Exploring the City of Biofilms: An Engaging Analogy-Based Activity for Students to Learn Biofilms

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Abstract

Multicellular biofilms constructed by microbes are key aspects of microbiology with significant implications in various fields, including medicine, environmental science, and biotechnology. While bacteria spend nearly all their lives in biofilms, many students do not study them in detail in a course setting. Consequently, students have misperceptions that microbes exist as free-living single-cell organisms and cannot understand the biofilm lifestyle accurately. Here, I present a comprehensive and engaging lab lesson plan designed for students to explore the concepts of biofilm lifestyle and compare biofilms to cities using a think-group-share strategy. Students are asked to individually define biofilms and relate them to living in a city, followed by forming small groups, and then discussing them as an entire class. The class will understand the different aspects of biofilms in each step of the life cycle from colonization to dispersal. Subsequently, the students will put their knowledge into practice by completing an activity where they must sort different functional activities into the following steps in the biofilm life cycle: colonization, formation, maturation, and dispersal. This analogy-based activity encourages comparative analysis and fosters long-term learning. I observed that students actively participated in the learning activity, which also cultivated a sense of class community during the sharing session. An end-of-module review activity six weeks later showed that the students could still recall the knowledge learned during the lesson. This lesson activity has several advantages: it is easy for the teacher to implement within 20–30 minutes and convenient for the students to engage with biofilm biology.

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Learning Goals

Students will:

◊ correlate their life experiences to what they are learning in course settings.
◊ practice peer learning and contribute to one another’s understanding.
◊ understand the stages, components, and activities of the biofilm life cycle.
◊ From the Microbiology Learning Framework:
  » How do microorganisms interact with their environment and modify each other?
  » Why do most bacteria in nature live in biofilm communities?

Learning Objectives

Students will be able to:

◊ define biofilm.
◊ define quorum sensing.
◊ explain the stages of the biofilm life cycle.
◊ explain quorum sensing and its role in the biofilm life cycle.
◊ predict the features of a biofilm and functions of biofilm components.
◊ analyze the roles of bacterial cells in a biofilm and its life cycle.
◊ From the American Society for Microbiology General Microbiology Learning Outcome Examples:
  » List the stages of biofilm formation and maturation.
INTRODUCTION

Microbial life is often characterized by two major lifestyles: free-swimming planktonic cells or sessile aggregates (biofilms). Biofilms are multicellular communities composed of microbes encased in a self-produced exopolymeric matrix (1). Biofilm formation is mainly controlled by quorum sensing (QS), which involves the exchange of chemical signals among microbes to communicate and coordinate group behavior based on population density (2). These biofilms play crucial roles in various contexts, from promoting bacterial survival to causing persistent infections in medical settings.

Biofilms are not limited to a single microbial species and they can involve complex communities of various microorganisms (3). In environmental ecosystems, biofilms can form on submerged surfaces, such as riverbed rocks, providing habitats for diverse microorganisms or participating in predator-prey interactions (4, 5). In humans, the microbiota in the organs and tissues play important roles in the normal functioning of human cells. Biofilms can also be engineered to perform productive tasks, such as bioremediation of pollutants (6–8) or prevention of pathogen colonization. However, biofilms can be harmful to humans and the functioning of society. Industrial pipelines can also fall victim to biofilm-based biofouling, causing blockages, reduced efficiency, and corrosion (9). Additionally, medical devices like catheters can become breeding grounds for harmful biofilms, leading to recurring infections in patients (10).

The life cycle of a biofilm involves five general stages (11, 12). It begins with attachment, where individual microbial cells adhere to a surface. As these cells multiply, they start producing exopolymeric substances (EPS), forming a protective matrix and initiating the growth phase. During biofilm maturation, the biofilm structure becomes more complex as various microbial species join the community. This is followed by the dormant phase, where growth slows due to nutrient limitations. In the final stage of dispersal, biofilm cells detach and spread to new surfaces, initiating the cycle anew (13, 14). Throughout these stages, QS plays a crucial role by coordinating gene expression and regulating the behavior of biofilm inhabitants (2), contributing to the overall adaptability and resilience of these microbial communities.

Biofilms are a rising topic in the field of microbiology, but they are mostly absent or minimally described in biology or microbiology curricula. Hence, many biology and medical science students may not have studied the topic formally or may not be familiar with applying their lived experiences in the city. If the definition and biofilm life cycle are presented without contextualization, students may simply memorize the information without fully comprehending the underlying concepts (15). However, rote learning via memorization seldom leads to long-term learning (16), resulting in information being forgotten after the end of the module (17). Given that learning is the ability to remember and apply the information even after a period of disuse, it is evident that pure memorization is an inefficient learning technique. Hence, collaborative learning is an effective solution for university students, as it helps students to retain knowledge better, practice problem solving skills, and increase their confidence (18). This also improves the engagement and communication of students and teachers in course settings and prepares students for their future careers (19).

Here, I employ a simple think-group-share activity in the microbiology course to help students associate the concept of biofilms with real-life experiences or prior knowledge in the context of city life. I will then ask the students to apply their newly acquired knowledge in an association activity that connects the biofilm life cycle to the rise and fall of a city. The activity can be implemented in a face-to-face, online, or hybrid classroom for a course in microbiology that focuses on biofilm physiology or biofilm applications. In this lesson article, I describe the activity which will enable the students to collaborate, learn from one another, and create a sense of community within the classroom. My activity can be used as a primer for other published lessons that either consist of laboratory experiments (20) or biofilm applications in clinical and environmental settings (21, 22). By providing an interesting and engaging introduction to deeper topics, my lesson plan contributes new educational materials that can be used in various courses involving general biology, microbiology, microbial ecology, and microbial diseases.

Intended Audience

The intended audience is college students in a biology course that covers microbiology topics but can also be applicable for high school or non-biology major students. The lesson has been tested in a small class of around 5–10 students and a large class of nearly 100 students. For a large class, online discussion software is encouraged to promote discussion among students. This lesson can be applied to any mode of classroom teaching (online, hybrid, or face-to-face).

Required Learning Time

This lesson requires 20–30 minutes in a single 60-minute class.

Prerequisite Student Knowledge

Students are required to have basic knowledge of biology and relevant information from their own lived experiences. While students should have basic knowledge of microbes, this lesson does not require prerequisite knowledge of biofilms. Student variation in knowledge and experience is expected to lead to productive group and class discussions.

Prerequisite Teacher Knowledge

The teachers are required to possess a basic understanding of microbiology, especially bacterial physiology. They also need to have some knowledge about the biofilm life cycle for the categorization activity of different stages in the life cycle. The teacher can also alter the activity for different topics where biofilms may participate (Table 1), such as chronic infections and biofilm engineering in wastewater treatment.

SCIENTIFIC TEACHING THEMES

Active Learning

In order to promote engagement and facilitate the construction of new knowledge, the lesson incorporates three active learning components: a think-group-share activity, a class brainstorming session, and finally a small group categorization activity. The implementation of such active learning techniques has been demonstrated to enhance student
motivation and engagement, foster confidence in students, and encourage active participation in class discussions (23). Furthermore, think-group-share activities have been found to enhance critical thinking skills (24). The categorization activity prompts students to engage in discussions and categorize different phases in the biofilm life cycle, enabling them to apply their prior knowledge to a novel task. This approach is more effective for learning compared to a presentation of answers to students (25). By integrating these activities into a single class period, students are afforded the opportunity to interact with all the necessary information required to meet the stated learning objectives.

Assessment
The main objective of this activity is to actively engage students with a new topic in microbiology. As such, the assessment focuses on participation and completion instead of summative assessment. Completed notes from both the think-group-share activity and the categorization worksheets are collected and reviewed. The categorization worksheets can be used for assessment, if desired. To aid in self-assessment, students are encouraged to participate in a post-activity discussion and refer to the lecture notes. The lecture notes allow them to identify any mistakes and seek clarification. Hence, the assessment approach adopted here is entirely formative, facilitating ongoing monitoring of students’ learning progress. Research has shown that formative assessments are linked to improved scores on subsequent summative assessments, such as tests and exams (26).

Inclusive Teaching
The lesson leverages the diverse lived experiences of students and incorporates them into the learning process. By connecting course topics to personal experiences, student interest and motivation are heightened, leading to more effective learning (27). For instance, students from different countries and majors may possess varied personal experiences, so this lesson encourages students to apply their prior personal knowledge and experiences. It shifts the focus from deficits (what students do not know) to growth, utilizing existing knowledge to facilitate the acquisition of new information.

Moreover, this lesson fosters inclusivity by providing students with various participation options: individual writing, small group discussions, and whole-class discussions. Inclusive teaching aims to ensure that no student feels excluded by offering structured opportunities for engagement so that all learners can benefit. Each student is encouraged to participate in a manner that suits their comfort level and confidence, thereby fostering a classroom community where everyone feels valued and heard. Consequently, students gain confidence and are more likely to participate in novel teaching methods in the future.

LESSON PLAN

Summary
This lesson is composed of three parts: (i) a think-group-share activity where students define the biofilm life cycle and city life individually followed by in groups; (ii) a brainstorming session where students discuss their answers together as a class; and (iii) a categorization activity where students sort different functional activities into steps in the biofilm life cycle (i.e., colonization, formation, maturation, dispersal, and recolonization/cycle restarts; Supporting File S1). View the notes section on each slide to see how the lesson is explained.

In Supporting File S2, a spreadsheet for the activity is supplied. The lesson can be finished in 20–30 minutes (Table 2), which is enough for students to discuss their answers with their peers.

Preparation

Face-to-Face Teaching
The teaching and learning materials should be prepared prior to the lesson. Ensure that the PowerPoint presentations (Supporting File S1) are functioning properly on the teaching computer. Print one copy of the worksheet (Supporting File S2) for every student or group. Adjust the materials accordingly to best suit the class, such as by changing the scenario (clinical or environmental settings) or adding additional informational slides.

Online Teaching
Supporting Files S1 and S2 should not be posted until after the completion of the activity, as they are designed for in-class discussion. It is important to become familiar with the use of an online meeting platform, such as Zoom or Microsoft Teams. To facilitate group discussions, group breakout rooms will be assigned to students at random or manually from the class list. The teacher can also move from one breakout room to another, to check on students’ progress or discuss ideas with students.

In-Class Activity

Comparing Biofilm Communities to a City
This activity has been implemented as an opening exercise for a lesson on microbial biofilms. Students participate in the exercises based only on their prior understanding of microbiology, and it is recommended not to teach the students about biofilms in advance. In this exercise, the students will offer their own experiences and background information, which enriches the group conversations. The students will be evaluated mostly on participation and completion instead of correctness. Remind students to avoid electronic devices, emphasizing that the purpose of this activity is to employ prior knowledge and group discussion.

Comparing Biofilm Communities to a City
This activity is conducted in a think-group-share format. First, display the prompt and instructions (Supporting File S1) to the class. Instruct the students to individually respond to the prompt, “Biofilms are multicellular communities made of microbes, similar to cities. Based on your life in the city, what do you think are the comparative features of the biofilms?” without consulting their peers or any reference materials. Allow five minutes for the students to contemplate the prompt and write down their answers. One minute prior to the end of the individual response, the students will be notified to conclude their writing.

Upon completion of the individual activity, instruct the students to form groups of three to four individuals. The number of students in each group can vary in classes of different sizes. The groups can be pre-assigned, random, or student self-selected. These groups will work together for the rest of this activity. Proceed to the second slide of the instructions
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(Supporting File S1) and inform the students that the designated speaker from each group will be expected to present their answers to the class. Instruct the groups to collate all their ideas and decide the answers that all group members agree upon. Allow each group to read out their answers for face-to-face teaching or type into the Chat function of an online meeting platform. After each group has shared their results, generate collective answers and show them to the class for reference.

Categorization Activity: Biofilm Life Cycle as a Process of Forming Cities
Engage in a brief discussion regarding the biofilm life cycle (Supporting File S1) and provide relevant examples to enhance understanding, if necessary. Instruct the students to relate city organization to the biofilm life cycle. Once the students are prepared to commence the activity, navigate to the eighth slide of Supporting File S1, which contains the same information as the worksheet (Supporting File S2). Allocate 5–10 minutes for student groups to finalize the assignment and address any queries during this time. If desired, collect the answer worksheets to further assess their accuracy.

Summary Activity
It is important to consolidate the students' knowledge about biofilms and the biofilm life cycle at the end of the activity. Different teaching techniques that are useful for large classes with different majors can be adopted. Employ a traditional lecture with visual aids, such as slides and diagrams of biofilms, as this will help most of the students visualize the concepts and understand the complexity of biofilms. Next, explore examples of biofilms in action, ranging from wastewater treatment to medical device infections. By discussing these examples, this will enable students from diverse backgrounds and interests to understand the broader impact and implications of biofilms in human health, industry, and the environment.

Optional End-of-Module Review Activity
This is an optional activity, which serves as both a review of previously covered material on biofilms and a demonstration of the students' progress in learning. It is intended to occur either towards the end of the course or after the completion of the biofilm section. Provide the questions via show of hands or the Poll function on an online meeting platform (Supporting File S3). These questions prompt students to define biofilms and associate various bacterial behaviors to the biofilm life cycle. To assess if the activity is useful, instruct the students to comment on their learning progress (Supporting File S3).

TEACHING DISCUSSION

General Observations
To date, this lesson had been conducted twice: once with a small class of 10 students and another time with a larger class of 100 students. All students from both classes present on the day of the lecture participated in the entire activity. The students demonstrated a high level of engagement and active involvement throughout the lesson. When approaching the ‘Comparing biofilm communities to a city’ activity, students generally identified the ways in which nomads and city dwellers live before associating how city life is comparable to life in a biofilm. For example, one common comparison was relating highly established telecommunications in a city to quorum sensing in biofilms. Students in the small class tended to write more detailed explanations, while students in the large class tended to write short and general definitions.

Summary of Student Responses
At the end of the think-group-share activity, student groups shared their collectively agreed-upon city-like properties of a biofilm. The students generally reached a consensus on associating biofilms with a city (Table 3).

Furthermore, the association of the biofilm life cycle with city activity proved engaging and sparked thoughtful group discussions, where participation was generally high. The biofilm life cycle was associated with the rise and fall of a city, where microbes colonized a suitable place first, followed by biofilm growth and maturation, and dispersal for migration to new areas. Working in groups enabled students to complete the activity even if everyone did not possess prior knowledge of biofilms. Based on our discussions and a review of the collected students’ materials, it became evident that the lesson’s learning objectives had been accomplished by the students.

Observations About the Optional Review Activity
At the conclusion of the module, the optional review activity was conducted to evaluate if the students could remember their knowledge about biofilms. The questions were provided to the students for completion, and the polls were used to test student knowledge. Additionally, I asked them to compare the ease of completing the activity now versus at the start of the activity. Many students expressed that they had more detailed answers, where they incorporated the knowledge they had acquired throughout the module and could answer the questions more easily. Students also exhibited enthusiasm while answering the questions, as this exercise served as a reminder of their significant growth and enabled the students to recognize the extent of their progress. Ultimately, this activity served to reinforce the accomplishment of the learning objectives outlined for this lesson.

Potential Improvements and Adaptations
If time is a constraint during lessons, this activity can be easily adapted to incorporate a homework assignment. Instead of allocating class time for students to associate the biofilm life cycle with city life, students can be assigned to complete this assignment independently at their own pace. In this adapted format, a subsequent class session should be allocated to discussing their answers in small groups and with the whole class. However, it is important to note that many students may be tempted to search for answers from external sources rather than relying solely on their background knowledge. To address this issue, you may consider requesting that students write a preliminary comparison of biofilms to city life.

Furthermore, this lesson can be expanded to encompass additional learning outcomes and serve as a platform for broader discussions on the different roles of biofilms in diseases and the environment. While the lesson primarily focuses on pathogenic disease, it can be generalized to any core topic where students can apply their personal experiences or background knowledge. Relevant questions such as “How is the biofilm life cycle important and relevant to infections?” or “How is the biofilm life cycle important in ecology, and what are the roles of biofilms in the environment?” can be posed to the students. Through subsequent activities or discussions
focusing on these questions, students can be prompted to apply, analyze, or evaluate further information. This lesson serves as an excellent tool to assess the existing knowledge of the class and can aid instructors in identifying common misconceptions regarding the core topics of biofilms and their life cycle.

**SUPPORTING MATERIALS**

- S1. City of Biofilms – Introduction slides
- S2. City of Biofilms – Worksheet
- S3. City of Biofilms – Review slides

**ACKNOWLEDGMENTS**

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Table 1. Different examples of the roles of biofilms that can be used according to teaching needs.

<table>
<thead>
<tr>
<th>Application</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Settings and Public Health</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic infection</td>
<td>Biofilms attach to human organs, tissues, and implants, leading to prolonged infections that are difficult to treat with antibiotics.</td>
<td>Bjarnsholt T. 2013. The role of bacterial biofilms in chronic infections. APMIS 121:1-58.</td>
</tr>
<tr>
<td>Environmental Settings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ecological Settings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotic interactions</td>
<td>Microbes interact with wildlife, such as animals and plants, where predator-prey interactions or symbiosis can occur.</td>
<td>Seiler C, van Velzen E, Neu TR, Gaedke U, Berendonk TU, Weitere M. 2017. Grazing resistance of bacterial biofilms: A matter of predators’ feeding trait. FEMS Microbiology Ecology 93.</td>
</tr>
</tbody>
</table>
Table 2. Biofilm physiology and life cycle lesson timeline.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Description</th>
<th>Estimated Time</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preparation for Class</strong></td>
<td>Class materials preparation</td>
<td>A worksheet for each student or group.</td>
<td>5 minutes</td>
</tr>
<tr>
<td><strong>In-Class</strong></td>
<td>Define biofilms based on analogy to a city. Individual work (Think-Group-Share)</td>
<td>1. Place the directions on the board. 2. Hand out worksheets (if using them).</td>
<td>5 minutes</td>
</tr>
<tr>
<td></td>
<td>Define biofilms based on analogy to a city. Group/class work (Think-Group-Share)</td>
<td>1. Students group in 3–4 persons and discuss their answers until the whole group agrees. 2. When groups are done with this, ask each group to read their answers. 3. Determine the answer the entire class agrees on and write it on the board. 4. Collect student worksheets for assessment.</td>
<td>10–15 minutes</td>
</tr>
<tr>
<td></td>
<td>Class brainstorming on the analogies comparing the biofilm life cycle to city life</td>
<td>1. Put up the instructional slides on the board. 2. Hand out analogy worksheet. 3. Give students time to complete and give them a chance to ask you questions. 4. Collect worksheets for assessment if desired.</td>
<td>5–10 minutes</td>
</tr>
<tr>
<td><strong>Optional End-of-Module Review Activity</strong></td>
<td>Review activity</td>
<td>1. Ask students to answer questions on biofilms and their life cycle. Put the questions on the board. 2. Allow students time to consider the questions. 3. Ask students to answer via raising hands or responding to an online poll.</td>
<td>10–15 minutes</td>
</tr>
</tbody>
</table>
Table 3. Students’ analogous comparison of a biofilm and a city.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Biofilm</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Most Common</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stability and settled life</td>
<td>Sessility and attachment to surfaces</td>
<td>Settling down in a fixed location, e.g., homes</td>
</tr>
<tr>
<td>Communication</td>
<td>Quorum sensing</td>
<td>Communication networks</td>
</tr>
<tr>
<td>Housing</td>
<td>Biofilm matrix</td>
<td>Buildings</td>
</tr>
<tr>
<td>Transport</td>
<td>Water channels and microbial motility</td>
<td>Roads and train networks</td>
</tr>
<tr>
<td>Common resources</td>
<td>Secreted metabolites or nutrients</td>
<td>Public goods, such as services</td>
</tr>
<tr>
<td>Waste</td>
<td>Storage of waste products</td>
<td>Refuse bins and landfills</td>
</tr>
<tr>
<td><strong>Less Common</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collective decision</td>
<td>Quorum sensing</td>
<td>Democracy</td>
</tr>
<tr>
<td>Motility of the masses</td>
<td>Bacterial colonization and dispersal</td>
<td>Emigration and migration</td>
</tr>
<tr>
<td>Protection</td>
<td>Barrier against predators and antimicrobials</td>
<td>Defense against invaders</td>
</tr>
</tbody>
</table>
REFERENCES


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