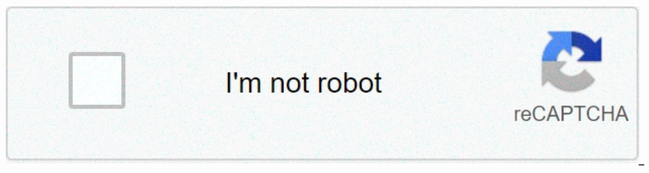

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Growth in a bacterial population worksheet answers

Bacterial Population Growth (page 3) (This chapter is 4 pages) © Kenneth Todar, PhD The bacterial growth curve In the laboratory, under favorable conditions, a growing bacterial population doubles at regular intervals. Growth is by geometric progression: 1, 2, 4, 8, etc. or 20, 21, 22, 23.....2n (where no the number of generations). This is called exponential growth. In reality, exponential growth is only part of the bacterial life cycle, and is not representative of the normal pattern of bacteria growth in nature. When a cool medium is inoculated with a given number of cells and population growth is monitored over a period of time, tracing the data will give a typical bacterial growth curve (Figure 3 below). Figure 3. The typical bacterial growth curve. When bacteria are grown in a closed system (also called batch culture), such as a test tube, the cell population almost always exhibits this growth dynamic: cells first adapt to the new medium (shift phase) until they can begin to divide regularly through the binary fission process (exponential phase). When their growth becomes limited, the cells stop dividing (stationary phase), until they finally show a loss of viability (death phase). Note the settings of the x and y axes. Growth is expressed as a change in the number of viable cells relative to time. Generation times are calculated during the exponential growth phase. Time measurements are in hours for bacteria with short generation times. Four characteristic phases of the growth cycle are recognized. 1. Shift phase. Immediately after cells are inoculation in the cool medium, the population remains temporarily unchanged. Although there is no apparent cell division occurring, cells can grow in volume or mass, synthesizing enzymes, proteins, RNA, etc., and increasing in metabolic activity. The duration of the shift phase apparently depends on a wide variety of factors, including the size of the inoculum; The time it takes to recover from physical damage or shock during the transfer; time required to synthesize essential coenzymes or divisive factors; and the time it takes to synthesize new (inductible) enzymes that are needed to metabolize the substrates present in the medium. 2. Exponential phase (newspaper). The exponential growth phase is a balanced growth model in which all cells regularly divide by binary fission, and develop by progression Cells divide at a constant rate depending on the composition of the growth medium and the incubation conditions. The exponential growth rate of a bacterial culture is expressed as generation time, also the doubling time of the bacterial population. Generation time (G) is defined as time (t) by generation (n -1.0) As a result, G-t/n is the equation from which generation time calculations (below) derive. 3. 3. Phase. Exponential growth cannot be sustained forever in batch culture (e.g., a closed system such as a test tube or vial). Population growth is limited by one of three factors: 1. depletion of available nutrients; 2. accumulation of inhibitory metabolites or finished products; 3. the depletion of space, in this case called a lack of biological space. During the stationary phase, if viable cells are counted, it is not possible to determine whether certain cells die and an equal number of cells divide, or whether the cell population has simply stopped growing and dividing. The stationary phase, like the shift phase, is not necessarily a quiescence period. Bacteria that produce secondary metabolites, such as antibiotics, do so during the stationary phase of the growth cycle (secondary metabolites are defined as metabolites produced after the active stage of growth). It is during the stationary phase that spore-forming bacteria must induce or unmask the activity of dozens of genes that may be involved in the sporulation process. 4. Death phase. If incubation continues after the population has reached the stationary stage, a phase of death follows, in which the viable cell population decreases. (Note that if counting by turbidimetric measurements or microscopic counts, the death phase cannot be observed.). During the death phase, the number of viable cells decreases geometrically (exponentially), essentially the reverse of growth during the notation phase. Growth rates and generation time As mentioned above, bacterial growth rates during the exponential growth phase, under standard nutritional conditions (culture medium, temperature, pH, etc.), define the generation time of the bacterium. Bacteria generation times range from about 12 minutes to 24 hours or more. The production time of E. coli in the laboratory is 15-20 minutes, but in the intestinal tract, the generation time of the coliform is estimated at 12-24 hours. For most known bacteria that can be grown, generation times vary from about 15 minutes to 1 hour. Symbiotes such as rhizobium tend to have longer generation times. Many lithotrophs, such as nitrifying bacteria, also have long generation times. Some pathogenic bacteria, such as Mycobacterium tuberculosis and Treponema pallidum, have particularly long generation times, and this is thought to be an advantage in their virulence. The generation times of some bacteria are shown in Table 2. 2. Generation time for some common bacteria under optimal growth conditions. Page 2 Bacterial Population Growth (page 4) (This chapter is 4 pages) © Kenneth Todar, PhD Continuous Culture of Bacteria The crops discussed so far for bacterial population growth are called batch cultures. As nutrients are not renewed, exponential growth is limited to a few generations. Bacterial cultures can be maintained in an exponential state over long periods using a continuous crop system (Figure 4), designed to relieve conditions that stop the exponential growth of batch crops. Continuous culture, in a device called chemostat, can be used to maintain a bacterial population at a constant density, a situation that is, in many ways, more similar to bacterial growth in natural environments. In a chemostat, the growth chamber is connected to a sterile medium tank. Once growth has begun, a cool environment is continuously provided from the reservoir. The volume of liquid in the growth chamber is maintained at a constant level by a kind of overflow drain. The cool medium is allowed to enter the growth chamber at a rate that limits the growth of bacteria. Bacteria grow (cells are formed) at the same rate as bacterial cells (and the spent medium) are removed by overflow. The rate of addition of the fresh medium determines the growth rate because the fresh medium always contains a limiting amount of an essential nutrient. Thus, chemostat relieves nutrient deficiency, the accumulation of toxic substances, and the accumulation of excess cells in culture, which are the parameters that initiate the stationary phase of the growth cycle. Bacterial culture can be cultivated and maintained at relatively constant conditions, depending on nutrient flow. Figure 4. Schematic diagram of a chemostat, a device for the continuous cultivation of bacteria. Chemostat relieves environmental conditions that limit growth by continuously providing nutrients to cells and removing used substances and used cells from the culture medium. Synchronized Growth of Bacteria The study of the growth of bacterial populations in batches or continuous cultures does not allow any conclusions about the growth behavior of individual cells, because the distribution of cell size (and therefore of cell age) among members of the population is completely random. However, the study of synchronous cultures can obtain information on the growth behaviour of individual bacteria. Synchronized cultures must be composed of cells that are all at the same stage of the bacterial cell cycle. Measurements on synchronized cultures are equivalent to measurements on individual cells. A number of intelligent techniques have been designed to obtain bacterial populations at the same stage of the cell cycle. Some techniques involve manipulating environmental parameters that encourage people to start or stop growth in the point of the cell cycle, while others are physical methods for selecting cells that have just completed the binary fission process. Theoretically, the smallest cells in a bacterial population are those that have just completed the process of cell division. The synchronous growth of a bacterial cell population is illustrated 5. Synchronous cultures quickly lose synchrony because not all cells in the population divide exactly the same size, age or time. Figure 5. The synchronous growth of a bacterial population. In careful selection of cells that have just divided, a bacterial population can be synchronized in the cycle of bacterial cell division. The synchrony can only be maintained for a few generations. CHAPTER FIN Previous Page Back to page 1 In this section, we will return to the questions asked in the first section on exponential and logarithmic functions. Remember that we are studying a population of bacteria undergoing binary fission. In particular, the population doubles every three hours. We would like to know the following: How many bacteria are present after 51 hours if a crop is inoculated with 1 bacterium? How many bacteria should a crop be inoculated if there are to be 81,920 bacteria present at the hour 42? How long would it take for an initial population of 6 people to grow to a size of 12,288 bacteria? Bacterial Culture Animation Why do we use exponential functions to answer these questions? The bacteria population in our example doubles every 3 hours. What exactly does that mean? Imagine you inoculating a fresh crop with N bacteria at 12:00 pm. At 3 p.m., you'll have 2N bacteria, at 6 p.m., you'll have 4N bacteria, at 9 p.m., you'll have 8N bacteria, and so on. If these cell divisions occur exactly at each of these time points, it is said that the cells develop synchronously. If this were the case, the growth process would be geometric. A geometric growth model predicts that the population increases at distinct times (in this example hours 3, 6 and 9). In other words, there is no continuous increase in the population. However, that is not what is really happening. Going back to our example above, imagine that you take a small sample of the crop every hour and count the number of bacterial cells present. If bacterial growth were geometric, one would expect to have N bacteria between 12:00 p.m. and 3 p.m., 2N bacteria between 3 p.m. and 6 p.m., etc. However, if you do this experiment in the laboratory, even under the best experimental conditions, that will not be the case. If you go a step further and make a graph with the number of bacteria on the Y axis and the time on the x axis, you will get a plot that looks much more like exponential growth than geometric growth. Why does bacterial growth look like exponential growth in practice? answer is because bacterial growth is not completely synchronized. Some cells divide in less than 3 hours; others will have a little more time to divide. Even if you start a culture with a single cell, synchronicity will only be maintained by a few cell divisions. A single cell will divide at a discrete point over time, and the resulting 2 cells will divide at about the same time, and the resulting 4 will divide again about the same time. As the population increases, the individual nature of the cells will result in a smoothing of the division process. This smoothing gives an exponential growth curve, and allows us to use exponential functions to make calculations that predict bacterial growth. Thus, while exponential growth might not be the perfect model of bacterial growth by binary fission, this is the appropriate model to use given the experimental reality. Now try to solve the 3 problems posed at the beginning of this section