SHORT COMMUNICATION

Modelling of Nutrient Gradients in a Bacterial Colony

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Oxygen and substrate gradients were simulated with a finite-volume method for a variety of bacterial colony sizes and substrate concentrations. A spherical segment geometry and a Monod relationship for simultaneous substrate and O₂ consumption were assumed. The results agreed well with previous theoretical and experimental estimates for gradients in microbial colonies.

INTRODUCTION

A microbial colony arises from one or a few cells. Modelling of this evolving ecosystem is complicated because of ranges in metabolic states and morphology, and colony growth depends on diffusion rates of essential nutrients.

Pirt (1967) considered physical and chemical factors affecting colony growth on solid media. Using data from macroscopic observations, his theory estimated that O₂ tension would be low at a depth of about 40 µm in an active colony. Wimpenny & Lewis (1977) measured the actual O₂ consumption rate for bacterial colonies via respirometry. Treating these data with the assumption of a spherical segment, the calculated thickness of the aerobic 'shell', or penetration depth of O₂, was 30–40 µm, depending on the bacterial species. Aerobic activity diminished as the colony aged. Wimpenny (1979) showed that with *Escherichia coli*, *Staphylococcus albus* and *Bacillus cereus* the spherical segment is a good approximation of colony shape.

Wimpenny & Coombs (1983) measured O₂ gradients in *B. cereus* colonies growing on agar and found contours similar to those observed in *Pseudomonas ovalis* colonies by Bungay *et al.* (1983). In general, steep O₂ gradients occurred near the colony surfaces, unless there was too little organic nutrient to create a strong demand. The extent of O₂ penetration varied with substrate concentration. The interior of colonies grown on dilute media showed abundant dissolved O₂, while those grown with supplemental glucose had significant regions of low O₂ tension (Bungay *et al.*, 1983). In this study we have used computer simulations to facilitate the analysis of O₂ contours for respiring organisms and to aid the design of experiments.

METHODS

A typical microbial colony is several millimetres in diameter but nutrients such as O₂ and glucose can diffuse through it in a few minutes. Based on relaxation times (e.g. Roels, 1983), a pseudo-steady state exists for nutrient gradients within the colony. The use of an unstructured kinetic model for nutrient uptake is appropriate in a computer simulation of discrete colony states.

The classic model of Monod (1942) is reliable for a single limiting nutrient; however, a colony system has two counterdiffusing limiting nutrients (substrate and O₂). Many biokinetic studies use a double Monod form (Bader, 1978), but this model has no experimental validation and is inconsistent when both nutrients are in short supply. In our simulation, uptake rates at a location within the colony were found from local determinations of the most limiting nutrient. This allowed selection of the lowest rate from two separate Monod expressions. A yield coefficient set the uptake rate of the excess species. Table 1 lists values for the parameters used in the simulation. These were averaged from various literature sources (Haas, 1981; Bungay *et al.*, 1969; Wimpenny, 1979). Determination of local kinetic parameters within colonies has not yet been reported, but spatial variation is likely.

Table 1. *Kinetic parameters and nutrient uptake*

Parameter	Value
Specific colony growth rate, μ_m	0.15 h ⁻¹
Biomass yield on substrate, Y_s	0.5 g (g substrate) ⁻¹
Biomass yield on oxygen, Y_{O_2}	1.5 g (g O ₂) ⁻¹
Dry cell density, ρ_x	1.1 g ml ⁻¹
Monod coefficient for substrate, K_s	4 × 10 ⁻⁶ g ml ⁻¹
Monod coefficient for O ₂ , K_{O_2}	4 × 10 ⁻⁶ g ml ⁻¹
Effective diffusivity of substrate, D_s	5 × 10 ⁻⁷ cm ² s ⁻¹
Effective diffusivity of O ₂ , D_{O_2}	2 × 10 ⁻⁶ cm ² s ⁻¹

As a first approximation, we assumed that the parameters in Table 1 do not change with position in the colony.

A well-defined system is important for the early stages of modelling. A major simplification was to define our system as a colony growing on a membrane over a well-mixed liquid medium so that the glucose concentration could be assumed constant at the base of the colony. Glucose diffuses up into the colony while O₂ diffuses in through its exposed surface. Diffusion of O₂ through the membrane was ignored in our simulation.

The numerical algorithm using a finite-volume method derived from the calculus of finite differences was advocated by Patankar (1980) on the grounds of simple physical interpretation. We modelled a colony as a segment of a spherical volume characterized by a certain height and radius. A subroutine connected our procedures to a packaged program available at Rensselaer Polytechnic Institute (RPI) for finite-volume analysis. The subroutine used the colony dimensions to calculate a differencing scheme in spherical coordinates, and it provided boundary conditions, the kinetic model, and the parameters in Table 1. All calculations were made on RPI's IBM 3081D mainframe computer. Details of the finite-volume subroutine in FORTRAN can be obtained from the authors.

RESULTS

Simulations were done with several combinations of colony size and external substrate concentration. Convergence of the numerical routines was rapid. Fig. 1 shows a series of simulated dissolved O₂ profiles for a colony 110 µm high and 5 mm radius. The varied parameter was external substrate (glucose) concentration (50, 500 and 5000 mg l⁻¹), i.e. a range from limiting to excess supply at the colony surface. The contours for substrate are shown in Fig. 2. O₂ was abundant where substrate was scarce, and became depleted (with steep gradients) where it was plentiful.

DISCUSSION

The contours in Fig. 1 are similar to contours from microelectrode studies. As external substrate concentration increases, there is a transition from a predominantly aerobic environment to one with significant anoxic regions. The penetration depth of O₂ in the presence of excess substrate (Fig. 1c) was 30–40 µm, which agrees with previous findings (Pirt, 1967; Wimpenny & Lewis, 1977). Simulations with smaller colonies showed an equivalent penetration depth (data not shown), hence smaller colonies have a larger proportion of aerobic activity. Increasingly anoxic environments in larger colonies probably contribute to toxic metabolite accumulation and eventual cessation of growth.

At present there are no experimental data to compare with the results shown in Fig. 2. Glucose microelectrodes have been constructed, but there are problems with stability and response time. Improved glucose sensors will reveal much more about colony growth.

Simulation of nutrient gradients is a first step in the development of a detailed colony growth model. Extending gradient analysis to the region of solid substrate below a colony is an important aim, but this requires a more elaborate numerical procedure that works with complicated geometry. We have had limited preliminary success with a finite-element implementation. Other aims are elucidation of kinetic and morphological parameters for colony systems. Factors that must be considered include spatial variation, product inhibition and the effects of age distribution. Computer simulations can have great value in organizing these experimental data and in exposing areas where further research is needed.

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Fig. 1. Simulated O_2 profiles for colonies of identical size exposed to a range of glucose concentrations: (a) 50 mg l^{-1} ; (b) 500 mg l^{-1} ; (c) 5 g l^{-1} . The solubility of O_2 was assumed to be 8.0 mg l^{-1} . Isopleth values are mg l^{-1} .

Fig. 2. Simulated glucose profiles for colonies of identical size exposed to a range of glucose concentrations: (a) 50 mg l^{-1} ; (b) 500 mg l^{-1} ; (c) 5 g l^{-1} . Isopleth values are g l^{-1} .

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