Quorum Sensing: Organisms Communicating and Coordinating

Overview

We live in an ever-changing world. Many people crave information about those changes. As a result, new means of communicating are continually evolving. People originally relied on word of mouth. With the invention of the printer, newspapers spread information to more people, more quickly. Later, telephones, television, cell phones and the Internet increased both the rate at which people could communicate and the number of people that the information reached. For many young people, it is hard to imagine a world without online social networks, cell phone texting, emails and television.

The cells in the human body and the cells in all living things also crave information. Cells are designed to both receive and send messages from their environment and others in their environment. From dusk to dawn, environmental parameters like temperature, pressure, and oxygen fluctuate in a periodic or arbitrary manner. Since the survival and well-being of organisms depends on how they respond to environmental change, it is important to be able to capture environmental cues and respond accordingly. For example, when the temperature drops, humans sense the temperature change and protect themselves by wearing warmer clothes. In addition, humans have developed means of communication, as simple as shouting to as complex as satellites, to alert as many people as possible of an upcoming event. Doing so has helped humans to survive life-threatening events, coordinate actions, and ultimately be more fit in uncertain environments.

Until recently, scientists thought that such means of communication and coordinated action was the characteristic of animals only. Recent discoveries, however, reveal that even the simplest organisms, bacteria, have a similar capacity to communicate with each other by secreting specialized, small molecules that are then received by other bacteria and interpreted accordingly. Bacteria are mainly using this form of communication to find out what the population density is in their neighborhood in order to decide whether or not to act, and, if they act, in what way. This ability is called quorum sensing, and it will be the focus of this unit.

Unit Goals and Objectives

Goal: Students will learn about a relatively new and applicable field of biology.
Objectives:
- Describe the basic cellular structure of a bacterium.
- Develop a simple understanding of how and why bacteria monitor their environment and communicate with other bacteria.
- Appreciate the significance of quorum sensing to living things.

Goal: Students will experience the excitement of applying mathematics to a current topic.
Objectives:
- Model exponential growth as a function of time.
• Manipulate algebraic equations to model interactive processes.
• Perform iterative calculations with numerical values written in scientific notation.

Goal: Students will experience how mathematical modeling simulates theoretical behavior of a proposed system.

Objectives:
• Use given parameters to create a mathematical model of changing population values and corresponding concentrations of cellular quantities that support the cells’ signaling mechanism.
• Develop an understanding of how unicellular bacteria can communicate and function in an almost multicellular way.
Lesson 1  The Microscopic World of Bacteria

Before quorum sensing is discussed, it is necessary to understand the organisms involved in the process. The study of microbes offers a unique lens for understanding more complex life forms. Microbiologists have come to realize that microbes have continuously been recycling and transforming the chemical elements of the planet on which we live, and the applications of microbiology include medicine, personal and public health, the environment, agriculture, the atmosphere and space, and industry.

What is a Microorganism? What is a Bacterium?

A microorganism or microbe is a form of life that is invisible to the naked eye, and microbiology is the study of the characteristics and behaviors of such organisms. The word microbe was first used in 1878, although the existence of invisible organisms was discovered centuries earlier. In tandem with the development of increasingly powerful magnifying lenses and the emergence of cell theories, seventeenth-century scientists explored the structure of plant tissue, human tissue, and body fluids. Dutch shopkeeper Antoni van Leeuwenhoek (1632-1723) examined scrapings from his tongue and created the earliest drawings of common bacteria in the human mouth.

The smallest forms of microbes are bacteria. Bacteria are single-celled microbes that have a circular strand of DNA not found in a nucleus and ribosomes for the production of proteins. Like all living cells, they are surrounded by a plasma membrane that regulates what enters and leaves the cell. Bacteria also have a cell wall for protection and support, and most have a capsule. The capsule offers protection against predators, but is also important in the adherence of bacterial cells to surfaces.[1] As such, bacteria are classified as prokaryotes, in contrast to eukaryotic cells like plant and animal cells that have a cell nucleus and a variety of membrane-bound organelles. Numerous examples of eukaryotic microorganisms exist. Yeast is an example of a microorganism that is classified as a fungus. Amoeba, paramecium, euglena, just to name a few, are examples of microorganisms classified as protists. All of these single-celled eukaryotes exhibit a much more complex cellular structure than bacteria. Despite their simplicity relative to the rest of the cellular world, bacteria are still able to carry out the complex behavior of quorum sensing.

Figure 1.1: Simple Drawing of a Bacterium.
Bacteria are the most numerous single-cell microbes, and the Earth’s soil holds the largest collection of bacteria. Bacteria display the greatest diversity in their ability to grow and multiply in varied environments. While it may not be pleasant to consider, the human body is home to trillions of microbes. An adult human has approximately 10 trillion cells, but the number of bacterial cells living in or on the same person is 10 times that number! If they could be gathered, the 100 billion bacteria that live on our skin would form a ball the size of a medium pea. The 15 trillion bacteria lining an empty digestive tract would fill a ten-ounce soup can to overflowing, and a single bowel movement can remove 100 trillion bacteria from the body.

The good news is that most bacteria are not pathogens, or disease-causing organisms. Many actually work to keep their human host, you, healthy. Since they live on and in a person, it benefits them if you stay healthy and continue to provide them with a place to live, food, warmth, and moisture. In 1885, German pediatrician Theodor Eschenrich isolated and identified “Bacterium coli” from the stool of newborns. This best-known of intestinal bacteria was renamed Escherichia coli and is recognized today as *E. coli* (Figure 1.2). In your intestines, *E. coli* normally helps with the digestion of food, the production of vitamins, and the prevention of other harmful bacteria growing in the same area. Each individual bacteria is rod-shaped.

Microorganisms are unicellular, meaning that they consist of just one cell. However, “investigators are finding that in many ways an individual bacterium is more analogous to a component cell of a multicellular organism than it is to a free-living, autonomous organism. Bacteria form complex communities, hunt prey in groups and secrete chemical trails for the directed movement of thousands of individuals.” Slime bacteria, myxobacteria, which are found predominantly in soil samples, are unique in that their whole lives are affected by cell-to-cell interactions. “What's more, they prey on other bacteria, feeding voraciously in wolf-pack fashion.” To provide a specific example, *Myxococcus xanthus* forms predatory spheres of millions of individual cells as a means of capturing prey in an aquatic environment thereby displaying the advantage of cells working together rather than individually.

A similar phenomenon has been observed in various species of microorganisms that form aggregates, called biofilms, and render them more resistant to antibiotics. It is precisely the
quorum sensing mechanism that bacteria utilize to communicate and coordinate their actions during biofilm formation. The topic of biofilm formation will be revisited again in the unit.

**Questions for Discussion**

1. What is a microorganism?

2. What is a bacterium?

3. Distinguish between the terms microorganism and bacterium.

4. How are eukaryotes and prokaryotes different? How are they alike?

5. What is meant when a bacteria is called a pathogen?

**Bacterial Growth**

Bacteria are the smallest cellular organisms, typically measuring only a few micrometers in length. When the environmental conditions are favorable, bacteria grow to a fixed size and then reproduce through asexual reproduction through a process that is called binary fission. During this process, the bacteria’s genetic material, which is just a circular piece of DNA, replicates and creates two identical copies, before the bacterium simply splits into 2 identical bacteria.

![Figure 1.3: A bacterium undergoing binary fission.](image)

**ACTIVITY 1-1 Bacterial Growth Simulation**

**Objective:** To introduce the phenomenon of bacterial growth through a simulation.

**Materials:**
- Handout QS-H1: Bacterial Growth Simulation Activity Worksheet
- Small cup of uncooked rice

1. In this simulation, each grain of rice represents a single bacteria cell. Each cell divides into two cells every 20 minutes. Place 1 grain of rice in the square on the table below labeled $t = 0$ min to represent the initial number of bacteria cells.

2. Twenty minutes later, the cell has divided into two cells. Model this cellular division by placing two grains of rice in the square labeled $t = 20$ min. Continue to model the cell division by
placing the appropriate number of grains of rice on each of the labeled squares through the square where \( t = 120 \) minutes.

3. Record the number of grains of rice in each cell and then continue to indicate how many grains you predict would be in the cells for \( t > 120 \) minutes. (Do not count out the rice grains.)

<table>
<thead>
<tr>
<th>( t )</th>
<th>0 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
<th>80 min</th>
<th>100 min</th>
<th>120 min</th>
<th>140 min</th>
<th>160 min</th>
<th>180 min</th>
<th>200 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N )</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.1: Rice Recording**

This pattern of growth is called **exponential growth** and can be modeled with a function in which the independent variable, time, appears in the exponent. This function is called an **exponential function**. The bacteria population, \( N \), can be expressed as a function of the number of minutes, \( t \), by \( N(t) = 1 \times 2^{(t/20)} \). The value 1 represents the initial number of cells, and the base of 2 designates that the value is doubling. The number of times that the population doubles is the number of 20-minute intervals that occur within \( t \) minutes, which, in this case, is determined by dividing \( t \) by 20. Other ways to represent this relationship include \( f(t) = 1 \times 2^{(t/20)} \) or \( y = 1 \times 2^{(t/20)} \).

4. Use the function \( N(t) = 1 \times 2^{(t/20)} \) to complete the table below, then compare your results to the numbers obtained in the simulation.

<table>
<thead>
<tr>
<th>( t )</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>120</th>
<th>140</th>
<th>160</th>
<th>180</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N )</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.2: Bacteria Population (N)**
Bacteria reproduction occurs asynchronously; this means that cell divisions do not instantly occur every 20 minutes. Thus, the number of bacteria cells is continually changing. In addition, bacteria die, therefore decreasing the number of cells. Even with these considerations, the function \( N(t) = 1 \times 2^{t/20} \) serves as a useful model for determining the number of cells for any number of minutes. For example, if \( t = 30 \) minutes, \( N(30) = 1 \times 2^{(30/20)} \) or simply \( 2^{1.5} \). A calculator can be used to approximate the value of \( N \) to be 3. For \( t = 90 \) minutes, \( N \) is approximately 23.

5. With the ability to use any number of minutes for the time, the number of bacteria can be represented as a continuous function, defined for \( t \geq 0 \). Graph this function on the axis below.

6. The function can also be altered to represent the growth for a different initial amount of cells. In the simulation, we began with one cell. Suppose we began the same cell division scenario at \( t = 0 \) with 3 grains of rice (representing three bacterial cells).

   a. How many grains of rice would be needed in each of the first few squares?

   b. Rewrite the function \( N(t) \) to reflect the initial condition \( N(0) = 3 \) to produce these values?

   c. What would the cell division scenario function \( N(t) \) be if \( N(0) = 100 \)?

**Growth Rate**

Consider an initial population of 100 bacterial cells that doubles every 20 minutes. After 20 minutes, there are 200 cells and after 40 minutes there are 400 cells. This situation is shown in Table 1.1 below. How would you describe the rate of growth? If you calculate the ratio of adding 100 cells in 20 minutes, that is an average growth rate of 5 cells per minute, but an additional 200 cells in the next 20 minutes indicates a growth rate of 10 cells per minute.
Table 1.3: Growth of Bacteria with $N(0) = 100$ and $N(t) = 100 \times 2^{(t/20)}$

**Questions for Discussion**

6. How should we complete the population chart shown in Table 1.3?

7. Complete the table. Round each answer to the nearest whole number.

8. What do you notice about the population values at $t = 10$ min and at $t = 30$ min? at $t=70$ min and $t=90$ min?

9. Select any two times 20 minutes apart and not shown on the table (e.g., 25 and 45). Determine the population at these times. Do they display the same relationship?

Growth rate describes how fast the population is growing and can be determined by using the function for the number of bacteria over time. Although the doubling phenomenon appears to hold for each 20-minute interval, the rate at which the population is growing is increasing over time. The average growth rate for any 20-minute interval can be calculated by dividing the change in population by the change in time.

In general, the average rate of change, as time changes from $t_1$ to $t_2$, can be determined using the difference quotient $\frac{N(t_2) - N(t_1)}{t_2 - t_1}$. Applying this formula to the growth that occurred during the first 60 minutes results in an average growth rate of $700/60$ or approximately 12 bacteria cells per minute.

**ACTIVITY 1-2 Rates of Change**

**Objective:** Explore and understand the difference between average and instantaneous rate of change.

**Materials:**

- Handout QS-H2: Rates of Change Activity Worksheet

1. Use the values for $N(t) = 100 \times 2^{(t/20)}$ from Table 1.3.
a. Complete the table below to calculate the **average rate of change** (or Average Growth Rate) for each of the following twenty-minute intervals.

<table>
<thead>
<tr>
<th>Interval</th>
<th>Change in time (min)</th>
<th>Number of bacteria at start of interval</th>
<th>Number of bacteria at end of interval</th>
<th>Difference Quotient</th>
<th>Average Growth Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>from 0 to 20 min</td>
<td>20</td>
<td>N(0) = 100</td>
<td>N(20) = 200</td>
<td>(200 - 100) /20</td>
<td>5 cells/min</td>
</tr>
<tr>
<td>from 20 to 40 min</td>
<td>20</td>
<td>N(20) = 200</td>
<td>N(40) = 400</td>
<td>(400 - 200) /20</td>
<td>10 cells/min</td>
</tr>
<tr>
<td>from 40 to 60 min</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td>20 cells/min</td>
</tr>
<tr>
<td>from 60 to 80 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40 cells/min</td>
</tr>
<tr>
<td>from 80 to 100 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80 cells/min</td>
</tr>
</tbody>
</table>

**Table 1.4: Growth Rate Calculations**

b. What do you notice about the average growth rates over time?

2. The difference quotient can be used to calculate the average rate of change for any time interval. By using smaller and smaller time intervals, one can approximate the rate of change at a given instant in time.
   a. Use your calculator to complete the chart below.
<table>
<thead>
<tr>
<th>Interval</th>
<th>Change in time</th>
<th>Number of bacteria at start of interval</th>
<th>Number of bacteria at end of interval (nearest tenth)</th>
<th>Difference Quotient</th>
<th>Average Growth Rate (to nearest tenth) cell/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>from 60 to 70 min</td>
<td>10 min</td>
<td>N(60) = 800</td>
<td>N(70) = 1131.4</td>
<td>(1131.4 - 800) /10</td>
<td>33.1 cells/min</td>
</tr>
<tr>
<td>from 60 to 65 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>from 60 to 62 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>from 60 to 61 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>from 60 to 60.5 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.5: Rate of Change Calculation

b. What do you notice about the values of the average growth rate as the time interval grows smaller?

3. The average growth rate approaches what appears to be a fixed value. This phenomenon relates to the calculus concept of limit. As the time interval used in the calculation becomes smaller, the sequence of average rates gets closer to a value that is referred to as the instantaneous rate of change. This value describes the rate at which the population is changing at an exact time (in this case, at 60 minutes). Calculus techniques produce a value of 27.72 cells per minute for the instantaneous rate of change when t = 60 minutes. What calculations could you do to more accurately approximate the instantaneous rate of change at 60 minutes?

4. Extension. Determine a method to graphically show the process of going from an average rate of change to an instantaneous rate of change. (Hint: Graph the function and plot the points you used in 2.a. above.)

Questions for Discussion

10. How do bacteria reproduce?

11. How would linear growth be different from exponential growth?

12. In a natural environment, could exponential growth occur indefinitely? Why or why not?

13. Describe the bacteria growth scenario that can be written as $N(t) = 5 \times 3^{(t/40)}$, where $N$ is the number of bacteria and $t$ represents the number of minutes.
Exponential Growth

It is interesting to note that bacterial growth has three phases. When a population of bacteria first encounters a new environment, either in the lab or in nature, it tries to adapt by expressing the genes that would allow the bacteria to grow optimally in that specific environment. For example, if there is just one carbon source (e.g., glucose, galactose, or lactose) available, then the bacteria will express the necessary genes to metabolize that nutrient and, thus, have energy to grow and reproduce. However, during this phase that is called the lag phase, the growth rate is very slow, because bacteria are struggling to adapt to the new environment and preparing the machinery (ribosomal proteins, enzymes, etc.) for fast growth.

Once this machinery is in place, bacteria enter a second phase that is called the exponential phase (also known as logarithmic or log phase). During this phase, cells grow very rapidly, following an exponential curve. A population can go from thousands to a billion cells in a matter of hours, as division time goes down to minutes. For example, E. coli has a doubling time of 20 minutes when conditions are favorable. Factors that may affect this are the food source and temperature.

How many E. coli cells there will be in 24 hours, if we start from just one cell? We can use the same function with an initial condition of \( N(0) = 1 \), therefore \( N(t) = 1 \times 2^{(t/20)} \). Since 24 hours = 1440 minutes, \( N(1440) = 1 \times 2^{72} \) or \( 4.72 \times 10^{21} \) which is over 4.7 sextillion cells (1 sextillion = \( 10^{21} \))!

However, all good things come to an end, and, unless the bacterial colony is constantly supplied with a fresh medium, the nutrients will start to deplete and toxic waste compounds will accumulate in the environment. At this stage, bacteria are stressed and stop growing as they are starved of nutrients. This final growth phase is called the stationary phase, and the exponential patterns for population and growth are replaced by other functions. Figure 1.4 shows the phases of the exponential growth curve.

**Figure 1.4:** An Exponential Growth Curve.
Questions for Discussion

14. Why is the growth rate slower during the lag phase than the exponential phase?

15. Why is the growth rate slower during the stationary phase than the exponential phase?

16. What variables may alter the growth rate during the exponential phase?
Lesson 2  A Conceptual Model of Quorum Sensing

By definition, a quorum is the minimal number of members of a group who must be present for the valid transaction of business. In terms of bacteria, it is the minimum number of bacteria that must be present before they change their behavior. For example, a certain number of bacteria must be present before a certain species will begin to glow or before a different species will go from benign to harmful.

Introduction to Quorum Sensing

Quorum sensing (QS) is a phenomenon that limits “these transactions” or behaviors to occurring only above a certain population density. It is a fundamental mechanism that enables communication among organisms such as bacteria and insects. QS is a mechanism of stimulus-and-response that enables organisms to perceive their environment and make decisions on that information in a decentralized way. Social insects, such as bees and ants, employ quorum sensing, and it has also been observed in simple microorganisms such as bacteria.

When honey bees (Apismellifera) want to make a new nest, a small portion of worker bees leave the swarm to scout for new sites. Once a new site is found and its quality is assessed, the worker bee returns to the swarm and recruits other workers to evaluate the new potential nest. When the visitors to that site have reached a quorum (15-20 bees), they return to the swarm and notify it that a new nest site has been found. This causes the entire swarm to fly to the new nest location. In a similar fashion, colonies of the ant Temnothoraxalbipennis use an analogous quorum-sensing process when they have to find a new nest site.

Scientists and researchers have studied QS extensively in the microscopic world and in bacteria specifically. A few decades ago, people thought of bacteria as living a life of solitude, unaware of their surroundings and without a definite plan other than to consume nutrients. Surprisingly, bacteria are capable of sophisticated behaviors of high complexity and have the mechanisms to carry them out efficiently. As a result, the simplest of all cellular organisms can communicate and cooperate to perform a wide range of multicellular behaviors, such as dispersal, foraging, biofilm formation, and “chemical warfare”. [5]

One example of quorum sensing is observed in biofilm formation. While the term may be new, everyone has experienced biofilms in one form or another. Perhaps the most familiar one is plaque, the biofilm that covers our teeth. Have you ever slipped in a stream or a pool because of the slime covering the rocks or the lining? The slime is due to a mass of green bacteria, a biofilm, growing over the surface. Have you ever had an acute ear infection? It was most likely caused by a biofilm. Biofilms can be beneficial and are used in water treatment systems and for cleaning up after soil groundwater has been contaminated by a spill. They may even be used to microbially extract minerals that have industrial uses. In other cases, they can be harmful and costly. Biofilms are associated with toxic algal blooms, clogging of pipes, fouling of machinery, and some diseases, such as bacterial endocarditis and Legionnaires’ disease.
In most environments, bacteria are observed living as individual cells passively floating in a fluid environment. They are said to be **planktonic** in this state. However, in certain environments, most bacterial species may quit their free-living, planktonic lives and begin to attach next to each other on a surface. These cells use short, hair like structures on their cell surface to adhere and anchor themselves permanently on a surface in their environment. Once this happens, these first colonists start to build an adhesive substance, called the **extracellular polymeric substance (EPS)** that holds the biofilm together and renders the recruitment of more bacteria easier. The cells are able to communicate via quorum sensing, and through growth and newly recruited cells, the biofilm grows bigger. Just as humans and other animals come together to form communities to improve protection from some external force, so do bacteria. Living as a group in a biofilm gives added protection. A good example of this is found in pathogenic bacteria. Living as a biofilm makes the bacteria as a whole much more resistant to antibiotics. The antibiotics will most likely affect the bacteria on the edges of the biofilm, but the bacteria in the center of the biofilm are less likely to come in contact with the antibiotic and therefore survive.

For quorum sensing to occur, bacteria must speak to each other. Whether they are bacterial cells or cells within your own body, when cells want to speak to each other, they do so using chemicals. Bacteria can produce these chemicals to “speak” to other bacteria and then release the chemical into the environment. Other bacteria recognize these chemicals. In this manner, bacteria can turn on group behaviors that improve their chances of survival. Bacteria often use chemical “words” that only other members of their own species can understand. This way the conversation can be very private. They can also use other chemical “words” that all bacteria, no matter what species they are, can understand. In this way, different populations of bacteria can converse and at least be aware of one another.

**Questions for Discussion**

1. Define quorum sensing in your own words.

2. Describe a behavior of humans that is analogous to quorum sensing.

3. State some of the behaviors that bacteria exhibit as a result of quorum sensing.
4. State two examples in which biofilms are harmful.

5. State two ways in which biofilms may be helpful.

6. What is the survival advantage to bacteria in forming a biofilm?

**Basic Mechanism of Quorum Sensing**

Quorum sensing works at a cellular level - cells sense their environment, integrate the quorum-sensing signal, and respond accordingly. Through quorum sensing, bacteria have evolved signaling networks that enable them to perceive the bacterial population density around them. In different bacterial species, quorum sensing can involve different biochemical molecules or regulatory sequences, but the *modus operandi* (way of operation) is surprisingly similar. The QS mechanism is a cell-to-cell signaling system that involves producing, secreting, and sensing small signaling molecules, which are called **inducers**. A special type of protein called an **enzyme** synthesizes this molecule. This enzyme can speed up the production of the inducer and is generically called **autoinducer synthase**.

Think of a single bacterium cell in a bacterial culture that wants to perform a specific action (for example, start to emit light – see Figure 2.3) once the density of the culture reaches a certain point. Assume that all bacteria in the culture produce the quorum-sensing inducer. To do this they must express or use the segment of DNA that codes for the enzyme to make **mRNA** which will then be read by the ribosomes to synthesize the autoinducer synthase. The autoinducer synthase in turn constructs the inducer molecules from essential materials that the cell obtains from the environment. If we make the analogy to the building of a table, then the enzyme is the carpenter, the cell is the factory that produces furniture, and the environment is the forest that provides the wood (essential materials) that the carpenter uses. For now, assume that the expression of these enzymes and, in turn, the production of the inducer molecules occur at a constant rate.

![Figure 2.2: Basic Model Of The Cellular Machinery Implicated In Quorum Sensing](image)

Inducer molecules are produced by cells and are moving freely in and out of the cells through diffusion. Once they are in, they can bind to receptor proteins R that exist in the cell. The
receptor-inducer complex can now perform specific functions that the receptor alone could not. One common function is to bind to specific positions in the DNA and then to activate the expression of downstream genes. In the scenario that is depicted here, the receptor-inducer complex activates the production of the Green Fluorescence Protein (GFP) that causes the cell to become fluorescent in high concentrations.

In bacterial systems, the inducer molecule is generally miniscule, so it can go in and out of the cell through the cellular membrane by diffusion. This will be the assumption of the model here, but keep in mind that there are cases where inducers need the help of special transporter proteins to get out of the cell. Another molecule that is important for quorum sensing is the receptor proteins R, which actually bind to the inducers where they are in close proximity to them. The likelihood of this binding event to occur is proportional to the concentration of inducer molecules and receptor proteins that are present in the cell. The higher the concentration of inducers and receptors, the higher the probability that these binding events will take place, and the more of the R-inducer complexes will be around. In fact, in some other systems, inducers don’t even get inside the cell, but are instead sensed by the receptor proteins that sit on the surface of the cellular membrane, bind to the inducers, and then propagate this signal downstream within the cell for further processing. These more complex systems will not be dealt with here, and it will be assumed that the inducers can move freely through diffusion.

The R-inducer complexes that form can perform various functions within the cell that neither the receptor proteins nor the inducer molecules could do alone. This is possible, because the binding of the inducer to the receptor protein changes the conformation of the latter and allows it to bind to other substrates, such as proteins, metabolites, or DNA sequences. One such function is binding to specific regions in the DNA that are called promoters and are located just before the region next to the gene. This is a process of high specificity, since the R-inducer complex recognizes and binds in a specific DNA sequence (about 10 nucleotides long on average) that is part of the promoter. This binding results in the activation or repression of the downstream gene’s expression. This means that the gene will (or will not, in the case of repression) be used to produce mRNA that, in turn, gets translated into a protein. In the example used in figure 8, this gene is the Green Fluorescence Protein (GFP) that causes the bacteria to absorb energy when a light source is available and then glow to green.

![Figure 2.3: Bacterium with Potential to be Luminescent](image)

The flasks above both contain *Vibrio fisheri*, a bacterium that has the potential to be luminescent. When found naturally free floating in the ocean, it does not luminesce due to its relative low
concentration (about $10^2$ cells/ml). See the flask on the left. But, when its concentration reaches a high cell density of about $10^{10}$ cells/ml, it will emit a blue-green light in a dark room. See the flask on the right. [6]

Questions for Discussion

7. What is an inducer?

8. How does the receptor molecule interact with the inducer and why?

9. What is an R-inducer complex?

10. What is the role of the promoter region of DNA in bacteria cells?

ACTIVITY 2-1 Bacteria: Bad or Good?

Objective: Research about benefits and harm of bacteria.

Materials:
Handout QS-H3: Bacteria: Bad or Good? Activity Worksheet

1. Using your personal knowledge, textbook, or the Internet, state three examples of how bacteria are useful or helpful to humans.

2. Using your personal knowledge, textbook, or the Internet, state three examples of how bacteria are harmful to humans.

3. From your group’s research choose one beneficial and one harmful example of bacteria-human interaction. Write a paragraph explaining each interaction.

Cases of Quorum Sensing in Nature

Quorum sensing in bacteria was first discovered in the bioluminescent marine bacterium Vibrio fischeri that colonizes the light organ of the Hawaiian squid Euprymna scolopes. When the bacteria achieve high colony densities, they are capable of bioluminescence, and the squid uses the light provided by the bacteria for masking its shadow and avoid its predators. [8] In this symbiotic relationship, both parties are happy. The squid has a lower likelihood of becoming the prey of another animal as it increases its stealth capacity, and the bacteria colonize an organ that is rich in nutrients and excellent for proliferation and growth.

Pseudomonas aeruginosa is a very versatile bacterium that can live in a wide range of ecological habitats such as soil, water, and vegetation. It is an opportunistic pathogen found in humans that causes a variety of infections in the urinary, gastrointestinal, and respiratory systems. It uses some weakness in a person’s defense system to cause an infection. In patients with HIV or cystic fibrosis, it can significantly raise the mortality rate. [1] It utilizes the quorum-sensing mechanism to coordinate adhesion, biofilm formation, and virulence factor expression that are necessary for the disease to progress in humans. [8] By forming biofilms, the bacteria become
more resistant to antibiotics since larger doses are necessary to disrupt the biofilm,[1] and, by monitoring their numbers and attacking all at once, they can increase their virulence. One small bacterium-releasing toxin will have little to no effect on a large organism such as a human. As the bacteria grow and divide, if they wait until there is a large enough number (monitoring their numbers by quorum sensing), then they can all release their toxins at once and overcome their host’s defense system.

Toxic spills are always an environmental concern. When an organic spill, such as an oil spill, occurs, the naturally occurring soil bacteria can use these chemicals as a food source. If these bacteria existed individually, their impact would not be as significant, but, because they have formed biofilms through quorum sensing, they have the potential to convert the toxic organic chemical into harmless products. This is referred to as bioremediation.[9]
Lesson 3  A Mathematical Model of Quorum Sensing

Introduction to the Model

In Lesson 2, the essential components - the inducer, the receptor and the R-inducer complex - in the quorum-sensing system were defined and the basic QS mechanism was described. This conceptual model was presented in words and diagrams, giving the reader a descriptive overview of microbiologists’ understanding about what happens inside cells during quorum sensing. In this lesson, another way to describe quorum sensing will be presented - a mathematical model.

It might seem strange to think of these microscopic entities and their interactions as having mathematical properties, but recall that a mathematical model is a simplified representation of a real-world entity and is often written as equations, formula and computer code. Such a quantitative model helps microbiologists understand how the cellular components that contribute to the phenomenon of quorum sensing change over time, is useful for making predictions about bacterial behaviors, and can be used to determine sensitivities to changes in the bacteria’s environment.

The range in magnitude of the numerical values that arise in this model is very wide. The number of bacteria present in an environment is typically expressed in the millions, billions or trillions! The environment in which these very large numbers of bacteria live is usually expressed in milliliters (0.001 L), microliters (1 × 10⁻⁶ L) and nanoliters (1 × 10⁻⁹ L). Although they increase over time, concentrations of receptors and inducers can start extremely small - often expressed in scientific notation with negative exponents. Thus, there is a delicate balance among the numerical values and their units in the mathematical calculations proposed in this model. While care should be given to “getting it right,” answers may vary, depending on the rules for rounding or the number of significant digits that are assigned to each value.

The dilemma of performing calculations on such a range of numerical values is literally “compounded” because, in most of the growth equations in this model, one answer is used to obtain the next one. This iterative process is an important mathematical tool for modeling and understanding growth over time - whether it is used to find bacteria populations or savings account balances! Further, the equations in this section’s model have been adapted from a set of differential equations, which in their typical form require calculus notation and processes to more accurately portray the growth of the quorum-sensing components.

To create a mathematical model, the concentrations of the different components will be considered, as will their rates of change. The concentration of a particular component C, typically denoting molecules per unit of volume (e.g., molecules/µL), will be represented in brackets as [C]. The microliter (denoted as µL) is one-thousandth of a milliliter and is often used when working with very small volumes. The concentration rate, which expresses the amount of change (difference) in concentration that occurs within a given time interval, is calculated as an average rate of change for that interval. For example, if the concentration of a component C changes from 10 molecules per µL at the start of the experiment (time equals to zero) to 100 molecules per µL an hour later, then the rate of concentration change is:
\[
\frac{\Delta [C]}{\Delta t} = \frac{[C]_{\text{after}} - [C]_{\text{before}}}{t_{\text{after}} - t_{\text{before}}} = \frac{100 - 10}{60 - 0} = \frac{3}{2} = \frac{1.5 \text{ molecules/µL}}{\text{minute}}
\]

where \([C]\) and \(t\) denote the concentration of component \(C\) and time, respectively, and \(\Delta\) denotes the “change in” those variables.

**Practice**

1. The concentration of a cellular protein changes from 5 molecules/µL at \(t = 0\) minutes to 60 molecules /µL when \(t = 10\) minutes. Find the rate of concentration change for this interval.

2. Rate of change can be calculated for decreasing quantities. Suppose that a protein’s concentration decreases rapidly from 125 molecules/µL to 84 molecules/µL in a 20-second interval. Determine the rate at which the concentration is decreasing on this interval.

3. Find the rate of concentration change for a protein that has a concentration of 0.015 molecules/µL at \(t = 14\) sec and a concentration of 0.121 molecules/µL at \(t = 35\) sec. Express your answer to the nearest thousandth.

**Representing The Changing Receptor And Inducer Concentrations**

As described in Lesson 2, the receptor proteins and inducer molecules lie at the heart of quorum sensing. While genes code for proteins, the proteins carry out the functions of the cell. Through the DNA and RNA, protein synthesis can occur at different rates, but proteins are also broken down. They may only last for minutes or be around for days. The balance between synthesis and degradation will determine the concentration of receptor proteins and the enzyme responsible for producing the inducer. This allows the bacteria to alter the level of a protein quickly in response to the stimuli in the environment.

This section addresses the production of bacteria as a mathematical model. There are two opposing phenomena that have to be taken into account to calculate the concentration of any molecule, either receptor or inducer: the production \((pC)\) and the degradation \((dC)\) of the molecule \((C)\) per unit time. The degradation or breakdown is usually a process that is independent of any variable other than time, while production may be dependent on the concentration of other molecules. In the case of the receptor protein \(R\) that is continually produced, its concentration rate can be expressed as follows:

\[
\frac{\Delta [R]}{\Delta t} = p_R - d_R \cdot [R]
\]

where \(p_R\) and \(d_R\) are the production and degradation rates of protein \(R\), respectively. These kinetic parameters may vary considerably depending on the quorum-sensing system and the particular bacterial species. Table 3.1 depicts some representative values for the parameters in our model.
**Parameter** | **Description** | **value**  
---|---|---
\( p_i \) | Inducer production rate (molecules per \( \mu L \) per min) | 0.01  
\( d_i \) | Inducer degradation rate (per min) | 0.001  
\( p_R \) | Receptor production rate (molecule per \( \mu L \) per min) | 0.05  
\( d_R \) | Receptor degradation rate (per min) | 0.005  
\( p_{GFP} \) | GFP production rate protein (molecule per \( \mu L \) per min). | 0.05  
\( d_{GFP} \) | GFP degradation rate (per min) | 0.005  
\( k_{fwd} \) | Forward rate of R-inducer binding (per mM per \( \mu L \) per min) | 10  
\( k_{rev} \) | Reverse rate of R-inducer binding (per \( \mu L \) per min) | 1

**Table 3.1:** Kinetic Parameters with Representative Values for the Quorum-Sensing Model

For example, if \( p_R = 0.05 \) molecules/\( \mu L \)/min and \( d_R = 0.005 \)/min, then this equation can be written as

\[
\frac{\Delta [R]}{\Delta t} = \frac{0.05 \text{molecules/} \mu \text{L}}{\text{min}} - \frac{0.005 \text{molecules/} \mu \text{L}}{\text{min}} \cdot [R]
\]

This equation reveals that the rate at which the concentration of \( R \) is changing is a function of the concentration of \( R \) and is determined by the difference between its rate of production (a constant 0.05 molecules/\( \mu L \) each minute) and the rate (expressed as a fraction of the current concentration) at which it is being degraded (0.005/min, which is the same as \( \frac{1}{2} \)% per minute).

Thus, if the receptor protein concentration at a certain time is 0.4 molecules/\( \mu L \), then the rate at which the concentration is changing at that time is 0.05 molecules/\( \mu L \)/min - (0.005/min)(0.4molecules/\( \mu L \)), or 0.048 molecules/\( \mu L \)/min.

An initial condition, such as the receptor protein concentration at \( t = 0 \), can be useful in solving for \([R]\) or for \( t \). In the current example, assume that the concentration is 0 molecules/\( \mu L \) at \( t = 0 \) minutes. Recalling the meaning of \( \frac{\Delta [R]}{\Delta t} \) and substituting 0.4 molecules/\( \mu L \) for \([R]\), one can solve for the time at which this concentration is attained.

\[
\frac{[R] - 0 \text{ molecules/} \mu \text{L}}{t - 0 \text{ min}} = \frac{0.05 \text{molecules/} \mu \text{L}}{\text{min}} - 0.005/\text{min} \cdot [R]
\]

Quorum Sensing  Student 21
\[
\frac{0.4 \text{ molecules/µL} - 0 \text{ molecules/µL}}{t-0 \text{ min}} = \frac{0.05 \text{ molecules/µL}}{\text{ min}} \cdot 0.005 \text{ (min)} \cdot \left( 0.4 \frac{\text{ molecules}}{\mu \text{L}} \right)
\]

\[
\frac{0.4 \text{ molecules/µL}}{t} = \frac{0.048 \text{ molecules/µL}}{\text{ min}}
\]

t \approx 8.3 \text{ minutes}

This example suggests that, when the initial concentration is 0 molecules/µL (at t = 0), the equation \( \frac{[R]_0}{t_0} = 0.05 - 0.005[R] \) can be solved for [R] as a function of t.

**ACTIVITY 3-1 Receptor Concentration**

**Objective:** Explore the behavior of the receptor concentration.

**Materials:**
- Handout QS-H4: Receptor Concentration Activity Worksheet
- Graphing Calculator

1. Solve the equation \( \frac{[R]_0}{t_0} = 0.05 \text{ molecules/µL/min} - (0.005/\text{min})[R] \) for [R] as a function of t.

2. Calculate the change in receptor protein concentration after 30 minutes, when the cell starts with zero receptor proteins initially. Use the parameters in Table 3.1 for the production and degradation rates. Verify by using the result of question 1.

3. Use a graphing calculator to investigate the behavior of the function found in question 1. Rewriting the result of question 1 as a calculator-ready function yields \( y = \frac{0.05x}{1+0.005x} \). After entering this function into Y1, you can perform a number of tasks.
   a. Create a table that displays the concentration as a function of time.
      i) Verify that the concentration (y) at x = 0 minutes is 0 molecules/µL.
      ii) Find the concentration of receptor proteins at x = 30. Compare your answer to the result in question 5 above.
      iii) Use the table to estimate the time for which the concentration is 0.4 molecules/µL. Change the interval value to produce a better estimate.

   b. Graph the equation in Y1. Make some observations about the behavior of the concentration based on the graph.

   c. In Y2, enter Y1/X. What does this new function represent?

   d. Observe values for Y1 and Y2 in a table. What is Y2 for x = 30 minutes? What is Y2 for x = 8.3 min?
e. How do these answers affect any conclusions about the rate of increase obtained from the graph?

4. Using the values in Table 3.1 for production and degradation rates, find the concentration of the receptor protein at \( t = 45 \) minutes when the concentration is 5.8 molecules/mL at \( t = 30 \) minutes.

You can similarly find that the concentration of the inducer molecule is described by the equation:

\[
\frac{\Delta [\text{inducer}]}{\Delta t} = p_i \cdot \delta - d_i \cdot [\text{inducer}]
\]

where \( p_i \) is the inducer production rate (molecules per volume per time unit), \( \delta \) (the density coefficient) represents the fraction of current bacterial concentration to the maximum concentration that can be achieved at that volume, and \( d_i \) is the degradation rate (per time unit).

**Question for Discussion**

1. The dependence of the inducer concentration on the density of the cells (through the density coefficient \( \delta \)) is an important factor of the quorum-sensing phenomenon. Why?

2. What is the largest possible value for density coefficient? Describe the bacterial population when that value is attained.

**Practice**

4. What is the density coefficient (\( \delta \)) for a bacterial population of \( 1.3 \times 10^7 \) cells if it exists in an environment capable of sustaining a population of \( 2 \times 10^{10} \) cells?
   \( \delta = \)

5. When the same population reaches a population size of \( 8.4 \times 10^9 \) cells, what is the density coefficient?
   \( \delta = \)

6. When the same population reaches a population size of \( 1.68 \times 10^{10} \) cells, what is the density coefficient?
   \( \delta = \)

7. A bacterial population doubles every 20 minutes. How does the density change in that 20-minute interval?

8. A certain environment is capable of sustaining a bacterial population of \( 2.2 \times 10^8 \) cells. If the density coefficient is 0.57, what is the current population?

9. Suppose that concentration of the inducer for the bacteria population described in question 8 was 0.02 molecules/\( \mu \)L five hours ago. Use the inducer concentration equation and the parameters from Table 3.1 to find the current concentration of the inducer.
11. In a bacterial solution, the concentration of the inducer is measured to be 1 molecule per µL. What is the density coefficient if the inducer concentration was measured to be 0.60 molecules per µL the previous day (i.e., 24 hours ago)? What is the size of the bacterial population if the maximum population size that can be achieved is $10^9$ cells? Use the parameter values given in Table 3.1.

**Developing a Population Model**

With the dynamics for the two primary components of the quorum-sensing phenomenon described in mathematical equations, we can develop a model for how the receptor and inducer concentrations change as a population grows. The following activity provides the opportunity to apply the equations and calculations in a given context.

**ACTIVITY 3-2 Simulating Bacteria Growth**

**Objective:** Gain an appreciation for the relationship between the receptor and inducer concentrations and bacteria population.

**Materials:**
Handout QS-H5: Simulating Bacteria Growth Activity Worksheet

Suppose that a certain bacteria divide synchronously every 30 minutes, and the bacteria live in a tube that holds 2 mL of rich media that can sustain a population of bacteria up to $10^8$ cells/mL. The culture started at time $t_0 = 0$ minutes, at which point 100 identical bacterial cells were inserted into the tube.

The receptor and inducer production equations developed above are shown below. You will use these equations to develop a table of values for a growing bacterial population. You will complete the four rows of the table as you address the four sets of questions and prompts in this activity.

$$\frac{\Delta[R]}{\Delta t} = \frac{0.05 \text{ molecules/µL}}{\text{min}} - \frac{0.005}{\text{min}} \cdot [R]$$

$$\frac{\Delta[\text{inducer}]}{\Delta t} = p_i \cdot \delta - d_i \cdot [\text{inducer}]$$

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Bacteria in the Tube</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density coefficient $\delta$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of receptor R (molecules/µL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of inducer (molecules/µL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.2: Bacteria Population**
**Bacteria Population**

1. What does the phrase “divide synchronously” mean?

2. Given that \( N(0) = 100 \), what function \( N(t) \) will model this population growth?

3. What is the maximum population that the tube will sustain?

4. Complete the first row of the table. When the population exceeds its maximum value, use the maximum population as the population value.

**Density Coefficient**

5. What is the density coefficient for a bacterial population that has reached its maximum?

6. How can the density coefficients for the population values in the table be determined?

7. Complete the second row of the table, calculating the density coefficients as the bacteria population grows over time.

**Receptor Concentration**

Even though it is reasonable to assume that there are receptors already present in the bacteria cells, for this activity let the initial concentration of the receptor protein be 0 molecules/µL at \( t = 0 \) min. Begin by reviewing the solution for question 5 in this section for finding the concentration of \( R \) when \( t = 30 \) min. Fill in the first two concentration values in row 3 of the table.

8. Set up the receptor production equation to model the change in concentration as time changes from 30 minutes to 1 hour. Use the parameters in Table 3.1 for the production and degradation rates.

9. Solve the equation to find \([R]\) at \( t = 60 \) min (1 hour). Enter the value in row 3 of the table.

10. Set up the appropriate forms of the receptor concentration equation to find successive values of the receptor concentration.

Use the same sequence of equation-solving steps to find the successive values. It is possible to solve the equation for \([R]\) for any time interval. Let \([R_{t2}]\) and \([R_{t1}]\) represent the concentrations at times \( t_2 \) and \( t_1 \), respectively. The receptor concentration equation becomes

\[
\frac{[R_{t2}] - [R_{t1}]}{t_2 - t_1} = 0.05 - 0.005[R_{t2}].
\]

Rewriting the time difference \( t_2 - t_1 \) as \( \Delta t \) and applying algebraic steps to combine the \([R_{t2}]\) terms yields an equation that can be used to find successive values.

11. Solve the equation \[
\frac{[R_{t2}] - [R_{t1}]}{t_2 - t_1} = 0.05 - 0.005[R_{t2}]
\] for \([R_{t2}]\).
12. Use the result of question 9 to verify the previously found receptor concentrations.

**Inducer Concentration**

The inducer protein concentration equation can be used in a similar way to determine successive concentration.

\[
\frac{\Delta [\text{inducer}]}{\Delta t} = p_{\text{inducer}} \cdot \delta - d_i \cdot [\text{inducer}] \quad \text{Rate of change in inducer concentration}
\]

In addition to the parameters from Table 3.1, the equation for the concentration of inducer for each time requires both the previous concentration value and the current density coefficient.

Assume that the inducer concentration is 0 molecules/µL at \( t = 0 \) min. This value may be entered in the table.

13. Set up the inducer concentration equation that would be used to determine the concentration of the inducer at \( t = 30 \) min. Remember to use the density coefficient for 30 min.

14. Solve the equation in question 11 for \([\text{inducer}]\) and enter the value in the table.

15. Continue to set up a sequence of equations that can be used to find the successive values for the inducer concentration required in the table.
Lesson 4  The Receptor-Inducer Complex

The receptor-inducer complex (hereafter referred to as the R-inducer complex and represented by $Ri$) is formed from the receptor and inducer molecules, according to the following reversible reaction:

$$ R + \text{inducer} \xrightarrow{k_{fwd}} Ri \xleftarrow{k_{rev}} $$

Note that this reaction can happen either in the forward or reverse direction, at rates $k_{fwd}$ and $k_{rev}$, respectively. Thus, the concentration of the R-inducer complex at any given moment is:

$$ [Ri] = k_{fwd} [R][\text{inducer}] - k_{rev}[Ri] $$

which, after solving for $[Ri]$, becomes

$$ [Ri] = \frac{k_{fwd} [R][\text{inducer}]}{1 + k_{rev}} $$

It is clear from the last equation that the lower $k_{rev}$ and the higher $k_{fwd}$ is, the higher the R-inducer concentration, with it having the maximum value if this reaction becomes non-reversible (i.e., $k_{rev}$ becomes zero).

The units that are assigned to the $k_{fwd}$ parameter require a brief explanation before proceeding. The abbreviation mM refers to a scientific unit of concentration called a \textit{milliMolar}, which, as the name suggests, is one-thousandth of a \textit{Molar}. One Molar denotes a concentration of 1 mole (or $6.02 \times 10^{23}$ molecules) per liter. A 1 Molar solution expresses a very high concentration for something on a biological level. For example, the concentration of the salts in seawater is usually given as approximately 0.6 M (or 600 mM). Stomach acid (with a pH between 1 and 2) has a concentration of 0.1 M and 0.01 M.

Further conversions reveal that 1 M is equivalent to $6.02 \times 10^{17}$ molecules/µL and, in turn, 1 mM = $1 \times 10^{-3}$ M or $6.02 \times 10^{14}$ molecules/µL. Thus, the $k_{fwd}$ parameter (10/mM/µL/min) displayed in Table 3.1 describes the rate at which the concentration of the binding molecules is changing over time in each µL of the environment. Therefore, a $k_{fwd}$ paremeter of 10 can be expanded for substitution and calculation purposes to 10/(6.02 × 10^{17} molecules/µL)/µL/min. Demonstrating that the units for the R-inducer concentration can be simplified to molecules/µL is left as a practice exercise.
R-Inducer and the Production of GFP

Since the receptor-inducer complex is necessary to attach to the promoter region of DNA coding for GFP (see Figure 2.2) and thereby begin the ultimate production of GFP (Green Fluorescent Protein), the concentration of GFP, \([\text{GFP}]\), can be given by:

\[
\frac{\Delta [\text{GFP}]}{\Delta t} = p_{\text{GFP}} \cdot [\text{Ri}] - d_{\text{GFP}} \cdot [\text{GFP}]
\]

where \([\text{Ri}]\) is the concentration of the R-inducer complex, and \(p_{\text{GFP}}\) and \(d_{\text{GFP}}\) are the production and degradation rates of the GFP (per unit time). Table 4.1 summarizes the model equations that describe this system.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Description</th>
</tr>
</thead>
</table>
| \[
\frac{\Delta [R]}{\Delta t} = p_{R} - d_{R} \cdot [R]
\] | Rate of change in receptor protein concentration |
| \[
\frac{\Delta [\text{inducer}]}{\Delta t} = p_{\text{inducer}} \cdot \delta - d_{i} \cdot [\text{inducer}]
\] | Rate of change in inducer concentration |
| \[
[Ri] = \frac{\frac{k_{fwd}}{1 + k_{rev}} [R][\text{inducer}]}{1 + k_{rev}}
\] | Rate of change in Receptor-inducer complex |
| \[
\frac{\Delta [\text{GFP}]}{\Delta t} = p_{\text{GFP}} \cdot \frac{k_{fwd}}{1 + k_{rev}} [R][\text{inducer}] - d_{\text{GFP}} \cdot [\text{GFP}]
\] | Rate of change in the GFP gene expression (output) |

Table 4.1: A Summary of the Basic Quorum-Sensing Model

Practice

1. Verify that the units for the \([\text{Ri}]\) calculation simplify to molecules/\(\mu\)L.

2. Suppose that \([R] = 1.3\) molecules/\(\mu\)L and \([\text{inducer}] = 2.91 \times 10^{-7}\) molecules/\(\mu\)L. Use the parameters from Table 3.1 and the equation above to determine \([\text{Ri}]\).

3. Consider a situation in which \(k_{rev}\) is 0 molecule/\(\mu\)L/min. Determine \([\text{Ri}]\) for \([R] = 1.3\) molecules/\(\mu\)L, \([\text{inducer}] = 2.91 \times 10^{-7}\) molecules/\(\mu\)L, and \(k_{fwd} = 10\). Why is this the largest \([\text{Ri}]\) value for these concentrations?

4. Referring to the table completed in Activity 3-2, what will be the concentration of receptor-inducer complex at 1 hour?

5. The GFP concentration in a bacterial population increased from 0 to 100 molecules/\(\mu\)L within 2 hours. Assuming that the receptor protein concentration remains constant at 10 molecules/\(\mu\)L and the kinetic parameters are given by Table 3.1, what is the inducer concentration?
Now that the equations for calculating [Ri] and [GFP] have been added to the model, the simulation that was begun in Lesson 3 can be extended to include these values.

**ACTIVITY 4-1 Iteration to Calculate Concentrations**

**Objective:** Use iterative equations and processes to calculate concentration levels.

**Materials:**
- Handout QS-H6: Iterations to Calculate Concentrations Activity Worksheet
- Completed table from Activity 3-2

1. Use the two equations shown below and the values provided in the table to complete the table.

\[
[Ri] = \frac{k_{fwd} [R][inducer]}{1+k_{rev}} \quad \frac{\Delta[GFP]}{\Delta t} = p_{GFP} \cdot [Ri] - d_{GFP} \cdot [GFP]
\]

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Bacteria in the Tube</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density coefficient δ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of receptor R (in molecules/µL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of inducer (in molecules/µL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of R-inducer (in molecules/µL)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of GFP (in molecules/µL)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.2:** Concentrations (0 to 24 hours)

2. Referring to the table completed in Activity 3-2, how many bacteria should be in the tube in order to see them glow with the naked eye if the [GFP] for this to happen is 300 molecules/µL? Will we be able to see them fluoresce?
Spreadsheet to Calculate Concentrations

Carrying out the iterative calculations required to complete the solution table for the activities can be time-consuming, as well as subject to errors. A consistent change in time (for example, every 30 minutes) would allow for more accurate values and a clearer picture of how the values are changing over time. In addition, it would be cumbersome to have to repeat the entire process for each row of each table for a different bacterium that has different population values and kinetic parameters. The iterative nature of these calculations makes a spreadsheet an ideal tool for performing the mathematical tasks.

The Model of Receptor and Inducer Concentrations Excel file that accompanies this unit replicates the calculations for bacteria population, density coefficient, receptor concentration, and inducer concentration. The spreadsheet has been constructed using the same equations that were developed in the previous activities and allows the user to enter the initial population, the maximum populations, and the kinetic parameters. The values are calculated for 30-minute intervals.

A second spreadsheet, the Model of R-inducer and GFP Concentrations Excel file, is also included in this unit. This spreadsheet extends the previous spreadsheet model by including the R-inducer and GFP concentration calculations as described in this lesson. The bacteria population values in the spreadsheet reflect that the population attains a maximum value at approximately 10.5 hours.

Microbiology

Making complete sense of the processes of quorum sensing at the cellular level, let alone adding its mathematical representations, can be an intimidating, yet awe-inspiring quest. This is the goal toward which biologists, mathematicians, and computer scientists continue to strive as they apply their tools to explain and predict the behavior of organisms, to expand their understanding of an emerging field in microbiology, and communicate new knowledge to the scientific world.

Bacteria use quorum sensing because quorum sensing helps them, in some way, to improve their chances of survival. In some cases, while these bacteria reap the benefits of quorum sensing, they may harm humans. In the case of bacteria that use quorum sensing to become more effective pathogens, either in man or in organisms that man relies on for food and other uses, combatting these bacteria could be enhanced through improved knowledge of their quorum-sensing mechanisms. Inhibiting their ability to use quorum signaling may be a weapon to defeat their spread.\textsuperscript{[10]}

Consider the equations that we have developed in this unit. If the inducer molecule production could be inhibited or the inducer could be destroyed, this would shut down the bacteria’s ability to communicate with each other. The same would be true if the receptor molecules could be blocked so that they can’t bind with the inducer. As a result, these bacteria are prevented from becoming pathogenic or from forming harmful biofilms. These same concepts could be used to reduce biofilm formation in pipes and on industrial equipment, thereby saving companies large amounts of money. On the other hand, in some cases, bacteria utilize quorum sensing and
humans benefit. When the end result of quorum sensing can improve water treatment systems, cleanup after toxic spills, or microbially extract minerals, enhancing the production of the inducer or being able to add an artificial inducer would be of great benefit. Clearly this field will continue to grow and increasing our understanding of it will help improve our lives.
Lesson 5    Extension: Applying Quorum Sensing

We can extend the basic model for quorum sensing by introducing the notion of a feedback loop. In the previous section, we assumed that the inducer is being produced by each bacterium at a constant rate. In most, if not all, cases of quorum sensing, the binding of the inducer to the receptor R causes the resulting R-inducer complex to further activate the production of the inducer molecule. As described in the previous section, this is achieved through the binding of the R-inducer complex to the promoter region of gene P that is responsible for the synthesis of the inducer molecules. In fact, in many systems, the P gene and the output genes (GFP in our case) are under the same operon (i.e. they are under the same promoter); thus, binding of the R-inducer complex to its promoter will increase the expression of both the inducer-producing gene P and the output gene GFP. In other words, the inducer ultimately increases its own expression and, for that reason, it is referred to it as an autoinducer. Figure 5.1.A depicts the positive feedback loop discussed here.

In some cases, as it is in the case of bacterium V. fisheri, the R-inducer complex engages in a second function. In addition to activating the expression of the inducer-producing and output genes, it also represses (i.e. decreases) the production of the receptor proteins R while binding to DNA. This is achieved by either the inclusion of common regulatory sequences in both R and P promoters, so that the R-inducer complex can recognize and bind, or by having the same promoter region for both gene sets in an approximate but opposing direction. This scenario is depicted in Figure 5.1.B. By including this negative feedback loop, the cell avoids becoming too sensitive to the inducer concentration, which would be increasing now that it produces more due to the positive feedback loop.

Figure 5.1: Extended Model of Quorum Sensing.
To add the positive feedback mechanism in our model, it is necessary to modify the rate of change in inducer concentration equation from Table 4.1, so that the rate of change for the inducer concentration is a function of the R-inducer complex concentration. As such, the equation becomes:

\[
\frac{\Delta [\text{inducer}]}{\Delta t} = p_i \cdot \delta + k_{\text{feedback}} [R_i] - d_i \cdot [\text{inducer}]
\]

where \( k_{\text{feedback}} \) is the coefficient that corresponds to the rate of inducer production due to the binding of the R-inducer complex. Similarly, to introduce the negative feedback, the rate of change in inceptor protein concentration equation is modified as follows:

\[
\frac{\Delta [R]}{\Delta t} = p_R - d_R \cdot [R] - k_{\text{negfeedback}} [R_i]
\]

where \( k_{\text{negfeedback}} \) is the rate coefficient that corresponds to the repression of the protein R expression due to the binding of the R-inducer complex to the promoter. The above system of equations can be simulated through computational methods to provide a better understanding of how the quorum system works. This is achieved by creating a computer program that iteratively calculates the values of all variables involved in the system (R, inducer, R-inducer complex, etc.) for a period of time. The simulation can be discrete (e.g. the values of the variables are calculated only every second of “simulation” time) or continuous (the variable values are calculated over the whole time interval that the system is simulated). Monte Carlo methods, computational techniques that employ repeated random sampling, are frequently used to simulate dynamical systems with a temporal component, such as the one analyzed here.

Figure 5.2 shows a simulation of a model of a bacterial colony that exhibits a quorum-sensing behavior. Each frame shows the progressive increase in the colony size and the amount of fluorescence in the colony (green color).

![Simulation of a bacterial colony](image)

**Figure 5.2:** Simulation Of A Bacterial Colony That Exhibits Quorum-Sensing Behavior

**Mechanistic Examples Of Quorum-Sensing Systems In Bacteria**

The model being used can now be applied to two examples of quorum sensing introduced in Lesson 2.
**Vibrio fischeri**

*Vibrio fischeri* is the bacterium that produces bioluminescence for a squid. The *V. fischeri* quorum-sensing system is shown in Figure 5.3. The autoinducer molecule in this system is a small chemical that is called acyl-homoserine lactone (AHL). AHL is produced by an enzyme that is called LuxI.[12][13] Another protein, LuxR, functions as the autoinducer receptor in the cytoplasm and as the DNA binding transcriptional activator. After the inducer is produced, it can diffuse in and out of the cell freely, and its concentration is increased with cell density.[14] It binds to LuxR spontaneously, and these events become more frequent with the increase in concentration of the inducer. The LuxR-inducer complex then activates the transcription of the luxICDABE operon that encodes luciferase which can generate bioluminescent light signals.[15] Furthermore, LuxI is also part of the operon and thus its expression is also increased as the inducer concentration increases, constituting the positive feedback mechanism that was just described. This allows the cell to switch into a “quorum-sensing” mode and produce light.

![Figure 5.3: Quorum Sensing In *Vibrio Fischeri*. Adapted from][16]

In Figure 5.3, LuxR (blue rectangle) corresponds to the receptor protein R that binds to the autoinducer molecule (red triangle) which is produced by the gene/protein LuxI (yellow ellipse). The LuxR-autoinducer complex binds to the promoter region of LuxICDABE (luciferase operon) and initiates the transcription of both LuxI and LuxICDABE proteins that are required for light production.

**P. aeruginosa**

Figure 5.4 depicts the quorum sensing system of this bacterium, which consists of two LuxIR circuits. In the first circuit, a protein called LasI produces an AHL autoinducer that binds to a receptor protein LasR (the receptor, producer and autoinducer proteins in *P. aeruginosa* are similar to those in *V. fischeri*). The LasR-inducer complex then binds to DNA and activates numerous targets, including the expression of the LasI protein (positive feedback, Seed et al. 1995). The complex also activates the second quorum-sensing circuit by expressing the rhlR and rhlI genes. The rhlI gene produces another inducer molecule that is similar but not exactly the same as the one produced by the LasI protein (this one is called C4-homoserine lactone, while...
the one produced by LasI is called 3OC12-homoserine lactone), and it only binds to the rhlR protein. The new rhlR-inducer complex then binds to DNA and activates its own set of target genes.

**Figure 5.4:** The Quorum-Sensing System In *P. Auruginosa* Adapted from [16]

In Figure 5.4, the system consists of two circuits, one based on the LasI and LasR genes, and another based on the RhlI and RhlR genes. It is not fully clear why the bacterium has evolved these two sub-systems, but recent microarray analyses show that this sequential architecture (i.e. the LasR/I sub-circuit activating the RhlR/I sub-circuit) allows for the temporally ordered activation of different sets of genes, and it may be critical for responding to early and late stage actions by the organism, especially during an infection.

**Extension**

1. Explain what the effect of the positive and negative feedback is to the quorum-sensing system’s behavior. Give an example of a system where the combination of positive and negative feedback can create a system that exhibits oscillatory behavior.

2. Referring back to Practice question 5 in Lesson 4, recall that the GFP concentration in a bacterial population increased from 0 to 100 molecules/µL within 2 hours and that the receptor protein concentration remained constant at 10 molecules/µL. Assuming that both $k_{\text{feedback}}$ and $k_{\text{negfeedback}}$ are equal and have a value of 0.01, use the kinetic parameters from Table 3.1 to:

   a. Determine the inducer concentration.

3. State similarities and differences between the two quorum-sensing systems (*Vibrio fischeri* and *P. auruginosa*) described above.
Glossary

**Autoinducer synthase** - the enzyme that catalyzes the production of the inducer molecules from essential materials that the cell obtains from the environment.

**Average rate of change** - term used to describe the change in a quantity per unit of time during a given time interval.

**Bacterium** - a type of single celled organism that does not have membrane bound organelles or an organized nucleus.

**Binary fission** - the most common form of reproduction in bacteria in which a single bacterium splits into 2 bacteria.

**Biofilm** - a protective mode of growth in which a complex aggregation of microorganisms formss on a solid substrate to survive a hostile environment.

**Capsule** - a protective outer shell found in some bacteria.

**Cell wall** - the supportive structure outside of the cell membrane. The chemical makeup of the bacterial cell wall is different to that of plants.

**Concentration** - the strength of a solution in terms of the amount of one substance in a unit amount of another substance (e.g. molecules/μL). The concentration of a component C is denoted at [C].

**Concentration rate** - the amount of change in concentration that occurs within a given time interval calculated as an average rate of change for that interval (e.g. 5 molecules/μL per minute).

**DNA** - the molecule that carries the genetic information in the cell by acting as the “blueprint” for protein synthesis.

**Enzyme** - specialized proteins that function as catalysts in biochemical reactions.

**Eukaryote** - single-celled or multicellular organisms that have cells with organized nuclei and membrane bound organelles.

**Exponential Function** - a function of the form $a \cdot b^x$.

**Exponential growth** - growth that can be modeled using an exponential function. Increase (or decay) is explained by the repeated use of a constant multiplier as opposed to a constant increase (or decrease).

**Exponential phase** - the stage of an organism’s population growth in which rapid growth, described by an exponential function, occurs.
**Extracellular polymeric substance** - adhesive substance that holds the biofilm together and renders the recruitment of more bacteria easier.

**Green Fluorescence Protein (GFP)** - a protein that glows green under fluorescent light.

**Inducer** - small molecules involved in cell-to-cell signaling by binding to receptor molecules.

**Lag phase** - the beginning stage of population growth in which the organism grows slowly as it acclimates itself to a new environment or other environmental conditions.

**Microbe** - a microscopic organism; a microorganism.

**Microorganism** - a living organism too small to be seen with naked eye but visible under a microscope.

**Microbiology** - the branch of biology that studies microorganisms and their effects on other organisms.

**Millimolar** - one thousandth of a molar.

**Molar** - a concentration of 1 mole (6.02 x 10^{23} molecules) per liter.

**Molecule degradation rate** (d_c) - the breakdown of the molecule C per unit time.

**Molecule production rate** (p_c) - the increasing of the molecule C per unit time.

**mRNA** - a nucleic acid made from DNA that carries the code for making a protein to the ribosomes.

**Operon** - a functioning unit of genomic DNA containing a cluster of genes under the control of a single regulatory signal or promoter.

**Parameter** - a variable that represents a particular characteristic in a mathematical model. For example, the production rate of the receptor protein is represented by the parameter p_R. The particular value of the parameter may depend upon the specific type of receptor.

**Pathogens** - an organism that can cause disease.

**Planktonic** - description of microorganisms that float or drift near the surface layer of a body of fresh or salt water.

**Plasma membrane** - the thin membrane that surrounds a cell. Proteins in the membrane help regulate what enters or leaves the cell.
**Prokaryote** - single-celled organisms that do not have an organized nucleus or membrane bound organelles.

**Promoter** - a segment of DNA that acts as a controlling element in the expression of the gene that follows.

**Protein** - a group of large molecules composed of amino acids that are important structurally and functionally.

**Quorum sensing** - a cell density dependent phenomenon in which microorganisms such as bacteria communicate and coordinate their gene expression/behavior through the accumulation of signaling molecules. When the signaling molecule accumulates to a sufficient concentration, the change in behavior occurs.

**Receptor protein** - a molecule that binds to the inducer. The complex of the two molecules initiates the production of a protein that will cause a change in behavior.

**Ribosome** - the organelle found in both eukaryotes and prokaryotes which catalyzes the synthesis of proteins.

**R-inducer complexes** - the result of the inducer and the receptor protein binding. The complex of the two molecules initiates the production of a protein that will cause a change in behavior.

**Stationary phase** - the stage of an organism’s population growth at which growth slows due to crowding and competition for resources.

**Transporter protein** - proteins in the cell membrane that assist in the movement of molecules through the cell membrane.
References


Quorum Sensing Student 39