## 4 The growth of yeast

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## 4.1 Description of the system

Growth is only exponential as long as the relative growth rate remains constant. This is usually so with yeast when it is grown under aerobic conditions with a sufficient supply of sugar and some other growth essentials. The sugar is then continuously consumed to provide the 'C skeletons' and the energy for the growth of new yeast cells and for maintenance of the yeast. The end-products,  $CO_2$  and  $H_2O$ , of the sugar broken down in the respiratory process do not pollute the environment of the yeast. However, if yeast grows under anaerobic conditions, one end-product of the respiratory processes is alcohol which may accumulate in the environment. This slows down and ultimately stops the development of yeast buds even when there is still enough sugar available for growth.

Growth curves for yeast that result under such conditions are given in Figure 4.1.



Figure 4.1. The growth of *Saccharomyces cerevisiae* and *Schizosaccharomyces 'Kephir'* in monoculture and in mixture. The observational data were obtained by Gause (1934) and the curves are simulated, as explained in the text. Note the difference in scales for the two graphs.

It should be noted that yeast once formed does not die because only the bud formation is affected by the alcohol. Two of the four growth curves are from an experiment of Gause (1934) with monocultures of the yeast species *Saccharomyces cerevisiae* and *Schizosaccharomyces 'Kephir'*. It is obvious that the initial relative growth rate and the maximum volume of yeast that is ultimately formed is highest for the first

P. A. Leffelaar (ed.), On Systems Analysis and Simulation of Ecological Processes, 41–50. © 1993 Kluwer Academic Publishers. Printed in the Netherlands. species.

Gause cultivated both yeast species not only in monoculture, but also in mixture. The results of this experiment are also presented in Figure 4.1 by the other two curves. A comparison of the growth of both species in mixture with their growth in monoculture shows that both affected each other in the first situation. It was proposed by Gause that this was due to the formation of the same waste product, alcohol, that affected the bud formation of both species. In this chapter we shall analyse whether this explanation is acceptable by constructing a model that simulates the growth of two species independently and in mixture under the assumption that the production of the same harmful waste product is the only cause of interaction.

#### 4.2 **Relational diagram**

The relational diagram for the yeast system is presented in Figure 4.2. There are three state variables; the amount of the first and second yeast species and the amount of alcohol. The lines of information flow show directly that the growth of yeast is supposed to depend on the amount of yeast, a relative growth rate and an auxiliary variable: a reduction factor. This reduction factor, in its turn, is given as a function of the amount of alcohol that is present. The relations are, of course, the same for both yeast species although numerical values of parameters and functions may be different.



The amount of alcohol increases by the rate of alcohol production of both species. The alcohol production of each species is supposed to depend on the growth rate of the species and on an alcohol production factor.

## Exercise 4.1

In Section 2.5 it is said that rates do not depend on each other in state determined systems. Why is the line of information flow between the rate of growth and the rate of alcohol production not in contradiction with this principle?

Relational diagrams should contain as few details as possible, otherwise they are very difficult to grasp and so defeat their purpose. In studying them, much emphasis should be given to aspects that are not incorporated. For instance, in the present scheme there are no loops that relate the alcohol production directly to the amount of yeast, indicating that the cost of maintenance of yeast cells is not accounted for. The amount of sugar is also not considered, because it is assumed to be always available in sufficient amounts.

## Exercise 4.2

Incorporate the aspect of limited food supply in the relational diagram.

#### Exercise 4.3

Compare the relational diagram of the continuous yeast culture fed by a sugar solution (Figure 2.9) with the one for the growth and interference of two interfering yeast species (Figure 4.2), and note two principal differences between the models.

## 4.3 Simulation

The growth of the first yeast species (Saccharomyces) is now simulated by stating that the amount of yeast equals

$$Y1 = INTGRL (IY1, RY1)$$
(4.1)

in which INCON IY1 = 0.45 is the initial amount of yeast in the arbitrary units, used by Gause, and the rate of yeast growth is given by

RY1 = RGR1 \* Y1 \* (1. - RED1) (4.2)

The relative growth rate is defined with PARAMETER RGR1 = .....

It was observed by Gause that in both species the formation of buds was completely stopped at some maximum alcohol concentration which is given as a percentage by PARAMETER MALC = 1.5. The dependence of the reduction factor on the alcohol concentration may now be obtained with an arbitrary function generator: RED1 = AFGEN(RED1T (ALC/MALC)). The most elementary assumption is that bud formation decreases linearly with increasing alcohol concentration, which is introduced with FUNCTION RED1T = (0,0,),(1,.1).

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Exercise 4.4

Express RED1 directly in ALC and MALC without using the function generator.

The alcohol concentration itself is the integral of the alcohol production rate which is zero at the initialization of growth:

$$ALC = INTGRL (IALC , ALCP1)$$
 (4.3)  
INCON IALC = 0.

and the alcohol production rate is proportional to the growth rate of yeast:

$$ALCP1 = ALPF1 * RY1$$
 (4.4)

Two values need to be determined now: the relative growth rate and the alcohol production factor. During the early stages of growth, RED1 is practically zero, so that the growth rate is equal to RGR1 • Y1. This allows a first estimate of RGR1 from the data in Figure 4.1 for the monoculture. ALPF1 follows from the observation that growth was terminated when the alcohol concentration equalled 1.5 percent and the amount of yeast about 13 units.

Exercise 4.5

AT C

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- a. What is a first estimate of RGR1 in the correct units, and how would you estimate the time step of integration?
- b. What is the value of ALPF1 in the correct units?
- c. Is this value only physiologically determined or does it also depend on the volume of water in the vessels with yeast?
- d. What is the value of IALC when not only the initial amount of yeast is introduced at initialization, but also the corresponding amount of alcohol?
  e. Estimate the same values for 2 states.
- e. Estimate the same values for Schizosaccharomyces, when it is known that the alcohol concentration at which the formation of buds is completely inhibited is also 1.5 percent.
- f. Which species has the larger alcohol production factor?

The structural equations that describe the growth of the second species (*Schizosaccharomyces*) are, of course, the same as those for the first, so that in a model for concurrent growth it suffices to write them twice: once with a 1 at the end of the relevant symbols and once with a 2. The equation that describes the alcohol concentration now becomes

ALC = 
$$1N'IGRL$$
 (IALC, ALCP1 + ALCP2) (4.5)

This equation holds on the condition that both species interfere only with each other through the production of alcohol.

Listing 4.1 shows the resulting simulation program with MALC identical for both species and the proper data. In the main program IY1 and IY2 are both set to 0.45 units, so that the growth in the mixture is simulated. The two monocultures are simulated in reruns.

FINTIM is set at 150 hours, but the two lines

FINISH ALC = LALC LALC = 0.99 \* MALC

are inserted to avoid unnecessary 'number-grinding', when the alcohol concentration is close to its maximum. This FINISH condition indicates that the simulation is terminated as soon as the alcohol concentration reaches 99 percent of its maximum value.

The relative growth rates and the alcohol production factors are chosen so that the results of the two experimental monocultures are matched as well as possible. A comparison of the mixtures (Figure 4.1) shows that the actual growth of *Schizosac*-charomyces is slightly more than the simulated growth.

Listing 4.1. A simulation program for the growth of two yeast species that interfere through the production of the same waste product (alcohol).

```
TITLE Mixed culture of yeast
INITIAL
INCON
          IY1
                 =0.45, IY2 =0.45, IALC =0.0
PARAMETER RGR1
                =0.21, RGR2 =0.06
PARAMETER MALC =1.5 , ALPF1=0.12, ALPF2=0.26
FUNCTION RED1T = (0.0, 0.0), (1.0, 1.0)
FUNCTION RED2T = (0.0, 0.0), (1.0, 1.0)
TIMER
          FINTIM=150., DELT=0.5, OUTDEL=2.0
OUTPUT
          Y1, Y2, ALC
PAGE
          GROUP = 2
METHOD
          RECT
LALC = 0.99*MALC
DYNAMIC
Y1
                                       )
          =INTGRL(IY1 , RY1
Y2
          =INTGRL(IY2, RY2
ALC
          =INTGRL(IALC, ALCP1 + ALCP2)
RY1
          =RGR1*Y1*(1.0-RED1)
RY2
          =RGR2*Y2*(1.0-RED2)
ALCP1
          =ALPF1*RY1
ALCP2
          =ALPF2*RY2
RED1
          =AFGEN (RED1T, ALC/MALC)
RED2
          =AFGEN (RED2T, ALC/MALC)
FINISH ALC=LALC
END
STOP
ENDJOB
```

Barring statistical insignificance, we must conclude that both species do not interfere with each other's growth through the production of alcohol only, as assumed in the model. It may be that *Schizosaccharomyces* produces some other waste product that is harmful for the other or that *Saccharomyces* produces a waste product that stimulates the other. These possibilities cannot be distinguished from each other without additional information. And as long as this is not available it is a futile exercise to simulate such suppositions.

These simulation programs are conveniently amended. For instance, the yeast cultures may be washed continuously with water that contains sufficient sugar.

### Exercise 4.6

a. Try to reason whether a similar effect could result from the supposition that the reduction functions for the species would not be given by

FUNCTION REDIT = (0., 0.), (1., 1.)FUNCTION RED2T = (0., 0.), (1., 1.)

but by, for instance:

FUNCTION REDIT = (0.,0.), (0.5,0.75), (1.,1.) (Saccharomyces) FUNCTION RED2T = (0.,0.), (0.5,0.25), (1.,1.)

(Schizosaccharomyces)

(4.6)

If this is too difficult, you may find the answer by simulation.

b. Try to reason why we should not proceed this way, and what way of tackling the problem would be more appropriate. Also reconsider Section 1.2 in this respect.

## Exercise 4.7

How would you reformulate the rate of change in the integral of the alcohol concentration, Equation 4.5, if the yeast cultures would be washed continuously with water that contains sufficient sugar?

Exercise 4.8

- Which type of system is represented by the mixed culture of the yeasts а. Saccharomyces and Schizosaccharomyces? (heuming Hepliable) Unique )
- b. Which type of model is represented by the FUNCTION RED1T  $\leq (0.,0.)$ , (1.,1.)? c. Can the yeast growth model 'Mixed culture of yeast' be called an 'explanatory dynamic model"? Explain your answer.

#### 4.4 Logistic growth

The form of the differential equation for the present problem will now be derived from the structural equations of the simulation program, but only for situations where the reduction factor is proportional to the alcohol concentration so that (1.-RED) may be replaced by (1.-ALC/MALC). Since the alcohol concentration is equal to the integral of the rate of change of yeast times the alcohol production factor, according to the Equations 4.3 and 4.4, it is then possible to rewrite Equation 4.2 in

$$dY/dt = RGR \cdot Y \cdot (1 - Y/Y_m)$$

in which Y is the amount of yeast, t is the time and  $Y_m$  stands for the maximum amount of yeast. This equation may be integrated and then becomes

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. . . . .

$$Y = \frac{Y_{\rm m}}{1 + K \cdot e^{-RGR \cdot t}}$$

Exercise 4.9

- Express Ym in MALC and ALPF. a.
- What are the values of Ym for both species of yeast? b.
- Show by differentiation that Equation 4.7 is an integrated form of Equation 4.6. c.
- Express the initial amount of yeast in the constant K and  $Y_m$  of Equation 4.7. d.
- Calculate the time course of the growth of Saccharomyces and compare the e. result with the simulated course.
- Why does the differential equation only hold for situations where the initial f. amount of yeast is very small, whereas the simulation program is generally valid?

The growth curve that is described by the differential equation and also presented by the simulated growth curves for the monoculture of yeast in Figure 4.1 is called the logistic growth curve. This S-shaped curve is symmetrical, but this symmetry hinges on the assumption of proportionality between the reduction factor of growth and the amount of growth that has been made. Especially Lotka (1925) and Volterra (1931) generalized the logistic differential equation for interfering species with the following set of differential equations:

$$dY_1 / dt = R_1 \bullet Y_1 \bullet (1 - A_1 \bullet Y_1 - B_1 \bullet Y_2)$$

$$dY_2 / dt = R_2 \bullet Y_2 \bullet (1 - A_2 \bullet Y_1 - B_2 \bullet Y_2)$$
(4.8)

In general this set of differential equations cannot be integrated into analytical expressions for  $Y_1$  and  $Y_2$  as functions of time and therefore it is wiser to leave such simplifying approaches alone and to formulate the problem directly in terms of a simulation model to study the dynamic behaviour.

#### Exercise 4.10

- Show to what extent the simulation model for mixed growth of yeast is covered by а. this set of differential equations.
- Express the constants  $R_1$ ,  $R_2$ ,  $A_1$ ,  $A_2$ ,  $B_1$  and  $B_2$  in the constants RGR1, b. RGR2, ALPF1, ALPF2 and MALC.
- Which constants of the differential equations are the same? C.
- Do they remain the same in situations where a species produces a waste product d. which is more harmful for the other species than it is for itself?

The equilibrium situation, however, i.e. the situation where the rates dY/dt in Equation 4.8 are zero, can be calculated similarly to the case of the continuous yeast culture fed by a sugar solution (Section 2.6). Figure 4.3 shows the equilibrium lines with  $Y_1$  and  $Y_2$  along the axes. Left of the lines the reduction factors, e.g.  $(1 - A_1 \cdot Y_1 - B_1 \cdot Y_2)$ , are positive. One can easily investigate whether an equilibrium is stable or unstable. Stability is defined as follows: when a system in equilibrium is disturbed and there is a reaction of that system that is directed towards the equilibrium value, the system is stable. If, however, the reaction of the system is directed

(4.0)



Figure 4.3. Competition between two species according to the equations of Lotka-Volterra (Equation 4.8). Full lines distinguish the areas of positive and negative growth rates. In the horizontally hatched area the growth rate of species  $Y_1$  is positive, in the vertically hatched area the growth rate of species  $Y_2$  is positive. Both growth rates equal zero where the lines cross. There, equilibrium exists.

away from the equilibrium after the disturbance, the system is unstable. For a thorough treatment of stability, the reader is referred to May (1973) and Edelstein-Keshet (1988).

Exercise 4.11

- a. Will there exist a stable or an unstable equilibrium according to Figure 4.3?
   b. Make a new figure where the application of the applicat
- b. Make a new figure where the equilibrium is opposite to the one in a).
- c. What is the ecological basis of these differences?

# 4.5 Summary and steps in model development

Experimental results from the literature (Gause, 1934) were analysed in terms of the qualitative (relational diagrams) and quantitative (differential equations, etc., and model building) methods introduced in Chapters 1 to 3.

It was illustrated that sometimes a rate is (stochiometrically) related to another rate. However, it should be born in mind that *mutual* dependance of rates should not occur in models: it would point to a violation of the hypothesis in systems analysis that the state of every system at every moment can be quantitatively characterized and that changes in a system can be described by means of mathematical equations.

The example clearly illustrates how models can and should be developed ideally, namely by separate calibration of model parameters on experimental results (here: the data of the monocultures of the species Saccharomyces cerevisiae and Schizosacchaspecies is affected by the formation of the hypothesis (here: that the growth of each results of the model for mixed growth is compared to fully independent experimental model. The procedure sketched is obviously iterative. It is summarized in Figure 4.4.



Figure 4.4. Model development for individual species, calibration of model parameters, coupling of the separate models through the hypothesis that is to be tested, and validation of the resulting mixed species model with fully independent experimental data for the mixed culture of the yeast species *Saccharomyces cerevisiae* and *Schizosaccharomyces 'Kephir'*.

Finally, from the differential equations of the example of 'mixed culture of yeast' the logistic growth equation was derived, that in its turn was generalized for interfering species according to Lotka and Volterra.

Three main phases in the development of models can be distinguished: model conceptualisation, programming and evaluation. Each phase may be elaborated in a number of steps.

For the conceptualisation phase, the following steps are noteworthy:

- definition of the problem;
- definition of the purpose or objectives of the study;
- definition and/or assessment of the boundaries of the system;
- choice of the level of detail to be considered, or choice of problem complexity (this strongly interacts with the objectives);
- development of qualitative relationships between system elements through e.g. relational diagrams. Here, the choice of the state variables and possible feedback loops become clear:
- development of model equations (differential equations, auxiliary equations, and forcing functions);
- explicit statement of model assumptions that underlie the individual model equa-

tions and the model as a whole.

Having arrived at this point one should be able to judge if it is still necessary to develop the full mathematical model: sometimes the conceptual model developed so far is sufficient to satisfy the objectives of the study.

For the model programming phase, the following steps are noteworthy:

- choice of the system of units for the different processes in the model (e.g. mol or g, m or cm, s or hour, etc.);
- grouping of the different processes in submodels (e.g. subroutines, see Section 7.3);
- writing the submodels and the main model;
- assessment of the time coefficients of the model equations;
- assessment of data that are necessary to parameterize the model (the number of parameters is strongly dependent on the level of detail considered and thus also depends on the objectives);
- assessing model integrity: does the model correctly represent the mathematical equations? This can be checked to a large extent by a dimensional analysis and by including material balances (conservation of mass).

In the model evaluation phase, the following steps are noteworthy:

- experimenting with the model: choice of parameter values (literature, new experiments), calibration, sensitivity analysis both with respect to model structure (model reaction on different model equations that could describe the same process) and model parameters (model reaction on changes in parameter inputs within the range of their uncertainty), judgement of model output, validation with independent experimental data on the level of the system as an entity;
- assessment of the model assumptions (these are interconnected with the assumptions made in the derivation of the equations);
- drawing conclusions from model behaviour with respect to the real system;
- documentation of the model, both with respect to technical aspects (list of abbreviations of symbols, correspondence of computer mnemonics with mathematical equations, description of the different routines) and to scientific aspects. The latter is usually not the problem, but the first is hardly ever done and thus needs attention;
- sometimes: simplification of the model, based on the increased physiological, chemical, physical and mathematical insight.

Though such enumerations can never be exhaustive, it reflects what we feel as the major steps in model development and it provides some insight in the skills a modeller should develop. Among other things it is clear that the modeller should endeavor a sound interaction between theoretical and experimental work.