofilms, Biofilms: recent advances in their study and control In: Evans L (ed), Harvvood Academic, Amsterdam, pp 19–34
53. Stehr G, Zorner S, Bottcher B, Koops HP (1995) Exopolymers - an ecological characteristic of a floc-attached, ammonia-oxidizing bacterium. Microb Ecol 30:115–126
54. Zartarian F, Mustin C, Bottero JY, Villemin G, Thomas F, Ailleres L, Champenois M, Grulois P, Manem J (1994) Spatial arrangement of the components of activated-sludge flocs. Water Sci Technol 30:243–250
55. Mayer R, Moritz C, Kirschner W, Borchard R, Maibaum J, Wingender HC (1999) Flemming, The role of intermolecular interactions: studies on model systems for bacterial biofilms. Int J Biol Macromol 26:3–16
56. Flemming HC, Wingender J (2001) Relevance of microbial extracellular polymeric substances (EPSs)-Part I: structural and ecological aspects. Water Sci Technol 43:1–8
57. Soto GE, Hultgren SJ (1999) Bacterial adhesins: common themes and variations in architecture and assembly. J Bacteriol 181:1059–1071
58. Mattick JS (2002) Type IV pili and twitching motility. Annu Rev Microbiol 56:289–314
59. Wall D (1999) Kaiser, Type IV pili and cell motility. Mol Microbiol 32:1–10
60. Sauer FG, Remaut H, Hultgren SJ, Waksman G (2004) Fiber assembly by the chaperone-usher pathway, Biochim. Biophys. Acta Mol. Cell Res 1694:259–267
61. Kostakioti M, Newman CL, Thanassi DG, Stathopoulos C (2005) Mechanisms of protein export across the bacterial outer membrane. J Bacteriol 187:4306–4314
62. Capitani G, Eidam O, Glockshuber R, Grütter MG (2006) Structural and functional insights into the assembly of type 1 pili from *Escherichia coli*, Microb. Infect 8:2284–2290

63. Krogfelt KA, Bergmans H, Klemm P (1990) Direct evidence that the FimH protein is the mannose-specific adhesion of *Escherichia coli* type 1 fimbriae. Infect Immun 58:1995–1998
64. Girard V, Mourez M (2006) Adhesion mediated by autotransporters of gram-negative bacteria: structural and functional features. Res Microbiol 157:407–416
65. Cotter SE, Surana NK, St JW (2005) Geme Iii, Trimeric autotransporters: A distinct subfamily of autotransporter proteins. Trends Microbiol 13:199–205
66. Hoiczyk A, Roggenkamp M, Reichenbecher A, Lupas J (2000) Heesemann, structure and sequence analysis of *Yersinia* YadA and *Moraxella* UspAs reveal a novel class of adhesins. EMBO J 19:5989–5999
67. Linke T, Riess IB, Autenrieth A, Lupas VAJ (2006) Kempf, Trimeric autotransporter adhesins: variable structure, common function. Trends Microbiol 14:264–270
68. Szczesny P, Lupas A (2008) Domain annotation of trimeric autotransporter adhesins - daTAA. Bioinformatics 24:1251–1256
69. El Tahir Y, Skurnik M (2001) YadA, the multifaceted *Yersinia* adhesin. Int J Med Microbiol 291:209–218
70. Müller NF, Kaiser PO, Linke D, Schwarz H, Riess T, Schäfer A, Eble JA, Kempf VA (2011) Trimeric autotransporter adhesin-dependent adherence of *Bartonella henselae*, *Bartonella quintana*, and *Yersinia enterocolitica* to matrix components and endothelial cells under static and dynamic flow conditions. Infect Immun 79:2544–2553
71. Riess T, Andersson SG, Lupas A, Schaller M, Schüfer A, Kyme P, Martin J, Wälzlein JH, Ehehalt U, Lindroos H and others. (2004). *Bartonella* adhesin a mediates a proangiogenic host cell response. J Exp Med 200:1267–1278
72. Zhang P, Chomel BB, Schau MK, Goo JS, Droz S, Kelminson KL, George SS, Lerche NW, Koehler JE (2004) A family of variably expressed outer-membrane proteins (Vomp) mediates adhesion and autoaggregation in *Bartonella quintana*. Proc Natl Acad Sci U S A 101:13630–13635
73. Hori K, Ishikawa M, Yamada M, Higuchi A, Ishikawa Y, Ebi H (2011) Production of peritrichate bacterionanofibers and their proteinaceous components by *Acinetobacter* sp. Tol 5 cells affected by growth substrates. J Biosci Bioeng 111:31–36
74. Hori K, Yamashita S, Ishii S, Kitagawa, Tanji Y, Unno H (2001) Isolation, characterization and application to off-gas treatment of toluene-degrading bacteria. J Chem Eng Japan 39(9):175–184
75. Ishii S, Unno H, Miyata S, Hori K (2006) Effect of cell appendages on the adhesion properties of a highly adhesive bacterium, *Acinetobacter* sp. Tol 5. Biosci Biotechnol Biochem 70(11):2635–2640
76. Ishikawa M, Shigemori K, Suzuki A, Hori K (2012) Evaluation of adhesiveness of *Acinetobacter* sp. Tol 5 to abiotic surfaces. J Biosci Bioeng 113:719–725
77. Ishikawa M, Nakatani H, Hori K (2012) AtaA, a new member of the trimeric autotransporter adhesins from *Acinetobacter* sp. Tol 5 mediating high adhesiveness to various abiotic surfaces. PLoS ONE 7(11):e48830
78. Corpe WA (1972) Microfouling: the role of primary film forming marine bacteria, International Congress on Marine Corrosion and Fouling, national bureau of Standards, Gaithersburg. pp. 598–609
79. Smith CS, Hinz A, Bodenmiller D, Larson DE, Brun YV (2003) Identification of genes required for synthesis of the adhesive holdfast in *Caulobacter crescentus*. J Bacteriol 185:1432–1442
80. Jones HC, Schmidt JM (1973) Ultrastructural study of crossbands occurring in the stalks of *Caulobacter crescentus*. J Bacteriol 116:466–470
81. Bodenmiller D, Toh E, Brun YV (2004) Development of surface adhesion in *Caulobacter crescentus*. J Bacteriol 186:1438–1447
82. Li CS, Smith YV, Brun JX, Tang (2005) The elastic properties of the *Caulobacter crescentus* adhesive holdfast are dependent on oligomers of N-acetylglucosamine. J Bacteriol 187:257–265
83. Tsang PH, Li G, Brun YV, Freund LB, Tang JX (2006) Adhesion of single bacterial cells in the micronewton range. Proc Natl Acad Sci. U. S. A. 103:5764–5768

Chapter 5
Microstructures of Biofilm

James Chapman

Abstract Biofilms are viewed as one of the most common form in which microorganisms exist naturally in nature. In particular, bacterial biofilms play important roles in industrial-based problems, disease and infection and have been studied in great detail. However, the intrinsic structure of a biofilm is not solidly understood where postulations on mechanisms, structure, chemical and biological nature and the community are still seldom reliably described. This chapter aims to highlight some of the most important structures contained in a biofilm—in particular microstructures. It is the microstructure of a biofilm which governs its make-up, integrity and functionality, all of which will be discussed to some level of detail.

Introduction

Bacteria are elementary, unicellular and are able to grow, divide, adapt and sense autonomously to surrounding environmental signals [1]. This self-sufficient behaviour allows bacteria to coordinate with other species to accomplish a symbiotic (not always) cooperative series of activities such as biofilm formation, bioluminescence, cellular communication (quorum sensing) and adhesion [2]. Coordination occurs through the quorum-sensing pathway and confers bacteria the capacity to recognise sophisticated measurements for the integrity of the bacterial community to co-exist on a given surface or substratum. Mainly, quorum sensing allows the recognition of population density through measurement of specific signalling molecules that members in the 'biofilm' secrete [3].

One of the most (not always) common processes that bacteria are able to achieve in a cooperative mode is through biofilm development. Biofilms are known communities of microorganisms that readily grow, attach to a surface and embed through the secretion of fasteners known as exopolymeric substances (EPS). From within the biofilm, the microorganisms grow protected from the environmental stresses and pressures such as salinity, desiccation, immune response attacks, protozoa ingestion and antimicrobial agents, to name a few. Crudely speaking, the understanding of

J. Chapman (✉)
School of Medical and Applied Sciences, Central Queensland University, Queensland, Australia
j.chapman@cqu.edu.au

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_5

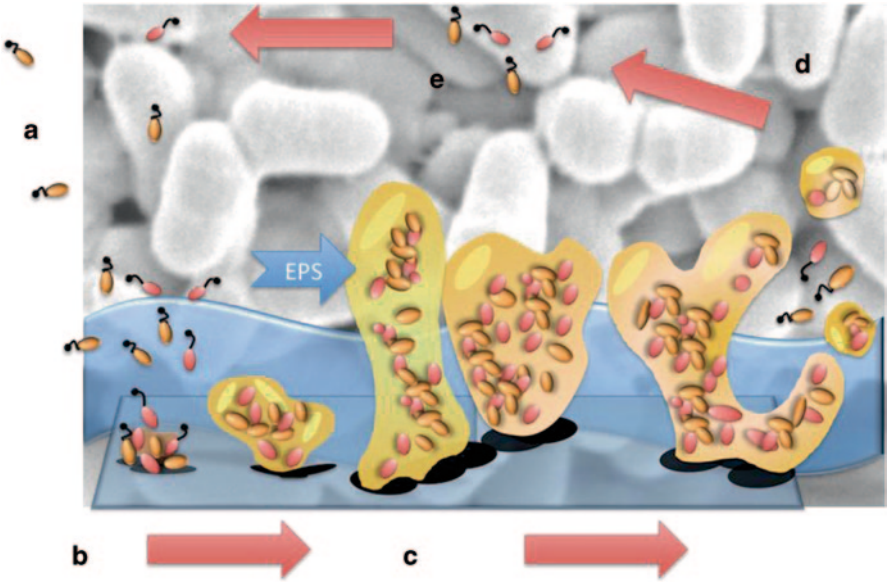


Fig. 5.1 The development of a biofilm and associated microstructures. **a** Planktonic organisms, **b** biochemical sorption to the surface of a material and subsequent bacterial colonisation, **c** adhesion and production of exopolymeric substances (EPS), **d** the detachment phase and **e** dispersion phase

biofilm formation comprises of five main events: biochemical adsorption of nutrients to the substratum, bacterial colonisation, irreversible adhesion to the surface, followed by cellular division and secretion of the EPS and finally, disassembly of the biofilm matrix and dispersion, Fig. 5.1. The development of a biofilm can occur from minutes to years to decades and therefore size distributions can be disparate depending on the environmental conditions, species present and the surface that is being dominated—although, all have similarities; a three-dimensional (3D) structure with microstructuring, Fig. 5.1.

One of the more important steps in biofilm development resides in the attachment phase where bacteria begin to divide and form microcolonies. At this point, the population density increases, as does the quorum signal concentration that can reach levels required to activate the maturation and disassembly of the biofilm in a controlled manner. This is of vast importance owing to its inherent ability to persist and survive. Recent evidence suggests that many bacterial species use quorum sensing to activate the disassembly process of the biofilm as a measure to ensure further colonisation. For this to happen, the biofilm dispersion allows the bacteria to escape, colonise new surface and substratum when nutrients become scarce and waste products and toxins accumulate.

Early in the twentieth century, researchers recognised that aqueous bound bacterial colonisations prefer a community-based surface-bound, sedentary lifestyle whereby these observations ignited an extensive and ever still increasing research effort for investigation. One of the founding fathers of biofilm research, J. W.

Costerton, pioneered the description of biofilms and the given associated microstructures [4].

This chapter has been written to describe some of the formation mechanisms of biofilm development along with the fluid mechanisms that influence environmental sustainability of a biofilm in certain conditions.

Biofilm Formation

Biofilm formation is a dynamic process, where it is now known that during the first stages of biofilm formation the structure of the biofilm is initially determined through; free living bacteria being brought to the solid surface through physical and chemisorption processes along with hydrodynamic forces. Extracellular organelles, such as flagella, curli, fimbriae or pili as well as outer membrane proteins have also been shown to help bacteria sense and interact with a given surface. These interactions can help cells overcome long-range repulsive forces along the surface. This process is critical in the formation of the biofilm structure and is influenced by the substrate surface (e.g. charge hydrophobicity etc.). It is at this stage that bacterial cells, usually held in Brownian motion, can be relatively easily removed through shear forces. Subsequently, once initial attachment has been made, flagellar arrest and the attached cells start to manufacture EPS. It is this process that promotes the transition of bacterial attachment from reversible to irreversible. The permanently attached cells are hard to detach owing to the EPS matrix. The secretion of eicosapentaenoic acid (EPA) is stimulated by chemical communication previously discussed as quorum sensing. The components of EPS vary between species and growth conditions, as cells begin to replicate and the EPS further accumulates, these micrometre communities grow into 3D structures as mature biofilms. Contained within a mature biofilm, adhesive EPS acts as a 'glue/adhesive' between the embedded cells to support the 3D nature of the biofilm. The complex arrangement allows the transport and removal of nutrients and waste products. Some intrinsic cues, such as cell death within biofilms, cells in some mature examples can also detach and return to bulk flow environments. The released cells then have the potential to reattach to any given surface and form new biofilms of a new environmental niche. It is the dispersion phase that is believed to be the crucial self-renewal of bacterial communities. Thus, cells within the biofilm community are less strongly affected than the suspended cells from changes in environmental conditions such as temperature, pH, nutrients, metabolic products and furthermore, toxic substances. An excellent visualised example of a biofilm has been obtained by Wallace and co-workers using scanning electron microscopy (SEM) to explore the intrinsic structure of the biofilm, Fig. 5.2. In this example, focused ion beam milling was used for observing the subsurface features of an unsaturated biofilm. Cross sections were precisely removed from the sample and allowed observation of the biofilm cell layers and the abundance of EPS matrix associated with them with clear definition and high resolution [5].

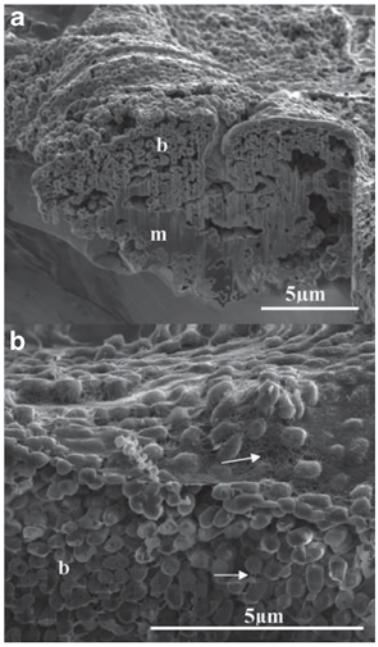


Fig. 5.2 A scanning electron micrograph of a 14 d biofilm, where clear layers and empty spaces are displayed in the structure. Vertical striation marks are also visible from the milling process on the sample. (Taken with permission from [5])

Biofilm Structure

Modern technological advances in microscopy and traditional analytical imaging have allowed a renaissance in biofilm characterisation and visualisation [6]. Of the more recent of these advances is the use of confocal scanning laser microscopy (CSLM). CLSM allows a non-destructive, in-situ analysis, living, fully hydrated without the need for fixation attributes to be realised. As such, CSLM comfortably analyses biofilms routinely in research nowadays using a wide range of specific fluorescent probes. It was the work of Claude Zobell noticing adherence of marine bacteria to surfaces [7]. The adherence and growth of these organisms included no implication of the complexity of a biofilm structure previously shown. It was not until the late 1980s that the structure was challenged showing the architectural communities and basic micro colonies formed in a fully hydrated biofilm matrix. Both the schematic understanding and real micrograph of the structures depicted in Figs. 5.1 and 5.2 demonstrate the complex structure of the biofilm. The basic microcolony and water channel architecture is affected by a number of external factors that affect the overall biofilm structure. While the structure of the biofilm is influenced by the biological factors; twitching, motility, quorum sensing, [8] growth and even deposition of EPS, other forces from the physical environment also shape a biofilm's structural integrity. They can be in the form of shear forces or even the hydrodynamics of the system.

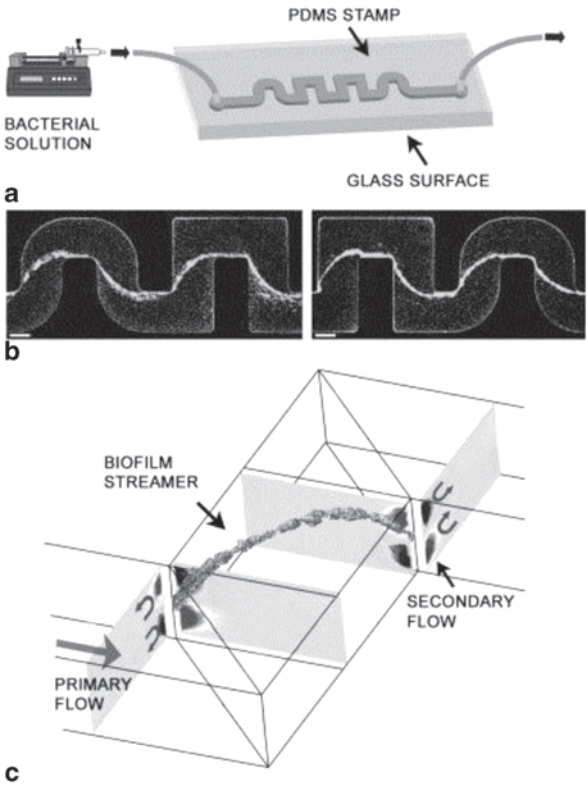


Fig. 5.3 Microfluidic experiment used to study biofilm formation under a continuous flow and constant nutrient condition. (Taken from [11])

Take high flow which is unidirectional, for example the influence of shear forces and cell clusters become elongated in the direction of the downstream to form a 'streamer' structure. Stoodley et al. found that by growing biofilms in turbulent environments a temporal complexity of biofilm behaviour was realised with time-lapsed imaging. This pioneering work gave vital information into the adaptability of a biofilm—reacting to different environmental conditions [9]. A more recent investigation on hydrodynamic force [10] on biofilm development from Rusconi et al. demonstrated that suspended thread-like biofilms develop in zigzag micro channels with varied radii and curvature [11, 12]. The authors postulated that through numerical simulations of a low Reynolds-number flow around these corners indicate the presence of a secondary vertical motion. The formation of streamers as suggested by Stoodley et al. has been found to be directly proportional to the intensity of the secondary flow around the corners, Fig. 5.3. Hydrodynamic conditions also control two very fundamentally linked parameters: mass transfer and drag. These parameters will significantly influence many of the processes associated with biofilm development. Although much of the hydrodynamic biofilm research has focussed upon using flow-cells with laminar conditions, the truest representation would be to grow biofilms in the field (or environment that they really persist in) with the

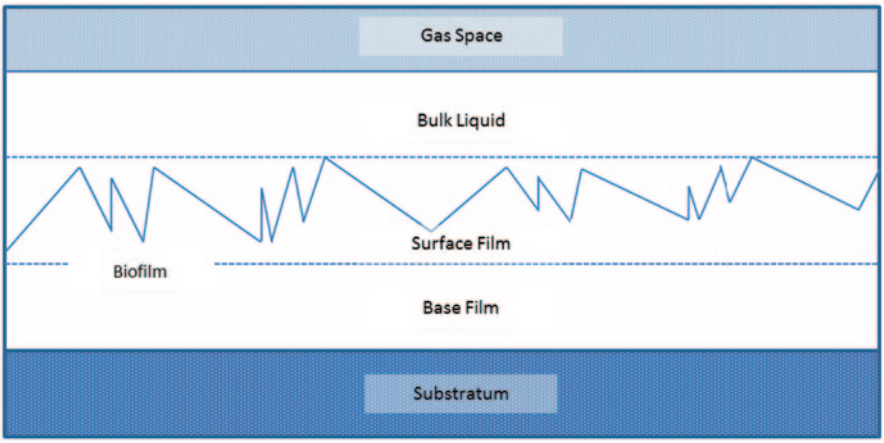


Fig. 5.4 The Wilderer-Characklis model, essentially depicted as a planar layered model but allowed for mathematical analysis to be realised on biofilm for quantitative analysis

use of complex imaging to visualise these enhancements [13] something, that in all honestly, will not be realised in exquisite detail for a number of years.

The 'Wilderer-Characklis' Model of a Biofilm

From the late 1980s the structure and function of biofilms was proposed as a model for structural identification rationale. Using an engineered approach, the biofilm system is divided into succinct compartments: the substratum, the biofilm, the bulk liquid and the possible gas spaces. The biofilm compartment was further subdivided into a base and surface film based around evidence in using SEM and other microscopies. The base film has been depicted as a homogenous planar layer of cells encased in the EPS matrix where the surface film is very rough, Fig. 5.4.

It is clear from this model that certain locations between interfacial parameters are somewhat subjective, nevertheless this led to some excellent conceptual models being developed using mathematical modelling. With closer examination of the Wilderer-Characklis model there is conclusive evidence that flow could be tracked through a biofilm microstructure. One way of achieving this is with the use of fluorescent particles using CLSM. Channels and flow velocity can be quantitatively determined as well as shear stress and hydrodynamic flow of a bulk liquid. This shear and mixing force have opposing influences on biofilm accumulation and resulting 'changing' structure. Increased shear force tends to also remove biofilm initiating a detachment phase as previously discussed in this chapter and will be discussed in depth in Chap. 6.

One overlooked parameter that can influence biofilm development is viscoelasticity. The property of viscoelasticity can induce short-term structural changes in the

biofilm make-up. At higher shear forces, viscoelasticity maintains the integrity of a biofilm. When an excessive load is applied, then the viscoelasticity of the biofilm allows it to flex, move and change to reduce the drag and allow the biofilm to be retained. Thus, the elastic limit of the biofilm is vitally important, and when this limit is reached then stress can influence the overall stability of the biofilm.

Biofilm microstructure therefore provides a solid protective scaffold that shelters viable cells from the prevalent shear forces and external stresses that biofilms encounter. The EPS produced by the microorganisms improves this shear stress resistance thus increasing the amount of biofilm immobilised and fixated on a given surface—reducing the amount of detachment and shedding. This increased stress resistance in a biofilm structure allows a wider range of nutrient fluctuations to be protected from, i.e. pH, dehydration osmoregulation than their planktonic counterparts.

The Biofilm Microstructure

This section will now discuss the intrinsic make-up of a biofilm where different sections of the biofilm will be discussed in terms of the microstructure. This discussion will be around a 3D argument as opposed to the planar model previously discussed using the Wilderer-Characklis model.

Microcolony Formation

Microcolonies are communities of cells three to five layers in depth. They are formed through the development of adhesins produced from the microorgnaisms that have adhered to the substratum. At a minimum, the clonal growth and cell-to-cell signalling are required to form and hold a microcolony firmly together. Some microorganisms exhibit more complicated characteristics where surface motility brings cells together to form the microcolony [14]. For example, *Staphylococcus aureus* the microcolony formation is mediated through the production of EPS. Several external environmental factors also influence the production of EPS for favourable microcolony formation; glucose, anaerobiosis, high osmoregularity, temperature increase, ethanol and limitations in iron concentrations [15].

Biofilm Microstructure

Following the initial stages of surface attachment, (and now) microcolony formation clear structures can be observed, Fig. 5.1. The structures are encased in EPS. The biofilms can be thick, homogenous, or they can be complex structures of pillars with water channels, protein signalling pathways and have been demonstrated to

allow nutrient and waste influx and efflux [16]. The overall microstructure present in this form can vary depending on the processes previous discussed such as regulation of microorganisms and physical shear forces. However, the structure can also vary owing to the regulation of the biofilm by bacteria. Mutants for instance, have been identified to affect the overall depth of the mature biofilm and furthermore the architecture—thus having ability to sense the population density of the biofilm.

The Depth of a Biofilm

The depth of a biofilm can be controlled or even reduced by catabolite repression through the common transcription factor CcpA [17]. The activity of CcpA is totally dependent on a state of phosphorylation that reflects the glycolytic activity in the cell—thus CcpA is active in reducing biofilm depth [18]. In *Escherichia coli* catabolite repression has been shown to inhibit biofilm formation. The exact step in biofilm formation that is affected by glucose is largely unknown, but glucose does not disrupt pre-established biofilms really suggesting that glucose is important in hindering the initial attachment phase of the biofilm development process.

The Overall Architecture

In the production of EPS and attachment of microorganisms surfactant production can occur. Certainly, with *Pseudomonas aeruginosa* surfactant production occurs with rhamnolipid production. This surfactant is required for the maintenance of pillar like structures in the biofilm microstructure [19]. Similarly, *P. aeruginosa* regulates lasl-lasR which is a quorum-system sensing transcriptional activator consistent with surfactant production and the maintenance of a biofilm structure—all of which contribute to the overall microstructure and maintenance of the biofilm.

Conclusions

Enormous interest is currently underway to improve the understanding of biofilm development because the biology supporting this process is anticipated to be instrumental for the development of new remediation measures. Early studies of microstructure biofilm growth have previously focused on the initial steps of biofilm identified surface adhesins responsible for the interaction with both biotic and abiotic surfaces. Subsequently, efforts were then aimed at understanding the regulation of the synthesis of biofilm matrix compounds to induce the synthesis of biofilm matrix EPS. More recently, studies have shown many bacteria use the signalling pathways to activate, in a coordinated manner, the dispersion of the biofilm

structure—which in some ways is the most important stage in survival (Chap. 6). The extent of biofilm architecture and microstructure accumulation in a given system is a complex integration of nutrient availability, hydrodynamics and microbial pressure. When the inter-relationships of the complex synergy in a biofilm are completely understood—then real concerted efforts in biofilm remediation will be quickly realised—until then we still have a problem.

References

1. Chapman J, Weir E, Regan F (2010) Period four metal nanoparticles on the inhibition of biofouling. Colloids Surf B Biointerfaces 78(2):208–216
2. Hellio C et al (2000) Inhibition of the development of microorganisms (bacteria and fungi) by extracts of marine algae from Brittany, France. Appl Microbiol Biotechnol 54(4):543–549
3. Costerton JW, Lappin-Scott HM (1995) Introduction to microbial biofilms. Microbial biofilms, pp. 1–12, Cambridge University Press
4. Costerton JW et al (1995) Microbial biofilms. Annu Rev Microbiol 49(1):711–745
5. Wallace PK, Arey B, Mahaffee WF (2011) Subsurface examination of a foliar biofilm using scanning electron- and focused-ion-beam microscopy. Micron 42(6):579–585
6. S. Gangadoo; A. Taylor-Robinson; J. Chapman (2014) Nanoparticle and biomaterial characterisation techniques. J Mater Technol (30) B1 pp. B44–B56
7. Lappin-Scott, Hilary M. "Claude E. Zobell–his life and contributions to biofilm microbiology." In *8th International Symposium on Microbial Ecology*, p. 6. 1999.
8. Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55(1):165–199
9. Stoodley, Paul, John D. Boyle, and Hilary M. Lappin-Scott. "Influence of flow on the structure of bacterial biofilms." (2000): 263-269.
10. Chapman J, Regan F (2012) Nanofunctionalized superhydrophobic antifouling coatings for environmental sensor applications—advancing deployment with answers from nature. Adv Eng Mater 14(4):B175–B184
11. Rusconi R et al (2011) Secondary flow as a mechanism for the formation of biofilm streamers. Biophys J 100(6):1392–1399
12. Rusconi R et al (2010) Laminar flow around corners triggers the formation of biofilm streamers. J R Soc Interface rsif20100096
13. Stoodley P et al (1998) Influence of hydrodynamics and nutrients on biofilm structure. J Appl Microbiol 85(Suppl. 1):19S–28S
14. Reisner A et al (2003) Development and maturation of Escherichia coli K- 12 biofilms. Mol Microbiol 48(4):933–946
15. Götz F (2002) Staphylococcus and biofilms. Mol Microbiol 43(6):1367–1378
16. O'Toole G, Kaplan HB, Kolter R (2000) Biofilm formation as microbial development. Ann Rev Microbiol 54(1):49–79
17. Stanley NR et al (2003) Identification of catabolite repression as a physiological regulator of biofilm formation by Bacillus subtilis by use of DNA microarrays. J Bacteriol 185(6):1951–1957
18. Seidl K et al (2008) Staphylococcus aureus CcpA affects biofilm formation. Infect Immun 76(5):2044–2050
19. Davey ME, O'toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64(4):847–867

Chapter 6
Detachment of Bacteria

James Chapman

Abstract The rheological properties of biofilms have a great impact on major processes such as transport of nutrients, light, biocides, water, biochemicals and cells. The knowledge of the mechanical properties of biofilms is essential in quantifying the overall process of biofilm development and bacterial survival (and proliferation)—one of the key processes in a biofilm lifecycle is detachment. It is therefore crucial to be able to predict the biofilm detachment and break up in response to internal and external forces that drive the biofilm cycle. This chapter aims to highlight some of the important processes in the biofilm detachment cycle. It will also draw on some new and old concepts on this age old battle of biofilm survival and proliferation.

Introduction

The population dynamics of biofilms are determined by the competition between organisms and the volume of substrate space. Within a biofilm the limitations in nutrients and space competition limit the base growth demonstrating that cells closest to the substrate are protected at a greater level whereas cells near the surface of the biofilm are removed more rapidly through detachment [1]. In Chap. 5, we observed the dynamics of a biofilm including its microstructure and architecture. The structure has also been shown to have mathematical representation and quantitation to understand the biofilm development process. Over the past few decades, mathematical models have been developed to enable us to understand the population dynamics, kinetics and ever-changing structure of a biofilm. For instance, Kisell et al. used a dynamic model to evaluate competition between autotrophic and heterotrophic incorporating mass transport kinetics, Monod kinetics as well as the determination of limiting electron acceptors or donors. [2] Where biofilms are dynamic, structurally complex and embed on to a given surface, the steps involved in sustainability are equally important to the biofilm's survival [3–5]. Recapping over Chap. 5, the steps involved in biofilm formation are: initial attachment of the microorganism cells to

J. Chapman (✉)
School of Medical and Applied Sciences, Central Queensland University, Queensland, Australia
e-mail: j.chapman@cqu.edu.au

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_6

the surface [6] and the irreversible binding and growth-dependent accumulation of the multilayers of cells by the production of exopolymeric substances (EPS) [7]. Lastly, followed by biofilm maturation with the development of the biofilm architecture in the matrix and finally, detachment, the generalised term used to describe the release of cells (either individually or in aggregates) from the biofilm—of which will be discussed in this chapter comprehensively.

Detachment

Detachment, and the process of detachment, is an important and pressing subject. In the context of public health, this is of particular significance as the dissemination of pathogenic bacteria, especially with hospital devices and related infection rates are due to this detachment process. Sometimes infection has also been driven through transport of bacteria in water systems where detachment is the associated common cause.

The detachment processes are separated into three different physical mechanisms and these are: sloughing [8], erosion and abrasion [9]. Sloughing is the apparent random loss of large sections of the biofilm architecture. Erosion refers to the continuous loss of single cells or of small clumps of cells owing to the physical forces or cell-mediated type events. Finally, abrasion is the removal of biofilm owing to the collision of external particles surrounding the biofilm surface.

Traditionally, the biofilm detachment process has been studied in flowing systems where the dominant mechanisms were perceived to have been local shear forces. [10] However, it is emerging that further mechanisms are now involved; including quorum sensing, matrix degrading enzyme action, increased expression of flagella [11] and downregulation of twitching motility, to name a few. Hydrodynamic forces also play a role in phenotypic responses. To supplement the importance of detachment mechanisms, Boyd and Chakrabarty have demonstrated that cells involving *Pseudomonas aeruginosa* biofilm development were important in determining the degree of cellular detachment in the system. [12] The authors reported that nonmucoid strains of bacteria detached up to 50 times more cells than mucoid strains, suggesting that the extracellular network of cells further cements the cells to the surface and therefore influences this rate of detachment.

The method of flow cytometry has allowed for the mechanistic biofilm detachment properties to be realised. *P. aeruginosa* has previously been grown under different fluid shear conditions, where low shear forces produced less dense biofilm architectures whereby the biofilm also detached at a much lower fluid shear. Higher shear conditions enabled detachment at higher fluid shear forces. Shear stress has been reported by Stoodley et al. to influence biofilm detachment and this sometimes occurred at a shear stress approximately twice the stress of the growth stage [13].

In most cases, mechanisms of biofilm detachment are poorly understood, and as such, are fundamental to the survival process of the biofilm. Critically, the importance of being able to quantify baseline levels of the detachment phase during

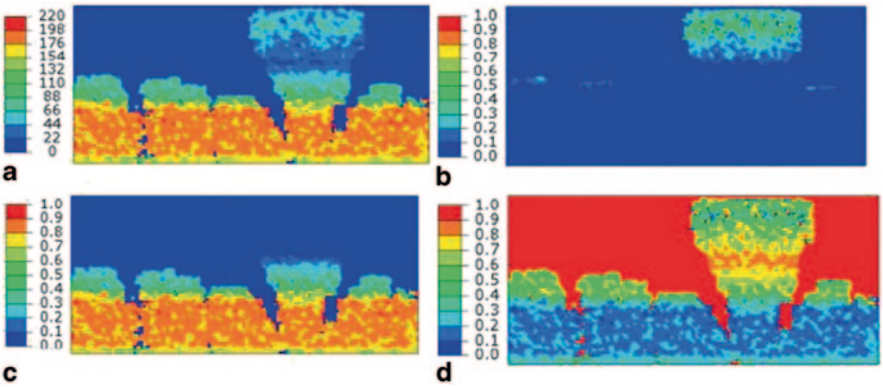


Fig. 6.1 Contours of (**a**) biofilm density in g COD x/l; (**b**) volume fraction c 1 of active biomass before deformation; (**c**) volume fraction c 3 of residual inert biomass profile before deformation; (**d**) volume fraction c 4 of void space before deformation. (Taken from [19])

a biofilm cultivation experiment in order to study the actual effect of remediation or mitigation techniques (i.e., increased shear, biocidal agents [3, 4, 14–16]) is extremely important. Previously, authors have demonstrated that detachment occurred only when the biofilm was fully formed—however, this is incorrect and detachment can occur at any time of the biofilm formation process. This suggests that biofilm formation is indeed the mechanism for survival in addition to proliferation [13].

Mechanical Detachment of Cells

The mechanical properties of biofilms are only ever obtained when subjecting the biofilm to a series of assays in static or dynamic conditions. These typically involve: tensile strength [17], elastic modulus, shear modulus and compression testing-based assays. Some of the parameters required to characterise the biofilm are: roughness, density, surface area and porosity. Density and porosity are closely linked and are certainly influential, varying from one biofilm to another where thickness is included [18]. Both density and porosity play roles in biofilm detachment and formation, when subjected to compression and tensile force, Fig. 6.1.

When an axial compression is made within the biofilm, the pores clearly collapse and thus the porosity decreases as demonstrated in Fig. 6.1. Characklis reported an increase in biofilm density with increased shear stress [20]. Furthermore, Wasche (2002) has demonstrated the empirical relationship between the biofilm density, nutrient loading and the Reynolds number—this omits any function of hydrodynamic mechanical forces on this compression situation [21].

However, hydrodynamic forces do need to be considered as an important driving force in the mechanical detachment processes of the bacterial cells. For example, Casey et al. have shown that detached particles when analysed regularly, stained,

filtered and examined under fluorescence microscopy, reproducibility is possible for detachment of old biofilms, as is for new. [22] This study distinguished between the erosion and sloughing formation following increments in shear stress [23].

Cell-Mediated Detachment

Cellular detachment can also be induced through communicative cell-to-cell processes. Despite the self-sufficiency of a biofilm, and the contained bacteria to coordinate efforts to minimise competitive stresses, cooperative efforts can co-exist in this complex matrix. Coordination can occur through cell-to-cell communication in the form of quorum sensing (QS), [24] as previously described in Chap. 5.

QS awards bacteria the capacity to recognise the overall population density through the continued measurement of signalling molecules that the secreted community of bacteria secrete. When the population density is high, the accumulated concentration of signalling molecules is sufficient to activate a driven response for detachment. The structure of QS-molecules typically have low molecular weights and belong to a large class of molecules inclusive of acyl-homoserine lactones (AHLs), furanosyl borate diesters (AI2) [25], *cis*-unsaturated fatty acids (DSF family signals) and certain peptide molecules [26].

A common process for bacteria to accomplish together—is in the formation of biofilm. The community of bacteria grow and detach as we have previously seen throughout Chap. 5 and 6, so far. When considering the relationship between biofilm developmental stages and the quorum-sensing pathway; once the biofilm development reaches a threshold level at what stage does QS participate in biofilm regulation? It is not until the later stages of biofilm maturation that detachment cues are forced and the communities of biofilm begin to 'take votes' on the overall biofilm architecture and composition. The biofilm dispersion process is therefore essential to allow bacteria to escape, colonise and generate new resource-managed niches. These processes are all driven to minimise toxicity of waste product accumulation and new nutrient sourcing. Various strategies for this dispersion event can be used for the following:

1. Ending the synthesis of biofilm matrix compounds
2. Disrupting the noncovalent interactions between the matrix components and
3. Degrading the matrix

Owing to the QS regulation and network process, these systems are extremely intricate and include several genes to affect the biofilm development. Some of these SQ signals have been summarised in Table 6.1.

The overall biochemical detachment process, in the form of QS, is of an extremely exciting stage in research. The main reason for this is that the biology, supporting the detachment process, will be anticipated to be crucial in developing new and emerging combat strategies for infection control [7] or indeed management of water systems that succumb to biofouling [14, 16].

Table 6.1 Summary table of biofilm detachment mechanisms regulated through quorum sensing networks

Bacteria	Biofilm dispersion stage	Surfactant used
P. aeruginosa	Pel exopolysaccharide	Rhamnolipids [27]
X. campestris	XagABC	N/A [28]
V. cholerae	Vps	Capsule [29]
S. aureus	PIA/PNAG	PSMs [30]

Enzyme-Based Activity

Thus far, we have seen that the detachment of adherent bacteria supported in the biofilm requires a set of general passive processes—particularly with some of the physical- and mechanical-based forces we have discussed. A particular discussion of this detachment process will continue with enzyme detachment processes. Some authors have identified that mixtures of enzymes such as proteases, polysaccharide lyases and cellulases could be used as inhibitory mechanisms for biofilm formation. The mechanisms that these particular enzymes use are centred on two main criteria: EPS degradation and direct biofilm attack. Additionally, fungi are able to degrade complex cell wall materials through the secretion of enzymes. For instance, fungal cultures have been used successfully to degrade exopolymeric substances in bacteria through the use of pectin. Interestingly, the complexity and depth of the biofilm matrix does matter in this process and thus is not the only route for removal [31]. Costerton et al. reported enzyme activity outside of the detachment and degradation process. For example, when biofilms form on the surfaces of insoluble-based nutrients such as cellulose, the initial adhesion process favours specific bacteria that can digest this sort of substrate—in this example, cellulolytic bacterium. The primary colonisers prior to biofilm formation therefore, have the ability to produce, cell-associated digestive enzymes that have the ability to attack the insoluble substrates. This of course is extremely site specific for the given substrate and bacterial colony [32].

Detachment Analysis

Although detachment is a stochastic process, it is caused by the local instabilities coupled with biofilm network activities. Some authors have been able to measure and quantify the rates and levels of detachment processes in biofilms. For instance mathematically, a biofilm can be thought of as a three-dimensional special evaluation, Fig. 6.2.

To explicate, Fig. 6.2: (a) represents a simplified depth of a biofilm with a random distribution and thickness, (b) the biofilm depends on the local dynamics of the system for this and is further broken down into (c) static biofilm conditions and

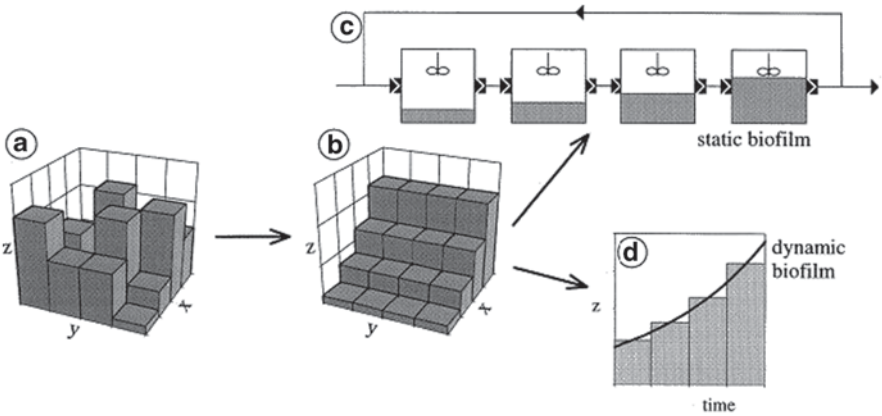


Fig. 6.2 Steps in transforming heterogeneous biofilm (**a**) to a one-dimensional biofilm assuming fluxes perpendicular to the substratum: (a) biofilm with a variable thickness distribution over the substratum; (**b**) thickness distribution of (**a**); in (**c**) a number. (Taken from [33])

(d) the dynamic biofilm system. For a static biofilm system, the location does not change with time. Thus it can be approximated by averaging all the *n*-elements. In the dynamic biofilm situation, the biofilm can be approximated by a single compartment with variable thickness over time, which has been brilliantly discussed by Morgenroth et al. [33]. The modelling concept of a biofilm has also been further examined by Phil Stewart and co-workers at the biofilm engineering centre—with cutting edge research methods and tools specifically designed to evaluate biofilm research [34].

Outside of mathematical modelling the detachment mechanisms of cells in biofilms, Boles et al. have created a reproducible flow cell experiment utilising confocal microscopy to measure detachment of cells. Prior to the cells detaching, the biofilm was scraped from the surface, homogenised and plated on LB agar plates. The authors have found that the variant and wild types of cells associated in the biofilm inhabit different regions in mixed biofilms. The detachment of the biofilm induces a revering pattern in biofilm growth—which was determined through red and green fluorescent dye. These findings prompted the authors to investigate rhamnolipid production that has subsequently been shown to decrease the adhesive interactions of the biofilm [35].

Conclusions

Some important considerations have been discussed in this chapter. Detachment is an exciting research topic for new and emerging synthesis of antimicrobial agents for cells and the subsequent detachment processes of bacteria for pathogenic transfer research.

There are multiple ways for biofilms, and in particular, bacteria to detach—some physical, some biochemical: shearing by fluid flow, cell lysis and enzymatic dissolution of matrix biomaterial. The importance of detachment needs to be emphasised as a critical process for biofilm architecture and the rate of biofilm accumulation and proliferation. The biofilm regulates the dissemination of bacteria from the contaminated surface. Therefore, we are in an important stage of biofilm and bacterial cell detachment responses. If scientists are able to decipher the detachment process then we would be able to design strategies for improved bacterial settlement resistance, biofilm proliferation. In essence, this would have the potential to save millions annually for issues caused by these processes.

References

1. Brackman G, et al (2009) Use of quorum sensing inhibitors to interfere with biofilm formation and development in *Burkholderia multivorans* and *Burkholderia cenocepacia*. Res Microbiol 160(2):144–151
2. Kissel JC, McCarty PL, Street RL (1984) Numerical simulation of mixed-culture biofilm. J Environ Eng 110(2):393–411
3. Chapman J, Regan F (2012) Nanofunctionalized superhydrophobic antifouling coatings for environmental sensor applications advancing deployment with answers from nature. Adv Eng Mater 14(4):B175–184
4. Chapman J, Weir E, Regan F (2010) Period four metal nanoparticles on the inhibition of biofouling. Coll Surf B Biointerfaces 78(2):208–216
5. Gangadoo S, Taylor-Robinson A, Chapman J (2014) Nanoparticle and biomaterial characterisation techniques. Mater Technol: Adv Biomater 30(B1):B44–B56
6. Costerton JW, Lappin-Scott HM (1995) Introduction to microbial biofilms. In: Lappin-Scott HM, Costerton JW (ed) Microbial biofilms, 1st ed. Cambridge University Press, New York, pp. 1–11
7. Gangadoo S, Chapman J (2014) Emerging biomaterials and strategies for medical applications: a review. Mater Technol : Adv Biomater 30(B1 2015):B3–B7
8. Rice S, et al (2005) Biofilm formation and sloughing in *Serratia marcescens* are controlled by quorum sensing and nutrient cues. J Bacteriol 187(10):3477–3485
9. Picioreanu C., van Loosdrecht MC, Heijnen JJ (2001) Two-dimensional model of biofilm detachment caused by internal stress from liquid flow. Biotechnol Bioeng 72(2):205–218
10. Peyton BM, Characklis W (1993) A statistical analysis of the effect of substrate utilization and shear stress on the kinetics of biofilm detachment. Biotechnol Bioeng 41(7):728–735
11. Watnick P, Kolter R (2000) Biofilm, city of microbes. J Bacteriol 182(10):2675–2679
12. Boyd A, Chakrabarty Aá (1994) Role of alginate lyase in cell detachment of *Pseudomonas aeruginosa*. Appli Environ Microbiol 60(7):2355–2359
13. Stoodley P, et al (2002) Biofilms as complex differentiated communities. Annl Rev Microbiol 56(1):187–209
14. Chapman J, et al (2014) Bioinspired synthetic macroalgae: examples from nature for antifouling applications. International Biodeterioration Biodegrad 86:6–13
15. Chapman J, et al (2010) Phthalate doped PVC membranes for the inhibition of fouling. J Membr Sci 365(1):180–187
16. Chapman J, et al (2013) Antifouling performances of macro-to micro-to nano-copper materials for the inhibition of biofouling in its early stages. J Mater Chem B 1(45):6194–6200
17. Ohashi A, et al (1999) A novel method for evaluation of biofilm tensile strength resisting erosion. Water Sci Technol 39(7):261–268

18. Battin T, Sengschmitt D (1999) Linking sediment biofilms, hydrodynamics, and river bed clogging: evidence from a large river. Microbial Ecol 37(3):185–196.
19. Laspidou C, et al (2014) Material modeling of biofilm mechanical properties. Math Biosci 251:11–15
20. Characklis W (1981) Bioengineering report: fouling biofilm development: a process analysis. Biotechnol Bioeng 23(9):1923–1960
21. Wäsche, S., H. Horn, D.C. Hempel (2002) Influence of growth conditions on biofilm development and mass transfer at the bulk/biofilm interface. Water Res 36(19):4775–4784
22. Casey E, Glennon B, Hamer G (2000) Biofilm development in a membrane-aerated biofilm reactor: effect of flow velocity on performance. Biotechnol Bioeng 67(4):476–486
23. Walker J, Marsh P (2007) Microbial biofilm formation in DUWS and their control using disinfectants. J Dent 35(9):721–730
24. Case RJ, Labbate M, Kjelleberg S (2008) AHL-driven quorum-sensing circuits: their frequency and function among the Proteobacteria. ISME J 2(4):345
25. Chen X, et al (2002) Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415(6871):545–549
26. Deng Y, et al (2010) Listening to a new language: DSF-based quorum sensing in Gram-negative bacteria. Chem Rev 111(1):160–173
27. Ueda A, Wood TK (2009) Connecting quorum sensing, c-di-GMP, pel polysaccharide, and biofilm formation in *Pseudomonas aeruginosa* through tyrosine phosphatase TpbA (PA3885). PLoS Pathog 5(6):e1000483
28. Dow JM, et al (2003) Biofilm dispersal in *Xanthomonas campestris* is controlled by cell–cell signaling and is required for full virulence to plants. Proc Natl Acad Sci U S A 100(19):10995–11000
29. Lee KJ, et al (2013) Role of capsular polysaccharide (CPS) in biofilm formation and regulation of CPS production by quorum—sensing in *Vibrio vulnificus*. Mol Microbiol 90(4):841–857
30. Periasamy S, et al (2012) How *Staphylococcus aureus* biofilms develop their characteristic structure. Proc Natl Acad Sci U S A 109(4):1281–1286
31. Orgaz B, et al (2006) Bacterial biofilm removal using fungal enzymes. Enzyme Microb Technol 40(1):51–56
32. Costerton JW, et al (1987) Bacterial biofilms in nature and disease. Annl Rev Microbiol 41(1):435–464
33. Morgenroth E, Wilderer PA (2000) Influence of detachment mechanisms on competition in biofilms. Water Res 34(2):417–426
34. Stewart PS (1993) A model of biofilm detachment. Biotechnol Bioeng 41(1):111–117
35. Boles BR, Thoendel M, Singh PK (2005) *Rhamnolipids* mediate detachment of *Pseudomonas aeruginosa* from biofilms. Mol Microbiol 57(5):1210–1223

Chapter 7
Genomics Approach

Hajime Ikegai

Abstract Biofilm is an essential strategic structure for microorganisms such as bacteria to grow stably in an aquatic environment. Its universal presence in the aquatic environment is a piece of evidence. In biofilm formation, microbes adhere to a surface of solid substrates using flagella or pili, which are microbial locomotors, and start proliferating. Quorum sensing is then induced by signal molecules termed autoinducers. Then large amounts of extracellular polysubstances are produced to form a biofilm in which sessile bacteria are encased.

Introduction

Bacteria living in the environment and many microorganisms residing in human beings, animals, and plants, form film aggregates (biofilm) from planktonic cells. These cells are on the surface of a solid substrate in water to allow them to evade various threats to their survival. Although biofilm has been recognized since the 1940s [1], it took more than 30 years to reveal that biofilm was composed of film polysaccharides and bacteria [2]. Furthermore, since 1987, a film bacterial population adhering to solid substrates has been referred to as biofilm to distinguish it from a population of planktonic cells [3]. Here we will discuss the meanings of biofilm formation for the survival of microorganisms, and the structure and functions of biofilms governed by genes and intracellular molecules.

Biofilm Formation

If you carefully observe a surface of various solid substrates that has been immersed in water for a long time, you may find dirty spots with adhesion of viscous substances. These dirty spots mostly consist of biofilms formed on the surface of solid substrates from adhesive matrix components such as extracellular polymeric sub-

H. Ikegai (✉)
National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: ikigai@chem.suzuka-ct.ac.jp

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_7

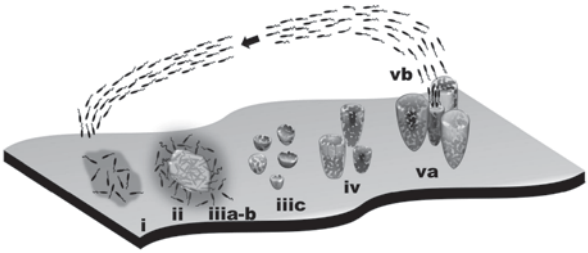


Fig. 7.1 A typical process of biofilm formation (i) attachment, (ii) growth, (iiia) EPS production, (iiib) occurrence of biofilm-forming bacteria, (iiic) microcolony formation, (iv) development of biofilm, (va) maturation of biofilm, and (vb) dispersion of planktonic cells

stances (EPS), aggregative fimbriae, and large adhesive proteins, with microscopic dirt attached to it.

Biofilms are not only formed by a single species of bacteria or fungi [4] but also covered with predatory protozoa, or algae, larger aquatic organisms. This can be observed on a surface of artificial structures and rocks found in a lake, river, brackish water, and the sea. Biofilms are also found in our surroundings. Wherever in a moist condition, such as a kitchen sink, bathroom floor, inner wall of a drainpipe, or the inside of an air conditioner and its drainage duct, then biofilms are formed on a hard surface. Biofilms are also formed on a surface and inside animal and plant bodies. Plaque formed on teeth or inside periodontal pocket between teeth and gums in the mouth and a furred tongue are typical examples. Additionally, some biota in the intestine, nasal cavity, and respiratory tract, are considered to form a biofilm.

As shown in Fig. 7.1, the process of biofilm formation has five stages: (i) attachment, (ii) growth, (iii) EPS production, (iv) development of biofilm, and (v) maturation of biofilm and dispersion of planktonic cells, in which the biofilm formation repeats rise and fall cyclically [5].

Advantage of Sessility

Sessility denotes a property of microorganisms to grow on the surface of solid inorganic or organic substrates. In microbes, attachment to the substrate surface takes place via both physical and biological reactions. In the former physical attachment, microbes are regarded as colloidal particles and physically attached to a solid surface solely via microscopic binding as an interfacial reaction between aqueous and solid phases.

In addition, around the time of microbial attachment to solid substrates, essential nutrients for microorganisms, such as glucose and amino acids, are available in the aqueous environment, but the quality and amount of nutrients therein are much less than those in a culture medium and far from sufficient. Organisms rather live in an oligotrophic condition, competing for nutrients with each other. Thus, the surface

of solid substrates where nutrients in water are concentrated by physical adsorption serves as an excellent growth environment for microorganisms. It is also an important point of sessility that microbial biofilms formed only on solid substrates offer great benefits to the growth of microorganisms. This nutrient adsorption step is considered as a preparatory stage prior to the attachment of microorganisms.

On the other hand, the latter biological attachment primarily attributes to sessile factors. These sessile factors are surface proteins of microorganisms, termed flagella or pili. While both are used for the attachment to solid substrates, they are also used as a locomotive apparatus of microbes. Both organs are essential for bacteria to grow and inevitably act as a pathogenic factor since they can advance the growth of microbes. Microbial motility is applied to escape threats and to acquire nutrients. This is especially true when nutritional substances are adsorbed to a surface of solid substrates as shown in Fig. 7.1(i), bacteria migrate aiming there. Sessile factors are also necessary for microbes to attach to a solid substrate even temporarily and anchor themselves to it for a steady uptake of nutrients. Thus, motility and sessility are inseparably bound.

Recently, structures and mechanisms of flagella and pili in *Pseudomonas aeruginosa*, which is prone to form biofilms, have been studied at a molecular level [6, 7]. Flagella are used for translocation towards concentrated nutrients under an oligotrophic environment. Regarding motilities of *P. aeruginosa* (cell colonies grown on an agar medium) they show distinct morphologies, so-called swimming and swarming, regulated by FlhF, which is one of the flagella-composing proteins. Motilities mediated by these apparatuses are used for translocation according to the concentration gradient of a chemical substance diffusing in an aqueous environment, toward a higher concentration if the substance is beneficial or toward a lower concentration to escape from a toxic substance [6].

The pilus is a tubular organ to mediate intercellular exchange of plasmid DNA (R- and F-factor). Other than this, pili found on bacteria like flagella, called type IV pili, provide motility (twitching, Ref. [8]) and sessility to bacteria.

Detachment of Bacterial Cells From Biofilms

Biofilm-forming bacteria do not always spend their entire life within the EPS, which is a primary component of biofilms, but some are to be dispersed from biofilm into the surrounding environment for new biofilm formation (Fig. 7.1, stage v). This dispersion can take place because the topmost layer of biofilms is always unstable with a loose structure, making it ready to detach from the body of biofilms any time by mechanical forces such as a water stream. Small biofilm clusters detached from the main body collapse and disperse planktonic cells into an aqueous environment. These planktonic cells may form new biofilms [9, 10].

Aside from the physical destruction of biofilms, partial detachment of a biofilm surface is also controlled by chemical signals secreted from biofilm-forming bacteria. In addition, detachment of biofilms is caused by not only external physical

forces but also by the synthesis of enzymes that decompose biofilms as well as signal molecules that induce detachment of biofilm clusters [11].

Biofilm Structure

Under microscopic observation, biofilm structures are found to be not smoothly flat but very rugged. Thickness of *P. aeruginosa* biofilms has been reported to range from 2 to 100 μm with the mean measurement of 33 μm [12]. Biofilms in the environment hold mixed microbial communities with different types of bacteria, fungi, algae, and even predatory protozoa sometimes, all together within EPS [13]. Bacteria and fungi such as *Candida* form biofilms because they act as beneficial barriers for protection from a wide variety of stresses, such as predators, antibiotics, changes of pH and temperature, and reduction of nutrition.

Drug Resistance of Biofilms

The biofilm structure is important for bacteria to avoid antimicrobial effects. Concentrations of antibiotics required to kill bacteria or inhibit their growth are 10 to even 1000 times higher in bacteria growing inside biofilms than in planktonic cells [13]. Biofilms composed of polymers including polysaccharides and proteins prevent antibiotics from reaching bacteria living in biofilms [14]. This resistance to antibiotics is found commonly in most bacteria regardless of whether they are gram-positive or gram-negative [13]. Deterioration of bacterial metabolic conditions, including depletion of nutrients, accumulation of metabolites, and lowered oxygen partial pressure, leads to activation of the *rpoS* gene and a decrease in bacterial intake of antibiotics, rendering bacteria less susceptible to antibiotics [15].

Bacterial Intercellular Communication via Substances Produced by Bacteria

It was in the late 1960s that studies on chemical substance-mediated communication between bacterial cells of the same species started [16]. This communication is not active when a bacterial population density is small but undertaken when the population reaches a certain density [17, 18]. This is because an increased number of bacteria results in decreased nutrients and increased metabolites. In order to avoid a decrease in the growth rate due to these conditional changes, once the population has reached a certain density, bacteria undertake synthesis and extracellular secretion of chemical substances termed autoinducers (AIs), which alter

intracellular metabolic activities. When the AI level increases, bacterial reuptake of AI and expression of genes for synthesis of proteins beneficial to their survival occur, and the synthesis of new proteins is initiated. This process is termed quorum sensing, and it enables bacteria to monitor their population size by detecting the AI concentration.

Autoinducers Induce Quorum Sensing

An AI of bioluminescence in the luminous marine bacterium *Vibrio fischeri* is *N*-(3-oxohexanoyl) homoserine lactone, an acyl-homoserine lactone (AHL) comprising homoserine lactone combined with an acyl chain (Fig. 7.2a, Ref. [19–21]). For accumulation of the AI in a quantity required to trigger bioluminescence, 10^7 cells/ml or more bacteria are necessary [22]. This AI is synthesized by an enzyme encoded by *luxI* gene in the gene cluster called *lux* operon. Quorum sensing with AHL regulated by *lux* operon is used in a wide variety of gram-negative bacteria, including *P. aeruginosa*, *Serratia*, *Yersinia*, and *Proteus* without being limited to *Vibrio* [23]. In gram-positive bacteria, oligopeptides (AIP;autoinducing peptide) play the role of AI (Fig. 7.2b, Ref. [19, 20, 24, 25]). Particularly, *agr* (accessory gene regulator), which governs expression of hemolysin in *Staphylococcus aureus*, is also involved in sessility and biofilm formation in gram-positive bacteria [26].

Quorum Sensing Controls Biofilm

Many studies on the relationship between biofilm formation and quorum sensing were conducted in *P. aeruginosa*, demonstrating that two genes called *lasI* and *rhlI* are deeply involved in it. It has been shown that *lasI* is responsible for the initial stage, in which bacteria adhere to solid substrates, form a biofilm, and then extend in two-dimensional directions, while *rhlI* is responsible for the subsequent thickening stage, in which the two-dimensional biofilm development nearly ends and three-dimensional expansion takes place [27]. In addition, quorum sensing via 4-quinolone, another substance used as an AI in *P. aeruginosa*, has been demonstrated to enhance biofilm formation [28].

To date, it has been revealed that biofilm formation is controlled by quorum sensing not only in *P. aeruginosa* but also in various other species of bacteria, including gram-negative bacteria such as *Escherichia coli* [29], *Campylobacter* [30], *Aeromonas* [31], and *Serratia* [32], and gram-positive bacteria such as *Bacillus subtilis* [33], *Staphylococcus aureus* [26], *Enterococcus* [34], and *Streptococcus mutans* [35]. Quorum sensing in these microbial species acts on expansion of biofilm structure and is also known to be involved in the deceleration of biofilm formation and destruction of biofilms.

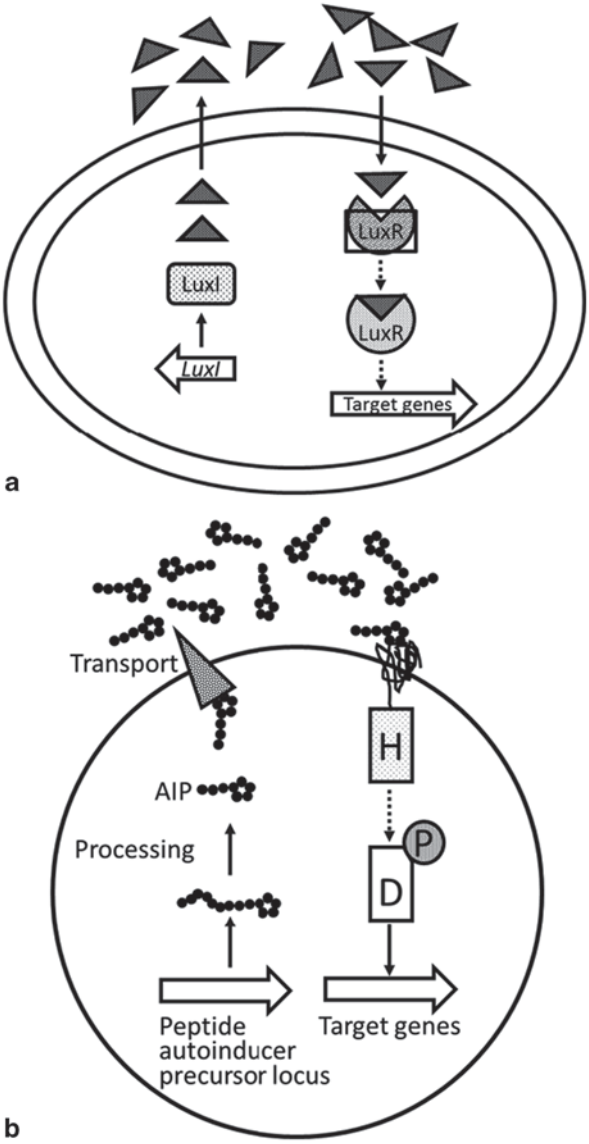


Fig. 7.2 Quorum sensing is regulated by autoinducers. **a** Quorum sensing in gram-negative bacteria. Acyl-homoserine lactone (AHL) which is an autoinducer synthesized by LuxI, indicated by triangles. When bacterial cell density increases and AHL reaches a specified signal concentration in and out of the cell, AHL is bound by LuxR and this LuxR/AHL complex activates the target gene. **b** Quorum sensing in gram-positive bacteria. Autoinducing peptide (AIP), which involves cleavage of a precursor protein coded by a peptide signal precursor locus, is transported out of bacterial cell via an ABC transporter. AIP is bound by membrane-bound sensor histidine kinases to a conserved histidine residue (H) and then the transcription of a target gene is activated by phosphorylation (P) of a conserved aspartate residue (D) [19]

References

1. Willkinson JF (1958) The extracellular polysaccharides of bacteria. Bacteriol Rev 22 46–73
2. Costerton JW, Geesey GG, Cheng GK (1978) How bacteria stick. Sci Am 238:86–95
3. Costerton JW, Cheng K-J, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ (1987) Bacterial biofilms on nature and disease. Annu Rev Microbiol 41:435–464
4. Hawser SP, Douglas LJ (1994) Biofilm formation by *Candida albicans* species on the surface of catheter materials *in vitro*. Infect Immun 62:915–921
5. Sauer K (2003) The genomics and proteomics of biofilms formation. Genome Biol 4:219.
6. Rashid MH, Kornberg A (2000) Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of *Pseudomonas aeruginosa*. Proc Natl Acad Sci U S A 97:4885–4890
7. Murray TS, Kazmierczak BI (2006) FlhF is required for swimming and swarming in *Pseudomonas aeruginosa*. J Bacteriol 188:6995–7004
8. Henrichsen J (1983) Twitching motility. Annu Rev Micribiol 37:81–93
9. Bester E, Wolfaardt GM, Aznaveh N, Greener J (2013) Biofilms' role in planktonic cell proliferation. Int Nat Mol Sci 14:21963–21982
10. Chew SC, Kundukad B, Seviour T, van der Maarel JRC, Yang L, Rice SA, Doyle P, Kjelleberg S (2014) Dynamic remodeling of microbial biofilms by functionally distinct exopolysaccharides. Mbio 5:e01536–14
11. Costerton JW (2007) The biofilm primer, Springer series on biofilm, 1st Edn. Springer-verlag, Berlin, pp 53-56
12. Stewart PS, Peyton BM, Drury WJ, Murga R (1993) Quantitative observations of heterogeneities in *Pseudomonas aeruginosa* biofilms. Appl Environ Microbiol 59:327–329
13. Donlan RM, Costerton JW (2002) Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167–193
14. Suci PA, Mittleman MW, Yu FP, Greenberg GG (1994) Investigation of ciproxacin penetration into *Pseudomonas aeruginosa* biofilms. Antimicrobial Agents Chemother 38:2125–2133
15. Dagostino L, Goodman AE, Marshall KC (1991) Physiological responses induced in bacteria adhering to surfaces. Biofouling 4:113–119
16. Kempner ES, Hanson FE (1968) Aspects of light production by *Photobacterium fischeri*. J Bact 95:975–979
17. Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria: the LuxR-Luxl family of cell density responsive transcriptional regulators. J Bact 176:269–275
18. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280:295–298
19. Bassler BL (2002) Small talk: cell-to-cell communication in bacteria. Cell 109:421–424
20. Nazzaro F, Fratianni F, Coppola R (2013) Quorum sensing and phytochemicals. Int J Mol Sci 14:12607–12619
21. Eberhard J, Burlingame AL, Eberhard C, Kenyon GL, Nealson KH, Oppenheimer NJ (1981) Structural identification of autoinducer of *Photobacterium fischeri* luciferase. Biochemistry 20:2444–2449
22. Pearson JP (2002) Early activation of quorum sensing. J Bact 185:2569–2571
23. Bainton NJ, Bycroft BW, Chhabra SR, Stead P, Gledhill L, Stewart GS (1992) A general role for the lux autoinducer in bacterial cell signalling control of antibiotic biosynthesis in *Erwinia*. Gene 116:87–91
24. Dunny GM, Leonard BA (1997) Cell-cell communication in gram-positive bacteria. Annu Rev Microbiol 51:527–564
25. Lyon GJ, Novick RP (2004) Peptide signaling in *Staphylococcus aureus* and other gram-positive bacteria. Peptides 25:1389–1403
26. Schertzer JW, Boulette ML, Whiteley M (2009) More than a signal: non-signaling properties of quorum sensing molecules. Trends Microbiol 17:189–195

27. Sauyer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG (2002) *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. J Bacteriol 184:1140–1154
28. Diggle SP, Winzer K, Chhabra SR, Worrall KE, Cámara M, Williams P (2003) The *Pseudomonas aeruginosa* quinolone signal molecule overcomes the cell density-dependency of the quorum sensing hierarchy, regulates rhl-dependent genes at the onset of stationary phase and can be produced in the absence of LasR. Mol Microbiol 50:29–43
29. Niu C, Robbins CM, Pittman KJ Osborn JL, Stubblefield BA, Simmons RB, Gilbert ES (2013) LuxS influences *Escherichia coli* biofilm formation through autoinducer-2-dependent and autoinducer-2-independent modalities. FEMS Microbial Ecol 83:778–791
30. Plummer PJ (2012) LuxS and quorum-sensing in *Campylobacter*. Front Cell Infect Microbiol 2:1–9
31. Sha J, Rosenzweig JA, Kozlova EV, Wang S, Erova TE, Kirtley ML, van Lier CJk, Chopra AK (2013) Evaluation of the roles played by Hcp and VgrG type 6 secretion system effectors in *Aeromonas hydrophila* SSU pathogenesis. Microbiology 159:1120–1135
32. Houdt RV, Givskov M, Michiels CW (2007) Quorum sensing in *Serratia*. FEMS Microbial Rev 31:407–424
33. Oslizio A, Stefanic P, Dogsa I, Mandic-Mulec I (2014) Private link between signal and response in *Bacillus subtilis* quorum sensing. Proc Natl Acad Sci U S A 111:1586–1591
34. Frank KL, Guiton PS, Barnes AMT, Manias DA, Chuang-Smith ON, Kohler PL, Spaulding AR, Hultgren SJ, Schlievert PM, Dunny GM (2013) AhrC and Eep are biofilm infection-associated virulence factors in *Enterococcus faecalis*. Infect Immun 81:1696–1708
35. Syvitski RT, Tian XL, Sampara K, Salman A, Lee SF, Jakeman DL, Li YH (2007) Structure-activity analysis of quorum-sensing signaling peptides. J Bacteriol 189:1441–1450

Chapter 8
General Biological Biofilm Observation and Evaluation

Hideyuki Kanematsu and Dana M. Barry

Abstract This chapter describes some general observation methods and techniques used by biologists to investigate biofilms. Scientists usually stain targets using pigments because some chemicals bind with important components of a bacterial cell. There are many dyeing processes devised by medical and biological researchers. Utilizing them, biofilm researchers have observed biofilm with a fluorescence microscope. This method leads to the observation not only of bacteria but also of exopolymeric substances. The dyeing technique has brought biologists merits for confocal scanning laser microscopy (CLSM), where biofilm has been visualized successfully. This special setup could be mentioned as the most advanced and successful visualization method to evaluate biofilm nowadays.

Introduction

As you could read many aspects brought about by biofilm in our daily lives, there are many phenomena relating to biofilm, which are beyond our expectations. Many of them have not had any appropriate countermeasures so far. To solve the problems from the industrial viewpoints, we should have appropriate evaluation tools. Since biofilm is originally produced by bacterial activities, it is related to medical science and biology deeply. Therefore, biological evaluation techniques have been developed fast. Since biofilm bacteria are much different from planktonic bacteria, the evaluation technique should differ from conventional biological methods. However, the methods most familiar to biologists are easy for them to handle. In this chapter some biological evaluation and observation techniques are described.

H. Kanematsu (✉)
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

D. M. Barry
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, US
e-mail: dmbarry@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_8

Dyeing [1]

Dyeing is the easiest method for biologists for the visualization of bacteria-related matters. This can be attributed to the truth that some bacteria components such as cells, organelles, etc. tend to bind with certain pigments. Many of the dyeing processes utilize this phenomenon. On the other hand, some other pigments tend to bind with enzymes. This is the other category. Even though biofilm bacteria are much different from planktonic ones in various ways, those in a biofilm can be stained easily as well as the planktonic ones. Generally, there are many dyeing processes in biology, as described below.

Gram's stain is one of the representative ways to stain bacteria. Usually, crystal violet or gentian violet is used. Bacteria stained in violet color are called gram-positive, while those free from dyeing are called gram-negative.

The Ziehl–Neelsen method is used for acid-fast bacillus. These bacteria are hard to stain by the conventional Gram's stain. Tubercle bacillus is one of the representative examples.

Hematoxylin stain has been the basic method to stain pathological specimens. Nuclei are stained in blue by hematoxylin, while eosin cell cytoplasm, fibers, and erythrocyte in pink. One can have a bird's-eye view of the organisms.

Masson trichrome stain is the dyeing process that dyes collagen fibril with aniline blue. Nuclei are stained in black-blue by iron hematoxylin and cell cytoplasms in red by acid fuchsin.

Romanovsky stain is a staining method based on methylene blue, methylene azure, and eosin. This is the basic and general way to stain pathological tissues.

Silver stain is used to detect proteins. Silver ion binds with proteins and then it is reduced by formalin and citric acid. As a result, proteins are stained in black.

Periodic acid-Schiff stain (PASS stain) is used often in organization studies and pathology. Glycol groups in polysaccharides are changed to aldehyde groups by periodic acid and reacted with basic fuchsin to be stained in red.

Papanicolaou stain is one of the basic dyeing ways for cellular specimens. Cells are stained by hematoxylin solution. This is one of the popular ways in medical practices to stain cancer cells.

Giemsa stain is one of the dyeing ways for specimens of blood. Azure-II-Eosin is mainly used to stain chromatin in cell nuclei in red-purple color. It can stain acidophilic and basophilic particles in cells differently.

A number of other methods exist to stain various matters relating to organisms. It is recommended that additional biological books be used, when the readers want to know more information.

One of the authors (Dr. Kanematsu) often uses crystal violet to stain bacteria in biofilm formed on materials. The pigment is basically a chemical containing basic triphenylmethane in the structure. The staining process is shown in Fig. 8.1. Using this method, he and his group found correlations between many metallic elements and materials and biofilm formation [2, 3].

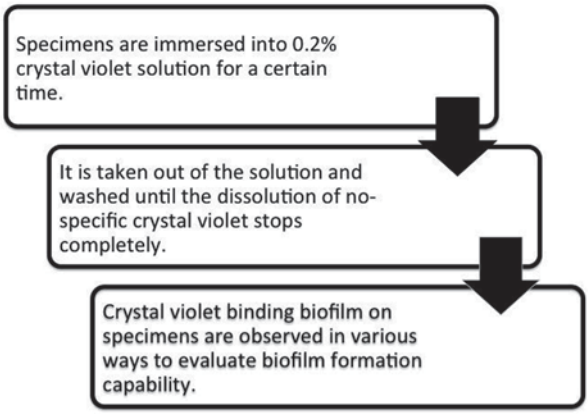


Fig. 8.1 An evaluation method with crystal violet for biofilm formation

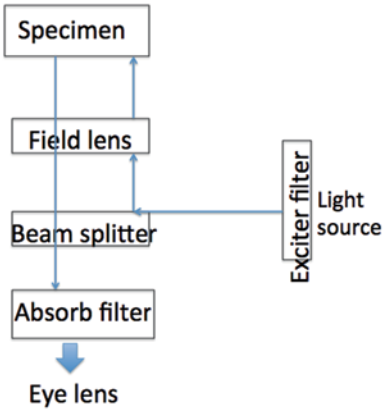


Fig. 8.2 Principle of a fluorescent microscope

Fluorescence Microscope [4]

The next step following dyeing processes is microscopic observation. Biologists often use fluorescence microscopes. This type of microscope is different from the usual optical microscopes. Figure 8.2 shows the schematic principle for a fluorescence microscope. In the fluorescent microscopic system, only a certain wavelength of light is irradiated on specimens, and the fluorescence from the specimen is analyzed. Ultrahigh voltage mercury lamps, xenon lamps, ultraviolet LEDs, laser beams, etc. are used for the light sources. The specimens are stained by low-molecular-weight organic compounds such as fluorescein and rhodamine, etc. in one case. They are often used as labels for proteins and nucleic acids. On the other hand, specimens are also stained by fluorescence proteins such as the green fluorescent protein (GFP). These are used to investigate static structures or dynamic ones for cells. When the light of a certain wavelength is irradiated on a specimen, some parts of the specimen

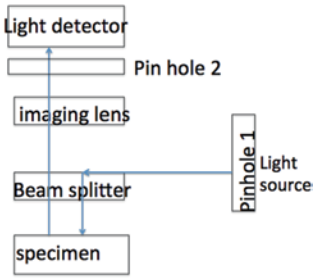


Fig. 8.3 Principle of a confocal scanning microscope

produce light. A certain wavelength of light could pass through filter(s) to reach the eye lens. When we come to think about the application to biofilm research, we can mention as merits that the fluorescence microscope could fix some types of bacteria in it and make it possible for us to observe exopolymeric substances (EPS).

Confocal Scanning Laser Microscope (CSLM) [5]

As an advanced observation, we can mention the confocal laser microscope. For comparison, the principle is shown in Fig. 8.3. As the reader will realize soon, pinholes are set behind the light source and the light detector. Since all of the pinholes are set at conjugated positions, the apparatus is called confocal microscopy. It means that the irradiated light from the light source (pinhole 1) can come into focus on the sample when the reflected light can come into focus on the light detector. When the light deviates from the confocal condition, it cannot pass through pinholes. In this way, one can get the high-resolution and high-contrast figures without any disturbance by scattering light. This optical system has been basically independent from dyeing. However, the dyeing could strengthen the contrast and clearness very much. When it is compared with the usual optical system, the depth of field (DOF) is much larger for CSLM. The DOF is defined as the range where the focus seems to be available around the exact focal point. Generally, it depends on the following three factors: aperture, focal point distance, and camera to subject distance. Since the system of CSLM has some pinholes, out of focus images can be eliminated and as a result, sharp and clear images could be obtained. The combination with a scanning function would lead to 3D images of biofilms. From the viewpoint, CSLM seems to have a large DOF, even though it has a thin DOF.

Due to these characteristics of the optical system, CSLM has been used for biofilm research successfully. Since biofilm is basically inhomogeneous concave–convex film-like matter, CSLM could provide us with the correct and concrete images of biofilms that are close to real existence and structures. In addition to that, this method does not require any special freeze dehydration since it is carried out in ambient air. Water is an important factor of biofilm, since more than 80 % of biofilm is water. When an important component must be removed from biofilm inevitably, the

image obtained after that can be quite different from the original one. Therefore, the CSLM could give us information about the mass transfer in biofilm. This method could make great contributions to clarify the structures of biofilms. The CSLM can be mentioned as the most successful evaluation technique for the visualization of biofilm.

References

1. Staining: Wikipedia, http://en.wikipedia.org/wiki/Staining
2. Kanematsu H, Ikigai H, Yoshitake M (2008) Antibacterial eco-plating based on HACCP and biofilm. Bull Iron Steel Inst Jpn 13(1):27–34
3. Kanematsu H, Ikigai H, Yoshitake M (2009) Evaluation of various metallic coatings on steel to mitigate biofilm formation. Int J Mol Sci 10(2):559–571
4. Pitts B (2008) Biological sciences—impact of biofilms in the real world. Microsc Microanal 14:1550–1551
5. Lewandowski Z, Beyenal H (2014) Fundamentals of biofilm research. Second Edition. CRC Press, Boca Raton

Part II
Biofilm and Industrial Problems

Chapter 9
Corrosion and Biofilm

Reza Javaherdashti

Abstract This chapter provides a review of how biofilm relates to corrosion. First of all, it presents corrosion as an electrochemical process. Then, the corrosion phenomenon is described from the viewpoint of electrochemistry. Next, microbiologically influenced corrosion (MIC) is discussed and related to biofilm. The process of biofilm formation is explained schematically and some concrete examples are provided. Finally, a mechanism is introduced for the correlation of biofilm and corrosion. In fact, biofilm is the key factor to differentiate MIC from the usual form of corrosion.

Introduction

Perhaps the summary of this chapter can be expressed in one sentence: "The contribution of biofilms to corrosion-that can be called as 'Microbiologically Influenced Corrosion' (MIC)—is electrochemical corrosion in nature and most probably happens via the establishment of electrochemical cells." No matter how simple and perhaps straightforward it may seem, the fact is that it is neither simple nor straightforward! Let us have a second look at what challenging points are "hidden" in this seemingly "simple and straightforward" statement:

1. For one thing, if "biofilm" means a "film" that is produced purely and totally by microbiological agents, the term "biofilm" is not correct!
2. There are at least seven ways to address MIC. Microbiologically influenced corrosion is just one of them. Which ones are correct, and thus useful for us and perhaps which one(s) may not be correct and rather misleading?
3. Why electrochemical corrosion? In fact, one may wonder what electrochemical corrosion is in the first place. Why, for example, we do not consider "high temperature corrosion"? What is it that relates biofilms and MIC to electrochemical corrosion and not to other corrosion processes?

R. Javaherdashti (✉)
ParsCorrosion Consultant, Perth, Australia
e-mail: javaherdashti@yahoo.com

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_9

4. What do we mean by "electrochemical cells"? How do they contribute to corrosion and thus MIC and how are they related to biofiolms?

In fact, if we want, we can ask more questions. These questions may shed some light on the subject, which is the link between biofilms and corrosion. However, these questions may also cause more confusion. Therefore, what we will do in this chapter is to follow a logical yet quite brief and to-the-point approach. For those who would like more details, it is advised to refer to the references given at the end of this chapter. Also, a short video on MIC and corrosion in practice may be viewed at the link given below:

http://www.youtube.com/watch?v=sk-r08OW9_0

We will start with a brief introduction/review of electrochemical corrosion and the concepts of electrochemical cells. Then, we will review the concept of biofilm formation and the involved mechanisms. Following this, the relationship between electrochemical corrosion and biofilm formation is redefined in the context of MIC.

Electrochemical Corrosion and Electrochemical Cells

Living cells (thus, bacteria) need water in liquid form to support their lives. Also, electrochemical corrosion needs liquid water to proceed. It then becomes sensible to expect that MIC will happen via electrochemical corrosion processes. In fact, when in the mid-1930s it was tried to explain MIC in terms of electrochemical corrosion, it was based on this expectation.

In any corrosion book, the author(s) may define electrochemical corrosion in their own words that while in essence is correct, it may not cover the whole idea of electrochemical corrosion. To avoid this, we will repeat ISO definition of corrosion verbatim as given in E. Mattson's book [1]. ISO 8044 defines (electrochemical) corrosion as "Physicochemical interaction (usually of an electrochemical nature) between a metal and its environment which results in changes in the properties of the metal and which may often lead to impairment of the function of the metal, the environment, or the technical system of which these form a part." This definition certainly has some very important aspects that we will briefly explain below:

a. It defines electrochemical corrosion. Therefore, it is of high significance to describe the link between biofilms and the corrosion phenomena.
b. It is certainly focused on metals and not non-metals: this implies that the term "corrosion" must be used for metals only and not for nonmetals such as concrete[1] or plastics.

[1] Please note that when we talk about reinforced concrete (RC) where there is a steel rebar inside a concrete, the word corrosion" should be used to address what will happen to the steel so that it will lose its mechanical integrity. What happens to the cementitious phase should be addressed as "degradation" or "deterioration".

c. Corrosion can not only affect the metal but also the environment and the system of which these two are a part. For instance, if a (metallic) subsea pipeline corrodes, not only will the pipe corrode and thus lose its mechanical integrity but also as a result of corrosion the properties of seawater may be changed by, say, releasing the toxic chemicals inside the pipe into the open sea. The study of mutual interaction of a corroding system with its surrounding environment is definitely a matter that is worth more detailed research [2] as it will add up to the measures to be taken to protect the environment.

Corrosion is a thermodynamic trend. It means that metals do "want" to corrode but why? In thermodynamics, there is a relatively well-known concept related to why a certain chemical reaction can be favoured: if the happening of that particular reaction is accompanied by lowering the overall energy, then it is thermodynamically favourable. One trivial example can be why water falls in a waterfall: it does so as to minimize its overall energy.

There are three important elements in any electrochemical reaction that must be present all together otherwise the chemical reaction (corrosion) will not take place.

I. Anode: It is the material (metal) that is, technically speaking, corroding. This metal creates two "electrical" species: it produces positively-charged ions (=cations) as well as negatively charged electrons. These electrons are actually produced when cations are created.
II. Cathode: If it is a metallic ion, it will be the one that receives the electrons generated at the anode. This way, it will create negatively charged ions (=anions). However, cathodes can be nonmetals as well. While practically there is always one anode at which an anodic reaction takes place, many cathodic reactions exist, based on the chemistry of the system. These cathodic reactions (in addition to reduction of metallic ions) are hydrogen evolution, oxygen reduction (in acidic solutions that results in water and in basic or neutral solutions that results in hydroxyl ion), metal precipitation and ionic reduction processes.
III. Conductive paths: For ions, this path is the medium through which the charges (anions and cations) are exchanged. This medium (normally liquid water) is technically called "electrolyte." We will also need a conductive path to let the generated and gained electrons travel. In practice this will be the metallic surface.

For a more detailed description of these elements, books on general electrochemistry and corrosion such as [3] and [4] in addition to the first reference given in this chapter are useful.

The next important topic is the electrochemical cells. In fact, we can categorize the electrochemical cells under a broader term: as P.R. Roberge puts it [5], a concentration cell is "An electrolytic cell, the emf of which is caused by a difference in the concentration of some component in the electrolyte. (This difference leads to the formation of discrete cathode and anode regions.)"

There are basically two types of such cells (we are not considering differential temperature cells here): differential aeration cells and differential concentration

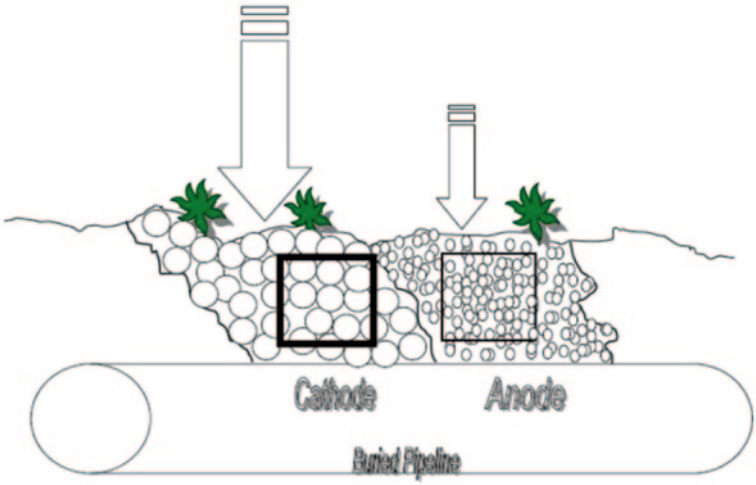


Fig. 9.1 Establishment of differential aeration cells due to size difference of soil particles. If not protected by proper coating, these cells can rapidly corrode the exterior of the pipe. The relative size difference in the *arrows* represents difference in oxygen partial pressure ingress

cells. As our approach here is based on making things simple and short, we will simply say that in a differential aeration cell, the anode is produced where the oxygen partial pressure is low relative to an adjacent spot where the oxygen partial pressure is high (and thus, it is a cathode). In addition, if the concentration of cations is different in two adjacent spots, the venue with lower cation concentration will act as an anode relative to a higher cation concentration [6].

Figure 9.1 shows schematically one of the practical important cases of corrosion that can be related to the formation of aeration differential electrochemical cells. As seen in this figure, due to the size difference of the soil particles (which is a common feature of different soils), oxygen penetration will not be the same all along the length of the pipe. This way, the spots of *high*- and *low*-oxygen partial pressure will be built up and thus formation of differential aeration cells in the form of anodes and cathodes will be inevitable. The moisture in the soil will take the role of the electrolyte and the metal itself will serve to transfer the generated electrons.

Microbiologically Influenced Corrosion (MIC)

There are at least seven ways to address MIC [7]. As expected, different authors address and define MIC in different ways. Of these, only six definitions are correct. However, "bacterial corrosion" is not a correct equivalent of MIC as all cases of MIC are not just linked to bacteria: We do have electrochemical corrosion happening due to the corrosive acts of fungi and algae as well. Bearing in mind that bacteria

are not the only cause of MIC, no matter what the definition, there are at least three principles in all MIC definitions:

1. MIC is an electrochemical process.
2. Microorganisms are capable of affecting the extent, severity and course of corrosion.
3. In addition to the presence of microorganisms, nutrients and water must also be present to initiate MIC.

The role of biofilms in this context will be defined: they are the only factor that will differentiate MIC from all other electrochemical corrosion processes. It follows, then, that if liquid water exists but there is no biofilm, the potential case of corrosion cannot be MIC but any other electrochemical process. This also explains why in studying MIC and the role of biofilm in it, one has to concentrate more on electrochemical corrosion: it is the liquid form of water that is needed here. In high temperature corrosion, for instance, where water cannot exist as a liquid, MIC may not be expected. In terms of industrial applications, in a power plant, the likelihood of getting MIC is much higher in cooling towers than in turbines.

Mechanism of Biofilm Formation

The term "biofilm" was reportedly [8] coined in 1978. It can be defined as "matrix-enclosed bacterial populations' adherent to each other and/or to surfaces or interfaces" [9]. However, as we will see later, biofilm by itself is a confusing term and in fact it is not a correct terminology at all!

What we can agree on about a biofilm build-up mechanism is at least two perspectives: (1) it is a very complicated procedure and many aspects of it are still under research and (2) it is a dynamic process and does not stop any time.

We can schematically show biofilm formation in Fig. 9.2

The first step is that the metallic surface (1) is exposed to an environment that does have microorganisms (2). These microorganisms will be from different species and groups. However, what makes them alike is that no matter how smooth the final finish of the surface, they will modify it by excreting a conditioning film that will allow them to, literally speaking, stick onto the surface (3). We schematically showed this by a change in colour (making the surface more dark).

It also follows that if after treatments such as welding, the surface is not made smooth and left as it is, this will give the microorganisms a better chance for finding suitable surfaces to "land on."

After stage (3), the biofilms start to become thicker (4) by not only attracting more microorganisms but also nonbiological "stuff" and debris so that based on organic matter of the biofilm's dry weight, the nonorganic content of biofilm is around 60–70 % of its dry weight [10]. As biofilm becomes thicker, transfer nutrients and gases to the root of the biofilm and the microorganisms there gradually become

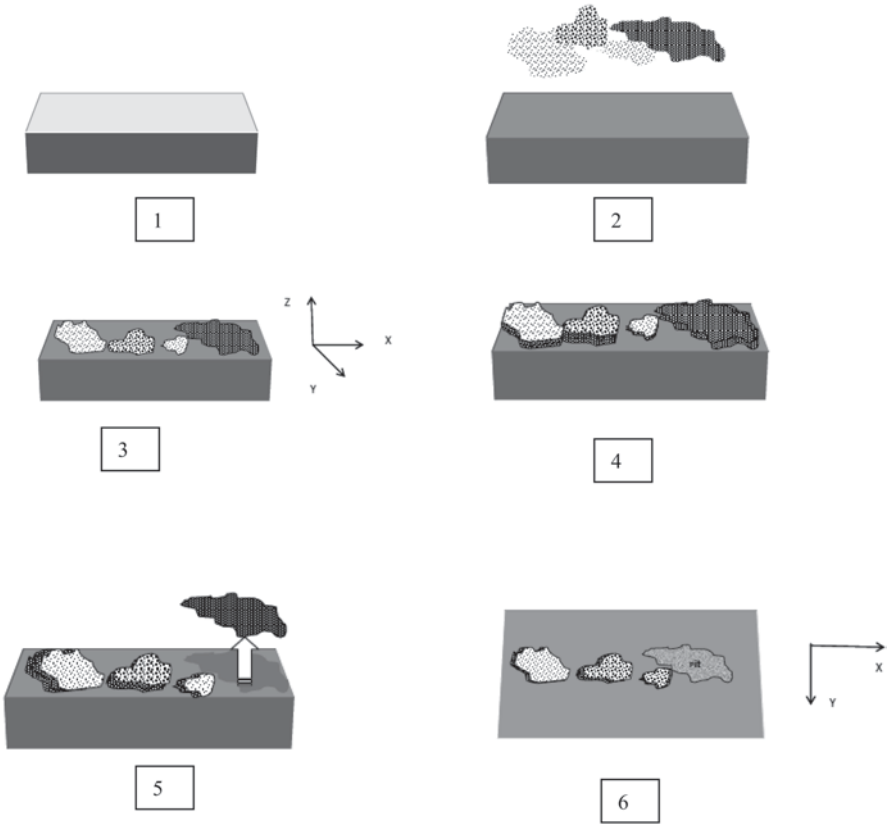


Fig. 9.2 Steps involved in biofilm build-up (*construction*) and deconstruction and how this process contributes to corrosion in the form of pitting

more difficult: typically getting a thickness of 12 microns will make the under-biofilm environment anaerobic (oxygen-free) [11].

Therefore with time the microorganisms at the biofilm–metallic surface interface will die out, but their bodies will stay there. These "zombies" will stay till environmental conditions change abruptly for a short period, for example, if a pump is activated for a short period, the shear forces thus produced will be sufficient to locally destroy a part of the biofilm patch (5).

The "blue print" of the biofilm that remains on the metal's surface may help in forming the morphology of pitting [12, 13] via mechanisms such as differential aeration electrochemical cells formation, as we will explain in Fig. 9.3. As seen in this figure, after being exposed to Sulphate reducing bacteria (SRB) culture for only 3 days, pitting is developed in a carbon steel sample. These pits are not of the same depth and the deepest of them has been designated.

In Fig. 9.4, another microorganism (Clostridia) on API XL 52 steel is shown where the biofilm formed has induced pitting. This serves to show that pitting in-

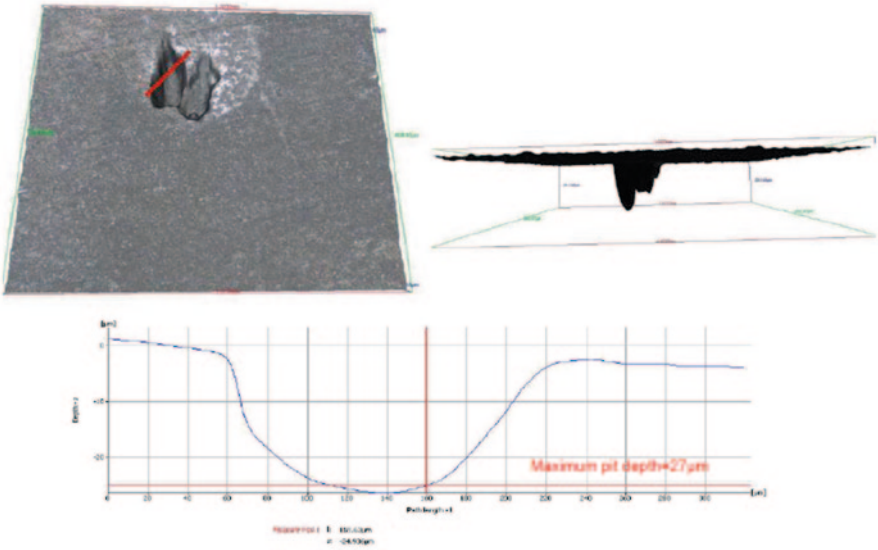


Fig. 9.3 (*Top, left to right*) 2D top view and 3D side view of pits growing in a carbon steel exposed to sulphate-reducing bacteria for 3 days.(*Bottom*) pit depth profile where the pit shown by the *red line* is the deepest pit formed under these conditions [14] © NACE International 2013

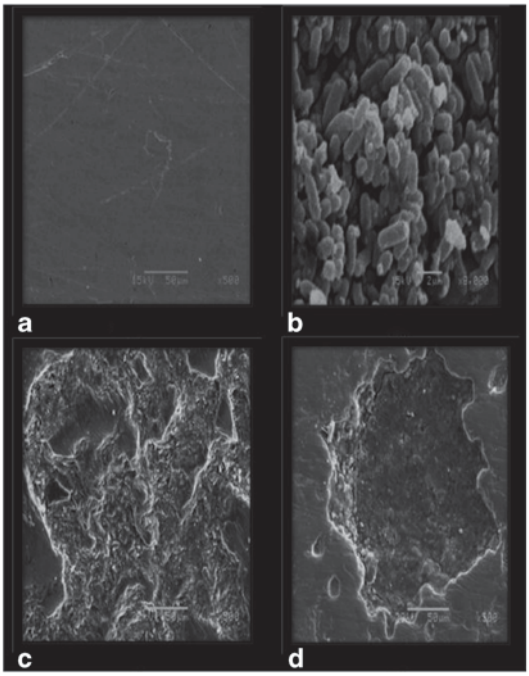


Fig. 9.4 (**a**) API XL 52 surface is shown before exposing to the bacterial culture of *Clostridium celerecrescens*. After biofilm is formed (**b**), localized corrosion will be induced by the bacteria (**c** and **d**) [15]

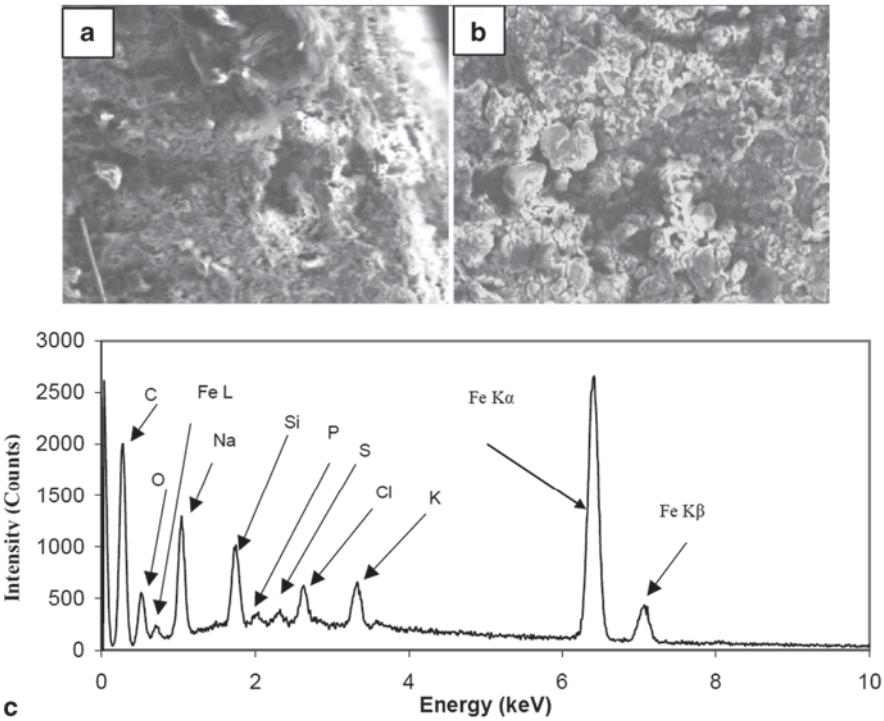


Fig. 9.5 (*Above*) two examples of biofilms formed on carbon steel by (**a**) SRB and (**b**) IRB. (*Below*) EDXA of the biofilm formed by IRB on carbon steel [16]

duced by biofilms does not belong to only one type of bacteria and under the right conditions, when biofilm is formed, pitting will follow.

As mentioned earlier, biofilms contribute to corrosion by establishment of concentration cells as follows:

- Concentration/accumulation of cations
- Concentration/accumulation of anions
- Concentration/accumulation of oxygen

While the first two can be categorized as "differential concentration electrochemical cells," the last one is in fact called "differential aeration electrochemical cell".[2] Figure 9.5 shows an example of biofilms formed by sulphate-reducing bacteria and iron-reducing bacteria and the abundance of elements in them.

As can be seen from the figure, the "patchy" and "fluffy" appearance of biofilms will help create areas of high- and low-oxygen partial pressure under the biofilm at

[2] One may add to these three factors also the effect of galvanic corrosion but in fact what happens there is that the corrosion products are entangled in the biofilm and come into contact with the main metal. As this contact, will be a direct contact between two metallic objects each with different electrical resistance, then it is likely that galvanic effect may happen. Even in this case, the metallic corrosion products have been accumulated under the biofilm.

the biofilm/metal interface. These areas can most probably contribute to the build-up of differential aeration cells. Also, as it is evident from the energy dispersive X-ray analysis (EDXA) of the biofilm, there will be accumulation of both cations and anions that in turn can lead to the establishment of further concentration cells. While the fabric of biofilms is itself acidic and may serve in metal binding, the metal concentration cells thus formed (as seen in the EDXA of the biofilm of Fig. 9.5) may increase corrosion by providing additional cathodic reactions [17]. It is then highly likely that via the activity of these electrochemical cells, corrosion will be initiated and pitting will occur. As pitting will serve as a stress-intensifying factor, it can be reasonably expected for the premature failure to happen in the material as a result of biofilm formation.

Summary

To settle on surfaces, bacteria need to produce substances that are collectively referred to as biofilm. Biofilms are complex structures and their functions are even more complex and confusing: they are complex structures because they are not static. They are always alternating from construction to deconstruction to re-construction. Understanding this dynamic feature of biofilms is a key factor in defining their functions: their "patchy" nature allows them to create different types of electrochemical concentration cells and thus accelerate corrosion. However, under certain conditions (if, for example, they are too thick), they can even slow down corrosion [18, 19]. This ability in both accelerating and decelerating corrosion is a function of the gradients formed across the biofilm boundary. Also, a rather entangled structure of biofilms may block poisonous substances such as silver and copper from reaching to the bulk of the bacterial population behind the biofilm [20, 21]. This ability may serve to explain why traditional MIC treatments such as application of biocides may fail in practice.

The role biofilms play in manipulating corrosion still needs to be further investigated. There are many things we still do not know about biofilms and their dynamics as well as the extent and severity of their contribution to either accelerating or decelerating corrosion. Research is still going on to find more effective biocides or more feasible ways to control biofilm formation and thus MIC. However, one should accept that what we know is probably just the tip of the iceberg!

References

1. Mattson E (1989) Basic corrosion technology for scientists and engineers. Chapter 3. Ellis Horwood, West Sussex
2. Javaherdashti R, Nikraz H (2010) A global warning on corrosions and environment: a new look at existing technical and managerial strategies and tactics. VDM, Germany
3. Uhlig HH (1971) Corrosion and corrosion control, 2nd edn. Wiley, West Sussex
4. Ahmad Z (2006) Corrosion engineering and corrosion control. Elsevier, Amsterdam

5. Roberge PR (2000) Handbook of corrosion engineering. McGraw-Hill, New York
6. Reference (iii)m Chap. 2.
7. Javaherdashti R, Nwaoha C, Tan H (2013) Corrosion and materials in oil and gas industries. CRC Press/Taylor & Francis, USA
8. Vu B, Chen M, Crawford RJ, Ivanova EP (2009) Bacterial extracellular polysaccharides involved in biofilm formation. Molecules 14(7):2535–2554
9. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. Annl Rev Microbiol 49:711–745
10. Beech I, Bergel A, Mollica A, Flemming H-C, Scotto V, Sand W (2000) Simple methods for the investigation of the role of biofilms in “, Brite Euram Thematic Network on MIC of Industrial Materials, Task Group1, Biofilm Fundamentals, Brite Euram Thematic Network No. ERB BRRT-CT98–5084
11. Al-Hashem A, Carew J, Al-Borno A (2004) Screening test for six dual biocide regimes against planktonic and sessile populations of bacteria, Paper No. 04748, CORROSION 2004, NACE International, USA
12. Little BJ, Lee JS, Ray RI (2006) Diagnosing microbiologically influenced corrosion: a state of the art review. Corrosion 62(11):1006–1017
13. De Romero M, Urdaneta S, Barrientos M, Romero G (2004) Correlation between *Desulfovibrio* sessile growth and OCP hydrogen permeation corrosion products and morphological attack on iron. Paper No. 04576, CORROSION 2004, NACE, Houston
14. Chen Y, He X, Howdyshell R, Howdyshell S, Bi-Min ZN, Cheng G, Castaneda H, Senko JM, Ju L-K (2013) Severe pitting corrosion caused by a starving sulfate-reducing bacterium surviving on carbon steel and effect of surface roughness. Paper No.2568, CORROSION 2013, NACE, USA
15. Ramos Monroy OA, Hernández Gayosso MJ, Ruiz Ordaz N, Zavala Olivares G, Juárez Ramírez C (2011) Corrosion of API XL 52 steel in presence of Clostridium celerecrescens. Mater Corrosion 62(9):878–883
16. Javaherdashti R (2010) MIC and cracking of mild and stainless steels. VDM, Germany
17. Little BJ, Lee JS (2009) Microbiologically influenced corrosion. In: Kirk-Othmer encyclopedia of chemical technology. John Wiley & Sons, Inc., Wiley Online LIberary, Hoboken. doi:10.1002/0471238961.micrlitt.a01
18. Javaherdashti R (2008) Microbiologically influenced corrosion-an engineering insight. Chapter 4.9. Springer, UK
19. Videla HA (1996) Manual of biocorrosion, pp 74–120 and 193–196. CRC, Boca Raton
20. Percival SL, Bowlera PG, Russellb D (2005) Bacterial resistance to silver in wound care. J Hosp Infection 60:1–7
21. Fischer WR, Wagner D, Peinemann H, Arens P, Tuschewitzki GJ, (1995) The influence of corrosion on biological parameters relevant for microbially influenced corrosion of copper. In: Angell P, Borenstein SW, Buchanan RA, Dexter SC, Dowling NJE, Little BJ, Lundin CD, McNeil MB, Pope DH, Tatnall RE, White DC, Ziegenfuss HG (eds) Proceedings of 1985 international conference on microbially influenced corrosion, pp 21/1–21/14. NACE, New Orleans

Chapter 10
Cooling Water

Dana M. Barry and Hideyuki Kanematsu

Abstract Cooling water is important for many industries. It is used to remove unwanted heat from equipment and processes that need to be cooled. Water is often used as a coolant because it is safe, easy to handle, nontoxic, and usually available at industrial locations. On the downside, using water as a coolant can encourage the growth of bacteria and biofilms as well as corrode metals in the systems. Therefore, to ensure a plant's productivity, cooling water systems need to be tested regularly for their chemical and biological content and frequently monitored for the presence of biofilms. Also when necessary, chemical treatment should be applied to the cooling water system for microbial control.

Introduction

Cooling water is used to remove unwanted heat. It absorbs heat from equipment and industrial processes that need to be cooled. Water is often used as a coolant because it is safe, easy to handle, nontoxic, and usually available in industrial locations. Sources of cooling water include fresh surface water from rivers and streams and ground water from wells. Also, salt water and wastewater (effluent from wastewater treatment) are used by some companies. Cooling water systems control temperatures by using heat exchangers. After the cooling water becomes heated, it must be cooled before it can be used again. Otherwise, a new source of water is needed.

Some disadvantages of using water as a coolant are that it can encourage the growth of bacteria on system surfaces and corrode metals used in cooling systems. Also, since minerals like calcium carbonate are relatively insoluble in water, they can precipitate and form scale deposits on the inside surfaces of the system's tubes

D. M. Barry (✉)
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, USA
e-mail: dmbarry@clarkson.edu

H. Kanematsu
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_10

Fig. 10.1 Natural draft cooling towers display the release of heat from the evaporation of cooling water

and pipes. Therefore, to ensure a plant's productivity, cooling water systems require the use of preventative measures, monitoring, and chemical treatment.

Cooling Water Systems

There are three basic types of cooling water systems: open recirculating systems, once-through systems, and the closed recirculating systems [1–3]. The open recirculating system generally includes pumps, heat exchangers, and a cooling tower. Pumps keep the water recirculating through heat exchangers. The cooling water, after picking up heat, moves to the cooling tower where heat is released from the water through the process of evaporation. See Fig. 10.1 [4]. The remaining water is collected at the base of the cooling tower for reuse. To counterbalance the loss of water through evaporation, there is an intake of make-up water.

Cooling towers are the main way of getting rid of heat in the open recirculating systems. They provide a way for air and water to come into close contact. Different styles of cooling towers exist. They are categorized by their kind of draft and the direction of airflow [5]. Natural draft towers are tall and rely on the density difference between air entering the tower and the warmer moist air inside the tower. Therefore, the warmer moist air (less dense) rises, while the denser cool air is drawn in at the bottom of the tower. Mechanical draft towers use fans to move air through the tower. In regards to airflow, counterflow towers move air in the opposite direction of the downward water flow, while cross-flow towers direct airflow in a direction that is horizontal to the downward flow of water.

For the once-through systems, cooling water only passes through heat exchangers one time and then it is discharged as waste. Therefore, large volumes of water are needed for these systems. See Fig. 10.2 for a diagram of a typical double pipe heat exchanger [6].

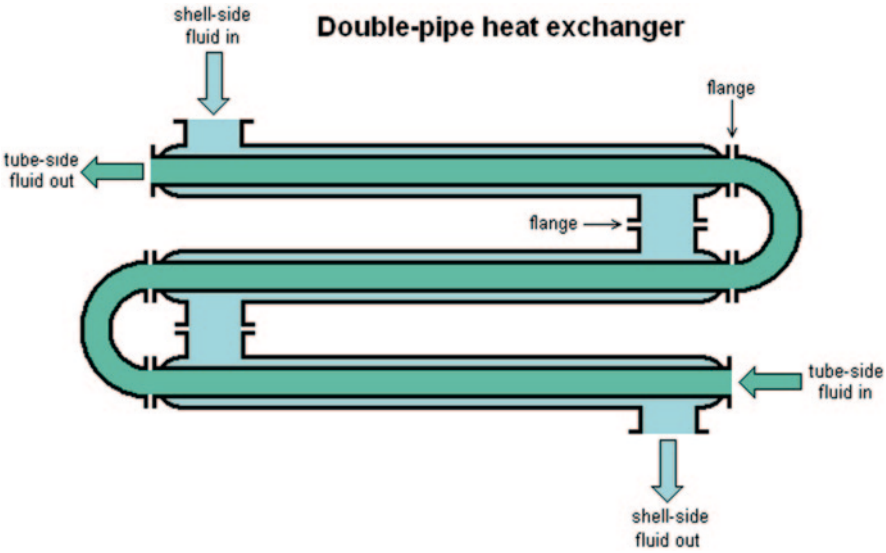


Fig. 10.2 Diagram of a double-pipe heat exchanger

On the other hand, the closed recirculating systems repeatedly use the same cooling water in a continuous cycle. To start, the water absorbs heat from an industrial process and then releases it to another heat exchanger.

Cooling Water Systems and Biofilms

Biofilms are detrimental to cooling water systems. They develop on the inside of metal pipes and cause clogging and corrosion. Clogging reduces a pipe's diameter and ability to exchange heat. A biofilm's insulating layers form barriers between recirculating cooling water and the surfaces of heat exchangers. Some biofilms, like those containing anaerobic sulfate-reducing bacteria, cause corrosion [7]. As sulfate is reduced by bacteria, a weak acid forms when the by-product (hydrogen sulfide) dissociates in water. This weak acid corrodes metals that the bacteria are attached to within the cooling system. Biofilms also raise health issues. Legionella, the bacteria responsible for Legionnaires' disease, can exist in cooling tower systems. *Legionella pneumorphila*, which produces a pneumonia-like infection in humans, can spread into the environment through aerosols generated in the cooling tower [8]. These problems and others result in reduced efficiency of a plant's heat exchangers and a lower life expectancy of its equipment. Also, they cause health concerns, affect the quality of a plant's products, and increase energy consumption and operational costs.

Biofilms are microorganisms that attach to moist surfaces by a self-produced extracellular polymeric substance (EPS) [9]. This sticky substance protects the cells within it and anchors them to materials such as metals and plastics. Biofilms differ in structure because variables such as temperature, pH, and the availability of nutrients affect their development. They can easily grow in cooling water systems because microorganisms may already be present in the cooling water. Also, bacteria and other organisms in the ambient air can be blown into the cooling tower.

Biofilms begin to form when microorganisms attach to a surface. At first this attachment is reversible. However, after a sufficient amount of time the attachment becomes more permanent. Biofilms grow by obtaining more microorganisms and by cell division. When the maturation state is reached, then cells are released from the biofilm colony so that the life cycle can continue on new surfaces.

Biofilm Detection and Treatment in Cooling Water Systems

Biofilms appear slimy on system surfaces and/or feel slimy when these surfaces are touched. They can be found in water samples that undergo wet chemistry tests. Such samples are periodically collected at the plant and analyzed in a laboratory for chemical and biological content. Also, a profile of many microorganisms found in cooling water samples can be obtained by using differential microbial analysis [10]. These tests differentiate many bacteria by growing them on agar plates and make use of microscopes to observe algae and fungi.

Microbes are able to grow quickly in cooling water systems, so it is essential to frequently check the systems. Online monitoring devices, such as biofouling sensors, are available and effective for this task [11]. They provide biofouling thickness measurements in real time on a continuous basis. These sensors are the result of advances in micro-electromechanical system (MEMS) technology. The application of this technology to biofouling utilizes the principles of thermal diffusion. Therefore, the biofouling sensors detect changes in thermal resistivity as a result of deposits on the sensor. Thicker deposits correspond to higher thermal insulation. Online monitoring devices provide information about biofilm levels and trends. This information and data about the water's chemical and biological content are useful in selecting treatments for microbial control. Also, the conductivity, pH, and hardness levels of the cooling water should be considered. Conductivity is a measure of water's ability to conduct electricity. The pH provides information about the acidity and basicity of the water. A pH of 7 is considered neutral, less than 7 is acidic, and more than 7 is basic. Controlling the pH is important for cooling water treatment programs. Hardness levels refer to the amount of ions like calcium in the water. Since some minerals (example: calcium carbonate) are relatively insoluble in water, they can precipitate and form scale deposits on pipes and system surfaces.

To start, pretreatment is recommended for water cooling systems. Some simple steps are mentioned. Clean all equipment to remove dirt and grease. Paint metal pipes and surfaces with corrosion inhibitors. Also make an effort to use cooling

water with few minerals to reduce the formation of scale deposits in the system. In addition, biofilters could be installed and used to improve the quality of recirculating cooling systems by reducing organic carbon content and bacterial numbers. Work carried out by Meesters, Van Groenestijn, and Gerritse support the use of biofilters. They showed that biofouling was lower in a recirculating cooling system with a biofilter as compared to an identical system without one [12].

Three different categories of chemicals are generally used for microbial control. They are applied in different concentrations and for various amounts of time depending upon the condition of a particular cooling water system. Treatments with high concentrations of biocides are used for shorter periods of time than those with weak concentrations. Chemical treatments involve oxidizing biocides, nonoxidizing biocides, and biodispersants [13]. Oxidizing biocides include gaseous chlorine, bleach, and bromine compounds. They kill organisms by oxidizing the cellular components of microoragnisms. Nonoxidizing biocides are organic compounds that destroy cells within the microorganism. Biodispersants are used to detach microbial deposits from a metal surface so that they can be washed away by the cooling water. They are effective in preventing the formation of biofilms.

References

1. http://www.gewater.com/handbook/cooling_water_systems/ch_31_open.jsp
2. http://www.gewater.com/handbook/cooling_water_systems/ch_30_through.jsp
3. http://www.gewater.com/handbook/cooling_water_systems/ch_32_closed.jsp
4. https://commons.wikimedia.org/wiki/. Didcot Power Station Cooling Tower, Zootalures, CC by—SA 3.0 (Owen Cliffe)
5. http://www.gewater.com/handbook/cooling_water_systems/ch_31_open.jsp
6. https://commons.wikipedia.org/wiki/heat_exchanger_diagrams#n. Double-Pipe Heat Exchanger, CC by—SA 3.0 (Turbojet)
7. Nalco cooling water treatment (Bulletin 34), Nalco Company (2005, 2009). http://www.nalco.com/ (Search “bulletin 34.”)
8. Meesters KPH, Van Groenestijn JW, Gerritse J (2003) Biofouling reduction in recirculating cooling systems through biofiltration of process water. Water Res 37:525
9. Percival S, Malic S, Cruz H, Williams DW (2011) Introduction to biofilms. In: Percival SL (eds) Biofilms and veterinary medicine. Springer-Verlag, Berlin
10. Nalco cooling water treatment (Bulletin 34), Nalco Company (2005, 2009). http://www.nalco.com/ (Search “bulletin 34.”)
11. O’Neal J, Guillemot G (2010) Online monitoring of biofilm in cooling tower systems. Anal Technol Suppl 39, http://www.advantagecontrols.com/downloads/misc/Online%20Monitoring%20of20Biofilm%20in%20Cooling%20Tower%20Systems.pdf.
12. Meesters KPH, Van Groenestijn JW, Gerritse J (2003) Biofouling reduction in recirculating cooling systems through biofiltration of process water. Water Res 37:525
13. Nalco cooling water treatment (Bulletin 34), Nalco Company (2005, 2009). http://www.nalco.com/ (Search “bulletin 34.”)

Chapter 11
Ships and Marine Structures

Daisuke Kuroda

Abstract Various types of ships and marine structures such as an oil tanker, cargo ship, mega float, and derrick, etc., are used to transport cargo and develop offshore oil fields, etc. However, severe corrosion and degradation attributable to the attachment of microorganisms and fouling organisms to their material surfaces have occurred in ships and marine structures. Severe corrosion that originated in microorganism adhesion and biofilm formation is called microbiologically influenced corrosion (MIC) (Li et al., Corros Rev 31:73–84, 2013). MIC is a very severe problem for the ship industry as it reduces the structural lifetime in combination with safety risks for crews or inspection personnel and increases maintenance costs. In addition, intercontinental move of an exotic organism can also occur by attachment of microorganisms and biofilm formation at the surface of the hull and ballast tank of a ship. Such a phenomenon will cause not only deterioration of a ship including the ballast tank and marine structures (Heyer et al., Corros Conf Expo 3:1754–1765, 2012; Heyer et al., Ocean Eng 70:188–200, 2013), but also disruption of an ecosystem (Murakami, J Jpn Ins Mar Eng 46:40–45, 2011). The corruption related to microorganism adhesion and fouling organism adhesion is called microfouling and macrofouling, respectively. In this chapter, I discuss several points about adhesion of microorganisms and fouling organisms to a ship and marine structures, and also antifouling technology.

Typical Types of Fouling Organisms Living in the Marine Environment

Figure 11.1 shows fouling organisms attached on the hull of a pleasure boat. Various types of fouling organisms such as oysters, barnacles, and Mediterranean mussels are observed. These marine organisms propagate themselves instantly as long as the environment is favorable. These marine organisms cause severe disruption of an ecosystem, because they have a high propagating power in various marine

D. Kuroda (✉)
National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: daisuke@mse.suzuka-ct.ac.jp

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_11

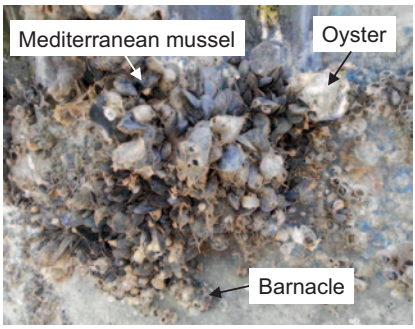


Fig. 11.1 Photograph of the typical fouling organisms. Oysters, barnacles, and Mediterranean mussels attached on the hull of pleasure boat

environments. Regulation for intercontinental organism transfer has been investigated by the international maritime organization (IMO). In addition, a carcass of these fouling organisms not only causes marine pollution including water pollution and stink, but also increases disposal costs. The biofilm formation is considered to be the major cause of macrofouling. Therefore, inhibition of microfouling and macrofouling in the ship and marine structure is very important in order to achieve reduction of maintenance costs and renovation of structural lifetime.

The Problem of Fouling Organism Adhesion to the Ship

Figure 11.2 shows the photographs of a large- and medium-sized cargo ship, respectively. The bottom plate and other parts of the ship were painted by using red-brown color and dark brown color, respectively. It is suggested that a common type of antifouling paint is used. Large- and medium-sized ships are generally fabricated using various types of steel such as high tensile strength steel [5]. Paint for the ship is, therefore, generally used to prevent the corrosion, microfouling, and macrofouling [3, 6]. The fuel consumption for the ship is increased in cases of microfouling

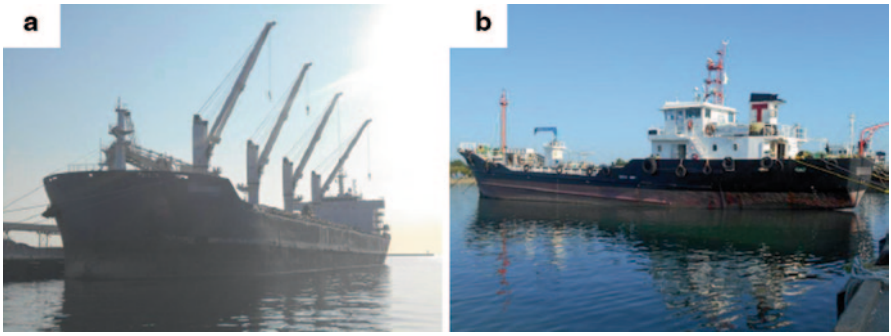


Fig. 11.2 Photographs of **a** a large-sized cargo ship and **b** medium-sized cargo ship

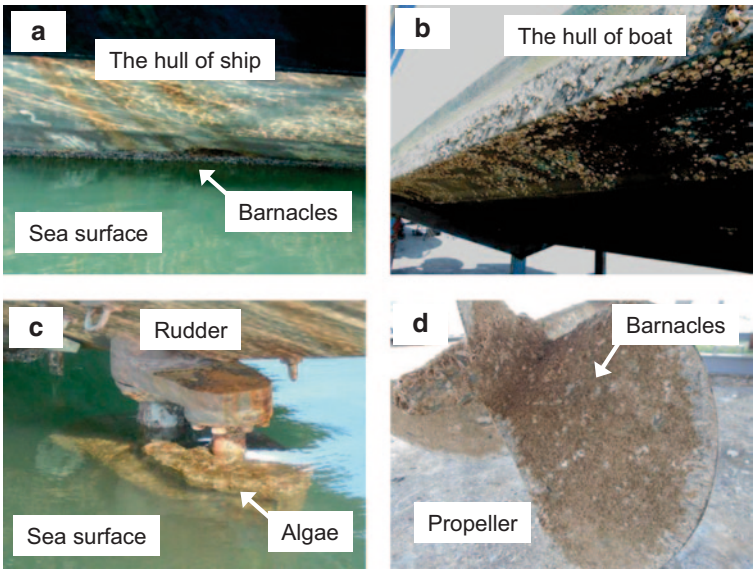


Fig. 11.3 Photographs of the microfouling and macrofouling developed at the **a**, **b** hull, **c** rudder, and **d** propeller of a ship. Attached organism is mainly a barnacle

or macrofouling because surface roughness, weight, and sailing resistance are increased by organism adhesion. These factors are closely related to increasing carbon dioxide, NOx, and SOx. In addition, adhesion and exfoliation of microorganisms of fouling organisms in the ballast tank and hull of a ship will cause not only a decrease of service life, but also disruption of an ecosystem. Therefore, various antifouling technologies have been developed and inspected to solve the industrial and biological problems mentioned above.

Figure 11.3 shows the photograph of the microfouling and macrofouling developed at each part of the ship during the summer season. Adhesion of algae and barnacles is mainly observed on the surface in each part. Each photograph has common characteristic features.

All the fouling organisms were attached near the sea surface or draft line of the ship. This indicates that microfouling and macrofouling occurred remarkably at the shallow water zone. This is because the sea coast area has abundant nutrients (bacteria) and dissolved oxygen for the marine organisms. The relation between the marine organism density and the depth of sea water or distance from coastline has been reported by De Palma [7]. Figure 11.4 shows a schematic diagram of the attachment ratio of marine organisms against the depth of water and distance from the coastline. The ratio of the attachment of organisms increases with decrease in the water depth and distance from coastline. The ratio of the attachment of marine organisms is also closely related to the surface velocity of the ship [7]. The adhesion of microorganisms and fouling organisms, therefore, occurs during anchorage. Figure 11.5 shows attached barnacles in the water intake of a medium-sized ship.

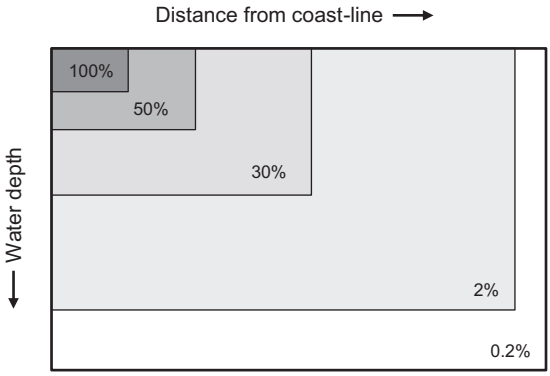


Fig. 11.4 Schematic diagram of attachment ratio of marine organism against depth of water and distance from coast-line

Fig. 11.5 Attached barnacles in the water intake

Adhesion of barnacles in the water intake will cause a decrease in engine performance, and increase the fuel consumption and risk of overheating. Reduction of water intake capacity due to macrofouling can also be a severe problem in the thermal power plant. Figure 11.6 shows the photograph of the bottom of a medium-sized ship. Barnacles of various sizes were observed on the painted bottom surface. On the other hand, a small amount of barnacle adhesion on the copper alloy component is observed. This phenomenon is applied to antifouling as described later.

Examination of Antifouling Technology for a Ship and Marine Structure

Marine antifouling paint contains copper and silicone [8–10] and thermal spray coatings with antifouling properties [4] that have been developed and tested in order to prevent the microfouling and macrofouling on the surface of ships. Both

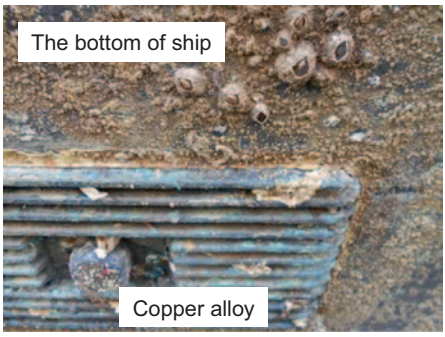


Fig. 11.6 Photograph of the bottom of a ship

technologies show the antifouling effect by dissolution of copper (Cu) or zinc (Zn) ion and other elements from the treatment layer to the marine environment. Therefore, an antifouling paint and antifouling spray coating layer that do not contain a toxic element such as Hg, Pb, tributyltin (TBT), etc., are needed. TBT causes environmental pollution and endocrine disruption. Therefore, TBT has not been used for ships and marine structures since 2008 [9].

The Problem of Fouling Organism Adhesion to the Marine Structure

The material for the marine structure is generally divided into two classes. One is a metallic material such as steel and the other one is concrete. The jack-up drilling rig is a typical marine structure, which has been used for the development of a submarine oil field (Fig. 11.7). The trench material consists of high tensile strength steel. Microbiologically influenced corrosion (MIC) also occurs in metallic marine structures. In addition, chloride iron generated from sea water attacks the marine structures. Therefore, resistance to MIC and galvanic corrosion is necessary for the marine structures as well as the ship. However, resistance to macrofouling is more important than resistance to corrosion in the marine structures.

This is because attached fouling organisms become resistance factors of the waves and wind. The floating power of floating type marine structures decreases with an increase in fouling organism adhesion. Figure 11.8 shows a photograph of the floating pier as a marine structure. Many fouling organisms are attached to a float and pillar. Antifouling paint is generally used for a marine structure to prevent corrosion, microfouling, and macrofouling. Resistance of corrosion and macrofouling in the marine structure is maintained by maintaining the antifouling paint. Antifouling paint on the surface of a marine structure, however, undergoes damage due to sea-urchins. Sea-urchins have strong mouth parts. Sea-urchins eat seaweed and barnacles with antifouling paint. In this case, there is a need to artificially scrape off the sea urchin from a marine structure.

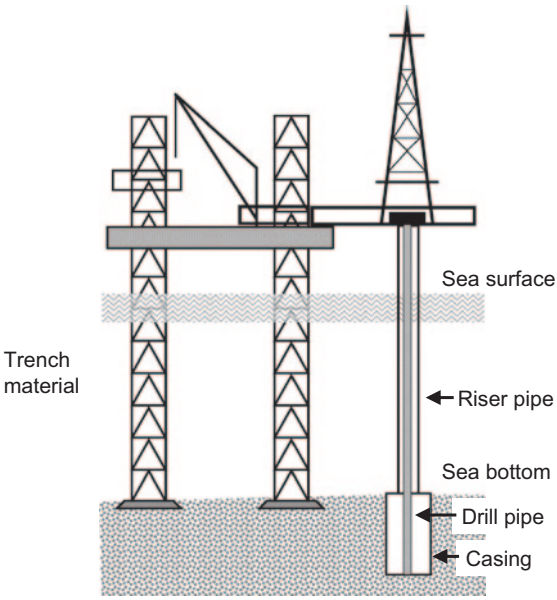


Fig. 11.7 A schematic diagram of the jack-up drilling rig

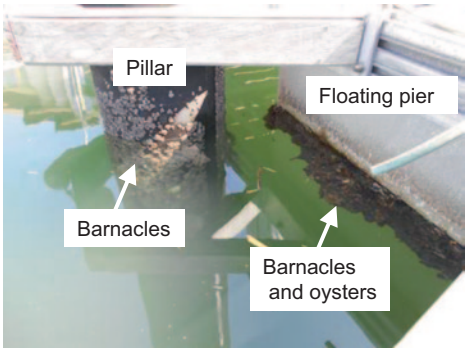


Fig. 11.8 Photograph of a floating pier. Attached fouling organisms were observed on the surface of the structure

Figure 11.9 shows a photograph of fouling organisms attached to a quay in summer season. Many barnacles are attached on the surface of a concrete quay. Chloride and oxygen will cause the deterioration of reinforcing steel in a concrete marine structure. Structural lifetime of a marine structure composed of concrete is improved by adhesion of fouling organisms due to fine and dense film formation. Fine and dense film prevent the diffusion of chloride and oxygen into the structure. In this case, deterioration of appearance and an occurrence of unpleasant odors are severe problems.

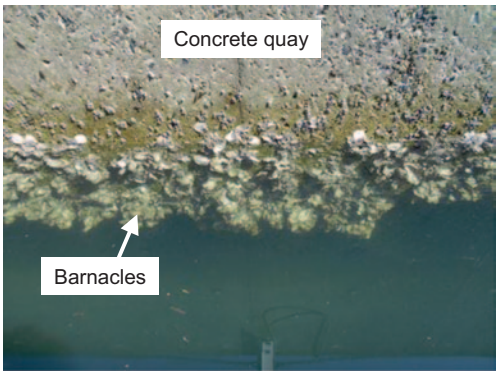


Fig. 11.9 Photograph of fouling organisms attached to a concrete quay

References

1. Li K, Whitfield M, Van Vliet KJ (2013) Beating the bugs: roles of microbial biofilms in corrosion. Corros Rev 31:73–84
2. Heyer A, Mol JMC, D'Souza F, De Wit JH, Ferrari G (2012) Study of MIC impact in a full-scale ship ballast tank. Corros Conf Expo 3:1754–1765
3. Heyer A, D'Souza F, Leon MCF, Ferrari G, Mol JMC, DE Wit JHW (2013) Ship ballast tanks a review from microbial corrosion and electrochemical point of view. Ocean Eng 70:188–200
4. Murakami K (2011) Corrosion protection and anti-fouling properties of thermal splayed metal coatings. J Jpn Ins Mar Eng 46:40–45
5. Masamura K, Oi K (2012) Steel products for energy industries. JFE Tech Rep 29:1–10
6. Marine Fouling and Its Prevention, Contribution No. 580 from the Woods Hole Oceanographic Institute Copyright 1952 by U.S. Naval Institute, Annapolis, Maryland George Banta Publishing Co., Menasha, WI, pp. 277–301
7. Nagatomo K, Matsushita H, Inui E, Miyoshi Y (1993) Studies on marine fouling of the bottom plates and propeller surface-I. J Shimonoseki Univ Fish 41:167–178
8. Yamamori N (2005) Anti-fouling paint in the future. Techno-Cosm 18:28–31
9. Masuoka S (2010) Bio-fouling by sessile organism and antifouling technology. Res Coat 152:47–51.
10. Chen C-l, Teo SL-M, Maki JS, Rittschof D (2013) Early marine bacterial film on a copper-based antifouling paint. Int Biodeterior Biodegrad 83:71–76

Chapter 12
Biofilm Formation on Medical Devices and Infection: Preventive Approaches

Sitaraman Krishnan

Abstract Biofilm formation on medical devices is a serious problem associated with deaths resulting from nosocomial (hospital acquired) infections. This chapter reviews strategies to control microbial adhesion to, and colonization of, medical surfaces using cell repellant and nonadhesive coatings, coatings that actively release antimicrobial compounds and biofilm inhibitors, antimicrobial coatings with tethered biocides, and coatings that promote competitive adherence of benign organisms. Antifouling materials such as PEGylated and zwitterionic polymers, silicone hydrogels, fluorinated amphiphilic polymers, natural polysaccharides, glycodendron-functionalized synthetic polymers, polymers tethered with antibiotics such as ciprofloxacin, antimicrobial cationic polymers and peptides, and functionalized polyhydroxyalkanoates are discussed. Controlled release coatings that deliver quorum sensing inhibitors such as 5,6-dimethyl-2-aminobenzimidazole, and antimicrobial species such as ceragenin, nitrofurantoin, nitric oxide, gallium and zinc complexes, silver ions, silver nanoparticles, and selenium nanoparticles are also reviewed.

Introduction

Biofilm formation on medical instruments is becoming a problem of increasing health and economic concern [1, 2]. Indwelling medical devices such as sutures, catheters, heart valves, vascular grafts, orthopedic implants, and intrauterine devices, are prone to biofilm formation [3], leading to a major risk of infection and patient morbidity. It has been estimated that, in the USA alone, there are approximately 1.7 million healthcare-associated infections (HAIs) each year, nearly 100,000 of which result in mortality [4, 5]. Catheter-associated urinary tract infection (CAUTI), central line-associated blood stream infection (CLABSI), and ventilator-associated pneumonia (VAP) are among the most common HAIs. Many of the HAIs are linked to biofilms of drug-resistant populations of pathogenic microorganisms. Common biofilm pathogens include bacteria such as *Staphylococcus aureus*,

S. Krishnan (✉)
Department of Chemical and Biomolecular Engineering, Clarkson University, Potsdam, NY, USA
e-mail: skrishna@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_12

Staphylococcus epidermidis, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*, and fungi such as *Candida albicans*, *Aspergillus fumigatus*, *Pneumocystis carinii*, and *Fusarium solani* [5, 6]. Topical devices such as contact lenses also form biofilms of pathogens on their surfaces [7, 8], leading to keratitis (infection of the cornea).

Mechanism of Biofilm Formation

The formation of bacterial biofilms on solid surfaces begins by the adhesion of free-floating (planktonic) bacteria to the surface, and aggregation of these cells into small groups of bacteria called microcolonies (see Fig. 12.1). The attachment of the cells to the surface activates genes required for the synthesis of extracellular polymeric substances (EPS), comprising polysaccharides and other biopolymers such as proteins, glycoproteins, glycolipids, and extracellular DNA [9]. The adhered cells produce and embed themselves in the EPS matrix, which protects them from the cellular and humoral immune reactions of the host [3]. As the biofilm matures, the undifferentiated microcolonies of the attached bacteria differentiate into phenotypes that are often profoundly different in gene expression than the planktonic cells [10]. The differentiation is triggered by accumulation, in the EPS matrix, of molecules such as *N*-acylhomoserine lactones that the individual bacterial cells produce. These quorum-sensing molecules mediate complex cell–cell interactions, leading to the formation of a mature biofilm with structure and metabolism that could sometimes be as complex as those in tissues of higher organisms [3]. The mature biofilm is generally formed within 1–2 weeks of initial colonization, and is visible as a slimy coating on the surface.

Planktonic bacterial cells release antigens and stimulate antibody production, and are therefore vulnerable to host immune responses. The sessile bacterial cells present in the biofilm, however, can withstand attack by the immune system. The bacterial cells embedded within a biofilm are significantly less susceptible to antibiotics

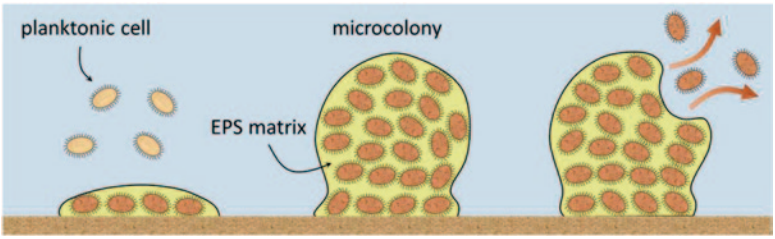


Fig. 12.1 Initial colonization and attachment (*left*), biofilm growth and maturation (*center*), and detachment of biofilm cells (*right*). Planktonic bacterial cells adhere to the biomaterial surface and form a biofilm consisting of microcolonies of aggregated cells surrounded by extracellular polymeric substances (*EPS*) of their own secretion. Biofilm cells are resistant to host immune responses and antibiotics. Shedding or detachment of cells from the biofilm can cause infection

compared with their free-floating counterparts [3]. The EPS matrix can retard the diffusion of antimicrobial agents, so that they are unable to reach underlying cells [11, 12]. The diffusion limited transport of antibiotics would be particularly relevant for thick biofilms and highly reactive antimicrobial oxidants such as hydrogen peroxide, hypochlorite, or nitric oxide. These cells would also exist in a slow-growing or non-growing state because of nutritional starvation, which could further lower their susceptibility to antimicrobial agents [13]. The third mechanism of biofilm resistance to antimicrobial agents is believed to be the formation of a unique biofilm phenotype (e.g., with multidrug efflux pumps, or with decreased permeability to antimicrobial compounds), as a specific response to growth on a surface. The biofilm phenotype and the planktonic phenotype may differ from each other by as much as 70 % in terms of the proteins expressed [14].

Detachment and dispersal of the sessile cells of a biofilm can occur by either chemical reactions, such as local hydrolysis of the EPS matrix, or physical fragmentation of a microcolony because of shear forces. Infection results when the host immune responses are unable to clear these planktonic cells that are shed by the biofilm. Most cases of biofilm formation on surgically implanted devices, such as mechanical heart valves or prosthetic hips, result in an infection. The biofilms can remain relatively stable for several months or years after the device is installed and could give rise to sudden occurrences (exacerbations) of device-related infections, caused by the same species that had originally colonized the device [14]. In such cases, surgical removal of the infected device becomes necessary.

Prevention and Control of Biofilms

Several approaches to prevent adhesion of microbial cells to surfaces (and, therefore, the formation of a biofilm) have been developed [1, 2, 15–21] and new strategies are emerging. These strategies include the use of

- Cell repellant and non-adhesive coatings
- Active release of antimicrobial compounds and biofilm inhibitors
- Antimicrobial coatings with tethered biocides
- Competitive adherence of benign organisms

This chapter reviews approaches to control biofilm formation on the surfaces of medical devices.

Cell Repellant and Non-adhesive Surfaces

Materials such as siloxane polymers (silicones) are used in the construction of urinary catheters and contact lenses. However, cells can readily adhere to surfaces of hydrophobic materials, such as polydimethylsiloxane (PDMS) elastomers, because

of van der Waals attraction and the hydrophobic effect. The functionalization of device surfaces with self-assembled monolayers (SAMs), polymer brushes, and polymer coatings that are capable of forming well-hydrated surfaces is an effective approach of preventing cell adhesion on these surfaces [22, 23]. The hydrophilic coatings can exert hydration repulsion on proteins and cells, inhibiting biofilm formation. SAMs of oligo(ethylene glycol) [24, 25], phospholipid-like zwitterionic molecules immobilized on a surface [26, 27], brushes of hydrophilic polymers such as poly(ethylene glycol) (PEG) and poly(sulfobetaine methacrylate) [28–30] are common examples of anti-biofouling coatings.

PEGylated surfaces are highly effective in biofilm prevention and are considered the "gold standard" for antifouling surfaces. The zwitterionic coatings, based on betaines such as phosphorylcholine, carboxybetaine, and sulfobetaine [31], or amino acids such as histidine, serine, and cysteine [32–34], mimic the phospholipid head groups of the cell membrane that provide resistance to protein adsorption and cell adhesion. All of these materials tend to be highly hydrated, that is, show strong intermolecular interactions with water molecules [35].

However, in the case of coatings prepared using ionic polymers, all of which form hydrated surfaces, the net surface charge of the coating also plays an important role in mediating interactions with proteins and cells [36]. Zwitterionic surfaces, charge-balanced amphoteric polymers, and polyelectrolyte multilayers, are quite effective in preventing cell adhesion [23, 35]. Cationic surfaces, on the other hand, generally promote cell settlement, and many of the cationic surfaces can also be biocidal in nature [37–39]. They can result in the disruption of cell membranes leading to cell death. The cytotoxicity of cationic polymers may not be selective to pathogenic cells and can affect even cells of the host tissue [40]. Interestingly, although sulfobetaine polymers are significantly negatively charged [36], they have been found to be quite effective as antifouling coatings against a wide range of proteins, with different isoelectric points (and, consequently, different net charges at physiological pH), and a wide range of microorganisms [35]. Evidently, electrostatic interactions are less important in the case of the zwitterionic polymers because of their ability to bind with a large number of water molecules, exerting strong hydration repulsion.

PEGylation of a surface is possible by dip-coating the device in poly(ethylene glycol) based multi-block surfactants [41] or copolymers [42, 43], or using other approaches such as surface-initiated polymerization [44, 22]. Silicone rubber samples coated with commercially available OptiChem crosslinked PEG-based coatings significantly slowed down *Staphylococcus* biofilm growth in an infected murine model [45]. Stainless steel surfaces coated with PEG-like coatings, using cold plasma and crown ether (12-crown-4) and triglyme as precursors, significantly reduced bacterial attachment and biofilm formation in the presence of a mixed culture of *Salmonella typhimurium*, *S. epidermidis*, and *Pseudomonas fluorescens* [46].

An enzymatically initiated surface radical polymerization was used to grow poly(sulfobetaine methacrylate) polymer brushes on silicone catheter surface [47]. The silicone surface was plasma activated (using O_2 plasma to generate surface hydroxyl groups) and aminated by reacting with (3-aminopropyl)triethoxy silane (APTES). The APTES functionalized surface was grafted with 3,4,5-trihydroxy-

benzoic acid (gallic acid, a natural phenolic compound) using laccase as the enzyme catalyst. Laccases are copper-containing oxidase enzymes that are capable of oxidizing aromatic compounds such as phenols and aminophenols. The tethered gallic acid residues were activated by laccase to phenoxy radicals, which initiated free radical polymerization of the sulfobetaine methacrylate monomer from the surface. In vitro tests with *P. aeruginosa* and *S. aureus* showed reduced biofilm formation compared with the unmodified urinary catheters.

Tanaka et al. [48] pioneered the development of silicone hydrogel materials, which are crosslinked polymers of hydrophobic siloxane monomers but capable of absorbing large amounts of water. Because of their tendency to absorb water (at least more than 50 % of their mass, that is, more than 33 % water content in the swollen gel), silicone hydrogels have significantly improved resistance to biofilm formation than conventional silicone elastomers. The siloxane monomers designed by Tanaka and co-workers, contained structural elements that improved thermodynamic compatibility with hydrophilic monomers such as *N*-vinyl pyrrolidone and dimethyl acrylamide, and prevented microphase separation of the polar and nonpolar components of the gel. Figure 12.2 shows the chemical structure of one such siloxane monomer, [methyl bis(trimethylsiloxy)silyl] propyl glycerol methacrylate, designated "SiGMA" by Tanaka et al. [48]. Hydrophilic siloxane macromonomers, such as α,ω-methacrylate terminated poly(ethylene oxide)-*block*-polydimethylsiloxane-*block*-poly(ethylene oxide), are also used in the preparation of silicone hydrogels [49].

Selan et al. [50] compared soft contact lenses, made from three different hydrophilic materials, for their susceptibility to biofilm formation by *S. epidermidis* and *P. aeruginosa*. The lens materials were (i) silicone hydrogel containing *N*-vinylpyrrolidone as the hydrophilic monomer (balafilcon A), (ii) a 2-hydroxyethylmethacrylate (HEMA) hydrogel, containing methacrylic acid comonomer, and 1,1,1-trimethylol propane trimethacrylate and ethylene glycol dimethacrylate crosslinkers (etafilcon A), and (iii) a HEMA hydrogel containing the zwitterionic monomer, 2-methacryloyloxyethyl phosphorylcholine, and ethyleneglycol dimethacrylate crosslinker (omafilcon A). They found that the phosphorylcholine functionalized contact lenses (omafilcon A) were more resistant to bacterial adhesion and colonization than the other two hydrogels.

Glycocalyx is a loose matrix of polysaccharide based fibers that overlays the cell wall and surrounds a bacterial cell. It enables bacteria to adhere to surfaces and also

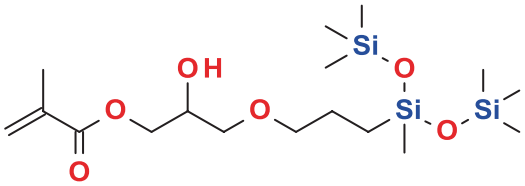


Fig. 12.2 Chemical structure of the Tanaka monomer, [methyl bis(trimethylsiloxy) silyl] propyl glycerol methacrylate

helps the bacteria evade detection by phagocytes. Glycocalyx is also present on the surface of vascular endothelial cells (the cells that line the inner surface of blood vessels). The endothelial glycocalyx contributes to the steric repulsion that prevents undesirable nonspecific adhesion of molecules and cells. Drawing inspiration from the nonadhesive properties of a glycocalyx, graft copolymers with oligosaccharide (glycodendron) side chains and poly(vinyl amine) backbone, were synthesized and found to possess antifouling properties [51]. Various polysaccharides including hyaluronic acid, heparin, and chitosan have been investigated as antifouling coatings [52–56]. Hyaluronic acid is of interest as an internal wetting agent in silicone contact lens [57, 58].

Coatings based on dendrimeric and hyperbranched polymers have been used to prevent cell adhesion on synthetic surfaces [59, 60]. Hyperbranched polyglycerol coatings showed excellent resistance to the adhesion of *E. coli*, *S. aureus*, *P. aeruginosa*, and *Aeromonas hydrophila* bacteria [61, 62]. In all of these coatings, surface hydration seems to be the key factor in preventing biofilm formation.

Amphiphilic copolymers that self-assemble to form surface domains of the nanometer scale show promise as anti-biofouling coatings [63–66]. Because of nanoscale compositional heterogeneity, these coatings can interfere with the adherence mechanisms of cells, preventing biofilm formation.

Active Release of Antimicrobial Compounds

Coatings that can actively release antimicrobial compounds or biofilm-inhibitory compounds can be used to prevent biofilm formation and device-related infections in patients [20, 67]. Active release coatings consisting of the PDMS elastomer and a ceragenin, a cholic acid derived antimicrobial agent that has a rapid, broad spectrum, and nonspecific method of attack on bacterial cell membranes [68], were evaluated in a sheep model of open fracture [69]. The ceragenin-releasing coatings were found to prevent *S. aureus* infection in 100 % of animals that were treated with them.

Controlled release of 5,6-dimethyl-2-aminobenzimidazole, a quorum-sensing inhibitor, from surfaces coated with poly(lactide-*co*-glycolide) films showed inhibition of *P. aeruginosa* growth and biofilm formation in vitro [70]. Coatings of poly(ethylene-*co*-vinylacetate) or poly(ethylene oxide)-*block*-polydimethylsiloxane that released the antibacterial agent, norfloxacin, inhibited adherence and growth of *E. coli*, *K. pneumoniae*, and *Proteus vulgaris* on surfaces of urinary catheters [43]. The incorporation of the antimicrobial compound, nitrofurantoin, in poly(L-lactic acid), using 3D printing, resulted in an inhibition of *S. aureus* biofilm colonization [71]. Foley urinary catheters impregnated with gaseous nitric oxide, demonstrated slow release of antibacterial NO over a 14-day period and were able to prevent biofilm formation on their luminal and exterior surfaces [72]. They were also able to inhibit the growth of *E. coli* within the surrounding media.

Gallium or zinc, complexed with protoporphyrin IX or mesoprotoporphyrin IX, were used as antimicrobial agents in coatings prepared using a segmented polyether

polyurethane polymer (BioSpan) [73]. Coatings cast from blends of the BioSpan polymer with a 1450-Da PEG, showed sustained release of the Ga- or Zn-complexes for at least 3 months, and demonstrated both in vitro and in vivo antibacterial activity against *S. epidermidis* and *P. aeruginosa*. The antimicrobial activity of the Ga and Zn is attributed to their interference in the bacterial iron metabolism. Microorganisms secrete iron chelators called siderophores to form complexes with Fe^{3+}. These complexes are taken up by the cells, by active transport mechanism, and used for cellular metabolism. Ga and Zn, being elemental analogs of Fe, will competitively bind to the bacterial siderophores. However, unlike Fe, they cannot undergo redox cycling within a cell and aid cell functions. Hence, competitive uptake of Ga or Zn by the bacterial cell will disrupt Fe metabolism, leading to cell death. Other chelators such as ethylenediamine tetraacetic acid and citrate have been found to inhibit microbial growth by disrupting surface adherence and preventing biofilm production [21].

Silver ions and silver nanoparticles are widely recognized for their ability to prevent biofilm formation [74–77]. However, their mechanism of antimicrobial action is still not well understood. It is known that silver nanoparticles have an enhanced biocidal ability as compared to aqueous silver ions at the same Ag concentration. Also, smaller nanoparticles, with larger surface-to-volume ratio, have higher antibacterial activity [74]. Silver nanoparticles were found to be a viable alternative to both, silver nitrate and the widely used clinical antiseptic, chlorhexidine, in preventing biofilm formation on dentin surfaces [78].

Sol-gel nanocomposite coatings of $AgCl–TiO_2$ nanoparticles were found to inhibit biofilm formation by *E. coli*, *S. epidermidis*, and *P. aeruginosa* [79]. The titania particles acted as good support matrix, facilitating sustained release of silver ions from the coatings. Anti-quorum sensing activity of the $AgCl–TiO_2$ nanoparticles was evaluated using the bacterial model *Chromobacterium violaceum* [80]. Silver released from the nanoparticles significantly reduced violacein production in a concentration-dependent manner, indicating inhibition of quorum sensing. Anti-quorum sensing activity was further confirmed by the absence of signaling molecule, *N*-(3-oxooctanoyl) homoserine lactone, during growth in the presence of the nanoparticles.

Selenium nanoparticles, 50–100 nm in diameter, were coated on the surface of polycarbonate and found to inhibit the growth of *S. aureus* [81].

Antimicrobial Coatings with Surface-bound Biocides

Coatings consisting of certain cationic compounds or polymers prevent biofilm formation by killing or inhibiting microorganisms after their adherence to the surface. Their mechanism of action is most likely related to membrane permeability alterations or membrane disruptions in the adhered cells. Surfaces of silicone rubber, covalently functionalized with the quaternary ammonium compound, 3-(trimethoxysilyl)propyldimethyloctadecylammonium chloride, were found to decrease infection rates when subcutaneously implanted in rats and seeded with *S. aureus* [82]. The

presence of hydrophobic polycationic polymer coatings, such as *N,N*-dodecylmethyl polyethyleneimine, on the surface of a metal implant, was effective in eliminating the clinical signs of infection in vivo in a sheep tibial fracture model [83]. Surfaces coated with the cationic polysaccharide, chitosan [52], showed excellent resistance in vitro to biofilm formation by *S. epidermidis*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *C. albicans*.

Robust antimicrobial coatings of 4-vinylpyridinium block copolymers, in which the nitrogen atoms of pyridine were quaternized using semifluorinated alkyl bromide, were highly effective against *S. aureus* colony formation [38]. The antimicrobial activity was found to depend on the concentration of the cationic pyridinium groups at the surface, that is, the surface charge density of the coatings. These block copolymer coatings, containing a polystyrene block, can be applied by solution casting, dip coating or spin coating, and can be blended with other polymers to impart antibacterial activity. Copolymers of 4-vinyl-*N*-hexylpyridinium bromide and dimethyl(2-methacryloyloxyethyl)phosphonate self-assembled to form ultrathin layers on titanium surfaces and inhibited the adherence and growth of *Streptococcus mutans* in vitro [84].

Copolymers composed of an antibacterial monomer containing ciprofloxacin, methyl methacrylate (MMA), and 2-perfluorooctylethyl methacrylate were prepared, and the surface properties and antibacterial performance of the copolymers and their blends with poly(methyl methacrylate) were investigated [85]. The fluorinated copolymers showed antibacterial activity toward *E. coli* and prevented the formation of biofilm for a prolonged period.

Dinjaski et al. [86] found that coatings prepared from a functionalized bacterial polyhydroxyalkanoate (PHACOS) selectively and efficiently inhibited the growth of *S. aureus* both in vitro and in vivo. They attributed the antibacterial activity to the thioester groups present in the side chains of the functionalized copolymer (see Fig. 12.3). The coatings elicited minimal cytotoxicity and inflammatory effects on murine macrophages and supported normal fibroblast adhesion.

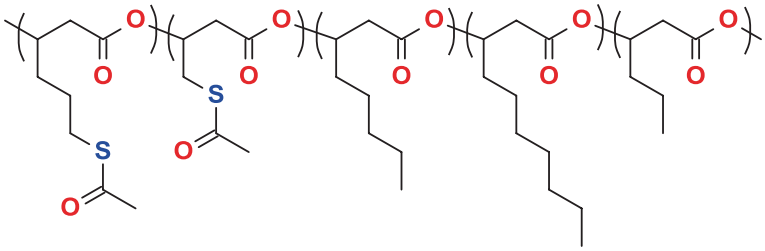


Fig. 12.3 PHACOS, a random copolymer composed of 3-hydroxy-6-acetylthiohexanoate (46.4 %), 3-hydro-4-acetylthiobutanoate (23.9 %), 3-hydroxyoctanoate (17.5 %), 3-hydroxydecanoate (10.3 %), and 3-hydroxyhexanoate (1.9 %) monomers

Antimicrobial Peptides

Coatings incorporating antimicrobial peptides (AMPs) can prevent biofilm formation on surfaces. AMPs such as magainins (found in the skin of African clawed frog *Xenopus laevis*), nisin (a bacteriocin peptide, produced by *Lactococcus lactis*, and commercially available as a food preservative), and LL-37 (a cathelicidin peptide, found in lysosomes of macrophages and polymorphonuclear leukocytes, that plays a critical role in immune response) have been used as antimicrobial agents in coatings [41, 87–89]. Films of poly(ethylene-*co*-vinyl acetate) incorporating nisin were prepared using film blowing of the melt and were found to be effective against *S. epidermidis* biofilm formation, even though temperatures as high as 120 °C were used in the production of the films [90].

Some AMPs could be immunogenic and cytotoxic. The attachment of AMPs to surfaces could potentially mitigate problems such as toxicity to host cells, immunogenicity, and short half-life (due to degradation by proteolytic enzymes in the body) associated with soluble peptides. AMPs, rich in the cationic amino acids, arginine, lysine, and tryptophan, were immobilized on PDMS and urinary catheter surfaces. The surfaces were pretreated with a radiofrequency argon plasma, dipped in a solution of allyl glycidyl ether in ethanol, and exposed to UV light for grafting of poly(allyl glycidyl ether) chains on the surfaces [91]. The epoxy groups of the resulting polymer brushes were used for surface-tethering of the AMPs. The peptide-coated surfaces were effective in preventing biofilm formation of *E. coli*, *S. aureus*, and *C. albicans*. Both the solution and immobilized peptides exhibited bacterial membrane disruption capabilities, but the latter showed a slower rate of kill. The AMP-coated surfaces showed no toxicity toward smooth muscle cells.

Polymer brushes were used to immobilize AMPs on titanium surfaces [92]. Copolymer brushes of *N,N*-dimethylacrylamide (DMA) and *N*-(3-aminopropyl) methacrylamide (APMA) were grown on the surfaces using surface-initiated atom transfer radical polymerization (see Fig. 12.4). The APMA mers were then functionalized with maleimide groups, which were used for covalently attaching a cysteinyl-

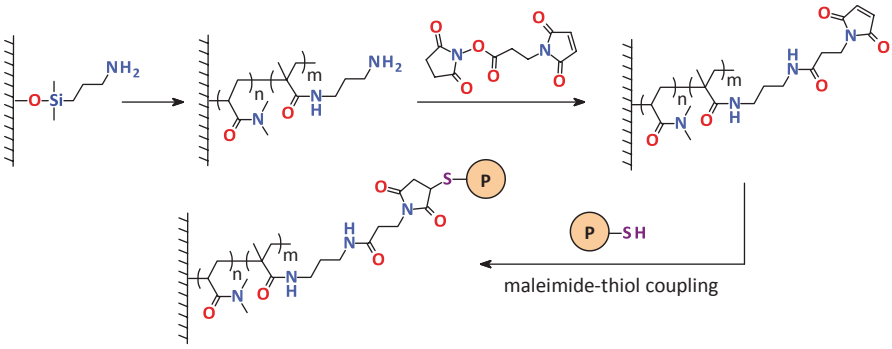


Fig. 12.4 Synthesis of copolymer brushes and peptide conjugation; P represents a peptide that contains a thiol group [92]

ated AMP, KRWRIRVRVIRKC. The AMP-coated titanium wires were implanted in rats and challenged with *S. aureus*. The tethered peptides maintained excellent antimicrobial activity. In vitro assays showed the ability of these coatings to prevent biofilm formation of *P. aeruginosa*. Furthermore, no toxicity to MG63 osteoblast-like cells, insignificant platelet activation and adhesion in human blood, and insignificant complement activation were observed.

Microporous calcium phosphate coatings and coatings of vertically aligned titania nanotubes have been developed as platforms for delivering AMPs at the surface of orthopaedic implants [93]. The HHC-36 AMP, KRWWKWWRR, designed through neural network algorithms [94], was loaded in these coatings by simple soaking, and the resulting surfaces were effective against biofilm formation of *S. aureus* and *P. aeruginosa* but were not cytotoxic for MG63 osteoblast-like cells. A multilayered coating, comprising of titania nanotubes, calcium phosphate, and a phospholipid (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine), was also developed for controlled release of this AMP and found to be promising in preventing device-related infection in orthopedic implants without being toxic to host cells [95].

An antifungal β-peptide, (ACHC-β^3Val-β^3Lys)$_3$, administered from polyelectrolyte multilayer films, prepared using poly(L-glutamic acid) (PGA) and poly(L-lysine) (PLL), was found to inhibit the growth and proliferation of *C. albicans* biofilms on the layer-by-layer coated surfaces [96]. The coatings consisted of 20 layers of the PGA/β-peptide/PLL trilayer, remained stable when incubated in culture media at 37 °C, and released AMP gradually into solution for up to 400 h. The luminal surfaces of polyethylene catheter tubes were coated with PGA/PLL multilayers, to produce ~700 nm thick multilayer coatings having the nominal structure, (PGA/PLL)$_{19}$(PGA) [97]. These coatings were loaded with the β-peptide, post-fabrication, by infusion. This approach resulted in extended intraluminal release of the AMP (over about 4 months) when incubated in physiological media.

Bacterial Interference

In this strategy, a preestablished biofilm of benign bacteria is used as a live, protective coating against pathogen colonization. This approach is similar to the well-established practice of using one protein, generally bovine serum albumin or milk proteins, to prevent nonspecific adsorption of other proteins (antibodies) on microplate surfaces in enzyme-linked immunosorbent assay (ELISA) [98]. Lopez et al. [99] activated PDMS surfaces with mild CO_2 plasma, and then attached poly(amidoamine) dendrimers to generated amino-functionalized PDMS surfaces. Carboxy-terminated mannose was then covalently attached to the surface to promote adhesion of benign *E. coli* 83972 cells that contain D-mannose-specific adhesin (FimH) in their fimbriae. This benign biofilm resulted in a significant reduction in the adherence of uropathogenic *E. faecalis*, quantified as the percentage of surface area covered by bacteria, compared with unmodified PDMS surface.

A related approach is the use of bacterial extracellular polysaccharides that inhibit biofilm formation by a wide spectrum of bacteria and fungi. These anti-biofilm polysaccharides may prove useful for the treatment and prevention of a variety of biofilm-related infections, particularly those caused by multispecies biofilms [100]. Novel coatings, incorporating the polysaccharides in synthetic polymers, can be developed.

Conclusion

The prevention of the formation of biofilms on surfaces is an active area of research and development, involving a collaborative effort between biochemists, microbiologists, immunologists, materials chemists, chemical engineers, and physicians. Of the several strategies of avoiding biofilm-related infection discussed in this chapter, the strategy of preventing microbial adherence and biofilm formation using cell repellent surfaces is the preferred choice. Antimicrobial coatings with tethered biocides or antimicrobial agents are generally effective only after the initial colonization of the surface. Breaking down a mature biofilm in to innocuous planktonic cells or combating an incidence of biofilm infection is a challenging task. The sessile cells protected within a biofilm are less susceptible to host immune responses and to antibiotics than the original planktonic cells. Hence, even initial colonization results in an increased propensity of infection. Because of the recent advances in polymer synthesis and thin film processing techniques, including surface-initiated polymerization and surface-grafting of molecules, it is now becoming feasible to coat medical devices with robust cell-repellent and nonadhesive biocompatible coatings that will integrate well with host tissues.

References

1. Francolini I, Donelli G (2010) Prevention and control of biofilm-based medical-device-related infections. FEMS Immunol Med Microbiol 59(3):227–238
2. Sousa C, Henriques M, Oliveira R (2011) Mini-review: antimicrobial central venous catheters—recent advances and strategies. Biofouling 27(6):609–620
3. Costerton J, Stewart PS, Greenberg E (1999) Bacterial biofilms: a common cause of persistent infections. Science 284(5418):1318–1322
4. Welsh CA, Flanagan ME, Hoke SC, Doebbeling BN, Herwaldt L (2012) Reducing health care-associated infections (HAIs): lessons learned from a national collaborative of regional HAI programs. Am J Infect Control 40(1):29–34
5. Donlan RM (2001) Biofilm formation: a clinically relevant microbiological process. Clin Infect Dis 33(8):1387–1392
6. Fanning S, Mitchell AP (2012) Fungal biofilms. PLoS Pathog 8(4):e1002585
7. McLaughlin-Borlace L, Stapleton F, Matheson M, Dart J (1998) Bacterial biofilm on contact lenses and lens storage cases in wearers with microbial keratitis. J Appl Microbiol 84(5):827–838

8. Imamura Y, Chandra J, Mukherjee PK, Lattif AA, Szczotka-Flynn LB, Pearlman E, Lass JH, O'Donnell K, Ghannoum MA (2008) *Fusarium* and *Candida albicans* biofilms on soft contact lenses: model development, influence of lens type, and susceptibility to lens care solutions. Antimicrob Agents Chemother 52(1):171–182
9. Flemming H-C, Neu TR, Wozniak DJ (2007) The EPS matrix: the "house of biofilm cells". J Bacteriol 189(22):7945–7947
10. Stoodley P, Sauer K, Davies D, Costerton JW (2002) Biofilms as complex differentiated communities. Annu Rev Microbiol 56(1):187–209
11. Stewart PS (1996) Theoretical aspects of antibiotic diffusion into microbial biofilms. Antimicrob Agents Chemother 40(11):2517–2522
12. Mah T-FC, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9(1):34–39
13. Fux C, Costerton J, Stewart P, Stoodley P (2005) Survival strategies of infectious biofilms. Trends Microbiol 13(1):34–40
14. Costerton JW, Cook G, Shirtliff M, Stoodley P, Pasmore M (2004) Biofilms, biomaterials, and device-related infections. In: Ratner BD, Hoffman AS, Schaer TP, Lemons JE (eds) Biomaterials science: an introduction to materials in medicine, 2nd edn. Elsevier Academic Press, London, pp 345–354
15. Pavithra D, Doble M (2008) Biofilm formation, bacterial adhesion and host response on polymeric implants—issues and prevention. Biomed Mater 3(3):034003
16. Denstedt JD, Cadieux PA (2009) Eliminating biofilm from ureteral stents: the holy grail. Curr Opin Urol 19(2):205–210
17. Rodrigues LR (2011) Inhibition of bacterial adhesion on medical devices. In: Linke D, Goldman A (eds) Bacterial adhesion. Advances in experimental medicine and biology, vol 715. Springer, Netherlands, pp 351–367
18. Khoo X, Grinstaff MW (2011) Novel infection-resistant surface coatings: a bioengineering approach. MRS Bull 36(05):357–366
19. Bruellhoff K, Fiedler J, Möller M, Groll J, Brenner RE (2010) Surface coating strategies to prevent biofilm formation on implant surfaces. Int J Artif Organs 33(9):646–653
20. Worthington RJ, Richards JJ, Melander C (2012) Small molecule control of bacterial biofilms. Org Biomol Chem 10(37):7457–7474
21. Raad II, Fang X, Keutgen XM, Jiang Y, Sherertz R, Hachem R (2008) The role of chelators in preventing biofilm formation and catheter-related bloodstream infections. Curr Opin Infect Dis 21(4):385–392
22. Krishnan S, Weinman CJ, Ober CK (2008) Advances in polymers for anti-biofouling surfaces. J Mater Chem 18(29):3405–3413
23. Vendra VK, Wu L, Krishnan S (2010) Polymer thin films for biomedical applications. In: Kumar CSSR (ed) Nanostructured thin films and surfaces. Wiley, Weinheim, pp 1–51
24. Hou S, Burton EA, Simon KA, Blodgett D, Luk Y-Y, Ren D (2007) Inhibition of *Escherichia coli* biofilm formation by self-assembled monolayers of functional alkanethiols on gold. Appl Environ Microbiol 73(13):4300–4307
25. Qian X, Metallo SJ, Choi IS, Wu H, Liang MN, Whitesides GM (2002) Arrays of self-assembled monolayers for studying inhibition of bacterial adhesion. Anal Chem 74(8):1805–1810
26. Lin P, Ting L, Lin C-W, Gu F (2014) Non-fouling property of zwitterionic cysteine surface. Langmuir 30(22):6497–6507
27. Chen S, Liu L, Jiang S (2006) Strong resistance of oligo(phosphorylcholine) self-assembled monolayers to protein adsorption. Langmuir 22(6):2418–2421
28. Yu B-Y, Zheng J, Chang Y, Sin M-C, Chang C-H, Higuchi A, Sun Y-M (2014) Surface zwitterionization of titanium for a general bio-inert control of plasma proteins, blood cells, tissue cells, and bacteria. Langmuir 30(25):7502–7512
29. Cheng G, Zhang Z, Chen S, Bryers JD, Jiang S (2007) Inhibition of bacterial adhesion and biofilm formation on zwitterionic surfaces. Biomaterials 28 (29):4192–4199
30. Saxer S, Portmann C, Tosatti S, Gademann K, Zürcher S, Textor M (2009) Surface assembly of catechol-functionalized poly(L-lysine)-*graft*-poly(ethylene glycol) copolymer on titanium

exploiting combined electrostatically driven self-organization and biomimetic strong adhesion. Macromolecules 43(2):1050–1060
31. Kudaibergenov S, Jaeger W, Laschewsky A (2006) Polymeric betaines: synthesis, characterization, and application. In: Abe A, Dušek K, Kobayashi S (eds) Supramolecular polymers, polymeric betains, oligomers. Advances in polymer science, vol 201. Springer, Berlin, pp 157–224
32. Ishii T, Wada A, Tsuzuki S, Casolaro M, Ito Y (2007) Copolymers including L-histidine and hydrophobic moiety for preparation of nonbiofouling surface. Biomacromolecules 8(11):3340–3344
33. Liu Q, Singh A, Liu L (2012) Amino acid-based zwitterionic poly(serine methacrylate) as an antifouling material. Biomacromolecules 14(1):226–231
34. Alswieleh AM, Cheng N, Canton I, Ustbas B, Xue X, Ladmiral V, Xia S, Ducker RE, El Zubir O, Cartron ML (2014) Zwitterionic poly(amino acid methacrylate) brushes. J Am Chem Soc 136(26):9404–9413
35. Chen S, Li L, Zhao C, Zheng J (2010) Surface hydration: principles and applications toward low-fouling/nonfouling biomaterials. Polymer 51(23):5283–5293
36. Wu L, Jasinski J, Krishnan S (2012) Carboxybetaine, sulfobetaine, and cationic block copolymer coatings: a comparison of the surface properties and antibiofouling behavior. J Appl Polym Sci 124(3):2154–2170
37. Carmona-Ribeiro AM, de Melo Carrasco LD (2013) Cationic antimicrobial polymers and their assemblies. Int J Mol Sci 14(5):9906–9946
38. Krishnan S, Ward RJ, Hexemer A, Sohn KE, Lee KL, Angert ER, Fischer DA, Kramer EJ, Ober CK (2006) Surfaces of fluorinated pyridinium block copolymers with enhanced antibacterial activity. Langmuir 22(26):11255–11266
39. Ding X, Yang C, Lim TP, Hsu LY, Engler AC, Hedrick JL, Yang Y-Y (2012) Antibacterial and antifouling catheter coatings using surface grafted PEG-*b*-cationic polycarbonate diblock copolymers. Biomaterials 33(28):6593–6603
40. Sellenet PH, Allison B, Applegate BM, Youngblood JP (2007) Synergistic activity of hydrophilic modification in antibiotic polymers. Biomacromolecules 8(1):19–23
41. Tai Y-C, McGuire J, Neff JA (2008) Nisin antimicrobial activity and structural characteristics at hydrophobic surfaces coated with the PEO–PPO–PEO triblock surfactant Pluronic® F108. J Colloid Interface Sci 322(1):104–111
42. Park JH, Cho YW, Kwon IC, Jeong SY, Bae YH (2002) Assessment of PEO/PTMO multiblock copolymer/segmented polyurethane blends as coating materials for urinary catheters: in vitro bacterial adhesion and encrustation behavior. Biomaterials 23(19):3991–4000
43. Park JH, Cho YW, Cho Y-H, Choi JM, Shin HJ, Bae YH, Chung H, Jeong SY, Kwon IC (2003) Norfloxacin-releasing urethral catheter for long-term catheterization. J Biomater Sci Polym Ed 14(9):951–962
44. Fan X, Lin L, Dalsin JL, Messersmith PB (2005) Biomimetic anchor for surface-initiated polymerization from metal substrates. J Am Chem Soc 127(45):15843–15847
45. Saldarriaga Fernández IC, Mei HC, Metzger S, Grainger DW, Engelsman AF, Nejadnik MR, Busscher HJ (2010) In vitro and in vivo comparisons of staphylococcal biofilm formation on a cross-linked poly(ethylene glycol)-based polymer coating. Acta Biomater 6(3):1119–1124
46. Denes AR, Somers EB, Wong AC, Denes F (2001) 12-crown-4 ether and tri(ethylene glycol) dimethyl ether plasma-coated stainless steel surfaces and their ability to reduce bacterial biofilm deposition. J Appl Polym Sci 81(14):3425–3438
47. Diaz Blanco C, Ortner A, Dimitrov R, Navarro A, Mendoza E, Tzanov T (2014) Building an antifouling zwitterionic coating on urinary catheters using an enzymatically triggered bottom-up approach. ACS Appl Mater Interfaces 6(14):11385–11393
48. Tanaka K, Takahashi K, Kanada M, Kato Y, Ichihara M (1979) Copolymer for contact lens, its preparation and contact lens made thereof. US Pat 4,139,692
49. Song M, Shin YH, Kwon Y (2010) Synthesis and properties of siloxane-containing hybrid hydrogels: optical transmittance, oxygen permeability and equilibrium water content. J Nanosci Nanotechnol 10(10):6934–6938

50. Selan L, Palma S, Scoarughi GL, Papa R, Veeh R, Di Clemente D, Artini M (2009) Phosphorylcholine impairs susceptibility to biofilm formation of hydrogel contact lenses. Am J Ophthalmol 147(1):134–139
51. Holland NB, Qiu Y, Ruegsegger M, Marchant RE (1998) Biomimetic engineering of non-adhesive glycocalyx-like surfaces using oligosaccharide surfactant polymers. Nature 392(6678):799–801
52. Myrick JM, Vendra VK, Krishnan S (2014) Self-assembled polysaccharide nanostructures for controlled-release applications. Nanotechnol Rev 3(4):319–346
53. Tenke P, Riedl CR, Jones GL, Williams GJ, Stickler D, Nagy E (2004) Bacterial biofilm formation on urologic devices and heparin coating as preventive strategy. Int J Antimicrob Agents 23:67–74
54. Abdelkefi A, Achour W, Ben Othman T, Ladeb S, Torjman L, Lakhal A, Ben Hassen A, Hsairi M, Ben Abdeladhim A (2007) Use of heparin-coated central venous lines to prevent catheter-related bloodstream infection. J Support Oncol 5(6):273–278
55. Hu X, Tan H, Li D, Gu M (2014) Surface functionalisation of contact lenses by CS/HA multilayer film to improve its properties and deliver drugs. Mater Technol 29 (1):8–13
56. Carlson RP, Taffs R, Davison WM, Stewart PS (2008) Anti-biofilm properties of chitosan-coated surfaces. J Biomater Sci Polym Ed 19(8):1035–1046
57. Weeks A, Morrison D, Alauzun JG, Brook MA, Jones L, Sheardown H (2012) Photocross-linkable hyaluronic acid as an internal wetting agent in model conventional and silicone hydrogel contact lenses. J Biomed Mater Res Part A 100(8):1972–1982
58. Weeks A, Subbaraman LN, Jones L, Sheardown H (2013) Physical entrapment of hyaluronic acid during synthesis results in extended release from model hydrogel and silicone hydrogel contact lens materials. Eye Contact Lens 39(2):179–185
59. Wei Q, Becherer T, Noeske PLM, Grunwald I, Haag R (2014) A universal approach to cross-linked hierarchical polymer multilayers as stable and highly effective antifouling coatings. Adv Mater 26(17):2688–2693
60. Zhou Y, Huang W, Liu J, Zhu X, Yan D (2010) Self-assembly of hyperbranched polymers and its biomedical applications. Adv Mater 22(41):4567–4590
61. Weinhart M, Becherer T, Schnurbusch N, Schwibbert K, Kunte HJ, Haag R (2011) Linear and hyperbranched polyglycerol derivatives as excellent bioinert glass coating materials. Adv Eng Mater 13(12):B501–B510
62. Moore E, Thissen H, Voelcker NH (2013) Hyperbranched polyglycerols at the biointerface. Prog Surf Sci 88(3):213–236
63. Kerstetter JL, Gramlich WM (2014) Nanometer-scale self-assembly of amphiphilic copolymers to control and prevent biofouling. J Mater Chem B. 2(46):8043–8052
64. Weinman CJ, Gunari N, Krishnan S, Dong R, Paik MY, Sohn KE, Walker GC, Kramer EJ, Fischer DA, Ober CK (2010) Protein adsorption resistance of anti-biofouling block copolymers containing amphiphilic side chains. Soft Matter 6(14):3237–3243
65. Martinelli E, Menghetti S, Galli G, Glisenti A, Krishnan S, Paik MY, Ober CK, Smilgies DM, Fischer DA (2009) Surface engineering of styrene/PEGylated-fluoroalkyl styrene block copolymer thin films. J Polym Sci Part A Polym Chem 47(1):267–284
66. Krishnan S, Ayothi R, Hexemer A, Finlay JA, Sohn KE, Perry R, Ober CK, Kramer EJ, Callow ME, Callow JA (2006) Anti-biofouling properties of comblike block copolymers with amphiphilic side chains. Langmuir 22 (11):5075–5086
67. Wenderska IB, Chong M, McNulty J, Wright GD, Burrows LL (2011) Palmitoyl-DL-carnitine is a multitarget inhibitor of *Pseudomonas aeruginosa* biofilm development. ChemBioChem 12(18):2759–2766
68. Epand RF, Pollard JE, Wright JO, Savage PB, Epand RM (2010) Depolarization, bacterial membrane composition, and the antimicrobial action of ceragenins. Antimicrob Agents Chemother 54(9):3708–3713
69. Williams DL, Haymond BS, Beck JP, Savage PB, Chaudhary V, Epperson RT, Kawaguchi B, Bloebaum RD (2012) In vivo efficacy of a silicone-cationic steroid antimicrobial coating to prevent implant-related infection. Biomaterials 33(33):8641–8656

70. Broderick AH, Breitbach AS, Frei R, Blackwell HE, Lynn DM (2013) Surface-mediated release of a small-molecule modulator of bacterial biofilm formation: a non-bactericidal approach to inhibiting biofilm formation in *Pseudomonas aeruginosa*. Adv Healthcare Mater 2(7):993–1000
71. Sandler N, Salmela I, Fallarero A, Rosling A, Khajeheian M, Kolakovic R, Genina N, Nyman J, Vuorela P (2014) Towards fabrication of 3D printed medical devices to prevent biofilm formation. Int J Pharm 459(1):62–64
72. Regev-Shoshani G, Ko M, Miller C, Av-Gay Y (2010) Slow release of nitric oxide from charged catheters and its effect on biofilm formation by *Escherichia coli*. Antimicrob Agents Chemother 54(1):273–279
73. Ma H, Darmawan ET, Zhang M, Zhang L, Bryers JD (2013) Development of a poly(ether urethane) system for the controlled release of two novel anti-biofilm agents based on gallium or zinc and its efficacy to prevent bacterial biofilm formation. J Controll Release 172(3):1035–1044
74. Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, Camargo ERd, Barbosa DB (2009) The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. Int J Antimicrob Agents 34(2):103–110
75. Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27(1):76–83
76. Secinti KD, Özalp H, Attar A, Sargon MF (2011) Nanoparticle silver ion coatings inhibit biofilm formation on titanium implants. J Clin Neurosci 18(3):391–395
77. Stobie N, Duffy B, McCormack DE, Colreavy J, Hidalgo M, McHale P, Hinder SJ (2008) Prevention of *Staphylococcus epidermidis* biofilm formation using a low-temperature processed silver-doped phenyltriethoxysilane sol–gel coating. Biomaterials 29(8):963–969
78. Besinis A, De Peralta T, Handy RD (2014) Inhibition of biofilm formation and antibacterial properties of a silver nano-coating on human dentine. Nanotoxicology 8(7):745–754
79. Naik K, Kowshik M (2014) Anti-biofilm efficacy of low temperature processed AgCl–TiO_2 nanocomposite coating. Mater Sci Eng C 34:62–68
80. Naik K, Kowshik M (2014) Anti-quorum sensing activity of AgCl–TiO_2 nanoparticles with potential use as active food packaging material. J Appl Microbiol 117(4):972–983
81. Wang Q, Webster TJ (2012) Nanostructured selenium for preventing biofilm formation on polycarbonate medical devices. J Biomed Mater Res Part A 100(12):3205–3210
82. Gottenbos B, van der Mei HC, Klatter F, Nieuwenhuis P, Busscher HJ (2002) In vitro and in vivo antimicrobial activity of covalently coupled quaternary ammonium silane coatings on silicone rubber. Biomaterials 23(6):1417–1423
83. Schaer TP, Stewart S, Klibanov AM (2013) Antibacterial coatings that inhibit biofilm formation on implants. US Pat 20130110237 A1
84. Pfaffenroth C, Winkel A, Dempwolf W, Gamble LJ, Castner DG, Stiesch M, Menzel H (2011) Self-assembled antimicrobial and biocompatible copolymer films on titanium. Macromol Biosci 11(11):1515–1525
85. Ni H, Jiang T, Hu P, Han Z, Lu X, Ye P (2014) Self-decontaminating properties of fluorinated copolymers integrated with ciprofloxacin for synergistically inhibiting the growth of *Escherichia coli*. J Biomater Sci, Polym Ed 25(17):1920–1945
86. Dinjaski N, Fernández-Gutiérrez M, Selvam S, Parra-Ruiz FJ, Lehman SM, San Román J, García E, García JL, García AJ, Prieto MA (2014) PHACOS, a functionalized bacterial polyester with bactericidal activity against methicillin-resistant *Staphylococcus aureus*. Biomaterials 35(1):14–24
87. Costa F, Carvalho IF, Montelaro RC, Gomes P, Martins MCL (2011) Covalent immobilization of antimicrobial peptides (AMPs) onto biomaterial surfaces. Acta Biomater 7(4):1431–1440
88. Joshi PR, McGuire J, Neff JA (2009) Synthesis and antibacterial activity of nisin-containing block copolymers. J Biomed Mater Res Part B Appl Biomater 91(1):128–134
89. Coad BR, Jasieniak M, Griesser SS, Griesser HJ (2013) Controlled covalent surface immobilisation of proteins and peptides using plasma methods. Surf Coat Technol 233:169–177

90. Nostro A, Scaffaro R, Ginestra G, D'Arrigo M, Botta L, Marino A, Bisignano G (2010) Control of biofilm formation by poly(ethylene-*co*-vinyl acetate) films incorporating nisin. Appl Microbiol Biotechnol 87(2):729–737
91. Li X, Li P, Saravanan R, Basu A, Mishra B, Lim SH, Su X, Tambyah PA, Leong SSJ (2014) Antimicrobial functionalization of silicone surfaces with engineered short peptides having broad spectrum antimicrobial and salt-resistant properties. Acta Biomater 10(1):258–266
92. Gao G, Lange D, Hilpert K, Kindrachuk J, Zou Y, Cheng JTJ, Kazemzadeh-Narbat M, Yu K, Wang R, Straus SK, Brooks DE, Chew BH, Hancock REW, Kizhakkedathu JN (2011) The biocompatibility and biofilm resistance of implant coatings based on hydrophilic polymer brushes conjugated with antimicrobial peptides. Biomaterials 32(16):3899–3909
93. Kazemzadeh-Narbat M, Kindrachuk J, Duan K, Jenssen H, Hancock REW, Wang R (2010) Antimicrobial peptides on calcium phosphate-coated titanium for the prevention of implant-associated infections. Biomaterials 31(36):9519–9526
94. Cherkasov A, Hilpert K, Jenssen H, Fjell CD, Waldbrook M, Mullaly SC, Volkmer R, Hancock RE (2008) Use of artificial intelligence in the design of small peptide antibiotics effective against a broad spectrum of highly antibiotic-resistant superbugs. ACS Chem Biol 4(1):65–74
95. Kazemzadeh-Narbat M, Lai BFL, Ding C, Kizhakkedathu JN, Hancock REW, Wang R (2013) Multilayered coating on titanium for controlled release of antimicrobial peptides for the prevention of implant-associated infections. Biomaterials 34(24):5969–5977
96. Karlsson AJ, Flessner RM, Gellman SH, Lynn DM, Palecek SP (2010) Polyelectrolyte multilayers fabricated from antifungal β-peptides: design of surfaces that exhibit antifungal activity against *Candida albicans*. Biomacromolecules 11(9):2321–2328
97. Raman N, Lee M-R, Palecek SP, Lynn DM (2014) Polymer multilayers loaded with antifungal β-peptides kill planktonic *Candida albicans* and reduce formation of fungal biofilms on the surfaces of flexible catheter tubes. J Controll Release 191:54–62
98. Xiao Y, Isaacs SN (2012) Enzyme-linked immunosorbent assay (ELISA) and blocking with bovine serum albumin (BSA)—not all BSAs are alike. J Immunol Methods 384(1):148–151
99. Lopez AI, Kumar A, Planas MR, Li Y, Nguyen TV, Cai C (2011) Biofunctionalization of silicone polymers using poly(amidoamine) dendrimers and a mannose derivative for prolonged interference against pathogen colonization. Biomaterials 32(19):4336–4346
100. Rendueles O, Kaplan JB, Ghigo JM (2013) Antibiofilm polysaccharides. Environ Microbiol 15(2):334–346

Chapter 13
Hygiene Problems and Food Industry

Satoshi Fukuzaki

Abstract In the food industry, the cleanliness of food-processing equipment is the most important in manufacturing safe and wholesome products. Cleaning is a primary operation for minimizing accumulation of soils and microorganisms in food industry settings. Cleaning and disinfection are complementary processes and are performed as a separate or a combined operation. The cleaning operation should precede a disinfecting operation because it is far easier to disinfect a clean surface than a soiled surface. Hygienic design and choice of construction materials of equipment are also important factors to prevent accumulation of soils and biofilm formation. Education and training of the staff are very important because food hygiene management is a man-mediated process. Hygiene conditions should be assessed periodically by a swab method. The results of swabbing become in turn scientific evidence for education of the staff.

Hygiene Problems

It is imperative that safe and high-quality products are manufactured and supplied for consumers. It is necessary to prevent any physical, chemical, and biological contamination of the product during the food-processing. Biological contamination is the most severe problem because it has a hazard to break out large-scale food poisoning events. Food residues that serve as nutrients and conditioning film can induce the subsequent adhesion and growth of microorganisms, resulting in the formation of biofilm [1]. Therefore, food residues on food-contact surfaces should be removed at the end of each set of processing operations by routine cleaning.

In general, food-processing equipment is intricately fabricated in consideration of requirements of hygiene, production efficiency, automation, and safety. In addition, a high degree of cleanliness is requested for food-contact surfaces. Poor-cleaning problems usually originate from incorrect decisions in the design of equipment.

S. Fukuzaki (✉)
Graduate School of Bioresources, Mie University, Tsu, Japan
e-mail: satoshi_fukuzaki@bio.mie-u.ac.jp

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_13

It is a main feature of food-processing equipment that there are many points to be considered in order to accomplish an effective cleaning operation.

In many factories, manual cleaning of equipment is performed routinely. The criteria of the degree of cleanliness are not always based on scientific data, and they rely on empirical data in many cases. It is likely that there are some blind spots that tend to be overlooked. Nowadays, a cleaning-in-place (CIP) system has been devised to clean the food-contact surfaces of vessels, equipment, and pipework in place, i.e., without dismantling. The CIP system can remove food residues together with microbes effectively. However, CIP is not a complete system so several problems concerning design of equipment and surface materials remain. The staffs have to understand the presence of dead areas of equipment and pipework in a CIP system. As shown below, cleanliness of equipment surfaces should be assessed periodically by a swab method. However, it is difficult to perform without discipline and training.

Basic Activities for Hygiene Management

When a hygiene management plan is put together in food industry settings, there are some programs that assist a solid foundation for the plan. These programs are often referred to as prerequisite programs. Prerequisite programs have been reconfirmed as the fundamental procedures to sustain hygienic conditions.

In general manufacturing industries, the basic activities for an increase in the efficiency of the manufacturing process, are arrangement and sweeping of the work space, elimination of unnecessary items, maintenance of dirt-less conditions, and education of the manufacturing staff. Here, the clean conditions mentioned are the situations where arrangement and order are kept. Although these activities are applicable to the food industry, it is of importance to note that the dirt free conditions mentioned above refer to visible clean conditions. In the hygiene management, it is also necessary to remove invisible soils and microorganisms. Only a dry-type sweeping operation is insufficient to prevent biological contamination.

For controlling microorganisms on food-contact surfaces, it is essential to perform a wet kind of cleaning using water as the cleaning medium because it can achieve far higher levels of cleanliness than a dry-type sweeping. Cleaning and disinfecting operations are complementary processes: neither alone can achieve the cleanliness to be desired.

Hygiene management is a man-mediated process. Contamination caused by mistakes made by the operating staff also often takes place. Therefore, education (which is referred to as discipline) and training of staff are very important because they affect the whole process. The processing hygiene is to some extent one of attitudes. In food and pharmaceutical industries, good manufacturing practice (GMP) norms provide common rules that should be followed [2]. A basic factor in the education is to make the staff understand not only “what to do” and “how to do” but also “why they must do so.”

Table 13.1 Four principles of control of microorganisms in the food industry

Principle	Physical method	Chemical method
Cleaning	Heat (thermal) action	Surface activity
		Organic surfactants
	Mechanical action	Chemical reaction
	Interfacial turbulence Friction/polishing force High pressure Foaming Ultrasonication	Alkalies, acids, oxidizing agents, sequestering/chelating agents, dispersing agents etc.
		Biological (enzyme) reaction
Disinfection	Heat treatment	Liquid agents
	High temperature (>100 °C) Low temperature (<100 °C) Wet-type (water-mediated) Dry-type (air-mediated)	Chlorine compounds, peroxygens, quaternary ammonium compounds, alcohols etc.
	Nonthermal treatment	Gaseous agents
	Ultraviolet irradiation Radiation irradiation (electromagnetic, particles) Ultra-high pressure Utrasonication	Ozone, formaldehyde, ethylene oxide etc.
		Solid agents
		Calcium hypochlorite, calcium oxide etc.
Biostasis	Low-temperature storage	Food additives
	Refrigerating and freezing	
	Low water activity	Modified atmosphere packaging
	High osmotic pressure	Oxygen absorber packaging
Physical separation	Substances	
	Wrapping/packaging (environmental microbes), gloves (hands), shoes (carry-over dirt), curtain (wind), window shade (light) etc.	
	Environment	
	Zoning (clean, semiclean, dirty room), color-based zoning, temperature, pressure, wet/dry floor, etc.	

Control of Microorganisms

There are four principles of the control procedure for microorganisms on a solid surface, i.e., cleaning, disinfection, biostasis, and physical separation, from substances and environments affecting the quality of foods (Table 13.1). It is necessary to combine these four principles effectively in each manufacturing site. The concept of using multiple intervention methods is analogous to hurdle technology where two or more methods are used to prevent microbial growth [3, 4].

Among these principles, cleaning is a primary operation. Food residues reduce the effectiveness of disinfectants and give rise to the subsequent growth of microorganisms. Therefore, it is desirable to remove food residues by cleaning operations as early as possible. If microorganisms on solid surfaces are completely removed by a cleaning operation together with soils, then a disinfecting operation does not have to be conducted. However, the complete removal of them is hardly achieved by cleaning alone. Therefore, a disinfecting operation should be performed to kill viable microbes remaining on the surfaces. Nevertheless, it is necessary to remove as much soil as possible and to decrease the number of microbes by cleaning operations. As a result, the load of the disinfection process can be reduced markedly.

Apart from a solid surface of equipment, surfaces of fresh and fresh-cut vegetables cannot be treated by strong detergents and heat-disinfection. These fresh products are usually washed with water and processed under low-temperature conditions. In this case, it is desirable to separate the facility into different hygiene areas, e.g., clean, semiclean, and dirty work zones, in order to minimize risks of microbial cross contamination. Finally, products are packaged in a physically separated room, e.g., clean room.

Design of Equipment

Equipment design plays the most important role in controlling soiling and biofilm formation. In general, it is difficult to gain the cleanliness to be desired when the design of equipment is inadequate for an effective cleaning even if any detergents and mechanical actions are used. Table 13.2 lists factors to be considered for an effective cleaning. These factors are also essential from the viewpoint of prevention of accumulations of microbes. In particular, it is necessary to avoid dead areas observed in pipework and equipment, where microbial growth is favored.

The choice of surface materials is of great importance when designing food-processing equipment. Materials used in the construction of the equipment must fulfill many requirements regarding corrosion resistance, mechanical strength, longevity, ease of fabrication, weldability, and nontoxicity [5]. All product-contact surfaces must be resistant to the product, as well as to all cleaning and disinfecting agents under operating conditions. In addition, the cleanability of equipment is a matter of great concern in hygienic plant operations.

From these requirements, austenitic stainless steels are widely used as construction materials for food machinery. One approach to improve the cleanability is to provide smoothness to the stainless steel surfaces by mechanical polishing and electropolishing [6, 7]. In order to achieve good cleanability, food-contact surfaces should have a roughness of less than 0.8 μm [7].

Glass also has good properties for food equipment because of its excellent cleanability, resistance to soiling, hardness, smooth surface, corrosion resistance, and transparency. However, it is weak against impact damage [8].

Table 13.2 Factors to be considered in design of equipment required for an effective performance of cleaning operation

Parts of concern	Factors to be considered
Structure	Avoidance of dead areas and legs
	Drainability
	Slope of pipework and tank bottom
	Air tightness, bacteria tightness (closed and aseptic equipment)
	Avoidance of sharp corners
	Hygienic fabrication of pipework (dismountable)
	Thermal insulation
	Correct installation of accessories and joints
Material	Corrosion resistance, chemical resistance, heat resistance
	Nonabsorbent material
	Mechanical strength, surface hardness, longevity
	Ease of fabrication, weldability
	Hydrophilicity and hydrophobicity
	Surface tension, surface charge (interfacial properties)
	Nontoxicity
Surface morphology	Surface finishing (surface roughness)
	Direction of polishing
	Nonporous

Rubbers are used as the sealing material such as gaskets and packings. The smooth surface of rubber is mechanically abraded or chemically deteriorated gradually during a long-term operation. The surface cracks on the rubber often result in the accumulation of microbes. Some microbes are also able to decompose rubber and use it as an energy source [2].

Assessment of Hygienic Conditions

Swabbing is the easiest and preferred method to both assess the cleanliness of equipment surfaces and search points where cleaning is poor. A swab method has many advantages as follows:

1) Easy and quick assessment
2) Available in actual food-manufacturing factories
3) Detection and quantification of invisible soils and bacteria
4) Applicable to various-shaped equipment

In swab method, viable bacterial cells, proteins, and adenosine triphosphate (ATP) are quantified as the index of the degree of contamination, because the presence of them indicates a poor cleaning and a hazard of biological contamination. At present,

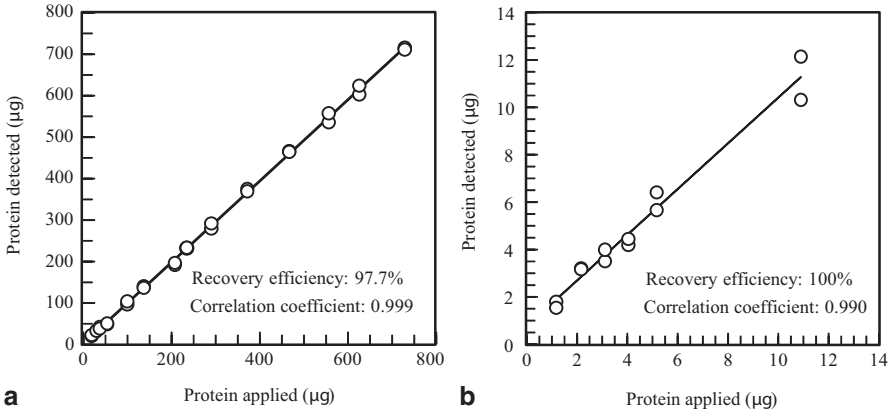


Fig. 13.1 Relationship between the amount of protein (egg albumin) applied onto a stainless steel plate and that of protein detected according to the standardized swab method. Adapted from reference [10]. **a** 20 ~ 730 μg egg albumin. **b** 1 ~ 11 μg egg albumin

however, there is a main problem that the amount of soils swabbed up by workers differs to a large extent from each other. This is due to a lack of standards for the swabbing operation. For the high performance of the swab method, it is desirable to standardize the swabbing operation and train workers to obtain sensitive, accurate, and repeatable results.

The standardization of a swab method begins with the selection of a quality swab stick. The criteria of ideal requirements of the swab stick as follows:

1) High recovery efficiency (affinity for soils, friction force)
2) Less-reactive to reagents of quantitative assay (less interference)
3) Absorptive head (appropriate porous structure)
4) Solvent compatibility (wetting and extraction solution)

Operating factors of the swab method have been examined for the quantitative assay of proteins or oil attached onto hard surfaces [9, 10]. It has been demonstrated that a swab stick comprised of a polyethylene (PE) head and polypropylene (PP) shaft was very useful for detection of protein on stainless steel in combination with Lowry-Folin assay [10]. Figure 13.1 shows the performance of the standardized swab method for the assay of protein (egg albumin) attached to stainless steel. Swabbing was conducted at 800–1400 mN for 20 s using 0.1 M NaOH as a wetting solution (60 μl: 85 % wetting level) for the swab head. The collected protein on the PE-head swab was eluted efficiently in 0.1 M NaOH containing 0.1 % sodium dodecyl sulfate as an eluent. As a result, the protein of at least 1 to 700 μg could be quantified with the recovery efficiency of 97 %. The wetting solution was found to be essential to recover the protein from stainless steel. These results indicate that the sensitive and accurate assay can be performed according to the standardized swabbing operation.

An effective swabbing technique is available to both monitor the effectiveness of the cleaning program and to validate the hygienic design of equipment. The results

of swabbing become scientific evidence to make the staff understand the real state of hygienic conditions.

References

1. Kumar CG, Anand SK (1998) Significance of microbial biofilms in food industry: a review. Int J Food Microbiol 42:9–27
2. Mattila-Sandholm T, Wirtanen G (1992) Biofilm formation in the industry: a review. Food Rev Int 8:73–603
3. Leistner L (2000) Basic aspects of food prevention by hurdle technology. Int J Food Microbiol 55:181–186
4. Leistner L, Gorris LGM (1995) Food preservation by hurdle technology. Trends Food Sci Technol 6:41–46
5. European Hygienic Equipment Design Group (1993) Hygienic equipment design criteria. Trends Food Sci Technol 4:225–229
6. European Hygienic Equipment Design Group (1993) Welding stainless steel to meet hygienic requirements. Trends Food Sci Technol 4:306–310
7. European Hygienic Equipment Design Group (1993) Hygienic design of closed equipment for the processing of liquid food. Trends Food Sci Technol 4:375–379
8. Dunsmore DG, Twomey A, Whittlestone WG, Morgan HW (1981) Design and performance of systems for cleaning product-contact surfaces of food equipment: a review. J Food Prot 44:220–240
9. Urano H, Omura H, Fukuzaki S (2010) Operating factors of the swab method for the quantitative assay of triolein attached onto hard surfaces (in Japanese). Bokin Bobai 38:715–722
10. Urano H, Omura H, Fukuzaki S (2010) Operating conditions and availability of the swab method for the quantitative assay of proteins attached onto stainless steel (in Japanese). Bokin Bobai 38:211–218

Chapter 14
Environmental Problems: Soil and Underground Water Treatment and Bioremediation

Hideyuki Kanematsu and Dana M. Barry

Abstract Bioremediation is environmental restoration technology using natural biological activities by organisms. When the contamination is not so severe and moderate, and when the time needed for the restoration can be taken and not rushed, then this method would be very appropriate. Usually, bioremediation is an environmentally friendly process. In addition, biofilm contains a lot of bacteria so the stability from various viewpoints is much higher than just the planktonic state. Therefore, biofilm will be used for bioremediation often in the future. Bioremediation by biofilm is divided into two types—in situ biofilm remediation and ex situ biofilm remediation. At any rate, biofilm remediation can achieve much higher efficiency as well as stability against toxicity, shear stress, ultraviolet rays, etc. Biofilm remediation is an advanced technology that should be investigated still further to be utilized for practical applications. When the structure, components, and characteristics of biofilm are clarified more, then applications for it will be established more firmly.

Introduction

Bioremediation [1] is defined as environmental restoration technology by microorganisms. As a well-known process in the category, we can mention the soil remediation technology and restoration technology for oiling in marine environments. The technology generally utilizes the metabolism of microorganisms. Therefore, bioremediation can be redefined as follows. Usually, planktonic bacteria just reduce harmful and toxic substances by their metabolism. On the other hand, biofilm can utilize extracellular polymeric substance (EPS) and operate biosorption functions.

H. Kanematsu (✉)
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

D. M. Barry
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, USA
e-mail: dmbarry@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_14

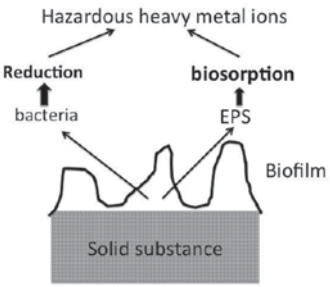


Fig. 14.1 The concept of bioremediation by biofilm

Definition of Bioremediation
It can be defined as a technology that can transform contaminants into less harmful forms by microorganisms and their produced substances.

The most effective merit for bioremediation is environmental friendliness. Many chemicals might destroy environments, even though such a method would bring prompt restoration. On the other hand, bioremediation would not destroy environments, but take time to change the harmful situation. Therefore, the usual remediation by chemicals is used for cases where the extent of contamination is relatively high and restoration is needed urgently. Bioremediation would be utilized in mildly contaminated environments and allowed to restore the places in a relatively long time. In addition, it might be used in a very difficult case, where other methods would not be possible (for example, the remediation of underground water). In this chapter, bioremediation by biofilm is referred to as biofilm remediation.

The contamination by heavy metals can be recovered and improved by two ways. One of them is to reduce the higher valence ions into lower ones and the other way is to have the hazardous metal elements absorbed into certain substances. The former technology has been investigated on full scale so far. In most cases, it utilizes the metabolism of bacteria. However, the importance of biofilm has been recently pointed out and the restructuring of the discipline is needed at this point. Biofilm can absorb many hazardous substances including heavy metallic ions as shown in Fig. 14.1. It may pave the new way and open the door to more versatile possibilities.

Why Is Biofilm Needed for the Bioremediation?

As described in former chapters, the bacteria in biofilm (biofilm bacteria) show different characteristics as compared to planktonic bacteria. Therefore, the remediation behaviors must be considered from different approaches. Even when the consideration is restricted to bacteria themselves, the density of bacteria is generally very high in the biofilm. It means that the effectiveness would be also very high for the remediation process. In addition to the performance, biofilm contains EPSs in them. They are composed of polysaccharides and proteins mainly. Many metal ions

tend to be combined with both the components in biofilm. It means that those metal ions can be separated into biofilm and removed from the contaminated places with biofilm.

Generally, remediation by biofilm has the following characteristics in addition to the general tendencies described above.

#1: Long time remediation is possible.

In biofilm, bacteria can continue to survive stably for a longer time, even though the characteristics from the viewpoint of metabolism might be pretty different, since the stability must be the main characteristic for biofilm bacteria. The existence probability is much higher in the biofilm state than in the planktonic state. Therefore bacteria prefer the former to the latter. It means the capability of bioremediation can continue for a relatively longer amount of time.

#2: Environmental friendliness.

This characteristic is true for general bioremediation. Also it is still available for biofilm remediation. Particularly, the bacteria in biofilm constitute complicated small bioecosystems. They are composed of unculturable bacteria. Most of them are harmful from the pathogenic viewpoint. Therefore, we can consider that the environmental friendliness is much higher.

#3: Availability for low concentration contamination

This is also a general characteristic for bioremediation. It is also true for biofilm remediation due to the same reason. Even though it might require a longer time, the low concentration of contamination can be restored well.

#4: High tolerance against toxicity and other stresses from outside.

Due to the existence of EPS as a component of biofilm, it can generally alleviate toxicity in biofilm [2]. Buchholz et al. [3] indicated that biofilm bacteria can resist up to 100 times the toxic concentration of a pollutant than the planktonic ones. It also alleviates and moderates the shear stress [4] and protects bacteria from ultraviolet light [5].

#5: Low cost to avoid the use of expensive chemicals

Compared to usual remediation, biofilm remediation does not require expensive chemicals. Usually, the cost for biofilm remediation is lower.

Classification of Biofilm Remediation [6]

Generally, biofilm remediation is classified into two main categories, in situ biofilm remediation and ex situ bioremediation. The former corresponds to the case where the process occurs in the site with the contaminants. On the other hand, the latter corresponds to the case where the restoration process occurs in a place away from the contaminated site.

In situ biofilm remediation can be classified further into four smaller categories, shown in Fig. 14.2. Natural attenuation utilizes natural phenomena where target substances would be absorbed into the coexistent ones naturally, evaporated into a gas phase, decomposed by bacteria, and so on. It can be defined as the natural

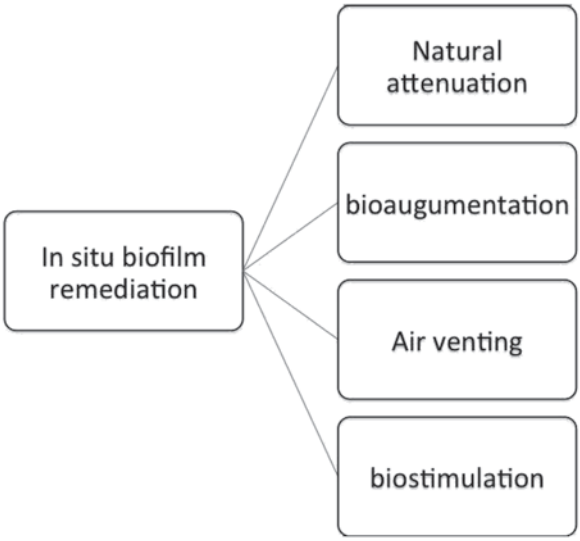


Fig. 14.2 The classification of in situ biofilm remediation

decrease and decay of a contaminant's concentration. The restoration of underground water often utilizes this process. On the other hand, bioaugmentation is the technology that would be carried out by adding bacteria into the target site artificially. Since the added bacteria would be an originally alien species in the target site, this process might bring about the collapse of the bioecosystem. Therefore, some countries established regulations or guidelines for the utilization. The further study and investigations should overcome the problem in future. Air venting is the restoration technique that allows air into the system to bring about the restoration process. Many organic substances can be decomposed favorably under aerobic environments, since they usually require a large amount of energy to break the chemical bonds within their inner structures. The air inspiring method not only promotes the decomposition of organics, but also helps gas bubbles take contaminants away with them. Biostimulation, the fourth in situ biofilm remediation method, is the restoration technology in the contaminated site where nutrients, enzymes, and something promoting metabolism and growth of bacteria would be added into the target site. As a concrete example, the remediation of the contaminated site by trichloroethene (TCE) can be mentioned. In such a case, lactate salts are usually added in the ecosystem. Since TCE is an electron donor, it can accelerate the metabolism and growth of *Dehalococoides enthenogenes* to dehalogenate TCE. Compared to a bioaugmentation process, the biostimulation has been practically industrialized and is more advanced.

The ex situ biofilm remediation process is generally carried out in sites away from the contaminated site. Many kinds of biofilm reactors have been proposed so far. Farhadian et al. [7] published a good review how ex situ biofilm reactors can eliminate monoaromatic pollutants such as benzene, toluene, ethylbenzene, and a

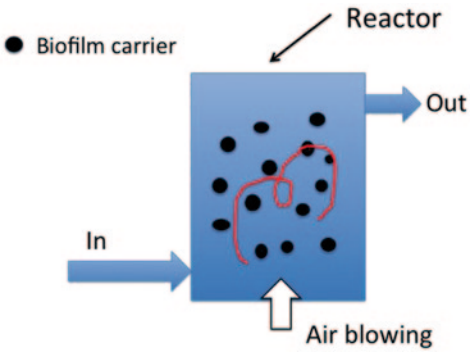


Fig. 14.3 Schematic illustration for a moving bed biofilm reactor

mixture of xylenes from groundwater. For example, Fig. 14.3 shows a schematic illustration for a moving bed biofilm reactor (MBBR). Polyethylene biofilm carriers can be moved around in the system by the inspiration of air. Each carrier has biofilm on the surface and can work to remove contaminants in the water phase. On the other hand, various types of membrane biofilm reactors (MBFR) have been developed and often utilized practically. The membrane is often composed of hollow fibers that are straw-like ones including tiny pores. The tiny pores can catch the contaminants with high efficiency. However, an MBFR develops biofilm on fibers. The biofilm can get the contaminants and separate them from the water phase. This type of ex situ process by MBFR is available for underground water. However, other slurry bioreactors are needed for soils and sediment. Various types of membranes have been developed for the purpose [8].

Possibility for Biofilm Remediation: —In the Case of Heavy Metal Ions

Heavy metal elements often violate environmental regulations to keep the quality of water phases. Particularly in the relation with plating and coating industries, the restoration technology is very interesting. The application of biofilm reactors is expected for them. Unfortunately, there are no concrete examples for biofilm remediation that have been used practically. However, there are some possibilities for some of them, since they can reduce the metallic ions to some extent by their metabolism.

Hexavalent chromium that has been issued very often can be reduced to the trivalent one by dissimilatory metal reducing bacteria (DMRB) [9]. *Methylococcus capsulatus*, one of the methane-producing microorganisms, can reduce hexavalent chromium to the trivalent one. *Pannonibacter phragmitetus*, anaerobic bacteria, can also reduce the hexavalent chromium. All of these bacteria form a biofilm in some situations artificially. They might be utilized for the future. If the EPS could absorb the hexavalent chromium, the biosorption effect might be added. Actually, Pal

et al. [10] found that *Stenotrophomonas* biofilms grown in the culture containing hexavalent chromium showed higher binding capability with hexavalent chromium. Gadd [11] suggests that EPS realized not only biosorption, but also the reduction of hexavalent chromium to trivalent chromium. These results will be understood correctly in the future, when the chemical forms of chromium binding with EPS are clarified.

Some actinides are now considered to be available for biofilm remediation. Concretely speaking, uranium (U), plutonium (Pu), and neptunium (Np) can be mentioned. The technology might be applied to nuclear leakage cases and the following contamination. In Japan, the nuclear energy plant in Fukushima was damaged by a huge Tsunami. The radiation contamination prevailed along the shore. In the USA, the radiation deriving from weapon production, nuclear energy plants, and incorrect mining management have contaminated underground water. Therefore, the bioremediation will hopefully be applied to those areas practically. The three actinides are metabolized and reduced by some bacteria. EPS also works for their biosorption for U and Pu. However, no concrete evidence relating to EPS has been found so far [6].

Kanematsu et al. [12] found that biofilm formed artificially in their laboratory biofilm reactor (LBR) could absorb iron, zinc, silicon, calcium, and some other elements. Their artificial biofilm was composed of viable but nonculturable (VBNC) bacteria derived from the ambient atmosphere. Therefore, it is hard to fix the precise mechanism. However, we should consider that the biosorption of EPS works, since no concrete bacteria have been known to metabolize the actinides so far. The results are still restricted to their LBR at this point. However, it could be applied to large scale experiments for ex situ biofilm remediation practices.

The biofilm remediation is still developing as new knowledge and information is obtained about biofilm. When the structure, components, and characteristics of biofilm are further investigated successfully and more detailed information is available, then a new development stage will appear.

References

1. Mohee R, Mudhoo A (2012) Bioremediation and sustainability: research and applications, 1st edn. Wiley-Scrivener, Austin, Texas, the USA
2. Flemming HC, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633
3. Buchholz F, Wolf A, Lerchner J, Mertens F, Harms H, Maskow T (2010) Chip calorimetry for fast and reliable evaluation of bactericial and bacteriostatic reliable evaluation of bactericidal and bacteriostatic treatments of biofilms. Antimicrob. Agents. Chapter 54, pp 312–319
4. Vu B, Chen MY, Crawford RJ, Ivanova EP (2009) Bacterial extracellular polysaccharides involved in biofilm formation. Molecules 14:2535–2554
5. Elasri MO, Miller RV (1999) Study of the response of a biofilm bacterial community to UV Radiation. Appl Environ Microbiol 65:2025–2031
6. Pastorella G, Gazzola G, Guadarrama S, Marsili E (2012) Biofilms: applications in bioremediation. In: Lear G, Lewi G (eds) Microbial biofilms -current research and applications. Caister Academic Press, pp 73–98

7. Farhadian M, Vachelard C, Duchez D, Larroche C (2008) In situ bioremediation of monoaromatic pollutants in groundwater: a review. Bioresour Technol 99(13):5296–5308
8. Robles-Gonzalez IV, Fava F, Poggi-Varaldo HM (2008) A review on slurry bioreactors for bioremediation of soils and sediments. Mirobial Cell Factories 7(5):5
9. Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156:609–643
10. Pal A, Paul A (2008) Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. Indian J Microbiol 48:49–64
11. Gadd GM (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84:12–28
12. Kanematsu H, Hirai N, Miura Y, Tanaka M, Kogo T, Itoh H (2013) Various metals from water by biofilm from an ambient germs in a reaction container. In Materials science and technology Montreal. Quebec, Canada. pp 2154–2161

Chapter 15
Energy Problems—Fuel Cell

Nobumitsu Hirai

Abstract Microbial fuel cells (MFCs) are one of the fuel cells which use the biological function of microorganisms. Biofilm formed on the electrodes of an MFC may affect the performance of the MFC. In this chapter there is a brief explanation of the principle of a fuel cell. Further, various kinds of MFCs are introduced.

Introduction: Energy Problems and Applications of Biotechnology

Issues such as nuclear power problems, global warming, rising prices for crude oil, and food problems are covered by the media almost every day. However, these can all be reduced to a trilemma of related issues involving the earth: economic development, resource/energy/food problems, and environmental problems. To resolve this trilemma, there is a strong need to establish a method of generating energy which is renewable and does not produce carbon dioxide. Candidates attracting attention in this field include not only natural energy such as solar and wind power, but also energy derived from biomass (organisms and their wastes). Combustion is the most typical method of using biomass as an energy source. Since biomass is organic material, it produces carbon dioxide when it is burned, but if the biomass comes form a plant, the carbon contained in the biomass derives from carbon dioxide absorbed from the atmosphere during photosynthesis in the growth process of the plant. Therefore, combustion of biomass is regarded as not increasing the amount of carbon dioxide in the atmosphere, i.e., it is taken to be "carbon neutral." In addition, the ultimate source of energy derived from biomass is solar energy taken in by the plant, and therefore the energy can be regarded as renewable if the energy conversion efficiency during the interim process is sufficiently high. For this reason, attention has been focused in recent years on energy generation processes using biomass, such as bioethanol [1], biohydrogen, and biofuel cells.

N. Hirai (✉)
National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: hirai@chem.suzuka-ct.ac.jp

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_15

Synthetic alcohol is ordinarily made through chemical synthesis, using ethylene gas (a natural resource) as the raw material, but ethanol made using biomass (normally plants) as a raw material is called bioethanol. Originally, most research focused on bioethanol derived from maize and sugarcane, but since these are foods, there are problems of interlinkage with global food problems, and recently the scope of research has expanded to ethanol derived from the lignocellulose-based biomass contained in materials such as wood. In addition, the mainstream methods for production of hydrogen are either hydrolysis of water, or steam reformation of natural gas, whose main component is methane. The term "biohydrogen" is used for hydrogen produced either using biomass as a raw material [2], or by employing the biological function of organisms in the manufacturing process (e.g., photodegradation reactions, fermentation reactions) [3]. In addition to being burned in an internal combustion engine or other system to produce energy, ethanol and hydrogen can also be used as a fuel for fuel cells. The following section provides a simple explanation of fuel cells.

Fuel Cells

A fuel cell is a device which uses hydrogen, organic material (e.g., alcohol) or the like as fuel, and employs an electrochemical reaction to convert the chemical energy produced by combination of the fuel with oxygen into electrical energy. A fuel cell has a fuel electrode (the "anode") and an air electrode (the "cathode"), and they are divided by a separator. If the fuel is hydrogen, the electrochemical reaction is the reverse of the water electrolysis reaction. As a concrete example, let us consider the case where the fuel is hydrogen. Figure 15.1 shows a schematic diagram of a fuel cell. In this case, the fuel is broken down at the anode, producing hydrogen ions (referred to below as "protons") and electrons.

$$H_2 \rightarrow 2H^+ + 2e^-$$

The produced protons diffuse in the separator and move to the cathode, but the electrons are first passed to the anode, and then to the cathode through an external

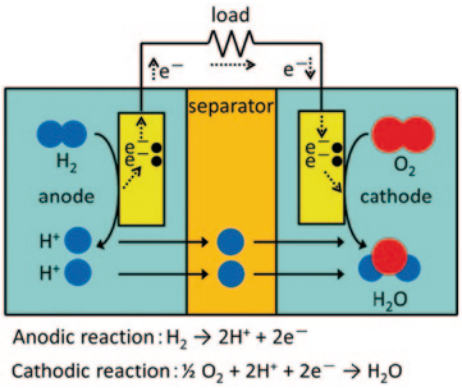


Fig. 15.1 Schematic diagram of a typical fuel cell

circuit. After that, water is obtained at the cathode by combining the protons and electrons with oxygen gas (an oxidizer) supplied to the cathode. Here, the flow of charged particles is completed.

$$2H^{+} + 2e^{-} + (1/2)O_2 \rightarrow H_2O$$

As a result, the mechanism is such that electric power is extracted when an electron passes through an external load. In this way, the protons and electrons produced at the anode are immediately recombined via oxygen gas at the anode. Therefore the oxygen gas which acts as the oxidizer is located only near the cathode, and is diluted near the anode. That is, it is necessary to maintain an anaerobic atmosphere near the anode and an aerobic atmosphere near the cathode.

A fuel cell itself is an extremely clean energy production system because it does not produce CO_2. In addition, while the efficiency of a heat engine at room temperature (25 °C) is 0, fuel cells have the major advantage of a theoretical efficiency of 83 % under the same conditions. The term "biofuel cell" generally refers to a fuel cell which uses the biological function of organisms in the fuel cell reaction process, but it is also sometimes used to indicate that the fuel (i.e., the raw material) is biomass. The next section explains microbial fuel cells which use the biological function of microorganisms within a biofuel cell.

Microbial Fuel Cells

First, the following provides a simple explanation of the cellular respiration (internal respiration) which is crucial when considering applications of microorganisms to fuel cells. Ordinarily, the term "respiration" refers to the process whereby animals, plants, and other organisms take the oxygen in air or water into their bodies from their respiratory organs or body surface, and expel carbon dioxide. Strictly speaking, this process is called external respiration. As examples of this, human beings carry out pulmonary (lung) respiration, while fish carry out branchial (gill) respiration. Next, the oxygen taken into the blood through external respiration is transported to cells and tissues in the body. After that, the protons and electrons obtained through the breakdown of nutrients (organic matter) are combined, and substances such as water are released. At this time, the oxygen acts as an electron acceptor (i.e., a recipient of electrons). This consumption of oxygen in the cell or tissues is called cellular respiration (internal respiration). Typical microorganisms also obtain the energy necessary for bodily activities by carrying out cellular respiration. Some microorganisms use substances other than oxygen as the electron acceptor during cellular respiration, and other microorganisms are known to use substances on the outside of the cell as electron receptors. The key points here are that: when a microorganism carries out cellular respiration, it must pass electrons to some sort of electron receptor; and if that electron acceptor is not oxygen, then cellular respiration can take place even without oxygen.

A microbial fuel cell (MFC) is a system which extracts energy by using the property of certain microorganisms whereby they pass electrons to a metal (electron

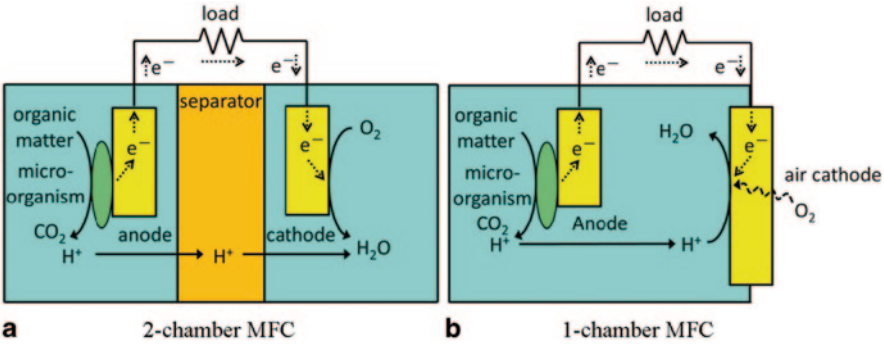


Fig. 15.2 Schematic diagram of typical MFCs. **a** 2-Chamber MFC. **b** 1-Chamber MFC

acceptor) directly or through mediators when breaking down the organic material which is their fuel in cellular respiration. The power generation mechanism of a typical MFC is as follows. In a condition of no or low oxygen, organic materials (carbohydrates) are broken down into carbon dioxide, protons and electrons, and the electrons are passed to the anode. (Care is necessary here. If the produced electrons are consumed before being passed to the anode, through combination with oxygen etc., then the electrons will not be passed to the electrode, and the system will not function as a fuel cell. Therefore, in an MFC, just like in an ordinary fuel cell, there is a need to maintain an anaerobic atmosphere near the anode, and an aerobic atmosphere near the cathode.) On the other hand, hydrogen ions move within an electrolyte and separator, receive electrons at the cathode, and then form water by combining with an oxidizer such as oxygen supplied near the cathode. In a typical MFC, platinum or carbon is used for the anode and cathode, and a cation exchange membrane is used as the separator.

Various forms of MFCs have been reported, but these can be roughly classified into two types, 2-chamber and 1-chamber, as shown in Fig. 15.2. As noted above, for all MFCs, it is necessary to maintain an anaerobic atmosphere near the anode and aerobic atmosphere near the cathode. In a 2-chamber MFC, the anode chamber and cathode chamber are partitioned by a separator with hydrogen ion permeability (Nafion membrane, etc.). An oxidizer such as oxygen is supplied only to the cathode chamber, and the anode chamber is kept in a low oxygen state. This type enables the oxygen concentration to be varied over a large range between the two chambers, thereby enabling higher energy recovery efficiency, but solubility of oxygen in water is low, and thus ordinarily the supply of oxygen to the cathode is a rate-determining process of the entire fuel cell reaction. Therefore, to increase the reaction rate, it is necessary to either provide ventilation to the cathode chamber, or to use an oxidizer other than oxygen such as potassium ferricyanide. The 1-chamber MFC is a structure proposed for mitigating the problem of supplying oxygen to this cathode. A membrane-type "air cathode" is used in a 1-chamber MFC. The air cathode has oxygen permeability, and thus oxygen can be supplied directly from the atmosphere. Therefore, oxygen which has permeated through from the atmosphere (outside the cathode) forms water by reacting with hydrogen ions on the catalyst

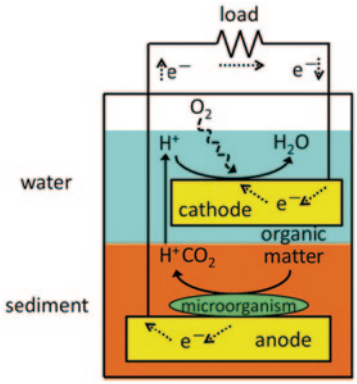


Fig. 15.3 Schematic diagram of a sediment MFC

coated onto the inside of the cathode. Unlike the 2-chamber type, there is no need to use a separator in this system, and since the internal resistance can be reduced, the obtained output tends to be high. However, it also has the disadvantage that excess oxygen which arrives near the anode is consumed by the microorganisms, thereby reducing the energy recovery efficiency to some extent.

Sediment Microbial Fuel Cell

A sediment microbial fuel cell (sediment MFC) is an MFC which uses the decomposition of organic material (biomass) by microorganisms in sediment [4]. This is achieved by placing the anode in the sediment of a lake, marsh, or the ocean, and the cathode in the water near the sediment. The principle of power generation is almost the same as the ordinary MFC shown in Fig. 15.3. In general, the atmosphere in sediment is anaerobic while the atmosphere in water is comparatively aerobic. Thus one major advantage is that the anode placed in sediment is naturally kept in an anaerobic atmosphere, and the cathode placed in water is naturally kept in an aerobic atmosphere. Therefore, no separator is necessary. Even with this method, the supply of oxygen to the cathode is the rate-determining process of the reaction. Therefore in order to increase the reaction rate, trials are being conducted of increasing the diffusion of oxygen by rotating the cathode [5]. In other trials, chitin and similar materials contained in the shells of crustaceans such as crabs and shrimps are being directly added to the anode [6]. Examples of applications have also been proposed as a power-supply-free and wire-free environmental monitoring sensor [7].

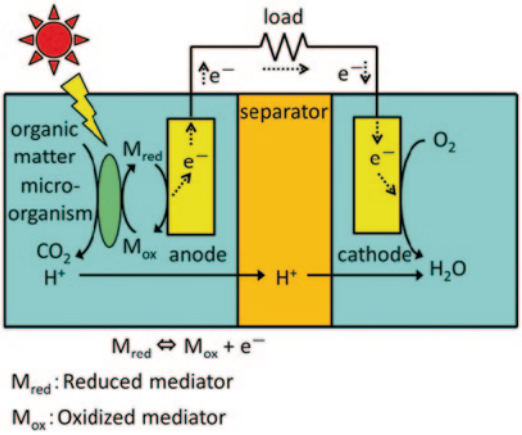


Fig. 15.4 Schematic diagram of a mediator photo MFC

Photo Microbial Fuel Cell

Decomposition of organic matter is not the only method which microorganisms use to obtain energy from the outside world. Microorganisms with photosynthetic pigments can obtain energy through photosynthesis. If "light energy" can be utilized by employing photosynthesis instead of fuel in an MFC, then the system can be called a "microbial photo cell." However, at present, no organism has been discovered which can directly convert light energy into electricity. Therefore, the following describes examples of research relating to photo microbial fuel cells (photo MFC) incorporating photosynthesis effects into an MFC.

1. Mediator Photo MFC [8] (Fig. 15.4)

A "mediator" is a chemical substance which can transfer electrons, i.e., mediate electron transfer. In a mediator photo MFC, the electrons obtained through photosynthesis by a microorganism with photosynthetic pigment are taken into the microorganism. Then they emerge from the microorganism and are passed to a metal anode. This is achieved by adding an artificial mediator to the electrolyte. This type of cell has the unique feature that it not only converts light energy to electrical energy when irradiated with light; it can also extract electrical energy when not irradiated with light using the organic matter accumulated inside the organisms during light irradiation. Tanaka et al. [9] have reported on fuel cells using 2-hydroxy-1,4-naphthoquinone. The problems with this method are that in many cases the mediators exhibit toxicity, and the cells have a comparatively shorter life.

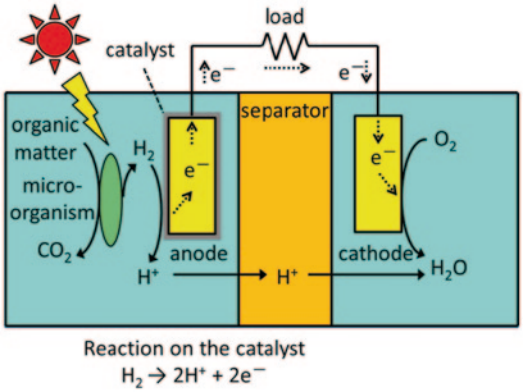


Fig. 15.5 Schematic diagram of an electrocatalytic photo MFC

2. *Electrocatalytic Photo MFC (Fig. 15.5)*

As was briefly explained in the introduction, biohydrogen can be produced by using the biological function of organisms. An electrolytic photo MFC is an MFC in which biohydrogen is produced from protons on an electrocatalyst located on the anode surface, via the photolytic reaction of an organism. The MFC then operates using that biohydrogen as fuel. Rosenbaum et al. [10] have achieved operation using a platinum electrode coated with conductive polymer as the anode, under low-sulfur conditions in a solution containing green algae. Optimization of the electrocatalytic material is a key point for this technique, and improvements are needed in terms of durability of catalytic activity and cost.

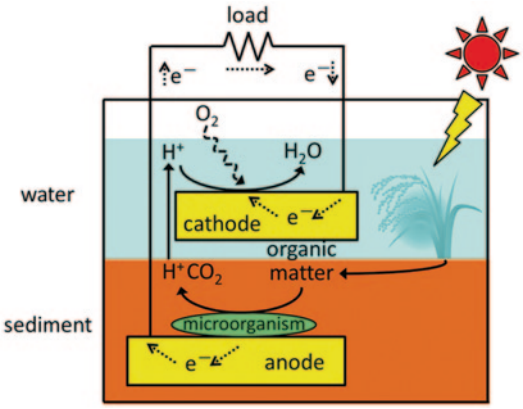


Fig. 15.6 Schematic diagram of a heterotrophic photo MFC

3. *Heterotrophic Photo MFC (Fig. 15.6)*

A heterotrophic photo MFC is an MFC in which organic matter is produced by microorganisms or plants with photosynthetic pigments. The cell operates by passing electrodes to the anode when another microorganism breaks down the organic matter through cellular respiration. He et al. [11] have experimentally shown, for sediment MFCs using lake sediment, that repeated cycles of light irradiation and nonirradiation result in electrical discharge, even if there is no additional supply of organic matter from the outside. They have also shown that these effects are the action of two types of microorganisms: microorganisms which have photosynthetic pigment and produce organic matter through photosynthesis, and those which break down the discharged organic matter and pass electrons to the anode.

Photosynthesis can be carried out not only by microorganisms with photosynthetic pigments, but also by ordinary plants. In addition, it is known that, when plants grow, they release various types of organic matter into the soil from their roots. Using this fact, experiments have been conducted by Schamphelaire et al. [12] in which rice plants were cultivated in the sediment of a sediment MFC. In this case, organic matter discharged from the rice plant roots into the sediment becomes nutrients for the microorganisms buried in the sediment which pass electrons to the anode, and the water filling the rice paddy for rice growing acts as the MFC electrolyte. It has been reported that, as a result, output is increased by seven times compared to the case where rice plants are not present.

4. *Supplying Oxygen to the Cathode Through Photosynthesis by a Microorganism*

Thus far, the explanation here has focused on the added effects of microorganisms on the anode reaction in a MFC, but this section will introduce the added effects of microorganisms on the cathode reaction. Powell et al. [13] have shown experimentally that green algae can function, via a mediator such as methylene blue, as an acceptor at the cathode of electrons which have come from the anode (i.e., as an electron acceptor).

Future Outlook

Thus far, an overview has been given of various MFCs, without being confined only to biofilms, but as is evident from the fact that MFCs operate via the action of microorganisms near the anode surface, it is believed that, in advancing research further in the future, the existence of the biofilms which have likely formed on the anode and cathode surfaces will be extremely important for further improving the performance of MFCs. The biggest issue will be increasing output power. Consider-

ing that, with heterotrophic photo MFCs, a major improvement in performance has been achieved over conventional types due to the combined effect of two or more types of organisms. There is likely potential for achieving a breakthrough in output power by looking closely at the combination effects of the multiple microorganisms which coexist in biofilms.

References

1. Menon V, Rao M (2012) Trends in bioconversion of lignocellulose: biofuels, platform chemicals & biorefinery concept. Prog Energy Combust Sci 38(4):522–550
2. Demirbas A (2007) Progress and recent trends in biofuels. Prog Energy Combust Sci 33(1):1–18
3. Levin DB, Pitt L, Love M, (2004) Biohydrogen production prospects and limitations to practical application. Int J Hydrog Energy 29(2):173–185
4. Reimers CE, Tender LM, Fertig S, Wang W (2001) Harvesting energy from the marine sediment−water interface. Environ Sci Technol 35(1):192–195
5. He Z, Shao H, Angenent LT (2007) Increased power production from a sediment microbial fuel cell with a rotating cathode. Biosens Bioelectron 22(12):3252–3255
6. Rezaei F, Richard TL, Brennan RA, Logan BE (2007) Substrate-enhanced microbial fuel cells for improved remote power generation from sediment-based systems. Environ Sci Technol 41(11):4053–4058
7. Donovan C, Dewan A, Heo D, Beyenal H (2008) Batteryless wireless sensor powered by a sediment microbial fuel cell. Environ Sci Technol 42(22):8591–8596
8. Rosenbaum M, He Z, Angenent LT (2010) Light energy to bioelectricity: photosynthetic microbial fuel cells. Curr Opin Biotechnol 21:259–264
9. Tanaka K, Tamamushi R, Ogawa T (1985) Biochemical fuel-cells operated by the cyanobacterium, *Anabaena variabills*. J Chem Technol Biotechnol 35B(3):191–197
10. Rosenbaum M, Schröder U, Scholz F (2005) Utilizing the green alga *Chlamydomonas reinhardtii* for microbial electricity generation: a living solar cell. Appl Microbiol Biotechnol 68(6):753–756
11. He Z, Kan J, Mansfeld F, Angenent LT, Nealson KH (2009) Self-sustained phototrophic microbial fuel cells based on the synergistic cooperation between photosynthetic microorganisms and heterotrophic bacteria. Environ Sci Technol 43(5):1648–1654
12. Schamphelaire LD, Bossche LVD, Hai SD, Höfte M, Boon N, Rabaey K, Verstraete W (2008) Microbial fuel cells generating electricity from rhizodeposits of rice plants. Environ Sci Technol 42(8):3053–3058
13. Powell E, Mapiour ML, Evitts RW, Hill GA (2009) Growth kinetics of *Chlorella vulgaris* and its use as a cathodic half cell. Bioresour Technol 100(1):269–274

Chapter 16
Bioreactors in Industries and Biofilm

Dana M. Barry and Hideyuki Kanematsu

Abstract Bioreactors are systems where chemical reactions (involving organisms or active substances derived from them) take place. Many types exist such as batch, continuous, photo, and membrane bioreactors. Industries use these reactors to make a variety of products including chemicals. A company's success at producing specific products depends in part on its ability to control various parameters (like temperature) and to use appropriate biofilms in the reactors. Biofilms are thick layers of cells used in bioreactors. They start to form when microorganisms like bacteria attach to a surface. After a certain amount of time, this attachment becomes somewhat permanent because the microorganisms produce a sticky polymeric material that anchors them. The resulting thick layers of cells are referred to as biofilms. Examples of biofilm and bioreactor use in industry are provided.

Bioreactors

A bioreactor can be referred to as a system where chemical processes (involving organisms or active substances derived from them) take place. These processes are carried out in vessels of various sizes that are often made of stainless steel. The vessels' reactions may include aerobic (with oxygen) or anaerobic (without oxygen) conditions.

Louis Pasteur's research on fermentation in the 1800s contributed to the development of bioreactors [1]. He demonstrated that each type of fermentation is linked to a specific microorganism(s). The process of fermentation is used to make many products such as breads, beer, and cheese. For beer, the microorganisms yeast and bacteria convert carbohydrates (e.g., the sugar glucose) to alcohol and carbon dioxide under anaerobic conditions. Bioreactors are desirable for industrial processes because they allow products to be produced in large volumes.

D. M. Barry (✉)
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, USA

H. Kanematsu
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_16

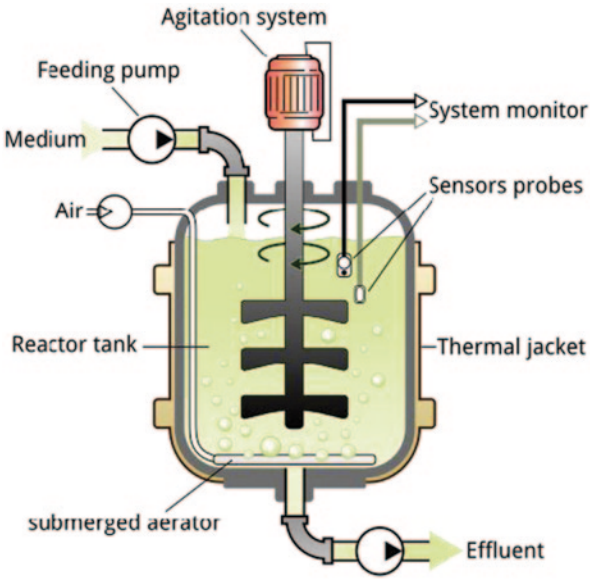


Fig. 16.1 Diagram of a typical batch type bioreactor

Many types of bioreactors exist. A few are presented and described below.

Batch Bioreactors These reactors involve the simplest mode of operation. They generally consist of a tank with an agitator, a heating/cooling system, a feeding pump for adding ingredients, an aeration system, and an effluent attachment. See Fig. 16.1 [2].

A bath reaction starts by adding ingredients to the reactor. The user calculates the amount of each ingredient needed to produce the desired product and the length of time the reaction will take. After the necessary ingredients are placed in the tank, the chemical reaction or fermentation process is allowed to proceed. Agitators are used for mixing. The heating/cooling system helps to control the temperature of the reactor's contents, because products in the reactor sometimes give off or absorb heat during processing. After the reaction is complete, the contents are removed. Then the reactor is cleaned, refilled with the necessary ingredients, and the process starts again.

Continuous Bioreactors Cells in these reactors continuously receive a culture medium (which may be sterile or composed of microorganisms) and the bioreactor fluid is continuously removed [3]. Therefore, the products and waste products continuously leave the reactor. Continuous bioreactors are more productive than batch bioreactors because they can operate for long periods of time without shutting down. This is due in part to the fact that the growth of bacteria in these reactors can be more easily controlled. Also, cells in these reactors can be immobilized to prevent their removal. A chemostat, which is sometimes used in the industrial manu-

facturing of ethanol, is an example of a continuous bioreactor [4]. A fresh medium is continuously added to it while the culture liquid is continuously removed. By changing the rate at which the medium is added to the bioreactor, one can control the growth rate of the microorganism.

Photobioreactors These reactors incorporate a light source and are used to grow organisms such as moss and algae [5]. Algae are being cultivated to produce fuels, generate animal feed, and to help reduce pollutants like carbon dioxide gas. For algae production, this reactor relies on the process of photosynthesis which is carried out by green algae. In the presence of sunlight, the chlorophyll-containing algae use carbon dioxide and water (both present in the reactor fluid) to produce the sugar glucose ($C_6H_{12}O_6$) and oxygen.

Membrane Bioreactors These systems combine a membrane for microfiltration or ultrafiltration with suspended growth reactors [6]. They are used for municipal and industrial wastewater treatment. One example is activated sludge treatment with membrane filtration [7, 8]. For this case activated sludge is considered to be sewage mixed with bacteria and protozoa that lead to its oxidation. The membrane used for filtering can be designed to remove contaminants such as nitrogen, phosphorus, bacteria, and suspended solids. It is either immersed in the bioreactor or exists as a separate device that is external to the reactor. The membrane bioreactor provides an option (for separating solids and liquids) to the conventional gravity-based method of settling tanks. The most serious problem with a membrane bioreactor is membrane fouling, which is caused by the membrane materials interacting with the wastewater components. Therefore, the membrane requires frequent cleaning.

Biofilm Use in Industrial Reactors

Biofilms are used in industrial reactors to produce a variety of chemicals and other products. They start to form when free-floating microorganisms such as bacteria attach to a surface (e.g., supports in reactors). If the cells remain at the surface for a certain amount of time, then the attachment becomes more permanent. This is because the microorganisms produce a sticky extracellular polymeric substance (EPS) that anchors them to materials like metals and plastics [9]. The resulting thick layers of cells are referred to as biofilms, which grow and mature by recruiting additional microorganisms and by cell division. These cells are used in industrial reactors.

Industrial operations prefer high reaction rates to improve productivity. Under optimized conditions of pH, temperature, substrate choice, medium components, etc. reaction rates can be increased by increasing the cell mass concentration in the reactor [10]. Methods to accomplish this include the use of permeable membranes (like those in membrane bioreactors) to retain cells and immobilized cell techniques such as cell adsorption where cells are attached to supports (substrates) in the reactors. Several examples are provided for biofilm use in industry.

Acetic Acid Production Acetic acid (CH_3COOH) is an important intermediate for chemical, detergent, wood, and food industries [11]. This weak acid can be produced by the traditional approach, which involves the conversion of fermentative alcohol using a particular type of acetic acid bacteria. Acetic acid can be made commercially by using *Acetobacter aceti, A. pasteurianus,* and *Gluconobacter oxydans* in a trickling-bed biofilm reactor [10]. To start, beech wood shavings are packed in the reactor. Then an alcohol solution is sprayed over the surface. This solution contains nutrients and bacteria to promote the growth of biofilm on the beech wood shavings. Liquid trickling to the bottom of the reactor includes the product, acetic acid. This liquid is cooled and pumped back to the top of the reactor to increase the concentration of acetic acid. As a result of using this trickling-bed biofilm reactor, about 90 % of the added alcohol is converted to acetic acid [10].

Ethanol Production A simplified method for making ethanol (C_2H_5OH) is provided. To start, plants use energy from the Sun along with carbon dioxide and water to make sugar. Then the sugar takes part in a fermentation process to make ethanol. A company named Proterro has eliminated the use of plants and is developing ways to produce fuel efficiently with light-harvesting bacteria (cyanobacteria) [12]. These bacteria imitate green plants and get energy from the Sun.

Proterro has a long, cylinder-shaped bioreactor that is inflated with gas rich in carbon dioxide. This reactor sits outdoors in Florida and is exposed to the Sun. It has hanging sheets of fabric (containing cyanobacteria) with water percolating through it. If this bioreactor proves to be economically feasible for producing sugar, then a commercial plant might be built with multiple reactors. These bioreactors would be directly hooked up to an ethanol fermenter. Proterro would take the carbon dioxide from the ethanol vats and pump it through the bioreactors so the cyanobacteria could use it to make sugar. Then the sugar water from these bioreactors could be pumped back to feed the yeast and make more ethanol. Proterro is hoping to develop a sustainable system for ethanol production.

Butanol Production Butanol ($C_4H_{10}O$) is used as a fuel. It can be produced from carbohydrates with various microbial cultures. A good way to make butanol is by using *Clostridium acetobutylicum* in a continuous immobilized cell reactor [10]. Start by packing the reactor with an adsorption support before inoculating it with the culture. This immobilizes the cells of *Clostridium acetobutylicum*. The culture forms biofilms (layers of cells) on the support allowing butanol to be produced.

Succinic Acid Production Succinic acid, $CO_2H\ (CH_2)_2COOH$, is used in the detergent, food, and pharmaceutical industries. Currently it is made commercially by petrochemical processes. However, to alleviate a dependence on oil, there is a demand for preparing it from microbial fermentation.

Yan et al. studied the use of a fibrous-bed bioreactor (FBB) in repeated batch fermentations to evaluate the efficiency of the FBB to make succinic acid from glucose with *Actinobacillus succinogenes* [13]. Their FBB had cotton fiber packed in the reactor. This proved to be effective for anaerobic cell-immobilized fermentation. As a result of this investigation, sufficient amounts of succinic acid were produced.

References

1. http://en.wikipedia.org/wiki/Louis_Pasteur
2. Yassine Mrabet, CC by -SA 3.0. http://en.wikipedia.org/wiki/bioreactor. Accessed 30 October 2009
3. Williams JA (2002, March) Keys to bioreactor selections. CEP Magazine 37–38. www.cep-magazine.org
4. http://en.wikipedia.org/wiki/chemostat
5. http://en.wikipedia.org/wiki/algae_bioreactor
6. http://en.wikipedia.org/wiki/membrane_bioreactor
7. Wastewater Management Fact Sheet (Membrane Bioreactors) (2007, Sept) United States Environmental Protection Agency. http://water.epa.gov/scitech/wastetech/upload/2008_01_23_mtb_etfs_membrane-bioreactors.pdf. Accessed September 2007
8. Chan YJ, Chong MF, Law CL, Hassell DG (2009) A review on anaerobic-aerobic treatment of industrial and municipal wastewater. Chem Eng J 155:1–18
9. Percival S, Malic S, Cruz H, Williams DW (2011) Introduction to biofilms. In: Percival SL et al (eds) Biofilms and veterinary medicine. Springer-Verlag, Berlin
10. Qureshi N, Annous B, Ezeji T, Karcher P, Maddox I (2005) Biofilm reactors for industrial bioconversion processes: employing potential of enhanced reaction rates. Microbial Cell Factories. http://www.microbialcellfactories.com/content/4/1/24. Accessed 2005. Doi: 10.1186/1475-2859-4-24
11. Awad H, Diaz R, Malek R, Othman NZ, Aziz R, El Enshasy H (2012) Efficient production process for food grade acetic acid by Acetobacter aceti in Shake flask and in bioreactor cultures. J Chem. Vol. 9 (2012), issue 4, 2275–2286
12. Krieger K (2014, Feb) A greener way to make biofuels. Phys Today
13. Yan Q, Zheng P, Dong J-J, Sun Z-H (2014) A fibrous bed bioreactor to improve the productivity of succinic acid by *Actinobacillus succinogenes*. J Chem Technol Biotechnol 89:1760–1766

Chapter 17
Fish Reef, Seaweed Bed, and Other Construction Applications

Hideyuki Kanematsu and Dana M. Barry

Abstract In this chapter, the biofilm is applied to an artificial fish reef. Seaweed beds and construction sites are explained too. An artificial fish reef is defined as an artificially made place where fish gather around to take nutrition. An artificial seaweed bed can be defined as the place artificially made where sea plants can grow easily. Some infrastructural materials such as concrete, used vessels, etc. would be used in both places. In those places, materials and particularly, the materials' surfaces should have characteristics to harmonize with their environments. To achieve the purpose, the application of biofilms would be the best, even though quite a few people have not realized the correlation yet. In this chapter, we describe what the artificial fish reef and seaweed bed are and how biofilm would correlate with the environmental friendliness of a material's surface and how it would lead to achieve the purpose.

Introduction

In many chapters of this book, harmful and detrimental effects by biofilm are introduced not only from the fundamental medical aspects, but also industrial and practical applications. However, there are some beneficial applications by biofilm. The environmental cleanup by biofilm (biofilm remediation, etc.) is one of them and explained in Chap. 14. In this chapter, we would like to focus on artificial fish reefs and seaweed beds. For both, the affinity and friendliness between organisms and inorganic materials would be very important at the initial stage.

H. Kanematsu (✉)
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

D. M. Barry
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, USA
e-mail: dmbarry@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_17

What Are Artificial Fish Reefs and Seaweed Beds?

Generally, at elevated portions of the beds of the sea, seawater tends to rise and bring nutrient salts near the surface area of the sea. Then phytoplanktons grow there, followed by zooplanktons. As a result, fish gather around the area to form a fish reef. When the process makes progress naturally, the fish reef is called a natural fish reef. On the other hand, it is called an artificial reef, when the elevated portions are formed and immersed by human beings.

On the other hand, a seaweed bed is defined as a community composed of marine algae, plants, weeds, etc. that are usually formed in coastal areas and on continental shelves. As for seaweed beds, they can be made with various advanced materials such as carbon fibers, etc. artificially to grow many kinds of sea plants. The purpose for both is similar and both structures need the affinity between substrate materials and organisms. From this viewpoint, the applications require a new kind of characteristic on materials' surfaces and that relate to biofouling capabilities and biofilm formation characteristics on materials' surfaces.

The artificial fish reef and seaweed bed are formed by immersing artificial materials into the sea. The most important materials are concretes. They are generally very stable and durable. Also they are the best habitat for fish. Fouling phenomena are easy to occur. The defect to use it as a fisher reef could be attributed to the heavy weight. In order to convey them to the site for the use or to immerse them into the sea, massive construction machines would be needed.

The second is a steel-hulled vessel. Particularly in the USA, many scraps have been utilized for the artificial fish reef. Among them, steel-hulled vessels occupy the large portion, particularly in the USA. There are some merits and demerits in using the vessels. The idea could be beneficial for the recycling of vessels for commercial and military use. One of the most critical demerits is removal of hazardous wastes from the vessels, such as polychlorinated biphenyls (PCBs), asbestos, lead, and some other metals. The detailed information can be read in the references [1].

Artificial Fish Reef/Seaweed Bed and Biofilm

As for the utilization of concrete, there are some trial projects for the application of slag produced by the iron- and steel-making process. And partly, the products have been used in practical applications. One of the main merits for the project is that slag usually contains lots of iron components. Iron tends to attract bacteria very strongly and exceptionally [2]. Therefore, those slags have been mixed into mortar for the purpose. One of the authors tackled with the problem in the past. Therefore, we would like to introduce the trial relating to biofilm partly [3, 4]. In the project, we utilized four kinds of mortars, as shown in Table 17.1. The concentration was measured by the inductively coupled plasma spectrometry (ICP-OES) and the concentrations of oxides were calculated from those measured values for metals.

Table 17.1 Slags utilized for the addition to mortars

Slag type	CaO	SiO_2	Al_2O_3	MgO	CaS	Cr_2O_3	MnO	TiO_2	FeO
Type1	35.4	36.3	13.5	6.87	0.22	2.07	3.31	1.98	0.23
Type2	33	44.1	5.39	7.68	0.33	3.29	4.09	1.02	0.74
Type3	20.5	20.2	20.1	6.21	–	1.72	4.07	–	26.8
Type4	47.1	30.5	10.5	5.81	2.53	1.27	0.47	0.44	1.17

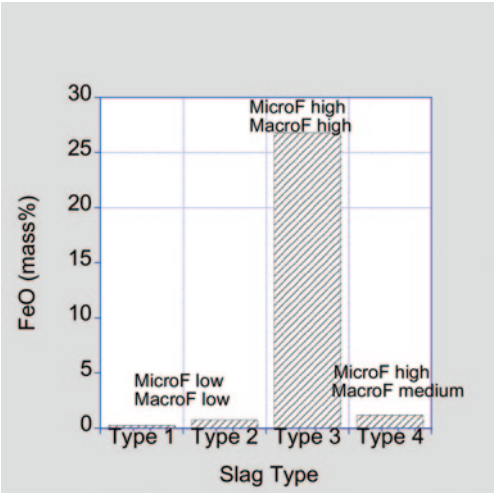


Fig. 17.1 Fouling behaviors for various mortars with steel-making slags and their iron contents

Type 1 and type 2 were oxidizing slags produced from the stainless steel-making process. Both did not contain much iron. On the other hand, type 3 and type 4 slags contained relatively more iron components. Type 3 was also an oxidizing slag. However, it was produced from the usual steel-making process. Type 4 slag was a reducing slag from the stainless steel-making process. All of these slags were wet-milled and mixed in mortar according to JIS A6206. The mortar specimens were immersed into the sea (Ise Bay, Japan) for a certain period (12 days). After the immersion, they were taken out of the sea and observed by naked eyes and scanning electron microscopy. On the other hand, all of those specimens were immersed into a circular-type laboratory biofilm reactor. The immersion into the real sea generally gives us information about macrofouling (the attachment of oysters, barnacles, etc.), while the immersion into a laboratory biofilm reactor (LBR) provides information for microfouling (bacteria and biofilm.) Fig. 17.1 shows the results of the experiments.

Usually, microfouling leads to macrofouling and both are related in series. Therefore, the mortar containing lots of iron components (type 3) attracted bacteria, followed by the formation of biofilm. Then it attracted larger organisms such as barnacles, oysters, etc. Type 4 slag-mixed mortar could form biofilm (MicroF) to a great extent. However, the extent seemed to be smaller than that for type 3. Therefore, macrofouling (MacroF) was to a medium extent. For type 1 and 2, both fouling categories were not so active, since they did not contain much iron.

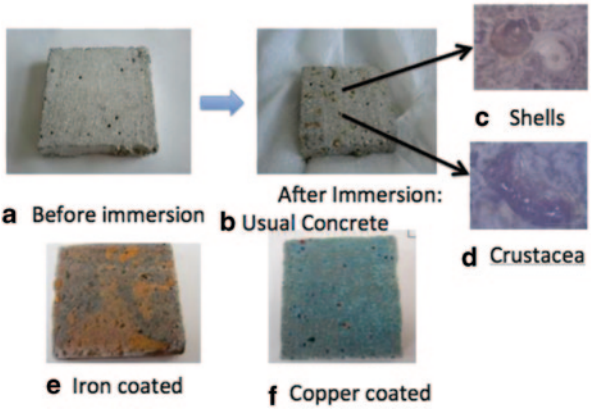


Fig. 17.2 Appearances for the concrete specimens immersed into a marine environment (Ise Bay) for 2 weeks

The experimental results suggest that the materials embedded in the sea could control the fouling behaviors, when they would contain some accelerating metals such as iron for biofilm formation. As already described above, macrofouling follows microfouling where bacteria and the biofilm play an important role. After microfouling occurs, larger organisms are attracted by the biofilm and finally, the place would be suitable for fish as fish reef or sea plants as seaweed bed. However, biofilm should be controlled properly, since too much dissolution of metals might violate environmental regulations, destroy the environment, or affect the balance of the ecology. Suitable dissolution of metals is desirable at all times. From the viewpoint, materials for fish reef, seaweed beds, etc. should contain the proper metallic elements at the proper levels, so that they would not destroy the balance of nature.

However, the materials for artificial fish reefs or seaweed beds might often be recycled or used industrial products such as used vessels, ships, etc. In such a case, it might be very hard for some suitable or controlling materials to be mixed with them. Then surface modification or treatment processes might work well. One of the authors investigated spray coating and its industrial application in the past [5]. In this experiment, the usual Portland cement was made. Then Ti, Fe-Cr, Sn, 50 %Ni-50 %Cr, 80 %Ni-20 %Cr, Cu, Zn, and Fe were spray-coated on the concrete specimens. Next they were immersed in the sea (also Ise Bay in Japan) for 2 weeks.

Figure 17.2 shows the results for the observation of various concrete specimens immersed in a marine environment. After the immersion, concrete specimens got macrofouling. Before that, the process for microfouling must have occurred. However, it was very hard to distinguish between the two types of fouling and the process reached the macrofouling stage very rapidly in this case. On the concrete surface, tiny shells, crustacean, etc. could be observed by a microscope (c), (d). When iron was spray coated on concrete, corrosion products (red brown iron rust, Fe_2O_3) were observed. Therefore, biofouling seemed to be inhibited to some extent, due to the cessation of iron dissolution. On the other hand, copper-coated concrete showed moderate macrofouling, even though it might get a chance to kill bacteria and to

Table 17.2 Fouling behaviors for spray-coated concretes in a marine environment

Coated material	Fouling (Yes) or nonfouling (No)
Ti	Yes
Fe-Cr	No
Sn	Yes
50 %Ni-50 %Cr	Yes
80 %Ni-20 %Cr	Yes
Cu	Yes
Zn	No
Fe	No

inhibit the growth of biofilm to some extent. Table 17.2 summarizes the results of those experiments. As shown in the table, surface modification could adjust and control the fouling properties. The technology will be utilized for the application.

Other Construction Applications

The importance of affinity between materials and organisms and also that of the unique characteristics at the interface have recently become known to us. Therefore, it is not so easy to mention some concrete examples for the topic. However, there are some cases to mention for construction materials, when we carefully consider the industrial problems from the viewpoint. A green construction method is one of them. Green construction method can be defined as greening technology for some construction materials and infrastructural materials such as concrete, bedrocks, glasses, etc. This technology can produce the situation where windows, floors, walls, ceilings (both outside and inside constructions), and other items can be greened. The green walls and interface give a warm and comfortable feeling, so that people could live, work, talk, etc. in the space comfortably. Turning the color into green for those infrastructural materials means that some kinds of plants would grow at the interface between the materials and ambient space. To achieve the purpose, those inanimate interfaces of materials must have the characteristics against environments. Here biofilm will be needed again. Generally, it is very hard to grow plants on inanimate materials' surfaces. However, if biofilm forms at the interface between the materials and ambient environment, then fungi, mosses, etc. would grow on them, which might lead to larger plants later, since plants would also need biofilm around the bottom parts to grow and survive. The bottom part is called rhizosphere. At this point, there is not much information available about the field. However, when we come to think about the environmental friendliness and harmonic content of materials' surfaces, the factor will be much more important in the near future. Then the application of the notion and knowledge about biofilm will get a chance to be applied to the system.

References

1. Commissions (2004) The Gulf and Atlantic states marine fisheries, Guidelines for Marinee Artificial Reef Materials
2. Kanematsu H, Ikigai H, Yoshitake M (2009) Evaluation of various metallic coatings on steel to mitigate biofilm formation. Int J Mol Sci 10(2):559–571
3. Kanematsu H, Masuda T, Miura Y, Hirai N, Kuroda D, Yokoyama S (2014) Various mortars for anti-fouling purposes in marine environments. AIP Conf Proc 1585:153–159
4. Masuda T, Kanematsu H, Hirai N, Kuroda D, Yokoyama S, Tanaka M (2013) Evaluation for biofouling on mortar containing various EAF Slags. CAMP-ISIJ 26:662–663
5. Kanematsu H, Kuroda D, Ohara C, Murakami K (2012) Metal Coating of concrete and biofouling in marine environment, in The 79th Annual Conference of the Electrochemical Society of Japan, The Electrochemical Society of Japan, Hamamatsu, Japan, p 346

Chapter 18
Contamination and Clean Surface of Materials

Hideyuki Kanematsu and Dana M. Barry

Abstract In this chapter, contamination on material surfaces is discussed from the viewpoint of the relationship between bacterial activity and contaminants. Contamination can be divided into three main groups: water soluble, oil-based, and solid contamination. However, in any of these cases, contaminants are completely inanimate substances. The bonding of the contaminants with material surfaces is not usually strong so the contaminants can be easily removed. When bacteria are attached to material surfaces, they grow, and then biofilm tends to form on these surfaces. Exopolymeric substances (EPS) in the biofilm are very sticky and make the contaminants stick to material surfaces. Some practical problems following the adhesion are predicted, introduced, and explained in this chapter. Also included is a concrete example that one of the authors investigated in a national project for Japan.

Introduction

Most industrial problems relating to biofilm can be boiled down to contamination. Contamination can be seen everywhere. In our daily lives, water stain is very often seen in plumbing products and places. Water stain is usually composed of calcium carbonate and silicon compounds, etc. The touch sensor screen used in various fields on a daily basis is well-thumbed and contaminated by fingerprints. In industrial activities (various production processes, etc.), corrosion resistant oil and lubricant oil are very often used. They might remain on surfaces as contamination. These oils are mostly inorganic, but even if they were organic, most of them are inanimate. They do not relate to bacteria at all at the initial stage. However problems could arise, if

H. Kanematsu (✉)
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

D. M. Barry
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, USA
e-mail: dmbarry@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_18

they remain stuck to material surfaces for an extended period of time. The sticking and fixing are strengthened by the existence of biofilm.

General Classification of Contamination

Generally, contamination can be divided into three groups. The first is the water soluble contamination. It is divided further into two subgroups. One of them is composed of the matters removable by water, e.g., sodium chloride. They can be removed only by water without using any chemicals. The other is composed of poorly soluble matters that could be removed with the addition of chemicals to change the pH.

The second main group is oil-based contamination. Members of this group do not dissolve in water, but in organic solvents instead such as benzene, ethanol, chloroform, etc. They can be further subdivided into three types: strong, medium, and weak polarized oil-based contaminations, according to their polar characteristics. For example, aliphatic acids, the strong polarized oil-based contamination, can be removed by surfactants. However, the effect of surfactants tends to decrease with the surfactant effect. This means that when the contaminant is completely non-polar, such as mechanical oil or carbohydrates, the surfactant would not work at all.

A contaminant that does not dissolve in water and does not dissolve in organic solvents is called solid contamination. It can be divided further into hydrophobic and hydrophilic contaminants. As for hydrophilic contaminants, silicon compounds can be mentioned as a typical example. Soot is a typical example for hydrophobic contaminants.

Biofilm and Contamination

All of the mentioned contaminations do not have any direct relation with bacteria and their activities. However, bacteria excrete sticky substances from themselves, when their number exceeds a certain threshold value. The phenomenon is called quorum sensing (QS) and the matter produced from the bacterial cells is called exopolymeric substance (EPS). Since EPS is sticky, it plays an important role to adhere contaminants to material surfaces just like starch. As a result, the inorganic or inanimate contaminants could be “tough dirt”. The daily phenomenon has not been familiar to us, unexpectedly, even though we could see many examples around us. For example, the dirtiness of glasses could lead to the deterioration of various aspects and aesthetic functions as well as reflection and transparency, etc. As for metals, biofilm decreases their corrosion resistance and accelerates the formation of scale. Therefore, the control of biofilm is very important in order to have materials with clean surfaces.

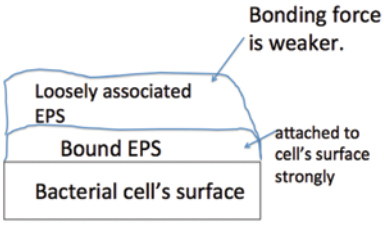


Fig. 18.1 Schematic sketch for EPS on materials

EPS and its Role for Contamination [1]

As described above, EPS is one of the main constituents that adhere contaminants to materials, as well as the contaminants themselves. Therefore, we should understand the essence of EPS at first to establish the countermeasure against the adhered contaminants. So what is EPS?

EPS is the collective term for exopolymeric substances, including polysaccharides, e-DNA, various proteins, etc., that exist in biofilm. As already explained in other chapters, biofilm is an inhomogeneous concavo-convex film-like matter. Usually, its shape is tower-like or mushroom-like. The framework for it is mainly made by EPS. From the viewpoint, EPS is the essential component for biofilm. At the beginning stage of research for biofilm, it was understood as the collective matter of polysaccharides. However, the image has been changed gradually. At this point, researchers think that EPS would contain lots of exocellular DNA (e-DNA). In some cases, the amount of e-DNA may be larger than that of polysaccharide. EPS contains matters excreted from bacterial cells. Therefore, each bacterium is surrounded by EPS. According to the recent analyses, EPS is classified into bound EPS and loosely associated EPS. As shown in Fig. 18.1, the Bound EPS layer exists in the area close to bacteria and the loosely associated EPS layer exists in the area away from bacteria. The former contains more proteins than polysaccharides, while the latter contains more polysaccharides than proteins. Polysaccharides have been investigated and found to be basically alginic acid. The protein may be basically e-DNA. EPS works not only as a glue, but also has other roles, which are summarized in Table 18.1.

Among those 14 actions of EPS in the table, #1, #3, #4, #5, and #6 are very important for the sticky contamination. At this point, it is not clear how much EPS in biofilm would relate contaminations and the following industrial cases. Therefore, many of them might just be predictions.

In most industrial productions, various machine oils have been used. Various pretreatment processes, such as degreasing, chemical cleaning, etc. are required inevitably to make the following production processes sound and workable. Conventionally, various types of chemical cleaning, electrolytic cleaning, etc. could be used to remove contaminants. However, these conventional methods do not anticipate EPS derived from bacterial activities. Practical engineers often encounter unknown and unpredictable contamination that cannot be solved by any conventional methods. Most of them may be attributed to the contaminants EPS would fix on material surfaces.

Nowadays, there are many daily devices around us that involve using touch panels and touch screens. This type of application is still expanding. It ranges from

Table 18.1 EPS of biofilm and their roles and characteristics [2]

Characteristics	Remarks
#1: Adherence to materials	EPS helps bacteria to overcome electric repulsion and attach to material surfaces
#2: Binding among bacterial cells	EPS contributes to the strong bond between bacteria
#3: Binding between biofilm and materials	EPS contributes to the strong bond between biofilm and materials
#4: Retention of water on material surfaces	EPS has the capability to keep water on material surfaces for a certain period
#5: Effect on host defense	EPS affects the correlation between bacteria and an immune system in the body
#6: Absorption of organic matters	EPS could take organic matters into biofilm and retain them. The phenomenon could lead to the separation and recovery of organic substances and environmental remediation
#7: Absorption of inorganic ions (metallic ion)	Inorganic ions could be incorporated into biofilm by ionic changes, etc. The phenomenon could be used for environmental remediation
#8: Structural change of biofilm	Biofilm could bring about its structural change and breakup, incorporating large molecules outside of it and digesting them
#9: Nutrition of bacteria in biofilm	Bacteria in biofilm always need nutrition to survive in oligotrophic environments. EPS could be nutrition for them
#10: Contribution to exchanges of gene information	EPS could contribute to the horizontal transfer of bacterial genes in biofilm
#11: Contribution to redox reactions in biofilm	Many kinds of redox reactions occur in biofilm. EPS becomes donors and acceptors to contribute to the reactions.
#12: Contribution to metabolic turnover	EPS seems to play a role in the process where surplus substances are excreted from biofilm as a result of metabolic turnover
#13: Contribution as carbon storage	EPS could incorporate lots of carbon in the environment into biofilm and work as carbon storage
#14: Contribution to bind enzymes to biofilm	EPS could contribute to the binding between enzymes and biofilm through various chemical reactions

automated teller machines, ticket vending machines, to smart phones, and tablet PCs. The touch panel is basically composed of displays and locator devices. The display part is composed of glass and polymer film. The top polymer film must be transparent and antifoggy. At this point, the contaminant-free film and glasses have been developed from the viewpoint of antifogging properties. The conventional antifogging properties have been approached from hydrophobic or hydrophilic viewpoints. However, this approach could not solve the problem completely. As explained already, bacterial activity and excreted EPS should be taken into consideration.

Another example can be seen in photovoltaic generation. One of the current important problems for the renewable energy system is to solve the fogging prob-

lem of glasses used for heliostats. Heliostat is a system where sunlight is reflected effectively so that the reflected light is then focused on a point to vaporize water so that turbines can be rotated by the vapor. This is followed by the generation of electricity. A technical problem to be solved is how the heliostat chases and catches the sunlight mechanically. Also the efficiency of reflection for the glass of a heliostat is very important. Fogging of glasses might lead to a serious decrease in efficiency of reflection. Conventionally, the fogging problems for heliostat glasses have been investigated also from the viewpoint of hydrophilicity and hydrophobicity. However, the problems still remain unsolved.

Kanematsu et al. pursued a study for contamination-free glass as part of a Japanese National Project in the past [3–7]. They tried to make clear why the glass was fogged by biofouling and confirmed that the decrease in transparency was brought about as a result. Various metals were investigated in their devised laboratory biofilm reactor (LBR). Metals that seemed to have resistance to biofilm formation (as determined in their laboratory work with the LBR) were then coated on glasses. The coated glasses were immersed into the LBR and their resistances against biofilm formation were investigated. According to their results, silver, copper, titanium, and tin could control the biofilm formation. Then the researchers devised a new composite coating composed of a silane compound matrix and dispersed nano-powders with those metals.

These are still just some theoretical predictions or experimental trials. However, the high quality material surfaces should be sought for in the future. Higher quality surfaces would be realized more effectively by controlling biofilm. From this viewpoint, this future-oriented technology should be investigated further.

References

1. Lewandowski Z, Beyenal H (2014) Fundamentals of biofilm research, 2nd edn. CRC Press, Boca Raton.
2. Flemming H-C, Wingender J (2010) The biofilm matrix. Nat Rev Microbiol 8:623–633
3. Kanematsu H, Okura Y, Hirai N, Miura Y, Itoh H, Tanaka M (2013) Condensation of some metal elements in biofilm. CAMP-ISIJ 26(1):417
4. Kanematsu H, Kougo T, Kuroda D, Ogino Y, Yamamoto Y (2013) Biofilm formation derived from ambient air and the characteristics of apparatus. J Phys: Conf Ser 433:012031, 1–6
5. Kanematsu H, Kogo T, Kuroda D, Itoh H, Kirihara S (2013) Biofilm formation and evaluation for spray coated metal films on laboratory scale. In Thermal Spray 2013—Innovative Coating Solutions for the Global Economy, ASM International: Busan, Republic of Korea, pp 520–525
6. Kanematsu H, Kogo T, Itoh H, Wada N, Yoshitake M (2013) Fogged glass by biofilm formation and its evaluation. In Proceedings of MS & T'13 Montreal, Quebec, Canada. pp 2427–2433
7. Kanematsu H, Hirai N, Miura Y, Tanaka M, Kogo T, Itoh H (2013) Various metals from water by biofilm from an ambient germs in a reaction container. In Materials Science and Technology. Montreal, Quebec, Canada. pp 2154–2161

Part III
Solving Industrial Biofilm Problems

Chapter 19
Chemical Cleaning

Satoshi Fukuzaki

Abstract Most cleaning processes proceed by the synergistic effect of physical and chemical actions. The role of chemical actions is to reduce the work requirements of a whole cleaning process. The cleaning using chemical action is referred to as chemical cleaning. The power of chemical cleaning for organic soils, including biofilms, on a hard surface is due to the actions of several detergent ingredients, such as alkaline constituents, ion-sequestering agents, surface-active agents, and oxidizing agents. Alkaline constituents are very effective in removing a variety of organic soils. Sodium hypochlorite is a convenient material to achieve both cleaning and disinfection in a single process. Surface-active agents can lower the surface tension of a chlorinated alkaline solution and confer penetrating power on the solution. For optimizing a cleaning process, it is required to combine two or more chemical agents effectively.

Thermodynamics of Adsorption

In any environment, organic macromolecules like proteins are spontaneously and irreversibly adsorbed onto a solid surface, forming a conditioning film. The conditioning film alters the physicochemical properties of the solid surfaces, and also plays a mediating role in bacterial adhesion [1]. Bacterial cells are also irreversibly adsorbed on a solid surface due to the production of extracellular polymeric substances (EPS). Finally, the formation of a biofilm occurs on any submerged surface. In the case of hydrophilic macromolecules and cells, the short-range forces involved in these irreversible adsorptions are ion–ion interactions, dipole–dipole interactions, hydrogen bonding, coordination bonding, and hydrophobic interactions. Due to these attractive forces, organic molecules and cells gain low-energy states, resulting in a decrease in the free energy of the system. The difference between the free energy levels of the system before and after adsorption is regarded as their energy of adsorption. The aim of a cleaning operation is to supply sufficient energy to the system in order to reverse the spontaneous adsorption process.

S. Fukuzaki (✉)
Graduate School of Bioresources, Mie University, Tsu, Japan
e-mail: satoshi_fukuzaki@bio.mie-u.ac.jp

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_19

Classification of Cleaning Energies

The energy of a cleaning solution is made up of mechanical energy (interfacial turbulence, high-pressure, etc.), thermal energy (temperature), and chemical energy provided by the ingredients of a detergent. Physical actions are recognized as being effective in removing biofilm. However, a large amount of energy must be supplied to the system without chemical energy. In addition, a complicated design of equipment often makes the physical removal of organic soils very difficult. The practical role of detergent is to reduce the energy required for a physical cleaning process. If suitable detergents are selected and used, the energy requirement will be reduced to only several percent of that needed for physical cleaning alone. Detergent ingredients effective in the removal of a biofilm are alkaline constituents, ion-sequestering agents, surface-active agents, and oxidizing agents.

Role of Detergents in Solid–Liquid Interfaces

The actions of a detergent at a solid–liquid interface are due to a comprehensive effect of five types of fundamental behaviors as follows:

1) Interfacial adsorption
2) Surface activity
3) Degradation
4) Displacement
5) Prevention of readsorption of soils

Detergent constituents in an aqueous solution can be adsorbed onto some interfaces in the system, i.e., soil–water, substrate–water, soil–substrate, and water–air. As a result, all the interfacial properties are largely changed, thereby lowering the interfacial or surface tension and improving the degree of solubility and dispersion of soils. Such a phenomenon is referred to as surface activity. The degradation of macromolecules such as proteins into low molecular weight fragments by detergents can accelerate the progress of cleaning. Finally, soils are displaced by detergents at a solid–liquid interface. The presence of detergents in a solution and on a solid surface prevents once-detached soils from their readsorption. At the end of chemical cleaning, detergent ingredients remaining on a solid surface should be removed from the system by rinsing with water. Requirements of an ideal detergent are both to have strong adsorption affinity to solid surfaces and to be rinsed out readily.

Alkaline Constituents

The primary purpose of using alkaline constituents is that they act as a source of hydroxide ion (OH^-). Hydroxide ions can be adsorbed both on most hydrophilic solid surfaces and on organic soils (= desorption of H^+), making their surfaces more

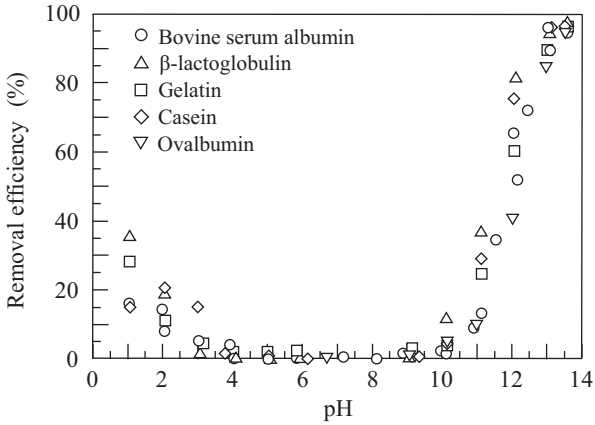


Fig. 19.1 Effect of the pH of cleaning solution on the removal of various kinds of proteins from stainless steel surfaces during batch cleaning at 40 °C [2]

negatively charged. As a result, OH^- affects the interfacial properties of solid surfaces and organic soils in an aqueous solution. In the case of a reaction of NaOH (sodium hydroxide) and fat soils (i.e., saponification reaction), OH^- degrades fats, transforming them into glycerol and sodium salts of carboxylic acids containing long chains of carbon atoms (soap), which in turn reduce the interfacial tension of the remaining fat–solution interfaces.

Figure 19.1 shows the effect of the pH of the cleaning solution on the removal efficiency of different kinds of proteins from stainless steel surfaces during batch cleaning [2]. At this point, these proteins are irreversibly adsorbed (i.e., they cannot be removed by a water rinse) on stainless steel. Although these proteins have different properties, e.g., thermal stability, solubility, and molecular structure, there are no significant differences in the relationship between the removal efficiency and the pH among these proteins. In the pH range of 4.0–9.0, only small or no removal of proteins was observed. At a pH greater than 10, the removal efficiency increases markedly with increasing pH. Although the amount of removed proteins increases slightly at a pH below 3.0, low pH solutions often induce the aggregation of proteins, resulting in persistent residual soils. Thus, most naturally occurring proteins are susceptible to caustic alkali cleaning. At high pH values, intramolecular electrostatic repulsion and swelling of the proteins can be enhanced, and the adsorbed proteins become more soluble and dispersible in the NaOH solution. In addition, the repulsive force between proteins and stainless steel surfaces becomes greater, and the attractive forces to hold protein molecules on stainless steel surfaces diminish, thereby accelerating the removal of the adsorbed proteins.

In general, the rate of proteineous soil removal by caustic alkali cleaning is enhanced with the increase in temperature. Figure 19.2 shows the effect of temperature on the rate of removal of proteineous soils from stainless steel in continuous cleaning with NaOH solution (pH 13) [3]. The rate of protein removal increases depending on the solution temperature. The combination of NaOH and heat stimulates the swelling of the soil layer and brings about the hydrolysis of proteins, thereby shortening lag time at the beginning of cleaning and accelerating the removal of

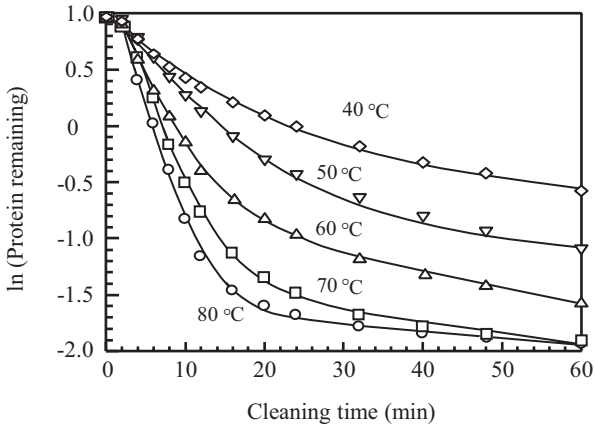


Fig. 19.2 Effect of temperature on the rate of protein removal from stainless steel surfaces during continuous alkali cleaning (pH 13) [3]

proteins. Kinetic analyses have shown that the first-order rate constant had an Arrhenius-type temperature dependence and increased from 1.4 to 1.6 times for every 10 °C rise in temperature [3, 4].

Ion-Sequestering and Chelating Agents

Most water contains appreciable quantities of multivalent cations such as calcium and magnesium. Dissolved multivalent cations are gradually accumulated in a biofilm. These cations contribute to cross-linkage between polymers in an EPS matrix and ensure the cohesion of the polymer layer. Cations also interfere with the efficiency of most anionic surface-active agents.

Ion-sequestering and chelating agents can bind the cations, forming soluble complexes. They include inorganic agents such as $Na_2P_2O_7$, $Na_5P_3O_{10}$, and $Na_6P_4O_{13}$, and organic agents such as sodium salts of gluconate, glucuronate, citrate, and ethylenediaminetetraacetate (EDTA). It has been demonstrated that EDTA in moderate alkaline solution was very effective in extracting EPS from anaerobic granular sludge [5, 6]. Multivalent cations are incorporated into an inner ring structure of EDTA, thereby breaking the cross-linkage of EPS matrix and releasing EPS polymers from bacterial aggregates. This action helps in the removal of a biofilm from a solid surface.

Sodium Hypochlorite

Sodium hypochlorite is manufactured by the absorption of gaseous chlorine in sodium hydroxide solution. The principal ingredients of a sodium hypochlorite solution are hypochlorite and sodium hydroxide. Most common commercial products of

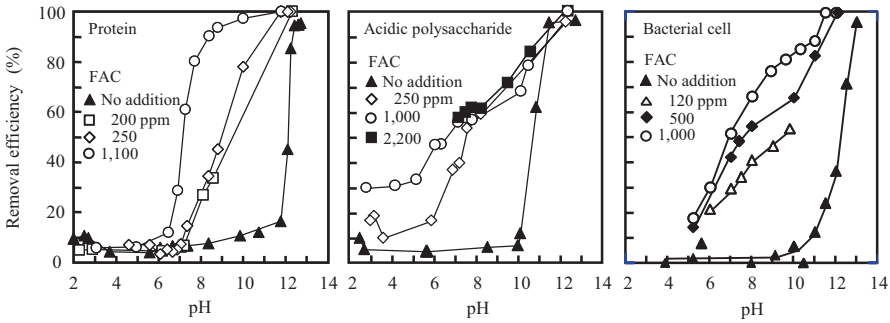


Fig. 19.3 Effects of the pH and FAC concentration of the cleaning solution on the removal of protein, acidic polysaccharide, and bacterial cells from hydrophilic solid surfaces during batch cleaning at 40 °C. (Adapted from references [7, 8])

sodium hypochlorite are strong base solutions containing 5–12 % of free available chlorine (FAC). In a diluted sodium hypochlorite solution, there is an equilibrium between HOCl ($pK_a = 7.5$) and OCl^- in the pH range of 5–10. The Cl atom in HOCl and OCl^- behaves as Cl^+, a strong electrophile, and combines with a pair of electrons in parts of the substrate that have high electron densities [2]. Among biological molecules, the C=C double bond, peptide bond (amide bond), amino groups, and thiol groups are susceptible to the electrophilic attack of Cl^+.

HOCl is believed to be the active species in the antimicrobial action. On the other hand, the OCl^- concentration is a key factor determining the actual FAC concentration required for the cleaning operation. This implies that the optimal pH region of the cleaning activity for sodium hypochlorite differs from that of its antimicrobial activity [2].

Figure 19.3 shows the effect of the pH and FAC concentration of the cleaning solution on the removal efficiency of protein, acidic polysaccharide, and bacterial cells from hydrophilic surfaces during a batch cleaning [7, 8]. The protein, polysaccharide, and bacterial cells are irreversibly adsorbed on the surfaces. In cleaning without NaOCl, i.e., with dilute HCl or NaOH solutions alone, the removal efficiency is less than 10 % in the pH range of 1.5–11, whereas it increases markedly at pH values more than 11. It is of interest to note that no significant amount of protein is removed in the pH range of 1.5–6.5 even in the presence of sufficient NaOCl. At pH values more than 6.5, the cleaning power of NaOCl becomes greater with increasing pH and it depends on the FAC concentration. Similarly to the result of protein removal, the efficacy of NaOCl for the removal of acidic polysaccharide and bacterial cells increases with increasing pH. The appearance of the cleaning action of NaOCl is clearly correlated with the degree of dissociation of HOCl to OCl^-.

In order to clarify the action of NaOCl, it is necessary to evaluate the contribution of NaOCl to the cleaning at pH values less than 10 in Fig. 19.3, where no significant cleaning action of HCl or NaOH alone is observed. Figure 19.4 shows the relationship between the removal efficiency and the OCl^- concentration [2]. It is noted that the removal of protein, acidic polysaccharides, and bacterial cells occurs

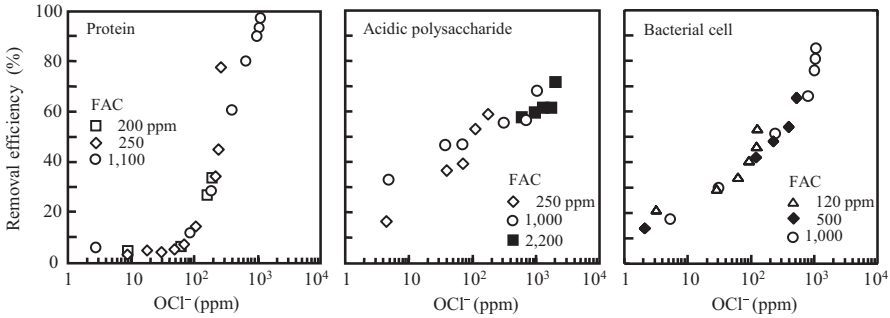


Fig. 19.4 The efficiency of the removal of protein, acidic polysaccharide, and bacterial cells as a function of the OCl^- concentration. [2]

in an OCl^--dependent manner. In other words, HOCl exerts little action in the removal of these organic soils even in the presence of sufficient FAC concentrations. It is thought that the primary function of OCl^- is to decompose the macromolecules into low molecular weight fragments by its oxidative action, and to displace them at solid–liquid interfaces [8].

At an alkaline pH region of more than 12, $OCl^-$and OH^- can synergistically enhance the efficiency and the rate of the removal of organic soils, especially, protein. High concentrations of OH^- cause the deprotonation of the N atom involved in the peptide bond, resulting in the increase in the electron density of the N atom. As a result, the peptide bond becomes more susceptible to the electophilic attack of Cl^+, thereby accelerating the fragmentation of the protein molecule, and hence the rate of desorption.

Combined Use of Sodium Hypochlorite and Surface-Active Agents

The high surface tension (γ) is one of disadvantages of NaOH and NaOCl solutions from the cleaning and disinfecting operations viewpoint. This is just a weak point of water in terms of the wettability. The property of high γ prevents the NaOH and NaOCl solution from wetting solid surfaces and soils, and from penetrating into the deposited soil layer. When OH^- and/or OCl^- are used as the main cleaning power, the aim of addition of surface-active agents is to reduce surface (interfacial) tension of the solutions to less than 40 mN/m. The marked decrease in the γ of NaOH and NaOCl solutions can improve the wetting ability of the solutions, thereby augmenting the efficiency of cleaning.

Figure 19.5 shows the effect of a surface-active agent on the rate of removal of bacterial cells from polyethylene terephthalate (PET) surface during cleaning with various cleaning solutions [9]. No significant desorption of cells is observed during cleaning with water or 0.02 % surface-active agent solution of pH 5. The number

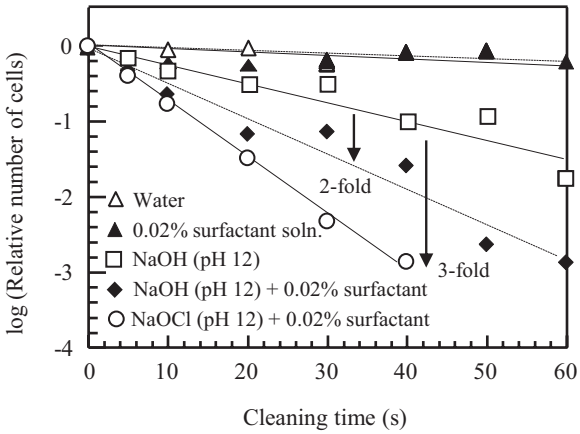


Fig. 19.5 The logarithmic relative reduction of the number of bacterial cells remaining on PET surface during alkali cleaning with or without surfactant and NaCl [9]

of cells on PET surface decreases according to a first-order process during cleaning with NaOH solution of pH 12. The addition of surface-active agent (0.02 %) alone or together with NaOCl (100 ppm) to the NaOH solution accelerates the rate of decrease in the number of adhering cells by twofold or threefold. In case of the cleaning process with NaOH solution containing surface-active agent and NaOCl, complete inactivation of cells remaining on PET surface has been demonstrated to be achieved for 50 s [9]. These data indicate that the cleaning using oxidative action of OCl^- in conjunction with OH^- and surface-active agent can completely disinfect the PET surface fouled with bacterial cells.

The technique of foam cleaning, combining surface-active agents, water, and compressed air, has been applied increasingly in the food industry. Foam cleaning has many advantages, e.g., lowering the interfacial tension, prolonging the contact time between detergents and soils to loosen all soils from equipment surfaces, low mechanical action, reducing the amount of detergent, and applicability to horizontal, vertical, and inverted surfaces, and to the parts of the machinery not accessible to human hands. Foam can suck up soils released from solid surfaces, which in turn makes rinsing easier. The use of alkaline solution containing NaOCl as the ingredient of foam cleaning is more suitable not only for the removal of organic soils but also for disinfection of microbes.

Effect of pH on Corrosion of Stainless Steel by Sodium Hypochlorite

As long as sodium hypochlorite is used as an alkaline solution with pH more than 10, the degree of corrosion of stainless steel is reduced considerably. Figure 19.6 shows the amounts of metal ion dissolved from an electropolished 304 stainless steel plate in sodium hypochlorite solutions (200 ppm) at various pH values. At

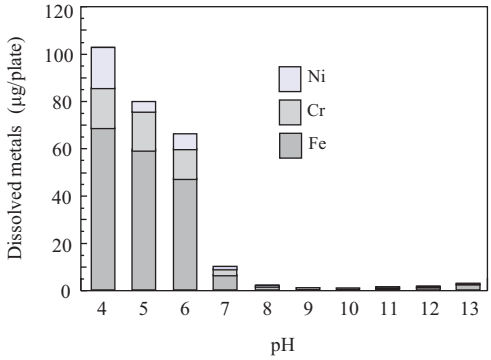


Fig. 19.6 Dissolved metal components from electropolished stainless steel plate (type 304) when immersed in pH-controlled NaOCl solutions at 60 °C for 8 h

pH values more than 10, only small amounts of metal ions (<2.5 μg/plate) are dissolved. This is attributed to the preferential adsorption of OH^- to a stainless steel surface because OH^- is a far stronger base ion than OCl^-. As a result, OH^- serves as an inhibitor of corrosion. On the other hand, the amount of metal ions increases markedly at a pH less than 7, and it reaches up to 100 μg/plate. In an acidic pH region, where the OH^- concentration is low, HOCl stimulates the cathode reaction by receiving electrons.

References

1. Kumar CG, Anand SK (1998) Significance of microbial biofilms in food industry: a review. Int J Food Microbiol 42:9–27
2. Fukuzaki S (2006) Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Sci 6:95–101
3. Takahashi K, Fukuzaki S (2003) Kinetic approach to the nonlinear curve of bovine serum albumin desorption from stainless steel surfaces. Biocontrol Sci 8:111–117
4. Jennings WG (1959) Circulation cleaning III. The kinetics of a simple detergent system. J Dairy Sci 40:17–40
5. Dolfing J, Griffioen A, van Neerven ARW, Zevenhuizen LPTM (1985) Chemical and bacteriological composition of granular methanogenic sludge. Can J Microbiol 31:744–750
6. Fukuzaki S, Nishio N, Nagai S (1995) High rate performance and characterization of granular methanogenic sludges in upflow anaerobic sludge blanket reactors fed with various defined substrates. J Ferment Bioeng 79:354–359
7. Fukuzaki S, Urano H, Yamada S (2007) Effect of pH on the efficacy of sodium hypochlorite solution as cleaning and bactericidal agents. J Surf Finish Soc Jpn 58:465–469
8. Urano H, Fukuzaki, S (2005) The mode of action of sodium hypochlorite in the cleaning process. Biocontrol Sci 10:21–29
9. Takahashi K, Fukuzaki S (2012) Removal and inactivation of bacterial cells adhering to polyethylene terephthalate surfaces during a combined alkali-chlorine cleaning (in Japanese). Bokin Bobai 40:405–413

Chapter 20
Physical Removal of Biofilm

Dana M. Barry and Hideyuki Kanematsu

Abstract Biofilms form plaque on teeth and contribute to slime and scale buildup that result in clogged pipes and drains. Clogged pipes display smaller diameters and exhibit a reduced ability for heat exchange and liquid flow. A company with such a problem could experience increases in energy consumption and operational costs. Also if not corrected on time, it may need to shut down for an extended period in order to replace the defective pipes. Therefore it is essential to clean pipes, drains, and water mains frequently, in order to protect them from scale and biofilm buildup. Physical cleaning methods exist for effectively removing biofilms. Some are presented and described in this chapter. They include flushing, swabbing, air scouring, pigging, and ultrasonic scaling (used to remove plaque on teeth).

Introduction

Biofilms are thick layers of cells that start to form when microorganisms such as bacteria attach to a surface, especially in the presence of moisture. After a sufficient amount of time, the attachment becomes somewhat permanent because these microorganisms produce a sticky polymeric slime that anchors them. As the biofilms grow, they can incorporate scale-forming minerals, which make them more difficult to remove.

Biofilms form plaque on teeth and contribute to slime and scale buildup that result in clogged pipes and drains. Clogged pipes display smaller diameters and exhibit a reduced ability for heat exchange and liquid flow. A clogged concrete water pipe is shown in Fig. 20.1 [1]. Companies with similar pipe problems could expe-

D. M. Barry (✉)
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, US
e-mail: dmbarry@clarkson.edu

H. Kanematsu
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_20

Fig. 20.1 Clogged concrete water pipe

rience increases in energy consumption and operational costs. Others with worse problems may need to shut down for an extended period in order to replace the defective pipes. Therefore, it is essential to clean pipes, drains, and water mains frequently, in order to protect them from scale and biofilm buildup. Physical cleaning methods exist for effectively removing biofilms. Some are presented and described.

Flushing

Biofilms can be removed from water systems by flushing. This technique involves discharging water (at appropriate velocities) through pipes to remove accumulated biofilms within the pipes. The required velocities depend on particle size, pipe diameter, and other variables. Conventional flushing is a routine carried out to remove contaminants. It can involve opening hydrants and leaving them open until the water quality improves. Examples include reducing the discoloration and turbidity of the water. This cleaning practice is best for bulk water turnover.

For a more systematic approach, unidirectional flushing (UDF) is used. Here the direction of flow is generally controlled by closing valves to isolate certain sections of pipe. This allows the water to enter from a cleaned main and to approach the exit from only one direction. Water hydrants are opened in a special sequence to

increase the velocity of water flowing through the pipes. Water velocities of about 6 ft/s (fps) or greater have removed microbes from smooth pipe surfaces [2]. The UDF flushing technique is relatively inexpensive and can be performed by several people. However, it uses lots of water, is unlikely to remove all of the biofilm, and is most suitable for pipes with 12 in. diameters or less [3].

Swabbing

The process of swabbing relies on the use of a compressible foam cylinder referred to as a swab. The swab can have a diameter up to 25 % greater than that of the pipe [4]. It is forced through a pipe using water pressure to remove biofilms. Swabs are generally inserted into pipes through existing hydrants. Before swabbing begins, all water services connected to the pipe are turned-off and preparations for retrieving the swab are made. If a swab travels too fast, then less material is removed and it tends to wear out faster. On the other hand if a swab seems to be taking too long, then it may be stuck in the pipe. In this case the direction of water flow should be reversed. This will assist in returning the swab to its starting position. Sometimes three or more swabs need to be sent through a pipe in order to adequately clean it.

Since a swab's diameter usually exceeds the inner diameter of a pipe, the swab is compressed during use. This increases frictional shear forces against the pipe wall and enhances biofilm removal [3]. Swabs can be either soft or hard. Soft foam swabs are beneficial in mains with tuberculation (mounds of corrosion products inside the pipe) or where changes may occur in a pipe's diameter. However, they tend to wear out quickly. Swabs made of hard foam are less compressible and most suited for water mains expecting minor reductions in diameter. In comparison to flushing, swabbing uses less water and is more effective at removing biofilm. On the downside, swabs can get stuck or break-up in a pipe.

Air Scouring

The method of air scouring generally makes use of a hydrant to inject filtered, compressed air into pipes. To start, a section of the water main between an entry and exit point is isolated. Then a continuous supply of water and air in appropriate proportions is used to form slugs of water in the pipe. These slugs are moved along by the compressed air at high velocity. The fast traveling slugs remove biofilm and sediment from the pipe. Once the exiting water looks clear, then service is restored to the isolated section. This method uses less water than flushing and swabbing. However, the task requires skilled workers to obtain the correct amounts of water and air.

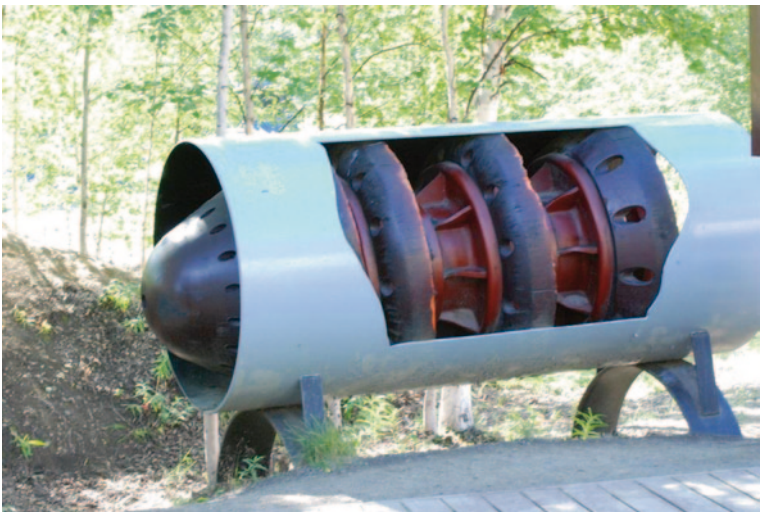


Fig. 20.2 A pig displayed in a cutout section of pipe

Pigging

This method generally uses a bullet-shaped cylinder called a pig to clean pipelines. Pigs, which tend to be harder and less flexible than swabs, are blown through a pipe. They fit tightly against the interior wall of the pipe and scrape the edges to remove debris and biofilm. To improve the pig's effectiveness for cleaning, brushes and/or blades can be added to it. Usually blades are better for deposit removal because the brushes may fill up with sediment. Pigging requires launching and retrieval of the pig and sometimes pipe relining. Figure 20.2 [5] shows a pig displayed in a cutout section of pipe. Today pipeline operators refer to any device made to pass through a pipeline for cleaning as a pig [6].

Ice pigging is a process where an ice–water slurry is pumped into a pipe to clean it by removing sediment and biofilm. This slurry generally has an ice fraction of at least 50% [7]. Ice pigging is an ideal process because if the ice gets stuck it will eventually melt with time.

Ultrasonic Scaling

Ultrasonic scalers are used by a hygienist to remove biofilm (bacterial plaque) from teeth. The scalers' mechanical action of high vibrational energy actually loosens, crushes, and removes tartar and biofilm. Dental patients get rid of this debris by thoroughly rinsing their mouths with cold water during and at the completion of this cleaning process. In addition to power scalers, hand-held scraping tools are

used to remove biofilm from teeth. Ultrasonic and scraping methods may be useful in cleaning pipes too.

References

1. Bidgee, concrete water pipe, CC By 3.0. http://en.wikipedia.org/wiki/water_pipe. Accessed 13 Oct 2008
2. Miller R, Friedman M, Koci D, Moore R (2013) Biofilm control: develop an effective strategy to preserve quality, ensure compliance. Opflow 39(9):13
3. Friedman M, Lakin B, Moore R (2012) Best practices cleaning mains: clean, pig, or dig. Opflow 38(8):12, 13
4. Vitanage D, Pamminger F, Vourtsanis T (2004) Maintenance and survey of distribution systems. In: Ainsworth R (ed) Safe piped water: managing microbial water quality in piped distributions systems. IWA Publishing, London, p 81
5. Harvey Barrison, pipeline pig, CC By-SA 2.0. http://en.wikipedia.org/wiki/pigging
6. Cote C, Rosas O, Sztyler M, Doma J, Beech I, Basseguy R (2014) Corrosion of low carbon steel by microorganisms from the 'Pigging' operation debris in water injection pipelines. Bioelectrochemistry 97:97. E pub, Accessed 20 Nov 2013. Copyright 2013 Elsevier B.V.
7. http://en.wikipedia.org/wiki/Ice_pigging

Chapter 21
Antibacterial Effect of Materials and Biofilm

Hideyuki Kanematsu and Dana M. Barry

Abstract In this chapter, antibacterial effect is explained. Some materials could show the effects. From the viewpoint of their mechanism, antibacterial groups can be divided into three classes. The first consists of metallic materials such as silver, copper, etc. From some concrete examples, the metallic ions dissociated from the metals themselves or their compounds work to show antibacterial effects. The second class is composed of photocatalytic compounds. In this case, produced radicals control the bacterial growth. The third class contains organic materials. Generally, organic materials cause impairment of cell membranes inside the peptidoglycan. The first consists of antibacterial metals could control biofilm formation, since they would inhibit bacterial growth on material surfaces. However, biofilm control is basically a broader and more complicated concept than the antibacterial effect for planktonic bacteria. Therefore, more versatile countermeasures could be proposed to control biofilm formation and growth.

Introduction

It is well-known that some materials (especially metallic ones) have antibacterial effects [1–4]. For example, in ancient Egypt, Greece, and Rome, silver and other metals like zinc, copper, etc., were used to clean wounds. Particularly silver has been used in wound dressings and for burn injuries so far. Why are some metals useful for antibacterial purposes? In this chapter, we describe what antibacterial effect is and how it works with some materials. Also the correlation between antibacterial effect of materials and biofilm is explained.

H. Kanematsu (✉)
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

D. M. Barry
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, US
e-mail: dmbarry@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_21

Antibacterial Effect and Its Definition [5]

In English, antibacterial effect does not really have many meanings and categories. However, Japanese (like one of the authors), are usually very sensitive to sanitary problems, hence, they have many kinds of categories and the corresponding technical terms for controlling bacteria. At least, five technical terms could be mentioned in Japan.

The first one is Sakkin (microbial effect). It means killing bacteria literally. However, the concept does not include any objects or extent of time. "No objects" means that you do not have to kill all bacteria on an object. Therefore, this concept never assures how much or how long the effect would work. It means just killing some bacteria.

The second is Mekkin (sterilization). It can be defined as the process of removing or killing all microbes and viruses from objects, irrespective if they are harmful or harmless. From the viewpoint of probability, it is impossible to make the bacterial number zero. Therefore, people often use Sterility Assurance Level (SAL) to judge the safety. Internationally, SAL should be smaller than 10^{-6} for sterilization.

The third is disinfection. It is defined as the process where the microbial number would be decreased to the harmless level. The process may be done by Mekkin (sterilization). However, the disinfection might be achieved without killing bacteria. Therefore, the disinfection should be differentiated from sterilization.

The fourth is Jokin. It means the decrease of bacterial number by removing them from the objects. It includes washing hands, filtering, etc. This concept does not have any objects or extents of time, etc.

The fifth is Kokin (antimicrobial effect). It means controlling the bacterial growth. However, the concept does not have any objects to control the growth or the extent of it, etc. Kokin's target is only bacteria. It does not target fungi and biofilms.

The aforementioned are the definitions of technical terms relating to antibacterial effect. As the definitions clearly show, controlling biofilm is different from killing and removing bacteria. The former concept is much larger than the latter. Unfortunately, the technical terms for antibacterial effect do not take biofilm bacteria into consideration. They deal with only planktonic bacteria in most cases. It seems to be a vital defect to control bacterial growth. From the viewpoint, Kokin, the fifth concept mentioned above seems to have a close relation with biofilm, since both concepts (Kokin and biofilm) involve the notion, "number of bacteria". To realize the Kokin effect, the number of bacteria must be decreased somehow. On the other hand, the number of bacteria should be controlled to inhibit the formation and growth of biofilm. Biofilm free surfaces should be the ones that are hard for bacteria to grow on.

The Mechanism of Antibacterial Effect [6]

From the viewpoint of mechanism for antibacterial effect, antibacterial materials can be divided into three groups: metals, photocatalytic substances, and organic matters.

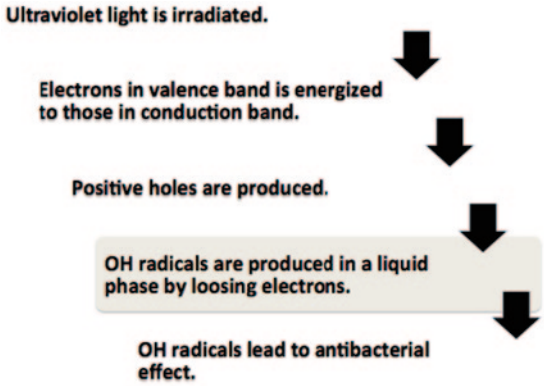


Fig. 21.1 Antibacterial process for catalytic compound

Antibacterial metal group, the first one, includes silver, copper, etc., that have been well known for their magical power to control bacteria. There are many mechanistic proposals to explain how some metals could show their antibacterial effects. The authors believe that dissolved metallic ion would react with some proteins on bacterial cells. Kanematsu et al. investigated the antibacterial effects of some generalized metallic elements and found that several metals such as Co, Cu, Mn, Ni, Ag, and Zn to have strong antibacterial effects [7]; and at the same time, they found some metals have weak or no antibacterial effects. From all the results, they could point out the two characteristics for antibacterial elements.

#1: The antibacterial effect could appear for gram-positive bacteria (*E-coli*, *Klebsiella pneumoniae*) more clearly, than gram-negative (*Staphylococcus aureus*) [6].

Gram-negative bacteria do not retain the crystal violet stain (Gram's stain), while gram-positive do. When we focus on the difference between two bacterial groups, the surface structures are the most different from each other. For example, gram-negative bacteria have an inner membrane and an outer one. The peptidoglycan layer is generally very thin too. On the other hand, gram-positive bacteria do not have outer membranes and the peptidoglycan layers are generally thick. Since the antibacterial effect is different between the two groups, it could be caused by the interaction between metals and surface components.

#2: Most of antibacterial metals tend to bind with amino acids [8, 9].

For example, His (histidine), one of the amino acids, binds with Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+} etc. On the other hand, Ag^{+} tends to react with Cys (Cysteine) and other thiol compounds containing Cys. When these metallic ions bind with proteins, then the higher-order structure of the protein would change. This would be followed by a change of functions.

From these two characteristics, the author and colleagues believe that some dissolved metallic ions would change the metabolic functions and then the antibacterial effect would appear.

On the other hand, some photocatalytic compounds show strong antibacterial effects. One of the representative compounds is titanium oxide. Figure 21.1 shows the schematic illustration about the mechanism for antibacterial effect by

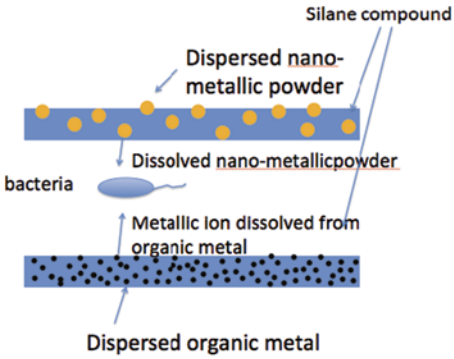


Fig. 21.2 The investigation by Kanematsu et al. for nanocomposite coating

photocatalysts. The photocatalytic compound is defined as the matter that shows catalysis by light. For example, titanium oxide has the valence band and conduction band, since it is a semiconductor. There is an energy gap between the two bands. When the ultraviolet light is irradiated (hv), anatase type TiO_2 gets energy from the light and the electrons in the valence band become energized to the conduction one. Then, a positive hole forms in the valence band. However, the positive hole in the valence band is usually unstable, since the positive hole is lacking an electron(s). Therefore, it tends to deprive electrons from hydroxide ions to disappear. In this way, OH radicals ($\cdot OH^-$) form. Generally, it has strong oxidation power and therefore, it shows the antibacterial effect. The same effect could be expected also for Fe_2O_3, WO_3, CdS, ZnS, etc. However, the antibacterial power seems to be the strongest for TiO_2. It has been used for many practical applications [10].

The third antibacterial materials group is organic matter. The antibacterial mechanism is quite different from those for metals or photocatalytic compounds. Generally, organic materials cause impairment of cell membranes inside the peptidoglycan. Based on the mechanism, many kinds of organic chemicals have been developed so far.

Antibacterial Effects and Antibiofilm

What is different between antibacterial effect and antibiofilm (or antifouling)? Both act as the control action against bacterial activity. However, antifouling or antibiofilm is usually a more complicated and broader concept than the antibacterial effect. As already described in Chap. 1, biofilm formation is composed of multiple steps. Antibacterial effect works well to control biofilm. Kanematsu et al. [11] studied copper powders dispersed in silane compound films and confirmed that they could control biofilm formation. In the same series of experiments, they also confirmed the antibacterial effect of silver organic compounds dispersed in the same silane compound film, as shown in Fig. 21.2. As shown in the figure schematically, antibacterial metallic ions would dissociate from the metallic powder or the organic

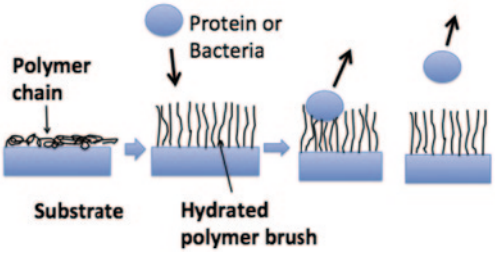


Fig. 21.3 Polymer brush surface and biofilm control

metal in the silane compound film to show antibacterial effects. Those specimens could obviously control the biofilm formation, since the antibacterial effect would inhibit the bacterial growth.

However, this is just a necessary condition, but never a sufficient one. In the same investigation described above, they also found that organic tin dispersed in the silane compound film also showed the antibacterial effect to some extent. By the same logic, the dissociated tin might get a chance to inhibit the bacterial growth. However, tin never shows any antibacterial effect with the international antibacterial test (Film Covering Method, ISO22196) [12]. Therefore, some other mechanisms to inhibit the bacterial growth for the element could be considered. For example, the smoothness of surface profile, electric charges, surface tension, hydrophilic–hydrophobic properties, water repellency, etc. could be mentioned. Either of them or some of them might be related to a corresponding step of biofilm formation and could control the growth. For other examples, polymer brush surfaces could be mentioned. The schematic illustration for the unique surface to control biofilm is shown in Fig. 21.3. Various processes have been proposed to produce polymer brushes [13, 14]. The produced polymer chain on the material surface could create a tightly packed highly hydrated brush in an aqueous environment. The brush could repel proteins and microbes from the surface. The former repelling would lead to the inhibition of conditioning film, and the latter would inhibit the bacterial attachment directly. At any rate, such a brush polymeric surface could control biofilm formation and it is a representative antifouling coating. It does not have any relation to the antibacterial effect.

All of these examples could show the similar and different characteristics for the antibacterial effect and the antibiofilm. As described above, biofilm formation is more complicated and a broader, multistep process. More holistic approaches are required to solve the problems.

References

1. Spear M (2010) Silver: an age-old treatment modality in modern times. Plast Surg Nurs 30:90–93
2. Atiyeh BS, Costagliola M, Hayek SN, Dibo SA (2007) Effect of silver on burn wound infection control and healing: review of the literature. Burns: J Int Soc Burn Inj 33(2):139–148. doi:10.1016/j.burns.2006.06.010

3. Silver S, Phung LT, Silver G (2006) Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. J Ind Microbiol Biotechnol 33(7):627–634. doi:10.1007/s10295-006-0139-7
4. Alexander JW (2009) History of the medical use of silver. Surg Infect 10(3):289–292
5. Kanematsu H, Yoshitake M (2015) "Nano Composite Coating for Antibacterial Purposes", Handbook of Nanoceramic and Nanocomposite Coatings and Materials, ed. by A.S.H.Makhlouf etc. Elsevier, in print.
6. Ikigai H, Kanematsu H (2011) Mechanism for antibacterial effects by metallic elements and their inhibition of bacterial infections. J Jpn Soc Biomater 29(4):232–239
7. Kanematsu H, Ikigai H, Yoshitake M (2008) Antibacterial eco-plating based on HACCP and biofilm. Bull Iron Steel Inst Jpn 13(1):27–34
8. Uchida M et al (2003) Silver ion and the reaction with various amino acids. Boukin-Boubai 31:695–704
9. Ikigai H, Ono T, Iwata M, Nakae T, Shimamura T (1997) EI Tor hemolysin of *Vibrio cholerae* O1 forms channels in planar lipid bilayer membranes. FEMS Micribiol Len 150:249–264
10. Kanematsu H, Ikegai H, Yoshitake M (2013) Patents for antibacterial metallic coating and its future trend in Japan. Res Invent: Int J Eng Sci 3(6):47–55
11. Kanematsu H, Kogo T, Sano K, Noda M, Wada N, Yoshitake M (2014) Nano-composite coating on glasses for biofilm control. J Mater Sci Surf Eng 1(2):58–63
12. Kanematsu H, Ikigai H, Yoshitake M (2009) Evaluation of various metallic coatings on steel to mitigate biofilm formation. Int J Mol Sci 10(2):559–571
13. Knetsch MLW, Koole LH (2011) New strategies in the development of antimicrobial coatings: the example of increasing usage of silver and silver nanoparticles. Polymers 3:340–366
14. Zhao BWJ (2000) Polymeric brushes: surface-immobilized macromolecules. Prrog Polym Sci 25:677–710

Chapter 22
Immersion Tests

Daisuke Kuroda

Abstract As you have seen in Chap. 11, adhesion of microorganisms is one factor of corrosion or deterioration of the ship and marine structure. Biofilm formation and adhesion of microorganisms cause microbiologically influenced corrosion (Li et al., Corros Rev 31:73–84, 2013). Service life of the marine structure which is composed of steel is shortened due to MIC. Biofilm formation also causes the adhesion of fouling organisms, including barnacles and oysters (Dobretsov et al., Biofouling 29:423–441, 2013). Fuel consumption of a ship is increased by adhesion of fouling organisms (Nagato et al., J Shimonoseki Univ Fish 41:167–168, 1993; Murakami, J Jpn Inst Mar Eng 46:40–45, 2011). On the other hand, the service life of the ship is reduced by adhesion of fouling organisms. Degradation of various structures is accelerated by the attachment of microorganisms and fouling organisms (Luciana et al., Int Biodeterior Biodegrad 63:607–614, 2009). The performance and durability of machines are also markedly reduced by adhesion of various organisms. Therefore, evaluation and observation of the adhesion behavior of the microorganisms and fouling organisms on the surface of each material in a real environment is very important for solving industrial biofilm problems. In this chapter, I outlined a typical immersion testing method. In addition, I also introduced the typical results of several immersion tests.

Importance of the Immersion Test in a Real Environment

The immersion test in a real environment is desirable in order to correctly evaluate the adhesion behavior of microorganisms and fouling organisms. It is quite hard to reproduce the same phenomenon as a real environment in the laboratory. Results of immersion tests strongly depend on the test environment. Sea, pond, water supply, draining piping, and heat exchangers, have been selected for immersion tests [6, 7]. Selection of seasonal and experimental periods are important for the correct evaluation, because the types and proportions of microorganisms on the material's surface change with the season and experimental period (immersion time in other

D. Kuroda (✉)
National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: daisuke@mse.suzuka-ct.ac.jp

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_22

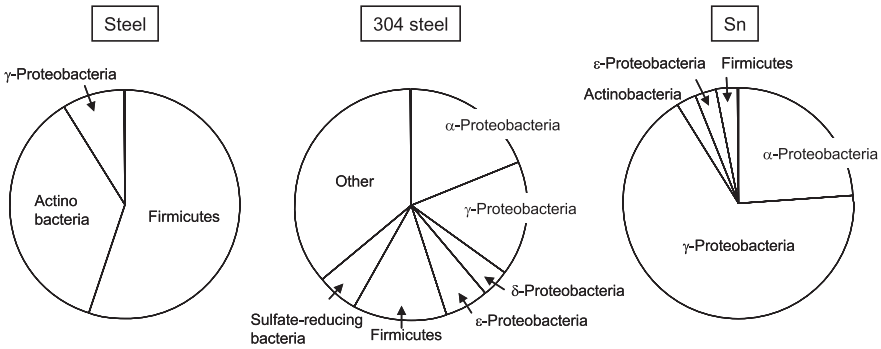


Fig. 22.1 The type and proportion of microorganisms on each metallic material which was immersed in a marine environment for 1 week. Immersion test was performed in the winter season

words). In addition, the types of attached microorganisms are affected remarkably by a variety of metallic materials. An example of this phenomenon is displayed in Fig. 22.1 [8]. Steel (rolled steel for a general structure, SS400), 304 steel and tin (Sn) were immersed in a marine environment for 1 week in the winter season. Types of microorganisms on the surface of these metallic materials were identified by using the 16S-ribosomal DNA phylogenetic analysis method. Types of microorganisms present change depending on the variety of metallic materials. The proportion of firmicutes on the surface of steel is higher than that of 304 steel and Sn. Sulfate-reducing bacteria (SRB) are observed on the surface of 304 steel. On the other hand, SRB are not observed on the surfaces of steel and Sn. Thus, the type of attached microorganism is affected remarkably by the variety of metallic material.

Change in type and proportion of microorganisms on the surface of Sn according to the immersion time is shown in Fig. 22.2 [8]. Figure 22.3 shows the appearance of each specimen after a 1 month immersion test. Adhesion of fouling organisms such as barnacles was not observed on the surface of each specimen, because it was immersed in winter. A variety of microorganisms were detected on the surface of Sn after an immersion for 1 week. On the other hand, only two types of microorganisms such as ε-proteobacteria and actinobacteria were detected after an immersion for 1 month, indicating that the type and proportion of attached microorganisms on the same material changed depending on immersion time. Thus the type and proportion of attached microorganisms are affected remarkably by immersion time. Short-term immersion tests or immersion tests in the winter season are selected to evaluate microorganism adhesion and biofilm formation. Long-term immersion or immersion tests in the summer season are mainly selected to evaluate fouling organism adhesion.

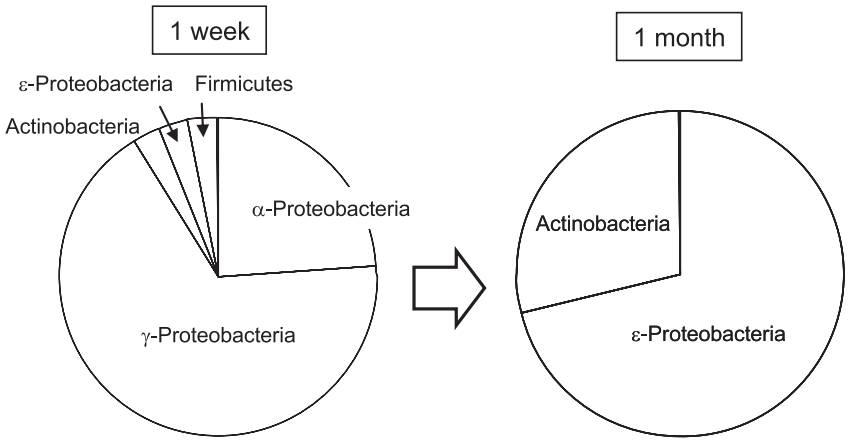


Fig. 22.2 Changes in the type and proportion of microorganisms on the surface of tin (Sn). Immersion test was performed in the winter season

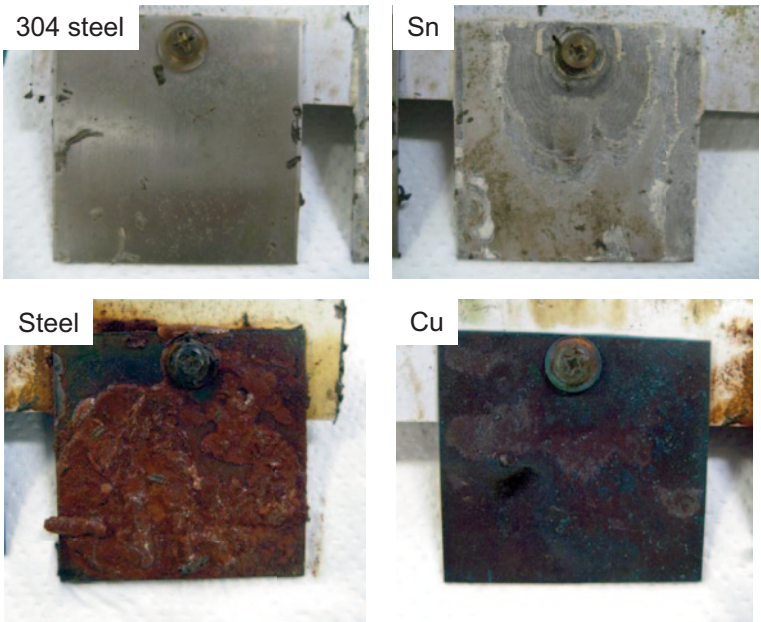


Fig. 22.3 Appearance of 304 steel, tin (Sn), steel and copper (Cu), after 1 month of immersion in a marine environment. Immersion test was performed in the winter season

Example of Immersion Test in Marine Environment [9]

I will introduce the results and one case of an immersion test in a marine environment. Figure 22.4 shows the appearance of specimens and specimen holders for a marine immersion test. Having the same surface roughness and the same size of specimen is desirable. Specimens shown in Fig. 22.4 were mirror polished by using silicone carbide emery paper and buff. Various types of plastic are generally used as a material for the specimen holder and setscrew in order to prevent the galvanic corrosion between metal specimens and specimen holders during immersion tests. Others have used polyvinyl chloride (PVC) and acryl resin. PVC is generally used as a reference material to evaluate the adhesion property of fouling organisms. Specimens were immersed in the sea using such a method as shown in Fig. 22.5. The immersion test was performed in the summer season for this study. Specimens were generally fixed to the floating pier in order to cope with changes in sea level, because the type of microorganism and environment change with changing depth from the sea surface. The continuous immersion test was performed under

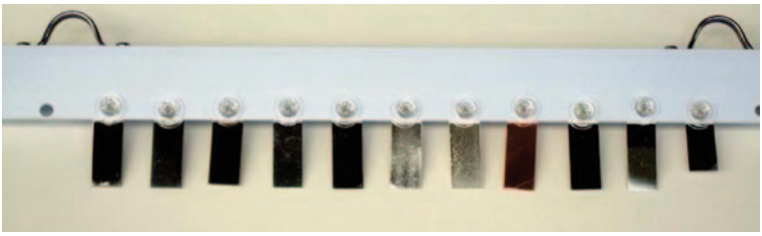


Fig. 22.4 Appearance of the specimens and specimen holders for a marine immersion test

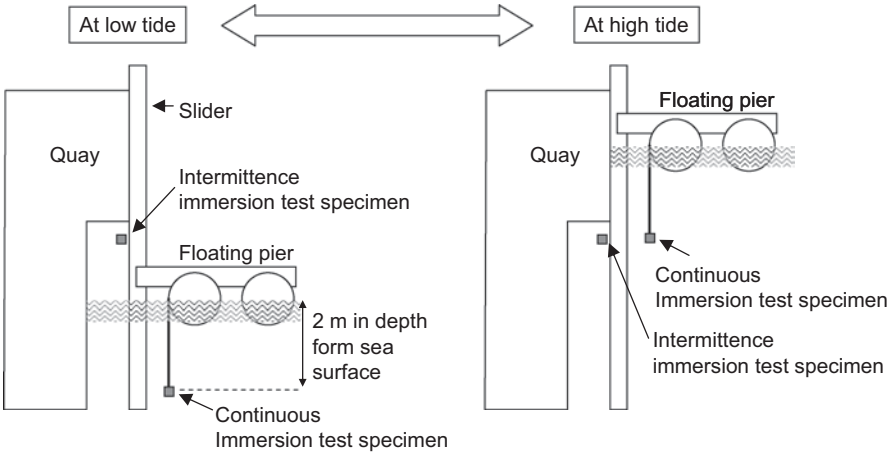


Fig. 22.5 Schematic diagrams of marine immersion test

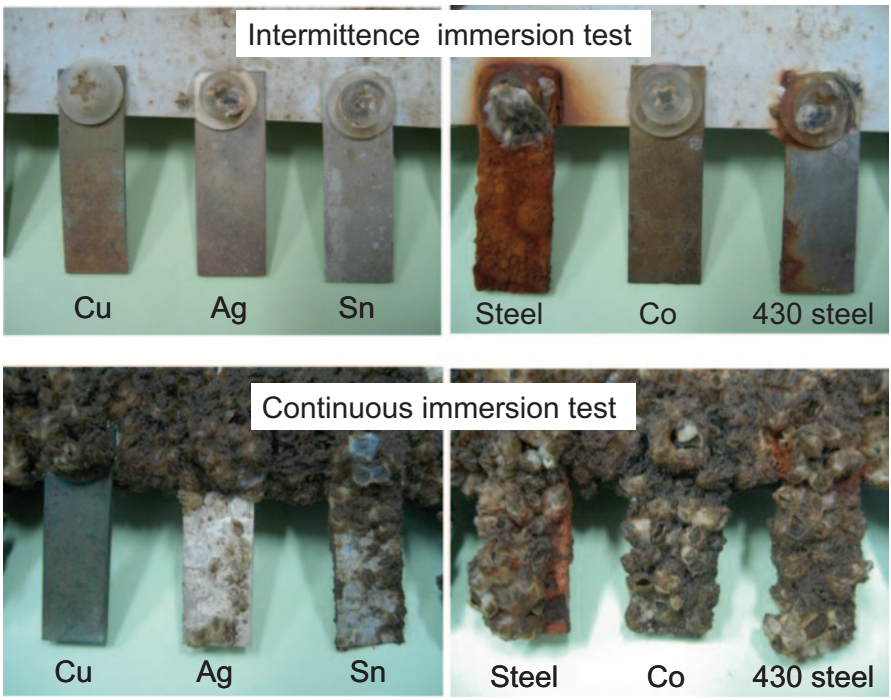


Fig. 22.6 Appearance of each metallic material after immersion tests under each condition. Immersion period and season are 1 month and summer, respectively

this condition. On the other hand, the intermittence immersion test was performed in order to evaluate the resistance to corrosion under severe corrosive conditions. In this case, a specimen was fixed to the quay. The extent of adhesion of microorganisms or fouling organisms in continuous immersion test conditions is higher than that in intermittent immersion test conditions.

Figure 22.6 shows the appearance of specimens such as copper (Cu), silver (Ag), Sn, cobalt (Co), steel, and 430 steel after the intermittence immersion test and continuous immersion test for 1 month. A large amount of barnacles are observed not only on the surfaces of Co, steel, and 430 steel but also on the surface of the PVC specimen holder after the continuous immersion test. In addition, the partial peering-off of barnacles is observed on the surfaces of Ag and Sn. Adhesion of barnacles is not observed on the surface of Cu. These results indicate that Ag, Cu, and Sn have highly inhibitory action against the fouling organism adhesion due to their antibacterial action. On the other hand, steel and 430 steel promote fouling organism adhesion due to the iron ion dissolved from the substrate. Biofilm formation and no fouling organism adhesion were observed on each specimen after intermittence immersion tests. This indicates that the type and proportion of attached organism changed with the experimental method. It is necessary to appropriately establish the test conditions to obtain precise results.

References

1. Li K, Whitfield M, Van Vliet KJ (2013) Beating the bugs: roles of microbial biofilms in corrosion. Corros Rev 31:73–84
2. Dobretsov S, Abed RMM, Teplitski M (2013) Mini-review: inhibition of biofouling by marine microorganisms. Biofouling 29:423–441
3. Nagato K, Matsushita H, Inui E, Miyoshi Y (1993) Studies on marine fouling of the bottom plates and a measure for prevention of marine fouling. J Shimonoseki Univ Fish 41:167–168.
4. Murakami K (2011) Corrosion protection and anti-fouling properties of thermal sprayed metal coating. J Jpn Inst Mar Eng 46:40–45
5. Luciana VR de M, Lucio S, Leila YR, Ricardo C (2009) The effect of biofouling on localized corrosion of the stainless steels N08904 and UNS S32760. Int Biodeterior Biodegrad 63:607–614
6. Kuroda D, Kanematsu H, Kamakura N Ito H, Ikigai H (2012) Adhesion of microorganisms on the surfaces of various metallic materials immersed in a cooling water tank of the package type cooling tower. Testu Hagane 98:109–116
7. Melchers RE, Jeffrey RJ (2013) Accelerated low water corrosion of steel piling in harbours. Corros Eng Sci Technol 48:496–505
8. Maseda H, Ikigai H, Kuroda D, Ogawa A, Kanematus H (2010) Immersion of iron and steel materials into marine environment at Ise gulf and gene analysis of attached microorganism. CAMP-ISIJ 23:668
9. Kuroda D, Kamakura N, Yokokawa S, Ikigai H, Kanematsu H (2011) Adhesion of the oceanic lives on metallic materials immersed in the gulf of Ise. Proceedings of the 55th Japan Congress on Materials Research, pp 212–213

Chapter 23
Artificial Biofilm Formation on the Laboratory Scale

Dana M. Barry, Hideyuki Kanematsu and Paul B. McGrath

Abstract Biofilms are collections of microorganisms that stick to each other on a variety of moist surfaces. These microbial communities are anchored to the surface by a glue-like extracellular polymeric substance (EPS). Biofilms grow, mature, and disperse cells in order to spread and colonize new surfaces. They can grow in different environments such as on floors, counter tops, food, human tissue, and ships. These films cause corrosion in metals and pose health risks to humans. Therefore, they are of interest to researchers who want to prevent and control them. Two different methods are presented for artificially producing biofilms at an accelerated rate in a laboratory setting. One involves an intermittent flow of water over various test materials while the other continuously exposes the items to moisture.

Biofilm Description

Biofilms consist of microorganisms attached to a surface and to each other through a self-produced extracellular polymeric substance (EPS). This sticky polymeric matrix protects the cells within it and anchors them to metals, plastics, and other materials. Biofilms are common in nature. They develop on the inner layer of metal pipes and lead to clogging and corrosion. Plaque on our teeth, which is responsible for cavities, is a good example of a bacterial biofilm. These films cause rocks in streams to become slimy. They also pose health risks to humans because they grow on food,

D. M. Barry (✉)
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University Potsdam, New York, US
e-mail: dmbarry@clarkson.edu

H. Kanematsu
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

P. B. McGrath
Department of Electrical and Computer Engineering, Clarkson University, Potsdam, NY, USA
e-mail: mcgrath@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_23

human tissue, and biomedical implants. Biofilms can be beneficial too. For example, they can be used to purify water by breaking down undesirable compounds.

The main component of a biofilm is water, which can make up to 90 % of its wet weight [1]. Water channels, which exist in some biofilms, allow them to obtain nutrients and to remove waste products. Biofilms differ in structure and composition depending upon their environment. Some contain minerals and corrosion particles [2]. Variables such as temperature, pH, availability of nutrients, and surface roughness, influence their development. Biofilms start to form when free-floating microorganisms become attached to a surface. If the cells remain at the surface for a sufficient amount of time, then the attachment becomes more permanent. This is because a sticky substance (EPS) is produced. It anchors microorganisms like bacteria to various materials and serves as a form of protection by encasing the initial colonizers. Biofilms grow by obtaining additional microorganisms and by cell division. After the maturation phase, cells are dispersed from the biofilm colony in order to continue their life cycle on new surfaces. Biofilms regulate colonization through quorum sensing (QS). This cell to cell communication through chemicals turns on certain bacterial activities [3].

Artificial Biofilm Formation in the Laboratory

Test Samples Intermittently Exposed to Moisture

Biofilms generally grow on solid surfaces in the presence of moisture and microorganisms. They act as a habitat for bacteria. Today there is a great concern about the spread of infections and the health risks of patients in hospitals. Almost 65 % of all hospital infections in humans originate from biofilms [2]. Also certain bacteria, protected by biofilms, are resistant to antibiotics [4]. Therefore, researchers Barry and McGrath decided to test hospital materials for biofilm formation. A special rotation disk process was designed to perform the laboratory experiment. It included a dip tank containing water and a rotating disk with multiple samples attached to it [5]. See Fig. 23.1 [6]. For each revolution of the wheel, the test samples were alternately dipped into the tank of water and exposed to microorganisms in the ambient air. This work was carried out to identify polymers (used in medical items) that are most resistant to water and biofilm formation [5]. Items with a high resistance to wetting should have a lower tendency for growing biofilms (particularly those that require moisture).

Samples were selected from manufactured medical products frequently used in hospitals. They included a nebulizer (polyvinyl chloride), a syringe (polypropylene), a pipet (polyethylene), cannula tubing (polyvinyl chloride), a suction catheter (polyvinyl chloride with added plasticizers), and a male external catheter (latex rubber-based). A small section of each product was attached to the rotating wheel. Then the six medical samples were simultaneously tested for wettability and bio-

Fig. 23.1 A simple cartoon simulates a rotating wheel with multiple samples attached to it

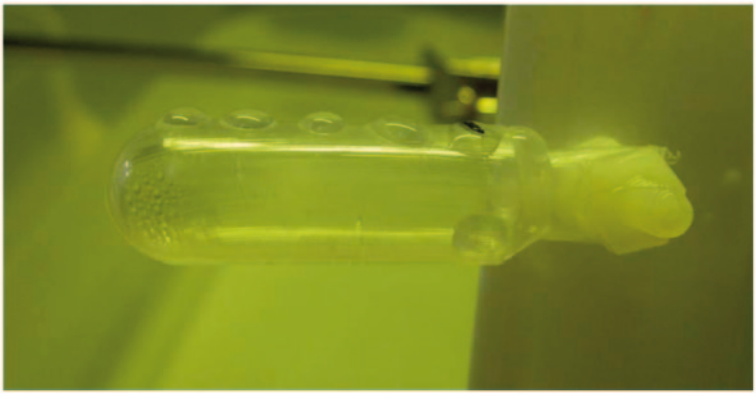


Fig. 23.2 Pipet sample (attached to the rotating disk) with water droplets after 3 weeks of testing

film formation. This process included a dip tank with deionized or distilled water and a disk designed to rotate at three revolutions per minute (RPM). The continuous motion of the wheel through water and air ensured a mix of microorganisms with water in the dip tank. This study lasted about 5 weeks. The water level in the dip tank was kept constant and the temperature, pH, and water conductivity were monitored regularly. Since the average pH value was 6.07, the overall experiment took place under slightly acidic conditions.

Wettability tests and biofilm evaluations were performed on the samples each week. The wettability test involved depositing sessile water droplets on the surface of each sample. Biofilm formation was observed by the naked eye, magnifiers, and microscopes. After the first week, the latex catheter displayed a wetted surface with a light coating that was visible to the naked eye. All of the other samples held water drops on their surfaces. At the end of week three, the pipet's surface appeared to be

about 90 % dry. See Fig. 23.2. The other samples had more and various degrees of wetting, while the latex catheter was wetted with an increased amount of surface coating (film).

At the completion of the experiment, SEM images were taken of the latex catheter test sample using a Field Emission SEM JEOL JSM-7400F. They clearly displayed spheres and rod-like shapes, which suggest two basic types of bacteria: the sphere (coccus) and the rod (bacillus). Next a Gram stain test was performed on the sample. The test results indicated the presence of both Gram-negative and Gram-positive bacteria.

Useful conclusions were made for the investigation of biofilm formation using medical materials. The special rotation disk process served as a successful method for accelerating wettability of sample surfaces and biofilm formation using an intermittent flow of water. The experiment showed polyethylene to be the most suitable polymer for use in a hospital environment. The next best polymer appeared to be polyvinyl chloride (used in the nebulizer and the cannula tubing samples) and the worst one was the latex rubber-based material [5].

In addition to the rotation disk process, drip flow reactors are used to simulate biofilms growing on surfaces without a continuous flow of water.

Other biofilm reactors exist. For each, cells can be adsorbed in a reactor without the use of chemicals [7]. To study the effect of biofilm formation on pressure drop, tubular reactors are often used. Packed-bed reactors are good for imitating flows in nature like porous media. Biofilm reactors generally operate in one of the three ways. They either use a recycle mode, a recycle method with a mixing chamber, or a no recycle format. If no recycling is involved, then the fluent enters the reactor and the effluent leaves.

Also static growth techniques allow biofilms to develop. However, they have limited amount of nutrients. Examples of this method include various spills left for long periods of time on surfaces such as floors and counter tops [8].

Test Samples Continuously Exposed to Moisture

Researchers in Japan developed a reactor for accelerating biofilm formation on various materials using a continuous flow of water [9]. They used a recycle method that included a mixing chamber. Clean water (provided by Suzuka City, Japan) was pumped at a rate of about 6 L/min. It flowed from a tank through an acrylic column holding the specimens and then back to the tank. A fan was used to blow ambient air into the tank so that germs and microorganisms could mix with the water. This technique allowed a continuous flow of water over the samples throughout the duration of the experiment. Some test items used were glass, stainless steel, and pure nickel sheets. After several days of immersion, they were removed from the system and examined. Observations obtained from an optical microscope with a 3D analysis function (Keyence VW-9000) displayed tower-like shapes, which correspond to biofilms on the substrates [9]. These findings confirmed the presence of biofilm on

all of the samples. The investigators used a low vacuum SEM-EDX (Hitachi Miniscope, TM-1000) to obtain additional information about the samples. They found out that the pure nickel sheets contained more biofilm than the glass specimens (ITO: Indium Tin Oxide and FTO: Fluorine-doped Tin Oxide) and that silicon, calcium, iron, and zinc, were incorporated into the biofilms [9, 10].

This reactor for biofilm formation was also used to form and study fogged glass [11]. Fogged glass causes poor visibility and can decrease the efficiency of a heliostat. A heliostat is an instrument consisting of a mirror mounted on an axis moved by clockwork so that a sunbeam is steadily reflected in one direction. For this investigation, dyeing by crystal violet solution confirmed the existence of bacteria causing biofilm. Also examination of the glass specimens with microscopes (Keyence VW-9000 and a low vacuum SEM-EDX Hitachi Miniscope, TM-1000) confirmed the existence of biofilms [11].

In addition, the researchers used their special reactor to grow biofilms on carbon steel (JIS SS400), aluminum sheets, and copper sheets [12]. Once again they used the same microscopes to confirm the existence of biofilms on these specimens.

References

1. Schmitt J, Flemming H-C (1999) Water binding biofilms. Water Sci Technol 39(7):77 (Elsevier).
2. Percival S, Malic S, Cruz H, Williams DW (2011) Introduction to biofilms. In: Percival SL et al. (eds.) Biofilms and veterinary medicine. Springer-Verlag, Berlin, p 44, 46
3. Costerton JW (1999) Introduction to biofilm. Int J Antimicrob Agents. PubMed.gov.
4. http://en.wikipedia.org/wiki/biofilm
5. Barry, DM, McGrath, PB, Rotation disc process for accelerated assessment of biofilm formation on medical materials, Materials Technology: Advanced Biomaterials, Vol. 30, Issue B1 (Feb. 2015) pp. 33–37
6. http://en.wikipedia.org/wiki/ferris_wheel#Mediaviewer/file.ferris_ups: Ferris Ups CC by-SA 3.0, En User: Ridetheory—drawn by En User: Ridetheory
7. Qureshi N, Annous B, Ezeji T, Karcher P, Maddox I (2005) Biofilm reactors for industrial bioconversion processes: employing potential of enhanced reaction rates. Microb Cell Factories 4:24. doi:10.1186/1475-2859-4-24
8. Garrett TR, Bhakoo M, Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. Prog Nat Sci 1053 (Elsevier Limited and Science in China Press) pp.1049–1056
9. Kanematsu H, Hirai N, Miura Y, Tanaka M, Kogo T, Itho H (2013) Various metals from water by biofilm from ambient germs in a reaction container. Proceedings of MS&T 2013, Montreal, Canada, pp. 2154–2161
10. Kougo T, Yamamoto Y, Naiki A, Ogino Y, Kanzaki T, Kanematsu H, Ikigai H, Itoh H (2013) Proposal for evaluation to biofilm formation on metal oxides in atmospheric exposure type circulation water systems. Mem Suzuka Natl Coll Technol 46:81–84
11. Kanematsu H, Kogo T, Itoh H, Wada N, Yoshitake M (2013) Fogged glass by biofilm formation and its evaluation. Proceedings of MS&T 2013, Montreal, Canada, pp.2427–2433
12. Kanematsu H, Hirai N, Miura Y, Itoh H, Kuroda D, Umeki S (2013) Biofilm leading to corrosion on materials' surfaces and the moderation by alternative electro-magnetic field. Proceedings of MS&T 2013, Montreal, Canada, pp.2761–2767

Chapter 24
New Evaluation Techniques for Biofilm in Materials Science

Hideyuki Kanematsu and Dana M. Barry

Abstract Biofilm and biofouling lead to various industrial problems and/or benefits. To solve the problems or to utilize them, it is very important for us to evaluate biofilm and biofouling properly and correctly. From materials science viewpoint, some analytical techniques familiar to the discipline would be better. A few of them include optical microscopy, scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDX), atomic force microscopy (AFM), etc. Even though many other spectroscopies might be applied to the evaluation of biofilm, some aforementioned representative techniques are described in this chapter. The following discussion will benefit the engineers, researchers, and students of materials science and engineering, who plan to use these techniques to solve problems.

Introduction

As described in Chap. 8, we already have many tools to analyze biofilm formation using biology and bacteriology. However, the biofilm generally forms on material surfaces, so the investigation of the entire process (biofouling) should also be approached from the areas of materials science and engineering. From this viewpoint, any conventional biological evaluation techniques are still not very useful to materials science engineers and researchers. The most friendly and easy-to-use tools for material scientists and engineers are microscopes, particularly the optical one. If the microscope is used more, the analysis and the countermeasure to control biofilm would be much advanced. When one would observe the biofilm on material surfaces, by using an optical microscope, it would often look like tiny water bubbles so that one might pass them over without noticing them as a biofilm. Researchers

H. Kanematsu (✉)
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

D. M. Barry
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, US
e-mail: dmbarry@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_24

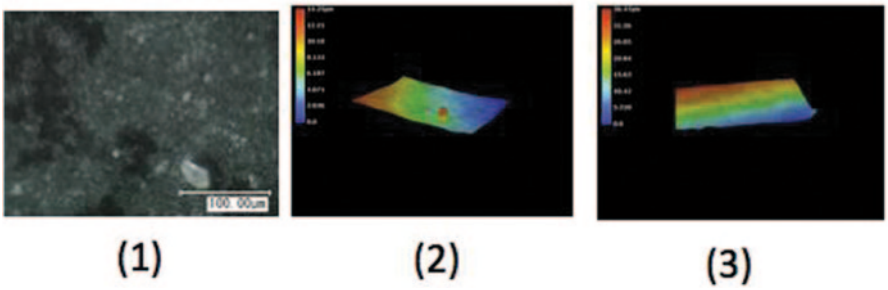


Fig. 24.1 Biofilm on a polymeric material observed by an optical microscope

need practice in order to properly investigate biofilms using optical microscopes. From the practical viewpoints, the evaluation technique should be easy to use, to evaluate properly, etc. Also the quantitative and qualitative characteristics should be very good. How should a simple image such as the water bubble provided by the optical microscope be modified for material scientists and engineers? We shall start this chapter with this basic question.

3D Images of the Optical Microscope

Microscopes are one of the powerful tools to visualize biofilms. As already described in Chap. 8, the use of pigments seems to be the easiest way to fix a biofilm. However, dyeing techniques and the use of fluorescence microscopes are hard to relate to. Therefore, it should be fine for us to modify the conventional way of using optical microscopes. In this chapter we would like to introduce some of our own examples. We begin with an optical microscope with a 3D function. At the focal point, the surface image of the specimen can be taken properly. Then the stage of the microscope is moved slightly around the focal point and some photos are taken. Next, all the images are superimposed into one image. In this way, the 3D image of a biofilm can be obtained. An example is shown in Fig. 24.1. One of the authors usually uses the 3D optical microscope (Keyence VW9000) shown in the figure. Since the biofilm usually has a tower-like or mushroom-like shape, the similar image should be obtained to fix biofilm formation. It would sometimes lead to a deviated result. Images in Fig. 24.1 show some of the typical biofilms confirmed by the optical microscope. The substrate was a polystyrene film and the biofilm formed on it artificially, when the specimen was immersed in a laboratory biofilm reactor (LBR) for a certain amount of time. Figure 24.1(1) shows the usual optical microscope. After being taken out of the LBR, the specimen was observed by using the optical microscope. Figure 24.1(1) shows the usual optical microscopic image. At this point, it is hard for us to judge which specimen would be a biofilm and which would not be. Then various parts of the specimen's surface were colored, depending on the height. For example, the red color corresponds to a higher place, while the blue

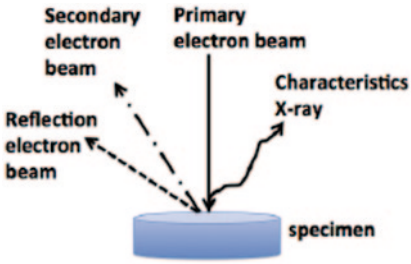


Fig. 24.2 Probe and signals for SEM-EDX

to a lower one. In this way, the 3D profiles could be presumed and grasped by the observer. Actually, the user could rotate the figure virtually and almost obtain the tower-like biofilm image, as shown in Fig. 24.1(2). On the other hand, Fig. 24.1(3) belongs to the specimen immersed in the LBR for a shorter amount of time. This photo shows only a monotonous gradient. Of all the figures, it shows only an inevitable tilt of the specimen. The biofilm image should involve a sea–island structure, as shown in Fig. 24.1(2). This technique is easy for us to use and intuitive in a way to understand.

Scanning Electron Microscopy (SEM-EDX)

Figure 24.2 shows the schematic principle of SEM. The probe for the analysis is an electron beam. When it is irradiated on material surfaces, it reacts with surface atoms. Then the secondary electron is emitted after the reaction, or the initial electron could be reflected after the reaction with surface atoms. In such a way, the reflected or secondary electron has a certain amount of energy relating to the surface atoms. Therefore, the secondary or reflected electrons basically create the surface image corresponding to the difference of elemental concentration and distribution. However, the irradiated electron beam also produces characteristic X-rays emitted from the surface area after the reaction between the irradiated electron beam and the surface atoms. Therefore, the X-ray also reflects the characteristics of surface elements. Basically, SEM microscopy evacuates air in the chamber, so that the electron beam would irradiate the specimen's surface correctly. On the other hand, the main component of biofilm is water. Therefore, the original shape would be lost inevitably, when it would be placed in the vacuum chamber of the SEM. However, the biofilm tends to incorporate some kinds of elements into it during its growth process, such as silicon, calcium, iron, zinc, etc. Since the image and X-ray energy by SEM-EDX originally reflect the component differences on material surfaces, the area where biofilm would exist could give graded effects. As a result, the biofilm area would take black color, while others would take white or gray, even though the shape of the biofilm might collapse. The extent of contrasts would become clearer with the biofilm growth. However, low vacuum SEM with EDX has been recently developed. Using this type of SEM-EDX, the biofilm is preserved relatively well in the vacuum chamber, even though in this case the original biofilm is collapsed.

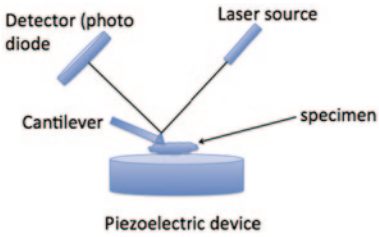


Fig. 24.3 Schematic principle of AFM

Atomic Force Microscopy (AFM)

Atomic force microscopy (AFM) is the technique used to obtain information about surface morphology and a profile in nanometer order and to visualize everything. Figure 24.3 shows the schematic principle of AFM. As shown in the figure, the AFM probe is set at the front edge of the cantilever. The probe is delicately attached to the surface of a specimen by minute forces and scanned with the feedback function. This way the amount of deflection for the cantilever would be kept constant by adjusting the distance between the probe and surface. Then the surface profile and situation could be visualized.

Generally, AFM measurement can be classified into three categories by the measurement process. The first is the contact mode where the probe moves along the surface of the sample. Corresponding signals are sent (as a result of this movement) so that a three-dimensional profile can be viewed. The second is the noncontact mode where the piezoelectric device senses the interatomic force between the cantilever and surface without any direct contact. The third is the taping mode where the probe vibrates up and down, tapping the surface and scanning the surface. This technique enables us to observe the surface with high accuracy and resolution.

Generally, the AFM has the following advantages. It does not need a vacuum environment in order to observe specimens. And in addition, it does not need any special and troublesome pretreatment for making measurements. These are merits for using the AFM to visualize biofilm. It is also possible to visualize bacteria through this. Since the existence of bacteria strongly suggests the presence of biofilm, the use of an AFM should be an excellent way to observe biofilm. Recently, various combinations have been developed for AFM use with other advanced analytical methods. They should be very useful for current and future work in evaluating and visualizing biofilms.

Gene Analysis (Community Analysis)

This method is not considered to include any techniques for materials science and engineering. However, the confirmation and the approach from the viewpoint relating to bacteria must be indispensable for biofilm research, since it is originally

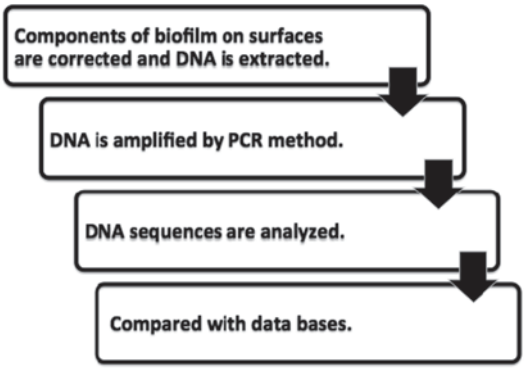


Fig. 24.4 Procedure for gene analysis (community analysis) for biofilms

produced by bacterial activity. As already described, the conventional culture technique is not appropriate for biofilm bacteria, even though it has had great contributions to the cases for planktonic bacteria. A biofilm is a complicated system and composed of many unknown bacteria, etc. as well as bacteria that cannot be cultured in the conventional way. Fortunately, the remarkable development of molecular biology made it possible for us to analyze the complicated system and bacteria based on the way of gene analysis. Figure 24.4 shows the general procedure for the community analysis.

First, the biofilm formed on specimens should be scraped and scoured, and collected for each specimen. Some chemicals might be added and heating process might be required. DNA is extracted after the pretreatments. For the extracted DNA, a certain area of rDNA (16srDNA for bacteria and 28SrDNA D2 for fungi) is amplified by the PCR method. In this way, DNA is refined. Then DNA arrangements are analyzed, using capillary electrophoresis with DNA sequencers. Being compared with libraries in databases, the related species are analyzed and the category that the bacteria belong to is fixed. This analysis makes it possible for us to identify the target bacteria in biological classifications. However, it would be very hard to fix the bacterial name completely in many cases.

This biological method might be very hard for students, engineers, and researchers for materials science and engineering. However, they can outsource the analysis to some institution. The combination of gene analysis and materials-scientific observations will elucidate a major information and knowledge about biofilms that is unknown and help us to explore the microbial world very well. All this data and information will assist in solving the problems produced by biofilm and those occurring in industries.

In this chapter, some representative methods to evaluate, analyze, and visualize biofilms are introduced and explained. All of them are used to understand biofilm as a whole entity. However, there are many other advanced analytical methods in materials science and engineering, for example, transmission electron microscopy (TEM) [1], ultraviolet visible spectroscopy (UV-VIS) [2], fourier transformation infrared spectroscopy (FTIR) [3], Raman spectroscopy [4], time-of-flight secondary

ion mass spectromicroscopy (ToF-SIMS) [5] etc., in addition to fluorescent microscopy and confocal scanning laser microscopy (CSLM)as already described in Chap. 8. These analyzes are already carried out for some special purposes and/or special laboratory cases. Readers interested in these techniques should refer to *Fundamentals of Biofilm Research* [6].

References

1. Reese S, Guggenheim B (2007) A novel TEM contrasting technique for extracellular polysaccharides in in vitro biofilms. Microsc Res Tech 70(9):816–822
2. Kanematsu H, Kogo T, Sano K, Noda M, Wada N, Yoshitake M (2014) Nano-composite coating on glasses for biofilm control. J Mater Sci Surf Eng 1(2):58–63
3. Cao B, Shi L, Brown NR, Xiong Y, Fredrickson KJ, Fomine FM, Marshall MJ, Lipton MS, Beyenal H (2011) Extracellular polymeric substances from *Shewanella* sp. HRCR-1 biofilms: characterization by infrared spectroscopy and proteomics. Environ Microbiol 13(4):1018–1031
4. Efeoglu E, Culha M (2013) Surface-enhanced Raman scattering for biofilm characterization. Spectroscopy 28(11):36–41
5. Hua X, Yu X-Y, Wang Z, Yang L, Liu B, Zhu Z, Tucker AE, Chrisler WB, Hill EA, Thevuthasan T, Lin Y, Liu S, Marshall MJ (2014) In situ moleular imaging of a hydrated biofilm in a microfluidic reactor by ToF-SIMS. Analyst 139:1609–1613
6. Lewandowski Z, Beyenal H (2014) Fundamentals of biofilm research, 2nd edn. Boca Raton: CRC Press.

Chapter 25
Future Scope

Hideyuki Kanematsu and Dana M. Barry

Abstract As already described in other chapters, biofouling and biofilm formation affect our daily lives in various ways. Being able to solve the daily problems that they cause may also help solve many industrial problems. For these purposes, it is very important for us to approach the problems from the viewpoint of materials science and engineering, especially since biofilms mostly form at the interface between organisms and materials. Therefore, it is also important for us, materials scientists, engineers, students, and teachers, etc., to understand biofilm and its related processes in order to investigate the topics in the right and appropriate way. This chapter presents topics for the future that will assist in achieving the purposes mentioned above.

Introduction

We have seen and tackled many topics relating to biofilm from the fundamental aspects to application cases. Particularly, we tried to introduce examples of biofouling processes and biofilms from the viewpoint of materials science and engineering. Therefore in this final chapter, it is very informative and meaningful to deal with the future topics for this field. As future topics, we would like to deal with four problems at this point. Two are related to the fundamental aspects of biofilms and the other two are related to their applications.

H. Kanematsu (✉)
Department of Materials Science & Engineering, National Institute of Technology, Suzuka College, Suzuka, Japan
e-mail: kanemats@mse.suzuka-ct.ac.jp

D. M. Barry
Clarkson University's Department of Chemical & Biomolecular Engineering and its Center for Advanced Materials Processing (CAMP), Clarkson University, Potsdam, New York, US
e-mail: dmbarry@clarkson.edu

© Springer International Publishing Switzerland 2015

H. Kanematsu, D. M. Barry (eds.), *Biofilm and Materials Science,*
DOI 10.1007/978-3-319-14565-5_25

Future Topics for Fundamental Aspects of Biofilm Problems

Since the notion of a biofilm was proposed in the late 1970s, the understanding of it has been making progress gradually. From the academic fundamental viewpoint, lots of fundamental information has been already clarified, as discussed in this book. When we think about the future, two topics can be mentioned as important agendas.

One of them is EPS (extracellular polymeric substance) and its role. As already described in Chap. 18, EPS has many kinds of roles and surface characteristics. Basically, it is the main component for biofilm shape and structure. To understand EPS more precisely would lead to a better understanding of biofilm itself and the surface phenomena relating to biofouling.

Polysaccharides have been considered as the main components for EPS. However, scientists have recently become aware of the fact that proteins occupy a portion of EPS, as already described in Chap. 18. As for the structure, it has been clarified pretty well. The ratio of polysaccharides and proteins changes according to the binding force between EPS and bacteria. However, we do not know precisely how the change would affect various interfacial phenomena, described in this book. This should be one of the future topics. Since the structure of EPS is usually very complicated, the analysis for EPS might be very hard in many cases. However, it would be worth investigating to solve the interfacial problems between biofilm and material substrates.

The other fundamental future topic is the redox reaction in the biofilm. Since EPS is one of the main components for biofilm, this topic might be related to EPS itself to some extent. However, the redox reaction involving EPS and many other components incorporated from the environment, or from the substrate, has not been fully clarified yet. Particularly for corrosion problems, this clarification would be an important topic for the future. To achieve the purpose, new ways of research should be devised. This endeavor might need to include new electrochemical measurements in addition to some new laboratory biofilm reactors.

Standard Evaluation Methods for Biofilm Formation

As described in Chap. 21, antibacterial effect of materials could be evaluated by a standard test. The name of the test is called Film Covering Method and standardized as ISO22196 [1]. It was basically established for plastic surfaces. However, it could be applied to many other industrial materials and their products. The following process is one of the modified examples, used for other metallic plates.

The bacteria that needs to be investigated for a certain purpose must be cultured in a bouillon of 18 ml for a certain time (eg., 18 h, at 35 °C). It is diluted, so that the bacterial number would be 10^5/ml. The suspension solution with bacteria of 400 μl is dropped (down) on the industrial specimen and then that spot of the specimen

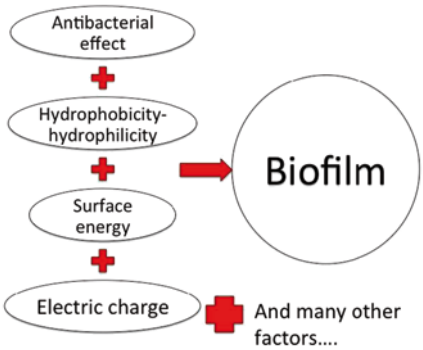


Fig. 25.1 The concept of biofilm and antibacterial effect

is covered with polyethylene film (4 x 4 cm). Next, the specimen is moved to a sterilized petri dish and kept at 35 °C for 24 h (relative humidity 99 %). Then the bacteria attach and grow on the specimen while the film is washed out to the petri dish. The bacterial suspension solution of 100 μl is inoculated with an agar media and cultured at 35 °C for one night. The following morning, the colony number of bacteria on the agar media is counted and evaluated for the antibacterial effect of the material [2].

This method already prevails and is often used as a modified version in many cases. If one wants to evaluate a new material and its antibacterial effect against a certain bacteria, he or she might be able to use it in their laboratory or ask research institutes to assist with the study. Probably, this is the only standardized method to check the antibacterial effect. As already described in Chap. 21, there is a certain close relationship between the antibacterial effect and the ability for biofilm formation. Therefore, one might get a chance to evaluate a biofilm's capability of forming for some industrial products by using this method to some extent. However, the concept of biofilm is larger than the antibacterial effect as shown schematically in Fig. 25.1. There are many factors relating to biofilm. Antibacterial effect is just a factor making a contribution to the biofilm. Therefore, antibacterial effect might not be appropriate to evaluate the biofilm formation characteristics in some cases. From this viewpoint, other appropriate standardized methods for industrial products will be needed to evaluate the biofilm formation capability.

Biofilm and Biomaterials

In many industrialized and advanced countries such as the USA, EU countries, and some advanced Asian countries including Japan, medical treatments have been making progress. Some advanced countries have rapidly aging societies. Therefore, in those societies infection problems are often very critical. For example, there are many hospital infection problems. Actually, about 100,000 patients lose their lives annually due to hospital infection problems. One of the main causes for these

problems is biofilms. Unfortunately, bacteria in biofilm have very strong resistance to antibiotics [3]. Therefore, materials that would not allow bacteria to grow (on surfaces) are required for use in medical environments. From this viewpoint, new and better medical materials need to be developed in the future. This task will be another important research topic for materials science and engineering.

Conclusions

This is the final section of our book, so our brief conclusions are presented. As we have introduced many aspects of biofilms, the concept and the discipline started just in the late 1970s. Since then, many revolutionary techniques and technology have been proposed and developed. However, this discipline is still developing. Firstly, the notion was proposed in the medical field. It prevailed there and then moved to the environmental and industrial fields. However, keep in mind that biofilm is generally produced at the interface between bacteria and materials. Therefore, properties and characteristics of the various materials should be investigated much more in order to help solve the problems. Also to enhance solution finding for industrial problems, materials scientists and engineers need additional knowledge, information, and new approaches. We hope that this book has clarified the meaning, importance, and relationship of biofilm and materials science, to essential topics involving medical, environmental, and industrial problems to name a few.

References

1. Technical Committee ISO/TC 61, Plastics, Subcommittee SC 6, "Ageing, chemical and environmental resistance": ISO 22196:2011(en), Measurement of antibacterial activity on plastics and other non-porous surfaces, 2011. http://www.poudrox.com/poudrox/wp-content/uploads/norme_ISO221961.pdf
2. Kanematsu H, Ikigai H, Yoshitake M (2008) Antibacterial eco-plating based on HACCP and biofilm. Bull Iron Steel Inst Jpn 13(1):27–34
3. Shunmugaperumal T (2010) Biofilm eradication and prevention—a pharmaceutical approach to medical device infections. Wiley, Hoboken