A MATHEMATICAL MODEL OF BACTERIAL GROWTH USING SOIL BACTERIAL COMMUNITIES

UN MODEL MATEMATIC AL CREŞTERII BACTERIILOR UTILIZÂND COMUNITĂȚILE BACTERIENE DIN SOL

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Abstract. *A bacterial growth model is presented starting from the Malthusian model of exponential growth. Considering the growth rate being a linear function, we can express it using the bacterial tolerance grade over different conditions. Resolving the obtained differential equation, we plot the growth of the bacterial population for the studied initial conditions. The numerical increase of soil bacterial population cultivated on common microbiological growth media (Potato dextrose agar – abbreviated PDA) is studied.*
Key words: mathematical model, bacterial growth

Rezumat. *Un model de creştere bacteriană este prezentat pornind de la modelul malthusian de creştere exponențială. Având în vedere că rata de creştere este o funcție liniară, o putem exprima folosind gradul de toleranță bacteriană în diferite condiții. Rezolvând ecuația diferențială obținută, am calculat creşterea populației bacteriene pentru condițiile inițiale studiate. Creşterea numerică a populației bacteriene din sol cultivată pe medii comune de creştere microbiologică (Potato dextrose agar – abreviată PDA) este studiată.*
Cuvinte cheie: model matematic, evoluția numărului de bacterii

INTRODUCTION

Bacteria are prokaryotic organisms and represent the most widespread form on the planet. Some bacteria positively affect human and animal life and others have a negative effect. That's why studying this form of life is a necessity. Part of this study is represented by the way to grow bacterial cultures, the factors that influence this growth, and the ability to predict numerical growth by making models as close to reality as possible.

There are four phases in increasing the growth of a bacterial population (fig. 1): (**A**) *adaptation to the environment* and maturation in order to prepare for the beginning of the division (does not increase the number of bacteria), (**B**) *exponential growth* of the number of bacteria by their periodic division, (**C**) the *stationary* phase (the rate of colony growth becomes zero primarily due to the limited development area and the amount of food that is inversely proportional to the population) and (**D**) the phase of *decreasing* of the number of colonies by their

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death caused by environmental toxicity produced by them and lack of food (Koch *et al.,* 1998; Monod, 1942)

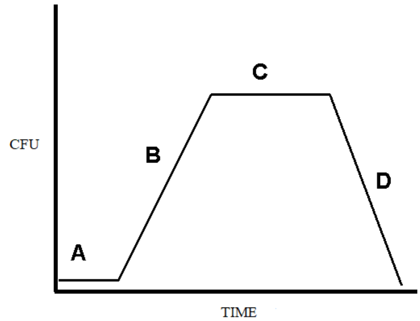


Fig. 1 Phases of the growth of a bacterial population

A mathematical model of the growth of bacterial communities provides a stylized framework in which we can quantitatively study the rate of increase or decrease in the bacterial population according to the conditions under consideration. Differential equations or differential equation systems are typically used to characterize the development of bacterial communities (Murray, 2001)

The mathematical model of the increase in the number of bacteria

It is known that each cell divides on average with a constant growth rate. Thus, the simplest mathematical model for increasing the number of bacteria is the Malthusian model (Koch, 2001; Burdujan, 1999; Smith and Waltman, 1995).

(1) $$\frac{dN}{dt} = r \cdot N,$$

where N represents the number of bacteria at a given time and $r > 0$ is a constant that signifies the *growth rate* that is specific to the type of bacterium. This model states that the rate of increase in bacterial counts is proportional to the number of cells (N) at a given time and the growth rate (r). In addition to equation (1), the initial conditions are also given

(2) $$N\ 0\ = N_0.$$

The solution of the model given by (1) and (2) is

(3) $$N\ t\ = N_0 e^{rt}$$

and the doubling time of the number of bacteria (i.e. $2N_0 = N_0 e^{rT}$) is calculated as being

(4) $$T = \frac{\ln 2}{r}.$$

This expresses the exponential increase in the number of bacteria. As long as $t \to \infty$ there is no upper barrier. This approach can be accepted if the field of development of the bacteria is unlimited compared to the size of the colony, the amount of food is unlimited and the toxicity caused by the increasing number of bacteria is neglected. Obviously, these conditions are not realistic. In fact, as bacteria multiply, the amount of food decreases and the degree of environmental toxicity increases. These considerations lead us to the next idea: the more the bacteria population increases, the faster the rate of growth decreases. Thus, we will further consider that growth rate is a function that depends on the number of bacteria:

$$(5) \qquad \frac{dN}{dt} = f \;\; N \;.$$

We can consider that equation (1) gives an intrinsic property of bacterial culture and equation (5) is the result of all environmental restrictions [4]. Real growth is a combination of the two equations, so the model will contain an equation of the form

$$(6) \qquad \frac{dN}{dt} = r \cdot N \cdot f \;\; N \;.$$

For simplicity, we will consider the function $f \;\; N$ as being linear, that is $f \;\; N \; = aN + b.$ In order to determine the coefficients a and b, we take into consideration some particular cases (Burdujan, 1999; Smith and Waltman, 1995).

If N is very small ($N = 1$), one can consider the area of the culture to be infinite (compared to the size of the bacterium) and thus the equation (1) shapes population growth well. In these conditions $f \;\; N$ is approximated by 1 (from equation (6)), thus $b = 1$.

We consider k, a new constant specific to each type of bacteria, namely, the *degree of tolerance* for decreasing the amount of food and the accumulation of toxins. If the bacteria exhibit great tolerance (k is big), the decrease of $\frac{dN}{dt}$ with the increase of N is slow, which is associated with a negative slope of the function f, thus $a < 0$. If the tolerance is low (k is small), then the decrease of $\frac{dN}{dt}$ is steep, so we can consider $a = -\frac{1}{k}$. Thus, the model obtained is

$$(7) \qquad \begin{cases} \dfrac{dN}{dt} = r \cdot N \cdot \left(1 - \dfrac{N}{k}\right). \\ N \;\; 0 \; = N_0 \end{cases}$$

The equation in this model is known in the literature as the logistic equation. This differential equation is with separable variables and the equilibrium is obtained by imposing $\frac{dN}{dt} = 0$, which leads to

$$(8) \qquad 0 = r \cdot N \cdot \left(1 - \frac{N}{k}\right)$$

with the solutions $N = 0$ and $N = k$. Thus, we can associate another meaning to the constant k as being the maximum number of bacteria that can occur in the population. Solving the system (7) we get the solution

$$(9) \qquad N(t) = k\left(1 - \frac{1}{1 + Ce^{rt}}\right),$$

where $C = \frac{N_0}{k - N_0}$. We have the growth rate of the population $\frac{dN}{dt} > 0$ when $0 < N < k$, thus the function $N(t)$ is increasing converging to k when $t \to \infty$. When $N > k$, the function $N(t)$ decreases toward k, for $t \to \infty$. The function graph (9) is called a growth curve and is in the form of the letter "S".

MATERIAL AND METHOD

In the experiment we used soil from V. Adamachi farm, apple plantation.

The required soil was taken from some points taken on the diagonal of the apple plantation plot, from the depth of 5-7 cm. For planting, the method of successive dilutions (tubes of 9 ml each of distilled and sterilized water, prepared in advance) was used. From the soil sample, one gram was taken and introduced into the first test tube resulting in 10^{-1} dilution. The solution was well homogenized and then, with a sterilized Pasteur pipette, 1 ml of solution was transferred to the second tube resulting in dilution of 10^{-2}. This procedure was performed until the 10^{-4} dilution was obtained.

From dilutions 10^{-3} and 10^{-4}, 1 ml of solution was taken and transferred into Petri dishes. Over 20 ml of PDA culture medium (Potato dextrose agar) was poured over this solution. We waited for the solidification of the medium (about 2 hours) after which they were placed in the incubator (28 degrees Celsius). For each dilution 5 pots were planted (fig. 1). From the moment of the first colonies, the number of colonies was determined on an hourly basis.

RESULTS AND DISCUSSIONS

The experiment resulted in two sets of data that we have accumulated in tables 1 and 2. These data were used to compare the theoretical and practical results (fig. 2).

Table 1

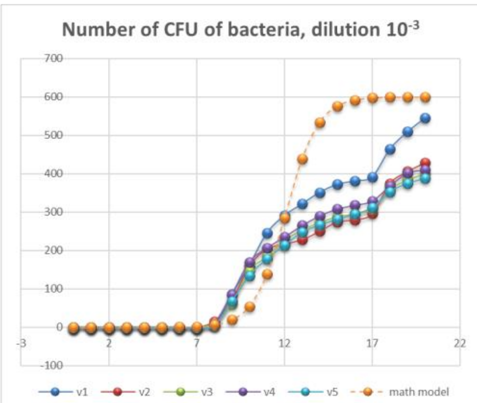
Results obtained using a 10^{-3} dilution

	1st det	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th
	8h	9h	10h	11h	12h	13h	14h	15h	16h	17h	18h	19h	20h
V1	10	86	161	245	291	321	351	372	381	392	464	509	545
V2	15	68	159	204	217	228	250	274	280	296	375	406	429
V3	8	61	148	184	226	253	273	288	296	309	359	384	400
V4	6	85	170	207	235	265	290	308	318	329	368	402	410
V5	4	67	134	178	213	247	266	281	294	312	353	374	387

Table 2

Results obtained using a 10^{-4} dilution

	1st det	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th
	8h	9h	10h	11h	12h	13h	14h	15h	16h	17h	18h	19h	20h
V1	8	9	10	11	12	13	14	15	16	17	18	19	20
V2	0	3	27	33	37	39	47	54	58	65	80	82	87
V3	0	5	46	60	64	76	85	94	97	106	115	118	122
V4	0	0	27	40	46	50	56	59	63	69	79	82	84
V5	0	4	36	40	44	49	54	59	61	68	82	87	89



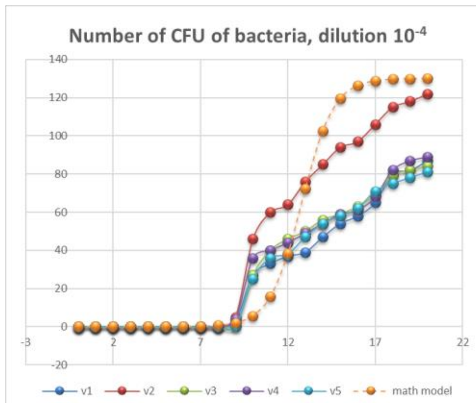


Fig 2 Bacteria number evolution (CFU 10^{-3} (left) and CFU 10^{-4}(right))

The values corresponding to the mathematical model were calculated according to solution (9):

$$N(t) = k\left(1 - \frac{1}{1 + Ce^{rt}}\right).$$

For time t we considered values between 0 and 20 hours. The growth rate r specific for these bacteria was calculated as the average of successive growth ratios, so we considered $r = 1.1$. The initial condition was taken as the dilution grade: for the first experiment $N_0 = 0.001$, and for the 10^{-4} dilution we considered $N_0 = 0.0001$. In order to compare the results (mathematical model / experimental data), the constant k was taken into consideration in relation to the maximum number of colonies of bacteria resulted in the two dilutions: at 10^{-3} we considered $k = 600$ and for 10^{-4} we considered $k = 140$. The constant C was calculated by the formula $C = \frac{N_0}{k - N_0}$.

CONCLUSIONS

1. Using the experimental data obtained we calculated the bacterial population growth rate and the growth of a bacterial population in the soil was simulated using the mathematical model (logistic equation)

2. The bacterial growth model follows the mathematical model. The data obtained by mathematical simulation and the data obtained by experimental measurement are substantially equal to the t student test (pv = 0.55) for a 10^{-3} and 10^{-4} dilution.

3. Only the first three phases of the development of a bacterial population were investigated, without taking into account the decreasing number of colonies.

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