

Bacterial Growth

H. L. Smith *

1 Simple Models

Bacteria are the dominant form of life on the planet. There are 10^5 cells in a milliliter of seawater, or on a square centimeter of our skin. There are ten times as many bacterial cells on our skin and in our large intestine as cells in our own body. We are nothing but a source of sustenance for bacteria. Humans have learned to harness bacteria and other microbes to produce products such as human insulin by genetically engineering. It is now easy to insert the human gene for insulin into bacteria and let them produce it in large industrial fermenters. So it is important to understand bacterial growth.

A “full-grown mother” bacterium typically divides into two equal-size daughter cells each of half its mother cells size. Thus, each daughter cell must double in size before it too can divide.

The growth of bacteria has been the subject of much study over the centuries, initially stimulated by bread, beer and wine making. See the reference works [1, 2, 5, 6, 7] for more recent scientific surveys. The simplest model is introduced in our calculus texts. If $N(t)$ is the size (e.g. weight in grams) of the bacterial population at time t , then the Malthusian model of exponential growth is given by

$$\frac{dN}{dt} = rN$$

together with some initial conditions $N(0) = N_0$. Parameter $r > 0$ is called the growth rate and must be measured. The solution

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

*Department of Mathematics and Statistics, Arizona State University, Tempe, AZ 85287, supported in part by NSF grant DMS 0107160

has the rather unrealistic feature of getting larger without bound as t increases. Basically, the model ignores that bacteria require resources to grow and that these resources are finite. The doubling time T for the bacteria is readily computed from

$$2N_0 = N_0e^{rT}$$

leading to

$$T = \ln(2)/r. \tag{1}$$

T , being easy to measure in the lab, allows computation of r . Exponential growth may be reasonable over short times but cannot continue indefinitely.

Exercise 1 The gut bacteria *E. coli* can divide every thirty minutes when nutrients are abundant. If they grow exponentially, how long would it take, starting with a single bacterium, to fill all the world's oceans with bacteria? Keeping in mind that bacteria have been present on earth for approximately 3.5 billion years, has there been enough time to fill the oceans? You may assume spherical bacteria of diameter 10^{-6} meters. An estimate of the volume of the world's oceans is 292,131,000 cubic kilometers.

In our first course in ordinary differential equations, the Logistic equation is usually mentioned. It takes the form

$$\frac{dN}{dt} = rN \left[1 - \frac{N}{K} \right]$$

where now besides r , there is a second parameter K , often called the “carrying capacity”.

The Logistic equation can be solved by separation of variables but it is not necessary to do so in order to understand the behavior of its solutions. The steady states or equilibrium solutions are obtained by setting $\frac{dN}{dt} = 0$ and solving for N :

$$0 = rN \left[1 - \frac{N}{K} \right]$$

obtaining $N = 0$ or $N = K$. Since $\frac{dN}{dt} > 0$ when $0 < N < K$ solutions $N(t)$ that start with $N(0)$ in this interval increase with t , converging to K as $t \rightarrow \infty$. $\frac{dN}{dt} < 0$ when $N > K$ so $N(t)$ decrease towards K as $t \rightarrow \infty$ when $N(0) > K$. In the former case, the shape of the graph of $N(t)$ versus t has the classic “S” shape. However, the logistic equation has the major disadvantage that the carrying capacity K cannot be measured other than by

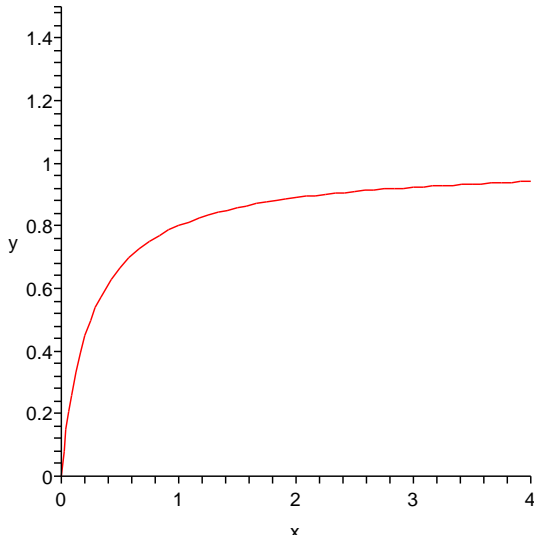


Figure 1: Plot of Monod function $\frac{x}{0.25+x}$

growing the organism until it stops growing. Moreover, there is no theoretical underpinning for it.

1.1 Contributions of Jacques Monod

In the 1930s and 1940s, Jacques Monod performed experiments on bacteria feeding on a single limiting nutrient (say glucose) in order to see if the Logistic equation accurately described bacterial growth. He found (see [3]) it did not and to describe his results we require some notation. If $S(t)$ denotes the concentration of the nutrient in the media (grams/liter) and $N(t)$ denotes the concentration of bacteria in the media (grams/liter) at time t , his experiments suggested that the specific growth rate

$$\frac{dN}{Ndt} = \frac{rS}{a + S} \quad (2)$$

seemed to best fit the data. The expression on the right is often called the Monod function. As a function of S it is monotonically increasing with limit r as $S \rightarrow \infty$. r is therefore called the maximum growth rate, despite the fact that it can never be reached, and a is called that half-saturation constant because when $S = a$, the right hand side of (2) becomes $r/2$, half the maximum

growth rate. Figure 1 plots a Monod function. The key feature of the Monod function is that the specific growth rate increases with nutrient concentration S as expected but it levels out high nutrient concentrations. Monod did not immediately note the similarity of his function to the Michaelis-Menten equation derived from enzyme kinetics, but later he acknowledged it.

Furthermore, Monod found that the rate of nutrient consumption by bacteria was opposite in sign to bacterial growth but proportional to it:

$$\gamma \frac{dS}{dt} = -\frac{dN}{dt}$$

which just means that

$$\frac{dN}{-dS} = \gamma.$$

A gain of dN units of bacteria requires γdS units of nutrient. The constant γ is called the growth yield; later research would show that it is not strictly constant but only approximately so. If N represented elephant biomass and S represented peanuts, then γ would be the ratio of elephant biomass to the mass of peanuts required to make an elephant.

For *E.coli*, a common bacteria that resides in the gut of mammals and that has been the subject of extensive research, grown on glucose at 30 degrees Celsius,

$$\begin{aligned} r &= 1.35 \text{ per hour} \\ a &= 0.004 \text{ gms/liter} \\ \gamma &= 0.23 \end{aligned} \tag{3}$$

If glucose is plentiful so that the specific growth rate is maintained at $r = 1.35$, equation (1) gives a doubling time of approximately half an hour.

These observations lead to the system of two equations for the nutrient S and bacteria N given by

$$\begin{aligned} \frac{dS}{dt} &= -\frac{1}{\gamma} N \frac{rS}{a+S} \\ \frac{dN}{dt} &= N \frac{rS}{a+S} \end{aligned} \tag{4}$$

Initial data must also be specified:

$$N(0) = N_0, \quad S(0) = S_0$$

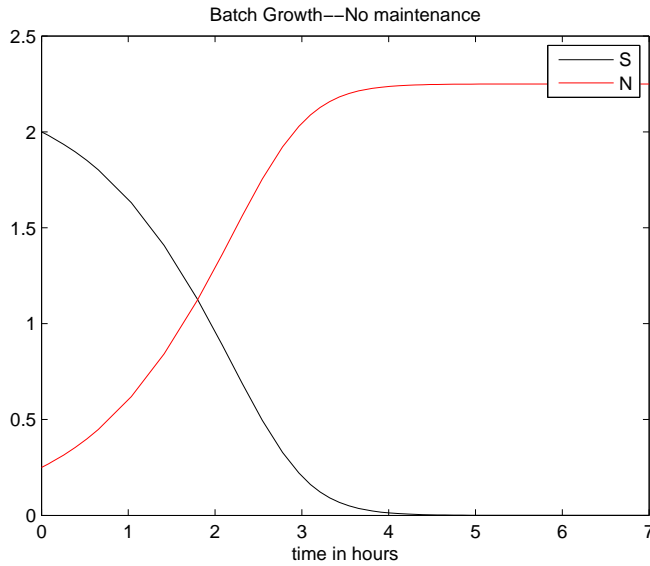


Figure 2: A solution $S(t)$ and $N(t)$ of equation (4)

If we multiply the first equation by γ and add it to the second, we get

$$\gamma \frac{dS}{dt} + \frac{dN}{dt} = 0$$

and thus by integration

$$\gamma S(t) + N(t) = \gamma S_0 + N_0.$$

This allows us to ignore $S(t)$ since we can always get it from $N(t)$ by

$$S(t) = S_0 + \frac{N_0 - N(t)}{\gamma}$$

We may substitute this into the equation for $\frac{dN}{dt}$ to obtain a single differential equation for $N(t)$

$$\frac{dN}{dt} = rN \left[\frac{\gamma S_0 + N_0 - N}{\gamma a + \gamma S_0 + N_0 - N} \right] \quad (5)$$

OK, so it is messy but the qualitative behavior of its solutions is similar to that of the logistic equation. Setting $\frac{dN}{dt} = 0$, we get the steady states

$$N = 0 \quad \text{or} \quad N = \gamma S - 0 + N_0$$

If $N_0 > 0$, then $\frac{dN}{dt} > 0$ along a solution and

$$N(t) \rightarrow \gamma S_0 + N_0, \quad t \rightarrow \infty.$$

Observe that the limiting value of bacterial biomass depends on the initial conditions S_0 and N_0 .

Figure 2 shows a typical solution using the data (3) with initial conditions $S(0) = 2 * a \text{ gm/l}$ and $N(0) = 0.25 * a * \gamma \text{ gm/l}$. The time scale is in hours on the horizontal axis; on the vertical scale the dimensionless quantities S/a and $N/a\gamma$ are plotted.

Equations (4) ignore the fact that bacterial cells require energy just to maintain cellular machinery and replace degraded proteins, even when they are not dividing. This “maintenance energy” is often modeled by assuming that the per unit mass maintenance energy is a constant m , resulting in the term $-mN$ being added to the second equation. The result is

$$\begin{aligned} \frac{dS}{dt} &= -\frac{1}{\gamma} N \frac{rS}{a+S} \\ \frac{dN}{dt} &= N \frac{rS}{a+S} - mN \end{aligned} \tag{6}$$

The maintenance term ruins our nice argument above which lead us to be able to solve for S in terms of N . However, as S is monotonically decreasing, it can be shown (Poincaré-Bendixson Theorem-there are no periodic orbits) that every solution converges to a steady state. Therefore, N converges monotonically to zero.

Figure 3 shows a typical solution using the data (3) and $m = 0.25$ with initial conditions $S(0) = 2 * a \text{ gm/l}$ and $N(0) = 0.25 * a * \gamma \text{ gm/l}$. The time scale is in hours on the horizontal axis; on the vertical scale the dimensionless quantities S/a and $N/a\gamma$ are plotted.

1.2 Chemostat

The chemostat is a well-stirred vessel of volume V with inflow F (liter/hour) of culture medium and fresh nutrient at concentration R (gms/liter) and with outflow of the well-mixed contents of medium, unused nutrient and bacteria, also at flow rate F so that the volume of fluid in the chemostat does not change. See figure 5. We wish to write differential equations for the concentration S of nutrient (gms/liter) and the concentration of bacteria N

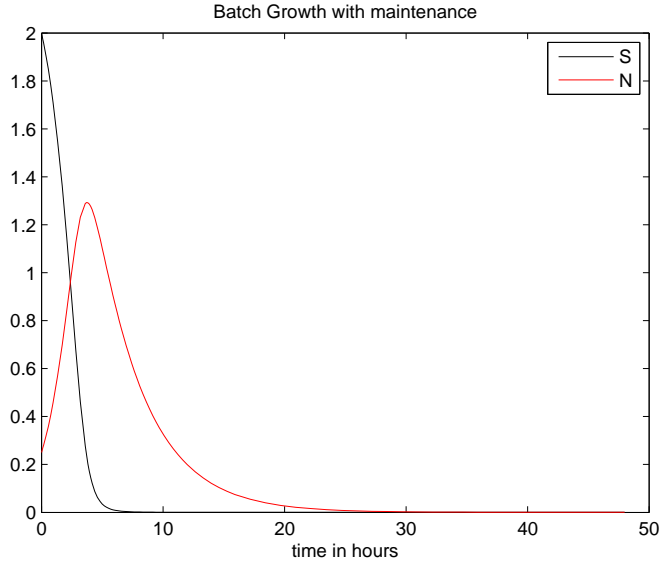


Figure 3: A solution $S(t)$ and $N(t)$ of equation (6)

(gms/liter) in the chemostat. It is helpful to deal with mass rather than concentrations in deriving the equations. The quantity VS gives the mass (gms) of nutrient and VN the mass of bacteria. VS gains due to fresh nutrient flowing in at rate FR gms/hour and loses due to unused nutrient flowing out at rate FS gms/hour and to the conversion of nutrient to microbial biomass at rate $VN \frac{1}{\gamma} \frac{rS}{a+S}$. Microbial mass VN increases due to growth at rate $VN \frac{rS}{a+S}$ gms/hour and decreases due to washout of biomass at rate FN gms/hour. Mathematically, this is expressed as the system of differential equations

$$\begin{aligned}
 VS' &= F(R - S) - \frac{1}{\gamma} VN \frac{rS}{a + S} \\
 VN' &= VN \frac{rS}{a + S} - FN
 \end{aligned}
 \tag{7}$$

Parameter

$$D = F/V$$

is called the dilution rate for the chemostat. If the chemostat were empty and we started to fill it with water at flow rate F then we'd fill it to volume V in $V/F = 1/D$ hours. Similarly if the chemostat were filled it would take time $1/D$ to empty it. For this reason, $1/D$ is the **mean residence time** of

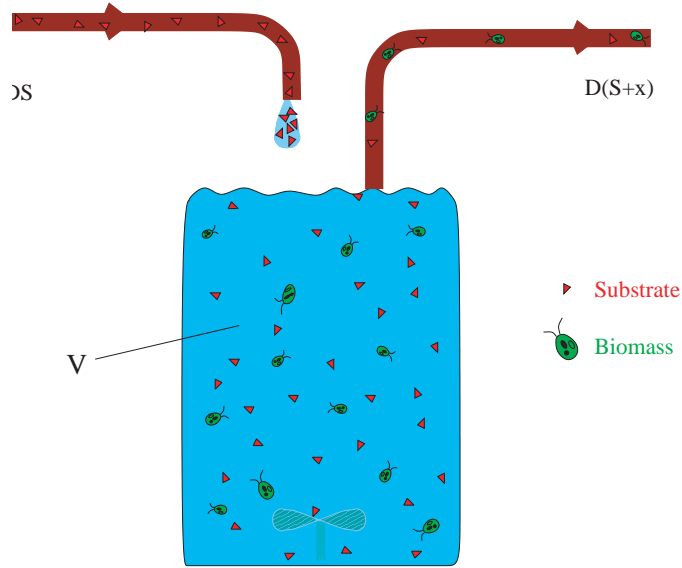


Figure 4: The chemostat: here x denotes our N .

a molecule or bacterial cell in the chemostat. Dividing out the volume V in the equations above, we get the chemostat equations

$$\begin{aligned} S' &= D(R - S) - \frac{1}{\gamma} N \frac{rS}{a + S} \\ N' &= N \left[\frac{rS}{a + S} - D \right] \end{aligned} \quad (8)$$

which require initial conditions:

$$S(0) = S_0, \quad N(0) = N_0$$

System (8) comes with five parameters D, R, γ, r, a , the first two of which can be determined by the experimenter while the last three are specific to the particular organism.

Equilibrium solutions satisfy

$$\begin{aligned} 0 &= D(R - S) - \frac{1}{\gamma} N \frac{rS}{a + S} \\ 0 &= N \left[\frac{rS}{a + S} - D \right] \end{aligned} \quad (9)$$

There are no more than two.

The washout equilibrium

$$S = R, \quad u = 0$$

always exists.

If $r > D$ then the equation

$$\frac{rS}{a+S} = D$$

has a unique positive solution

$$S = \lambda := \frac{aD}{r-D}$$

N can be determined by setting $\frac{rS}{a+S} = D$ in the first equation yielding the equation

$$0 = D\left(R - \lambda - \frac{1}{\gamma}N\right)$$

Solving for N gives

$$N = \frac{R - \lambda}{\gamma}$$

which is positive provided that $R > \lambda$, or equivalently since the function $S \rightarrow \frac{rS}{a+S}$ is increasing, provided

$$\frac{rR}{a+R} > D \tag{10}$$

We put $S = R$ and $S = \lambda$ into the function to get (10). The inequality (10) has a nice interpretation.

In summary, if (10) is satisfied, there is “survival equilibrium” given by

$$S = \lambda := \frac{aD}{r-D}, \quad N = \frac{R - \lambda}{\gamma}. \tag{11}$$

We are going to establish the following result.

Theorem 1 *If*

$$\frac{rR}{a+R} \leq D$$

then $(S(t), N(t))$ converges to the washout equilibrium as $t \rightarrow \infty$. If (10) holds, then $(S(t), N(t))$ converges to the survival equilibrium provided that $N(0) > 0$.

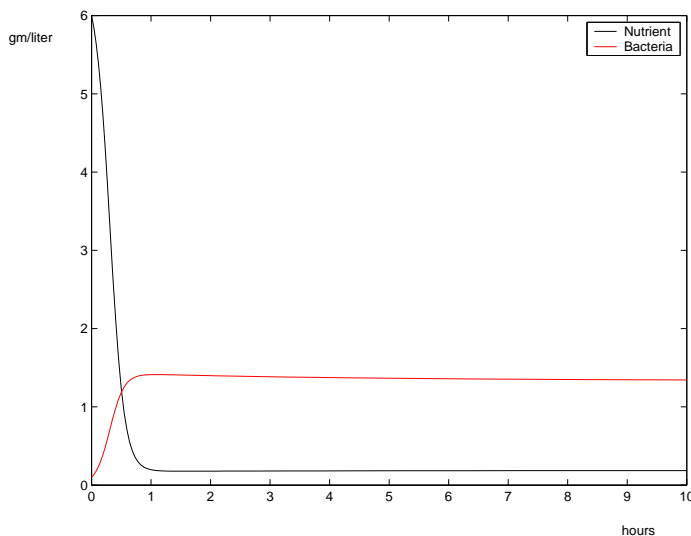


Figure 5: A solution $S(t)$ and $N(t)$ of equations (8)

Figure 4 depicts a solution of (8) with data as in (3) and with $D = 0.25$, $R = 6$ with initial conditions $S(0) = 6$ and $N(0) = 0.1$.

Monod developed the theory of the chemostat but the name chemostat is attributed to the paper of Novick and Szilard [4].

Some interesting features of microbial growth in a chemostat are:

1. The bacteria cannot survive in the chemostat unless the dilution rate is less than the maximal growth rate r and the concentration R of nutrient in the feed supply exceeds the value λ . For this reason, λ is referred to as the “breakeven” concentration since at $S = \lambda$, the growth rate exactly matches the washout rate D .
2. Unlike the Logistic equation, the Monod equations give a clear mechanism for the deceleration of the growth rate, namely nutrient depletion.
3. For any growth rate $g \in (0, r)$, we can choose control parameters D and R so that, at the survival equilibrium for the chemostat, the bacteria grow exponentially at rate g . Simply choose $D = g$ and select R so that (10) holds.
4. The survival equilibrium is completely independent of the initial conditions S_0 and N_0 . The system “forgets” its initial conditions. Contrast

this with batch growth discussed in the previous section.

5. The chemostat can be viewed as a laboratory model of a waste water treatment plant where microbes are used to break down wastes, of a lake containing algae, fed and drained by streams, and of an industrial fermenter in which genetically engineered organisms produce desired products such as vaccines, hormones, antibodies and enzymes. A thorough understanding of microbial growth in the chemostat translates to better understanding of these natural environments or industrial processes.

An engineer might wish to maximize the flux of bacteria exiting the chemostat. The flux F is given by

$$F = DN = D \frac{R - \lambda}{\gamma} = \frac{D(a + R)}{\gamma(r - D)} \left[\frac{rR}{a + R} - D \right]$$

where $0 \leq D \leq \frac{rR}{a+R}$. If R is fixed but the engineer can adjust D in this interval, maximize the flux. Note that the flux is zero for D at both end point of the interval, guaranteeing that a positive maximum occurs at an intermediate dilution rate.

We now give arguments in support of the Theorem stated above. It is convenient to introduce some notation to simplify our computations. We let

$$f(S) = \frac{rS}{a + S}.$$

We want to determine the stability of the two equilibria. First, we compute the jacobian of the right hand side of (8) at a general point (S, N) :

$$\begin{bmatrix} -D - \frac{f'(S)N}{\gamma} & -\frac{f(S)}{\gamma} \\ Nf'(S) & f(S) - D \end{bmatrix}$$

For the washout equilibrium, we put $S = R$ and $N = 0$ to obtain the matrix

$$\begin{bmatrix} -D & -\frac{f(R)}{\gamma} \\ 0 & f(R) - D \end{bmatrix}$$

which has eigenvalues $-D$ and $f(R) - D$. If $f(R) < D$, then the washout equilibrium is asymptotically stable. But if (10) holds, then $f(R) > D$ so the washout equilibrium is an unstable saddle point.

For the survival equilibrium, we use (11) to get

$$\begin{bmatrix} -D - \frac{f'(S)N}{\gamma} & -\frac{f(S)}{\gamma} \\ Nf'(S) & 0 \end{bmatrix}$$

It has a negative trace and positive determinant since $f'(S) > 0$ so the survival equilibrium is asymptotically stable whenever it exists.

If we multiply the first of equations (8) by γ and add to the second, we find that

$$(\gamma S + N)' = D[\gamma R - (\gamma S + N)]$$

which we can integrate to find $\gamma S + N$

$$\gamma S(t) + N(t) = R\gamma + (\gamma S_0 + N_0 - R\gamma)e^{-Dt}.$$

We see that $\gamma S(t) + N(t) \rightarrow R\gamma$ as $t \rightarrow \infty$. Since $S(t) \geq 0$ and $N(t) \geq 0$, this proves that solutions are bounded and approach the line $\gamma S(t) + N(t) = R\gamma$ in the (S, N) -plane. Notice also that if the initial data (S_0, N_0) lie on the line, then the solution remains on the line for all t ! The line

$$\gamma S(t) + N(t) = R\gamma \tag{12}$$

is invariant. We can study solutions that start (and hence stay) on the line by solving for $S = R - \frac{N}{\gamma}$ and substituting this into the N equation to get

$$N' = N \left[f \left(R - \frac{N}{\gamma} \right) - D \right]$$

where, of course, $\frac{N}{\gamma} < R$ in order that $S \geq 0$. Since f is increasing, it is easy to see that if $f(R) \leq D$, then solutions with $0 < N_0 < R\gamma$ converge to zero. On the other hand, if $f(R) > D$, then $f \left(R - \frac{N}{\gamma} \right) - D$ is positive for $N < \frac{R-\lambda}{\gamma}$ and negative for $N > \frac{R-\lambda}{\gamma}$. Hence $N(t) \rightarrow \frac{R-\lambda}{\gamma}$ as $t \rightarrow \infty$ if $N_0 > 0$.

If $f(R) \leq D$ then there is only the washout equilibrium. There can be no periodic orbits (how could one lie on a line?) so every solution converges to the washout equilibrium by the Poincaré-Bendixson Theorem.

If $f(R) > D$, then both equilibria exist but only the survival equilibrium is stable (it is asymptotically stable). Again, there can be no periodic orbits since every solution trajectory approaches the line (12) in the (S, N) -plane.

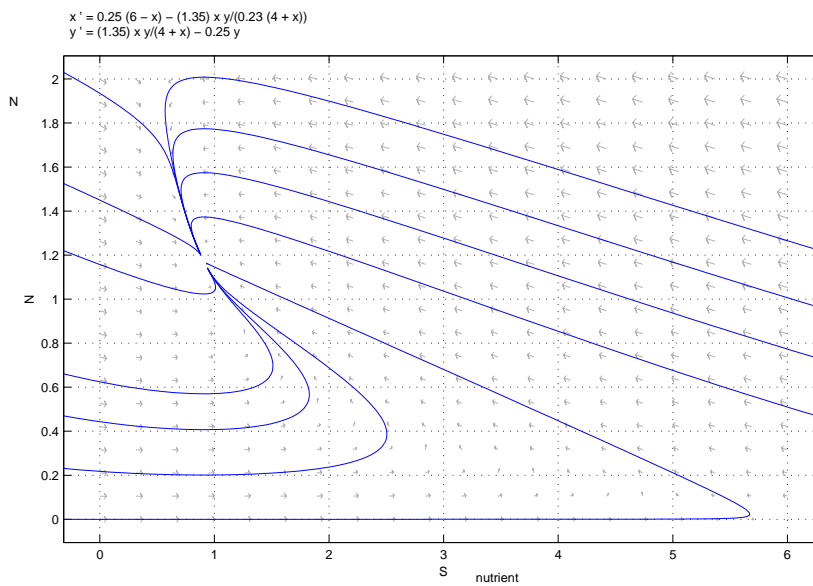


Figure 6: Direction Field and some trajectories of equations (8)

The only trajectories that can converge to the washout equilibrium belong to the S -axis. The Poincaré-Bendixson Theorem implies that all solutions with $N(0) > 0$ converges to the survival equilibrium.

Figure 5 depicts some trajectories in the phase plane in case $f(R) > D$.

References

- [1] J.P.Grover, Resource Competition, Population and Community Biology Series 19, Chapman and Hall, New York, 1997.
- [2] A.L. Koch, J.A. Robinson, G.A. Milliken, Mathematical Modeling in Microbial Ecology, Chapman & Hall, New York 1998.
- [3] J. Monod, Recherches sur la croissance des cultures bacteriennes. Paris:Herman, 1942.
- [4] A. Novick and L. Szilard, Description of the Chemostat, Science 112(2921), 715-6.

- [5] N.S. Panikov, *Microbial Growth Kinetics*, Chapman & Hall, London, 1995.
- [6] H.L. Smith and P. Waltman, *The Theory of the Chemostat*, Cambridge University Press, Cambridge, UK, 1995.
- [7] D. Tilman, *Resource Competition and Community Structure*, Princeton University Press, Princeton, 1982.